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BIOMARKERS
Defective Mismatch Repair and Microsatellite Instability in Solid Tumors
# Table of Contents

- Biology of Mismatch Repair and Genome Instability
- Identification of Defective Mismatch Repair and Microsatellite Instability
- Endometrial Cancer and Defective Mismatch Repair/ Microsatellite Instability
- Pan-Tumor and Defective Mismatch Repair/Microsatellite Instability
- Defective Mismatch Repair/Microsatellite Instability and Immune Checkpoint Blockade
Biology of Mismatch Repair and Genome Instability
Defective MMR occurs following genetic mutations

Consensus definition¹

MMR is a highly conserved DNA repair pathway used to reverse single-base mismatches or short insertions and deletions that occur within microsatellites/tandem repeat regions.

Four proteins, codified by homonym genes, that play a critical role in this process are MLH1 (mutL homolog 1), MSH2 (mutS homolog 2), MSH6 (mutS homolog 6), and PMS2 (postmeiotic segregation increased 2).

MLH1, MSH2, MSH6, and PMS2 function in heterodimers, namely MLH1-PMS2 and MSH2-MSH6.

Inactivation of these genes, which can occur as a result of germline and/or somatic mutations or epigenetic silencing, results in a defective MMR (dMMR) mechanism.

MMR, mismatch repair; mut, mutation.

DNA MMR Restores DNA Integrity

MMR Pathway Activation After Polymerase Error¹,²


The figure is reproduced with the permission of GSK. Martin A, Scharff MD. Nat Rev Immunol. 2002;2:605-614.
Microsatellites Are Repetitive DNA Sequences Within the Genome

Consensus definition

Also named short tandem repeats

Repetitive DNA sequences that are distributed along the genome, in both coding and noncoding regions

Microsatellites are tracts of repetitive DNA motifs (range in length from 1 to 6 or more base pairs), typically repeated 5-50 times

Repeats are typically consistent throughout an individual but are highly polymorphic among different individuals (number of repeats of sequence varies between individuals).

Repetitive nature renders them particularly sensitive to DNA mismatching errors, which can occur during DNA replication or induced by medical treatment (e.g. DNA alkylating agents, cisplatin).

Microsatellite instability results from defective DNA repair mechanisms

Consensus definition¹

- MSI is a condition of genetic hypermutability resulting from defective DNA MMR
- MSI is characterized by clustering of mutations in microsatellites typically consisting of repeat length alterations
- The presence of MSI represents phenotypic evidence that MMR is not functioning normally

MMR, mismatch repair; MSI, microsatellite instability.

Key Points – Biology of Mismatch Repair and Genome Instability

• MMR is defined as a highly conserved mechanism used to restore DNA integrity after the occurrence of mismatching errors\(^1\)
  
  – Four proteins that play a critical role in this process include MLH1, MSH2, MSH6, and PMS2

• Inactivation of these genes, which can occur as a result of germline and/or somatic mutations or epigenetic silencing, results in dMMR\(^1\)

• Microsatellites are repetitive DNA sequences (1-6 bases) distributed in both coding and noncoding regions of the genome that are sensitive to DNA mismatching errors\(^1\)

• MSI is a condition of genetic hypermutability resulting from defective DNA dMMR, which indicates that MMR is not functioning normally\(^1\)

---

dMMR, defective mismatch repair; MMR, mismatch repair; MSI, microsatellite instability.

Identification of Defective Mismatch Repair and Microsatellite Instability
A number of tests are available to assess MMR/MSI status

**MMR**

- Tumor tissue staining for protein expression of 4 MMR genes: MLH1, MSH2, MSH6, and PMS2

**MSI**

- Assesses the proportion of alterations in a predetermined panel of microsatellite repeat markers that indicates the loss of MMR activity

- Provides a pan-cancer approach that results in a full mutational signature as an output

---

IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NCI, National Cancer Institute; NGS, next-generation sequencing; PCR, polymerase chain reaction.

**Recommendations for dMMR detection**

**Immunohistochemistry (IHC, Recommendation A)**

- IHC is the preferred test
  - Using antibodies recognizing the 4 MMR proteins: MLH1, MSH2, MSH6, and PMS2
- IHC detects the presence or absence of MMR proteins
  - An abnormal IHC test shows at least one of the proteins “not detected”

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Recommendations from the European Society of Medical Oncology (ESMO)
IHC, immunohistochemistry; MMR, mismatch repair
Recommendations for MSI Testing

Polymerase Chain Reaction (PCR, Recommendation B)¹

- When IHC results are unclear, confirmatory molecular analysis is recommended
  - PCR is the first-line of molecular analysis:
    - 2 possible panels –
      - (i) Bethesda/NCI panel (testing for 5 unique microsatellite loci) consists of 2 mononucleotide (BAT-25 and BAT-26) and 3 dinucleotide (D5S346, D2S123, and D17S250) repeats,¹² and
      - (ii) Promega panel (testing for 7 unique microsatellite loci) consists of 5 poly-A mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, and NR-27) as well as two pentanucleotide loci (used for specimen identification)¹²
    - Both panels have been/are being used to assess MSI in clinical trials. Molecular tests guarantee the highest values of specificity and sensitivity in MSI testing¹


PCR compares the length of nucleotide repeats in tumor cells and normal cells

Recommendations from the European Society of Medical Oncology (ESMO)
MSI, microsatellite instability; NCI, National Cancer Institute; PCR, polymerase chain reaction.

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Microsatellite Instability Phenotype Classifications:¹,²,³

- **MSS** (microsatellite stable) – tumors without microsatellite instability
- **MSI-L** (low microsatellite instability) – tumors showing MSI at only one of the predefined loci*.
- **MSI-high** (high microsatellite instability) – tumors showing MSI at ≥2 of the predefined biomarker loci from the Bethesda/NCI or Promega panels or >30% of the loci if >5 markers are tested
  - Occurs in the context of heritable cancer syndromes or sporadically
  - Represents global genomic instability and decreased DNA damage signaling

* Using the Promega method tumors are classified as MSS/MSI-H. The Promega system can help resolve cases of MSI-L into either MSI-H or MSS.

### Commonly Used Microsatellite Markers and Classifications

<table>
<thead>
<tr>
<th>Microsatellite marker</th>
<th>Repeat type</th>
<th>Chromosomal location (gene near marker/GenBank number)</th>
<th>Bethesda/NCI panel</th>
<th>Promega panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT-25</td>
<td>Mononucleotide</td>
<td>4q12 (c-kit, intron 16)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>BAT-26</td>
<td>Mononucleotide</td>
<td>2p 16.3p21 (hMSH2 gene, intron 5)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>NR-21</td>
<td>Mononucleotide</td>
<td>2p16 (MSH2)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>NR-24</td>
<td>Mononucleotide</td>
<td>5q21/22 (APC)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MONO-27</td>
<td>Mononucleotide</td>
<td>17q111.2-q12 (BRCA1)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>D2S123</td>
<td>Dinucleotide</td>
<td>2p16 (MSH2)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>D5S346</td>
<td>Dinucleotide</td>
<td>5q21/22 (APC)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>D17S250</td>
<td>Dinucleotide</td>
<td>17q111.2-q12 (BRCA1)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Penta C</td>
<td>Pentanucleotide</td>
<td>21q22.3 (AL138752)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Penta D</td>
<td>Pentanucleotide</td>
<td>9p 12-13.3 (AC003656)</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

¹ The Bethesda Panel represents international criteria for the determination of microsatellite instability in colorectal cancer.

Next-generation sequencing (NGS, Recommendation C)\(^1\)

- NGS represents another type of molecular test to assess MSI that should be conducted only in selected centers devoted to these techniques
  - Advantages include the possibility of coupling MSI analysis with the determination of tumor mutational burden
  - Sophisticated bioinformatics protocols are necessary to use NGS as a method for MSI\(^2\)
  - Laboratories using NGS testing for MSI should have validated the assay for use in the cancer in which it is being used\(^2\)

MSI, microsatellite instability; NGS, next-generation sequencing


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NGS can identify MSI but also total tumor mutational burden

Mutation load: 10
TMB: 15.83
I index (%): 10

Mutation load: 18
TMB: 28.13
I index (%): 11.1
Key points: identification of dMMR/MSI

- Immunohistochemistry is recommended as the preferred test, using antibodies directed against 4 MMR proteins\(^1\)
- If results are unclear, further testing based on PCR analysis of selected DNA repeat sequences is recommended\(^1\)
- NGS represents another type of molecular test to assess MSI that should be conducted only in selected centers devoted to these techniques\(^1\)

DNA, deoxyribonucleic acid; MMR, mismatch repair; MSI, microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction.

Endometrial Cancer and Defective Mismatch Repair/Microsatellite Instability
dMMR/MSI-H Occurs in Endometrial Cancer

- Approximately 30% of endometrial cancers harbor dMMR/MSI-H$^{1-3}$
  - dMMR and MSI-H populations are biologically similar and have been shown to have >95% concordance when assessed by respective IHC, PCR or NGS assays$^{4,5}$
- Lynch Syndrome, caused by germline mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6, and PMS2, accounts for ~5% of all endometrial carcinomas$^1$
- The prognostic value of dMMR/MSI-H in endometrial cancer remains unclear$^6$

![Endometrial cancer](image)
NCCN Clinical Practice Guidelines In Oncology (NCCN Guidelines®) for MMR/MSI testing for EC

• IHC and MSI analyses are screening tests that are typically performed on endometrial cancer tissue to identify individuals at higher risk for having Lynch Syndrome\(^1\)

• Universal testing of endometrial carcinomas for mismatch repair (MMR) proteins/microsatellite instability (MSI) is recommended\(^2\)

• For recurrent endometrial cancer, NCCN recommends dMMR/MSI-H testing if not previously done\(^2\)

---

IHC, immunohistochemistry; dMMR, deficient mismatch repair; MSI, microsatellite instability; MSI-H, high microsatellite instability

\(^1\) Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment Colorectal. V.1.2021. © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed [May 3, 2021]. To view the most recent and complete version of the guideline, go online to NCCN.org.\(^2\) Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Uterine Neoplasms. V.3.2021. © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed [July 14, 2021]. To view the most recent and complete version of the guideline, go online to NCCN.org
National Comprehensive Cancer Network® (NCCN®) Recommendations

NCCN Guidelines®¹
Principles of Molecular Analysis

- NCCN Guidelines recommend universal testing of endometrial tumors for defects in the MMR pathway (e.g. MLH1, MSH2, MSH6)
  - Testing may be performed on the initial presurgical biopsy or D&C material or the final hysterectomy specimen
  - MLH1 loss should be further evaluated for promoter methylation to assess for an epigenetic process rather than a germline mutation
- Genetic counseling should be offered to the following patients:
  - Patients with all other MMR abnormalities
  - Patients without MMR defects but who have a significant family history of endometrial and/or colorectal cancer
- NCCN Guidelines also support the use of ancillary studies to complement morphological assessment of histologic tumor type:
  - POLE mutations
  - MMR/MSI
  - Aberrant p53 expression

IHC, immunohistochemistry; MMR, Mismatch Repair; dMMR, deficient Mismatch Repair Deficient; D&C, dilatation and curettage; EC, endometrial cancer; MSI, microsatellite instability; POLE, DNA polymerase epsilon.

¹ Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Uterine Neoplasms. V.3.2021. © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed [July 14, 2021]. To view the most recent and complete version of the guideline, go online to NCCN.org
SGO Recommendations

SGO Clinical Practice Statement¹
Screening for Lynch Syndrome in EC

- All women diagnosed with endometrial cancer should undergo clinical screening
  - Review of personal and family history
  - And/or molecular screening for Lynch syndrome
- Two main strategies for assessing Lynch syndrome
  - Germline testing recommended for women at an increased risk for Lynch syndrome defined by clinical criteria, but women who do not have a suggestive family history may not be identified by clinical criteria
  - Universal molecular tumor testing for either all endometrial cancers or cancers diagnosed at < 60 years old regardless of personal or family history
- IHC for MLH1, MSH2, MSH6, and PMS2 expression is recommended as it is the most cost-effective and widely available
- Tumors that show loss of MLH1 on IHC should undergo further testing for MLH1 hypermethylation

IHC, immunohistochemistry; MMR, Mismatch Repair; dMMR, deficient Mismatch Repair Deficient; D&C, dilatation and curettage; EC, endometrial cancer; MSI, microsatellite instability; POLE, DNA polymerase epsilon.

Key points: Endometrial Cancer & MMR/MSI

• Approximately 30% of endometrial cancers harbor dMMR/MSI-H<sup>1</sup>
• Guidelines recommend universal testing of endometrial cancers for defects in the MMR pathway<sup>2,3</sup>
  • IHC is recommended as it is the most cost-effective and widely available<sup>3</sup>
• For recurrent endometrial cancer, Guidelines recommend dMMR/MSI-H testing if not previously done.<sup>2</sup>

IHC, immunohistochemistry; dMMR, deficient mismatch repair; MSI-H, high microsatellite instability; NGS, next-generation sequencing

dMMR, mismatch repair deficient; MMR, mismatch repair; MSI, microsatellite instability.
Tumor Agnostic Drug Development
Recent biomarker-driven, tissue-agnostic clinical trials represent a significant paradigm shift in precision cancer medicine.

Indications for cancer treatments are based on a common biomarker across different types of tumors rather than a location in the body where the tumor originated.

Molecular testing then becomes an essential element of treatment planning.

Regulatory Timeline For Tumor-Agnostic Drug Development (US)

<table>
<thead>
<tr>
<th>Year</th>
<th>Approval Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>Pembrolizumab MSI-H/dMMR approval</td>
</tr>
<tr>
<td>2018</td>
<td>Larotrectinib NTRK fusion+ approval</td>
</tr>
<tr>
<td>2019</td>
<td>Entrectinib NTRK fusion+ approval</td>
</tr>
<tr>
<td>2020</td>
<td>Pembrolizumab TMB-H approval</td>
</tr>
</tbody>
</table>

Common features typical of a Basket Trial

1) Cancers are enriched for one or more molecular alterations

2) These alterations have a reasonable likelihood of predicting response to a particular therapy based on preclinical functional and/or computational modeling

3) These alterations are found across a variety of cancers

GARNET Trial Design

<table>
<thead>
<tr>
<th>Part 2B Expansion cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1: dMMR EC</td>
</tr>
<tr>
<td>A2: MMRp EC</td>
</tr>
<tr>
<td>E: NSCLC</td>
</tr>
<tr>
<td>F: Non-endometrial dMMR basket</td>
</tr>
<tr>
<td>G: PROC</td>
</tr>
</tbody>
</table>

dMMR, deficient mismatch repair; EC, endometrial cancer; MMRp, mismatch repair proficient; NSCLC, non-small cell lung cancer; PROC, platinum-resistant ovarian cancer

4. Study of TSR-042, an Anti-programmed Cell Death-1 Receptor (PD-1) Monoclonal Antibody, in Participants With Advanced Solid Tumors - Full Text View - ClinicalTrials.gov
Pan-Tumor and Defective Mismatch Repair/Microsatellite Instability
Lynch Syndrome and dMMR/MSI-H

- Lynch syndrome is characterized by inherited mutations in genes coding for MMR proteins, whereby patients have a higher lifetime risk of developing certain cancers\(^1\)
- Colorectal cancer (CRC) and endometrial cancer (EC) are the most common cancers in Lynch syndrome\(^2,3\)
  - **CRC**: individuals with Lynch syndrome have a 50-80% lifetime risk of developing CRC compared to 2% in the general population.
  - **EC**: Women with Lynch syndrome carry a 40-60% lifetime risk of developing EC
- Individuals with Lynch syndrome also carry an increased risk of developing other gastrointestinal cancers (i.e., gastric), gynecologic cancers (i.e., ovarian), and other solid tumors (Figure)

Lynch Syndrome and dMMR/MSI-H (cont’d)

- Microsatellite instability (MSI) is a hallmark of Lynch syndrome-related tumors but is not specific to it.¹
- Most MSI/dMMR tumors (~80%) are sporadic; only 15–20% of MSI/dMMR tumors can be attributed to Lynch syndrome.¹,²

CRC, colorectal cancer; EC, endometrial cancer; MSI, microsatellite instability; MSS, microsatellite stable

MMR deficiency is well recognized as the predominant cause of MSI within endometrial and colorectal cancers.

### Estimated Prevalence of MSI-H by Solid Tumor Type

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Percentage of MSI-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial</td>
<td>26%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>13%</td>
</tr>
<tr>
<td>Gastric</td>
<td>11%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>11%</td>
</tr>
<tr>
<td>Esophageal</td>
<td>4%</td>
</tr>
<tr>
<td>Cervical</td>
<td>4%</td>
</tr>
<tr>
<td>Prostate</td>
<td>3%</td>
</tr>
<tr>
<td>Bile Duct</td>
<td>3%</td>
</tr>
<tr>
<td>Liver</td>
<td>3%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3%</td>
</tr>
<tr>
<td>Uveal</td>
<td>2%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>2%</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>2%</td>
</tr>
<tr>
<td>Mesotheloma</td>
<td>2%</td>
</tr>
<tr>
<td>Breast</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

- In the US, the overall prevalence of MSI-H and dMMR across different solid tumors (all-stages) has been estimated at ~20% and ~14%, respectively.\(^1\)
- The highest prevalence of MSI-H has been noted in Lynch-syndrome associated tumors, including endometrial and colorectal.\(^1\)-\(^3\)
- dMMR/MSI-H has also been reported in other gynecologic cancers (ovarian), gastrointestinal cancers (gastric, esophageal\(^2\)) and at a low prevalence (<3%) in other solid tumors (Figure).\(^1,4\)

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MMR deficiency is well recognized as the predominant cause of MSI within endometrial and colorectal cancers.

**Estimated Prevalence of MSI-H by Solid Tumor Type**

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<tr>
<td>Ovarian</td>
<td>11%</td>
</tr>
<tr>
<td>Esophageal</td>
<td>4%</td>
</tr>
<tr>
<td>Cervical</td>
<td>4%</td>
</tr>
<tr>
<td>Prostate</td>
<td>3%</td>
</tr>
<tr>
<td>Bil Duct</td>
<td>3%</td>
</tr>
<tr>
<td>Liver</td>
<td>3%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3%</td>
</tr>
<tr>
<td>Uveal</td>
<td>2%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>2%</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>2%</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>2%</td>
</tr>
<tr>
<td>Breast</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

**Estimated Cancer Prevalence in the US**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Estimated New Cases in 2021</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial</td>
<td>59,913</td>
</tr>
<tr>
<td>Ovarian</td>
<td>21,410</td>
</tr>
<tr>
<td>Cervical</td>
<td>14,480</td>
</tr>
<tr>
<td>Colorectal</td>
<td>149,500</td>
</tr>
<tr>
<td>Gastric (stomach)</td>
<td>26,560</td>
</tr>
<tr>
<td>Esophageal</td>
<td>19,260</td>
</tr>
<tr>
<td>Prostate</td>
<td>248,530</td>
</tr>
<tr>
<td>Liver and intrahepatic bile duct</td>
<td>42,230</td>
</tr>
<tr>
<td>Thyroid</td>
<td>44,280</td>
</tr>
<tr>
<td>Uveal</td>
<td>3,320</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>60,430</td>
</tr>
<tr>
<td>Wilms</td>
<td>600</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>3,000</td>
</tr>
<tr>
<td>Breast</td>
<td>281,550</td>
</tr>
</tbody>
</table>

Prevalence of MSI-H varies by Tumor Stage

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>MSI-H (%) Stages 1-2</th>
<th>MSI-H (%) Stages 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gynecological Tumors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial Cancer</td>
<td>27%</td>
<td>26%</td>
</tr>
<tr>
<td>Ovarian Cancer</td>
<td>17%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Gastrointestinal Tumors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>20%</td>
<td>9%</td>
</tr>
<tr>
<td>Gastric Cancer</td>
<td>13%</td>
<td>10%</td>
</tr>
<tr>
<td>Esophageal Cancer</td>
<td>NR</td>
<td>18%</td>
</tr>
</tbody>
</table>

• Across solid tumors, subgroup analyses indicated that early stage disease tend to have higher MSI-H prevalence (Stage I/II cancers, 15%) than later stages (Stage III, 9%; Stage IV, 3%)\(^2\) However this varies by tumor type

Acknowledging molecular alterations in Ovarian Cancer could allow for more personalized treatment strategies for patients:

- Women with pathogenic \textit{BRCA1} or \textit{BRCA2} variants are eligible for treatment with PARP inhibitors:
  - These agents have evidence of benefit after front-line response to platinum chemotherapy and as treatment in recurrent settings.

- Women with dMMR tumors may benefit from treatment with PD-1 inhibitors.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{prevalence_of_msi-h_in_ovarian_cancer_by_tumor_histology}
\caption{Prevalence of MSI-H in Ovarian Cancer by Tumor Histology.}
\end{figure}

dMMR Pan-tumor in Clinical Guidelines
Universal MMR Testing

- Colorectal cancer
- Gastric cancer
- Endometrial cancer
- Small bowel adenocarcinoma
The NCCN Guidelines recommend universal MMR testing in CRC, EC, gastric cancer, and small bowel adenocarcinoma. MMR testing is also recommended for certain patients with other solid tumors (e.g., ovarian, esophageal, EGJ).

**Gynecologic Cancers (EC\textsuperscript{a}, ovarian)**
- Test all EC and endometrioid carcinoma; test refractory/relapsed ovarian cancer
- Available at [nccn.org](http://nccn.org)

**GI Cancers (CRC\textsuperscript{a}, gastric, esophageal, small bowel)**
- Test all CRC, all newly diagnosed gastric cancers and small bowel adenocarcinoma
- Consider testing locally advanced, recurrent, or metastatic esophageal and EGJ cancers who are candidates for PD-1 inhibitors
- Available at [nccn.org](http://nccn.org)

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\textsuperscript{a}Lynch-Syndrome Cancer

NCCN Guidelines Treatment Recommendations

The NCCN Guidelines recommend PD-1 inhibitors for the neoadjuvant (CRC), 1L (CRC, SBA), and subsequent-line (CRC, EC, ovarian, gastric, esophageal, EJC, SBA) treatment of dMMR solid tumor patients.

Gynecologic Cancers (EC, ovarian)

- Recurrent, metastatic, or high-risk dMMR EC: pembro, nivo, or dostarlimab
- Recurrent dMMR ovarian cancer: pembro
- Available at nccn.org

GI Cancers (CRC, gastric, esophageal, small bowel)

- NAT/1L dMMR mCRC: pembro or nivo ± ipi
- Subsequent-line dMMR mCRC: pembro, nivo, or nivo + ipi
- 2L or subsequent in unresectable locally advanced, recurrent, or metastatic dMMR gastric, esophageal, or EGJ cancer (where local therapy is not indicated): pembro
- Initial or subsequent therapy in small bowel adenocarcinoma: pembro or nivo ± ipi
- Available at nccn.org

1L, first line; 2L, second line; dMMR, mismatch repair deficient; EC, endometrial cancer; EGJ, esophagogastric junction; GI, gastrointestinal; ipi, ipilimumab; mCRC, metastatic colorectal cancer; NAT, neoadjuvant therapy; nivo, nivolumab; PD-1, programmed death-1; pembro, pembrolizumab; SBA, small bowel adenocarcinoma

Lynch-Syndrome Cancer

**Ovarian**

ASCO Guideline – *Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer*

Select Recommendations

**Recommendation 1.2.** Women diagnosed with clear cell, endometrioid, or mucinous ovarian cancer should be offered somatic tumor testing for mismatch repair deficiency (dMMR) (Type: evidence based, benefits outweigh harms; Evidence quality: intermediate; Strength of recommendation: moderate).

**Recommendation 1.3.** Testing for dMMR may be offered to women diagnosed with other histologic types of epithelial ovarian cancer (Type: evidence based, benefits outweigh harms; Evidence quality: intermediate; Strength of recommendation: moderate).

**Recommendation 2.2.** Women diagnosed with recurrent epithelial ovarian cancer with identified dMMR should be offered FDA-approved treatment under their labeled indication based on these results. dMMR qualifies for FDA-approved treatment (Type: evidence based, benefits outweigh harms; Evidence quality: intermediate; Strength of recommendation: moderate)

- The identification of a dMMR phenotype or genotype presents an opportunity for treatment with pembrolizumab in the setting of recurrent disease, regardless of tissue of origin.
- The value of testing for the mismatch repair (MMR) phenotype is the tissue-agnostic FDA approval of pembrolizumab for patients with microsatellite instability–high (MSI-H) or dMMR recurrent solid tumors. This provides another treatment option for patients with recurrent ovarian, fallopian tube, or primary peritoneal cancers that are MSI-H/dMMR.

Colorectal Cancer

ASCO Guidelines: dMMR Testing

ASCO Guideline – Hereditary Colorectal Cancer Syndromes

- Tumor testing for DNA mismatch repair (MMR) deficiency with immunohistochemistry for MMR proteins and/or MSI should be assessed in all CRC patients. As an alternate strategy, tumor testing should be carried out in individuals with CRC younger than 70 years, or those older than 70 years who fulfill any of the revised Bethesda guidelines.

ASCO Guideline – Molecular Biomarkers for the Evaluation of CRC

- While testing of CRC for MMR has been recommended for all patients with CRC as a workup test to evaluate for possible Lynch syndrome, guidelines for use of MMR as a predictive biomarker of response to therapy have not been reported.
- Recent molecular biomarker data have shown the importance of microsatellite instability (MSI) testing, a marker of deficient mismatch repair (dMMR), for the selection of patients for immunotherapy.
- Clinicians should order mismatch repair status testing in patients with colorectal cancers for the identification of patients at high risk for Lynch syndrome and/or prognostic stratification.

## Late-Stage Colorectal Cancer

**ASCO Guideline – Treatment of Patients With Late-Stage Colorectal Cancer**

Select ASCO Guideline: PD-1 dMMR place in therapy

<table>
<thead>
<tr>
<th>Rec</th>
<th>Population</th>
<th>ASCO Resource Levels: Maximal</th>
<th>Strength of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-Line Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8</td>
<td>Any RAS status and dMMR or MSI-H and patients not candidates for intensive chemotherapy</td>
<td>Immune checkpoint inhibitors&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Recommendations on Second-Line Systemic Colorectal Metastatic Treatment</strong></td>
<td></td>
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</tr>
<tr>
<td>3.7</td>
<td>dMMR or MSI-high</td>
<td>Immune checkpoint inhibitors (if not previously given)</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Recommendations on Third-Line and Fourth-Line Systemic Colorectal Metastatic Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>dMMR/MSI-H</td>
<td>Immune checkpoint inhibitors (if not previously given)</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

<sup>a</sup>Qualifying statement for first-line immunotherapy: At the time of this writing, the US Food and Drug Administration had not approved the use of immune checkpoint inhibitors (eg, single-agent pembrolizumab or nivolumab or the combination of nivolumab plus ipilimumab) in first-line treatment of patients with mCRC.

Defective Mismatch Repair/Microsatellite Instability and Immune Checkpoint Blockade
Immune Checkpoint Blockade in MSI Tumors

- Somatic mutations in tumors can be recognized by the immune system\(^1\)
- MSI tumors carry 10-100 times as many somatic mutations compared with normal cells\(^1\)\(^-\)\(^3\)
  - PD-1 responsive cancers often have high mutational burden or carry a high mutational volume, owing to exogenous exposure to carcinogens\(^1\)
    - Smoking (lung cancer); UV light (melanoma)
- MSI tumors have prominent lymphocyte infiltrates, priming them for immune-mediated activity\(^1\)\(^,\)\(^4\)


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Proposed Checkpoint Blockade: Mechanism of Action in MSI-High Tumors\textsuperscript{1,2}

- Strongly expresses PD-1, PD-L1, CTLA-4, LAG-3, and IDO
- Increases cytotoxic T lymphocyte (CD3+/CD8+) invasion
- Increases presence of type 1 T-helper cells and expression of chemokines
- Increases presence of memory T lymphocytes

Determining dMMR/MSI Status in Patients

- MSI-high/dMMR occurs in a variety of malignancies
- MSI and/or MMR testing is recommended by NCCN and ESMO clinical practice guidelines
- Multiple tests are available to assess MSI/MMR status across all solid tumors
- Diagnostic testing for MSI/MMR can identify patients likely to respond to treatment with PD-1/PD-L1 inhibitors