Microsatellite Instability Testing

<table>
<thead>
<tr>
<th>Overview</th>
<th>Overview of DNA repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Describe</td>
<td>Describe Mismatch Repair System</td>
</tr>
<tr>
<td>Discuss</td>
<td>Discuss Lynch Syndrome</td>
</tr>
<tr>
<td>Review</td>
<td>Review Testing Methods Frequently Used for MSI Testing</td>
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<tr>
<td>Present</td>
<td>Present Validation of Idylla MSI System</td>
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</table>
Learning Objectives

• Describe the two main testing strategies for MSI testing
• Identify the reasons/clinical applications for performing MSI testing and what and MSI-High vs. Microsatellite Stable (MSS) results means
• Analyze the performance of three MSI testing technologies (IHC + 2 molecular testing methods)
Humans are constantly bombarded and attacked by environmental toxins that damage DNA: Chemicals, Radiation, Sunlight

- Cancer is relatively rare
- Humans have the ability to repair DNA damage effectively
- Inherited and acquired defects with DNA repair result in increased risk of cancer
Three fundamental mechanisms for repairing DNA damage

**Repair of DNA DAMAGE**

- Nucleotide excision repair pathway
- Homologous recombination DNA repair
- Mismatch repair (Lynch Syndrome)

People with inherited mutations in genes that repair DNA have greatly increased risk for developing cancer
Nucleotide Excision Repair (NER)

- Removes DNA damage induced by UV light
- UV results in bulky DNA adducts (eg, thymine dimers)
- NER removes short segment of damaged DNA lesion
- DNA polymerase and ligase fill-in the gap

Xeroderma Pigmentosum

- Risk of cancer on sun-exposed skin 1000X
- Defective repair of UV damage to pyrimidines
- Multiple genes contribute to disease
- XPC and XPD (ERCC2)

- Basal Cell Carcinoma
- Corneal scars
- Hyperpigmentation
DNA Repair by Homologous Recombination

- Double strand DNA breaks are extremely genotoxic
- Ineffective repair leads to chromosomal instability and cancer
- Accurate repair is mediated through Homologous Recombination

BRCA1
Risk of ovarian and prostate cancer

BRCA2
Risk of ovarian, prostate, pancreas, biliary, stomach, melanoma, lymphoma

- Fanconi anemia (multi-genic)
- Bloom syndrome ($BLM$ gene)
- Ataxia-Telangiectasia ($ATM$ gene)
Mismatch Repair (MMR): MLH1, PMS2, MSH2, MSH6

- Mismatch repair genes (MMR)
  - MLH1, MSH2, MSH6, PMS2
- Proofread and repair mismatches during replication
- Defective in MMR genes leads to accumulation of mutations in genome

Some mutations occur in critical genes becoming the initiating event in a patient’s cancer

- Results in Microsatellite Instability Phenotype (MSI)
What are Microsatellites?

- Microsatellites are short repeated regions of DNA
  - 1 – 6 nucleotides units
  - Units repeated 5 – 50 times
  - Distributed throughout the genome

- Examples:
During DNA replication repetitive regions (microsatellites) are prone to polymerase ‘slippage’

- pMMR: Cells with proficient mismatch repair machinery correct mistakes
- dMMR: Cells with deficient mismatch repair acquire mistakes leading to microsatellite instability (MSI)
Defective MMR function results in MSI

**Mistake Made**

-1 nucleotide

**MSS (Mistake Repair)**

- **pMMR = Mistake Repaired**
  - nucleotide added

**dMMR = Mistake Not Repaired**

**MSI**
dMMR/ MSI creates high probability for mutations in cancer genes

<table>
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<tr>
<th>Gene</th>
<th>Function of encoded protein</th>
<th>Wild-type coding sequence</th>
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<th>Stomach</th>
<th>Endometrium</th>
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<td>WISP-3</td>
<td>growth factor</td>
<td>A₈</td>
<td>X</td>
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</tbody>
</table>

Prevalence of MSI across different cancer types – survey of 39 cancer types

MSI-H% in most common cancer types:

- Colorectal Cancer: 15-20%
- Gastric Cancer: 10-20%
- Endometrial Cancer: 20-30%
- Ovarian Cancer: 2-10%
Loss of MMR Function

Sporadic

Hereditary

Random accumulation of mutations during subsequent cell divisions

Mutation(s) in important gene(s)

Further accelerate tumor development

Cancer
Lynch Syndrome (LS)
(\textit{Hereditary Nonpolyposis Colorectal Cancer})

Inherited predisposition to developing cancer
Caused by genetic defect in DNA mismatch repair genes (MMR) 
\textit{MLH1, MSH2, MSH6, PMS2, EPCAM}

Types of Cancers
   - Most common: Colorectal, endometrial
   - Others: extra-intestinal cancer

2-3\% of Colon cancer occurs in LS patients

Cancer occurs at an earlier age (40’s versus 60’s)
Synchronous: occurring at the same time
Metachronous: occurring at different times
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Lifetime Risk with MMR gene mutation</th>
<th>Average age of presentation (years)</th>
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</thead>
<tbody>
<tr>
<td>Colon</td>
<td>28-80%</td>
<td>44</td>
</tr>
<tr>
<td>Endometrial</td>
<td>30-50%</td>
<td>46</td>
</tr>
<tr>
<td>Small intestine</td>
<td>4-7%</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>2-13%</td>
<td>56</td>
</tr>
<tr>
<td>Ovarian</td>
<td>3-13%</td>
<td>42.5</td>
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<tr>
<td>Hepatobiliary tract</td>
<td>2%</td>
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<tr>
<td>Upper genitourinary</td>
<td>1-12%</td>
<td></td>
</tr>
<tr>
<td>Brain (glioblastoma)</td>
<td>1-4%</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>2-6%</td>
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<tr>
<td>Upper genitourinary</td>
<td>1-12%</td>
<td></td>
</tr>
</tbody>
</table>

About 1 in 35 CRC patients have LS

High risk for second primary cancer (16% in 10 years)

Better prognosis and survival rates
2 synchronous tumors

Mucinous Features and Chronic Inflammation

Chronic Inflammation

Signet Ring Features
Why is MSI testing performed?

Characterization of Cancer

- Prognostic Stratification
- Lynch Syndrome Screening
- Predictive Value

Clinical Practice Guidelines:

- “Tumor screening for MMR deficiency is appropriate for all CRC and endometrial cancers...”

- “Clinicians should order MMR status testing in patients with colorectal cancer for identification of patients at high risk for Lynch syndrome and/or prognostic stratification”

1. NCCN Guidelines “Gentic/Familial High-Risk Assessment: Colorectal” Version 3.2017
Patients with MSI have a more favorable prognosis:

MSI tumors have a decreased likelihood to metastasize

A review of 31 studies reporting survival on 12,782 patients with MSI tumors show a favorable prognosis

1. NCCN Guidelines “Gentic/Familial High-Risk Assessment: Colorectal” Version 3.2017
MMR/MSI predicts response to PD-1 inhibition

- MSI tumors ~1000-2000 mutations per cell.
- Many become tumor-specific neoantigens
- Tumors block anti-tumor immunity via PD-L1::PD-1 binding
- PD-1 therapy restores antitumor immunity
MMR/MSI predicts best response to PD-1 inhibition

Cancer patient who has progressed on prior therapies

- MSI and/or MMR Testing
- MSI-H or MMR
- MSS

- Patient Receives Immunotherapy
- Patient Receives Alternate Therapy

Graphs showing biochemical and radiographic responses:
- Biochemical Response: Mismatch repair-proficient colorectal cancer, Mismatch repair-deficient colorectal cancer, Mismatch repair-deficient noncolorectal cancer
- Radiographic Response: Mismatch repair-proficient colorectal cancer, Mismatch repair-deficient colorectal cancer, Mismatch repair-deficient noncolorectal cancer

References:
- Le, Science, 357(6349):409-413, 2017
MMR/MSI predicts response to PD-1 inhibition

Le, NEJM, 372:2509-2520, 2015
Le, Science, 357(6349):409-413, 2017
5-FU ADJUVANT THERAPY in CRC

• Reduced response to 5-FU based chemotherapy in dMMR tumours
• Improved response of MSI-CRC tumors to combination chemotherapy with oxaliplatin and irinotecan in comparison to 5-FU based agents.
• According to the National Comprehensive Cancer Network (NCCCN), MMR testing should be considered for all patients with stage-II disease, as stage-II MSI tumors have a good prognosis and may not benefit from chemotherapy.


MMR/MSI Testing: IHC or MSI

- Normal
- Tumor

- MLH1
- PMS2
- MSH2
- MSH6

Microsatellite Stable
- INTACT MMR
- AAAAAAAAAAAAAAAAAAAAAAAAAAAA
- Microsatellite Stable

Microsatellite Instability
- DEFECTIVE MMR
- AAA
- Microsatellite Instability

Normal - BAT25

Tumor - BAT25

<table>
<thead>
<tr>
<th>Normal</th>
<th>Normal- BAT25</th>
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<tbody>
<tr>
<td>NR-27</td>
<td>NR-21</td>
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<tr>
<td>NR-24</td>
<td>BAT-25</td>
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<td>BAT-26</td>
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Tumor

<table>
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<tr>
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<tbody>
<tr>
<td>NR-27</td>
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<td>NR-21</td>
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Clinical Analysis of MMR: Immunohistochemistry
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<td>PMS2</td>
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**NO MUTATION**

![Images of IHC staining](attachment:images)

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**MSH6 depends upon MSH2 for stability**

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<tr>
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<th>MLH 1 IHC</th>
<th>PMS2 IHC</th>
<th>MSH2 IHC</th>
<th>MSH6 IHC</th>
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<td><img src="PMS2.png" alt="Image" /></td>
<td><img src="MSH2.png" alt="Image" /></td>
<td><img src="MSH6.png" alt="Image" /></td>
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Most common pattern and frequently seen in somatic MSI CRC

PMS2 depends upon MLH1 for stability

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# Patterns of Staining

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<th>MLH1</th>
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<th>MSH2</th>
<th>MSH6</th>
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<td>+</td>
<td>+</td>
<td>Sporadic cancer</td>
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<tr>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>LS or sporadic cancer</td>
<td>BRAF V600E MLH1 hypermethylation MLH1 mutation</td>
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<td>+</td>
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<td>Need to rule out LS</td>
<td>MSH2 mutation</td>
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<tr>
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<td>Need to rule out LS</td>
<td>PMS2 mutation</td>
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</table>
Challenges with IHC
Role for BRAF V600E testing to rule out LS in MLH1 deficient CRC

1. **MSI**
   - **LOSSE**
   - **MMR or MSI Test**
     - **INTACT**
       - MSS
     - **LOSS**
       - MMR or MSI Test

2. **MLH1 IHC**
   - **LOSS MLH1**
     - **BRAF V600E**
       - **YES**
         - Sporadic
       - **NO**
         - **MLH1 Methyl**
           - **YES**
             - Lynch Syndrome
           - **NO**
             - Genetics

3. **INTACT: MLH1**
   - **LOSS: MSH2, MSH6, PMS2**
BRAF V600E negatively affects prognosis in CRC

<table>
<thead>
<tr>
<th>MMR</th>
<th>BRAF</th>
<th>Prognosis</th>
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<tbody>
<tr>
<td>Deficient</td>
<td>WT</td>
<td>Good</td>
</tr>
<tr>
<td>Deficient</td>
<td>Mutant</td>
<td>Intermediate</td>
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<tr>
<td>Proficient</td>
<td>WT</td>
<td>Intermediate</td>
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<tr>
<td>Proficient</td>
<td>Mutant</td>
<td>Worse</td>
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Conventional MSI Testing and Reporting

Technology Type:
- Most labs use commercial kits or LDT
- PCR followed by capillary electrophoresis

Test Result Outcomes:
- MSS: 0 markers have MSI
- MSI-L: 1 marker has MSI
- MSI-H: ≥ 2 markers have MSI

Technique Basics:
Determine if MSI is present at microsatellite loci
Mononucleotide markers: NR-21, NR-24, BAT-25, BAT-26, MONO-27
LDTs normally use a similar set of markers
Normal or Tumor Cell with Intact MMR System: Repairs the mismatch error

Mispairing: DNA Slippage & Loss of TTTTT

Intact MMR repairs deletion

Microsatellite length is maintained (24 A’s)
Tumor Cell with DEFECTIVE MMR System: Mutations accumulate

Loss of TTTTT

Deficient MMR
No repair of deletion
MSI is observed

Microsatellite is shortened by −5 bp (19 A’s)
Microsatellites are sensitive markers of defective MMR function.

Normal tissue:

```
GCTTTTAGGFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFGTCCTTAG
CGAAAATCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGGAATC
```
20 A's

Tumor tissue:

```
GCTTTTAGGFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFGTCCTTAG
CGAAAATCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGGAATC
```
15 A's
Examine 5 mononucleotide microsatellite markers
Two tissue samples are tested: Normal and Tumor
Compare the lengths of the tumor and normal microsatellites
Testing of Normal and Tumor Tissue

Rarely, individuals are heterozygous: 2 differently sized microsatellite alleles
Case 1

41 year old male with rectal adenocarcinoma
Microsatellite stability is observed in all 5 markers.

MSS
Case Example 2

- 47 year old female
- Adenocarcinoma in proximal colon
- Poorly differentiated with mucinous features
Microsatellite Instability at 5 out of 5 loci
Traditional PCR Based Diagnostic Testing

Pre-analytical

- Indication for testing

Tumor
Normal

Surgical Pathologist
Molecular Pathologist

Analytical (1-2 days)

- DNA Prep
- PCR
- Analysis
- Review

Molecular Technician
Reagents
Equipment
Computers

Post-analytical

Clinical Interpretation
Idylla real time PCR system: Rapid Molecular Diagnostic Testing

**Pre-analytical**
- Indication for testing
- Tumor

**Analytical (2-3 hours)**
- DNA Prep
- Testing
- Test Analysis
- Molecular Technician
- Reagents
- Equipment
- QC review
- Analytics
- Computers
- Software
- Database
- IT Support

**Post-analytical**
- Clinical Interpretation

**Tissue** → **On-board** → **Results**

Surgical Pathologist
Molecular Pathologist
The Idylla MSI Test

**Key Characteristics**

1. MSI detection based on 7 novel biomarkers
2. Results available in 150 minutes
3. Less than 2 minutes of hands-on time
4. Directly on FFPE tissue sections
5. No need for normal tissue sample
6. PCR based assay

**Specimen Requirements**

- 5 µm FFPE glass mounted tissue section
- 10 µm FFPE tissue section (CURLS)
- Neoplastic cell content
  (if < 20%, macro-dissection needed)

**Idylla MSI Biomarkers**

7 homopolymers frequently mutated in MSI-H cancers

- ACVR2A
- SULF1
- SEC31A
- BTBD7
- MRE11
- DIDO1
- RYR3

These biomarkers are different from the Bethesda markers
Idylla™ MSI Result Report

The Idylla™ MSI Assay will make an individual mutation call for each of the 7 biomarkers:
- Mutation Detected
- No Mutation Detected
- Invalid

The Idylla™ MSI Assay will also make an overall MSI determination:
- **MSI-H** → ≥2 of the 7 markers are mutant
- **MSS** → <2 of the 7 markers are mutant
- **Invalid** → >2 of the 7 markers are invalid
Validation Design:
- 50 CRC FFPE samples were analyzed by Idylla MSI and MCW MSI and MMR IHC
- All samples analyzed by three methods

Study Results:
- MSI results were available for 50 samples – Overall concordance was 100% (50/50)
  - PPA = 100% (40/40)
  - NPA = 100% (10/10)
- Comparison to IHC: 100% (10/10) samples were concordant
- Overall Failure Rates:
  - MCW Assays = 0%
  - Idylla = 0%
Idylla™ MSI Data Overview: All Centers

Over 3,000 clinical samples tested to date

<table>
<thead>
<tr>
<th>Study</th>
<th>Format</th>
<th>Cancer Type</th>
<th># Samples</th>
<th>Comparison Technology</th>
<th>Overall Concordance</th>
<th>Invalid Rates (Idylla vs. Promega)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCW, 2018</td>
<td>Abstract</td>
<td>CRC</td>
<td>N=50</td>
<td>LDT</td>
<td>100%</td>
<td>0%</td>
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<tr>
<td>Claes B. et al. ASCO 2015</td>
<td>Abstract</td>
<td>CRC</td>
<td>N = 70</td>
<td>Promega</td>
<td>98.3%</td>
<td>9% vs 16%</td>
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<tr>
<td>De Craene B. et al. ESMO 2017</td>
<td>Poster</td>
<td>Gastric</td>
<td>N = 85</td>
<td>Promega</td>
<td>100%</td>
<td>0% vs 10.6%</td>
</tr>
<tr>
<td>Maertens G. et al. ESMO 2017</td>
<td>Poster</td>
<td>CRC</td>
<td>N = 201</td>
<td>Promega</td>
<td>93.6%</td>
<td>4% vs 11.9%</td>
</tr>
<tr>
<td>De Craene B. et al. ASCO 2018</td>
<td>Online Abstract</td>
<td>CRC</td>
<td>N = 348</td>
<td>Promega</td>
<td>96.1%</td>
<td>3.4% vs 3.4%</td>
</tr>
</tbody>
</table>
Summary of Idylla™ MSI Test

>95% concordance of the 7 novel MSI biomarkers with commercial and LDT PCR tests and IHC

- Fast and reliable information on MSI status
- Unbiased result reporting
- Significantly lower failure rate compared to standard of care molecular methods
- No need for normal tissue samples

The MSI Test is currently in development. Product characteristics mentioned are anticipated but not yet validated.

(1) De Craene B. et al. Idylla MSI in gastric samples. ESMO 2017 poster 697P
(2) Maertens G. et al. Idylla MSI in CRC. ESMO 2017 poster 138P
(3) Data based on internal research data
Thank you very much!

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