FEBRUARY PULMONARY PATHOLOGY JOURNAL CLUB
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1. Lung Biopsy Findings in Severe Pulmonary Illness Associated With E-Cigarette Use (Vaping): A Report of Eight Cases. Am J Clin Pathol 2020; 150:30-39 – *Accompanied by an Editorial Vaping-Associated Lung Injury Nonspecific Histopathologic Findings Necessitate a Clinical Diagnosis by Boland and Aesif.*

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I. ARTICLES FOR DISCUSSION
1. Calculating the Tumor Nuclei Content for Comprehensive Cancer Panel Testing. Mikubo et al. JTO 2020; 15: 130-137 – Discussion by Matt Cecchini
Aim
To determine the accuracy of tumor cellularity estimations by pathologists compared with calculated cellularity from sequencing results and to improve the accuracy of pathologist estimation by training and feedback.
Material and methods
- 9 pathologists evaluated the tumor cell content of 18 tumors before and after a training session.
- The training session used a set of seven tumors as standards with an explanation on the analysis.
- The gold standard for the true tumor cellularity was based on the mutant allele frequency and the cellularity determined by the pathologist at Foundation medicine institute (FMI) that was blinded to the results.
- The same cases were reviewed in one day with the training session as a washout in between (using separate tumors). The order of tumors was randomized in each session.

Results

- The cellularity of the tumor was determined using the allele frequency of common oncogenes and tumor suppressors corrected if these mutations typically occur in a heterozygous state. The tumors had a calculated allele frequency from 15 – 70%.
- In the first round of review the pathologists had calculated a statistically significant higher cellularity from both the (FMI) and the allele frequency. There was no difference between the FMI pathologists and the calculated mutant allele frequency.
- After completion of the training the estimated allele frequency by the pathologists was not significantly different from the FMI or calculated allele frequency.
- There was a large range of tumor cellularity calculated by the pathologists in some cases spanning the cutoff of acceptable cellularity.
- This range continued but the standard deviation improved after the training.
- The tumors with the largest standard deviation and ranges were the colonic adenocarcinomas and pancreatic tumor.
- In the first round the pathologists tended to calculate the tumor cellularity based upon the tissue area occupied by tumor and failed to account for the additional normal cells including lymphocytes, vessels and fibroblasts that results in over-estimation of the tumor cellularity.

Conclusion

- The group of pathologists tended to overestimate the tumor cellularity for molecular testing. However, the training using standardized controls improved the accuracy of cellularity estimates by pathologists.
- Despite the training there remains a large range of pathologist calculated tumor cellularity which spans cut-off ranges for the assay.

2. Rapid clinical mutational testing of *KRAS*, *BRAF* and *EGFR*: a prospective comparative analysis of the Idylla technique with high-throughput next-generation sequencing. Van Haele et al. J Clin Pathol 202; 73: 35-41 – Discussion by Matt Cecchini

Aim

Prospective study to investigate the concordance between the Idylla platform and gold standard next generation sequencing for the detection of EGFR, KRAS and BRAF mutations in non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and melanoma.

Material and methods

- Prospective collection of 48 cases from a single institution in Belgium over a 6-month period in 2017.
- Cases with a diagnosis of melanoma, NSCLC or CRC that required mutational analysis to establish a personalized treatment plan were included. Cases with insufficient tissue were excluded.
- Discrepant cases were tested with a third method using the Cobas 4800 (Roche) at a third party accredited laboratory.

- The Idylla platform is a fully automated system that uses cartridges that have all the required reagents to process tissue samples (FFPE, cytology or fresh) using high – intensity focused ultrasound followed by fluorescent-based PCR amplification, with the following tissue requirements; BRAF: 50% and EGFR/KRAS: 10% tumor cellularity in an area between 50 and 600 mm^2 on 5 μm sections or between 25 and 300 mm^2 on a 10 μm section.
- NGS sequencing was performed on a MiSeq/NextSeq system using 6-12 sections to meet the required 50 ng of DNA and a tumor cell percentage of 10% to detect hotspot mutations using the TruSight tumor26 kit (Illumina)

Results
- Table 1 shows an overview of previous studies on the Idylla platform.
- 45 FFPE cases and 3 cytology cases fixed in CytoRich Red (alcohol based).
- Mutations were identified in;
    - 8 of 13 cases of melanoma with no discordances,
    - 9 of 18 cases of CRC, one discordance (mutation only identified by Idylla)
    - 6 of 17 cases of NSCLC, one discordance (Ile740_Lys745dup identified on NGS)
- Discordant CRC case KRAS p.(Gln61His) c.(183A>T) mutation only identified on Idylla could be false positive or false negative in other assays.
- Discordant NSCLC rare insertion of 18 base pairs in exon 19 (seen in 1% of NSCLC) sensitizing mutation in EGFR not designed to be detected by the Idylla assay.
- Turnaround time 90 – 150 min for Idylla and 3 days for NGS

Conclusion
- Idylla platform can provide rapid mutational testing for clinically actionable mutations that shows high concordance with NGS sequencing for the tested mutations in NSCLC, melanoma and CRC.

3. NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls. Solomon et al. Mod Pathol 2020; 33:38-46

Background
- *NTRK* fusion in solid tumors is a therapeutic target, regardless of tumor origin.
- 3 trk receptor genes, *NTRK1, NTRK2, NTRK3,* that can be involved..
- First examples were *ETV6-NTRK3* fusions in colorectal carcinoma, infantile fibrosarcoma, mesoblastic nephroma and secretory carcinomas breast and salivary glands.
- Screening is challenging as there are 3 genes, numerous partners and various breakpoints along the genes with various assays like DNA sequencing of the *NTRK* introns, RNAseq and pan-trk IHC

Aim
Report the sensitivity, specificity and pitfalls of the various clinically validated assays.

Method
- Jan 1st 2014- March 30th, 38,905 tumor samples
- DNA sequencing (MSK-IMPACT) of 468 genes including all 3 *NTRK*. Any novel structural variant was confirmed with RNA seq
- RNA seq (MSK-fusion panel custom) multiplex PCR of 15 genes including the 3 *NTRK* of select cases
- Definition of positive case – RNA seq with in-frame fusion involving the kinase domaine of *NTRK* , or if not RNA seq, by DNA *NTRK* fusion of the kinase domain with a known fusion partner.
- IHC with Pan-Trk (EPR17341 clone Abcam). Positive if at least 1% tumor cells positive, any pattern of staining. 66 confirmed cases from molecular and negative controls including 16 cases that were DNA "positive" and RNA "negative"

Results
- 87 of 38,905 samples (33,997 patients) (0.2%), 9 lung cancers (of 3993, 0.23%), mutually exclusive of MAPK pathways alterations.
- For DNA seq overall sensitivity was 81.1% and specificity 99.86%
- For IHC, 66 positive and 317 negative cases tested.
    - Sensitivity for *NTRK3* 79.4% sometimes weak and focal staining
    - Sensitivity for *NTRK1,2* 96.9%
    - Overall specificity 81.1%
    - Lung sensitivity 87.5 and specificity 100%

Conclusion
Largest series and excellent study. Great reference to have at hand.

4. "Interchangeability" of PD-L1 Immunohistochemistry Assays: a Meta-Analysis of Diagnostic Accuracy. Torlakovic et al. Mod Pathol 2020; 33:4-17

Aim
Assess the performance of various PD-L1 IHC based on their diagnostic accuracy as defined during clinical trials, looking at FDA approved assays versus LTD

Material and methods
- Systematic review of the literature on PD-L1 IHC assays
- Reached out to authors to get additional data to allow for sensitivity, specificity and PPV and NPV.
- Meta-analysis using various statistical methods
- Clinically acceptable defined as a sensitivity and specificity of 90% and greater.

Results
- Each study had often multiple test comparisons within a single study.
- Table 1A and 1B shows the Sensitivity and Specificity for all compared assays using the FDA approved assay as the gold standard comparator. Table 1A is focused on NSCLC.

- For PD-L1 IHC 22C3 pharmDx, 22C3 LDT had the best performance for both cut-offs, meeting the definition of clinically acceptable. PD-L1 28-8 pharmDx and Ventana SP262 was only acceptable for the 50% cut-off
- For PD-L1 IHC 28-8 pharmDx, Ventana SP263 was considered clinically acceptable for the 1% cut-off although specificity was 87% overall
- For Ventana PD-L1 (SP263) no substitute candidate identified.
- All the 3 clones above are very sensitive but lose specificity so perhaps not as "interchangeable" as not developed for the same clinical outcome.
- SP142 confirmed as being of too low sensitivity to be recommended for use.

Conclusion

- This study suggests that a laboratory can develop an accurate LDT as long as it is used for the same clinical intent as the FDA approved assay and it is appropriately performed and validated.
- LDT with this 'spirit of the law' is better than substituting FDA approved assays.

II. Articles for notation
Neoplastic
1. Detection of rare and novel EGFR mutations in NSCLC patients: Implications for treatment-decision. Sousa et al. Lung Cancer 2020; 139:35-40
In this study, the authors compared Sanger sequencing to the commonly used Cobas or Idylla methods of detecting EGFR mutations and identified new mutations in 3% of their study population not detected by the other 2 methods. The clinical significance of their finding is not entirely clear as response to the EGFR inhibitors is not completely known but it does highlight the possibility of missing significant mutations in a small group of lung cancer patients.

2. Longitudinal Monitoring of Somatic Genetic Alterations in Circulating Cell-Free DNA During Treatment with Epidermal Growth Factor Receptor–Tyrosine Kinase Inhibitors. Iwama et al. Cancer 2020; 126: 219-227 – *Accompanied by Editorial Liquid Biopsy as a Follow-Up Tool: Comment on Longitudinal Monitoring of Somatic Genetic Alterations in Circulating Cell-Free DNA During Treatment With Epidermal Growth Factor Receptor–Tyrosine Kinase Inhibitors by Malapelle et al.*
Prospective study of detection of EGFR mutations and other mutations in cfDNA at baseline and various time points in 100 patients with lung cancer with EGFR mutations. This study showed low sensitivity of cfDNA to detect EGFR mutation at baseline by either droplet PCR or NGS, but if present associated with worse outcome, question of test sensitivity or configuration of the tumor at detection of shedding DNA (higher sensitivity with extra thoracic metastasis)? It showed the ability of this test to detect progression as there was an increase in the number of alleles in patients with tumor progression. It also showed the ability of detecting new mutations, including TM970 in predicting resistance to treatment. This is an important study further suggesting NGS on cfDNA as an important role in monitoring patients on targeted therapies.

3. High MET Overexpression Does Not Predict the presence of MET exon 14 Splice Mutations in NSCLC: Results From the IFCT PREDICT.amm study. Baldacci et al. JTO 202; 15:120-124

The authors studied the role of MET IHC expression as a surrogate to detect exon 14 mutations in MET as over 120 different mutations described making it challenging to identify using usual clinical strategies for detecting mutations. As with other studies, the authors confirmed IHC for MET is not a good surrogate and does not predict for mutations.

4. Molecular profiling of thymoma with myasthenia gravis: Risk factors of developing myasthenia gravis in thymoma patients. Lee et al. Lung Cancer 2020; 129: 157-164

The authors identified 4 genes –HIF3A, GFPB1,KLF15, PDK4, through high throughput RNA seq, and confirmed QT-PCR, with high expression in thymomas of patients with MG compared to those without MG. Furthermore, the AUCs of the combination of HIF3A and IGFPB1 were >0.958 for both RNAseq and QT-PCR, suggesting a role for these genes in inducing MG. This correlated with IHC expression. Interesting study. No clear clinical application at this time.

5. EURACAN/IASLC Proposals for Updating the Histologic Classification of Pleural Mesothelioma: Towards a More Multidisciplinary Approach. Nicholson et al. JTO 2020; 15:29-49

Manuscript to be aware of as this will change how we practice. The proposals are akin to what we saw happen with lung adenocarcinoma. The goal was to standardize and provide more useful information for patient care. By increasing the complexity i.e. % various patterns, % of cytologic features etc, unfortunately will likely result in more confusion due to known inter-observer variation. As this may be, we are likely to see these changes in the next WHO. However there were recommendations that will more standardized and less prone to inter-observations including: 1-Pathologic staging was recommended for extended pleurectomy and EPP. 2- % of sarcomatoid versus epithelioid to be included in biphasic MM. 3- The concept of MM in situ is introduced and to be considered as an addition to future classifications. 4- Recommendation to grade epithelioid MM as low versus high grade based on nuclear grade, mitosis and necrosis. There was also good discussion on acquisition of high quality specimens for diagnosis, ancillary studies and future research in clinical trials, and collection of "normal" as controls in surgical specimens.
Recommendations were also made for radiologists, medical oncology and surgery.

6. Pulmonary Myoepithelial Tumors With Exuberant Reactive Pneumocytes Proposed Reclassification of So-called Pneumocytic Adenomyoepithelioma. Yuan et al. Am J Surg Pathol 2020; 44: 140-147

In this study, the authors suggest that cases of pneumocytic adenomyoepithelioma are in fact examples of myoepithelial tumors, primary or metastatic, with exuberant entrapped pneumocyte proliferation. Molecular studies with *PLAG1* or *HMGA2* for salivary glands, or *FUS* or *EWSR1* for myoepithelial tumors may be helpful to the diagnosis.

7. Joint use of the radiomics method and frozen sections should be considered in the prediction of the final classification of peripheral lung adenocarcinoma manifesting as ground-glass nodules. Wang et al. Lung Cancer 2020; 139: 103-110

Interesting study assessing the role of radionomics and its comparison to FS diagnosis to assist surgeons in determining appropriate surgery for AIS-MIA-IA. Overall, radionomics and FS diagnosis are comparable when predicting in final diagnosis but radionomics proved useful when a FS diagnosis was not possible, or if there was discordance between FS and radionomics, radionomics was more accurate, and this was mostly seen with MIA which had the highest rates of inaccuracy between FS and permanent diagnosis.

Non-neoplastic
1. Lung Biopsy Findings in Severe Pulmonary Illness Associated With E-Cigarette Use (Vaping): A Report of Eight Cases. Mukhopadhyay et al. Am J Clin Pathol 2020; 150:30-39 *– Accompanied by an Editorial Vaping-Associated Lung Injury Nonspecific Histopathologic Findings Necessitate a Clinical Diagnosis by Boland and Aesif.*
This study complements the work published by the Mayo Group in the N Engl J of Med. As with the prior study, all 8 cases showed various features of acute lung injury, including DAD, OP, AFOP based on description. And specifically no features of exogenous lipoid pneumonia.

Case Report
1. PLEKHM2-ALK: A novel fusion in small-cell lung cancer and durable response to ALK inhibitors. Li et al. Lung Cancer 2020; 146-150
ALK rearrangement reported in SCLC in only 2 prior case reports and both were ALK-ELM4 fusions. In this case report, using NGS (no other specific information as to the method), the authors identified a novel fusion with ALK in an extensitve SCLC, which correlated with positive IHC for ALK. Furthermore, the patient was treated with Crizotinib as maintenance therapy following the usual chemo-radiation regimen, with a longer survival than typical.

Review articles
1. Biomarkers for Immune Checkpoint Inhibition in Non-Small Cell Lung Cancer (NSCLC). Bodor et al. Cancer 2020; 126:260-270
Excellent review that goes beyond PD-L1, with nice overview of TMB, gene expression signatures and serum-based biomarkers as potential ancillary tests in identifying responders.