Lynch Syndrome screening: What do we need to know in 2019?

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58 yo male with ascending colon mass
MLH1

PMS2

Isolated loss of MSH6

MSH2

MSH6
Question 1:

Which of the following is true of the MMR IHC results?

A. This pattern is diagnostic of Lynch Syndrome
B. This pattern is diagnostic of sporadic MMRD carcinoma
C. Defects in MSH6 can be either somatic or germline
D. This tumor likely arose from a sessile serrated polyp
Question 1:

Which of the following is true of the MMR IHC results?

A. This pattern is diagnostic of Lynch Syndrome
B. This pattern is diagnostic of sporadic MMRD carcinoma
C. Defects in *MSH6* can be either somatic or germline
D. This tumor likely arose from a sessile serrated polyp
RESULTS
- IHC: Isolated loss of MSH6, preserved expression of MSH2, MLH1 and PMS2

METHOD
Immunohistochemical staining (IHC) is used to determine the presence or absence of protein expression for MLH1, MSH2, MSH6, and PMS2. Lymphocytes and normal epithelium exhibit strong nuclear staining and serve as positive internal controls for staining of these proteins.

INTERPRETATION
These results indicate loss of normal Deoxyribonucleic Acid (DNA) mismatch repair function within the tumor. Isolated loss of MSH6 expression is frequently associated with the presence of a germline (heritable) mutation in MSH6. Thus, this individual, and other family members, are at increased risk for having an inherited colon cancer syndrome due to defective DNA mismatch repair (Lynch syndrome).

It is important to note that these results do not distinguish between somatic and germline mutations. Germline testing of MSH6 on an additional blood sample may help distinguish between these two possibilities and provide the opportunity for predictive testing for at-risk family members.

Additional information regarding this testing may be obtained by ordering a consultation through the inherited cancer clinic. (480-342-6263)

CAUTIONS
Test results should be interpreted in context of clinical findings, family history, and other laboratory data. If results obtained do not match other clinical or laboratory findings, please contact the laboratory for possible interpretation. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
Germline testing for MSH6 was negative. What does this mean?
Outline:

• Pathways to colon cancer
• Definition of Lynch Syndrome and goals of screening
• Principles of MMR IHC as a screening tool
• Issues with MMR IHC interpretation
• “Lynch-like” syndrome
Subtypes of colorectal carcinoma

Comprehensive molecular characterization of human colon and rectal cancer

The Cancer Genome Atlas Network
Nature 2012

Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions

Felipe De Sousa E Melo, Xin Wang, Mamiz Jansen, Evelyn Fessler, Anne Trinh, Laura P.M.H. de Rooij, Joan H de Jong, Onno J. de Boer, Ronald van Leersum, Maarten F. Bijlsma, Hans Rodermond, Maartje van der Heijden, Carel J M van Noesel, Jurriaan B. Tuynman, Eveline Dekker, Florian Markowitz, Jan Paul Medema & Louis Vermeulen
Nat Med 2013

A colorectal cancer classification system that associates cellular phenotype and responses to therapy

Nat Med 2013

Proteogenomic characterization of human colon and rectal cancer

Nature 2014
Subtypes of colorectal carcinoma

- These classification schemes are not very practical
- The Jass classification scheme is more useful to pathologists
Pathologists’ view of Colon Cancer: Modified Jass classification

Conventional Pathway
- FAP
- Germline APC
- Sporadic
- APC or beta-catenin
- RAS
- SMAD 4
- P53
- CIN
- CIMP -
- CIN +
- Overall Proportion: 1-2%

Serrated Pathway
- Traditional Serrated
  - KRAS/BRAF mutated
  - CIMP +/−
  - CIMP +
  - MSS
  - 5-10%?

- Sessile Serrated
  - BRAF mutated
  - CIMP +
  - MSI-H
  - 8-12%

Lynch
- Germline DNA mismatch repair gene alteration
- Overall Proportion: 3-5%

CpG Island Methylation (CIMP)
Chromosomal vs Microsatellite Instability (CIN / MSI)

Overall Proportion
- Conventional: 60-70%
- Serrated: 8-12%
- Lynch: 3-5%
Why does molecular classification matter?

• Prognostic implications

• Predictive of response to certain treatments

• Provides a framework for screening for Lynch syndrome
Figure 1. Kaplan-Meier survival curves for disease-specific survival.

<table>
<thead>
<tr>
<th>Type</th>
<th>Number at risk</th>
<th>Years since diagnosis</th>
<th>Number at risk</th>
<th>Years since diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>100</td>
<td>78  71  66  56  43  14</td>
<td>Type 2</td>
<td>55  38  24  23  21  20  4</td>
</tr>
<tr>
<td>Type 2</td>
<td>55  38  24  23  21  20  4</td>
<td>Type 3</td>
<td>353  268  223  191  160  130  37</td>
<td>Type 4</td>
</tr>
<tr>
<td>Type 4</td>
<td>631  541  473  433  370  300 113</td>
<td>Type 5</td>
<td>50  43  39  37  32  26  10</td>
<td>Type 5</td>
</tr>
<tr>
<td>Other</td>
<td>155  120  103  96  84  65  22</td>
<td>Other</td>
<td>155  120  103  96  84  65  22</td>
<td></td>
</tr>
</tbody>
</table>
Mucinous growth
Poor differentiation
Medullary growth/TILs
Crohn’s-like reaction
Lynch Syndrome Screening: Goals

- Identify MSI-H tumors
- Separate out sporadic MSI-H tumors
- Identify those patients that need germline testing
- Identify deleterious mutations in MMR genes
- Identify affected family members
- Enroll affected individuals in lifelong screening program
Lynch Syndrome Screening: Goals

- Identify MSI-H tumors
- Separate out sporadic MSI-H tumors
- Identify those patients that need germline testing
- Identify deleterious mutations in MMR genes
- Identify affected family members
- Enroll affected individuals in lifelong screening program
Lynch Syndrome Definition

• Germline mutations in DNA mismatch repair (MMR) genes:
  • *MLH1* (50%)
  • *MSH2* (40%)
  • *MSH6* (7%-10%)
  • *PMS2* (<5%)

• Deletions in *EPCAM/TACSTD1* (1-3%)
  • Result epigenetic silencing of the *MSH2* gene by hypermethylation
## Lynch Syndrome: Cancer Risk

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>General Population Risk</th>
<th>Lynch Syndrome Risk</th>
<th>Mean Age of Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>4.8%</td>
<td>52%-82%</td>
<td>44-61 years</td>
</tr>
<tr>
<td>Endometrium</td>
<td>2.7%</td>
<td>25%-60%</td>
<td>48-62 years</td>
</tr>
<tr>
<td>Stomach</td>
<td>&lt;1%</td>
<td>6%-13%</td>
<td>56 years</td>
</tr>
<tr>
<td>Ovary</td>
<td>1.4%</td>
<td>4%-12%</td>
<td>42.5 years</td>
</tr>
<tr>
<td>Hepatobiliary tract</td>
<td>&lt;1%</td>
<td>1.4%-4%</td>
<td>Not reported</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>&lt;1%</td>
<td>1%-4%</td>
<td>~55 years</td>
</tr>
<tr>
<td>Small bowel</td>
<td>&lt;1%</td>
<td>3%-6%</td>
<td>49 years</td>
</tr>
<tr>
<td>Brain/central nervous system</td>
<td>&lt;1%</td>
<td>1%-3%</td>
<td>~50 years</td>
</tr>
<tr>
<td>Sebaceous neoplasms</td>
<td>&lt;1%</td>
<td>1%-9%</td>
<td>Not reported</td>
</tr>
</tbody>
</table>
Who to screen for Lynch Syndrome

• **Universal screening of all patients with CRC**
  – Endorsed by the following organizations:
    • National Comprehensive Cancer Network (NCCN), EGAPP (working group sponsored by the CDC), American Society of Medical Oncology (ASCO), US Multi-Society Task Force on Colorectal Cancer, American College of Gastroenterology (AGA)

• **Selective Screening of all patients <70 years of age & in patients >70 years fulfilling revised Bethesda guidelines** (misses up to 5% of patients with Lynch syndrome)
  – Endorsed as an option by the following organizations:
    • National Comprehensive Cancer Network (NCCN) and the American Society of Medical Oncology (ASCO)
Molecular Biomarkers for the Evaluation of Colorectal Cancer

Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology

Antonia R. Sepulveda, MD, PhD,1 Stanley R. Hamilton, MD, PhD,2 Carmen J. Allegra, MD,2 Wayne Grody, MD, PhD,4 Allison M. Cushman-Vokoun, MD, PhD,7 William K. Funkhouser, MD, PhD,8 Scott E. Kopetz, MD, PhD,8 Christopher Lieu, MD,9 Noralaine M. Lindor, MD,10 Bruce D. Minsky, MD,2 Federico A. Monzon, MD,11 Daniel J. Sargent, PhD,12 Veena M. Singhi, MD,13 Joseph Willis, MD,14 Jennifer Clark, SCT, MB(ASCP),15 Carol Colasacco, MLS,16 R. Bryan Rumble, MSc,17 Robyn Temple-Smolkin, PhD,18 Christina B. Ventura, MT(ASCP),18 and Jan A. Nowak, MD, PhD14

Recommendation

• Mismatch repair status testing in patients with colorectal cancers should be performed for the identification of patients at high-risk for Lynch syndrome and/or prognostic stratification.

• Testing can be performed by immunohistochemistry or by MSI DNA-based testing.
Flip the paradigm: Tumor sequencing

Validation of a targeted next-generation sequencing approach to detect mismatch repair deficiency in colorectal adenocarcinoma


David J. Papke Jr.¹ · Jonathan A. Nowak² · Matthew B. Yurgelun² · Alexander Frieden¹ · Amitabh Srivastava¹ · Neal I. Lindeman¹ · Lynette M. Sholl¹ · Laura E. MacConaill³ · Fei Dong¹

- 275 gene panel (training set of 243 CRC)
- 298 gene panel (validation set of 436 tumors)
- 13 indels per Mbp in MMRD vs. 0.45 indels/Mbp per tumor MMRP

- Training set: ≥ 3 indels/Mbp identified 22 of 23 MMRD and 218 of 218 MMRP tumors (96% sensitivity and 100% specificity)

- Validation set: ≥ 3 indels/Mbp identified 44 of 46 MMRD and 388 of 290 MMRP tumors (96% sensitivity and 99% specificity)
Screening for Lynch Syndrome

• Who to screen has been answered: Universal Screening is the best (for both CRC and Endometrial carcinoma)

• Many issues remain
  • Correct interpretation of MMR IHC
    • Unusual MMR IHC staining patterns
    • Pitfalls in interpretation
  • How do you set up a successful program?
  • Should we screen other GI tract carcinomas? Polyps?
  • MMR IHC and other tests suggest LS but germline testing is negative, now what? Does pathology play any role in this scenario?
MMR Immunohistochemistry

- Defective MMR genes results in **loss of immunohistochemical expression**
- All 4 antibody testing (MLH1, PMS2, MSH2 and MSH6)
  - If >10% of tumor nuclei demonstrate expression, then protein expression is preserved.
  - If <10% of tumor nuclei demonstrate expression, then protein expression is equivocal. Repeat stain, or reflex to MSI PCR.
- Must see **complete lack of staining** to call loss of expression.
• Dimers of obligate and secondary partner.

• Loss of obligate partner results in proteolytic degradation of the respective secondary partner.

• Loss of secondary partner still results in intact expression of obligate partner as it can bind to other partner proteins preventing degradation.
MMR Proteins: Basic Biology

- Loss of MSH2 and MSH6 IHC expression
- MSH2 mutation
- MSH6 mutation
- Obligate: MSH2
- Secondary: MSH6
- Intact MSH2 with loss of MSH6
# MMR IHC as a screening tool

<table>
<thead>
<tr>
<th>IHC result</th>
<th>Most likely defective gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of MLH1 and PMS2</td>
<td></td>
</tr>
<tr>
<td>Loss of MSH2 and MSH6</td>
<td></td>
</tr>
<tr>
<td>Isolated loss of MSH6</td>
<td></td>
</tr>
<tr>
<td>Isolated loss of PMS2</td>
<td></td>
</tr>
</tbody>
</table>

- **Loss of MLH1 and PMS2**: Seen in sporadic MSI-H and Lynch syndrome.
- **Loss of MSH2 and MSH6**: Concerning for Lynch syndrome but not diagnostic.
- **Isolated loss of MSH6**
- **Isolated loss of PMS2**
Need additional testing
Pathways to MMR Deficiency

Sporadic MSI-H

- Sessile serrated polyp

- **BRAF V600E**
- CpG island methylation
  (*MLH1* promoter)

Lynch Syndrome

- Adenoma

- Lynch syndrome

**Deficient DNA Mismatch Repair (MSI-H/MMRD)**
Patient with Colorectal Carcinoma

MMR IHC

- Loss of MSH2/MSH6, Isolated loss of MSH6, or Isolated loss of PMS2

- Preserved Expression of all 4 MMR proteins

- Loss of MLH1/PMS2 expression

- BRAF Mutation Testing

- BRAF V600E Mutation Positive

- Wild-type BRAF Mutation

- MLH1 Promoter Hypermethylation Analysis of Tumor

- Referral to Genetic Counseling

- No further testing required

- Positive for MLH1 Promoter Hypermethylation in Tumor

- Referral to Genetic Counseling

- Negative for MLH1 Promoter Hypermethylation in Tumor

- Germline MMR Gene and/or EPCAM Mutation Testing

- Preserved Expression of all 4 MMR proteins

# MMR IHC as a screening tool

<table>
<thead>
<tr>
<th>IHC result</th>
<th>Most likely defective gene</th>
</tr>
</thead>
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<tr>
<td>Loss of MLH1 and PMS2</td>
<td>$MLH1$</td>
</tr>
<tr>
<td>Loss of MSH2 and MSH6</td>
<td>$MSH2$ or $EPCAM$</td>
</tr>
<tr>
<td>Isolated loss of MSH6</td>
<td>$MSH6$</td>
</tr>
<tr>
<td>Isolated loss of PMS2</td>
<td>$PMS2$ or $MLH1$</td>
</tr>
</tbody>
</table>

*Rarely, other patterns can be seen*
Unusual MMR IHC Patterns

- **Punctate/speckled nuclear MLH1**
  - Typically seen with concurrent PMS2 loss and \textit{BRAF V600E} mutation/\textit{MLH1} promoter hypermethylation.
  - Likely a technical issue with staining protocol.

Don’t interpret as isolated loss of PMS2
Unusual MMR IHC Patterns

- **Nucleolar MSH6 or Membranous MLH1**
  - Should not be taken as evidence of intact expression. MSI PCR should be performed.
  - Likely a technical issue with staining protocol.

Nucleolar MSH6  
Membranous MLH1
Unusual MMR IHC Patterns

Clonal/Subclonal Loss of MLH1 and PMS2

- Large areas of tumor show abrupt loss of expression
Unusual MMR IHC Patterns

- **Clonal/Subclonal Loss of MLH1 and PMS2**
  - Large areas of tumor show abrupt loss of expression
  - Result of differential *MLH1* hypermethylation within these different areas

Unusual MMR IHC Patterns

- Decreased MMR expression after neoadjuvant therapy

Decreased MSH6 in 20% of treated tumors

Decreased expression of all MMR proteins after treatment

Decreased expression correlated with proliferation
Unusual MMR IHC Patterns

Same tumor, different areas
Unusual MMR IHC Patterns

- Concurrent Loss of MLH1, PMS2, and decreased MSH6
  - Decreased MSH6 (<5% expression) is most often due to secondary *somatic* mutation of a coding microsatellite within the *MSH6* gene.

Figure 1  Immunohistochemical staining showing scanty MSH6 staining in a colonic adenocarcinoma; a and b represent two areas from one tumor where there is distinct nuclear staining for MSH6, but the staining is present only in a limited number of tumor cells. Note the presence of tumor infiltrating lymphocytes that stain positively for MSH6. This tumor has intact expression of MLH2 and complete loss of MLH1 and PMS2 (staining not shown).

Unusual MMR IHC Patterns

- “Isolated loss of MSH2” with patchy but convincing staining for MSH6

Most patients will have *MSH2* mutations similar to those with complete loss of MSH2 and MSH6

**MMR IHC as a screening tool**

### Most common patterns

<table>
<thead>
<tr>
<th>IHC result</th>
<th>Most likely defective gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of MLH1 and PMS2</td>
<td><em>MLH1</em></td>
</tr>
<tr>
<td>Loss of MSH2 and MSH6</td>
<td><em>MSH2</em> or <em>EPCAM</em></td>
</tr>
<tr>
<td>Isolated loss of MSH6</td>
<td><em>MSH6</em></td>
</tr>
<tr>
<td>Isolated loss of PMS2</td>
<td><em>PMS2</em> or <em>MLH1</em></td>
</tr>
</tbody>
</table>

- Punctate MLH1, PMS2 loss
- Nucleolar MSH6
- Membranous MLH1
- Clonal loss of MLH1/PMS2
- Punctate MLH1, PMS2 loss
- Reduced MSH6
- Concurrent loss of MLH1, PMS2 and focal MSH6
40 yo with ascending colon tumor: MMR IHC

- MLH1
- MSH2
- PMS2
- MSH6
40 yo with ascending colon tumor: MMR IHC

What are your next steps?

- Repeated PMS2 x3:
  - Same result
  - Performed MSI testing by PCR
    - Only 3 of 5 loci were evaluable
    - 2 of 3 were unstable (MSI-H)
40 yo with ascending colon tumor: MMR IHC

- LS-associated cancers
- Polyposis
- Café-au-lait macules
- Loss of affected MMR protein expression in tumor and normal
- PMS2 and MSH6 are the most affected

CONSENSUS GUIDELINES

Recommendations on Surveillance and Management of Biallelic Mismatch Repair Deficiency (BMMRD) Syndrome: A Consensus Statement by the US Multi-Soci Colorectal Cancer

Carol Durno,1 C. Richard Boland,2 Shlomi Cohen,3 Jason A. Dominitz,4,8 Frank M. Giardiello,6 David A. Johnson,7 Tonya Kaltenbach,8 T. R. Levin,8 David Lieberman,10 Douglas J. Robertson,11,12 and Douglas K. Rex13

Table 1. Estimated Penetration and Age of Onset Neoplasms in BMMRD

<table>
<thead>
<tr>
<th>Organ</th>
<th>Estimated penetration, %</th>
<th>Age at diagnosis, median (range), y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-bowel adenomas</td>
<td>50</td>
<td>12 (10–20)</td>
</tr>
<tr>
<td>Colorectal adenomas</td>
<td>&gt;90</td>
<td>9 (6–15)</td>
</tr>
<tr>
<td>Small-bowel cancer</td>
<td>10</td>
<td>28 (11–42)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>70</td>
<td>16 (8–48)</td>
</tr>
<tr>
<td>Low-grade brain tumors</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>High-grade brain tumors</td>
<td>70</td>
<td>9 (2–40)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>20–40</td>
<td>5 (0.4–30)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>10–40</td>
<td>8 (2–21)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>&lt;10</td>
<td>(19–44)</td>
</tr>
<tr>
<td>Urinary tract cancer</td>
<td>&lt;10</td>
<td>(10–22)</td>
</tr>
</tbody>
</table>

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Screening for Lynch Syndrome: Goals

- Identify MSI-H tumors
- Separate out sporadic MSI-H tumors
- Identify those patients that need germline testing
- Identify deleterious mutations in MMR genes
- Identify affected family members
- Enroll affected individuals in lifelong screening program
Setting up a screening program

• Approach 1: Provide results to the surgeon who would decide who to refer to genetic counseling.
• Approach 2: Provide results to surgeon and GC. GC would contact surgeon and not patient.
• Approach 3: Provide results to surgeon and GC. GC would contact patient directly
Setting up a screening program

Approach 3 is superior
Screening for Lynch syndrome

• Who to screen has been answered: Universal Screening is the best (for both CRC and Endometrial carcinoma)

• Many issues remain
  • Correct interpretation of MMR IHC
    • Unusual MMR IHC staining patterns
    • Pitfalls in interpretation
  • How do you set up a successful program?
  • Should we screen other GI tract carcinomas? Polyps?
  • MMR IHC and other tests suggest LS but germline testing is negative, now what?
Question 2:

Screening which of the following has the highest yield for detection of Lynch Syndrome?

A. Gastric adenocarcinoma
B. Cholangiocarcinoma
C. Colonic adenomas in patients < 40 yrs
D. Small bowel adenocarcinoma
Screening which of the following has the highest yield for detection of Lynch Syndrome?

A. Gastric adenocarcinoma  
B. Cholangiocarcinoma  
C. Colonic adenomas in patients < 40 yrs  
D. Small bowel adenocarcinoma
Small bowel adenocarcinomas

Table 3. Standardized incidence ratios (SIRs) and corresponding 95% confidence intervals (CIs) of primary extracolonic cancers following colorectal cancer for carriers of mismatch repair gene mutation*

<table>
<thead>
<tr>
<th>Site of cancer</th>
<th>O</th>
<th>E</th>
<th>Median age at diagnosis, y(min–max)</th>
<th>Median no. of years from colorectal cancer to following cancer diagnosis (min–max)</th>
<th>SIR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both sexes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney etc.†</td>
<td>25</td>
<td>1.99</td>
<td>60 (35–78)</td>
<td>14 (1–40)</td>
<td>12.54</td>
<td>(7.97 to 17.94)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>18</td>
<td>2.49</td>
<td>65 (54–84)</td>
<td>11 (2–34)</td>
<td>7.22</td>
<td>(4.08 to 10.99)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>17</td>
<td>0.23</td>
<td>55 (31–67)</td>
<td>13 (1–28)</td>
<td>72.68</td>
<td>(39.95 to 111.29)</td>
</tr>
<tr>
<td>Stomach</td>
<td>9</td>
<td>1.59</td>
<td>69 (55–79)</td>
<td>19 (1–38)</td>
<td>5.85</td>
<td>(2.32 to 9.69)</td>
</tr>
<tr>
<td>Hepatobiliary tract†</td>
<td>7</td>
<td>1.18</td>
<td>62 (39–73)</td>
<td>6 (2–13)</td>
<td>5.94</td>
<td>(1.81 to 10.94)</td>
</tr>
<tr>
<td>Brain</td>
<td>5</td>
<td>1.15</td>
<td>68 (62–80)</td>
<td>16 (10–33)</td>
<td>4.36</td>
<td>(0.79 to 9.55)</td>
</tr>
<tr>
<td>Hematopoietic tissue</td>
<td>5</td>
<td>1.61</td>
<td>57 (41–76)</td>
<td>12 (2–18)</td>
<td>3.11</td>
<td>(0.63 to 6.10)</td>
</tr>
<tr>
<td>Lung</td>
<td>4</td>
<td>9.48</td>
<td>57 (48–65)</td>
<td>13 (1–18)</td>
<td>0.42</td>
<td>(0.10 to 0.91)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>1.62</td>
<td>65 (46–67)</td>
<td>13 (9–23)</td>
<td>1.86</td>
<td>(0.00 to 4.31)</td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>0.11</td>
<td>68 (64–71)</td>
<td>3.5 (3–4)</td>
<td>17.99</td>
<td>(0.00 to 45.41)</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>19</td>
<td>9.25</td>
<td>64 (55–77)</td>
<td>14 (4–33)</td>
<td>2.05</td>
<td>(1.23 to 3.01)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrium</td>
<td>45</td>
<td>1.12</td>
<td>50 (35–69)</td>
<td>8 (1–34)</td>
<td>40.23</td>
<td>(27.91 to 56.06)</td>
</tr>
<tr>
<td>Breast</td>
<td>20</td>
<td>11.34</td>
<td>60 (43–79)</td>
<td>16 (1–23)</td>
<td>1.76</td>
<td>(1.07 to 2.59)</td>
</tr>
<tr>
<td>Ovary</td>
<td>6</td>
<td>1.43</td>
<td>52 (48–61)</td>
<td>10 (1–26)</td>
<td>4.19</td>
<td>(1.28 to 7.97)</td>
</tr>
</tbody>
</table>
• MMRD only in duodenum and jejunum
• 9/14 MMRD were associated with Lynch syndrome (14% of all SB adenoCA in this series)
Colonic polyps in Lynch Syndrome

- dMMR by IHC in 79% of LS-adenomas
- 27/29 (93%) with villous component
- 47/65 (73%) w/o villous component
- 12/12 (100%) with HGD
- No diff between <10mm and >10 mm

- 18/36 (50%) of adenomas, 0/21 HP, 0/2 SSPs
- >8mm were more likely to demonstrate dMMR

- dMMR by IHC in 79% of LS-adenomas
Loss of MSH2

LS patient with MSH2 mut

SSL

Loss of MSH2

TA
Should we screen adenomas?

- Identified 76 patients with adenomas < 45 y
- 64 patients had tissue available and only 1/64 probable LS patient was identified

---

### Advanced Colorectal Adenomas in Patients Under 45 Years of Age Are Mostly Sporadic

Vladimir M. Kushnir · ILKe Nalbantoglu · Rao Watson · Jonathan Goodwin · Elyas Safar · Reena V. Chokshi · Riad R. Azar · Nicholas O. Davidson


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### Routine Molecular Analysis for Lynch Syndrome Among Adenomas or Colorectal Cancer Within a National Screening Program

Anne Goverde,1,2 Anja Wagner,2 Marco J. Bruno,1 Robert M. W. Hofstra,2 Michael Doukas,3 Marcel M. van der Weiden,2 Hendrikus J. Dubbink,2 Winand N. M. Dinjens,3 and Manon C. W. Spaander1

Gastroenterology 2016;155:1410–1415

#### Table 2. Results of Molecular Diagnostics

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, y (IQR)</th>
<th>Male gender, n (%)</th>
<th>MMR deficiency</th>
<th>MHL1 promoter methylation</th>
<th>Germline MMR mutation</th>
<th>Somatic MMR mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients included for IHC</td>
<td>456</td>
<td>67 (63–71)</td>
<td>296 (65)</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>56</td>
<td>69 (63–72)</td>
<td>36 (64)</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Advanced adenoma</td>
<td>370</td>
<td>66 (62–71)</td>
<td>237 (64)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Villous component</td>
<td>186</td>
<td>65 (61–69)</td>
<td>124 (67)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High-grade dysplasia</td>
<td>42</td>
<td>67 (63–74)</td>
<td>30 (73)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4–10 nonadvanced adenomas</td>
<td>30</td>
<td>67 (63–74)</td>
<td>23 (77)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Screening for Lynch syndrome

• Who to screen has been answered: **Universal Screening** is the best (for both CRC and Endometrial carcinoma)

• Many issues remain
  • Correct interpretation of MMR IHC
    • Unusual MMR IHC staining patterns
    • Pitfalls in interpretation
  • How do you set up a successful program?
  • Should we screen other GI tract carcinomas? Polyps?
  • MMR IHC and other tests suggest LS but germline testing is negative, now what?
Germline testing for MSH6 was negative. What does this mean?
“Lynch-Like” Syndrome

- Deficient DNA mismatch repair protein expression with *no deleterious germline mutation* in mismatch repair genes or *EPCAM* and, if MLH1-deficient, no evidence of *BRAF* mutation or *MLH1* promoter hypermethylation.

- Also called “Suspected Lynch Syndrome” by Win and colleagues (*Gut* 2015;64:101-10)

- Accounts for between 2.5% and 3.9% of patients with colorectal carcinoma.

- ~30% of patients with abnormal MMR protein expression within their tumor concerning for Lynch syndrome will have *negative germline mutation studies*. 
1. Anxiety for patients as they are uncertain if they have a genetic disease with ramifications for their health and the health of their family.

2. Intensive lifelong screening protocols for patients with Lynch syndrome.
   a) Should it be applied to patients with “Lynch-like syndrome”?
   b) Most patients with “Lynch-like syndrome” have opted to follow a screening protocol as if they have confirmed Lynch syndrome.
Somatic MMR Gene Mutation

Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors

Isabelle Sourrouille, Florence Coulet, Jeremie H. Lefevre, Chrystelle Colas, Melanie Eyries, Magali Strzelecka, Armelle Bardier-Dupas, Yann Parc, Florent Soubrier

BRIEF REPORTS

Somatic Mutations in MLH1 and MSH2 Are a Frequent Cause of Mismatch-Repair Deficiency in Lynch Syndrome-Like Tumors


Gastroenterology 2014;146:643-646
(Next-generation sequencing)

J Pathol 2014;234:548-559
(Next-generation sequencing)

(Sanger sequencing)

Gastroenterology 2014;147:1308-1316.
(Next-generation sequencing)
Somatic MMR Gene Mutation in Colorectal Carcinoma in Patients with “Lynch-like Syndrome”

<table>
<thead>
<tr>
<th>Somatic Alteration #1</th>
<th>Somatic Alteration #2</th>
<th>Loss of MLH1 &amp; PMS2 (N=45)</th>
<th>Loss of MSH2 &amp; MSH6 (N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMR gene deletion/frameshift/insertion/duplication</td>
<td>MMR gene deletion/frameshift/insertion/duplication</td>
<td>13%</td>
<td>41%</td>
</tr>
<tr>
<td>MMR gene deletion/frameshift/insertion/duplication</td>
<td>MMR gene loss of heterozygosity (LOH)</td>
<td>58%</td>
<td>27%</td>
</tr>
</tbody>
</table>

% of “Lynch-like” carcinomas with biallelic somatic mutations explaining the abnormal MMR IHC results

71% 68%
Solving Lynch-Like cases: Current state

Incorrect MMR IHC Results

Biallelic somatic MMR gene alterations

Germline mutation present but not detected with current methods

Other Possibilities:
Somatic mosaicism
MUTYH

Solving cases of “Lynch-like Syndrome”

MMR Deficient Colonic Crypts: A Novel Indicator of Lynch Syndrome

• First reported by the same research group in Heidelberg, Germany (Kloor et al. and Staffa et al.)
  • Between 25% to 32% of patients with Lynch syndrome had MMR-deficient normal appearing crypts. Correlated with length of mucosa

• Can the identification of MMR deficient crypts help identify patients with Lynch syndrome?
• Analyzed the following:
  • Normal mucosa from 52 patients with Lynch syndrome (LS) with known germline pathogenic variants and colorectal carcinoma
  • Normal mucosa from 30 MSS cancers and 30 sporadic MLH1 deficient colorectal cancers
• LS: IHC for known affected MMR gene
• MSS: IHC for all 4 MMR proteins
• Sporadic MLH1 deficiency: IHC for MLH1
DNA mismatch repair protein deficient non-neoplastic colonic crypts: a novel indicator of Lynch syndrome

Rish K. Pai1 · Beth Dudley2 · Eve Karloski2 · Randall E. Brand2 · Neil O’Callaghan3,4 · Christophe Rosty3,4,5,6 · Daniel D. Buchanan3,4,7 · Mark A. Jenkins8 · Stephen N. Thibodeau9 · Amy J. French9 · Noralane M. Lindor10 · Reetesh K. Pai11

Solitary MSH6 deficient crypt in patient with germline pathogenic MSH6 variant

Group of MSH6 deficient crypts in patient with germline pathogenic MSH6 variant
MSH2 deficient crypts in patient with germline $MSH2$ pathogenic variant
### MMR Deficient Colonic Crypts in normal mucosa

**Table 3** Patients with Lynch syndrome stratified by the presence of DNA mismatch repair (MMR) protein-deficient colonic crypts

<table>
<thead>
<tr>
<th>Clinicopathologic features</th>
<th>Lynch syndrome with MMR-deficient non-neoplastic colonic crypt N (%)</th>
<th>Lynch syndrome without MMR-deficient non-neoplastic colonic crypt N (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>18</td>
<td>34</td>
<td>NA</td>
</tr>
<tr>
<td>Mean Age in years (range)</td>
<td>57 (27–79)</td>
<td>51 (28–80)</td>
<td>0.1</td>
</tr>
<tr>
<td>Gender, Male/Female</td>
<td>10 (56) / 8 (44)</td>
<td>17 (50) / 17 (50)</td>
<td>0.9</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Colon</td>
<td>14 (78)</td>
<td>18 (53)</td>
<td>0.08</td>
</tr>
<tr>
<td>Left Colon / Rectum</td>
<td>4 (22)</td>
<td>16 (47)</td>
<td></td>
</tr>
<tr>
<td>MMR IHC pattern in carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact expression of all 4 proteins</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0.7</td>
</tr>
<tr>
<td>MLH1 and PMS2 Loss</td>
<td>4 (22)</td>
<td>9 (26)</td>
<td></td>
</tr>
<tr>
<td>MSH2 and MSH6 Loss</td>
<td>7 (39)</td>
<td>17 (50)</td>
<td></td>
</tr>
<tr>
<td>Isolated MSH6 Loss</td>
<td>4 (22)</td>
<td>4 (12)</td>
<td></td>
</tr>
<tr>
<td>Isolated PMS2 Loss</td>
<td>3 (17)</td>
<td>3 (9)</td>
<td></td>
</tr>
<tr>
<td>Germline Mutation Analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>MLH1</em> pathogenic variant present</td>
<td>4 (22)</td>
<td>9 (27)</td>
<td>0.6</td>
</tr>
<tr>
<td><em>MSH2</em> pathogenic variant present</td>
<td>7 (39)</td>
<td>18 (53)</td>
<td></td>
</tr>
<tr>
<td><em>MSH6</em> pathogenic variant present</td>
<td>4 (22)</td>
<td>4 (12)</td>
<td></td>
</tr>
<tr>
<td><em>PMS2</em> pathogenic variant present</td>
<td>3 (17)</td>
<td>3 (17)</td>
<td></td>
</tr>
<tr>
<td>Mean length of colonic mucosa evaluated by IHC in millimeters (range)</td>
<td><strong>136 (21–336)</strong></td>
<td><strong>88 (5–244)</strong></td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>Mean estimated number of colonic crypts evaluated by IHC (range)</td>
<td><strong>1508 (233–3733)</strong></td>
<td><strong>981 (56–2711)</strong></td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>
MMR Deficient Colonic Crypts in normal mucosa

• How to increase sensitivity?

• Estimated frequency of MMR deficient crypts:
  • Based on our initial data: 1 MMR-deficient crypt per ~1000 colonic crypts
  • Evaluation of 3250 crypts would yield a 95% probability of detecting at least one MMR protein-deficient crypt.

Sensitivity (%)

Length (cm, 1cm = 125 crypts)

Based on Bernoulli trial using expected frequency of crypts with loss
MMR Deficient Colonic Crypts in normal mucosa

1 MMR-deficient crypt per ~1000 colonic crypts
Hypotheses:
1. MMR deficient non-neoplastic crypts can be detected from biopsies of normal colorectal mucosa obtained during colonoscopy.
2. Detection of MMR deficient crypts can help identify patients with Lynch syndrome.
50 patients undergoing screening colonoscopy

- 33 patients with Lynch syndrome: 22 with a cancer history, 11 with no cancer history
- 13 patients without Lynch syndrome (10 MSS CRC, 2 with biallelic $MLH1$ somatic mutations, and 1 with $MLH1$ hypermethylation).
- 4 patients with germline variants of uncertain significance (2 $MSH2$ and 2 $MSH6$)

8 jumbo forcep biopsies procured from each patient

- 4 biopsies from right colon and 4 biopsies from left colon
- The biopsies were sectioned at 100 µm intervals to include 8 total sections per biopsy in order to evaluate >3250 crypts.
## Clinicopathologic Features

<table>
<thead>
<tr>
<th>Clinicopathologic Features</th>
<th>Lynch Syndrome with MMR Deficient Crypts</th>
<th>Lynch Syndrome without MMR Deficient Crypts</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>23</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>Median Age in years (IQR)</td>
<td>56 (19)</td>
<td>46 (29)</td>
<td>0.07</td>
</tr>
<tr>
<td>Gender, Male/Female (%)</td>
<td>3 (13) / 20 (87)</td>
<td>4 (40) / 6 (60)</td>
<td>0.08</td>
</tr>
<tr>
<td>Lynch syndrome Type (%)</td>
<td></td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>Affected</td>
<td>15 (71)</td>
<td>6 (29)</td>
<td></td>
</tr>
<tr>
<td>Unaffected</td>
<td>8 (67)</td>
<td>4 (33)</td>
<td></td>
</tr>
<tr>
<td>Germline Mutation Analysis (%)</td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>MLH1 pathogenic variant present</td>
<td>5 (71)</td>
<td>2 (29)</td>
<td></td>
</tr>
<tr>
<td>MSH2 pathogenic variant present</td>
<td>10 (63)</td>
<td>6 (38)</td>
<td></td>
</tr>
<tr>
<td>MSH6 pathogenic variant present</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td></td>
</tr>
<tr>
<td>PMS2 pathogenic variant present</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td></td>
</tr>
<tr>
<td>EPCAM pathogenic variant present</td>
<td>1 (100)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

MMDd crypts were not seen in non-LS patients
58 F with germline *PMS2* c.943C>T pathogenic variant

Multiple PMS2 deficient non-neoplastic colonic crypts in both the right and left colon
56 F with germline $MLH1$ splice site c.208-3C>G likely pathogenic variant

Solitary MLH1 deficient non-neoplastic colonic crypt in the right colon
41 M with germline $MSH6$ c.3226 C>T pathogenic variant

Solitary MSH6 deficient non-neoplastic colonic crypt in the right colon
Variants of uncertain significance (VUS)

- More than 700 VUS have been reported in the InSIGHT database
- More VUS will be identified as LS screening

If MMR-deficient normal crypts = Lynch syndrome, then this simple test can help classify VUS
### Variants of uncertain significance (VUS)

<table>
<thead>
<tr>
<th>Case</th>
<th>Cancer History</th>
<th>Germline MMR Gene Variant of Uncertain Significance (VUS)</th>
<th>Age/Sex</th>
<th>Number of MMR Deficient Crypts</th>
<th>Location of MMR Deficient Crypts</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>Uterine</td>
<td>MSH6 c.3385T&gt;C</td>
<td>63/F</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>30</td>
<td>Colon</td>
<td>MSH6 c.3227G&gt;A</td>
<td>67/F</td>
<td>1</td>
<td>R colon</td>
</tr>
<tr>
<td>31</td>
<td>Colon</td>
<td>MSH2 c.166G&gt;A</td>
<td>59/F</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>32</td>
<td>Colon</td>
<td>MSH2 c.1865C&gt;A</td>
<td>59/M</td>
<td>1</td>
<td>L colon</td>
</tr>
</tbody>
</table>

**MSH2 c.1865C>A VUS**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Protein</th>
<th>Concensus InSiGHT Classification</th>
<th>Classification Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2</td>
<td>c.1865C&gt;A</td>
<td>p. (Pro622Glu)</td>
<td>Class 3: uncertain</td>
<td>2016/04/20 v1.9</td>
</tr>
</tbody>
</table>
No Difference in Colorectal Cancer Incidence or Stage at Detection by Colonoscopy Among 3 Countries With Different Lynch Syndrome Surveillance Policies

Gastroenterology, 2018 Nov;155(5):1400-1409

No history of CRC

History of CRC

- Different colonoscopy intervals between 1 and 3 years. No difference in CRC detection
- Suggests screening does not improve CRC detection?
- Precursor lesions are not endoscopically visible?
- MMR-deficient crypts may contribute to CRC in Lynch without progressing through a visible adenoma? Flat adenoma or directly to CRC?
Lynch Syndrome Carcinogenesis

Bypass the visible adenoma to develop carcinoma?

Eradication by normal cell turnover or possibly an immune mechanism
Neoantigens produced by MMRd crypts may induce an inflammatory response and subsequent crypt elimination.
Pathways to MSI-H

**SPORADIC**
- Sessile serrated lesion
  - BRAF V600E
  - CpG island methylation (MLH1 promoter)
- Adenoma
- Biallelic somatic MMR gene mutation

**INHERITED**
- Adenoma
- Lynch Syndrome
- MMRd Crypt?

**Deficient DNA Mismatch Repair (MSI-H) Carcinoma**
Collaborators:

• Reet Pai, UPMC
• Randall Brand, Beth Dudley, Eve Karloski, UPMC
• Laney Lindor, Mayo AZ
• Steve Thibodeau and Amy French, Mayo Rochester
• Dan Buchanan, Univ of Melbourne, Australia
• Cristophe Rosty, Envoi Pathology, Brisbane Australia
Outline:

• Pathways to colon cancer
• Definition of Lynch Syndrome and goals of screening
• Principles of MMR IHC as a screening tool
• Issues with MMR IHC interpretation
• “Lynch-like” syndrome