Articles for Discussion


**Context:** Detection of ALK-gene rearrangements in NSCLC is mainly performed by fluorescence in-situ hybridization (FISH). The question was raised if FISH might be replaced by immunohistochemistry (IHC) in a reliable and reproducible manner across different laboratories.

**Methods:** 15 NSCLC (constructed into 2 TMA’s, see figure 1) were independently tested for ALK-IHC protein expression by 16 participating institutes. These institutes underwent calibration of their staining instruments plus training of the observers for ALK IHC interpretation. Each laboratory utilized the VENTANA ALK-D5F3 IHC assay. The 15 samples were pre-tested with FISH and displayed unequivocal ALK break-positivity (6×) and negativity (7×), as well as ALK positive-“borderline” character (2×), which was RT-PCR-confirmed.

**Results:** (see figure 2 for examples of IHC staining with accompanying FISH results)
- All seven ALK FISH-negative cases were homogenously scored as ALK-IHC negative.
- All 16 participants scored the two ALK positive-“borderline” samples as unequivocally positive according to their protein expression.
- Concordant IHC interpretation was also noticed in four of six unequivocal ALK break positive cases.
  - In two of the six, some observers described a weak/heterogeneous ALK-IHC staining which in a routine diagnostic setting would have resulted in follow up ALK-testing by FISH and/or PCR.

**Take home points:**
- This study shows for the first time that predictive semiquantitative IHC reveals reliable and reproducible results across several labs when methodology and interpretation are strictly defined and the pathologists are uniquely trained and offer a proposed testing algorithm (see figure 3).
- The authors comment that there can be issues with FISH testing, as it is expensive, time consuming and requires special equipment. There can be technical issues, material based issues and signals can be subtle and difficult to interpret leading to false positives or negatives.
- The application of validated ALK IHC assays and its comparison to ALK-FISH is highly needed in future clinical trials to see if ALK-IHC could potentially serve as a pre-screening tool or perhaps even as a stand-alone test at least in cases displaying an unequivocally staining pattern as well as an alternative predictive test in samples with reduced FISH interpretability.
Figure 1. Preparation and testing modality of the TMA’s. Each of the 2 TMA’s was cut into 43 slides. Slides 1, 2, 22, 42, and 43 were tested by means of ALK-FISH in Berlin (B) and Heidelberg (HD). Slides 5, 23, and 39 were stained by H&E and were electronically available as scans.

Figure 2. 10 NSCLC cases stained with H&E, ALK-IHC (10x objective), and FISH (63x objective). Two ALK-negative cases with challenging IHC due to positivity of macrophages and necrosis are shown in cases 4 and 9. The latter displays a single green signal pattern (SGS) in FISH. At the bottom the interpretations of all 16 observers (negative vs positive) is given. Note the heterogeneous staining pattern in case 11.
Figure 3. ALK-IHC-testing proposal.
Solitary fibrous tumour (SFT) can be found in any part of the body, but appear most frequently in the pleura, the lower extremities or the retroperitoneum. The clinical behaviour of SFTs is difficult to predict and they may behave aggressively even if there are no obvious features of malignancy. SFTs present with a wide range of histological features including bland fibroblastic morphology with a patternless architecture, variable cellularity and pleomorphism, branching thin-walled ‘staghorn’-shaped vessels, and perivascular hyalinization. All of these features can be present, to some extent, in many other tumors - up to 15% of all soft tissue sarcomas may show HPC-like features. IHC profile is relatively non-specific -CD34, 80%; CD99, 70%; bcl-2, 30%) making it often a diagnosis of exclusion. Recently, a highly recurrent fusion on chromosome 12q13 spanning the NAB2 and STAT6 loci has been described as a genetic hallmark alteration in SFTs and HPCs. NAB2 and STAT6 act as transcriptional regulators. In SFTs, the early growth response (EGR)-binding domain of NAB2 is fused to the activating domain of STAT6, resulting in transcriptional deregulation of EGR-controlled genes.

Figure 4. A, Illustration of chromosome 12 and a map of region 12q13–15 with the relative positions of

NAB2, STAT6, CDK4, and MDM2. The reading orientation of a gene is indicated by the direction of the apex. B, The assumed mechanism of NAB2–STAT6 fusion in SFTs starts with the inversion of STAT6, which is followed by fusion of the activation domain of STAT6 with the directly adjacent NAB2. C, Amplification of chromosomal regions including CDK4 and MDM2 sometimes involves contiguous genes such as STAT6, and might contribute to the increased STAT6 expression in some DDLS cases.

However, direct detection of NAB2–STAT6 fusion is complicated – FISH cannot be used as the loci are too close and there are too many breakpoints for PCR-based methods to be practical to detect NAB2–STAT6 fusion. Previous studies have suggested that NAB2–STAT6 fusion leads to nuclear accumulation of STAT6.

**Aims:** To test the reliability of STAT6 IHC in SFTs of soft tissue

**Methods:** 35 pleural SFTs and 654 other mesenchymal tumours were selected.
- 270 tissue samples were analysed by the use of tissue microarrays (TMAs), with at least two representative 0.6-mm cores being taken from each case. 419 samples were analysed on whole tissue sections. The anti-STAT6 antibody used was a polyclonal rabbit, clone S-20; sc-621 from Santa Cruz Biotechnology. STAT6 IHC was evaluated for nuclear staining intensity in tumour cells using a three-level score: 1, faint/weak staining; 2, moderate staining; and 3, strong specific staining with a score of < 2 being rated negative. **In those cases with STAT6 expression in endothelial cells or in lymphocytes, score 1 for neoplastic cells was adjusted to the STAT6 intensity of these non-neoplastic cells.**
- A Duolink proximity ligation assay (PLA) was performed to validate NAB2–STAT6 fusion at the protein level in 9 DDLSs and 5 SFTs, using primary antibodies against NAB2 (monoclonal mouse, clone 1C4) and STAT6.
- The same subsets were also assessed for copy number variants (CNVs) with the Illumina Infinium Human Methylation 450 array using genomic DNA isolated from formalin-fixed, paraffin-embedded material of representative tumour areas using the Allprep DNA/RNA FFPE Kit (Qiagen).

**Results:**

**IHC:**
- 34/35 SFTs showed strong nuclear STAT6 expression
- 5/68 DDLSs, 2/130 undifferentiated pleomorphic sarcomas and 1/63 cases of nodular fasciitis showed moderate to strong nuclear STAT6 expression.

In the positive non-SFT cases, nuclear STAT6 staining was predominantly homogeneously distributed, was of moderate intensity as compared with SFTs, and was often more intense in highly pleomorphic cells. Many cases across all entities presented with subtle STAT6 expression restricted to the cytoplasm.
Figure 1. Representative images of tumours with nuclear immunohistochemical expression of STAT6 on the left side (A,C,E,G) and STAT6-negative counterparts on the right side (D,F,H). A, Solitary fibrous tumour (SFT) with distinctive nuclear STAT6 expression. B, Histological SFT mimic with diffuse cytoplasmic STAT6 staining of moderate intensity. C, Dedifferentiated liposarcoma (DDLS) with nuclear and cytoplasmic staining in pleomorphic tumour cells. D, DDLS without STAT6 expression. E, Undifferentiated pleomorphic sarcoma (UPS) showing a number of tumour cells with moderate to strong nuclear expression of STAT6. F, UPS without STAT6 expression. G,H, Nodular fasciitis (NF) with moderate to strong STAT6 expression (G) and a STAT6-negative counterpart (H).
Table 1. Types of tumour studied, and results of STAT6 immunohistochemistry

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>N*</th>
<th>Nuclear STAT6</th>
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</thead>
<tbody>
<tr>
<td>Solitary fibrous tumour</td>
<td>35</td>
<td>34 (97%)</td>
</tr>
<tr>
<td>Dermatofibroma</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Dermatofibrosarcoma protuberans</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Desmoid-type fibromatosis</td>
<td>26 (26)</td>
<td></td>
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<tr>
<td>Nodular fascitis</td>
<td>63 (63)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Low-grade fibromyxoid sarcoma</td>
<td>6 (6)</td>
<td>0</td>
</tr>
<tr>
<td>Low-grade myofibroblastic sarcoma</td>
<td>18 (18)</td>
<td>0</td>
</tr>
<tr>
<td>Myxofibrosarcoma</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Well-differentiated liposarcoma</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Myxoid liposarcoma</td>
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</tr>
<tr>
<td>Dedifferentiated liposarcoma</td>
<td>68</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Pleomorphic liposarcoma</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Malignant peripheral nerve sheath tumour</td>
<td>46 (16)</td>
<td>0</td>
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<tr>
<td>Synovial sarcoma</td>
<td>54 (32)</td>
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<tr>
<td>Leiomyosarcoma</td>
<td>35 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal stromal tumour</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated pleomorphic sarcoma</td>
<td>130 (91)</td>
<td>2 (2%)</td>
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<tr>
<td>Haemangiomia</td>
<td>21</td>
<td>0</td>
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<tr>
<td>Angiosarcoma</td>
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<tr>
<td>Alveolar soft part sarcoma</td>
<td>8 (2)</td>
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<tr>
<td>Clear cell sarcoma of tendons and aponeuroses</td>
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<tr>
<td>Epithelioid sarcoma</td>
<td>8 (2)</td>
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<tr>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>689</strong></td>
<td><strong>42</strong></td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate tumours examined on tissue microarray.
**PLA:** NAB2-STAT6 fusion protein present in 5/5 SFTs, but signal was also detected in 5/9 DDLSs. SFTs showed nuclear signals in almost all tumour cells, suggesting close molecular proximity of both antigens (<30 nm), as expected for fusion proteins (Figure 2A). The positive DDLSs showed multiple nuclear dots in the pleomorphic tumour cell component (Figure 2B) and 3/5 also showed nuclear STAT6 accumulation.

Figure 2. NAB2 and STAT6 proximity ligation gives dot-like signals if both antibodies bind in close proximity (approximately <30 nm). A, SFT with nuclear dot-like signals in almost all tumour cells. B, DDLS with nuclear dot-like signals in a fraction of pleomorphic tumour cells. C, DDLS without a nuclear signal.

**Chromosomal copy number alterations:** Copy number analysis showed an overall low frequency of chromosomal imbalances in SFTs, but complex karyotypes in DDLSs, including amplification of STAT6 and MDM2 loci.

**Discussion:** A ‘gene dosage’ effect likely responsible for STAT6 expression in DDLSs. Region 12q13–15 harbours well-known oncogenes amplified in DDLSs, - MDM2 CDK4. STAT6 amplification was later confirmed in STAT6-positive DDLSs by FISH analysis.

**Take home message:** Thoroughly conducted study which supports the conclusion that the detection of nuclear relocation of STAT6 with IHC is a promising diagnostic marker that helps to discriminate SFTs from histological mimics.

**Context:** The oncofetal protein IMP3 is expressed during the early phases of embryogenesis with functions implicated in cell growth and cell migration. While epigenetically silenced after birth, IMP3 re-expression is noted in several malignancies and its re-expression has been correlated with decreased survival and increased risk of progression and metastases, and it is also considered a marker of preinvasive lesions.

**Objective:** To evaluate the expression of IMP3 in a series of neuroendocrine tumors of the lung, and correlate the results with mitotic count, Ki67 index and with clinical data and follow up. Also to confirm IMP3 involvement in stem cell processes by correlating its expression with Nanog and Oct3/4 (stem cell markers).

**Methods:**
- Using TMA’s, IHC for IMP3, Ki67, Nanog and Oct3/4 was performed and scored on a total of 74 neuroendocrine tumors (46 typical carcinoids, 9 atypical carcinoids, 13 large cell neuroendocrine carcinomas and six small cell carcinomas).
- IHC scoring as follows: IMP3 (focal = ≤30% vs diffuse = >30%); Nanog and Oct ¾ (0 (0% of cells), 1+ (≤30%), 2+ (31-60%), 3+ (≥61%); Ki-67 (grouped as ≤4%, 4-20%, >20%).
- All medical records were reviewed to obtain patients’ data, including age at diagnosis, sex, tumor location, tumor size and histological classification.

**Results:**
- IMP3 expression: in 50% of small cell carcinomas, 84% of large cell neuroendocrine carcinomas, 55% of atypical carcinoids and 10% of typical carcinoids.
- IMP3-positive cases showed significantly decreased overall and disease-free survival time compared with IMP3-negative cases.
- Nanog was expressed in 50% of small cell carcinomas, 31% of large cell neuroendocrine carcinomas, 33% of atypical carcinoids and 15% of typical carcinoids, and 68% of IMP3-positive tumors were also enriched for Nanog expression.
- Oct3/4 expression could not be detected in all the analyzed series.
- When combining Ki67 and IMP3 expression we demonstrated that all the cases with a Ki67 index higher than 4% were also IMP3-positive, and their simultaneous expression was a poor prognostic factor.

**Take Home Points:**
- IMP3 is a marker of poor outcome in lung neuroendocrine tumors (see figure 1).
- IMP3 correlation with Nanog expression suggest an implication of IMP3 in stem cell processes (see figure 3 for staining examples).
- IMP3 association with a Ki67 labeling index higher than 4% stratifies a subset of atypical carcinoids with a higher risk of recurrence and mortality (see figure 2).
- No difference in focal vs diffuse IPM3 staining with DFS and OS.
- NO statistically significant correlations observed with other demographic or clinpath parameters.
FIGURE 1. Cox proportional-hazards regression of IMP3-positive and -negative cases for disease-free survival (A, p = 0.0010) and overall survival (B, p = 0.0042).

FIGURE 2. Cox proportional-hazards regression of IMP3 and Ki67 values for atypical carcinoids: cases IMP3-negative and Ki67 < 4% are classified as "negative," and cases IMP3-positive and Ki67 > 4% are classified as "positive" with a statistical significance regarding the overall survival (A, p = 0.04) and disease-free survival (B, p = 0.01).

FIGURE 3. IMP3 and Nanog brown, cytoplasmic (IMP3) and nuclear/cytoplasmic (Nanog) staining in a typical carcinoid (A and B, respectively), in an atypical carcinoid (C and D, respectively) and in a large cell neuroendocrine carcinomas (E and F, respectively). Regarding the Nanog staining, in this case of typical carcinoid it was scored as 1+ (B), in the case of atypical carcinoid as 3+ (D) and in the case of large cell neuroendocrine carcinomas as 2+ (TMA spot: 4+, insert: 20×).

&


I decided to discuss these case histories as they fascinate me and I like the sleuthing that goes into solving these riddles given that “non-specific” inflammatory lesions are the bane of my life.

**Metal turners in a large, modern factory producing specialised machine parts.**

**Patient #1:**

- 2 year history of severe breathlessness that improved when he was not at work
- A high resolution CT scan - widespread ‘mosaic’ pattern of attenuation indicative of small airflow obstruction.
- A diagnosis of occupational extrinsic allergic bronchioloalveolitis and recommended that he change his work.
- After 12 months working elsewhere in the same company, away from the machine shop, his dyspnoea was greatly improved but had not disappeared.

**Patient #2:**

- For 2 years a patient in a specialist interstitial lung disease clinic with a diagnosis of chronic hypersensitivity pneumonitis.
- Positive findings of an autoimmune screen - an ‘autoimmune’ aetiology
- A high level of serum-specific IgG antibodies to Aspergillus species, therefore thought that exposure to ‘mould at home or work’ might be relevant
- While continuing to work he had been treated with pulsed methylprednisolone, cyclophosphamide, prednisolone, mycophenolate and N-acetyl cysteine with little evidence of success.
- On being informed that his illness was in all probability caused by his occupation, he chose not to return to work. Six months later, without any specific treatment, his lung function measurements had started to improve.

3 other patients found on systematic review of employees

Machining or ‘turning’ metal parts on a lathe is a skilled occupation used in the manufacture of a very wide variety of components. Metal shaping and grinding commonly involves the use of MWF (in the UK also known as ‘coolant’, ‘cutting fluid’ or ‘suds’) to lubricate the process, to control its temperature and to carry away the waste metal. The machines are generally enclosed and may be exhaust-ventilated to reduce—but rarely eliminate—the escape of MWF mist into the atmosphere. MWF is collected and recirculated, often with several machines sharing a common ‘sump’ or reservoir. New machines are capable of being operated continuously for 24 h and used a far higher volume of MWF. These changes probably led to far higher concentrations of MWF mist in the air of the shop floor.
A high index of suspicion is required in EAA due to MWF exposure as the symptoms are often non-specific and may be progressive, rather than clearly work-related. In some cases the presenting symptoms have been predominantly constitutional, with general malaise and unexplained weight loss. Long delays in reaching the correct diagnosis are not uncommon because symptoms are often attributed to asthma, COPD or to recurrent chest infections; or, as here, the diagnosis has been otherwise explained.

Over the last decade, MWF exposure has become the most commonly reported cause of occupational EAA in the UK, responsible for approximately half of all cases.

An outbreak in the Stoke-on-Trent area of North Staffordshire, UK, in July 2012, the first case presenting on July 18, 2012 with symptoms of a pneumonic illness. By the end of the outbreak at the beginning of August, a total of 20 cases had been confirmed and 2 eventually died.

The public health enquiry undertaken by the UK Health Protection Agency eventually identified three different local retail sites in the southern area of Stoke-on-Trent that the affected patients had visited. A discount warehouse that had a hot tub on open display. A swab taken from the tub identified the same L. pneumophila strain as in the sputum of the confirmed cases. All 20 patients, in fact, confirmed visiting the store at some point within the 2 weeks prior to the onset of their acute illnesses.

The hot tub was in fact positioned at the front of the store close to the tills and exits, meaning that a large number of people would have come into contact with it.
Articles for Notation

Neoplastic


**Objective** - To evaluate the potential role of immunohistochemistry (IHC) as a screening tool to identify candidate cases for FISH analysis and for ALK inhibitor therapy in NSCLC.

**Design** - FISH and IHC for ALK and mutational analysis for EGFR and KRAS in 523 NSCLC specimens. 344 whole-tissue sections (65.8%), 149 small biopsies (28.5%), and 30 cell blocks (5.7%) from pleural effusions. (77.6%) adenocarcinoma (ADC); (11.5%) squamous cell carcinoma; (5.7%) NSCLC, not-otherwise specified; (2.7%) large cell carcinoma; (1%) large cell neuroendocrine carcinoma; (0.8%) adenosquamous carcinoma; (0.8%) sarcomatoid carcinoma. IHC analysis with the monoclonal antibody D5F3 (Ventana), FISH analyses on paraffin sections of tumor tissues using a break-apart probe (Vysis LSI ALK dual color, Abbott) which hybridizes to the band 2p23 on either side of the ALK gene breakpoint. Also performed a Mass ARRAY-based analysis (Sequenom, San Diego) in 11 samples to detect EML4-ALK rearrangement.

**Results** - Of the 523 NSCLC specimens, 20 (3.8%) were positive for ALK rearrangement by FISH analysis. Positive ALK by FISH (20) exhibited ADC (17; 85%), 1 (5%) had sarcomatoid carcinoma, 1 (5%) had adenosquamous carcinoma, and 1 (5%) had NSCLC, not-otherwise specified, with neuroendocrine differentiation. No squamous histotypes were detected. A predominant solid pattern in 9 cases (53%), 5 cases (29%) predominant acinar pattern, and the 3 other cases (18%) predominant papillary pattern, signet ring features in 6 patients. **ALK rearrangement and EGFR and KRAS mutations were mutually exclusive.** Of 523 tumor samples analyzed, 18 (3.4%) were ALK(+) by IHC, 18 samples (3.4%) had concordant IHC and FISH results, and 2 ALK(+) cases (0.3%) by FISH failed to show ALK protein expression. In the latter 2 FISH-positive cases, no ALK variant RNA-transcripts or variants 1, 2, and 3 of EML4-ALK were detected. One patient underwent crizotinib treatment, but did not show any response. ? is the detected rearrangements by FISH not transcribed or translated., or the rearranged ALK protein not expressed or at an undetectable level. Alternately, an ALK fusion partner different from EML4 (such as TFG or KIF5B) could explain the difference between the results obtained at the DNA level and those obtained at the mRNA/protein level.

**Take home message** – Yet another paper favoring IHC over ALK FISH analysis and suggests that only ALK IHC is necessary at all. If FISH is positive and IHC negative may be unimportant as the transcription of the protein is the only relevant factor in determining whether the patient responds to therapy or not.

**Objective:** To report a case of pulmonary adenocarcinoma with concomitant EGFR mutation and ALK rearrangement with a literature review to look for other cases showing this phenomenon.

**Results:**
Authors case report:
- 68-year-old Caucasian male (never-smoker) with acinar pattern pulmonary adenocarcinoma, stage 4 (bilateral tumor nodules). Initially treated with first line cisplatin and pemetrexed, had stable disease. 8 months after diagnosis, was found to have EGFR mutation in exon 21 (L858R), subsequently treated with the TKI erlotinib and had stable disease for over 3 years until symptoms reappeared. Patient re-biopsied and shown same EGFR mutation PLUS ALK rearrangement (then retrospectively found on initial biopsy). Patient then treated with 3rd line therapy of ALK inhibitor crizotinib and remained asymptomatic with reduction in nodules in July 2014 (when paper was submitted).
- Of note, the two mutations were found within the SAME tumor cells (not in different clones).

**Literature Review:**
- Authors found 42 other cases with concomitant EGFR and ALK mutations, and in those who received TKI inhibitors or crizotinib (in various combinations), up to 70% had partial or good responses.
- The majority of patients with dual mutations had adenocarcinomas (34 in total), were non-smokers and mainly Asiatic (28 cases). Gender made no difference.

**Take Home Message:**
- The authors note that EGFR/ALK double-positive NSCLC seems to show a therapeutic advantage from combinations of different treatments.
- Even though these double mutations are uncommon (1-1.5%), the possibility of a therapy advantage should broaden our scope to simultaneously look for multiple mutations that were previously thought to be mutually exclusive.


**Context:** There is a growing trend to move away from surgically staging lung cancer and to more minimally invasive approaches like EBUS-FNA. However problems can arise when while molecular analysis is attempted on the limited EBUS cytology samples.

**Objectives:** EGFR mutations are commonly tested by PCR based techniques on formalin-fixed paraffin-embedded (FFPE) samples including cell blocks (CB) that may fail due to the absence of tumor cells. The cell pellet from cytology specimens obtained at the time of EBUS-FNA represents an alternative resource for additional tissue. The authors redirected testing to the FNA cell pellet versus the paraffin embedded cell block for the detection of EGFR mutations.

**Method:** 39 cytology samples from patients with NSCLC referred for EGFR testing were analyzed using the EGFR rotor-gene Q (RGQ) PCR assay (Qiagen), for internal validation. Then a consecutive series of 228 EBUS FNA samples were tested.
**Results:** The ASPCR assay demonstrated acceptable intra-assay, inter-assay and inter-lot reproducibility, sensitivity, and specificity. Of the consecutive series, only 6/228 (2.6%) failed analysis (5 due to insufficient DNA yield). Of 228 EBUS FNA cell pellets tested 32 (14.0%) demonstrated clinically relevant mutations.

**Take Home Points:**
- By using the cell pellet for testing instead of the paraffin embedded CB, ASPCR can reliably detect EGFR gene mutations in FNA preparations from patients with NSCLC obtained at EBUS.
- Limitations of using LBC for molecular testing is that specimen may still be paucicellular.
- DNA extraction from LBC specimens will need validation in the future.
- May need to re-consider doing more than 3 needle passes to meet requirements for future expanded molecular testing.


**Objectives:** Noninvasive, real-time, inexpensive detection and monitoring of EGFR mutations in patients with NSCLC is highly desirable.

**Methods:** A novel core technology, electric field-induced release and measurement (EFIRM), which relies on a multiplexible electrochemical sensor that can detect EGFR mutations directly in bodily fluids.

**Measurement and Main Results:** EFIRM was developed for the detection of the EGFR mutations in vitro and correlated with tumor size from xenografted mice. In clinical applications, demonstrated that EFIRM could detect EGFR mutations in the saliva and plasma of 22 patients with NSCLC. Finally, a blinded test was performed on saliva samples from 40 patients with NSCLC. The receiver operating characteristic analysis indicated that EFIRM detected the exon 19 deletion with an area under the curve of 0.94 and the L858R mutation with an area under the curve of 0.96.

**Conclusions:** “Our data indicate that EFIRM is effective, accurate, rapid, user-friendly, and cost effective for the detection of EGFR mutations in the saliva of patients with NSCLC. We termed this saliva-based EGFR mutation detection (SABER)”.

**Take home message:** the authors describe cancer-derived microvesicles which can alter the contents of secreted microvesicles of salivary gland cells and therefore found it readily believable that tumor derived-DNA with an EGFR mutation could be detected in the saliva. I don’t understand the technology but more studies are needed and, if this holds up to scrutiny, would be a real breakthrough.


**Context:** Insulin-like growth factor-1 receptor (IGF1R) is a tyrosine kinase membrane receptor that is overexpressed in many cancers and while its exact role is not completely understood, there is in vitro evidence that high IGF1R protein levels and gene copy number (GCN) predict sensitivity to IGF1R
inhibition by monoclonal antibodies, therefore IGF1R may be a potential therapeutic target.

**Objective:** To determine the incidence of alterations in IGF1R GCN and protein expression in primary and metastatic NSCLC in different histological subtypes and to investigate any correlations with clinicopathological features.

**Methods:** Using tissue microarray sections from a **retrospective** cohort of 309 surgically resected NSCLCs (138 adenocarcinomas (ADC), 114 squamous cell carcinomas (SCC), 54 large cell carcinomas (LCC) and 3 others (2 spindle cell carcinomas and 1 pleomorphic carcinoma) IGF1R gene copy number status was evaluated by chromogenic SISH and IGF1R protein expression was evaluated by immunohistochemistry. The results were then compared with clinicopathological features, including EGFR and KRAS mutational status (in the adenocarcinomas) and patient survival.

**Results:**
- IGF1R gene copy number status was positive (high polysomy or amplification) in 29.2% of NSCLC, and 12.1% exhibited IGF1R gene amplification and high IGF1R expression in 28.3%.
- There was a modest correlation between IGF1R gene copy number and protein expression (r=0.2, p<0.05).
- Alterations of IGF1R gene copy number and protein expression in primary tumours were significantly associated with alterations in lymph node metastases (p<0.01).
- High IGF1R gene copy number and protein expression was significantly higher in squamous cell carcinomas compared with other subtypes of NSCLC (p<0.05).
- There were no other associations between IGF1R status and other clinicopathological features including patient age, gender, smoking status, tumour size, stage, grade, EGFR or KRAS mutational status or overall survival.

**Take Home Message:**
- High IGF1R gene copy number and protein overexpression are frequent in NSCLC’s, particularly in squamous cell carcinomas (similar to other studies).
- IGF1R is not a prognostic marker in NSCLC.


**Context:** Mutant BRAF is a driver oncogene found in 2% of lung adenocarcinomas and represents a target for therapy.

**Objective:** To examine the clinical characteristics and course of patients with lung adenocarcinomas with BRAF mutations.

**Methods:** To identify lung adenocarcinoma patients with BRAF mutations by using a mass spectrometry-based PCR genotyping assay of hot spot BRAF mutations (V600, D594, and G469). Patient characteristics (including age, sex, race, stage at initial diagnosis of BRAF mutant disease, date of resection, treatment history, and smoking history, were recorded) and treatment outcomes were analyzed. Overall survival (OS) was compared with stage-matched patients with KRAS and EGFR mutant lung adenocarcinomas.
Results:
- 63 patients had BRAF mutant lung adenocarcinomas between 2009 and 2013 (V600 = 36; non-V600 = 27).
- Most with BRAF mutations were smokers (92%), although patients with V600 mutations were more likely to be light/never-smokers compared with patients with non-V600 mutations (42% vs 11%; p = 0.007).
- Of the 32 patients with early-stage disease, six (19%; 95% C.I. 7%–36%) developed second primary lung cancers harboring KRAS mutations.
- Patients with advanced V600 mutant lung adenocarcinomas had a better survival from diagnosis compared with those with non-V600 mutant lung adenocarcinomas (3-year OS: 24% vs 0%; p < 0.001).

Take Home Points:
- This is the largest series of patients with BRAF mutant lung cancers described.
- Most patients were heavy smokers.
- 19% of patients with early-stage BRAF mutant lung cancers developed second primary lung cancers harboring KRAS mutations.
- Patients with advanced lung adenocarcinomas harboring V600 mutations have an improved OS compared with those with non-V600 mutations.
- While this is the largest series to date, the numbers are still relatively small to make firm conclusions.

7. Seo AN et al. FGFR1 amplification is associated with poor prognosis and smoking in non-small-cell lung cancer. Virchows Arch 2014;465:547-558.

Context: FGFR1 amplification has been identified as an important therapeutic target in NSCLC (via pathway inhibition, especially in SqCC). It has been cited as an independent negative prognostic factor, and has been associated with a smoking history in lung SqCC, however the data from previous studies on the clinical implications of FGFR amplification in NSCLC are inconsistent.

Objective: To critically compare scoring criteria of FGFR1 amplification in NSCLC used in previous studies and investigate the associations of FGFR1 amplification with clinicopathological characteristics and its prognostic significance.

Methods: Retrospective analysis that evaluated FGFR1 gene copy number (GCN) in 369 cases of surgically resected NSCLC from May 2003 to June 2008, using five previously reported criteria and investigated associations between clinicopathologic parameters and FGFR1 amplification. FGFR1 status was obtained using FISH on TMA sections. FGFR1-IHC was used and levels of membranous and cytoplasmic staining were scored.

Results:
- FGFR1 amplification was found in 32/369 (8.7%) of NSCLC and was more frequent in SqCC (18.0% in SqCC, 3.0% in adenocarcinoma; p < 0.001) and in smokers (p < 0.001).
- On univariate analysis, FGFR1 amplification was significantly associated with shorter overall survival (OS, 58.6 vs 80.0 months; p = 0.033) and shorter disease-free survival (DFS, 58.5 vs 80.0 months; p = 0.042) in patients with SqCC, but this was not statistically significant on multivariante
analysis (OS: hazard ratio [HR] = 1.79, 95% confidence interval [CI] = 0.83–3.87, p = 0.139; DFS: HR = 1.73, 95% CI = 0.93–3.21, p = 0.081).

**Take Home Points:**
- FGFR1 amplification is more common in smokers and in patients with SqCC.
- Patients with FGFR1 amplification show a tendency for adverse outcome, but FGFR1 amplification is not an independent prognostic factor in SqCC.
- Correlation between FGFR1 GCN and protein expression is not significant (rho=0.08; p=0.123).
- Findings suggest that cigarette smoking is responsible to FGFR1 amplification and oncogenic pathway activation.
- Authors hypothesize that FGFR1 amplification may not be a driver alteration in lung cancer but may play a role in its progression.


**Objective:** To characterized the histologic and immunophenotypic features of pulmonary mucoepidermoid carcinoma and assess the significance of disease defining translocation, t(11;19)(q21;p13) in pulmonary MEC.

**Methods:** 43 pulmonary mucoepidermoid carcinomas were re-reviewed and graded according to the Brandwein grading system for mucoepidermoid carcinoma (4 were excluded due to differing opinions between re-review and the pathology report). TTF-1, napsin A, p40 and p63 immunostains were scored and FISH was used to detect the MAML2 rearrangement.

**Results:**
- 25 cases with available tissue blocks tested for immunohistochemistry and were positive for p63; 23 also expressed p40.
- In six cases, the p63 score was higher than p40.
- TTF-1 and napsin were uniformly negative in all 25 cases.
- MAML2 rearrangement was identified by FISH in each of the 24 cases tested (3 low, 19 intermediate, 2 high grade).
- Clinical history was available in 29 patients (15 men) (median age, 48 years) with follow-up in 24 (median, 8.4 years). Five patients died of unrelated causes; one developed metastatic pulmonary mucoepidermoid carcinoma.

**Take home points:**
- Most pulmonary mucoepidermoid carcinomas are of morphologic intermediate grade.
- Features helpful in distinguishing pulmonary MEC from others include: a) location: central/endo- or peribronchial location, b) IHC expression: p63 expression and lack of TTF-1 and napsin A expression, c) cytogenetics: MAML2 rearrangement, d) morphologic characteristics including scattered mucous cells, and lack of keratinization.
- p63 might be superior to p40 in aiding the diagnosis of pulmonary mucoepidermoid carcinoma.
- Separating MEC from its mimics is critical because of prognostic (prognosis of mucoepidermoid carcinoma is usually superior) and therapeutic implications (see table 2).

Context: The Lungscape project was designed to address the impact of clinical, pathological, and molecular characteristics on outcome in resected NSCLC.

Objective: To describe the outcome, including overall survival (OS), as reported in the IASLC database, as well as the relapse-free survival (RFS) and time to relapse (TTR), according to pathological stage, histology, and clinical parameters for 2449 patients with resected NSCLC.

Methods: A decentralized biobank was created to clarify the outcome resected stage I to III NSCLC by clinically, pathologically, and molecularly characterized subgroups. Selection criteria for participating centers included sufficient number of cases, tissue microarray building capability, and documented ethical approval. Patient selection was based on availability of comprehensive clinical data, radical resection between 2003 and 2009 with adequate follow-up, and adequate quantity and quality of formalin-fixed tissue.

Results:
- 15 centers contributed 2449 cases from 2003 to 2009 with complete information on medical history, histology, and pathological TNM staging.
- Most patients with status alive at last follow-up have been followed for more than 3 years, except for 43 (with follow-up between 2 and 3 years).
- 5-year overall survival (OS) was 69.6% and 63.6% for stages IA and IB, 51.6% and 47.7% for stages IIA and IIB, and 29.0% and 13.0% for stages IIIA and IIIB, respectively (p < 0.001).
- Median and 5-year relapse-free survival (RFS) was 52.8 months and 47.3%, respectively.
- Distant relapse was recorded for 44.4%, local for 26.0%, and both for 16.9% of patients.
- Based on multivariate analysis for the OS, RFS, and time to relapse, the factors significantly associated with all of them are performance status and pathological stage.

Take Home Points:
- Lungscape is the first large series reporting on NSCLC surgical outcome measured by OS but also by RFS and time to relapse and including multivariate analysis by significant clinical and pathological prognostic parameters.
- This study provides a massive database for molecular analyses with disease outcomes (ALK translocations have been reported) and there are numerous other projects underway to look forward to...
Background: Chromosomal rearrangements leading to fusion of ROS1 with a number of different partners create a constitutively active kinase that activates the MAP kinase, STAT3, and phosphoinositide 3-kinase (PI3K) pathways. In NSCLC, ROS1 rearrangements are more likely to be found in younger patients, those with adenocarcinoma, and those without a history of tobacco use. Crizotinib is a small-molecule tyrosine kinase inhibitor of anaplastic lymphoma kinase (ALK), ROS1, and another proto-oncogene receptor tyrosine kinase, MET.

Methods: 50 patients with advanced NSCLC who tested positive for ROS1 rearrangement in an expansion cohort of the phase 1 study of crizotinib. ROS1 fusion partners were identified with the use of next-generation sequencing or reverse-transcriptase-polymerase-chain-reaction assays.

Results: The objective response rate was 72% (95% confidence interval [CI], 58 to 84), with 3 complete responses and 33 partial responses. The median duration of response was 17.6 months (95% CI, 14.5 to not reached). Median progression-free survival was 19.2 months (95% CI, 14.4 to not reached), with 25 patients (50%) still in follow-up for progression. Among 30 tumors that were tested, 7 ROS1 fusion partners were identified: 5 known and 2 novel partner genes. No correlation was observed between the type of ROS1 rearrangement and the clinical response to crizotinib.

Conclusions: In this study, crizotinib showed marked antitumor activity in patients with advanced ROS1-rearranged NSCLC. ROS1 rearrangement defines a second molecular subgroup of NSCLC for which crizotinib is highly active.

Take home message: The overall response rate of 72% was very encouraging given that response rates to cytotoxic chemotherapy in patients with previously treated NSCLC are generally <10%. This large response rate was seen despite a relatively small trial – 50 patients. ROS1 testing is here to stay.
Background: The National Lung Screening Trial (NLST) showed that screening with low-dose computed tomography (CT) as compared with chest radiography reduced lung-cancer mortality. This study examined the cost-effectiveness of screening with low-dose CT in the NLST.

Methods: Mean life-years, quality-adjusted life-years (QALYs), costs per person, and incremental cost-effectiveness ratios (ICERs) for three alternative strategies: screening with low-dose CT, screening with radiography, and no screening were examined. Estimations of life-years were based on the number of observed deaths that occurred during the trial and the projected survival of persons who were alive at the end of the trial. Quality adjustments were derived from a subgroup of participants who were selected to complete quality-of-life surveys. Costs were based on utilization rates and Medicare reimbursements.

Results: As compared with no screening, screening with low-dose CT cost an additional $1,631 per person (95% confidence interval [CI], 1,557 to 1,709) and provided an additional 0.0316 life-years per person (95% CI, 0.0154 to 0.0478) and 0.0201 QALYs per person (95% CI, 0.0088 to 0.0314).
corresponding ICERs were $52,000 per life-year gained (95% CI, 34,000 to 106,000) and $81,000 per QALY gained (95% CI, 52,000 to 186,000). However, the ICERs varied widely in subgroup and sensitivity analyses.

Conclusions: Screening for lung cancer with low-dose CT would cost **$81,000 per QALY gained, but modest changes in assumptions would greatly alter this figure**. The determination of whether screening outside the trial will be cost-effective will depend on how screening is implemented.

**Take home message:** The jury is still out on whether this is a cost-effective way to go


**Background:** For laboratories is to meet needs of molecular diagnostics there must be reliable methods and processes in place to ensure timely and accurate reporting on which patients treatment will be based.

**Objectives:** The authors offer a framework for the minimum requirements for the management of molecular pathology to provide reliable service. As part of the framework, they incorporate the recommendations from the CAP, IASLC and AMP and other agencies, along with the ISO15189 guidance.

**Take Home Messages:**
- The paper is a nice overview of the QA/QC elements to consider in the management of molecular pathology laboratories, from pre-analytical considerations to laboratory accreditations and reporting.
- Would also be a good review for residents taking their board exams!

**Non-neoplastic**


The International Society for Heart and Lung Transplantation, American Thoracic Society, and European Respiratory Society convened a committee of international experts to describe and/or provide recommendations for 1) the definition of BOS, 2) the risk factors for developing BOS, 3) the diagnosis of BOS, and 4) the management and prevention of BOS. A pragmatic evidence synthesis was performed to identify all unique citations related to BOS published from 1980 through to March, 2013. The expert committee discussed the available research evidence upon which the updated definition of BOS, identified risk factors and recommendations are based. The committee followed the GRADE (Grading of Recommendation, Assessment, Development and Evaluation) approach to develop specific clinical recommendations. The term BOS should be used to describe a delayed allograft dysfunction with persistent decline in forced expiratory volume in 1 s that is not caused by other known and potentially reversible causes of post-transplant loss of lung function. The committee formulated specific recommendations about the use of systemic corticosteroids, cyclosporine, tacrolimus, azithromycin and about re-transplantation in patients with suspected and confirmed BOS. The diagnosis of BOS requires the careful exclusion of other post-transplant complications that can cause delayed lung allograft dysfunction, and several risk factors have been identified that have a significant association with the
onset of BOS. Currently available therapies have not been proven to result in significant benefit in the prevention or treatment of BOS. Adequately designed and executed randomised controlled trials that properly measure and report all patient-important outcomes are needed to identify optimal therapies for established BOS and effective strategies for its prevention.

**Take home message:** Parts of this are a useful reference when confronted with a bronchial biopsy in post-transplant patients.


**Rationale:** Beryllium continues to have a wide range of industrial applications. Exposure to beryllium can lead to sensitization (BeS) and chronic beryllium disease (CBD).

**Objectives:** The purpose of this statement is to increase awareness and knowledge about beryllium exposure, BeS, and CBD.

**Methods:** Evidence was identified by a search of MEDLINE. The committee then summarized the evidence, drew conclusions, and described their approach to diagnosis and management.

**Main Results:** The determinants of progression from BeS to CBD are uncertain, but higher exposures and the presence of a genetic variant in the HLA-DP β chain appear to increase the risk. Periodic evaluation of affected individuals can detect disease progression (from BeS to CBD, or from mild CBD to more severe CBD). Corticosteroid therapy is typically administered when a patient with CBD exhibits evidence of significant lung function abnormality or decline.

**Take home message:** A good reference for beryllium related disease.


Interesting rare lesion. Doesn’t appear to be associated any other abnormalities.

**Miscellaneous**


**Comment** on article by Bass et al.

Objective - To evaluate the impact of preanalytical factors associated with the formalin fixation and paraffin embedding process on downstream morphological and molecular endpoints. The existing literature using the National Cancer Institute's Biospecimen Research Database for published reports investigating the potential influence of preanalytical factors associated with the formalin fixation and paraffin embedding process on DNA, RNA, protein, and morphological endpoints.

Conclusions - Based on the literature evidence, the molecular, proteomic, and morphological endpoints can be altered in formalin-fixed, paraffin-embedded specimens by suboptimal processing conditions. While the direction and magnitude of effects associated with a given preanalytical factor were dependent on the analyte (DNA, RNA, protein, and morphology) and analytical platform, acceptable conditions are highlighted, and a summary of conditions that could preclude analysis is provided.

Take home message: Yet more on quality control issues associated with FFPE tissue but an important topic since tests on these tissues are only as relevant as the quality of the materials used.


Objectives: Formalin-fixed, paraffin-embedded unstained archived diagnostic tissue sections are frequently exchanged between clinical laboratories for immunohistochemical staining. The manner in which such sections are prepared represents a type of preanalytical variable that must be taken into account given the growing importance of immunohistochemical assays, especially predictive and prognostic tests, in personalized medicine.

Methods: Recommendations were derived from review of the literature and expert consensus of the Canadian Association of Pathologists-Association canadienne des pathologistes National Standards Committee for High Complexity Testing/Immunohistochemistry.

Results: Relevant considerations include the type of glass slide on which to mount the unstained sections; the thickness of the tissue sections; the time from slide preparation to testing; the environment, particularly the temperature at which the unstained sections will be maintained prior to testing; the inclusion of on-slide positive control tissue where possible; and whether patient identifier(s) should be included on slide labels.

Take home message: a useful reference for those responsible for relevant studies with overlap with previous article.


Context - Laboratories must validate all assays before they can be used to test patient specimens, but currently there are no evidence-based guidelines regarding validation of immunohistochemical assays.
Objective - To develop recommendations for initial analytic validation and revalidation of immunohistochemical assays.

Design - The College of American Pathologists Pathology and Laboratory Quality Center convened a panel of pathologists and histotechnologists with expertise in immunohistochemistry to develop validation recommendations. A systematic evidence review was conducted to address key questions. Electronic searches identified 1463 publications, of which 126 met inclusion criteria and were extracted. Individual publications were graded for quality, and the key question findings for strength of evidence. Recommendations were derived from strength of evidence, open comment feedback, and expert panel consensus.

Results - Fourteen guideline statements were established to help pathology laboratories comply with validation and revalidation requirements for immunohistochemical assays.

Conclusions - The parameters for cases should take into account intended use and should be sufficient to ensure that the test accurately measures the analyte of interest in specimens tested in that laboratory. Recommendations were also provided for confirming assay performance when there are changes in test methods, reagents, or equipment.

Take home message: Yet another quality control type paper which provides a useful reference (and Dr. Paul Swanson, senior author has just joined us here in Calgary - had to mention this!).


Context - The interpretation of scanned whole-slide images (WSI) offers some theoretical advantages for long-distance, consultative diagnosis in surgical pathology. Few WSI validation studies have focused on difficult consultation cases.

Objective - To test intraobserver variability of WSI interpretations in cases that had been submitted for consultation using the same hardware and software configuration selected by a client.

Design - The 217 cases (approximately 20 nearly consecutive cases received in consultation for each of 11 subspecialty groups) were scanned, uploaded to an image-distribution application, and interpreted by 26 pathologists who had reviewed the microscope slides an average of 47 days earlier. Independent pathologists identified and classified discrepancies between microscope slide and WSI diagnoses.

Results - There were 2 major discrepancies (0.92%) and 8 minor discrepancies (3.7%). One major discrepancy reflected atypical versus nonatypical endometrial hyperplasia; the other related to reactive squamous changes versus carcinoma. Strengths of the study include the large sample size, the many pathologists involved, the degree of difficulty of the cases, and the duplication of scanning and software configuration projected to be used by a client. Although the average 43-day washout exceeds the 2-week interval recommended by an expert panel of the College of American Pathologists, an important limitation in this study was that pathologists commonly remember consultation cases for a long time.
**Take home message** – Very interesting study. Perhaps they should have got the pathologists to look at the cases on slides again too and see if any discrepancies. Not sure if we all “commonly remember consultation cases for a long time”, it depends on how unusual and interesting the case is for me at least.

**Pop quiz: how many of you are looking forward to exclusive digitization of slides?**