Interactive Molecular Pathology 2014

The American Society for Investigative Pathology (ASIP) Companion Meeting at the USCAP 2014 Annual Meeting

Chairs/Moderators:
Dani S. Zander, MD
Penn State College of Medicine/Milton S. Hershey Medical Center, Hershey, PA

Karen L. Kaul, MD, PhD
NorthShore University HealthSystem, Evanston, IL

Presentation materials available online at: www.asip.org/meetings/Companion.cfm
The American Journal of Pathology
The Journal of Molecular Diagnostics

Read the Articles...
Take the Exams...
Earn CME & SAM Credit!

NEW THIS YEAR! In 2014 the ASIP AJP CME Program in Pathogenesis will be made freely available to ASIP Members! Visit www.asip.org for complete details!

The ASIP provides two annual Journal CME Programs: The American Journal of Pathology (AJP) CME Program in Pathogenesis offers you the opportunity to earn up to 48 CME credits per year while renewing and updating your knowledge in the pathogenesis of disease. The Journal of Molecular Diagnostics (JMD) CME Program in Molecular Diagnostics offers you the opportunity to earn up to 48 CME credits per year while renewing and updating your knowledge in molecular diagnostics. These programs are approved for Self-Assessment Module (SAM) credit with The American Board of Pathology.

JMD 2014 CME Program in Molecular Diagnostics

The JMD 2014 CME Program in Molecular Diagnostics is an annual program consisting of a series of at least 48 questions based on selected articles in the 2014 issues (Volume 16) of The Journal of Molecular Diagnostics (JMD). Bimonthly exams, consisting of at least 8 questions that are based on selected articles appearing in each issue of the Journal, are available online on the Journal website for registered participants.

To receive CME credit for this journal-based CME activity, participants must achieve a score of at least 75% on each bimonthly exam and complete a Post-Test Evaluation. All exams must be completed by December 31, 2014 to receive CME credit. Participants will earn 4 AMA PRA Category 1 Credit(s)™ for the successful completion of each bimonthly exam.

CME Accreditation Statement:
This activity (“JMD 2014 CME Program in Molecular Diagnostics”) has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

The ASCP designates this journal-based CME activity (“JMD 2014 CME Program in Molecular Diagnostics”) for a maximum of 48 AMA PRA Category 1 Credit(s)™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

SAM Credit
The JMD 2014 CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 48 SAM credits. Physicians should only claim credit commensurate with the extent of their participation in the activity. After successfully completing the bimonthly CME exams as described above, participants may separately apply for SAM credit by completing the SAM application available for download on the ASIP website at: http://www.asip.org/CME/documents/JMD/JMD.SAM.Application.pdf. All SAM applications must be received in the ASIP office by December 31, 2014 in order for participants to receive SAM credit.

For more information regarding SAM credits, please contact the ASIP Education Office by phone at (301) 634-7440; email journalcme@asip.org, or mail your inquiry to 9650 Rockville Pike, Suite E-133, Bethesda, MD 20814.

ASIP 2014 AJP CME Program in Pathogenesis

The ASIP 2014 AJP CME Program in Pathogenesis is an annual program consisting of a series of at least 48 questions based on selected articles in the 2014 issues (Volume 184) of The American Journal of Pathology (AJP). Monthly exams, consisting of at least 6 questions based on selected articles appearing in each monthly issue of the Journal, are available online on the Journal website for registered participants.

To receive CME credit for this journal-based CME activity, participants must achieve a score of at least 75% on a monthly exam and complete a Post-Test Evaluation. All exams must be completed by December 31, 2014 to receive CME credit. Participants will earn 4 AMA PRA Category 1 Credit(s)™ for the successful completion of each monthly exam.

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ASIP Companion Meeting at USCAP 2014
Interactive Molecular Pathology 2014
Sunday, March 2, 2014, 1:30 PM - 5:00 PM

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Mark E. Sobel, MD, PhD, Executive Officer, American Society of Investigative Pathology, Bethesda, MD

Challenges and Opportunities

Education
- Physicians, trainees, payors, ourselves

Standardization of diagnostic and clinical pathways
- Informatics tools:
  - Clinical decision support
  - Clinical utility → reimbursement

Management of referred testing

Evolving Role of the Pathologist

- Morphology
- Molecular pathology
- Flow cytometry
- Immunohistochemistry

Cancer Drugs with Companion Pharmacogenic Tests

Find a Cancer Mutation
Search Clinical Trials

Select Disease
Select gene variant to see its significance within that disease and related clinical trials
Goals of this session

To provide a case-based, interactive learning experience in molecular pathology, in which participants choose methods of evaluation appropriate for specific clinical scenarios, interpret test results, integrate results into the clinical context to provide a meaningful clinical consultation, recognize potential pitfalls, and learn about costs, quality assurance, and proficiency testing needs.

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Mark E. Sobel, MD, PhD, American Society for Investigative Pathology

The chairs/moderators have no conflicts of interest to disclose.

The evolution of molecular pathology
• 1980s: Southern blots and cloning gene probes
• 1990s: PCR
• 2000: Real-time PCR and arrays
• 2010: Targeted therapy and Next-gen sequencing

Potential of Molecular Pathology
• Risk/disease prevention
• Diagnosis
• Prognosis
• Treatment choice in cancer
• Pharmacogenomic testing
• Infectious disease identification
• Microbiome

Opportunities and challenges
• Rapidly advancing field
  − Variable knowledge base of clinicians and pathologists
  − Consensus (utilization) guidelines needed
  − Complex results
  − In house vs. sendout
• Changing Technologies
  − PCR to NGS?
• Reimbursement

Are we prepared?
Slide courtesy of Rich Haspel, TRIG
Genomics curricula

- TRIG (PRODS and ASCP)
  - 4 Lectures, online tools, questions
- Stanford Curriculum
  - Ten comprehensive lectures
- AMP T & E committee
  - MGP Genomics Curriculum
- AMP Task force updating resident curriculum
- NCHPEG, other professional organizations
- Medical School Curricula

Genomic tumor testing oversight?

- Pre-analytic case review?
- Post-analytic tumor boards
- Outcome tracking

Reimbursement

- New molecular codes in 2013 on lab fee schedule
- AMA Coding Caucus considering several new CPT codes for NGS in early 2014
- Will include several syndromic gene panels, Whole exome and whole genome analysis
- Tiered levels of reimbursement
- Value studies later in 2014?
- Lab or Physician fee schedule?

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ASIP Companion Meeting at USCAP: Bladder Cancer

Kevin C. Halling, MD, PhD.
Director, Molecular Anatomic Pathology
Mayo Clinic
Rochester, MN

Financial Disclosure

- I am a co-inventor on the patent for the UroVysion™ probe set and along with Mayo Clinic, receive royalties from its sale
- I receive grant funding from Abbott Molecular to develop FISH assays for the detection of tumor cells in cytologic specimens

Case History

- 55 year old man with microhematuria.
- Long smoking history
- Cystoscopy-negative
- Cytology-suspicious but not diagnostic for malignancy

FISH Findings

CEP 3 CEP 7
CEP 17 LSI 9p21

What Kind Of FISH Abnormality is Present?

a) None
b) Tetrasomy
c) Polysomy
d) Trisomy 7
e) 9p21 Loss Alone

25% of the non-inflammatory/non-squamous cells showed this abnormality
What Should You Do Next?

a) Ignore the FISH result, it is a false positive?
b) Repeat the FISH result in 3 months?
c) Random biopsies x 6?
d) Fluorescence cystoscopy?

e) Evaluate the upper tract for a tumor

Any of the above are reasonable except for a. With 25% of the cells exhibiting polysomy this is very unlikely to represent a false positive FISH result. The negative cystoscopy could be due to a tumor that is not visible by cystoscopy (e.g. CIS) or could be due to presence of an upper tract tumor. The upper tract should be evaluated if a tumor cannot be found in the bladder. Random biopsies or fluorescence cystoscopy of the bladder may reveal a tumor that is not visible by white light cystoscopy. At the very minimum the FISH result should be repeated 3 months later.
Correlations of % Abnormal Cells with Muscle Invasive Bladder Cancer

Which of the Following Patients Are Good Candidates for UroVysion Analysis?

a) Patient with grade 3 T1 bladder cancer who has just completed BCG therapy
b) 35 yo female with microhematuria but no previous history of bladder cancer and no smoking history
c) 65 yo male with microhematuria and 50 year pack history of cigarette smoking
d) A and C
e) None of the above

Impact of Disease Prevalence on PPV

<table>
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<tr>
<th>Disease Prevalence</th>
<th>PPV of a Test with 80% Sensitivity and 95% Specificity by Disease Prevalence</th>
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<tr>
<td>1 per 10^1</td>
<td>64%</td>
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<tr>
<td>1 per 1000</td>
<td>14%</td>
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<tr>
<td>1 per 1,000</td>
<td>2%</td>
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<tr>
<td>1 per 10,000</td>
<td>0%</td>
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<tr>
<td>1 per 100,000</td>
<td>0%</td>
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1 For example, smokers over age 50 with microhematuria
2 For example, general population

Questions & Discussion
What’s New in the Molecular Pathology of Myeloproliferative Neoplasms (MPN’s)? (it’s not just JAK2 anymore)

Richard D Press MD, PhD
Dept of Pathology
Oregon Health & Science University

Case #1

- 67 yo white female
- cold–induced finger pain and discoloration (microvascular?); no overt thrombosis/bleeding episodes
- no other significant PMH; on no drugs
- Persistent thrombocytosis: 750k and rising for past 6 months ; not treated (yet)

Essential Thrombocythemia (ET) or reactive thrombocytosis?

- No JAK2 V617F mutation
  - Clonal ET marker in ~60% of ET cases
  - JAK2 positivity would define WHO-confirmed ET
- No evidence for reactive causes: infection, inflammation
- Neither hematologist nor patient enthusiastic about a bone marrow biopsy
- Another test on the same blood sample clinched the diagnosis; and this test was...

a mutation in the CALRETICULIN gene

Two articles published: Dec 10, 2013

Somatic CALR Mutations in Myeloproliferative Neoplasms with Nonmutated JAK2

Somatic Mutations of Calreticulin in Myeloproliferative Neoplasms
CALR in 67% of JAK2-neg ET


53%
32%
Calcium-binding ER chaperone protein (NEG-charged C-term)
Frameshift mutations (POS-charged C-term)
Δ-52 bases
Mutation spectrum is heterogeneous across CALR exon 9
(PCR-sizing will detect all variants)
Consistent insertion or deletion to generate the identical +1 frame-shifted positively-charged protein that lacks the C-terminal Ca-binding and ER retrieval signals

CALR mutations predict a more indolent clinical course

ET: CALR+ pts (vs JAK2+) had:
- Higher plat cnt
- Longer overall survival
- Lower risk of thrombosis

PMF: CALR+ pts (vs JAK2+) had:
- Higher plat cnt
- Longer overall survival

Klampfl, et al. NEJM 2013 369:2379

What about our patient?

- 52-bp deletion in CALR exon 9: most common mutation in MPN’s
- CALR mutation confirms an abnormal hematopoietic clone
- Does she still need a bone marrow biopsy?
- 2008 WHO MPN diagnostic criteria requires the demonstration of bone marrow “megakaryocyte proliferation with large & mature morphology”
- Nevertheless, the hematologist was satisfied with an “ET” diagnosis in the absence of a BM, and the patient was treated with hydroxyurea and aspirin

Calreticulin: Key Points

- ~90% of patients with ET and PMF will have mutations in either JAK2, CALR, or MPL (MPL mut’s are rare)
- Suggests reflex CALR mutation testing for evaluation of JAK2-negative ET and PMF
- JAK2 vs. CALR mutant ET and PMF has prognostic value
- JAK2 and CALR testing can help distinguish reactive vs. neoplastic thrombocytosis
- CALR mutation test can help with a MPN diagnosis in patients with equivocal PMF marrow findings
- Revised WHO diagnostic criteria will likely incorporate the presence of a CALR mutation as a major diagnostic criteria for ET & PMF (just like JAK2)

Case #2

- 73 yo male w/ persistent leukocytosis
- No clinical symptoms (no drugs)
- 45-50k WBC count (over last 6 months)
  - mostly PMN’s; normal Hgb & plat’s
- No overt infection/inflammation
- Mild splenomegaly
- JAK2 and BCR-ABL negative (blood)
Peripheral Blood
49k WBC’s
60% PMN’s; 35% bands
(1% mono’s; no blasts)
Hgb 13.3
Plat 339k

Bone marrow
Hypercellular
M:E ratio ~20:1
Mostly mature myeloids
No dysplasia

Chronic Neutrophilic Leukemia (CNL)
or Reactive Neutrophilia?

• WHO diagnostic criteria for CNL:
  – Rare non-classic myeloproliferative neoplasm
  – >25k wbc (>80% segs/bands; <10% immature)
  – Hypercellular BM with increased segs and bands (normal maturation); <5% blasts
  – No dysplasia (in contrast with atypical CML)
  – No reactive granulocytosis or other MPN

• A single additional lab test was performed to clinch the diagnosis, and this test was...

Maxson et al NEJM 368: 1781 (May 9, 2013)

• Mutations in the gene for colony-stimulating factor 3 receptor (ligand = granulocyte colony stimulating factor) in 8 of 9 patients with CNL
  – 2nd report: CSF3R mutations in 12/12 CNL patients*
• Vast majority of mutations were T618I (membrane proximal) that activate downstream JAK2 signaling
• Ruxolitinib, a Jak2 kinase inhibitor, induced a marked durable hematopoietic response in one T618I CSF3R patient (and in a mouse model)

*Pardanani, Leukemia 27: 1870 (2013)

What about our patient?

• CSF3R T618I mutation was detected in the blood
• CSF3R mutations are quite specific for CNL
  – Only very rarely seen in other myeloid neoplasms
• The CSF3R mutation confirms an abnormal hematopoietic clone, and, together with the persistent mature granulocytosis, confirms the diagnosis of CNL

• Should CSF3R mutation screening be routinely used to distinguish reactive from neoplastic granulocytosis?
• Should ruxolitinib therapy be tried in all T618I CSF3R cases?

Summary

2013 saw the discovery of two new disease-specific molecular biomarkers for myeloproliferative neoplasms that will be very useful for:
  – Assigning a specific MPN diagnosis
  – Distinguishing MPN from reactive thrombo- or leuko-cytosis

Calreticulin mutations in ET and PMF (common syndromes)
  • Very common (present in 70-90% of JAK2-negative cases)

CSF3R mutations in chronic neutrophilic leukemia
  • T618I mutation in the vast majority of these rare cases
ASIP COMPANION MEETING: NP CASE

Arie Perry, M.D.
Director, Neuropathology

CLINICAL HISTORY

- 34-yo man with new onset seizures
- Presented with confusion acutely while traveling
- Head MRI: focally enhancing, centrally hemorrhagic/necrotic left frontal lobe mass
- Near total resection performed for diagnosis and therapy
DDx

- Glioblastoma (GBM), WHO IV
- GBM with an oligo component (GBM-O), WHO IV
- Anaplastic Oligoastrocytoma (AOA), WHO III
- Anaplastic Oligodendroglioma (AO), WHO III
**ADDITIONAL DATA**

- CEP7/EGFR FISH: no EGFR amplification/nl copy numbers
- MGMT MS-PCR: Methylation detected (Methylation Score: 73.82; Ref: Methylated if ≥ 2.00)

**DX: GBM-O, WHO GRADE IV**
(Comment: Genetically favorable molecular profile, similar to AO, WHO GRADE III)
CLINICAL COMPARISON

- AO (1p/19q codeletion 80%; IDH mutations)
  - Average survival 15 years with 1p/19q loss if treated with combined PCV chemo and radiation
  - What about chemo alone up front?
- SC-GBM (IDH-, EGFR-AMP 70%, -10q 95%)
  - Average survival 1 year
  - Typically treated with combined radiochemotherapy
  - Different set of clinical trials than the high-grade oligodendrogliomas

BIOMARKER CONCEPTS

- Types
  - Diagnostic
  - Prognostic
  - Predictive
  - (Elucidate Biology)
- Practicality issues
  - Cost and ease of implementation
  - IHC vs. FISH vs. PCR vs. genomics
  - Reimbursement

OLIGODENDROGLIOMA 1p19q FISH

MECHANISM OF 1p/19q CODELETION

CANDIDATE TUMOR SUPPRESSOR GENES

- Exome sequencing experiment
- CIC gene (homolog of the Drosophila gene capicua) on chromosome 19q
- FUBP1 gene [encoding far-upstream element (FUSE) binding protein] on chromosome 1p

From: Griffin CA et al., J Neuropathol Exp Neurol 65:988, 2006
From: Raghavan et al. JNEN 62:530, 2003
From: Jenkins R, Cancer Res 2006; 66: 9852
From: Bettegowda C et al., Science 333:1453, 2011
Fig. 1 Hypothesized pathways of adult glioma development.


*Somatic TERT mutations in primary GBMs and oligos.
Case Presentation:

A 52 year old man presents with a complaint of having a “lump” in his neck. He states that he first noticed it a few months ago and thinks it has recently gotten larger. He has no other symptoms. He has a history of hypertension which is well controlled with medication. He does not consume alcohol and has never smoked. Physical examination reveals an approximately 3cm x 2cm fixed right submandibular mass. No skin lesions are observed and no thyroid nodules are palpable. Examination of his oral cavity reveals healthy dentition and no lesions. Furthermore, no lesions are identified on fiberoptic laryngoscopy. A fine needle aspiration of the mass is performed and the patient is referred for a CT scan of the head and neck. Fine needle aspiration yields a diagnosis of metastatic squamous cell carcinoma (Figures 1-3). The referring physician requests HPV testing.

Data:

On the cell block, immunohistochemistry for p16 was performed. Results are shown in Figure 4.

Interpretation:

What is the HPV status of the patient’s tumor?
Questions:
1. What is the clinical utility of performing HPV testing on this case?
2. What methods are available for determining the HPV status of the patient’s tumor and what sample types are required?
3. How do the various methodologies compare with regards to sensitivity and specificity?
4. What is the anticipated turn around time for each method? Cost?
5. What are the potential pitfalls of each method?
6. Can p16 immunohistochemistry be used alone and, if so, is there a risk?
7. What other virus is important in head and neck cancer and what is the typical testing algorithm?
8. Is there any overlap between these two viruses or the tumors typically associated with them?

Q1: What is the clinical utility of performing HPV testing on this case?
- HPV status can aid in
  - Localizing primary tumor
  - Determining prognosis
  - Predict response to chemoradiation
- Stratification for clinical trials
- Shouldn’t change management decisions

Q1: What is the clinical utility of performing HPV testing on this case?
- NCCN Guidelines Head and Neck Cancers Version 2.2013
  - Occult Primary with squamous or undifferentiated histology
    - HPV-16 testing is suggested
    - EBV testing is suggested
  - Cancers of Oropharynx
    - Tumor HPV testing is recommended
    - Initial workup

Q2. What methods are available and what sample types can be used to determine HPV status?
- In situ hybridization
  - Cytology slides
  - Cytology cell blocks
  - Histology slides
- PCR
  - Paraffin embedded tissue
  - FNA smears
  - FNA needle rinses
- p16 Immunohistochemistry (immunocytochemistry)
  - FNA smears
  - Cytology cell blocks
  - Histology sections

Q2. What methods are available and what sample types can be used to determine HPV status?
- In situ hybridization
  - HPV-16 only?
  - Probe cocktail?
- PCR
  - DNA versus E6/E7 mRNA?
  - HPV-16 only or multiplex?
Q3. How do the various methodologies compare with regards to sensitivity and specificity?

- E6/E7 mRNA = gold standard
- PCR for HPV DNA = sensitive and specific
- IHC for p16 = sensitive but lower specificity
- ISH for HPV = specific but lower sensitivity

Q4. What is the anticipated turn around time for each method? Cost?

- How fast is your immunohistochemistry lab?
- One day TAT achievable for all methods.
- Most expensive = PCR
- Most affordable = p16 IHC

Q5. What are the potential pitfalls of each method?

- False Positives
  - HPV not etiologic to the cancer
  - Variant staining patterns / misinterpretation
  - PCR contamination
- False Negatives
  - Test lacks sensitivity
  - HPV type other than that being tested
  - Variant staining patterns / misinterpretation
  - Poor DNA or RNA quality

Q6. Can p16 immunohistochemistry be used alone and, if so, is there a risk?

- Yes, but causes beyond HPV can cause p16 to be overexpressed.
- Regardless, p16 overexpression may be sufficient to indicate better prognosis

Q7. What other virus is important in head and neck cancer and what is the typical testing algorithm?

- EBV
- Test lymphoepithelial carcinomas of the head and neck as well as occult primaries with undifferentiated histology
- EBER RNA in situ hybridization is standard assay

Q8. Is there any overlap between these two viruses or the tumors typically associated with them?

- HPV-positive lymphoepithelial carcinomas have been described
- Non-endemic populations
Molecular Anatomic Pathology Application: Variants of Squamous Carcinoma and Viral Associations

There are variants of Head and Neck squamous cell carcinoma that have a unique pathway of tumorigenesis in that they do not progress through the usual dysplasia-carcinoma pathway. In these tumors, carcinogenesis is linked to viral pathogenesis. Epstein Barr Virus (EBV) and Human Papillomavirus (HPV) are two viruses strongly associated with variants of head and neck carcinoma.

The viral associated tumors are interesting because of their pathogenesis, but also because of the interesting epidemiology. Many of these tumors appear to be increasing in their incidence, not just in the United States, but worldwide. As smoking rates decrease, there is not a good explanation for the increased incidence other than the viral pathogenesis. Some speculation has been made as to whether this incidence will decrease, after the HPV vaccination takes effect in the appropriate population (with the obvious lag that will occur simply because of the age of onset of the head and neck tumors).

Basaloid squamous cell carcinoma

Basaloid squamous cell carcinoma (BSCC) is a tumor that has a propensity to occur in the oropharyngeal areas, with a particular disposition for the tonsil and tongue base region. However, it can affect just about any location in the head and neck mucosal sites. Patients with BSCC tend to present in the 6th and 7th decades with these tumors.

BSCC has a pathology that is characteristic. It can present with any size tumor, but small tumors are particularly notorious, especially in the tonsil, and can be difficult to detect. The tumor can have a surface component, but it also has a tendency to grow sub-mucosally for some distance and have overlying normal appearing mucosa. Even when the tumors are small, they can have bulky metastatic disease in the neck [1]. These nodes can be cystic and often will have extracapsular invasion.

The primary BSCC tumors will often invade in large sheets and nests, instead of the single infiltrating cells or very small angular tongues of conventional keratinizing SCC. The tumor cells have a typically basaloid morphology, with dark clumped chromatin, polarization at the base, and a high apoptotic and mitotic rate [2]. There can be central comedo-type necrosis.
as well. The typical BSCC will usually have some minor foci of keratinization, but this is often discovered as abrupt keratinization and is usually a minor component. Other features that can be present are a spindle cell component or other variants that are mixed or co-mingled with BSCC [3, 4]. Occasional tumors will exhibit deposition of basement membrane type hyalinized material, similar to what is seen in adenoid cystic carcinoma. In fact, in these cases, the differential diagnosis actually does include solid type or de-differentiated adenoid cystic carcinoma. This can be resolved using immunohistochemistry (IHC) (see below) [5].

BSCC is positive for typical keratins associated with tumors derived from mucosa [6, 7]. In the differential diagnosis with adenoid cystic carcinoma, a single stain can be very helpful. p63 is strongly and diffusely positive in BSCC, while it will be essentially negative or very isolated in solid type adenoid cystic carcinoma [5]. Because of the association with HPV, many BSCCs will also be positive for p16 (see below discussion).

BSCC and other tumors of the tonsil and tongue base are frequently associated with HPV [8-10]. HPV-associated SCCs are thought to have a very different etiology and prognosis from the typical smoking induced SCCs of the head and neck. HPV positive squamous cell carcinomas tend to present earlier, to have a lower frequency of second primary tumors (in contrast to the high frequency in patients who have a smoking induced field effect), and they tend to have a different treatment response to the typical chemoradiation therapy treatments [11-13]. For these reasons, it is important to identify SCCs that are related to HPV [11, 14].

**Table: Differences between HPV and smoking associated SCC of the head and neck**

<table>
<thead>
<tr>
<th></th>
<th>HPV Positive</th>
<th>HPV Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td>• 5 years younger</td>
<td>• Typical ages</td>
</tr>
<tr>
<td></td>
<td>• Non-smokers/non-drinkers</td>
<td>• Tobacco and alcohol</td>
</tr>
<tr>
<td><strong>Site</strong></td>
<td>Tonsil &amp; Tongue base</td>
<td>All locations</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>Poorly differentiated, non-keratinizing, basaloïd</td>
<td>Keratinizing SCC</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td>• p53 inactivated by E6</td>
<td>• p53 inactivated by mutation</td>
</tr>
<tr>
<td></td>
<td>• Rb inactivated by E7</td>
<td>• Rb inactivated by cyclin D1 amplification</td>
</tr>
<tr>
<td></td>
<td>• p16 over-expressed</td>
<td>• Inactivation of p16</td>
</tr>
</tbody>
</table>
HPV assays can also be used diagnostically in certain circumstances. The most frequent application of HPV tests for diagnosis is when there is a metastatic tumor in the neck and no known primary can be identified [15-18]. The finding of HPV or p16 positivity in this metastatic tumor can lead to targeted biopsies of the oropharynx, since such a high percentage of HPV associated tumors are primarily centered in the tonsil and/or tongue base [16, 17]. In some cases, a primary tumor is never found, but the oropharynx might even be treated with radiation for HPV positive metastases.

**Detection of HPV in Anatomic Pathology Material**

There are a number of viruses that are known to be associated with tumorigenesis. The most common are EBV and HPV. Of course, HPV is particularly well known for its association with carcinomas of the uterine cervix. In fact, HPV testing of cervical smear specimens has become standard of care for the management of certain subsets and age groups of women.

The most common subtype of HPV in oropharyngeal carcinomas is HPV 16 [19]. HPV types 18, 31, and 33 can also be seen. HPV related oropharyngeal squamous cell carcinomas have a different prognosis than HPV negative tumors [20]. They have a decreased rate of second primary tumors, less local recurrence, and better survival rates [20]. They also show improved response to chemoradiation therapy [21, 22].

Detecting HPV can be done with several different assays, including PCR based assays, in situ hybridization (ISH), and immunohistochemistry (IHC) [23]. The advantages of ISH are that the virus can be localized to the tumor cell nuclei and that false positives are reduced because it is not overly sensitive [24]. Integrated virus can even be distinguished from episomal virus by its dot-like positivity. Another advantage of ISH is that it can be performed in most pathology labs and only requires a light microscope for interpretation. IHC for p16 overexpression is a surrogate marker that can be used to suggest HPV positivity. HPV positive cases are associated with strong and diffuse p16 immunoreactivity. Variant patterns of staining can occur and these may be difficult to interpret. IHC for p16 lacks the specificity of ISH. Since p16 is also a tumor suppressor gene, alterations in expression will not always be due to HPV. IHC testing for p16 expression has the advantage of being readily accessible to most pathology labs. Practicing pathologists are comfortable with the interpretation of IHC, no additional equipment or expertise is required, and the test can be performed with a rapid turn around time. PCR assays can be designed to detect single HPV types or wide spectrum virus. An advantage of PCR is the increased sensitivity. However, the high sensitivity may lead to clinical false positives if latent virus is detected (super-infection not implicated in the etiology of the disease). PCR also has a longer turn around time and requires additional technical skills and equipment.
Indications for HPV testing in a clinical setting include diagnostic, therapeutic, and prognostic implications. HPV testing can help suggest the site of origin for a neck metastasis from an unknown primary. HPV testing can be used to distinguish a second primary from a metastatic tumor focus. HPV testing may be necessary for enrollment in clinical trials evaluating different courses of therapy. Finally, given the improved prognosis of HPV related tumors, many patients will request this information.

**Lymphoepithelial carcinoma**

Lymphoepithelial carcinoma (LEC) is the descriptive name for the tumor that has also been referred to as “Nasopharyngeal undifferentiated carcinoma”. It has been variously named in the past as Regaud and Schmincke’s tumor, undifferentiated nasopharyngeal carcinoma (in the nasopharynx) and lymphoepithelioma. Nasopharyngeal LEC or those that involve Waldeyer’s ring (base of tongue, palatine tonsils and adenoids) are more common in Southeast Asia and North Africa, where the incidence may be as high as 30-80/100,000 [25]. In the U.S. and Europe the incidence is well below 1/100,000. In Asia, there is a bimodal age distribution, with the largest peak in the 6th decade and the smaller peak between 10 and 25 years of age [26]. The most common presentation is a mass in the neck, with possible cranial nerve involvement and resulting neurologic symptoms.

In non-nasopharyngeal sites, LEC is quite rare. In several large series of all laryngeal carcinomas, LEC represented less than 0.2% of the cases studied. In our experience, the most common non-nasopharyngeal site is the larynx, and particularly the pyriform sinus.

The histologic features of LEC are unique. The cells in these tumors are large, and pleomorphic. The oval to round nuclei are vesicular and may contain large, distinct nucleoli. The cells grow in syncytial sheets that have prominent infiltrating lymphocytes. The lymphocytes in these lesions are polyclonal and therefore are considered to be reactive in nature. Two types of lesions have been described: pure LEC and mixed LEC with a component of conventional SCC. No differences in prognosis have been described, though this tumor is rare and large series have not been reported.

LEC in the nasopharynx is highly associated with EBV, particularly in endemic areas, such as Southeast Asia and North Africa [27]. EBV is fairly easy to identify in tissue samples by using RNA in situ hybridization, where a probe identifies the EBER RNA in the cells and gives a positive signal [28, 29]. There are other ways as well, including polymerase chain reaction and immunohistochemical stains. These are not considered to be the standard approach, however, and are not as reliable as the EBER assay [28].
In non-endemic areas, EBV is present in approximately 1/3 of LEC cases [30, 31]. Tumors in non-endemic areas are more often smoking related. The age distribution in western countries is unimodal (6th decade predominance), which supports the fact that it parallels conventional squamous cell carcinoma. Importantly, recent evidence from studies of LEC in non-endemic regions suggests a linkage to HPV, as well [32-34]. This is particularly true in the lesions that are involving the tonsil/tongue base region, either in the primary site or by local extension. These HPV associated LEC tumors have all the typical morphological features of EBV-associated tumors of the nasopharynx, but HPV can be detected via the usual route.

Table: *Two papers that demonstrated p16 staining and HPV in situ hybridization results for LECs that occurred in non-endemic populations [32, 34].*

<table>
<thead>
<tr>
<th></th>
<th>P16</th>
<th>HPV 16 ISH</th>
<th>HPV PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singhi</td>
<td>22/22 (100%)</td>
<td>19/22 (86%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Carpenter</td>
<td>14/15 (93%)</td>
<td>8/14 (57%)</td>
<td>6/6 (100%) (ISH -)</td>
</tr>
</tbody>
</table>

Genetic predisposition to nasopharyngeal LEC has been suggested by correlation between development of tumors and certain HLA profiles [35, 36]. The at-risk HLA types appear to be different from region-to-region. Diet has also been implicated as having a role in the development of nasopharyngeal LEC. Salted fish and preserved foods containing nitrosamines and herbal teas are a few of the suspected agents [37-39].
References

Molecular Diagnostics: Lung Cancer

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USCAP Annual Meeting
ASIP Companion Society Meeting
March 2, 2014

Lung Cancer Discussion: Overview

• Case presentation: clinical and histologic
• Request for Molecular Testing
  – Which molecular tests should be performed?
    • All at once, or sequentially?
  – How should molecular testing be performed?
    • Impact of the sample on this decision
• Review of molecular results
  – Data analysis and interpretation
  – Clinical implications
  – How to communicate results to clinical team?
• What’s in the future?

Case Presentation:

• 52 yr old female
• Symptoms (months):
  – Headaches
  – Light/dark sensitivity
  – Chronic dry cough
• MRI:
  – Enhancing dural mass

• Differential Dx:
  – Tumor
  – Meningioma
  – Lymphoma
  – Metastasis
  – Primary CNS tumor
  – Granuloma
  – Sarcoidosis
  – Tuberculosis
  – Chronic meningitis
  – Wegener’s

Case presentation: Brain Biopsy

• Adenocarcinoma
  – Lung
  – Colon
  – Other
Case Presentation

- Follow-up imaging studies
  - lung mass
  - Additional masses: Lung (x2), liver (x2), bone
- Non-small Cell Lung Cancer, Stage IV
- Now what?

“And some molecular testing”

- What to test?
  - EGFR
  - ALK
  - KRAS
  - ROS1
  - RET
  - MET
  - ERBB2
  - FGFR1
  - BRAF
  - …


EGFR testing

- Which mutations should be tested?
- How should testing be performed?
- How should results be interpreted?

Targeted EGFR Testing: exon 19

Targeted EGFR testing: codon 858

Sanger sequence: wild type control
Case: **EGFR Exon 18**

Case: **EGFR Exon 19**

Case: **EGFR Exon 20**

Case: **EGFR Exon 21**

**What if **EGFR** is wild type?**

- **EGFR** false negative?
- **KRAS**?
- **ALK**?
- Others

**Low tumor content: Standard Sanger Sequence**
Low tumor content:
sequence after PNA-clamped PCR

**KRAS testing**
- Which mutations should be tested?
- Why test for KRAS?
- How to test for KRAS?

Lung case 2: KRAS exon 2

**ALK testing**
- Which mutations should be tested?
- Why test for ALK?
- How to test for ALK?

Lung case 3: ALK FISH

What if EGFR, ALK, KRAS all wild type?
Molecular Microbiology: Whole genome sequencing of microorganisms in the clinical laboratory

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Director, Molecular Diagnostics Laboratory
Co-Director, Microbiology Laboratory
Department of Pathology and Genomic Medicine
Houston Methodist Hospital

Case History

You receive a phone call from a very concerned transplant surgeon:

“All of my patients are infected with multi-drug resistant Klebsiella pneumoniae. There is a hospital-wide outbreak!”

How do we respond? How do we investigate?

Consult 1: Infection Control Team

• Multi-drug resistant K. pneumoniae have been recovered from 3 transplant patients in the Surgical ICU.
• Over the same timeframe, similar organisms were recovered from 4 other patients in our hospital.

How do we determine if the K. pneumoniae strains recovered from these 7 patients are clonally related?

Consult 2: Microbiology Laboratory

• The work cards show identical MALDI-TOF spectra, Gram stain morphology, etc.
• The antibiograms are identical, with the same pattern as most other multi-drug resistant strains recovered in our hospital.

What is the next step in our investigation?

Consult 3: Molecular Diagnostics Laboratory

• Multilocus sequence typing (MLST) had been performed as part of a research project.
• Each strain was determined to have the same sequence type, the most common in our region.
  • Not helpful

What is the next step in our investigation?

Whole genome sequencing of microbes in our clinical laboratory

• Species assignment for slow growing, difficult to cultivate or difficult to identify organisms.
  Loong et al, J Clin Microbiol, 2013
• Real-time investigation of nosocomial infections and outbreaks.
• Study the molecular basis of severe, unusual or interesting infections.
  Wright et al, Arch Pathol Lab Med, 2011
• Understand bacteria strain genotype – patient disease phenotype relationships.
  Olsen et al, Proc Natl Acad Sci, 2010
**Genome Sizes**

- **Virus:** few kb -> ~200 kb
- **Bacterium:** ~1 Mb -> 6 Mb; haploid
- **Saccharomyces:** 12.1 Mb
- **C. elegans:** 97 Mb
- **Fruit fly:** 180 Mb
- **Human:** 3,000 Mb; ~20,000 genes

**Workflow and cost for whole genome sequencing of microbes**

- Extract genomic DNA (Automated, Qiagen) → 4 hours
- Prepare sequencing libraries (Automated, NexteraXT kit) → 4 hours
- Perform sequencing reaction (Illumina MiSeq) → 6-24 hours (overnight)
- Analyze data (Custom bioinformatics process) → 1-6 hours

Colonies on a plate to analyzed genomic data: <48 hours
Total reagent cost: ~$1,200

**Consult 3: Microbial Genomics Laboratory**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Specimen</th>
<th>Disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>kp1</td>
<td>Abdominal fluid</td>
<td>expired</td>
</tr>
<tr>
<td>kp2</td>
<td>Blood, Lower resp. tract</td>
<td>rehab facility</td>
</tr>
<tr>
<td>kp3</td>
<td>Lower resp. tract</td>
<td>expired</td>
</tr>
<tr>
<td>kp4</td>
<td>Urine</td>
<td>inpatient</td>
</tr>
<tr>
<td>not avail</td>
<td>Lower resp. tract</td>
<td>expired</td>
</tr>
<tr>
<td>kp5</td>
<td>Lower resp. tract</td>
<td>inpatient</td>
</tr>
<tr>
<td>kp6</td>
<td>Lower resp. tract, CSF</td>
<td>inpatient</td>
</tr>
</tbody>
</table>

We performed whole genome sequencing to investigate the possible clonal relatedness of strains recovered from these 7 patients.

**Whole genome sequencing identified three distinct lineages**

Phylogenetic tree based on single nucleotide polymorphisms (SNPs) in the sequenced strains relative to the reference strain.

Strain key:
- **same location/ different service**
- **outbreak patients**
- **different location/ different service**

Epidemiologically linked strains kp3 & kp4 are distantly related!

**Case Summary**

- Low resolution techniques were not able to distinguish the 7 strains.
- High quality sequence data was generated and analyzed within 48 hours.
- Three distinct lineages of multi-drug resistant *Klebsiella pneumoniae* were identified in the study set.
- Importantly, the *Klebsiella pneumoniae* strains recovered from the two transplant patients were determined to be distantly related.
- The whole genome sequence data rapidly informed our institutional response to a perceived outbreak.
  - Reemphasized hand hygiene and PPE by health care workers
  - Reevaluated isolation criteria in the Surgical ICU
LUNG CANCER

1. Which of the following samples would be most appropriate for molecular diagnostic testing for mutation in EGFR exons 19 and 21?
   a. B-plus fixed, paraffin-embedded lymph node with metastatic lung adenocarcinoma
   b. Formalin-fixed, paraffin-embedded cell block from a fine needle aspiration of a lymph node, with metastatic lung adenocarcinoma comprising ~40% of the cells
   c. Formalin-fixed, paraffin embedded, decalcified bone biopsy with metastatic lung adenocarcinoma comprising 85% of viable cells
   d. Formalin-fixed, paraffin-embedded lobectomy with a 3cm squamous cell carcinoma of the lung
   e. Fresh-frozen wedge resection showing recurrent lung adenocarcinoma after initial response to treatment with erlotinib, in a patient previously shown to have an L858R mutation in EGFR

2. Which of the following genes is most critical for cytogenetic testing in adenocarcinomas of the lung?
   a. ALK
   b. BRAF
   c. EGFR
   d. ERBB2
   e. FGFR3

3. Testing for which gene is valuable in lung adenocarcinoma not because it is a therapeutic target, but rather because mutations discovered would exclude other more treatable genetic changes?
   a. ALK
   b. EGFR
   c. FGFR1
   d. KRAS
   e. ROS1

4. Which gene is most likely to be mutated in combination with another genetic alteration and, therefore, may be less attractive as a therapeutic target in lung cancer?
   a. ALK
   b. BRAF
   c. EGFR
   d. ERBB2
   e. PIK3CA
MOLECULAR MICROBIOLOGY

1. A public health official requests your consultation on an outbreak of severe Methicillin-Resistant *Staphylococcus aureus* infections in a local nursing home. Your response is that whole genome sequencing of the implicated strains is:
   a. capable of confirming the clonal outbreak and defining transmission pathways.
   b. challenging due to the complexity and size of bacterial chromosomes.
   c. labor-intensive and cost-prohibitive for use in outbreak investigations.
   d. less informative than multi-locus sequence typing or pulse field gel electrophoresis.
   e. performed only by specialized microbial research centers.

HEAD AND NECK CANCER

1. Which method allows for direct visualization of HPV DNA in the nuclei of carcinoma cells?
   a. in situ hybridization
   b. p16 immunohistochemistry
   c. PCR for HPV type 16 DNA
   d. PCR for wide spectrum HPV DNA
   e. RT-PCR for HPV E6/E7 RNA

2. You diagnosed metastatic squamous cell carcinoma in a cervical lymph node from a 47 year old male non-smoker. Immunohistochemistry for p16 was performed and results are shown in the image. Where is the most likely anatomic site of the patient's primary tumor?

   a. Larynx
   b. Nasopharynx
   c. Oral Cavity
   d. Oropharynx
   e. Ventral tongue
MOLECULAR NEUROPATHOLOGY: GLIOMAS

1. In the differential diagnosis between anaplastic oligodendroglioma and a classic, primary (de novo) glioblastoma, the following is most typical:
   a. Anaplastic oligodendroglioma will be IDH1-R132H immunoreactive, 1p/19q codeleted, EGFR non-amplified, and PTEN (chromosome 10) intact
   b. Anaplastic oligodendroglioma will be IDH1-R132H immunopositive, 1p/19q intact, EGFR amplified, and PTEN (chromosome 10) intact
   c. Anaplastic oligodendroglioma will be IDH1-R132H immunonegative, 1p/19q intact, EGFR amplified, and PTEN (chromosome 10) deleted
   d. GBM will be IDH1-R132H immunonegative, 1p/19q deleted, EGFR amplified, and PTEN (chromosome 10) intact
   e. GBM will be IDH1-R132H immunopositive, 1p/19q codeleted, EGFR non-amplified, and PTEN (chromosome 10) intact

2. An anaplastic oligodendroglioma is diagnosed in a 7 year old girl. The most likely molecular finding is:
   a. 1p 19q codeletion
   b. IDH1-R132H mutation
   c. EGFR amplification
   d. PTEN gene mutation
   e. There is no likely molecular finding for a patient of this age

GENOMIC HEMATOPATHOLOGY

1. An asymptomatic healthy elderly man with persistent thrombocytosis for 4 months (600-700k platelet count) is tested for the presence of the JAK2 V617F mutation to rule out a myeloproliferative disease. No V617F mutation is detected. What reflex molecular test should be performed on the same DNA sample to provide the most sensitive algorithm for distinguishing essential thrombocytemia (ET) from benign reactive thrombocytosis?
   a. BCR-ABL fusion gene detection
   b. Calreticulin mutation analysis
   c. Colony stimulating factor 3 receptor (CSF3R) mutation analysis
   d. JAK2 exon 12 mutation analysis
   e. Repeat the JAK2 V617F assay

2. An asymptomatic healthy elderly woman with persistent leukocytosis for 3 months (35-40k leukocyte count; 70% granulocytes, 25% bands; normal hemoglobin & platelets) undergoes a bone marrow biopsy to rule out a myeloid neoplasm. The bone marrow is hypercellular with predominantly mature myeloid cells, <1% blasts, and no evidence of dysplasia. Molecular studies reveal a mutation in the CSF3R gene (T618I). The most likely diagnosis is:
   a. Chronic myeloid leukemia (CML)
   b. Chronic neutrophilic leukemia (CNL)
   c. Atypical chronic myeloid leukemia (aCML)
   d. Reactive granulocytosis
   e. Pre-fibrotic primary myelofibrosis (PMF)
BLADDER CANCER

1. FISH analysis was performed on a urine sample from a 55 year old man with microhematuria. The patient had a long smoking history. What type of FISH abnormality is present in the cells shown.

   a. There is no FISH abnormality
   b. Tetrasomy
   c. Polysomy
   d. Trisomy 7
   e. 9p21 Loss Alone

2. 25% of the non-squamous and non-inflammatory cells in a patient’s urine sample exhibit polysomy with the UroVysion probe set. The patient’s cystoscopy however shows no evidence of tumor. What should you recommend to the urologist?
   a. Ignore the FISH result, it is a false positive
   b. Evaluate the upper urothelial tract for tumor and if that is negative for tumor consider doing random biopsies of the bladder
   c. Repeat the FISH result in 1 year
   d. Repeat the FISH result in 2 years
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