90 A Practical Approach to Molecular Diagnostics in Gynecologic Neoplasms

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AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
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90 A Practical Approach to Molecular Diagnostics in Gynecologic Neoplasms

Recent advances in molecular genetics have contributed to our understanding of the molecular pathogenesis of gynecologic malignancies. As a result, there is an increase demand for integrating molecular diagnostic testing and morphologic interpretation to provide diagnostic, prognostic, therapeutic information for patient management. This session explores the molecular markers that are currently useful in the diagnosis of the commonly encountered gynecologic malignancies and those that may have a significant impact on the management of women with these diseases. The presentation of unknown cases will illustrate the practical application and clinical implications of the molecular tests that are discussed. Topics will include HPV testing in cervical lesions, microsatellite instability analysis in screening patients with Lynch Syndrome, the use of DNA genotyping in gestational trophoblastic diseases, and mutation analyses (Ki-ras, BRAF and p53) for ovarian and endometrial cancers.

- To identify salient molecular alterations and markers of gynecologic neoplasms.
- To choose appropriate molecular diagnostic tests for both common and uncommon gynecologic neoplasms.
- To consult clinicians on the significance and implications of the results of molecular diagnostic tests in reference to the morphologic interpretation.

FACULTY:

Pei Hui MD
David Chhieng MD

Practicing Pathologists
Molecular Pathology
Molecular Pathology
3.0 CME/CMLE Credits

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Practical approach to molecular diagnostic in gynecologic neoplasms

Part I

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Department of Pathology
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Adjunctive Testing in Gynecologic Cytology

- With LBP, residual cells and fluids are available following the preparation of slides for adjunctive testing
  - Micro-organisms
  - Biomarkers for cervical cancer
  - Molecular markers for genetic diseases

Testing for Microorganisms

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Method</th>
<th>Regulatory Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV</td>
<td>Hybrid Capture II (Digene)</td>
<td>FDA approved (ThinPrep)*</td>
</tr>
<tr>
<td></td>
<td>Third Wave Invader (Cervista)</td>
<td>FDA approved (ThinPrep)*</td>
</tr>
<tr>
<td></td>
<td>PCR (Amplicor)</td>
<td>FD approved (ThinPrep)*</td>
</tr>
<tr>
<td>Chlamydia/gonorrhea</td>
<td>Hybrid Capture II</td>
<td>FDA approved (ThinPrep)*</td>
</tr>
<tr>
<td></td>
<td>Viper (BD)</td>
<td>FDA approved (ThinPrep)*</td>
</tr>
<tr>
<td></td>
<td>PCR (Amplicor, Aptima)</td>
<td>FDA approved (ThinPrep and SurePath)</td>
</tr>
<tr>
<td>Herpes Simplex</td>
<td>PCR</td>
<td>Not FDA approved, requires CLIA validation</td>
</tr>
<tr>
<td>Group B Streptococci</td>
<td>Nucleic acid amplification</td>
<td>Not FDA approved, requires CLIA validation</td>
</tr>
<tr>
<td>Candida, Trichomonas,</td>
<td>Hybrid Capture (BD Affirmis)</td>
<td>Not FDA approved, requires CLIA validation</td>
</tr>
<tr>
<td>Gardinerella</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What is HPV

- A member of papovavirus family
- 8 KB circular single DNA strand, encapsulated by a capsule
- 6 early genes (E genes)
- Regulatory region code for replication process
- 2 late genes (L genes) for the capsule protein
- Requires a human host cell for replication

Why are we concerned with HPV

- One of the most common sexually transmitted infections worldwide
- By age 50, at least 80% of women who are sexually active will have had HPV infection
- Estimated incidence of HPV infection in US—6.2 million per year and prevalence of 20 million
- HPV is the major cause of cervical cancer

Low risk Vs High risk HPV types

- Low risk HPV type never associated with HSIL and cancer
- High risk HPV type associated with cancer and its precursors
- Based on the effectiveness of the HPV E6 & E7 gens at transforming host cells—low risk HPV has little transforming potential
- Low grade diseases (CIN1 and HPV cytopathic changes) do not imply low-risk type—80% of low grade lesions still associated with high risk HPV types
Oncogenic genes of HPV

- 2 genes: E6 and E7
- HPV DNA breakage occurs in E2 gene region → upregulation of E6 and E7 genes
- E6 interferes with host p53 gene function
- E7 interferes with host Rb gene function
- Disabling both host tumor suppressor genes

Approaches to HPV Detection

- Cytology and histology
  - Unable to distinguish different types of HPV
- IHC and EM
  - Detect viral particles and genus-specific antigens
  - Insensitive
- HPV serology
  - Limited value
  - Does not distinguish between present and past infections
  - Take weeks or months for antibodies to become detectable
  - Antibodies remained detectable for many years
  - Useful for epidemiologic studies and vaccine clinical trials

Types of HPV Detection Techniques

- Rely on the detection of viral nucleic acids
  - Direct probe methods
  - Signal amplification
  - Target amplification
    - Southern
    - ISH
    - Hybrid Capture
    - Third Wave Invader
    - Type specific PCR
    - Real time PCR
HPV Testing in Routine Clinical Labs

Qualities of HPV tests
- Easy to perform
- Capable of detecting many HR HPV types
- Validated and standardized
- Reproducibility
- High sensitivity
- High specificity
- Cost effective
- Commercially available

Examples of commercially available HPV detection assays
- ISH assays (Ventana)
- HPV solution hybridization assay (Digene Hybrid Capture II)
- Third Wave Invader assay (Cervista, Hologic)
- HPV PCR Assays (Roche HPV amplior assay)

Sample Collection for HPV Detection

- Fresh, frozen, or fixed tissues and cervical scrapes obtained for routine or liquid based cytology
- Collected using a cytobrush or Cervex-Brush
- Transport media
  - e.g. PreservCyt (Hologic), SurePath (BD), Digene collection media (Qiagen)
  - These media preserve cell morphology and DNA/RNA molecules
  - Stored at room temp for up to 21 days
  - Cells collected in PreservCyt and SurePath also suitable for ISH

In-situ hybridization

- Positive staining indicates presence of HPV
- Staining patterns differ depending if HPV DNA is episomal or integrated

Integration: multiple, opaque, small blue round foci of staining within the nucleus
Episomal: dense, opaque, blue oval structure in the central portion of the target cell
HPV Solution Hybridization Assay

- Digene Hybrid Capture II (HC II) assay
- For both low risk (6, 11, 42, 43, 44) and high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68)
- Using signal amplification and chemiluminescence detection
- Can also be used for detection of Chlamydia trachomatis and Neisseria gonorrhea

Interpretation of HC II Results

<table>
<thead>
<tr>
<th>RLU/CO Unit</th>
<th>Test Result</th>
<th>Result Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.0</td>
<td>Negative**</td>
<td>HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 not detected</td>
</tr>
<tr>
<td>≥ 1.0*</td>
<td>Positive</td>
<td>HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 detected</td>
</tr>
</tbody>
</table>

*If RLU/CO between 1.0 and 2.5, specimen must be retested (FDA requirement)
**did not rule out other low risk HPV types

Third Wave Invader Assay (Cervista)

- Another signal amplification
- Marketed under the name Cervista
- For detection of HR HPV and genotyping 16 and 18
- Utilize a proprietary isothermal process to enhance the signal
- FDA approved for ThinPrep
**Digene HC II assay Vs Cervista**

<table>
<thead>
<tr>
<th>Digene HC II assay</th>
<th>Cervista HR HPV assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detect 13 HR types</td>
<td>Detect 14 HR types</td>
</tr>
<tr>
<td>Gray zone—requires repeat</td>
<td>No gray zone</td>
</tr>
<tr>
<td>Longest track record &amp; FDA approval</td>
<td>Recently FDA approved (2009)</td>
</tr>
<tr>
<td>More data in literature</td>
<td>Limited data in literature</td>
</tr>
<tr>
<td>No information of individual subtype</td>
<td>Genotyping for 16/18 available</td>
</tr>
<tr>
<td>Larger batch size: 88</td>
<td>Smaller batch size (28)</td>
</tr>
<tr>
<td>Risk of false negative on acellular samples</td>
<td>Built in control—minimize false negatives</td>
</tr>
<tr>
<td>Relatively lower sensitivity—recommendations for patient management based on</td>
<td>Relatively higher sensitivity—? Increase detection of clinically irrelevant HPV infection</td>
</tr>
</tbody>
</table>

**Polymerase Chain Reaction**

- 2 types of PCR assays
  1. HPV type-specific PCR primers based on sequence variations in the E6 and E7 genes
  2. General primer PCR assays using consensus primer
     - Detecting up to 40 different HPV genotypes
     - Deletion in the L1 genes → false negatives

**Value of HPV testing in Clinical Practice**

- Improve the sensitivity of detecting HSIL
- Reduce the referral rate for colposcopic examination and cervical biopsies in patient with equivocal abnormalities e.g. ASC-US
- Follow up after treatment of CIN—prediction of treatment failure
- Combination of cytology and HPV testing for primary screening of women older than 30 years (cotesting)
HR HPV testing for triage of ASC-US

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>% of women tested positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 2+</td>
<td>CIN 3+</td>
<td>CIN 2+</td>
</tr>
<tr>
<td>93%</td>
<td>96%</td>
<td>62%</td>
</tr>
</tbody>
</table>

- Sensitivity of HC2 14% higher than repeat cytology (ASC+) for detection of CIN 2+
- HC2 and repeat cytology showed a similar specificity

Cuzick et al. Vaccine 2008;26S:K29

- Benchmark for positive HPV rate in ASC-US: 35% to 45%

Performance of HR HPV testing (HC2)

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>% of women tested positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 2+</td>
<td>CIN 3+</td>
<td>CIN 2+</td>
</tr>
<tr>
<td>97%</td>
<td>97%</td>
<td>31%</td>
</tr>
</tbody>
</table>

- Reflex HPV testing for patients with LSIL not recommended

Cuzick et al. Vaccine 2008;26S:K29

Triage for LSIL with HR HPV testing

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>% of women tested positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 2+</td>
<td>CIN 3+</td>
<td>CIN 2+</td>
</tr>
<tr>
<td>97%</td>
<td>97%</td>
<td>31%</td>
</tr>
</tbody>
</table>

Cuzick et al. Vaccine 2008;26S:K29

- % of patients with LSIL positive for HPV
  - <30 years: 89%
  - 30-49 years: 70%
  - 50-64 years: 51%

Moss et al. BMJ 2006;332:83

- % of patients with LSIL positive for HPV
  - <30 years: 90%
  - 30 years +: 70%

Sherman et al. JNCI 2002;94:102
Triage for LSIL

- ASCCP does not recommend reflex HPV testing for patients with LSIL
- Viral clearance in 18%-45% over 6-12 months; up to 60% in those age 30+
- Some advocate delayed HPV testing and/or repeat cytology after 6-12 months
- Requires good compliance

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Follow up outcomes and high risk HPV status among various age groups in patients with LSIL

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>HR HPV Status</th>
<th>Follow up result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>CIN 1/HPV</td>
<td>CIN 2+</td>
</tr>
<tr>
<td>Younger than 30 years</td>
<td>19 (50.0%)</td>
<td>18 (47.4%)</td>
<td>1 (2.6%)</td>
</tr>
<tr>
<td></td>
<td>103 (23.0%)</td>
<td>278 (62.2%)</td>
<td>66 (14.8%)</td>
</tr>
<tr>
<td></td>
<td>122 (25.2%)</td>
<td>296 (61.0%)</td>
<td>67 (13.8%)</td>
</tr>
<tr>
<td>30 years or older</td>
<td>61 (47.7%)</td>
<td>66 (51.6%)</td>
<td>1 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>67 (18.3%)</td>
<td>250 (68.3%)</td>
<td>40 (13.4%)</td>
</tr>
<tr>
<td></td>
<td>127 (24.3%)</td>
<td>316 (63.9%)</td>
<td>50 (10.1%)</td>
</tr>
<tr>
<td>Overall</td>
<td>80 (48.2%)</td>
<td>84 (50.6%)</td>
<td>2 (1.2%)</td>
</tr>
<tr>
<td></td>
<td>170 (20.9%)</td>
<td>528 (64.9%)</td>
<td>315 (14.2%)</td>
</tr>
<tr>
<td></td>
<td>250 (25.5%)</td>
<td>612 (62.5%)</td>
<td>117 (12.0%)</td>
</tr>
</tbody>
</table>

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HR HPV testing in Women with AGC

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases (%)</th>
<th>HPV + (%)</th>
<th>HPV – (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative for Dysplasia</td>
<td>905 (77%)</td>
<td>108 (12%)</td>
<td>399 (39%)</td>
</tr>
<tr>
<td>CIN 2+</td>
<td>105 (10%)</td>
<td>96 (9%)</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>AIS &amp; BC adenocarcinoma</td>
<td>91 (4.8%)</td>
<td>27 (9%)</td>
<td>45 (5%)</td>
</tr>
<tr>
<td>EM adenocarcinoma</td>
<td>14 (6.5%)</td>
<td>15 (5%)</td>
<td>19 (6%)</td>
</tr>
<tr>
<td>Extravaginal malignancy</td>
<td>4 (0.6%)</td>
<td>4 (0.6%)</td>
<td>4 (0.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>661</td>
<td>233 (35%)</td>
<td>429 (65%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rate of HPV associated disease</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>90%</td>
<td>79%</td>
<td>97%</td>
<td>97%</td>
</tr>
</tbody>
</table>

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Rodriguez et al. JNCI 2008;100:513

HR HPV testing in Women with ASC-H

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases (%)</th>
<th>HPV + (%)</th>
<th>HPV – (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 1/negative</td>
<td>516 (65%)</td>
<td>215 (42%)</td>
<td>301 (48%)</td>
</tr>
<tr>
<td>CIN 2+</td>
<td>279 (35%)</td>
<td>236 (85%)</td>
<td>43 (15%)</td>
</tr>
<tr>
<td>Total*</td>
<td>795</td>
<td>451 (58%)</td>
<td>344 (42%)</td>
</tr>
</tbody>
</table>

Rate of HR HPV + Sensitivity Specificity PPV NPV
56% (55% - 77%) 85% 94% 52% 88%

- Based on the meta-analysis of the following 4 studies:

Performance of HR HPV testing (HC2)

<table>
<thead>
<tr>
<th>Predicting treatment failure</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>% tested positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>94% (91%-98%)</td>
<td>75% (69%-80%)</td>
<td>32% (33%-40%)</td>
<td></td>
</tr>
</tbody>
</table>

- Treatment failure occurs in 10% of patients
- Sensitivity of HPV testing to detect CIN 2+ significantly higher than that of repeat cytology at cut-off ASCUS (ratio 1.16)
- Specificity of HPV testing not significantly lower (ratio 0.96)
- Reassurance for patients with negative colposcopy or biopsy if HPV testing is negative; positive HPV testing requires careful examination of the EC canal to rule out AIS or its precursor lesions

Primary screening with HR HPV testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>% to Colpos</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV only</td>
<td>97.4%</td>
<td>94.3%</td>
<td>7.6%</td>
<td>100.0%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Pap only</td>
<td>56.4%</td>
<td>97.5%</td>
<td>8.5%</td>
<td>99.8%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Pap with reflex HPV</td>
<td>53.8%</td>
<td>98.7%</td>
<td>44.9%</td>
<td>99.8%</td>
<td>1.6%</td>
</tr>
<tr>
<td>HPV with reflex Pap</td>
<td>53.8%</td>
<td>99.0%</td>
<td>24.4%</td>
<td>99.8%</td>
<td>1.6%</td>
</tr>
<tr>
<td>HPV &amp; Pap cotesting</td>
<td>100.0%</td>
<td>92.4%</td>
<td>5.5%</td>
<td>100.0%</td>
<td>7.9%</td>
</tr>
</tbody>
</table>

- Based on 10,000+ women age 30-69
- Used conventional Pap
- HPV by HC2
- Primary endpoint was detection of CIN 2+
Long term follow up

<table>
<thead>
<tr>
<th></th>
<th>Risk of developing CIN 2+ after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
</tr>
<tr>
<td>Normal Pap (Con)</td>
<td>0.33%</td>
</tr>
<tr>
<td>Negative HPV (HC2)</td>
<td>0.09%</td>
</tr>
</tbody>
</table>

- A negative HPV test offers twice the protection of a negative Pap of a negative Pap for at least 5 years and still more predictive of lower risk at 9 years than a negative cytology
- Vice versa, an initial positive HPV test is far more predictive of increased risk than an initial positive Pap up to 9 year follow up

Correlation of HR HPV and cytology results

- Cotesting is now recommended in the US

<table>
<thead>
<tr>
<th>HPV result</th>
<th>Cytology result</th>
<th>Risk of CIN 3 or above</th>
<th>Suggested management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>&lt;0.1%</td>
<td>Repeat cytology alone in 1-2 year</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>2% to 10%</td>
<td>Rescreening every 3 years</td>
</tr>
<tr>
<td>Negative</td>
<td>ASC, ASC-H, LSIL, AEC, &amp; AGC</td>
<td>2% to 10%</td>
<td>Rescreening every 3 years</td>
</tr>
<tr>
<td>Positive</td>
<td>ASC, ASC-H, LSIL, AEC, &amp; AGC</td>
<td>&gt;10%</td>
<td>Immediate colposcopy</td>
</tr>
<tr>
<td>Positive/Negative</td>
<td>HSIL</td>
<td>&gt;40%</td>
<td>Immediate colposcopy</td>
</tr>
</tbody>
</table>

HPV Genotyping

10 year cumulative incidence rates of CIN 3+

<table>
<thead>
<tr>
<th>HPV 16+</th>
<th>HPV 18+</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.2%</td>
<td>13.6%</td>
</tr>
</tbody>
</table>

- for HR HPV but –ve for 16 and 18
- for HR HPV

-ve for HR HPV 0.8%
Incidence of HR-HPV + and Negative Cytology

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location of study</th>
<th>HR-HPV + rate (%)</th>
<th>Patient age range</th>
<th>Pap type</th>
<th>HPV test method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrall, 2010</td>
<td>US</td>
<td>5.4</td>
<td>≥ 30</td>
<td>LBP</td>
<td>HC II</td>
</tr>
<tr>
<td>Castle, 2009</td>
<td>US</td>
<td>4.0</td>
<td>≥ 30</td>
<td>Conventional</td>
<td>HC II</td>
</tr>
<tr>
<td>Datta, 2008</td>
<td>US</td>
<td>8.1</td>
<td>30-65</td>
<td>Conventional &amp; LBP</td>
<td>HC II</td>
</tr>
<tr>
<td>Sargent, 2008</td>
<td>UK</td>
<td>4.1</td>
<td>30-64</td>
<td>LBP</td>
<td>HC II</td>
</tr>
<tr>
<td>Bulkman, 2007</td>
<td>Netherlands</td>
<td>5.2</td>
<td>29-56</td>
<td>Conventional &amp; LBP</td>
<td>PCR</td>
</tr>
<tr>
<td>Ronco, 2006</td>
<td>Italy</td>
<td>9.6</td>
<td>30-60</td>
<td>Conventional</td>
<td>HC II</td>
</tr>
<tr>
<td>Cuzick, 2003</td>
<td>UK</td>
<td>6</td>
<td>30-60</td>
<td>Conventional</td>
<td>HC II</td>
</tr>
<tr>
<td>Petry, 2003</td>
<td>Germany</td>
<td>5.9</td>
<td>≥ 30</td>
<td>Conventional &amp; LBP</td>
<td>HC II</td>
</tr>
</tbody>
</table>

Follow up results patients with negative cytology and HPV cotesting

<table>
<thead>
<tr>
<th></th>
<th>HR HPV + (n=146)</th>
<th>HR HPV - (2,573)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up rate</td>
<td>82% (n=120)</td>
<td>43% (n=1,334)</td>
</tr>
<tr>
<td>Cytology (± biopsy)</td>
<td>74% (n=108)</td>
<td>52% (n=1,334)</td>
</tr>
<tr>
<td>NILM</td>
<td>86% (HPV + 45%)</td>
<td>95% (HPV + 3%)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>10% (HPV + 82%)</td>
<td>3% (HPV + 27%)</td>
</tr>
<tr>
<td>LSIL</td>
<td>9%</td>
<td>1%</td>
</tr>
<tr>
<td>HSIL</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Biopsy (± cytology)</td>
<td>42% (n=60)</td>
<td>1% (n=8)</td>
</tr>
<tr>
<td>ASC i</td>
<td>34% (27.5%)*</td>
<td>22% (±7.5%)*</td>
</tr>
<tr>
<td>CIN 1</td>
<td>5% (2.5%)*</td>
<td>1% (0.5%)*</td>
</tr>
</tbody>
</table>

* Percentage based on patients with follow up

Thrall et al. Am J Clin Pathol. 2010;133:894

Percentage of woman with normal cytology tests and positive for HR HPV by age group and for all women 30 and older

Thrall et al. Am J Clin Pathol. 2010;133:894
## Genotyping in Women with ASC-US

**Performance of Cervista HPV 16/18 genotyping compared with histologic FU results (CIN 2+)**

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;30</th>
<th>≥30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of CIN 2+</td>
<td>6.6%</td>
<td>4.1%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>70%</td>
<td>67%</td>
</tr>
<tr>
<td>Specificity</td>
<td>62%</td>
<td>80%</td>
</tr>
<tr>
<td>NPV</td>
<td>96%</td>
<td>97%</td>
</tr>
<tr>
<td>PPV</td>
<td>15%</td>
<td>22%</td>
</tr>
</tbody>
</table>

(Einstein et al. Cancer Epidemiol Biomarkers & Prev. 2011; 20: 1185)

## Genotyping in Women with ASC-US

**HR HPV Result**

<table>
<thead>
<tr>
<th>HPV genotyping</th>
<th>Absolute risk of harboring CIN 2+</th>
<th>Likelihood ratio of harboring CIN 2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>16 and/or 18 +</td>
<td>17.1% (44/257)</td>
</tr>
<tr>
<td>Positive</td>
<td>16 &amp; 18 -</td>
<td>4.0% (20/500)</td>
</tr>
<tr>
<td>Negative</td>
<td>NA</td>
<td>0.9% (5/555)</td>
</tr>
</tbody>
</table>

Prevalence of CIN 2+ is 5.3%

(Einstein et al. Cancer Epidemiol Biomarkers & Prev. 2011; 20: 1185)

## Genotyping in Women with LSIL

**HPV type**

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Positive</th>
<th>Incidence of CIN 2+</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>24%</td>
<td>45%</td>
<td>42%</td>
<td>43%</td>
<td>4.44</td>
</tr>
<tr>
<td>18</td>
<td>9%</td>
<td>30%</td>
<td>10%</td>
<td>90%</td>
<td>1.46</td>
</tr>
<tr>
<td>31</td>
<td>10%</td>
<td>44%</td>
<td>18%</td>
<td>44%</td>
<td>3.79</td>
</tr>
<tr>
<td>33</td>
<td>6%</td>
<td>44%</td>
<td>10%</td>
<td>44%</td>
<td>3.77</td>
</tr>
<tr>
<td>16/18</td>
<td>30%</td>
<td>27%</td>
<td>49%</td>
<td>39%</td>
<td>3.94</td>
</tr>
<tr>
<td>16/18/31</td>
<td>42%</td>
<td>40%</td>
<td>70%</td>
<td>40%</td>
<td>5.99</td>
</tr>
<tr>
<td>16/31/33</td>
<td>30%</td>
<td>21%</td>
<td>25%</td>
<td>26%</td>
<td>2.66</td>
</tr>
</tbody>
</table>

HPV genotyping archived with PCR using the general primer pair GP%+/BioGP 6a.

Chlamydia and Gonorrhea

- 3% and 0.5% of women tested positive for CT & GC, respectively
- CDC & ACOG recommend annual screening of all sexually active women aged ≤25 years, older women with risk factors, and all pregnant women
- Culture—gold standard
- Materials collected into LBC transport media not suitable for culture
- FDA approved CT/GN molecular tests for endocervical swab, ureteral swabs, voided urine, and LBP
- Convenient—avoid missed opportunities for screening
- False positives and false negatives: Rare

Trichomonas, Candida, & Gardnerella V.

- Candida, Trichomonas, and bacterial vaginosis account for 90% of infectious vaginitis
- 90% of BV involves Gardnerella vaginalis
- Molecular testing using vaginal swab: BD Affirm VPIII
- The detection thresholds of the Affirm VPIII set above the levels of normal flora → only detect clinically relevant cases

<table>
<thead>
<tr>
<th></th>
<th>Candida</th>
<th>Trichomonas</th>
<th>Gardnerella v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affirm Vs Wet mount</td>
<td>11% vs 7%</td>
<td>7% vs 3%</td>
<td>45% vs 14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affirm Vs Pap test</td>
<td>13% vs 11%</td>
<td>3% vs 0.5%</td>
<td>44% vs 15%</td>
</tr>
</tbody>
</table>

Affirm more likely to detect coinfection by 2 or more organisms than Pap test: 7% vs 1%


Cervical Carcinoma Markers

- Reduce both false negative and false positive rates
- Increase interobserver agreement
- Predict biologic aggressiveness

<table>
<thead>
<tr>
<th>Target</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>P16</td>
<td>Immunocytochemistry</td>
</tr>
<tr>
<td>ProEx C</td>
<td>Immunocytochemistry</td>
</tr>
<tr>
<td>DNA ploidy</td>
<td>FISH</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Immunocytochemistry</td>
</tr>
<tr>
<td>Epigenetic markers</td>
<td>PCR</td>
</tr>
</tbody>
</table>

p16INK4a

- A tumor suppressor protein
- Normal function—prevent cells from dividing in the absence of appropriate signal
- Deletion or inactivation of p16 results in tumor progression in many types of cancer
- In cervical carcinogenesis, p16 is over expressed in cervical dysplasia and cancer

p16

Mechanism of p16\textsuperscript{INK4} over-expression in pre-cancerous and cancerous cells.
p16 positivity in Pap tests according to histology

<table>
<thead>
<tr>
<th>% of p16 positive</th>
<th>CIN 1</th>
<th>CIN 2</th>
<th>CIN 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% (0.4-9.6%)</td>
<td>38% (23-52%)</td>
<td>68% (44-92%)</td>
<td>82% (72-92%)</td>
</tr>
</tbody>
</table>

- Definition of p16 positivity: nuclear ± cytoplasmic
- Cutoff values: variable ranging from focal to diffuse


Efficacy of p16 immunostaining in Predicting CIN 2+ and CIN 3+ (n=136)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 2+</td>
<td>79</td>
<td>85</td>
<td>91</td>
<td>68</td>
</tr>
<tr>
<td>CIN 3+</td>
<td>90</td>
<td>71</td>
<td>71</td>
<td>90</td>
</tr>
</tbody>
</table>

Gup et al. AJCP. 2011;135: 212.
p16 in cytology

<table>
<thead>
<tr>
<th>Cytological Category</th>
<th>NILM</th>
<th>ASC-US</th>
<th>LSIL</th>
<th>HSIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of p16 positive</td>
<td>12%</td>
<td>45%</td>
<td>45%</td>
<td>89%</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(7-17%)</td>
<td>(35-54%)</td>
<td>(37-57%)</td>
<td>(84-95%)</td>
</tr>
</tbody>
</table>

- Definition of p16 positivity: nuclear ± cytoplasmic
- Cutoff values: variable ranging from any staining to >30% of atypical cells.


Efficacy of p16 immunostaining in Pap with ASC-US/ASC-H

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>p16</th>
<th>% of CIN 2+ in FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (%)</td>
<td>59 ± 35</td>
<td>66 ± 18</td>
<td>35 ± 20</td>
<td>33% (7-50%)</td>
</tr>
</tbody>
</table>

- Advantage: Help to locate few potentially dysplastic cells in a background of normal cells


p16 in Cytology

- Non-dysplastic cells stain for p16
  - Endometrial and endocervical cells
  - Atrophic or metaplastic epithelia
  - Make interpretation difficult because of lack of cellular context
- Only score p16 positive cells that also demonstrate nuclear abnormalities
- Variable definition of p16 positivity and cutoff value

Genetic Disease Carrier Detection

- Cystic fibrosis
- Fragile X Syndrome
- Muscular dystrophy
Cystic Fibrosis

- 1:29 Caucasians carrying a CF mutation
- 1 infant out of every 3,000 live births affected by CF
- If both parents are carriers, 25% of chance of their child being born with CF
- ACOG recommends screening for CF to all couples seeking preconception or prenatal care
- Offering screening prior to pregnancy allows client more reproductive choices
- ACOG recommends a screening panel of 23 common CF mutations

Carrier Rates: Cystic Fibrosis

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Carrier Frequency</th>
<th>Detection Rate</th>
<th>Carrier risk after negative test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern European Caucasian</td>
<td>1/29 – 1/29</td>
<td>85-90%</td>
<td>~1 in 256</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>1/26 – 1/29</td>
<td>97%</td>
<td>~1 in 930</td>
</tr>
<tr>
<td>Southern European Caucasian</td>
<td>1/29</td>
<td>70-80%</td>
<td>~1 in 97 to 1 in 140</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1/46</td>
<td>97%</td>
<td>~1 in 105</td>
</tr>
<tr>
<td>African American</td>
<td>1/65</td>
<td>72%</td>
<td>~1 in 232</td>
</tr>
<tr>
<td>Asian</td>
<td>~1/90 (7)</td>
<td>~20% (7)</td>
<td>Not available</td>
</tr>
</tbody>
</table>

CF Carrier Results

- Many tests detect a majority but not all carriers
  - Detection rates differ by ethnicity
  - Negative results do not eliminate risk
  - Positive results using LBP may be derived from sexual partner due to sperm contamination—repeat testing using sample from buccal swab for the patients and her sexual partner
- Different mutations may confer different risks
  - Example: CFTR R117H mutation and 5’ allele
- Genetic consultation should be offered to carriers
Practical approach to molecular diagnostic in gynecologic neoplasms
Part II

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Associate Professor
Department of Pathology
Yale University School of Medicine

The authors declare no financial interest associated with this presentation

Course Outline

1. HPV Testing (Dr. Chhieng)
   - Cervical Cancer
2. Microsatellite Instability Testing (MSI)
   - Endometrial Cancer
3. DNA Genotyping Diagnosis
   - Hydatidiform moles
   - Gestational trophoblastic tumors
   - Others
4. Emerging Molecular Testing of Gynecologic Cancers
   - Her2/neu testing of uterine serous carcinoma for Trastuzumab therapy
   - Her2/neu testing of vulvar Paget’s disease for Trastuzumab therapy
   - OncoSNP, BRCA gene mutations, MSI testing and genomic profiling of ovarian cancers
Lynch Syndrome (HNPCC)

- Germline mutation in one of DNA MMR genes
- Autosomal dominant
- No unique phenotypical markers
- Clinical diagnostic problems
  - Small families
  - Incomplete family history
  - Incomplete penetrance

Amsterdam Criteria 1991

- Presence of histologically verified CRC in at least three relatives, one of whom is a first degree relative of the other 2.
- The presence of disease in at least two successive generations
- Age of onset of CRC of less than 50 years in at least one of the individuals
- Exclusion of FAP diagnosis

Bethesda Guidelines 1996

- Meet the Amsterdam criteria
- 2 or more HNPCC-related cancers
- 1st degree relative with CRC &/or HNPCC-related cancer (or adenoma), 1 diagnosed <45 years (or adenoma <40 years)
- CRC or endometrial cancer diagnosed < 45 years
- Right-sided CRC with undifferentiated (solid/colloid) pattern, diagnosed < 45 years
- Signet-ring-cell-type CRC, diagnosed <45 years
- Adenoma diagnosed < 40 years
Revised Bethesda Guidelines 2004

- CRC in patients <50 yrs of age
- Synchronous or Metachronous colorectal or other HNPCC-associated tumors regardless of age
- CRC with MSI-H morphology in patients <60 yrs of age
- CRC with one or more 1st degree relative with HNPCC-related tumors, 1 diagnosed <50 yrs
- CRC in 2 or more 1st or 2nd degree relatives with HNPCC-related tumor, regardless of age

Tumor Spectrum of Lynch Syndrome

- Colorectal cancers
- Endometrial cancers
- Upper GI: small bowel, stomach and pancreas
- Ovarian cancers
- Sebaceous adenoma or carcinoma and keratoacanthoma (Muir-Torre Syndrome)
- GU cancers: bladder, ureter and renal pelvis
- Brain: glioblastoma (Turcot Syndrome)

Diagnosis

- Family history in those families meeting the above criteria and with tumor microsatellite instability (MSI)
- Molecular genetic testing for germline mutation in mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2) or EPCAM
Mismatch Repair Genes (MMR)

Multi-protein complex/single base pair and small loop mismatch repair

hMLH1, hMSH2, hMSH6, PMS2 and hMSH3

Both somatic and germline mutations found in variety of cancers

Tumors with defective MMR featured by unique genetic instability - Microsatellite instability (molecular marker)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exons</th>
<th>Amino Acid</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>19</td>
<td>756</td>
<td>&gt;200</td>
</tr>
<tr>
<td>MSH2</td>
<td>16</td>
<td>934</td>
<td>&gt;170</td>
</tr>
<tr>
<td>MSH6</td>
<td>10</td>
<td>1360</td>
<td>&gt;30</td>
</tr>
<tr>
<td>PMS2</td>
<td>15</td>
<td>862</td>
<td>rare</td>
</tr>
<tr>
<td>EPCAM</td>
<td>9</td>
<td>314</td>
<td>1-2.8% LS</td>
</tr>
</tbody>
</table>
Screening Testing: IHC and MSI

Tests performed on tumor tissue: To screen for the probability of Lynch syndrome; and To identify which gene likely to have a causative germline mutation.

Method of Detection of DNA Mismatch Repair Gene Defect

A: Microsatellite Instability PCR Testing
- BAT25 (mononucleotide)
- BAT26 (mononucleotide)
- D5S346 (dinucleotide)
- D17S250 (dinucleotide)
- D2S123 (dinucleotide)

B: Immunohistochemistry Testing for the Presence of MMR proteins
- MLH1
- MSH2
- MSH6
- PMS2

Immunohistochemistry of MMR proteins

Must be nuclear staining
Internal control cells (lymphocytes) must be present
Classic staining patterns:

<table>
<thead>
<tr>
<th>Protein Combination</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1/PMS2</td>
<td>Loss</td>
</tr>
<tr>
<td>MSH2/MSH6</td>
<td>Loss</td>
</tr>
<tr>
<td>MSH6</td>
<td>Loss alone</td>
</tr>
<tr>
<td>PMS2</td>
<td>Loss alone</td>
</tr>
</tbody>
</table>
Advantages of IHC testing
- 92% sensitivity in detection of MMR germline mutation [Shia 2008].
- Tumor tissue/cell correlation
- MMR gene specific detection (for downstream target sequencing)

Limitations of IHC testing
- False negative or positive results [Shia 2008].
- Some missense germline mutations will lead to false negative result [Wahlberg et al 2002, Bellizzi & Frankel 2009].
Microsatellite Instability Testing

What are microsatellites?
- Short repetitive sequences of DNA (1-6 base pair, up to 40 repeats)
  - $AAAAAAAAAAAA \ (A)_{11}$
  - $GTTGGTTGGTG \ (GT)_{11}$
  - $CTGCTGCTGCTG \ (CTG)_{13}$
  - $ACTCACTCACTCC \ (ACTC)_{11}$
- Abundant and randomly scattered throughout genome

What are Microsatellites?
- Highly polymorphic (length and number variations from person to person)
- Inheritable and stable in normal cells
When a microsatellite is unstable

- A somatic gain or loss of repeat units creates a change in length from the germ-line microsatellite allele

\[(\text{CA})_6 \Rightarrow (\text{CA})_8 \text{ or } (\text{CA})_5\]

Clonal expansion of an altered microsatellite in a neoplasm

- Must compare the tumor to normal tissue to detect differences!

Defective Mismatch Repair

Genomic Allele = (CA)_4

\[
\begin{align*}
\text{C-A-C-A-C-A} & \quad \text{G-T-G-T-G-T-G-T} \\ \text{Deletion} & \quad \text{C-A-C-A-C-A} \quad \text{G-T-G-T-G-T-G-T} \\ \text{Insertion} & \quad \text{C-A-C-A-C-A-C-A} \quad \text{G-T-G-T-G-T-G-T-G-T} \\
\end{align*}
\]

M Clark MD 2006

Why microsatellites become unstable

- **Lynch Syndrome**
  - Germline mutations of MSH2, MLH1, MSH6, PMS2
  - Defective DNA-mismatch repair
  - Increased rates of early onset colorectal, endometrial, ovarian, and gastric cancer

- **Spontaneous tumors**
  - Biallelic methylation of the promoter sequence of MLH1 (Epigenic silencing of MLH1)
  - Defective DNA-mismatch repair
Defective DNA mismatch repair: nucleotide alterations in length of microsatellite sequences (slippage mutations)

Microsatellite Instability (MSI): The presence of novel bands in tumor cells compared to matched normal tissue

Steps for MSI testing

1. DNA is extracted from normal and tumor tissue
2. Five loci are amplified using fluorescent multiplex PCR
3. Amplified DNA is analyzed using capillary electrophoresis
4. Results are analyzed
Microsatellite Stable

Microsatellite Unstable (MSI-H)

Microsatellite Instability (MSI) Testing:
Scoring (5 Markers)

- MSS - Microsatellite stable (no instable)
- MSI-L - low Microsatellite instable (one marker)
- MSI-H - High microsatellite instable (two or more markers)
Advantages of MSI testing

- >93% sensitivity for germline MMR gene mutation [Shia 2008].
- Detection of false negative IHC tumor positive (non-functional protein)
- Highly reproducible [Zhang 2008].

Limitations of MSI testing

- Lack of MMR gene specificity
- High complex testing (microdissection and molecular analysis)
- False negativity (small number tumor cells or MSH6 germline mutation low levels of MSI [Shia 2008].

Genetic Epidemiology of Colorectal Cancer

- Sporadic (~75%)
- Familial (~25%)
- HNPCC (~5%)
- MAP (~1%)
- FAP (~1%)
Colon Cancers with Defective MMR

<table>
<thead>
<tr>
<th>Frequency</th>
<th>15-20% sporadic CRC and Majority of tumors in Lynch Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Features</td>
<td>Predominantly right sided</td>
</tr>
<tr>
<td></td>
<td>More in females</td>
</tr>
<tr>
<td></td>
<td>Early stage of disease</td>
</tr>
<tr>
<td></td>
<td>Better prognosis</td>
</tr>
<tr>
<td>Tumor Characteristics</td>
<td>Poorly differentiated</td>
</tr>
<tr>
<td></td>
<td>Signet ring cell type</td>
</tr>
<tr>
<td></td>
<td>Inflammatory infiltrate</td>
</tr>
<tr>
<td></td>
<td>Near diploid DNA content</td>
</tr>
</tbody>
</table>

My GI colleagues do not want to miss any Lynch Syndrome patients!

| Prevalence                         | Lynch syndrome accounts for approximately 1%-3% of colon cancers, and 0.8%-1.4% of endometrial cancers [Kowalski et al 1997, Chadwick et al 2001, Cunningham et al 2007]. |
|                                   | The population prevalence of Lynch syndrome can be estimated at 1/440 [Chen et al 2008]. |
In 2011, 46,470 new cases/ 8,120 death in the United States
Most prevalent gynecologic malignancy (2nd to breast)
It's incidence remained stable at 20/100,000 women per year. Incidence of uterine cancer is 60% higher among whites. Mortality is 30% higher among African American
Perimenopausal and postmenopausal women. Abnormal vaginal bleeding. Office biopsy or curettage to confirm.
Over 80% are endometrioid adenocarcinomas.

- Endometrial cancer (EC) is the most common extraintestinal malignancy in Lynch syndrome (LS) and often is the sentinel malignancy.


- Endometrial cancer is the most common cancer in women with Lynch Syndrome.

Endometrioid Adenocarcinoma
FIGO grade 2 with loss of MLH-1 and PMS2

According to the National Comprehensive Care Network (2011), testing for Lynch Syndrome is advised for individuals who fit any of the following:
- Meets revised Bethesda guidelines or Amsterdam criteria
- Diagnosed with endometrial cancer under age 50
- Known Lynch syndrome in the family
Histologic Subtypes of Endometrial Cancers with MSI-H (MD Anderson Study 2009)

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sporadic and &lt;50 yrs</td>
<td>41/42</td>
<td>97.6%</td>
</tr>
<tr>
<td>B. Sporadic MLH1 methylated</td>
<td>25/26</td>
<td>96.2%</td>
</tr>
<tr>
<td>C. Lynch Syndrome</td>
<td>43/50</td>
<td>86.0%</td>
</tr>
<tr>
<td>SC, CC, MMMT (all MSH2 mutated)</td>
<td>7/50</td>
<td>14.0%</td>
</tr>
</tbody>
</table>

Meyer LA, et al. Cancer Control 2009, 16(1)

Test for Defective MMR gene Function by IHC and MSI

- Negative result: STOP
- Positive result: Genetic counseling and test for germline mutation (MLH1, MSH2, HSH6, PMS2 and EPCAM)

- MSI-H status assessed by polymerase chain reaction is an indicator of poor prognosis in FIGO 1, but not in FIGO 2-4 endometrial endometrioid adenocarcinomas.

Endometrial cancer surveillance is less well established than that for colon cancer. Because many endometrial cancers can be diagnosed at early stages on the basis of symptoms, women should be educated about the signs of endometrial cancers. Currently the National Comprehensive Cancer Network (NCCN) does not recommend any specific screening for endometrial or ovarian cancer [NCCN 2011].

<table>
<thead>
<tr>
<th>Screening</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>Every 5-10 years beginning at age 50-60 years, or at the younger age of cancer diagnosis in the family, whenever colonoscopy is scheduled.</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Every year beginning at age 50-60 years.</td>
</tr>
<tr>
<td>Vaginal examination</td>
<td>Every year beginning at age 50-60 years.</td>
</tr>
<tr>
<td>History and physical examination</td>
<td>Every year beginning at age 50-60 years.</td>
</tr>
<tr>
<td>Colon/rectum resection</td>
<td>Generally not recommended for primary prophylaxis, but if cancer is diagnosed, the preferred procedure is a subtotal colectomy.</td>
</tr>
<tr>
<td>Methylene blue or topical methylene blue</td>
<td>Unused as an option after endoscopy is complete.</td>
</tr>
</tbody>
</table>


Conclusions

Detection of MMR defect is essential for the initial screening of Lynch Syndrome proband in a family at risk.

Both colorectal and endometrial cancer specimens are appropriate for screening of Lynch Syndrome.

Pathologists play an important role in facilitating and screening by IHC and MSI molecular testing.

Joint efforts of surgeons, oncologists, pathologists and genetic counselors are important.
Emerging Molecular Testing of GYN Cancers

- Her2/neu testing of uterine serous carcinoma for Trastuzumab therapy
- Her2/neu testing of vulvar Paget disease for Trastuzumab therapy
- OncoSNP, BRCA gene mutations, MSI testing and Genomic profiling of ovarian cancers

Emerging Molecular Testing of GYN Tumors

Her2/neu testing of uterine serous carcinoma for Trastuzumab therapy
Table 1. HER-2/neu Receptor Gene Status in Endometrial Carcinomas: A Tissue Microarray Study

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>HER-2/neu Test Positive</th>
<th>FISH Test Positive</th>
<th>HER-2/neu/FISH Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPSC</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>78</td>
</tr>
<tr>
<td>Endometroid Carcinoma</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>199</td>
</tr>
<tr>
<td>Normal-appearing Endometrial Samples*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>73</td>
</tr>
</tbody>
</table>

UPSC: uterine papillary serous carcinoma

* Normal-appearing endometrial samples: proliferative endometrium (27 cases); secretory endometrium (19 cases); atypical endometrium (15 cases); and atypical simple or complex hyperplasia (11 cases).


Table 2. Uterine papillary serous carcinomas with HER-2/neu protein overexpression and/or gene amplification (Tissue Microarray Study, N = 11)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Stage</th>
<th>Grade</th>
<th>HER-2/neu</th>
<th>FISH</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>7</td>
<td>71</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>10</td>
<td>66</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>11</td>
<td>66</td>
<td>D3</td>
<td>IIA</td>
<td>2</td>
<td>2.0</td>
<td>Cervical Cancer</td>
</tr>
</tbody>
</table>

D3 = death of disease

Overall 21.6% of uterine papillary serous carcinomas harboring HER-2/neu protein overexpression and/or gene amplification.

USCs of African-American patients had a significantly higher rate (50%) of elevated HER-2/neu gene status.

Mechanisms such as transcription up-regulation may be responsible for the protein over-expression in a subset of USC.

HER-2/neu protein overexpression and/or gene amplification are very rare in Type I endometrioid carcinomas.

Further prospective studies are needed to ascertain whether USCs with an elevated HER-2/neu status are susceptible to target trastuzumab treatment.


Emerging Molecular Testing of GYN Tumors

Her2/neu testing of vulvar Paget Disease for Trastuzumab therapy (>50% overexpressing HER2)


Emerging Molecular Testing of GYN Cancers

Ovarian Cancers

- OncoSNP
- BRCA gene mutations
- MSI testing
- Genomic profiling

High grade serous carcinoma: p53 mutations
Clear cell carcinoma: ARID1A and PIK3CA mutations
Endometrioid carcinoma: CTNNB1, ARID1A and PIK3CA
Mucinous carcinoma: Ki-Ras mutations

Emerging Molecular Testing of GYN Cancers

**Ovarian Cancers**

- BRCA gene mutations - Prophylactic BSO-TAH (>=40yrs)

Mean age of ovarian cancer:

- General population: 59 yrs
- Hereditary ovarian cancers: 52 yrs
- Lynch Syndrome: 45 yrs

- **Ovarian cancer.** The risk for ovarian cancer varies by the MMR genes in which the germline mutation occurs. The risk to heterozygotes for an $MLH1$ mutation has been found to be 4%-6%, while $MSH2$ heterozygotes have an 8%-11% risk. The mean age of diagnosis of Lynch syndrome-associated ovarian cancers is 42.5 years. Approximately 30% of Lynch syndrome-associated ovarian cancers are diagnosed before age 35 years [Watson et al 2008].

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Emerging Molecular Testing of GYN Cancers

**Ovarian Cancers - OncoSNP**

Dr. Weidhaas at Yale identified single-nucleotide polymorphisms (SNPs) in one of the let-7 (miRNA tumor suppressor) target sites in the 3' UTR of the oncogene KRAS. Characterized in 2,433 people representing 46 human populations, this let-7 target SNP (or 'oncoSNP') was identified in up to 20.3% of non-small cell lung carcinoma (NSCLC) patients and 5.8% of the world population. It is also associated with BRCA1/2-negative hereditary breast and ovarian cancer (HBOC), and a strong argument was made for the oncoSNP as a genetic marker of cancer risk.

[Link to MitraDx logo]
Emerging Molecular Testing of GYN Cancers

Ovarian Cancers - Genomic Profiling

RESULT: pathways of importance (figures 3a/b/d)

HOTNET:
most frequently Δ pathways

PARADIGM:
FOM1 87% [transcriptional level only]
RESULTS: transcriotional subtype determination (Figure 2a)

4 HGS-OvCa subtypes were identified

<table>
<thead>
<tr>
<th>IMMUNOREACTIVE</th>
<th>PROLIFERATIVE</th>
<th>DIFFERENTIATED</th>
<th>MESENCHYMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑CXCL11/10</td>
<td>↑HMGA2</td>
<td>↑NUCLE1/1</td>
<td>↑HOX</td>
</tr>
<tr>
<td>GANC</td>
<td>SORL1</td>
<td>FAPI</td>
<td>ANGPTL1/2</td>
</tr>
<tr>
<td>PCNA</td>
<td>RDH10</td>
<td>SLPI</td>
<td></td>
</tr>
<tr>
<td>MUC16/1</td>
<td>HOX1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These subtypes were reproducible through a public dataset. Survival did NOT differ across these groups.

NATURE 474:609-615, 2011

RESULTS: pathways of importance

A defect in genes important for homologous recombination occurred in nearly 50% of tumors

<table>
<thead>
<tr>
<th>Gene</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1/2</td>
<td>20%</td>
</tr>
<tr>
<td>EMSY</td>
<td>8%</td>
</tr>
<tr>
<td>PTEN</td>
<td>7%</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>5%</td>
</tr>
<tr>
<td>RAD51</td>
<td>3%</td>
</tr>
<tr>
<td>ATM/R</td>
<td>2%</td>
</tr>
</tbody>
</table>

PARP inhibitors should prove efficacious in HGS-OvCa.

NATURE 474:609-615, 2011

SUMMARY

• Fist large-scale integrated genomic analysis of epithelial ovarian cancer
• HGS-OvCa is mutationally distinct from clear cell, endometrioid, and mucinous ovarian cancers
• Homologous recombination pathways are altered by mutation, hypermethylation or copy number in 50% of cases
• Towards rational drug design
  - development of novel agents to target RB, Ras/PI3K, FoxM1, Notch pathways
  - PARPi & agents against the 22 genes amplified in HGS-OvCa
RESULTS: copy number analysis – pharmacologic agents

Pharmacologic agents are already in development for 22 of the somatic copy number alterations (SCNA).

Genotyping by Short Tandem Repeat (STR) Polymorphism Analysis
Principles
Tissue Identity Testing
Diagnosis of Molar Pregnancy
Diagnosis of Trophoblastic Tumors

GENOTYPING
Measurement of the genetic variation between members of a species
Short Tandem Repeat (STR) Polymorphism

- Repetitive DNA sequences from 2 to 15 base pairs
- Typically non-coding intron regions
- Highly pleomorphic and genetically stable
- Forensic analysis - tetra or pentanucleotide repeats
- Combined DNA Index System (CODIS) - FBI Lab 1980s

![Diagram of STR Polymorphism with Paternal and Maternal Alleles]
AmpFLSTR Identifiler™ (ABI)

- Single multiplex PCR assay at 15 microsatellite loci allowing maximal allelic polymorphism
- Short amplicons: 100-360 bp

Capillary Electrophoresis
Species Specificity - Primates only

Human, Gorilla, Chimpanzee, Orangutan, Macaque

0.5 to 1.25 ng of DNA ≈ 80 to 180 human diploid cells
Principle
Tissue Identity Testing
Diagnosis of Molar Pregnancy
Diagnosis of Solid Tumors

Case 1: Does this cancerous piece belong to the patient?

YNHH: 43 year-old female had a right breast core biopsy (shown here). Left breast had a poorly differentiated carcinoma diagnosed 5 years earlier.

A) Mixing up two specimens in pathology or physician’s office
B) Floaters (grossing, embedding, sectioning and staining)
C) Vanishing cancer syndrome!
D) The physician wants the pathologist to be happy!
CASE 1: Cancerous Floater

43 year-old female had a right breast core biopsy (shown here). Left breast had a poorly differentiated carcinoma diagnosed 5 years earlier.

Vanishing Cancer Syndrome
Principle
Tissue Identity Testing
Diagnosis of Molar Pregnancy
Diagnosis of Solid Tumors

Very early complete mole (VECM)
Buza N, Hui P. Gestational Trophoblastic Diseases: Histological Diagnosis in the Molecular Era (invited review). Diag Histopath. 2010, Sep 17. [Epub ahead of print]

Early complete mole

- Normal pregnancy
- Partial mole
- Hydropic villi
Differential Diagnosis of Partial Mole

- Hydropic villi/missed abortions
- Common chromosomal abnormalities (trisomy 16,18,21)
- Early complete mole
- Beckwith-Wiedemann syndrome (mesenchymal dysplasia)
- Twin (complete mole with coexisting normal fetus)

15 to 20% complete mole patients require chemotherapy and 5% develop choriocarcinoma. **0.5 to 2% partial mole patients require chemotherapy and 0.1 to 0.5% develop choriocarcinoma.**

Surveillance Program (both complete and partial moles)

- Contraception with biweekly hCG for 56 days until normal
- If normal: 4 weekly hCG for 6 months
- If abnormal: continue biweekly hCG until normal and then 4 weekly hCG for 6 months
- In all cases, 4 weeks urine hCG or 10 weeks serum hCG following any future pregnancy
- All patients requiring chemotherapy is monitored by lifetime hCG follow-up


PARTIAL HYDATIDIFORM MOLES: A Mess!

Underdiagnosis: One in every 100 pregnancies is a partial mole (Genest 2001) and most are “missed abortions”

Overdiagnosis: Very common (1/3 of diagnosed partial moles are not by histology and ploidy analysis). Over-treatment with significant medical and psychological impact.
Not all triploid gestations are partial moles!

- 2/3 are true partial moles with paternal disomy (genetic partial mole)
- 1/3 are digyne, monoandric, non-molar gestations

(Redline 1998, Zaragoza 2000, Genest 2001)

P57 Immunohistochemistry

Normal Villi  Complete Mole  Partial Mole
Hippocrates: impure water leading to dropsy of uterus

Aetius of Amida: molar hydatidosa

Historical timeline: Gestational Trophoblastic Disease

Li & Hertz: methotrexate therapy of choriocarcinoma

McGrath & Surani: different roles of parental genomes in fetal and placental development

Marchand: HM and choriocarcinoma derived from villous trophoblasts

Countess Margaret gave birth to “365 Children”

Kajii & Ohama: androgenetic nature of complete mole

1977

1956

1984

1995

Gold Standard

Gold Standard

Hui P. Expert Review of Molecular Diagnosis 10(8):1023, 2010

Normal Gestation

Endometrium

Chorionic Villi

Allelic Ladder

Hui P. Gestational Trophoblastic Disease: Diagnostic and Molecular Genetic Pathology, Springer, 2011
Partial Hydatidiform Mole

Hui P. Expert Review of Molecular Diagnosis 10(8):1023, 2010

Dispermic Partial Mole (90%)

Monospermic Partial Mole (10%)
33 year-old with tubal pregnancy. DNA index (ploidy analysis) is 1.40 (near triploid). Histological diagnosis is in favor of PHM.

Trisomy 18 (D18S51) confirmed by DNA genotyping.
Rule out molar gestation!

Digynic—monoandric Triploid Non-molar Gestation
High Risk of Persistent GTN in Heterozygous Complete Mole


Principle
Tissue Identity Testing
Diagnosis of Molar Pregnancy
Diagnosis of Solid Tumors

Non-gestational Choriocarcinoma

22 year-old female with a hemorrhagic mass involving fallopian tube and broad ligament.

Epithelioid trophoblastic tumor v.s Squamous cell carcinoma

51 year-old female with 21.5 cm right ovarian mass with no evidence of metastatic v.s primary ovarian carcinoid tumor.