Screening for Lynch Syndrome in Endometrial Carcinoma: Practical Considerations
Financial Disclosures

- NONE!
Lynch Syndrome: Definition

- Autosomal dominant genetic disorder that confers an increased risk of cancer
  - 2-3% of endometrial carcinomas are Lynch syndrome associated
  - In women, endometrial cancer and colorectal cancer are equally likely to be the sentinel cancer
Lynch Syndrome: Genetics

- Germline mutation that leads to loss of function of a mismatch repair (MMR) protein
Lynch Syndrome: Mismatch Repair

- The MMR system recognizes and repairs base pair mismatches. The consequence of impaired MMR is an increased mutation rate with biallelic loss.
  - Recognition
    - MutS-alpha heterodimer: **MSH2** and **MSH6**
    - MutS-beta heterodimer: MSH2 and MSH3
  - Repair
    - MutL-alpha heterodimer: **MLH1** and **PMS2**
    - MutL-beta heterodimer: MLH1 and PMS1
    - MutL-gamma heterodimer: MLH1 and MSH3
  - EpCAM
    - Upstream from MSH2, large deletions in 3’ end cause silencing of MSH2
Lynch Syndrome: Molecular Phenotype

- Microsatellite instability
  - MSI testing
- Loss of protein expression
  - Immunohistochemistry
Lynch Syndrome: Molecular Phenotype

- Microsatellite instability
  - Lynch syndrome carcinomas show high frequency microsatellite instability (MSI-H)
  - Microsatellites are regions of repetitive nucleotide sequences
  - These regions are particularly prone to DNA mismatches
  - Mismatch leads to lengthening or shortening of the microsatellite
Lynch Syndrome: Molecular Phenotype

- **Microsatellite instability**
  
  - Testing employs PCR to amplify regions containing microsatellites in tumor *and* normal tissue. Amplification products are separated by capillary electrophoresis
  
  - MSI-High is defined by instability in ≥30% of examined microsatellites (or 2 loci in a 5 marker panel)
  
  - MSI-Low is defined by instability of <30% of examined microsatellites
  
  - MSS (Microsatellite stable) is defined by no instability
Not All MSI-H Tumors are Lynch Associated!!!

- Sporadic MSI-H colorectal and endometrial carcinomas
  - Silencing of MLH1 due to promoter methylation
  - Sporadic MSI-H colorectal carcinomas
    - BRAF V600E present in sporadic but *not* Lynch associated carcinomas
  - Sporadic MSI-H endometrial carcinomas
    - Lack the BRAF V600E mutation so must assess for promoter methylation
MSI Caveats

- MSS and MSI-L can be seen in cases with germline mutation of MSH6
- Sensitivity is likely affected by the composition of microsatellite markers used
- Mononucleotide repeats are more sensitive
Lynch Syndrome: Molecular Phenotype

- Loss of protein expression
  - Detected by immunohistochemistry for MLH1, PMS2, MSH2, MSH6 performed on tumor
    - Four patterns
      - No staining for MLH1 and PMS2 due to loss of MLH1
      - No staining for PMS2 due to loss of PMS2
      - No staining for MSH2 and MSH6 due to loss of MSH2 (or EPCAM)
      - No staining for MSH6 due to loss of MSH6
Lynch Syndrome: Molecular Phenotype

Colonic adenocarcinoma: IHC pattern consistent with loss of MLH1

MMR IHC Caveats

- Lack of clearly defined and agreed upon criteria for interpretation

- United Kingdom National External Quality Assessment Service: 2008 Recommendations
  - *Normal*: similar staining intensity in tumor and internal control
  - *Abnormal*: no staining in tumor with positive staining in internal control, or patchy or weak staining in tumor compared to internal control
MRR IHC Caveats

<table>
<thead>
<tr>
<th>Gene</th>
<th>Overbeek et al. 2008 % of cases with consensus interpretation (5/7 pathologists)</th>
<th>Klarskov et al. 2010 % of cases with consensus interpretation (5/6 pathologists)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>89%</td>
<td>83%</td>
</tr>
<tr>
<td>PMS2</td>
<td>96%</td>
<td>91%</td>
</tr>
<tr>
<td>MSH2</td>
<td>93%</td>
<td>94%</td>
</tr>
<tr>
<td>MSH6</td>
<td>87%</td>
<td>76%</td>
</tr>
</tbody>
</table>

- Klarskov et al. identified weak staining as primary cause of lack of consensus
- Stratified weak staining into
  - Technically weak – weak staining in tumor and internal control
  - Biologically weak – weaker staining in tumor compared to control
    - 19 tumors showed a biologically weak pattern, 8 carried a disease predisposing mutation

Klarskov et al. Human Pathology 2010;41:1387-1396.
MLH1 stain in tumor with MLH1 mutation

MLH1 stain in tumor without MLH1 mutation

MLH1 stain in tumor with MLH1 mutation

PMS2 stain in tumor with MLH1 mutation

Klarskov et al. Human Pathology 2010;41:1387-1396.
Findings from the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) Working Group

• Clinical Validity
  – MSI
    • MLH1 or MLH2
      – Sensitivity 80-91%, Specificity 90%
    • MSH6 or PMS2
      – Sensitivity 55-77%, Specificity 90%
  – IHC
    • Sensitivity 83%, Specificity 89%
    • Regardless of MMR protein
  – BRAF V600E
    • Absent in virtually 100% of Lynch syndrome individuals
    • Present in 68% of individuals without Lynch syndrome

Identification of Lynch-Related Carcinomas

- Targeted vs. universal screening?
- Which screening strategies to implement?
Lynch Syndrome: Clinical Phenotype

- Positive family history, prior cancer history, younger age at diagnosis
  - Amsterdam Criteria (1999)

- Tumor site
  - Endometrial carcinoma - lower uterine segment (LUS)
    - In a series of 1,009 cases, 10 (29%) of the 35 (3.5%) LUS endometrial carcinomas were Lynch Syndrome associated*

Lynch Syndrome: Histologic Phenotype

  - 102 endometrial carcinoma cases; 52 MSI-H & 50 non MSI-H
    - Host inflammatory response
    - Cytologic grade
    - Architecture
    - Invasion pattern
    - Metaplasia
    - Necrosis
    - LVI
    - Background endometrium
  - Tumor infiltrating lymphocytes (OR 3.1) and peritumoral lymphocytes (OR 2.8) only features that were significant predictors of MSI status
Lynch Syndrome: Histologic Phenotype

Endometrioid FIGO 1 with peritumoral lymphocytes

Lynch Syndrome: Histologic Phenotype

Endometrioid FIGO 1 with tumor infiltrating lymphocytes

Lynch Syndrome: Histologic Phenotype

# Lynch Syndrome: Histologic Phenotype

## Histologic type in Lynch Syndrome associated tumors

<table>
<thead>
<tr>
<th></th>
<th>Broadus et al., 2006, N=50</th>
<th>Carcangiu et al., 2010, N=23</th>
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<tbody>
<tr>
<td></td>
<td>Endometrioid</td>
<td>Non-endometrioid</td>
</tr>
<tr>
<td>MLH1</td>
<td>3 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>MSH2</td>
<td>40 (80%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>MLH1 &amp; MSH2</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

Recommendations for Lynch Syndrome Screening in Endometrial Carcinoma

- **NCCN**
  - Patient < 50 or meets Amsterdam or Bethesda Criteria

- **Society of Gynecologist Oncologists**
  - “In summary, all women who are diagnosed with endometrial cancer should undergo systematic clinical screening for Lynch syndrome (review of personal and family history) and/or molecular screening. Molecular screening of endometrial cancers for Lynch syndrome is the preferred strategy when resources are available.”
Lynch syndrome Screening Strategies in Endometrial Carcinoma

- Buchanan DD et al. Journal of Clinical Oncology 2014;32:90-100
  - Comparison of the frequencies of MLH1, PMS2, MSH2, and MSH6 mutations in LS associated endometrial carcinoma
  - Comparison of the performance characteristics of various screening strategies
  - Validation of MLH1 promoter methylation as a negative predictive marker for Lynch syndrome
**Lynch syndrome Screening Strategies in Endometrial Carcinoma**

Performance characteristics of selected clinical criteria and tumor tests

<table>
<thead>
<tr>
<th>Criteria</th>
<th>% Fulfilling Criteria</th>
<th>% of Mutation Carriers Fulfilling Criteria</th>
<th>Positive Predictive Value</th>
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<tr>
<td></td>
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<tr>
<td>Amsterdam II</td>
<td>3.6%</td>
<td>14.3%</td>
<td>12.0</td>
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<tr>
<td>Bethesda</td>
<td>36.3%</td>
<td>76.2%</td>
<td>6.3</td>
</tr>
<tr>
<td>Age &lt; 60</td>
<td>40.8%</td>
<td>85.7%</td>
<td>6.3</td>
</tr>
<tr>
<td>MMR IHC loss &lt;60</td>
<td>10.2%</td>
<td>85.7%</td>
<td>25.4</td>
</tr>
<tr>
<td>MMR IHC loss &lt;60, MLH1 unmethylated</td>
<td>5.6%</td>
<td>85.7%</td>
<td>46.2</td>
</tr>
</tbody>
</table>

**N=698** **N=21**

**Negative predictive values ranged from 97.3-100.0%**

Buchanan DD et al. Journal of Clinical Oncology 2014;32:90-100
Proposed Algorithm for Endometrial Carcinoma

Buchanan DD et al. Journal of Clinical Oncology 2014;32:90-100
Towards a Rational, Comprehensive Approach to Genetic Screening For Lynch Syndrome and Beyond

- A well-coordinated interdisciplinary approach is essential to success
  - To address questions of informed consent
  - To ensure implementation of reporting and referral protocols that result in appropriate patient follow up
- Prospective research
  - To engage in an on-going assessment of the clinical utility and cost effectiveness of adopted testing
Lynch Syndrome Screening in Endometrial Carcinoma

QUESTIONS???
Interobserver variability in MMR immunohistochemistry interpretation in Lynch associated carcinomas.

Klarskov et al. Human Pathology 2010;41:1387-1396.