ARTICLES FOR DISCUSSION
1- The pulmonary histopathologic manifestations of the anti-PL7/antithreonyl transfer RNA synthetase syndrome. Yousem et al. Hum Pathol 2014; 45:1199

Background
- The highest incidence of ILD in CTD occur with antisynthetase syndromes, characterized by varying incidence of myositis, arthritis, Raynaud, skin lesions including mechanic’s hands.
- ASS is associated with specific autoAbs, the most common being anti-Jo1 (20-30%) which has a reported incidence of 70% for ILD.
- The second most common autoAb is PL7 (3-17%) also with high incidence of ILD but not well characterized histologically.

Methods
- The U of Pitts has a CTD registry with 3 decades of prospectively enrolled patients.
- Mined the registry from 1985-2009 for ASS with Anti-PL7 who had a pathology specimen.
- Classified ILD according to the 2002 ATS/ERS consensus classification of ILD

Results
- 8 patients; 5W:3M mean 54 yo, cough in 5, SOB in 3. 7 with surgical bx, 1 with TBBx
- HRCT 5 with UIP, 3 with NSIP
- Concordance between HRCT and pathology in 5 cases
  o 4 UIP
  o 1 NSIP
- 1 case of HRCT dx as UIP had OP, but on a TBBX however the FU indicated ANED at 120mos which would support a dx of OP rather than UIP
- 2 cases of HRCT dx as NSIP, 1 had OP and 1 had LIP
- Total 4 UIP, 2 OP, 1 NSIP and 1 LIP
- FU 15-132 mos ave 64 mos and FU in keeping with the histologic dx

Conclusion
- In their series, UIP is the most common dx. In contrast to the few cases reported in the literature with 4/ 5 cases of NSIP. Bias of patients biopsied versus not? The authors don’t report on the other non-bx specimen for comparison.
2- B7-H1 Expression in Malignant Pleural Mesothelioma is Associated with Sarcomatoid Histology and Poor Prognosis. Mansfield et al. JTO 2014; 9:1036

Background
- TILs in MM are associated with improved survival
- B7-H1 aka PD1-L1 is a negative co-stimulatory molecule which inhibits T-cell activation and immune tolerance by binding to the PD-1 receptor
- Many tumors express B7-H1 and this expression has been associated with poor prognosis

Aim
- Look at the effect of B7-H1 expression on the survival of MM

Methods
- All treated MM at Mayo Clinic 1987-2003
- IHC for B7-H1
- Statistical analysis

Results
- 106 patients with 104 dead at last FU. 1 alive with no recurrence at 10 years and 1 lost to FU
- 42 (40%) with B7-H1 expression, med 35% cells
- All sarcomatoid MM (16 of 17 cases except 1 desmoplastic) expressed B7-H1
- Positive cases had less surgery, higher stage
- TILs similar between positive and negative cases
- 3 patients with multiple samples and B7-H1 expression concordant between samples
- Positive cases with shorter survival 5mos med vs 14.5 mos (p<0.0001)
  - Epitheloid positive cases with shorter survival 14 vs 6 mos (NS)
- In multivariate B7-H1 and sarcomatoid histology remained significant

Conclusions
- Importance of this finding relates to immune modulatory therapies
  - Currently early phase clinical trials including in lung cancer looking at targeting B7-H1/PD-1 axis with promise
  - This therapeutic strategy could be applied to MM
3- The prognostic value of architectural patterns in a study of 37 type AB thymomas. Vladislav et al. *Mod Pathol* 2014; 27:863

**Background**
- Histologic subtype associated with behavior
- Recent suggestion that a subset of type A with worse outcome based on cytologic atypia.
- One of the authors noted 2 different architectural patterns to the AB subtype and hypothesized these could have different outcome

**Methods**
- In Indiana University, a thorough database and pathologic material kept on all patients treated for thymoma. The database was queried for spindle cell thymomas with >10% lymphocytes. 37 cases with complete information retrieved.
- All slides reviewed by 2 pathologists and cases divided into 2 subtypes based on different criteria (Table 2) mainly the morphology of the spindle cell (fibroblast like versus plump) and the lymphoid stroma

<table>
<thead>
<tr>
<th>Histological features</th>
<th>AB type 1</th>
<th>AB type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrotic bands</td>
<td>No/inconspicuous</td>
<td>Yes/predominant</td>
</tr>
<tr>
<td>Reticular growth pattern</td>
<td>Yes/predominant</td>
<td>No/inconspicuous</td>
</tr>
<tr>
<td>Type of cells in intervening septae</td>
<td>Tumor cells</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>Stromal lymphocytes</td>
<td>Sparse</td>
<td>Variable</td>
</tr>
<tr>
<td>Character of the spindle cells</td>
<td>Elongated</td>
<td>Plump</td>
</tr>
<tr>
<td>Nuclear features</td>
<td>Bland</td>
<td>Irregular with small nucleoli</td>
</tr>
<tr>
<td>Cytologic atypia</td>
<td>No</td>
<td>Maybe present</td>
</tr>
</tbody>
</table>

**Results**
- 37 patients with various stage (I to IVB) and treatment reviewed.

<table>
<thead>
<tr>
<th></th>
<th>Type I N=18</th>
<th>Type II N=19</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>Stage I-II</td>
<td>18</td>
<td>10</td>
<td>0.0011</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Max size</td>
<td>14</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>16- 89%</td>
<td>12- 63%</td>
<td>NS</td>
</tr>
<tr>
<td>Adjuvant TX</td>
<td>2- 11%</td>
<td>7- 37%</td>
<td>NS</td>
</tr>
<tr>
<td>Relapse</td>
<td>0</td>
<td>4- 21%</td>
<td>0.047</td>
</tr>
<tr>
<td>Metastasis</td>
<td>0</td>
<td>1-5%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Authors state not enough cases for a multivariate analysis

**Conclusion**
- I can’t believe this got published in Mod Path. Seems that stage not histologic subtype likely determined the outcome in these cases….And not enough case to make any conclusions
Type AB thymoma is not a mixed tumor of type A and type B thymomas, but a distinct type of thymoma. Miki et al. Virchows Arch 2014; 464:725

**Background**
- Type AB thymoma generally regarded as a mixture of type A and type B thymomas but not been extensively investigated
- Metaplastic thymoma is a rare histological variant characterized by a histological biphasic growth pattern of type A thymoma-like areas and metaplastic mesenchymal areas

**Aim**
- To examine the morphological and immunohistochemical nature of AB thymomas.

**Methods**
- 1990-2011 45 thymomas: 10 A, 19 AB, 15 B1 and 1 metaplastic
- IHC AE1/AE3, Vimentin, EMA, Claudin-1, Fascin, HLA-DR, CD99, Langerin, E-cadherin

**Results**
- Type A thymomas (n=10):
  - Tumor cells + for AE1/AE3, claudin-1, and E-cadherin
  - Tumor cells negative for vimentin, EMA, and HLA-DR
  - No fascin+ cells r/o thymic cortical dendritic macrophages, mature medullary dendritic cells (mmDC). Immature fascin− HLA-DR+ immature DC scattered in tumor
  - No CD99+ or CD99− T lymphocytes observed.
- Type B1 thymomas (n=15)
  - In the cortical areas, numerous CD99+ thymocytes and in medullary area CD99 mature T lymphocytes present
  - AE1/AE3 show tumor cells mainly present in cortical areas, fewer in the medullary areas
  - Tumor cells in cortical area negative for HLA-DR and claudin-1; in medullary area thymic cells strongly positive for HLA-DR and weakly positive for claudin-1.
  - In the cortical areas, numerous fascin+ TCDM
  - Numerous mature DC in medullary areas
- Metaplastic thymoma (n=1)
  - Spindle cells look like type A, AE1/AE3+, vimentin−, EMA−,claudin-1+, E-cadherin+
  - The fibroblast-like spindle cells negative for AE1/AE3, claudin-1, and E-cadherin and positive for vimentin and EMA.
  - No fascin− HLA-DR+ immature DC
  - Small areas with fascin+ HLA-DR+ mmDC and CD99− mature T lymphocytes like thymic medulla - “medullary” islands
- Type AB thymomas (n=19)
  - Variable mixture of lymphocyte-poor type A thymoma-like components and lymphocyte-rich type B short spindle cells
The immunophenotype of the short spindle cells was similar to that of the tumor cells of type A thymoma. No TCDM, mature medullary DC, or immature DC were detected within the A-like components.

In 14 cases, the type A-like components were composed solely of elongated fibroblast-like spindle cells like in metaplastic thymoma with similar immunophenotype = “metaplastic subtype AB thymoma.”

In the two subtypes, the type B-like components composed of cortical and medullary areas.

So 2 subtypes of type AB thymoma – 1) a “true” mixture of type A- and type B1-like components and 2) a mixture of type B1-like components and metaplastic mesenchymal components, and constituting the major subtype (14 of 19). And the B component of AB appears different than in B1 (spared the whole details of the distribution of epithelial cells, mature/immature DC etc)

**Conclusions**

- No lack of minutia in this publication (and spared us many of the details) but makes a point of morphologically and immunophenotypically distinct AB thymomas. Does not address the clinical significance of this finding.
- And the only overlap with the other article is the nature of the spindle cells – plump vs fibroblasts 😊
5- Prognostic Factors for Cure, Recurrence and Long-Term Survival After Surgical Resection of Thymoma. Safieddine et al. JTO 2014; 9: 1018

Background
- Several studies show that Masaoka stage and complete surgical resection are independent prognostic factors for thymoma
- Although WHO adopted for histologic classification, it still remains controversial as an independent prognostic factor for thymoma

Aim
- To do a retrospective study of a large cohort of thymoma to further study the association of WHO histology as well as tumor size, Masaoka and completeness of resection with survival.

Methods
- Retrospective cohort of patients 1986-2010 with a prospectively collected database which otherwise translated to no re-review of the pathology and diagnosis basically taken from reports – not all slides available for review
  - 200/262 had a WHO histologic classification
  - 24 Bernatz
  - 19 “benign” thymoma
  - 19 thymic carcinoma not included in survival analysis
- Surgical resection labeled as microscopically complete or incomplete
- Masaoka staging used

Results
- 262 51%W, med 55yo, 39% myasthenia
  - Med FU 7.5yrs
  - Med tumor size 5.4cm
- Masaoka stage: I, 65 (25%); II, 123 (47%); III, 45 (17%); and IV, 10 (4%)
- WHO (for the 200) type A, 14 (7%); AB. 44 (22%); and B1, 30 (15%); B2 77 (38.5%); B3 35 (17.5%)
- 200 (83%) R0; 43 (17%) with 41 R1 and 2 R2
- 166 adjuvant radiotherapy, 4 adjuvant chemoradiotherapy, and 14 neoadjuvant chemoradiotherapy
- OS 95% at 5 years, 91% at 10 years, and 91% at 15 years. Don’t mention DFS
- Recurrence in 12; disease-related death in 4
- On univariate analysis
  - Masaoka stage and incomplete resection associated with statistically increased risk of recurrence
  - Larger tumor size greater than 7 cm and increased age also statistically significantly
- On multivariate analysis
  - Only increased Masaoka stage and size remain statistically significant associated
  - Gender, WHO histology and adjuvant radiotherapy not significant

Conclusions
Size came out as an independent prognostic marker (and we found the same in our study that we are still trying to get published). So probably something that should be considered in a staging model.

My pet peeve about this is with JTO not the article per se, the editor rejected a month ago one of our thymoma study stating that unfortunately for us as the new WHO was coming out our study could not be published, yet they publish this study which not only does not use a future unpublished classification but includes non WHO classified tumors.

ARTICLES FOR NOTATION

Original article

1- Tumor/ Stromal Caveolin-1 Expression Patterns in Pleural Mesothelioma Define a Subgroup of the Epithelial Histotype With Poorer Prognosis. Rhigi et al. Am J Clinic Pathol 2014; 141:186

Background

- MPM epithelioid type has a better prognosis than other types.
- Recent meta-analysis of gene expression profiling in MPM showed 78 highly significant genes including Caveolin-1 (CAV-1).
- CAV-1 is a membrane protein with potential pathogenetic role in IPF and systemic sclerosis fibrosing lung disease, and marker of EMC in neoplasms.

Aim

- To assess the expression of CAV1 in MPM and potential prognostic role.

Methods

- 131 MPM – Group A 63 (54 epithelioid, 9 biphasic), Group B – validation group 45 (37 epithelioid and 8 biphasic), Group C- 23 sarcomatous and 40 pleuritis
- Meso cell lines 2 epithelioid and 1 biphasic RNA expression
- IHC for CAV-1 along with calretinin, D2-40, WT-1, CD31, V, SMA for dual labeling with CAV-1
- For the tumor cells, CAV-1 %cells X intensity for a score of 0-300
- For the stromal cells, CAV-1 scored 0-3

Results

- N (for tumor)-CAV1 score
  - Epithelioid MPM 70/91 CAV1+ med score 40
  - Biphasic MPM 17/17 CAV1+ med score 130
  - Sarcomatous MPM 23/23 CAV1+ med score 220
  - P<0.0001

- Cell lines
  - Expression of CAV1 greater in biphasic than epithelioid cell lines
- S (for stromal cells)-CAV1 observed in the epithelioid MPM (61/91)
  - The N-CAV1 score higher in these S-CAV1 MPM but NS
  - These “stromal”cells are + for CK, D2-40, WT1, SMA, focal calretinin and low Ki-67

- All cases of pleuritis neg for CAV1
The S-CAV1+ epithelioid MM worse outcome (med survival 17mos vs 37.8mos p<0.001) than the S-CAV1 neg cases, more similar to the outcome of biphasic MPM

Conclusions
- Interesting finding especially of these S-CAV1+ epithelioid MPM. We have all had these epithelioid MPM with strong keratin positive spindle cells that appear benign and thought to be reactive so not called biphasic. These authors suggest perhaps the early transition into a biphasic MPM with outcome more akin to biphasic than other epithelioid MPM


Background
- Currently more than 10 variants of EML4-ALK fusions and more than 4 different partners for ALK reported.
- FISH is the current standard and potentially can detect all variants but more challenging than IHC
- IHC most routine for all pathologists but many Ab, different artefacts.
- RT-PCR fraught with challenges including getting good RNA from FFPE tissue and requiring multiplex because of all the variants

Aim
- To compare all 3 methodologies

Methodologies
- 2 groups of cases 1- 36 prospectively collected AD and 2- 10 AD with known ALK rearrangement (in results the denominator becomes 11 not clear why)
- Methodologies
  - IHC 3 clones ALK1, 5A4, D5F3
  - FISH 2 probes the breakapart from Abbott (and apparently the gold standard) and the split probe from DAKO
  - RT-PCR

Results
- IHC
  - 1/36 IHC+ with all 3 Ab (and confirmed by FISH and RT-PCR)
  - 9/10 + with 2 Ab and 10/10 with D5F3
  - So for D5F3 sensitivity and specificity 100%, signal usually 3+ with occasional 2+
  - For the other 2 clones, sensitivity 91% specificity 100% usually 1,2+ signal
- FISH
  - 1/36 FISH+ by both probes
  - 1/11 (?) FISH not possible, 10/10 both probes 100% sensitive and specific
- RT-PCR
  - Failed in some specimens and did not detect in 2
Sensitivity of 88% specificity of 100%

Conclusions
- IHC with D5F3 was technically 100% possible and 100% sensitivity and specificity
- Outperforms even FISH in that FISH technically failed in 1 although sensitivity and specificity was 100% for both probes.

3- Choline Phosphate Cytidylyltransferase-a Is a Novel Antigen Detected by the Anti-ERCC1 Antibody 8F1 With Biomarker Value in Patients With Lung and Head and Neck Squamous Cell Carcinomas. Vaezi et al. Cancer 2014; 120:1898

Background
- Platinum is the cornerstone of tx in advanced NSCLC and H&N SQCC but problem of resistance.
- ERCC1 is essential to DNA repair and low expression shown in multiple studies and clinical trials to be associated with better outcome thought to be due to greater drug sensitivity. However, a few recent studies have raised doubts to the value of ERCC1 as a predictive marker.
- The Ab used for ERCC1 is 8F1 and it appears to not be specific and would target another nuclear protein. This could explain the discrepancies in the studies regarding the value of ERCC1
- In H&N SQCC, a study showed that a more specific Ab to ERCC1 (FL297) is a better predictive biomarker than 8F1

Methods
- IHC with various Ab against ERCC1, anti CCT-a
- Cell line studies both for protein detection and functional studies
- Validation on 187 early stage NSCLC and 80 H&N SQCC
- Statistics

Results
- First identified the second nuclear protein targeted by 8F1 – methods used appear sound. That protein is CCT-a. 8F1 levels don’t vary as long as one of the 2 proteins is expressed.
- Confirmed the specificity of the anti-ERCC1 Ab and CCT-a on the cell lines and that 8F1 staining is influenced by both proteins.
- In NSCLC, showed that CCT1-a is expressed more in SQCC versus AD and LCC while ERCC1 expression is independent of cell type.
  - CCT-a and not ERCC1 was associated with DFS. HR0.41 (88.2 mos vs 54.5mos)
- In H&NSQCC, the expression of 8F1 is a result of CCT-a more than ERCC1
  - CCT-a associated with survival HR0.34

Conclusion
- Interesting twist as this study suggest that CCT-a and not ERCC truly the predictive marker and what was really measured with the 8F1 Ab
- The study focused only on early stage and thus as CCT-a as a prognostic marker and not a very impressive HR. Would need to do on higher stage platinum treated patient to study if CCT-a is the predictive marker.

**Background**
- The research on pathogenesis, etiology and therapy of pulmonary fibrosis is hampered by the lack of a good animal model.
- Pleuroparenchymal Fibroelastosis was added to the new classification of ILD as a rare, mostly idiopathic fibrotic process of the lung. There has been association of PPFE with connective tissue disease, genetic predisposition and other.
- One hypothesis is that PPFE is the result of recurrent infections
- Donkeys suffer from pulmonary fibrosis (DPF) which is morphologically similar to PPFE

**Aim**
- Comprehensive review of DFP to confirm that it is similar to PPFE

**Methods**
- 32 donkey lung 19 with gross evidence of fibrosis, 13 without
  - Formalin inflated, routinely processed and 3 medical and vet pathologists with experience with lung disease blinded to any information. Classified as features similar to PPFE as described by Ready et al, or not
- HRCT performed on 18 inflated ex-vivo lungs, 11 with fibrosis and 7 without. Images reviewed blindly by radiologists with classification as c/w PPFE or not
- Cases classified as c/w PPFE if BOTH pathology and radiology c/w PPFE
- PCR for herpes virus done on 4 fibrotic and 4 normal lungs
- X-ray diffraction done in 4 fibrotic cases

**Results**
- 10/19 had fibrosis c/w PPFE – all 10 had a chronic bronchiolitis and 3 had granulomatous inflammation
- Herpes virus identified in 6/8 specimens processed (don’t specify which 2 were negative)
- Small numbers of particles, Silica and talc, found in x-ray diffraction

**Conclusion**
- Interesting observation...a potential animal model?
- Meaning of herpes virus needs to be determined


**Background**
- PTFE is the most common fluoropolymer used in plastics and with a constant high rate of market growth
- Previous reports have described inhalational exposure lung diseases such as fume fever, pulmonary edema and interstitial pneumonia.
- Furthermore, granulomatous disease 2ary to Teflon injection in cases of vocal cord paralysis have also been reported.

**Aim**
• To report the findings in 3 Chinese men with a definite PFTE exposure in a work place, spray PFTE on pans which are heated 380-420 C, and then sandpapered, with no regulation only wearing paper mask

Results
• 3 men, 2 with occasional chronic cough, 1 with persistent productive cough
• PFTs unremarkable
• CT scan show bilateral centrilobular nodules in all plus GGOs in 1
• All 3 had wedge resections:
  o 2 with foreign body type granulomatous inflammation airway centered
  o 1 with granulomatous inflammation with more a lymphangitic distribution
  o All had foreign material in the granulomas
    ▪ Transparent material birefringent
    ▪ Electron dense on TEM
    ▪ Peak fluorine on SAM but also C, Al, Si
    ▪ PFTE confirmed material by Fourrier transform infrared spectrum analysis

Conclusions
• Another pattern of PFTE occupational exposure, probably result of gross inhalation, which we may not see here. But something to keep in mind.


Background
• IGF1R linked to cell proliferation, migration and apoptosis. Overexpression described in many cancers including NSCLC
• Several clinical trials looking at inhibitors of IGF1R and need for predictive markers
• A number of SNPs in IGFR1 described and associated with cancers
• No activating mutations reported

Aim
• To assess for the prevalence of mutation in IGFR1, selected SNPs and protein overexpression and to correlate with clinicopathologic features.

Methods
• 304 NSCLC with peripheral blood and tissue
• Mutational analysis by PCR on 10 AD and 10 SQCC
• Genotyping with selected SNPs in 304
• IHC for IGFR1 – quantification was done on 181 patients that had clinical and genotyping data

Results
• Mutational analysis
  o Total of 8 mutations
  o 4 known SNPs
  o 3 intronic point mutations and 1 silent mutation
• Genotyping did not reveal much positive findings except for better OS for AD with 1 homozygous SNP versus the heterozygous but not for DFS. In multivariate, only stage was independent prognostic feature

• IHC
  o Only significant finding was higher membranous staining in SQCC vs AD

**Conclusion**

• Basically a negative study. The authors claim they identified activating somatic mutations for the 4 not previous SNP but they did not look for these mutations in the patients’ blood so they can’t conclude this with certainty

7- Frequent Coamplification and Cooperation between C-MYC and PVT1 Oncogenes Promote Malignant Pleural Mesothelioma. Riquelme et al. *JTO* 2014; 9:998

**Background**

• Need to therapeutic target in the treatment of MPM
• A potential one is amplification of 8q24 which harbors C-MYC and PVT1
• CMYC transcription factor amplified in 30% of cancers
• PVT1 is a candidate oncogene adjacent to CMYC in noncoding RNA and with several miRNA. Both CNGs and overexpression implicated in various cancers

**Aim**

• Assess for the presence of CNG and amplification of these genes in MPM and study their function

**Methods**

• 75 MPM, 37 epithelioid, 26 biphasic, 12 sarcomatoid
• 12 cell lines of MPM and normal
• SNPs and CNG with Affy
• FISH qPCR to validate CNG
• RNA and miRNA expression
• Functional assays

**Results**

• Cell lines
  o 3/5 cells lines had CNG of 8q24 confirmed by FISH and qPCR
  o CNG of CMYC in 6/12 by FISH and 5/12 by qPCR – 2/12 with CMYC amplification
  o CNG of PVT1 in 5/12 by FISH and qPCR- same cell lines with CNG of CMYC
  o mRNA expression of CMYC in 5 cell line and of PTV1 in one
  o miRNA for PTV1 detected in 7/12 cell lines
  o Knockdown cell lines for PTV1 showed increase apoptosis, decrease cell proliferation in 1 of 2 cell lines and for CMYC decrease apoptosis and decrease cell proliferation. And increase sensitivity for cisplatinum for both knockdowns.
  o CMYC knockdown decreases PTV1 expression
Apoptosis assay showed increased pro-apoptotic and decreased anti-apoptotic gene expression in the PTV1 knockdown versus balanced expression for the CMYC knockdown.

- Tumors
  - FISH 11/75 with CNG of CMYC, not in sarcomatoid subtypes
  - FISH 11/75 with trisomy for CMYC, not in epithelioid subtypes
  - Confirmed by qPCR
  - No correlation with any clinicopathologic features
  - qPCR showed CNG for PVT1 in 12/30 8 with CMYC CNG and 3 without
  - RNA expression significant differences in both genes between tumor and normal

Conclusions
- CMYC appears to increase proliferation and decrease sensitivity to cisplatinum
- PTV1 appears to do the same and inhibit apoptosis
- CMYC appears to regulate PTV1 not the other way around as previously thought
- CMYC and PTV1 CNG (not amplification so title misleading) common in MPM, usually co-expressed

8- Sample Features Associated with Success Rates in Population-Based EGFR Mutation Testing. Shiau et al. JTO 2014; 9:947

Background
- Therapeutic targets have become central to lung cancer testing. As 70% present with advanced stage, small biopsy specimens routinely used for dx
- These specimens may be suboptimal for molecular testing as some methods require significant amount of tissue
- A centralized testing model could potentially increase quality of tissue collected and avoid second procedures
- The authors’ center was part of a pan-Canadian effort for uniform testing

Aim
- To retrospectively study the sample-related characteristics that correlated with test success
- Assess the mutation frequency rate in an epidemiologically unselected patient population

Methods
- Analysis come from consecutive EGFR testing over 24 mos in patients with locally advanced NSCLC eligible for first line TKI tx
- Preanalytical data from H&E slides. If no tumor cells on H&E, dx of SQCC or duplicate specimen, cases excluded
- EGFR and seemed to have assessed only Ex19 del and Ex21 L858R mutation

Results
- Results from 2293 unique patients – 1780 histology and 513 cytology (2651 samples submitted for testing, 358 excluded) from 69 different laboratories
  - Complete patient characteristic in 1884 patients
• 124 (5.4%) inconclusive because of failed PCR amplification
  o Similar for histology and cytology specimens
  o No differences from lab of origin or time between collection and testing
• Tumor cellularity with higher success rate in tissue
  o Lobectomy better than FNBrx 1ary or met or resection of met
  o Core from 1ary highest test failure 10.1%
  o 30% more tumor, regardless of area size, yielded best success 95.6% (vs 88.7%)
  o 2mm² + tumor higher (95.5 vs 82.2%)
• No significant differences in cytology samples for cellularity
• For multiple tested specimens- done when 1st failed, the rate of success on second specimen was 83.3% with 8.5% higher cellularity (but NS)
• EGFR mutation in one of the 2 exons tested in 20.6%
  o Women, Asian, non-or light-smokers with higher mutation rate
  o TTF-1+, well/mod diff AD and non mucinous predictive of EGFR mutation
  o Detection in broad range of area (as low as 0.5 mm²) and tumor cellularity (as low as 2.5%) in biopsies
  o In cytology no predictive features
  o Similar test success in biopsies from metastasis with the exception of bone
  o Cytology better than histology for pleural, mediastinal and distant LN

Conclusion
  • No matter what specimen, unless there is no tumor, worth trying!
  • Nothing really new…

9-Multiplexed Molecular Profiling of Lung Cancer Using Pleural Effusion.
Akamatsu et al. JTO 2014; 9:104

Background
  • Malignant pleural effusion more easily accessible and repeatedly, so potentially good source for molecular testing
  • Feasibility of multiplex molecular testing in pleural effusion not fully investigated

Aim
  • From a prospective genotyping study of lung cancer patients, multiplex molecular profiling performed in pleural effusions

Methods
  • Multiple gene mutations, amplification and fusion genes were analyzed using pyrosequencing, qRT-PCR and RT-PCR on pleural effusions
  • State DNA extracted from FFPE samples also but don’t mention RNA so not clear performed same experiments on both type of specimens

Results
  • 92 (of 845 patients) had pleural effusions; 8 excluded because not a NSCLC.
  • 102 samples for 84 patients analyzed.
    o 35 patients (42%) had a genomic abnormality detected in the pleural effusion
    o 80/84 had their cytology reviewed and 63 had a malignant effusion while 17 had a negative effusion
• 30/63 with genomic An
• 3/17 with genomic An
  o Concordance rate between pleural effusions and FFPE samples was 88%

Conclusions
• Although the idea good, methodology not clear. Did all the 84 patients have effusion and FFPE samples? And how did they detect the amplification and fusion proteins on the FFPE?
• We know the concordance rate but we don’t know if more findings in the effusion versus paraffin? We don’t know the false negative rate? Which abnormalities were different between the effusion and the FFPE? And do they explain the 3/17 as false positive?


Background
• Endobronchial metastasis from non-pulmonary solid tumors are thought to be rare with few large series and quite variable reported incidence

Aim
• Assess frequency of endobronchial metastasis and describe clinicopathologic findings in large cohort

Methods
• Cases identified from 2 large Italian practices between 1992 and 2009.
• 174 consecutive cases were identified through endoscopy and diagnosis confirmed from endobronchial biopsies.

Results/Conclusions
• In one practice, endobronchial metastasis represented 4% of all endobronchial procedures (a bias in that we don’t know if represents all endobronchial lesions some of which assumed as met and not biopsied?)
• 5% may be the first manifestation of the disease mostly renal cell carcinoma
• Table 1 shows the rest of the findings and nothing really new in terms of symptoms and common laries

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex and age</strong></td>
<td></td>
</tr>
<tr>
<td>Male (mean age: 69 years)</td>
<td>94 (54)</td>
</tr>
<tr>
<td>Female (mean age: 66 years)</td>
<td>80 (46)</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>42 (24)</td>
</tr>
<tr>
<td>Cough</td>
<td>38 (22)</td>
</tr>
<tr>
<td>Variable</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>30 (17)</td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>20 (12)</td>
</tr>
<tr>
<td>Dysphonia</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Not available</td>
<td>24 (14)</td>
</tr>
</tbody>
</table>

**Primary tumors**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>52 (30)</td>
</tr>
<tr>
<td>Colon-rectum</td>
<td>42 (24)</td>
</tr>
<tr>
<td>Kidney</td>
<td>24 (14)</td>
</tr>
<tr>
<td>Stomach</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Prostate</td>
<td>8 (4.5)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>8 (4.5)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Endometrium</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Liver</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Small bowel</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Ovary</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Bladder</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Solitary fibrous tumor</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Vagina</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Cervix</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Liposarcoma spermatic cord</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

**Type of metastasis**

<table>
<thead>
<tr>
<th>Metastasis Type</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metachronous</td>
<td>154 (89)</td>
</tr>
<tr>
<td>Synchronous</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Anachronous</td>
<td>9 (5)</td>
</tr>
</tbody>
</table>
11- A genetic sequence variant (GSV) at susceptibility loci of 5p15.33(TERT-CLPTM1L) is associated with survival outcome in locally advanced and metastatic non-small-cell lung cancer (NSCLC). Azad et al. Lung Cancer 2014; 84:289

**Background**
- Beyond the usual clinicopathologic factors and somatic mutations, heritable genetic components such as GSV play a role in cancer susceptibility and likely cancer outcome.

**Hypothesis**
- GSV associated with lung cancer risk may affect patient survival

**Methods**
- 231 stage III and 313 stage IV NSCLC
- GSV selection based on literature search. GSV chosen if associated with cancer risk (power 80%), functional significance and involved in cancer pathogenesis
- Genome DNA from blood
- Statistics for OS and DFS, with exhaustive multivariate for all potential confounding prognostic markers

**Results**
- 20 candidate GSVs in 12 genes.
- Only one remained statistically significant after the multivariate analysis: GSV located in the TERT-CLPTM1 on 5p15.33 for both OS and DSG with HR 0.75 and 0.74

**Conclusion**
- Potential prognostic marker although survival curve differences not dramatic.

12- Non-terminal respiratory unit type lung adenocarcinoma has three distinct subtypes and is associated with poor prognosis. Sumiyoshi et al. Lung Cancer 2014; 84:281

**Background**
- TRU type of AD well defined as peripheral AD with cell morphology similar to type II pneumocytes or Clara cells, TTF-1+, EGFR mutated.
- Non-TRU type of AD not so well defined. Possibly the central AD, solid, TTF-1-. Possibly the mucinous AD with bronchiolar epithelium, TTF-1 neg, EGFR wild type with MUC5B and MUC5C

**Aim**
- To define the non-TRU AD looking at TTF-1, MUC5s, EGFR and K-RAS

**Methods**
- 337 consecutive AD with complete surgical resection, no neo-adj tx.
- Morphologically divided these in TRU and non-TRU as well as the proposed classification of AD
- TMA for IHC TTF-1, MUC5B, MUC5c
- EGFR and K-RAS mutational analysis.
- Statistical analysis with outcome

**Results**
- 244 (not sure what happened to the difference from 337) patients.
Table provides the details of the patients. The main difference between the morphologic TRU and non TRU are:
  o TRU more TTF-1+ (not all), less MUC5+ (although some are), more EGFR mutated (although many are not), less KRAS mutated (although some are), all are non-mucinous, rarely solid or MP.
  o So right there huge overlap and certainly morphologic TRU not a uniform group except non-mucinous.
• Hierarchical clustering based on the IHC and mutational analysis creates 5 groups (we don’t know the n for each group).
  o TRU1 = All TTF-1+, no MUC5, no EGFR
  o TRU2 = all TTF1+, no MUC5, EGFR mutated
  o Combined= TTF1+ and MUC5+
  o Bronchiolar type = All MUC5+, KRAS mutated, no EGFR, no TTF1
  o Null-type = negative for all, wild type for all
• Although these 5 groups tend to be more in one morphologic group, there is overlap, not surprisingly
  o TUR morphology= 61 TRU1, 93 TRU2 but also 40 combined, 3 bronchiolar and 4 null
  o Non TUR morphology = 1 TRU1, 2 TRU2, 16 combined, 16 bronchiolar and 0 null
• Correlation with DFS and OS
  o No difference in DFS and OS between TRU1 and TRU2 so combined as 1 group
  o 5-yr DFS for TRU 75.2%, combined 61.7%, bronchiolar 53.6%, null 40.0%
  o In multivariate, stage and 5 subgroups remain independent
  o Non-TUR group associated with increased risk for recurrence

Conclusions
• Findings are predictable and simply combine known poor prognostic factors such as poor differentiation (null type), k-ras mutation or known good prognostic factors such as EGFR mutation
• The groups TRU and non TRU are not pure, too many overlapping features.


Background
• Currently FISH is the gold standard to detect EML4-ALK with many other methods studied that do not surpass it in sensitivity, applicability and cost. However, some limitations to FISH, small inversion of ALK hard to detect, one rearrangement at a time, counting of cells…
• NGS to detect ALK using FFPE tissue would be valuable but challenges related to FF tissue included small fragments of DNA. Possible to look at ALK rearrangements by determining the differential expression of the 3’ 5’ portions of ALK mRNA since only the 3’ involved and thus increased in expression with 5’
expression low unchanged. This was proven with different assay such as microarray and qRT-PCR

**Aim**
- To assess for EML4-ALK using amplicon RNA NGS

**Methods**
- 32 samples of FFPE of different types: pleural effusions, FNA, FNBx, BBx, surgical biopsies and surgical specimens. N as controls
  - 11 with ALK rearrangements by FISH and RT-PCR with various partners
  - 21 without ALK- 8 with EGFR, 6 with k-ras and 7 wild type for all
- Cells lines of AD with and without ALK rearrangements.
- FISH, RT-PCR, qRT-PCR and amplicon NGS

**Results**
- Experiments on cell lines to prove concept and determine thresholds for number of reads looking at the 3’ and 5’ ends
- Using >100 reads of the 3’ as a cutoff, their sensitivity and positivity on the 32 samples was 100%. One case of k-ras mutated showed increased 3’ reads but below the 100 reads.
- Confirmed by qRT-PCR
- Analytical sensitivity of the test determined at 1-5%

**Conclusion**
- Promising NGS test for rearrangements. The main advantage for such as test is the possibility of looking for multiple rearrangements at once (instead of one at a time like FISH)

14- T790M mutation is associated with better efficacy of treatment beyond progression with EGFR-TKI in advanced NSCLC patients. Wei et al. *Lung Cancer* 2014; 84: 295

**Background:**
- Acquired resistance of EGFR-TKI can occur through many different mechanisms, the most frequent T790M secondary mutation in EGFR
- Association between T790M mutation and OS controversial.
- Progression under EGFR-TKI can be divided into 3 groups: local progression, gradual progression and dramatic progression
- Continued treatment with TKI and other modality may be of benefit but no biomarker to identify patients that may or may not benefit

**Aim**
- Study the prevalence of T790M mutation on advanced NSCLC and EGFR mutation (without T790M) at 1ary dx
- Evaluate the association of T790M mutation with efficacy of continuous treatment of TKI after acquired resistance

**Methods:**
- All patients with stage IV disease and acquired resistance willing to have a re-biopsy post treatment.
- RECIST guidelines use for progression and classified into the 3 groups 1- local progression defined as solitary progression with no more than 3 lesions 2- gradual
progression as minor increment in tumor burden 3- dramatic progression as rapid increment in tumor burden or cancer-related symptoms. The first 2 groups received continuous TKI plus other treatment and the 3rd group was switched to chemo only

- EGFR mutational analysis

**Results:**

- In 2 years, 369 with acquired resistance – 54 re-biopsy
  - 22 local progression
    - Local radiation therapy plus TKI
  - 19 gradual progression
    - Chemotherapy (variable regimens) plus TKI
    - Objective response 26% and disease control rate 63%
  - 13 dramatic progression
    - Chemotherapy alone – various regimens
    - ORR 23% and DCR 54%

- 29 T790M+ and 25 T790M- with no difference in gender, histology, smoking, TKI therapy and type of progression
- PFS associated with gender and pathology not T790M
- In patients treated with continuous TKI (ie groups 1 and 2), in univariate T790M associated with PFS and OS and in multivariate remains a lower risk at HR 0.352 – this translates into 4 mos difference in survival

**Conclusion:**

- The authors state that they have shown that patients with T790M and continuous TKI treatment have longer PFS and OS compared to those who don’t have the mutation (by 4 mos which for Stage IV lung cancer is the usual “good” benchmark). What is not clear is the role of the continuous TKI treatment for both of these groups.


**Background**

- Next generation sequencing with massive parallel sequencing of millions of DNA or RNA fragments now allows for the possibility of better understanding the genomics of cancer
- Few studies on RNA-seq in NSCLC with limited data

**Aim**

- RNA-seq in NSCLC of male smokers

**Methods**

- Frozen tumor and adjacent N from resected NSCLC from 88 men.
- RNA-seq performed
- Validation using IHC on TMA of SQCC and AD from both men and women, smoker and never smoker
- Pathway analysis

**Results**

- 54 AD and 34 SQCC; 45 Stage I, 41 II, 2 III. Not clear if actually all smokers but seems like it with average 35 pack-yrs
• Has many RNA reads in the NSCLC as the N tissues
• Identified top 20 upregulated genes: SPP1 Secreted phosphoprotein, KRT6, Chromobox homolog, KRT17, KRT 5, GPX2 Glutathione, KRT15, KRT16, MMP11 Matrix metallopeptidase, MPRSS4 Transmembrane protease, serine 4, SOX2 SRY (sex determining region Y)-box 2, GJB2 Gap junction protein beta 2, S100 calcium binding protein, Ubiquitin-conjugating enzyme, CRABP2 Cellular retinoic acid binding protein 2, TOP2A Topoisomerase, MMP1 Matrix metallopeptidase, Desmplakin, Plakophilin 1 (ectodermal dysplasia/skin fragility syndrome), TYMS Thymidylate synthetase
  o Keratins upregulated in the SQCC not AD
  o SPP1 upregulated in both
  o CBX3, GJB2, CRABP2 and DSP never reported in NSCLC
• Identified top 20 downregulated genes: SFTPC Surfactant protein, CLDN18 Claudin 18, ADH1B Alcohol dehydrogenase 1B (class I), beta polypeptide, SFTPA2 Surfactant protein, SFTPA1 Surfactant protein A1, FABP4 Fatty acid binding protein 4, adipocyte, MFAP4 Microfibrillar-associated protein 4, PGC Progastricins (pepsinogen C), INMT Indolethylamine N-methyltransferase, WiFi1 WNT inhibitory factor 1, CLEC3B C-type lectin domain family 3, member B, MARCO Macrophage receptor with collagenous structure, FHL1 Four and a half LIM domains 1, HBA1 Hemoglobin, alpha, TCF21 Transcription factor 21, CYP4B1 Cytochrome P450, family 4, subfamily B, polypeptide 1, CAV1 Caveolin 1, caveolae protein, CLIC5 Chloride intracellular channel, TNNC1 Troponin C type 1, UPK3B Uroplakin 3B
• IHC for the 4 new genes: CBX3 + in 90%, CJB2 in 23%, CRABP2 in 72% and DSP in 18% in both AD and SQCC, but stat sign for GJB2 and CRABP2 more in AD than SQCC
• Found 7 up and 7 down regulated pathways

Conclusions
• Possibly identification of new genes in NSCLC which will need further confirmation and exploration as to their potential role

16- Idiopathic pulmonary fibrosis is strongly associated with productive infection by herpesvirus saimiri. Folcik et al. Mod Pathol 2014; 27:851

Background
• G-herpesviruses can cause pulmonary fibrosis in horses and donkeys that clinically resemble human IPF
• Murine herpesvirus-68 (MHV68),3 a g-herpesvirus closely related to herpesvirus saimiri, has been used to cause pulmonary fibrosis in mice and was successfully arrested with antiviral therapy
• Herpesvirus saimiri can cause subclinical infections in humans.

Aim
• To assess the etiologic role of herpesvirus saimiri in IPF

Methods
• Samples:
  o Patients samples: 21 cases of IPF as defined by ATS/ERS classification – open lung biopsies
Mean age 60.6 yo; 14 M:7W
- Controls, 21 cases of focal lung fibrosis of known cause – open lung biopsies; 7 fibrosis-related adenocarcinoma, 5 of lung fibrosis associated with emphysema, 9 of interstitial pneumonitis and fibrosis of known viral etiology including measles (one case), adenovirus (three cases), hantavirus (three cases), and rotavirus (two cases).
- Additional negative and positive controls, infected Jurkat cells with herpesvirus saimiri
  - ISH for herpesvirus saimiri on all 42 cases and on the “majority” of cases for EBV, CMV and HSV1 and II
  - IHC
    - EBV-LMP, KSHV, CMV
    - Cyclin D1, thymidylate synthase, IL-17, and dihydrofolate reductase as associated with productive infection by herpesvirus saimiri
  - Co-expression Analysis for 2 targets using a previously described method
  - RT-PCR for Cyclin D1 human and viral

**Results**
- 21/21 IPF positive for herpesvirus saimiri by ISH (and 13 cases negative for all other viruses)
  - 0/21 control cases positive for herpesvirus saimiri by ISH
  - Infected and non-infected cells were appropriately + and –
- The positive cells were mostly reactive pneumocytes covering the fibroblastic foci; rare positive pneumocytes seen in more normal lung
  - The positive cells were often atypical, multinucleated (?)
- The target was nuclear s/o productive infection confirmed by associated expression of cyclin D1, TS, DHR, IL in the same positive epithelial cells. The staining is weaker in the control cases and not all 4 co-expressed like in IPF.
  - This was assessed with their co-expression study and probes from cloned portions of the HVS
  - Correlation between ISH of virus and the viral cyclin D
- Also sequenced the viral DNA in the IPF tissue

**Conclusions**
- Basically show productive herpesvirus saimiri in all their cases of IPF, by multiple ways (and with all controls being negative) implying that IPF always caused by this virus? Seems almost too much?
- But the good news would be that antiviral therapy could be tried in these patients as it seems to work in the mice model…

**Case reports**
1- A Man in His 40s With Frequent Bronchitis, Localized Wheezes, and Hemoptysis. Skaria et al. *Chest* 2014; 145: 1426
  - Case presentation with nice illustration CT, bronchoscopy and histology of tracheobronchial amyloidosis.

2- Multiple Pulmonary Chondroid Hamartoma. Fan et al. *JTO* 2014; 9:1053
• Image of the month. A 30 yo man with innumerable lung nodules (worth looking up the CT scan….unfortunately the gross image underwhelming) many with central calcifications of the RU-ML. The surgical resection confirms that these nodules are hamartomas.

• 43 yo W with history of invasive ductal carcinoma. At FU an incidental anterior mediastinal mass, PET+, was identified. She underwent a biopsy. 2 mos later the mass had spontaneously regressed. 11 mos later free of disease.
• The biopsy show multifocal nodular expansion of the cortex and medulla. The nodules are comprised of histiocytes with features of Langerhans cells. IHC with S100 prot, CD1a and Langerin confirms this.
• BRAF mutation was detected by PCR and by IHC, supporting this to be a neoplastic process rather than reactive.