
Purpose:
- To evaluate protein expression of mutation specific, monoclonal anti-EGFR antibodies (SP111 for E746_A750 [exon 19 del], SP125 for L858R [exon 21 point mutation], Ventana; 85-90% of EGFR mutations).
- To correlate protein expression with gene mutations and clinical outcome

Methods:
- Surgical resection specimens from 240 patients with primary lung adenoCa; TMAs
- IHC scoring: 0-no or weak staining in <10% tumor cells (at 40x), 1-weak in ≥10% (40x), 2-moderate in ≥10% (10-20x), 3-strong in ≥10% (5x)
- EGFR mutation analysis by direct sequencing and/or pyrosequencing

Results:
- 52.9% men, 57.5% smokers, acinar predominant most prevalent
- EGFR mutation in 46.3% (55.9% 19del including 30.6% E746_A750 del; 41.4% L858R)
- Interobserver variability for E746_A750del-specific ab: κ=0.86 (if score ≥2 considered +), κ=0.95 (score 3+), κ=0.43 (all scores)
- Interobserver variability for L858R-specific protein ab: κ=0.73 (if score ≥2 considered +), κ=0.97 (score 3+), κ=0.48 (all scores)
- E746_A750 del-specific protein – cytoplasmic and/or membranous, + in 34 cases (16.7%); score 1 (14), 2 (16), 3 (10)
- L858R-specific protein – cytoplasmic and/or membranous, 2 cases nuclear staining, + in 58.3% (score 1 (83), 2 (38), 3 (19)
- Intratumoral heterogeneity in 3% (E746_A750 del) and 4.7% (L858R)
- Correlation protein expression - mutation:
  - E746_A750-mutated cases (n=34): IHC 3+ (29.4%), 2+ (41.2%), 1+ (23.5%), 0 (5.9%).
  - E746_A750-wt cases (n=28): IHC score 0 (93%), 1+ (4%), 2+ (4%); the cases with score 1+ & 2+ (n=2) had L747 T751 deletion in exon 19
  - L858R-mutated cases (n=46): IHC 3+ (41.3%), 2+ (39.1%), 1+ (13.0%), 0 (6.5%).
  - Sensitivity, specificity, PPV, and NPV for IHC (cutoff score 2): E746 A750 del: 70.6%, 99.0%, 92.3%, 95.3%. 19 del in total: 40.3%, 99.4%, 96.2%, 82.7%. L858R: 80.4%, 89.7%, 64.9%, 95.1%.
  - If score 3 is cutoff – specificity and PPV for both ab is 100%
- If IHC scores of 2 & 3 considered positive: No difference in outcome between IHC-EGFR+ and – groups and between EGFR mutant and wt groups. For EGFR wt group: patients with EGFR-IHC + had longer PFS (p=0.033); no statistical difference in multivariate analysis

Take Home Points:
• Both ab have high specificity (100% if score 3 is cutoff) but low sensitivity; in part because not all mutations are detected including some that might be susceptible to TKIs
• CAP guidelines no longer accept limited testing for 2 major mutations in clinical practice
• If IHC score 3+: Patient-candidate for EGFR-TKI treatment if tissue samples are unavailable for additional EGFR-mutational testing. All others need more tissue for mutation analysis

Background & Purpose:
- Pulmonary interstitial emphysema (PIE) is rare; air gains access to the pulmonary interstitium in a “lymphangitic” pattern and elicits a foreign body giant cell reaction. Most commonly associated with mechanical ventilation in preterm infants with RDS. Might lead to air leak, massive air embolism, chronic lobar emphysema. Rare in adults
- To examine lung explants for presence of PIE and correlate with clinical features

Methods:
- All bilateral pneumonectomy explants for lung transplantation
- At least 2 sections/lobe, each bronchus, representative LNs
- Histologic features of PIE: elongated or round, air-filled spaces of variable size and shape, distending interlobular septa and bronchovascular bundles. Spaces frequently lined by foreign body giant cells and histiocytes.

Results:
- 55 pts, double lung transplantation, mean age 54 (range, 19-70); 49% female
- UIP (26%), CTD (10%), COPD (15%), miscellaneous (9%), HP (9%), CF (8%), PHT (6%), NSIP (6%), sarcoid (2%)
- 19/55 (36%) showed PIE including 70% of UIP cases
- PIE-cases: No age predilection but male predominance (p=0.001)
- No difference in incidence of PIE based on hx of ventilator use (38% of patients with ventilator use had PIE vs 35% of patients without ventilator use)
- In cases of PIE-74% had hx of ventilator use or prior bx; in cases w/o PIE – 47% (p=0.061)
- PIE seen in all lobes, mostly in >1 location (6 cases only 1 location, 2 cases in all locations), most frequent in LUL, least frequent in RML
- Histologic features: elongated and/or angulated spaces, most commonly around bronchovascular bundles (Fig. 3). Cyst-like spaces lined by scant to prominent foreign body giant cells with macrophages, surrounded by variable fibrosis and eos (4 cases prominent eos)
- IHC in some cases (D2-40, CD68, keratin, SMA, CD34) – no complete epithelial and endothelial lining, and variable lymphatic endothelial staining
- PIE only mentioned in 3 path reports

Take Home Points:
- PIE is present in the adult explanted lung at relative high frequency (36%)
- PIE is most commonly associated with UIP
- No significant association with mechanical ventilation in adults (in contrast to infants)
- Mimics: granulomatous infection or granulomas of HP, congenital pulmonary lymphangiectasis, cystic lymphangioma, OP, fibroblastic foci of UIP
- Most patients have hx of ventilator use and/or prior bx
- **Importance of this finding? Do I miss it all the time?**

Purpose:
- To examine the mutational status of known oncogenic genes including EGFR, KRAS, HER2, BRAF, PIK3CA, AKT1, DDR2, ALK, RET in SQCC with <10% glandular component (SQCC with minor glandular component-SQCC-mGC) and SQCC w/o glandular component

Methods:
- SQCC diagnosis confirmed by H&E and IHC: Well-diff SQCC stained with TTF1 (clone 8G7G3/1), CK7; mod- poorly diff Ca stained with p63, CK5/6, TTF1, CK7.
- Positive TTF-1 or CK7 staining = mod-strong staining in <10% tumor cells. If >10% tumor cells stain with TTF-1 and/or CK7+ → case was excluded.
- Pure SQCC = diffuse p63 and/or CK5/6+ and TTF-1-CK7-. Molecular analysis by PCR

Results:
- 310 cases (95 SQCC-mGC, 215 pure SQCC)
- 61/95 (64.2%) SQCC-mGCs: TTF-1+ and/or CK7+; 34/95 (35.8%) TTF-1-CK7- (H&E diagnosis). 215 pure SQCCs - TTF-1-CK7-.
- 36/310 (11.6%) had mutations. 26/95 (27.4%) SQCC-mGC had 28 mutations: EGFR (n=10), KRAS (7), PIK3CA (3), EGFR/PIK3CA (1), KRAS/PIK3CA (1), BRAF (1), HER2 mutation (1), EML4-ALK fusions (1). 22/61 (36.1%) TTF-1+ and/or CK7+ cases had mutations: EGFR (n=8), KRAS (6), BRAF (1), HER2 (1), EGFR / PIK3CA (1), KRAS / PIK3CA (1), PIK3CA (2), EML4-ALK fusions (2). 4/34 (11.8%) TTF-1-CK7- SQCC-mGCs had mutations: EGFR (n=2), 1 KRAS (1), PIK3CA (1). 10/215 (4.7%) pure SQCC had mutations: PIK3CA (n=7), AKT1 (1), DDR2 (1), EGFR (1).
- SQCC-mGCs had significantly higher rate of mutations (27.4%) than pure SQCCs (4.7%). Significantly higher rate of EGFR mutations in women, never smokers, SQCC-mGC or in peripheral lung. Significant higher rate of KRAS mutations in SQCC-mGCs.
- Multivariate analysis: Minor glandular component = independent factor to predict EGFR mutations.
- No differences in RFS and OS between patients with EGFR mutation vs KRAS, PIK3CA, wt.
- 5 pts with SQCC received EGFR-TKI at time of relapse. Only the pt with EGFR mutation had partial response, patients with wt EGFR had stable disease or progression.

Take home points & Comments:
- SQCC-mGC appears relatively common (1/3 of all SQCC), some have targetable mutations
- Should minor glandular component be reported in SQCC?
- Mutations may only be present in glandular component (microdissection was not performed) – larger outcome studies are important to identify efficacy of targeted therapy in these pts
Purpose:
- To investigate the effect of the presence of a micropapillary (MP) or solid pattern on outcome in patients with invasive pulmonary adenoCa
- To determine clinical and radiologic predictors that could provide suggestions regarding the presence of a histopathologically proven MP or solid pattern

Methods:
- Completely resected solitary invasive lung adenoCa ≤3cm; no neoadjuvant therapy
- WHO, pattern according to IASLC/ATS/ERS proposal in 5% increments
- 511 patients (279 males), median age 61, median F/U 77 months (range, 10.1-255.8)
- 10% lepidic-predominant, 52% acinar, 12% papillary, 21% solid, 5% micropapillary
- Chest CT and PET-CT within 2 weeks from surgery
- Pathologic review by 2 pulmonary pathologists together on one scope – consensus

Results:
- Classification according to the presence/absence of any (≥1%) MP or solid (S) subtypes: 17% MP-/S-, 40.5% MP+/S-, 38.4% MP-/S+, 4.1% MP+/S+
- Median % MP in 228 pts - 2.5%, solid in 217 pts - 25%
- MP-/S- smaller, less invasive, less frequent adjuvant chemo, disease progression or death than MP+/S- or MP-/S+. Females more likely to have MP-/S-
- Lepidic predominant tumors - good prognosis; solid- & MP-predom. - poor prognosis
- MP-/S- tumors had better DFS than MP+/S- or MP-/S+; MP+/S+ had worst OS; OS and DFS of MP tumors were worse than tumors w/o MP
- DFS (not OS) worse for S+ tumors vs S- tumors
- In the MP subgroup, solid subtype was strong predictor for OS
- tumor stage and MP - independent factors for OS (pts with MP had lower OS)
- Multivariate analysis: stage > I, tumor size ≥2.5 cm, solid mass, SUVmax ≥7 - independent predictors for presence of MP or S subtype; Multivariate analysis: tumor size ≥ 2.5cm, ill-defined tumor margins - predictors for MP subtype; male gender, SUV max ≥7 and solid mass – predictive of solid subtype
- 4-factor combination of stage ≥ 2, tumors size ≥ 2.5 cm, SUV max ≥7, absence of GGO component predicted presence of MP or solid subtype in 100%.

Take Home Points:
- MP - risk factor for poor prognosis; validity of solid subtype for poor prognosis not proven
- MP subtype (≥1%) identified in 44.6% of resected lung adenoCa
- Imaging might be able to predict the presence of MP or S subtype
Background & Purpose:
- Response rate of Crizotinib app. 60%; median PFS of 8-10 mos in ALK-rearranged NSCLC.
- App. 1/3 of ALK-rearranged NSCLC have relapse (acquired mutation within ALK TK domain, amplification of ALK fusion gene, some with unchanged ALK fusion gene). Only limited treatment options after failure of crizotinib.
- Ceritinib (Novartis) - oral, small molecule, ATP-competitive, TKI of ALK. In enzymatic assays, 20x more potent than crizotinib against ALK. Marked antitumor activity against both crizotinib-sensitive and crizotinib-resistant tumors in ALK-rearranged NSCLC in xenografts.
- Phase 1 study of ceritinib to determine safety, maximum tolerated dose (MTD), pharmacokinetic properties, and antitumor activity in patients with advanced, ALK-rearranged NSCLC and other cancers harboring ALK alterations.

Methods:
- Locally advanced or metastatic Ca with ALK rearrangements (FISH).
- Patients with prior treatment with one or more ALK inhibitors were eligible.
- Treatment until disease progression, unacceptable level of toxicity, or withdrew consent.
- 94% NSCLC, 3% breast Ca, 1% each: alv. rhabdomyosarc, IMT, ALCL, rectal adenoCa.

Results:
- 130 pts; 94% with NSCLC were postchemo; 68% had prior crizotinib
- Toxic events: diarrhea, vomiting, nausea, dehydration, elevated ALAT, hypophosphatemia; all resolved on intermittent treatment cessation.
- MTD – 750 mg
- 4 cases of ILD that were possibly related to ceritinib, resolved with discontinuation of drug and standard treatment.
Among 114 patients with NSCLC (received at least 400mg ceritinib/day): 58% response rate 58% (95% CI, 48-67), median PFS 7 months (95% CI, 5.6-9.5). 80 pts who had prior crizotinib 56% RR (95% CI, 45-67), median PFS 6.9 months (95% CI, 5.6-9.5).

- 8 pts with tumors other than NSCLC – 2 had response: ALCL, IMT
- 19 NSCLC-pts with disease progression during crizotinib treatment had re-biopsy before treatment with ceritinib - all had ALK rearrangement, 2 also had ALK gene amplification, 5 had secondary resistance mutations in ALK TK domain, 12 had no additional genetic alteration. Tumor regression was observed in all these pts regardless of molecular status; (6/7 pts with ALK gene amplification or mutation; 7/12 pts without ALK alteration)

**Take Home Points:**
- Ceritinib seems active in most ALK rearranged NSCLC independent of prior crizotinib
- Crizotinib-resistant tumors may remain ALK-dependent; crizotinib may have subtherapeutic inhibition of target, which may be overcome by more potent and structurally distinct ALK-inhibitors; or ceritinib may inhibit an unknown kinase
Articles for notation - Neoplastic


Background & Purpose:
- Neoadjuvant chemo followed by EPP and adjuvant hemithoracic intensity-modulated radiation therapy (IMRT) achieved a cumulative 3-yr survival of 53% with median survival of 59 months in patients with ypN0 disease who completed all 3 modalities. Success was limited by distant failures, most commonly abdominal peritoneal cavity, contralateral lung
- inadvertent tumor spillage at time of EPP → Neoadjuvant tumoricidal and/or tumorostatic effects to prevent distant seeding → New protocol with short accelerated course of high-dose hypofractionated radiation followed by EPP
- Seamless phase I/II study testing safety and feasibility of Surgery for Mesothelioma After Radiation Therapy (SMART)

Methods
Study Schema
Inclusion criteria: ≥18 yo, performance status 0-2, good PFTs, new histologic diagnosis of MPM previously untreated, clinical stage T1-3N0M0, suitable for combined modality therapy, able to give informed consent

Results:
- 25 patients (of 138 patients seen with disease). All completed IMRT and EPP
- IMRT well tolerated, only fatigue, nausea, esophagitis
- EPP: resection of diaphragm, pericardium, parietal pleura and lung; goal: complete macroscopic tumor resection
- Surgical complications in 52%; main: atrial fibrillation; no patient died within 30 days of surgery; one patient (4%) died from empyema 88 days postop; no patient developed bronchopleural fistula
- All but 1 pt (stage IB) were stage III (n=11) and IV (n=13) on final path; 5 (of 13, 38%) with ypN2 underwent 3-6 cycles of adjuvant chemo
- Median F/U (all) 23 months (range, 6-51), 3-yr cumulative OS 58%. 3-yr survival better for epithelial than biphasic.
- Epithelioid MPM: 3 yr - cumulative survival 84%, DFS 65%. All with ypT3 and ypT4 N2-neg disease were alive at last F/U; only one (ypT4N0) developed recurrence
- Recurrence (n=11) (7 biphasic, 4 epithelioid): ipsilateral chest (n=2), ipsilateral chest and distant sites (n=3), distant sites (n=6). Distant sites: retro-peritoneal LNs, peritoneal cavity, liver, contralateral lung parenchyma, contralateral pleura.

Take Home Points
- EPP after a short accelerated course of high-dose hemithoracic radiation is feasible; radiation well tolerated
- Remarkably good outcomes with patients with epithelioid phenotype
- Longer F/U and larger patient population needed
- Disadvantage: Patients must undergo EPP after IMRT to avoid potential radiation-induced pulmonary toxicity → requires coordination and multidisciplinary approach

**Purpose:**
- To investigate the use of molecular testing on cytology specimens and to identify cytologic features associated with lung adenoCa harboring specific mutations or gene rearrangements

**Methods:**
- All cytology cases of NSCLC with mutation studies for \textit{EGFR} or \textit{KRAS} or \textit{ALK} FISH
- Specimens: FNA, pleural fluid (Diff Quick, pap, ThinPrep (fluids), cell block (needle rinse))
- \textit{EGFR} and \textit{KRAS} mutations by sequencing, \textit{ALK} by FISH

**Results:**
- 160 pts: 97 (60%) primary lung FNAs, 63 (40%) mets
- 62 (39%) had mutations: 31% \textit{KRAS}, 14% \textit{EGFR} (12 \textit{EGFR} exon 19, 3% \textit{ALK}, 0.6% \textit{EGFR} exon 19 and \textit{ALK}
- Patients with \textit{EGFR} mutation were more likely to be female (p=0.04)
- Most wt cases (61%), \textit{EGFR}+ cases (65%), \textit{KRAS}+ cases (59%) were from primary lung tumors; most \textit{ALK}+ cases (75%) were from mets
- 95% of \textit{EGFR}+ cases had an original cytologic diagnosis of lung adenoCa vs 49% of \textit{KRAS}+ cases and 75% of \textit{ALK}+ cases; all others: NSCLC-favor adenoCa (25% \textit{ALK}+ cases, 28% \textit{KRAS}+ cases, 5% \textit{EGFR}+ cases) or NSCLC-NOS (23% \textit{KRAS}+ cases)
- IHC (performed in 94% cases): All \textit{EGFR}+ cases – diffuse/strong TTF1/CK7+, p63 or p40- and CK5/6- (1 case had CK5/6); 2 \textit{ALK}+ cases – diffuse TTF1/CK7+, p63/p40-, CK5/6-. \textit{KRAS}+ cases - focal p63/p40- (30%) and TTF-1- (13%)
- Cytologic re-review in 79% cases: all 3 \textit{ALK}+ cases - acinar or glandular growth; 88% \textit{EGFR}+ cases - glandular, papillary or mixed glandular-papillary growth, 45% \textit{KRAS}+ - poorly differentiated growth, no acinar or papillary growth
- Mean nuclear grade higher in \textit{KRAS}+ & \textit{ALK}+ cases than in \textit{EGFR}+ cases (2.2 & 2.3 vs 1.9).
- Eosinophilic granular cytoplasm more in \textit{ALK}+ & \textit{KRAS}+ cases than in \textit{EGFR}+ cases (100% & 32% vs 6%). Most \textit{EGFR}+ cases showed vacuolated, glassy cytoplasm. Necrosis more common in \textit{ALK}+ & \textit{KRAS}+ cases than in \textit{EGFR}+ cases (67% & 65% vs 12%)
- 16 mutated cases had histologic F/U: 88% adenoCa, 12% NSCLC-NOS (both \textit{KRAS}+)

**Take Home Points**
- More \textit{ALK}+ in mets (more aggressive nature and high stage at diagnosis?)
- Cytologic differences between \textit{ALK}+ and \textit{KRAS}+ cases and \textit{EGFR}+ cases → cytology might help in prioritizing or triaging molecular tests in cases with limited available material

Purpose:
- To compile unselected data of two independent groups performing parallel analysis of ALK rearrangements by FISH and ALK protein detection by IHC;
- To compare FISH results with IHC results

Methods:
- 3244 consecutive NSCLC (2596 adenoCa, 303 SQCC, 14 adeno-squam Ca, 331 NOS).
- FISH – Vysis probe, examined ≥ 100 non-overlapping tumor nuclei. Positive if ≥ 15% nuclei exhibit break apart or isolated red signals (deletion of the 5’ALK region);
- IHC: ALK ab from Abcam (clone 5A4). Scoring: negative; 1+ (faint, and/or doubtful heterogeneous staining), 2+ (moderate), 3+ (intense homogeneous staining); 1+-3+ considered +

Results:

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<th>Positive</th>
<th>Negative</th>
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<th>Total</th>
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<tr>
<td>Total</td>
<td>116</td>
<td>2647</td>
<td>481</td>
<td>3244</td>
</tr>
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FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NC, noncontributive analysis or technical failure; NSCLC, non–small-cell lung cancer.

- 4.6% (150) were ALK+ by FISH and/or IHC; predominant female (p<0.0001) with 2.2 fold increased risk to have ALK alteration
- In adenoCa-ALK+ 5%; NSCLC-NOS 4.2%; SQCC 1% (n=3) (ALK-FISH+ in n=2)
- ALK+ cases also had KRAS (14) or EGFR (8) mutations; EGFR mutations identified in 2 ALK-FISH+ case; KRAS mutations identified in 8 ALK-FISH+ cases
- 53% of “ALK+” cases were FISH and IHC positive; 96% stained 2+ or 3+
- If IHC had been the first technique used 36 FISH+ cases would have been missed.
- 17 FISH+/IHC- cases were also stained with D5F3 ab (cell signaling); 5 were +

Take Home Points:
- The algorithm of IHC for ALK first followed by FISH might not work for all labs. Validation should be performed in each lab.
- Differences in antibody clones, lab techniques, specimen fixation, staining techniques
• ALK rearrangement might present in SQCC? Example was not demonstrated but a certain population (female never smoker) might be tested.

• Possibility of mechanisms other than ALK rearrangement leading to ALK expression in the discordant FISH-NSCLC. ALK amplification and point-activating mutations have been shown to be associated with ALK expression and response to ALK inhibitors in other tumors.

Background & Purpose:
- No GLUT-1 expression in most normal epithelia and benign epithelial tumors, but has been shown to be expressed in variety of epithelial malignancies.
- To evaluate the usefulness of GLUT-1 immunoreactivity in differentiating reactive from malignant mesothelial proliferation.

Methods:
- Unstained slides contributed by international mesothelioma panel;
- H&E and anti-GLUT1 (rabbit IgG, DAKO) staining performed at single institution
- Cases evaluated by 2 independent pathologists at single institution blinded to original diagnosis (interobserver reproducibility? histologic criteria for assessment of malignancy?)
- GLUT-1 (membranous and/or cytoplasmic): 0; 1+ (1-25% of tumor), 2+ (26-50), 3+ (≥ 51)

Results

<table>
<thead>
<tr>
<th>GLUT-1 positive (%)</th>
<th>Staining score</th>
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<tr>
<td>Mesothelioma, all subtypes</td>
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<td>MM-S</td>
<td>21/29 (72%)</td>
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<td></td>
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<tr>
<td>MM-E</td>
<td>21/41 (50%)</td>
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<tr>
<td>Biphasic</td>
<td>3/3 (100%)</td>
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<tr>
<td>Desmoplastic</td>
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<td>Reactive proliferations, all</td>
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<tr>
<td>FP</td>
<td>0/29 (0%)</td>
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<tr>
<td>MH</td>
<td>0/31 (0%)</td>
</tr>
</tbody>
</table>

- Sensitivity 58%, specificity 100%
- Predilection for peri-necrotic tumor (comment: looks non-specific in figure)
- No difference in GLUT-1 staining based on pattern (tubular, papillary, tubulopapillary, micropapillary, trabecular, solid) (comment: micropapillary looks negative)

Take Home Points:
- If GLUT-1 is expressed – malignant meso; if negative, still could be malignant
- Authors seem less convinced of their results because they conclude that p16 deletion by FISH provides the only definite evidence of malignancy

**Background & Purpose:**
- >20 variants of EML4-ALK fusion genes and 4 more fusion partners have been identified
- To identify the aberrant expression of huntingtin interacting protein 1 (HIP1)-ALK and the chromosomal translocation for generating this fusion gene in NSCLCs.

**Case:**
- 38 yo Korean woman, non-smoker, 2.5 cm adenoCa – acinar predominant pattern; 1 (of 9) LN+
- ALK+ by IHC (clone 5A4, Novocastra), cytoplasmic, with higher density in sub-membrane region
- FISH for ALK-break apart probe (Abbott): split 5’-3’signals and isolated 3’ signals
- No EGFR or KRAS mutations
- PCR confirmed novel fusion gene HIP1-ALK
- t(2;7)(p23;q11.23)
- Crizotinib binding side present
- Patient treated with Crizotinib – no recurrence or met after 15 months

**Take Home Points:**
- HIP-ALK novel fusion transcript in ALK+ lung cancer
- Patients may respond to ALK inhibitors

Purpose:
- To evaluate clinical utility of TTF-1 and napsin A IHC to distinguish primary from metastatic lung adenoCa among pts with extrapulmonary malignancy and pts with primary lung adenoCa without extrapulmonary malignancy.
- To investigate the frequency of primary lung adenoCa in pts with history of extrapulmonary adenoCa who have a new lung nodule

Methods:
- FNA and/or needle core bx
- Patients divided into (1) with and (2) without history of extrapulmonary malignancy
- Cytologic and morphologic comparison of current nodule with previous malignancy
- Follow up resections reviewed if available
- Pts divided into 5 groups: (1) primary lung adenoCa with hx of extrapulmonary adenoCa, (2) primary lung adenoCa with hx of extrapulmonary non-adenoCa, (3) metastatic adenoCa, (4) primary lung adenoCa w/o hx of extrapulmonary malignancy, (5) metachronous lung adenoCa with hx of previous lung adenoCa
- TTF-1 and Napsin A (no clones provided)

Results:
- 168 cases: 61 met Ca – 2 focally TTF+ (colon, pancreas); 1 focally Napsin A+ (breast).
- TTF-1: Sensitivity 89.4%, specificity 93.9%, PPV 98.1%, NPV 72.1%
- Napsin: Sensitivity 93.3%, specificity 94.7%, PPV 93.3%, NPV 94.7%
- If hx of extrapulmonary malignancy – 47.4% of new lung nodules were primary lung adenoCa
- If hx of extrapulmonary adenoCa – 40.2% of new lung nodules were primary lung adenoCa
- Patients with primary lung adenoCa – 38.5% had hx of extrapulmonary malignancy
- Primary sites of previous extrapulmonary adenoCa in pts with primary lung adenoCa and hx of extrapulmonary adenoCa: breast (35.8%), colon (13.2%), prostate (11.3%), endometrium (5.7%), ovary (5.7%)
- Primary sites for metastatic adenoCa: colon (32.8%), breast (28.1%), pancreas (7.8%), prostate (7.8%), endometrium (6.3%), ovary (6.3%)
- Smoking incidence higher in pts with primary lung adenoCa than met adenoCa (P<0.005)
- Higher incidence of multiple pulmonary nodules in pts with met adenoCa than primary lung adenoCa (p<0.005)
- Patients with smoking hx, multiple nodules and no hx of extrapulmonary malignancy had a higher chance of having primary lung adenoCa

Take Home Points:
- High frequency of primary lung adenoCa in patients with known extrapulmonary malignancy or extrapulmonary adenoCa (almost 50%)
- No discussion on morphologic comparison, especially in cases that were TTF-, napsin-
- No numbers on difficult cases in which phenotype of primary lung adenoCa and met are known to overlap (certain renal tumors, thyroid carcinoma, etc)

**Purpose:**
- To correlate clinically relevant morphologic, immunophenotypic and molecular characteristics of adenoCa with outcome

**Methods:**
- Surgical resection specimens of invasive adenoCa, Caucasian cohort
- Morphologic classification according to 2004 WHO and recent IASLC/ATS/ERS classification; staged according to TNM and UICC
- Mean follow up 48.2 months; 90.7% smokers; 62.1% male
- TMAs; H-score for IHC reporting, no clones reported on antibodies
- Molecular analysis by sequencing; ALK by IHC, if positive followed by FISH

**Results:**
- 416 cases (97.9% conventional with 7.5% lepidic, 43.9% acinar, 5.2% papillary, 6.2% micropapillary, 37.2% solid predominant; 2.1% invasive mucinous adenoCa)
- *ALK* translocation in 1.4%, *KRAS* (37.6%), *EGFR* (15.5%), *BRAF* (4%)
- Double mutations: *KRAS/KRAS* (1.9%, n=8), *EGFR/EGFR* (1.4% (n=6), *EGFR/BRAF* (0.5%, n=2), *ALK translocation/KRAS* (n=1)
- Staging differs with prominent growth pattern
- Predominant growth pattern correlates with smoking (acinar, solid, papillary more likely in smokers than lepidic or micropapillary) (P<0.001)
- TTF-1 more prevalent in low stage tumors; Napsin associated with growth pattern (micropapillary had highest expression, solid and lepidic more likely to be negative)
- *EGFR* more frequent in females; *KRAS* more frequent in males
- TS expression higher in older patients; ERCC1 expression higher in smokers
- Smokers had higher rates of *KRAS*, lower *EGFR*
- *KRAS* more frequent in invasive mucinous adenoCa; Higher *EGFR* in lepidic and micropapillary; *BRAF* predominantly in micropapillary but not papillary or lepidic
- TS and ERCC1 expression higher in TTF-1+ adenoCa, associated with ALK translocation and wt *KRAS*
- TTF-1 and napsin associated with longer survival; TTF-1 is stage- and pattern-independent predictor of OS; TS is predictor of OS and DFS (only in univariate analysis); *BRAF* negative prognostic factor for DFS

**Take Home Points:**
- Studies might need to be done on other ethnicities; larger studies for multivariate analysis necessary; Interobserver variability might change things

Purpose:
- To evaluate TTF-1 in primary invasive breast carcinomas and primary pulmonary carcinomas as controls using both 8G7G3/1 and SPT24 clones
- To analyze staining pattern and its clinicopathologic significance

Methods:
- TMA, 0.6 mm cores for breast carcinoma, 1.0 mm cores for lung carcinomas
- Positive nuclear and cytoplasmic staining - defined as moderate to strong staining in either the nuclei or cytoplasm of the tumor cells.

Results:
- **Nuclear** staining
  - Table 1. Nuclear TTF-1 staining with 8G7G3/1 and SPT24 done in invasive breast carcinoma and pulmonary carcinoma

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Total cases</th>
<th>8G7G3/1 +ve (%)</th>
<th>SPT24 +ve (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>1123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive breast carcinoma of no special type</td>
<td>925</td>
<td>10.1%</td>
<td>0</td>
<td>/</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>/</td>
</tr>
<tr>
<td>Others</td>
<td>164</td>
<td>0</td>
<td>0</td>
<td>/</td>
</tr>
<tr>
<td>Total</td>
<td>1123</td>
<td>10.09%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>208</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>125</td>
<td>70.2%</td>
<td>70.4%</td>
<td>1.000</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>6</td>
<td>75%</td>
<td>62.5%</td>
<td>1.000</td>
</tr>
<tr>
<td>Bronchoalveolar carcinoma</td>
<td>15</td>
<td>59.8%</td>
<td>81.3%</td>
<td>0.500</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>/</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>3</td>
<td>33.3%</td>
<td>33.3%</td>
<td>1.000</td>
</tr>
<tr>
<td>Sarcomatoid carcinoma</td>
<td>1</td>
<td>100%</td>
<td>100%</td>
<td>0.839</td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>70.2%</td>
<td>70.2%</td>
<td></td>
</tr>
</tbody>
</table>

- TTF-1+ breast case – diffuse, moderate-strong, histologic grade 2, ER+, PR+, Her2-
- Specificity, sensitivity, PPV, NPV for 8G7G3/1 for lung adenoCa when compared to breast Ca: 99.9%, 71.6%, 99.3%, 95.0%; for SPT24: 100%, 70.7%, 100%, NPV 95.1%
- **Cytoplasmic**: 44 invasive breast Ca (8G7G3/1) but not by SPT24. No cytoplasmic expression in any lung Ca. Cytoplasmic expression correlated with high TNM stage, PR-, Her2 overexpression, shorter DFS and OS. Multivariate analysis: TTF-1 expression was independent poor prognostic factor for DFS and OS

Take Home Points:
- For distinction breast from lung Ca, both 8G7G3/1 and SPT24 clones highly specific but relatively low sensitivity
- SPT24 not expressed in breast Ca (previous reports showed SPT24 expression in rare breast Ca)
- Cytoplasmic TTF-1 (clone 8G7G3/1) might be prognostic marker in breast Ca

**Purpose:**
- To compare Hispanic and non-hispanic US patients with lung adenoCa for incidence of mutations with therapeutic agents either available or in development using a highly multiplexed genotyping assay.

**Methods:**
- Target genes: *KRAS, MET, BRASF, mTOR (FRAP1), STAT3, JAK2, PIK3CA, AKT1-3, PTEN*.
- Multiplex PCR

**Results:**
- 85 specimens from 83 patients (40 Hispanic, 43 non-Hispanic white)
- Hispanic: 13% non-smoker, 33% smoker, 55% unknown smoking status, median age at diagnosis 63 years
- Non-Hispanic white: 19% non-smoker, 49% smoker, 33% unknown smoking status, median age at diagnosis 62 years
- *KRAS* mutations in 13% of all cases; 15% of Hispanic, 12% of non-Hispanic white. *KRAS* mutations in codons 12, 13, 59 (Hispanic); codons 12, 61 (non-Hispanic white)
- No correlation between smoking status and *KRAS* mutation status
- *PIK3CA* mutations in 5% Hispanic, no in non-Hispanic white
- No other mutations identified

**Take Home Points:**
- No significant differences in frequency and presence of targetable mutations in lung adenoCa between Hispanic and non-Hispanic white (*EGFR* mutation, *ALK* rearrangement were not investigated) (previously shown that 33% of NSCLC in Hispanic have *EGGFR* mutation)
- Codon 59 *KRAS* mutations might be more common in Hispanics than non-Hispanic whites, but needs to be confirmed by larger studies

Background & purpose:
- Type A thymoma are postulated to originate from thymic epithelial cells or cells differentiating towards thymic epithelial cells; however, type A component in type AB thymoma appears to be genetically distinct from that in type A thymoma
- Type B component resembles epithelial cells at the corticomedullary junction; its normal counterpart is not clear.
- β5t = proteasomal β subunit - expressed exclusively in cortical thymic epithelial cells; involved in development and repertoire formation of thymocytes. Previously shown that β5t is expressed in most cases of type B thymoma and some cases of AB thymoma.
- To analyze β5t expression in type AB thymoma and to examine whether β5t expression correlates with histologic features of type AB thymoma

Methods
- Rabbit polyclonal antisera for human β5t raised against the peptide encompassing resides 285 to 300 of human β5t.
- Mean age 53 years (range,13-77), 14 females
- Classification of B component: b-1 = B-component with spindle to oval tumor cells; b-2= round to polygonal tumor cells, b-3, round to polygonal tumor cells accompanied by medullary differentiation

Results:
- 20 cases of AB thymoma stained with anti-β5t
- No expression of β5t in A component
- β5t expression in B-component of 12/20 cases; most frequently in b-2 and b-3 components; ratio of β5t-positive cases was consistently increased from b-1 → b-2 → b-3 (27%, 47%, 80% respectively)
- Ratio of TdT-positive lymphocytes (predominantly found in B-areas) also increased from b-1 → b-2 and b-3 (60%, 80%, and 80%)
- Expression of β5t in type AB thymoma correlates with the morphology of tumor cells and the abundance of TdT-positive lymphocytes.
- No correlation between expression of β5t and clinical parameters

Take Home Points:
- Type B component of type AB thymoma has the potential of cortical differentiation

Purpose:
- To investigate whether thymomas could be characterized using differentiation (cortical and medullary) and maturation (early and late stages) of their epithelial component as criteria.

Methods:
- Cortical markers: anti-PRSS, anti-β5t, anti-catepsin V
- Medullary markers: anti-CD40, anti-claudin-4, anti-AIRE, anti-desmin
- Thymic epithelial cell (TEC) maturation markers: anti-AIRE, involucrin, CK10

Results:
- Type B1 – cortical differentiation identical to normal thymus, some medullary features are moderately reduced/missing; terminal differentiation slightly impaired (30% cases)
- Type B2 - cortical signature identical to normal thymus and B1 thymomas, but even greater reduction of medullary features. Terminal differentiation only in 40% of cases
- Type B3 - only abortive cortical features, almost complete absence of medullary differentiation. Both, medullary differentiation and terminal maturation are disturbed.
- Type A - abortive medullary features, no cortical differentiation. mTEC markers negative
- Type AB - complex, cortical and medullary differentiation at single-cell level
- Thymic SQCC - traces of differentiation along the cortical and/or medullary lineage

Take Home Points:
- Most thymoma have epithelial cell clusters with both cortical and medullary differentiation
- Groups: (1) cortical and medullary differentiation (type B1, B2, AB) and (2) only abortive lineage-specific differentiation (B3, A thymomas, thymic SQCC)
- Types B1, B2, AB can be further differentiated by degree of terminal mTEC maturation
- Thymomas derive from common progenitor cell with maturation defects
- Results support morphological distinction of currently recognized thymoma subtypes.
Bari MF et al. BAI3, CDX2 and VIL1: a panel of three antibodies to distinguish small cell from large cell neuroendocrine lung carcinomas. Histopathology. 2014. 64: 547-56.

**Purpose:**
- To improve classification of SCLC and LCNEC by using gene expression profiling of microdissected tumor cells to identify discriminating markers that might aid in the diagnosis

**Methods:**
- 173 cases, biopsies and resections; consensus diagnosis if submission and re-review diagnosis were the same (n=147; 71 LCNEC, 76 SCLC); others were “indeterminate” (n=26)
- TMAs, 1mm cores
- qRT-PCR was performed
- genes which showed a significant difference on qRT-PCR or showed detection in ≥75% cases of one tumor type and no detection in ≥75% of the other tumor type → IHC studies

**Results:**
- 21 frozen samples – 4 SCLC, 4 LCNEC, 7 BL (background lung) had a RIN (RNA integrity number) >5 – used for gene expression profiling; hierarchical clustering separated SCLC, LCNEC and BL into 3 distinct clusters; 888 differentially expressed genes (DEG) identified, (556 upregulated in SCLC compared to LCNEC and BL; 332 upregulated in LCNEC compared to SCLC and BL). Direct comparison between tumor types identified 63 DEGs between SCLC and LCNEC and 48 genes between LCNEC and SCLC
- qRT-PCR: 20 (of 23) genes with a similar fold change to expression array. CDX2, CD99, VIL1, ATP5B, FAM134B, FGG genes showed significant differences at the qRT-PCR level between SCLC and LCNEC. BAI3, LHX2, KCNK10 showed detection in ≥75% of SCLC samples, not detected ≥75% of LCNEC. TCF4 detected in 5/8 SCLC but not in LCNEC. CDX2, CD99, VIL1 (for LCNEC) and BAI3, LHX2, KCNK10 and TCF4 (for SCLC) were selected for validation.
- BAI3, CDX2, CD99, VIL1 antibodies showed all or none expression pattern between SCLC and LCNEC, all others had some positivity in both (not further studied).
- BAI3+ (nuclear) in 89% SCLC, neg or cytoplasmic in LCNEC. CDX2 + nuclear and VIL1 + cytoplasmic in 56 and 70% LCNEC, often just focal. Sensitivity, specificity: CDX2 0.56, 0.9; VIL1: 0.7, 0.87; BAI3 cytoplasmic 0.29, 0.99; BAI3 nuclear 0.89, 0.76; if CDX2 and VIL1 combined (either marker positive), 0.81, 0.81
- CD99 - strong stromal staining in SCLC and LCNEC, distinction between stromal and tumor cells difficult
- Indeterminate cases: 58% had IHC pattern indicating either SCLC or LCNEC, all others showed no staining (11.5%) or w pattern where markers of both tumors were present (31%).

**Take Home Points:**
- 3-antibody panel had relatively high sensitivity and specificity
- Most labs don’t have those markers

Purpose:
- To develop an assay for detection of ALK alterations in FFPE samples based on qRT-PCR.

Methods:
- qRT-PCR – primers targeting 5’ and 3’ portions of ALK transcript (exons 4/5 and 24/25)
- FISH – breakapart probe specific for ALK locus (Zytolight)
- IHC – rabbit monoclonal ab (clone D5F3, New England Labs)

Results:
- ALK qRT-PCR assay: ALK rearrangement → overexpression of 3´ portion of ALK encoding kinase domain (exons 20–29); 5’ portion (exons 1–19) unexpressed → two amplicons targeting 5’ and 3’ portions of the ALK transcript separately.
- FFPE samples with known ALK status (FISH): 1 t(2;5) + lymphoma, 2 NSCLC with ALK translocations, 10 wt NSCLC. For EML4-ALK and full-length ALK transcripts, cell lines H2228 and RH30 used as positive controls, respectively. Unbalanced ALK transcript expression was seen in all ALK translocated cases, all had 3´ exons but not 5´ exons. In all wt cases, neither 5´ nor 3´ exons were expressed.
- 652 NSCLC screened by qRT-PCR (tumor content ≥ 10%). 523 interpretable. 4.6% expression of ALK 3’ portion above cutoff level (0.3), 5’ portion not expressed. 6 additional tumors (1.1%) showed full-length ALK transcript.
- 198 samples for FISH – 182 interpretable. 19/22 cases with 3’ expression and 19/19 with 3’ expression > 0.4 were ALK rearranged. 1 case, FISH showed translocation accompanied by a partial deletion of the 3’ part of ALK – qRT-PCR showed expression of 3’ ALK portion.
- 154 qRT-PCR-negative NSCLC with interpretable FISH: 152 FISH neg; 2 FISH+ (unbalanced, yet low expression of the 3’ portion (0.22; 0.15, respectively)).
- 6 NSCLC with full-length transcript were negative for genomic ALK rearrangements. In 4/6 additional ALK gene copies detected in >25% of the tumor cells.
- qRT-PCR assay, accurately typed 97% of tumors analyzed by FISH and strongly suggested rearrangements of ALK in 3 tumors with insufficient/ambiguous FISH analysis. qRT-PCR identified cases with full-length ALK overexpression not detectable by FISH.
- Relative expression of 3’ ALK portion was independent of sample size
- ALK protein expression: ALK IHC+ in all 21 cases with ALK rearrangement (FISH). Expression levels of 3´ portion of ALK transcript did not correlate with intensity of IHC. 5 cases with up-regulated full-length transcription – 4 weak and 1 no ALK protein expression.
- All cases with ALK expression and/or FISH positive were adenoCa or 1 large cell Ca
- 6 cases with full-length ALK – expression, 1/6 had heterozygous nucleotide substitution within kinase domain (S1220Y) – also present in the benign lung → germline origin, however, expression of ALK transcripts was restricted to tumor cells.

Take Home Points:
- qRT-PCR is inexpensive, rapid, relatively high-throughput; reliable in small bxs with 10-30% tumor cells.

Background & purpose:
- No guidelines for margin distance from primary tumor for wedge resection for small tumors.
- To characterize the association between the margin distance in patients who had undergone wedge resection for small (< 2cm) NSCLC and local recurrence.

Methods:
- Adults – wedge resection of primary NSCLC < 2cm
- Local recurrence = primary outcome. Local recurrence (Martini and Melamed) = tumor of same histologic type occurring within same lobe (or draining hilum and/or mediastinum) or tumor of same histologic type in a different lobe or lung with carcinoma in the lymphatics common to both, and/or extrapulmonary metastases.
- Only tumors with confirmed negative margins; margins had to be ≥1mm free of tumor.
- Margin distance stratified: 1-5 mm, 6-10 mm, 11-15 mm, 16-30 mm
- Multivariable Cox regression analysis used to model the interval to local recurrence. Adjusted for covariates: COPD, preop FEV1, smoking status, diabetes, tumor size, tumor lobe location, location within the hemithorax, surgery type (thoracotomy vs VATS), whether LN had been sampled.
- Nothing about TNM stage!!!!

Results:
- Overall 1-year local recurrence rate – 5.7%, 2-year 11%, 3-year 16.4%
- Increased margin distance - associated with lower risk of recurrence (p=0.033), with diminished additional benefit beyond a margin distance of app. 15 mm.
- After adjustment for confounding factors (see methods), patients with a margin distance of 2 mm had a 54% greater risk of local recurrence than patients with 5 mm margin; patients with 10 mm margin had 45% lower risk, patients with 15 mm margin had 59% lower risk than patients with 5 mm margins of local recurrence

Take Home Points:
- Margin distance is important for risk for local recurrence in wedge resection of small tumors
- Ideal margin cannot be established
Articles for notation - Neoplastic


Purpose:
- Report of 6 cases of coexistent Crohn dis and pulmonary GPA, 4 cases of extrapulmonary GPA and 3 previously published cases with extrapulmonary GPA.

Methods:
- Exclusion of cases in which ulcerative colitis and GPA overlapped, in which only one of the diagnoses were conclusive and the other merely suspected.

Results:
- 13 patients including 2 consultation files, 3 from literature
- Median age at the time of diagnosis – Crohn dis, 29 years, GPA, 35 years
- Crohns preceded GPA in 11 cases by median of 6 years (range 0-13)
- GPA involved lungs in 6 cases, upper airways (n=9), kidney (n=3)
- Neuritis (n=4), eye involvement (n=3), pyoderma gangrenosum (n=1)
- PR3-ANCA+ (n=6), -(n=2), unavailable (n=5)
- MPO-ANCA – (n=6), unavailable (n=7)
- GPA in remission in all cases with available clinical data
- Crohn limited to colon (n=4), ileocolonic (n=5), jejenum (n=1)
- Path findings: Surgical lung biopsy (n=4): all had classical features of GPA (parenchymal necrosis, giant cells, vasculitis, large irregularly shaped [geographic] areas of necrosis constituting necrotizing granulomatous inflammation in an inflammatory background of fibrosis, or fibrinous or organizing pneumonia)
- Neutrophilic microabscesses (n=3), few palisaded granulomas (n=1). No sarcoid-like granulomas. GMS, Ziehl-Neelsen negative in all cases
- Sino-nasal mucosa showed granulomatous inflammation, with eos, necrosis, and microabscesses with cellular debris and giant cell reaction (n=1)

Take Home Points:
- GPA coexistent with Crohn-very unlikely but both can mimic the presentation of each other.
- Granulomatous colitis with disease in head & neck region or pulmonary system should prompt consideration of GPA as a separate second disease needing appropriate therapy
- Sulfasalazine toxicity might mimic pulmonary GPA – distinction from GPA might be challenging
- C-ANCA/PR3-ANCA almost universally present in severe GPA, maybe negative in limited GPA. In UC usually p-ANCA. PR3-ANCA in pts with IBD (UC or Crohn) should prompt search coexisting GPA but does not establish diagnosis.
- DD: Extraintestinal Crohn, extrapulmonary GPA
Review Articles


Review of various morphologic patterns and clinical features of spindle cell thymoma.

Clinical features:
- Up to 22% of all thymoma, mean age 6th decade (range, 7-83 years), no sex predilection;
  maybe associated with autoimmune disease, most commonly myasthenia gravis; presentation
  with chest pain, cough, dyspnea, or asymptomatic; might be found incidentally on imaging
  for unrelated problems

Gross features: 2.5 – 18 cm, solid or cystic. Grossly identifiable cystic spaces range from
microscopic to >3.5 cm; hemorrhage and necrosis not common

Histologic features – nice table with type, histologic features and diff diagnosis
- Diffuse spindle cell thymoma
- Micronodular thymoma with lymphoid B-cell hyperplasia
- Desmoplasic spindle cell thymoma
- Ancient (sclerosing) thymoma
- Spindle cell thymoma with prominent papillary and pseudopapillary features
- Spindle cell thymoma with hemangiopericytoma-like pattern.
- Spindle cell thymoma with neuroendocrine-like pattern
- Spindle cell thymoma with neuronal pattern: meningothelial or schwannoma-like patterns.
  Classical verocay bodies are not seen.
- Angiomatoid spindle cell thymoma
- Cystic thymoma

IHC and molecular findings: Table 2.
- Tumor cells: Diffuse + for pancytokeratin, CK5/6, Pax8; variably for CK7, synaptophysin,
calretinin, Bcl2, SMA. Occasional TTF-1.
- Lymphoid component: CD3 and CD5+ mature T cells, small proportion of immature
  CD99+/CD1a+ T cells.

Differential diagnosis
- Depends on morphology, includes spindle cell thymic carcinoma.

Treatment and prognosis: considered malignant, complete surgical resection is best
Background & purpose:
- Clinical implications of Ki67 not clear.
- To assess role of Ki-67 for clinical purposes based on analysis of a large number of articles

Methods:
- Articles dealing with the 1999 and 2004 WHO or equivalent systems, English literature
- 2067 lung NE tumors retrieved corresponding to 25 independent studies.

Question 1: Are there relevant technical issues to Ki-67 IHC and evaluation of results?
- Yes. No uniform methodology for IHC and evaluation of results; most studies used monoclonal MIB-1 antibody on FFPE after antigen retrieval procedures; antibody dilutions vary widely
- Ki-67 LI should be calculated in surgical specimens by counting at least 2000 consecutive tumor cells in hot spot fields at x 40 of 2mm²
- All tumor cells showing nuclear staining for Ki-67 were considered positive regardless of staining pattern (diffuse, speckled, nucelolar, mitosis featuring)
- Variably used mean vs median
- Reproducibility studies encouraging: < 1.5% variability, Ki-67 LI better reproducible than mitotic count
- For experienced pathologists, manual counting of Ki-67 LI upon visual inspection or eyeball estimation differs little from more sophisticated methods.

Question 2: Is there a diagnostic role for Ki-67 LI in lung NE tumors?
- No. Current classification based upon morphology alone, but a practical utility for this marker has been emerging for separating TC/AC from high-grade NE tumors in limited diagnostic material. Thresholds up to 25-30% of Ki-67 LI - quoted as useful adjunct to exclude PD NE tumors (exceedingly high proliferation index); thresholds of <3% would support a diagnosis of low-grade NE tumor; thresholds 3-30% indicate indeterminate tumors that most often consisted of AC with very few PD tumors.

Question 3: Is there a prognostic role for Ki-67 LI?
- Possibly. Ki-67 proposed as prognostic factor in excised specimens of TC and AC (cutoff values 2.5 – 5.8%), not always independent of morphology. No recommendation at this time because of conflicting results and no agreed-upon cutoff to stratify these tumors.

Question 4: Is there an established role for Ki-67 LI in tumor grading?
- No. Existing data do not support a recommendation to apply to the lung the grading systems devised for NE tumors in other anatomical sites.
- Ki-67 correlates closely but not perfectly with mitotic count

Question 5: Is there a predictive role for Ki-67 LI in therapeutic decisions?
- No. No randomized trials to show that Ki-67 LI in lung NE tumors may guide therapy.

Take Home Points:
- Ki-67 LI currently does not play a role in diagnosing NE tumors in the lung or clinical guidance for therapy or prognosis.

This review summarizes current practice of molecular testing and reviews future methods in molecular testing of lung carcinoma.

- Emphasizes the need to subclassify NSCLC; recommends that number of NOS cases should not be ≥5%
- According to recent CAP/IASLC/AMP guidelines, only EGFR and ALK testing is recommended for adenoCa and mixed lung cancers with an adenoCa component (e.g. pleomorphic carcinoma, carcinosarcoma, adenosquamous), large cell carcinoma with adenoCa immunophenotype; testing not recommended for SQCC and small cell carcinoma
- Discusses positive predictors of response to EGFR-TKI and ALK inhibitors.
- EGFR mutations are discussed (table with all mutations that occur in at least 1% of lung adenoCa).
- EGFR inhibitor-acquired resistance is discussed together with EGFR mutations that might be implicated in resistance. Clinical testing for EGFR-TKI resistance is not standardized but discussed
- Discussion of other biomarkers that occur less frequent and testing is not standard yet: ROS1, KIF5B-RET
- Table on targetable genetic abnormalities in NSCLC other than EGFR and ALK and their occurrence in SQCC and adenoCa
- Short discussion on IHC (EGFR, ALK, BRAF, ROS) and nice table that includes antibodies, specificity and sensitivity
- Discussion of role of NGS for molecular testing of lung cancer
Purpose: To summarize all potential biomarkers in lung cancer and to discuss biomarkers that are currently not part of the routine practice but might be in the future.

- Discussion on how histology predicts response to chemotherapy mainly because non-SQCC carcinoma are eligible for mutation screening and respective treatment.
- Assessment of expression of specific genes might in the future provide information predictive for response to chemo including thymidylate synthase (TS); genes involved in DNA repair of cytotoxic drug-induced damage in tumor cells such as ERCC1 in relation to the response to cisplatin; ribonucleoside-diphosphate reductase large subunit (RRM1) in relation to response to gemcitabine or 5-FU.
- Discussion of markers for targeted therapy including EGFR and EGFR-resistance, ALK, Her2, BRAF, RET, ROS1, DDR2, KRAS, PIK3CA, FGFR1, MET, PTEN, NUT.
Case Reports


Case:
- 56-yo male admitted with 2nd episode of 100-200 mL hemoptysis over a 48-hr period. Was on Ticagrelor (platelet aggregation inhibitor) and aspirin, had been commenced 10 days earlier after coronary artery stenting. No other symptoms or bleeding history
- Coarse crackles bilaterally throughout lower lung fields; Hgb normal, INR 1.1, PTT 27.8s, fibrinogen 4.3 g/L
- CT: bilateral GGOs consistent with peribronchovascular pulmonary hemorrhages. Elevated diffusing capacity.
- Ticagrelor was paused, patient underwent bronchoscopy – edematous airways, frank blood within lower lobes. No bx because of ongoing bleeding.
- Hemoptysis recurred with recommencement of Ticagrelor– change to clopidogrel
- Surgical lung biopsy and elective CABG to allow cessation of clopidogrel: wedge shaped lesions with intervening areas of preserved architecture. Within areas of lung injury, alveolar septal walls show variable fibrous thickening and contain a mild, chronic inflammatory infiltrate. Early and established fibrosis, patchy bronchiolization of the airways. No intraalveolar hemorrhage
- Given the clinical history, the pathology was most in keeping with drug-induced lung injury

Take Home Points:
- Ticagrelor = P2Y12 receptor antagonist - acts by binding to adenosine diphosphate noncompetitively, inhibiting the prothrombotic effects of platelet adenosine diphosphate binding.
- Dyspnea is common site effect
- Pulmonary hemorrhage has not been reported