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BIOMARKERS
Defective Mismatch Repair and Microsatellite Instability in Endometrial Cancer

Table of Contents

Biology of Mismatch Repair and Genome Instability

Defective MMR occurs following genetic mutations

Consensus definition[1]

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**

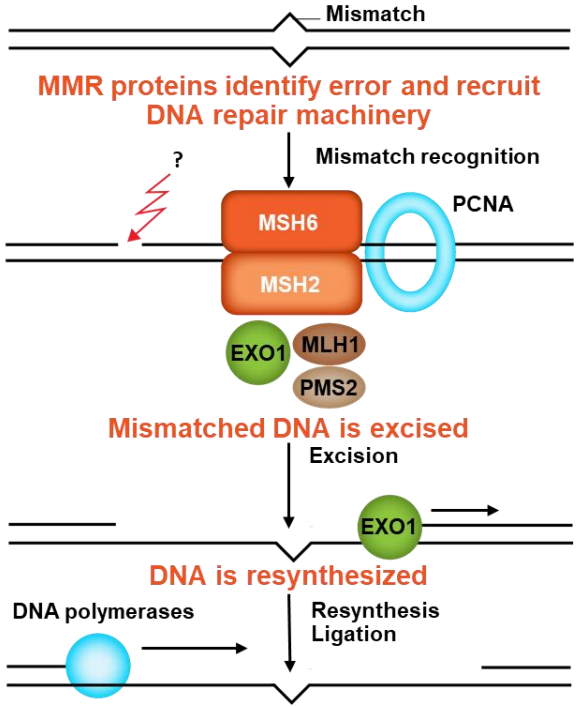
Inactivation of these genes, which can occur as a result of germline and/or somatic mutations or epigenetic silencing, results in a defective MMR (dMMR) mechanism

MMR, mismatch repair; mut, mutation.

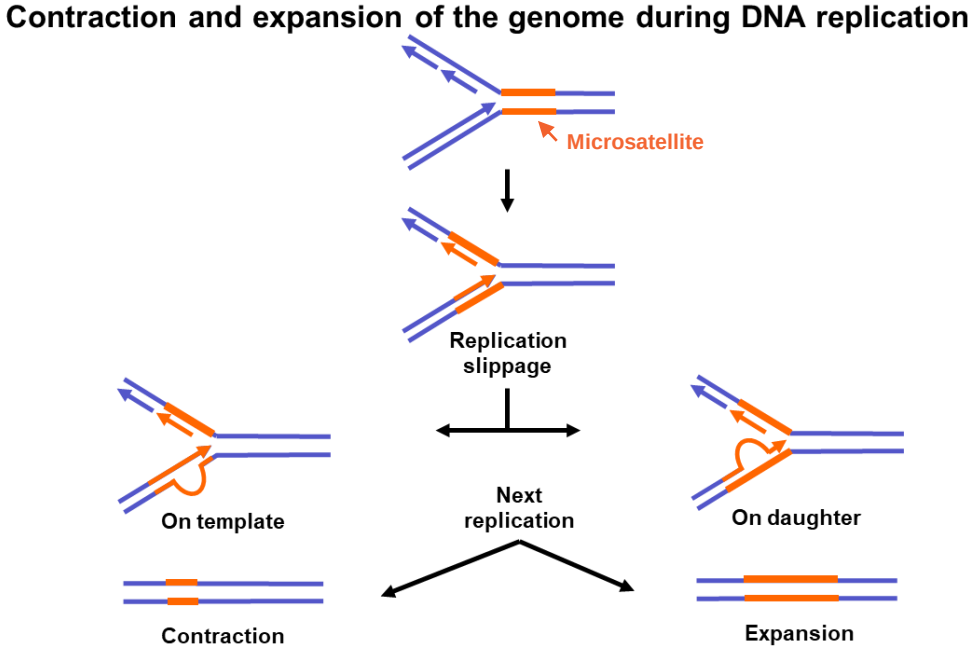
1. Luchini C et al. *Ann Oncol*. 2019;30:1232-1243.

DNA MMR Restores DNA Integrity

MMR Pathway Activation After Polymerase Error[1,2]



The figure is reproduced with the permission of GSK. Martin A, Scharff MD. Nat Rev Immunol. 2002;2:605-614

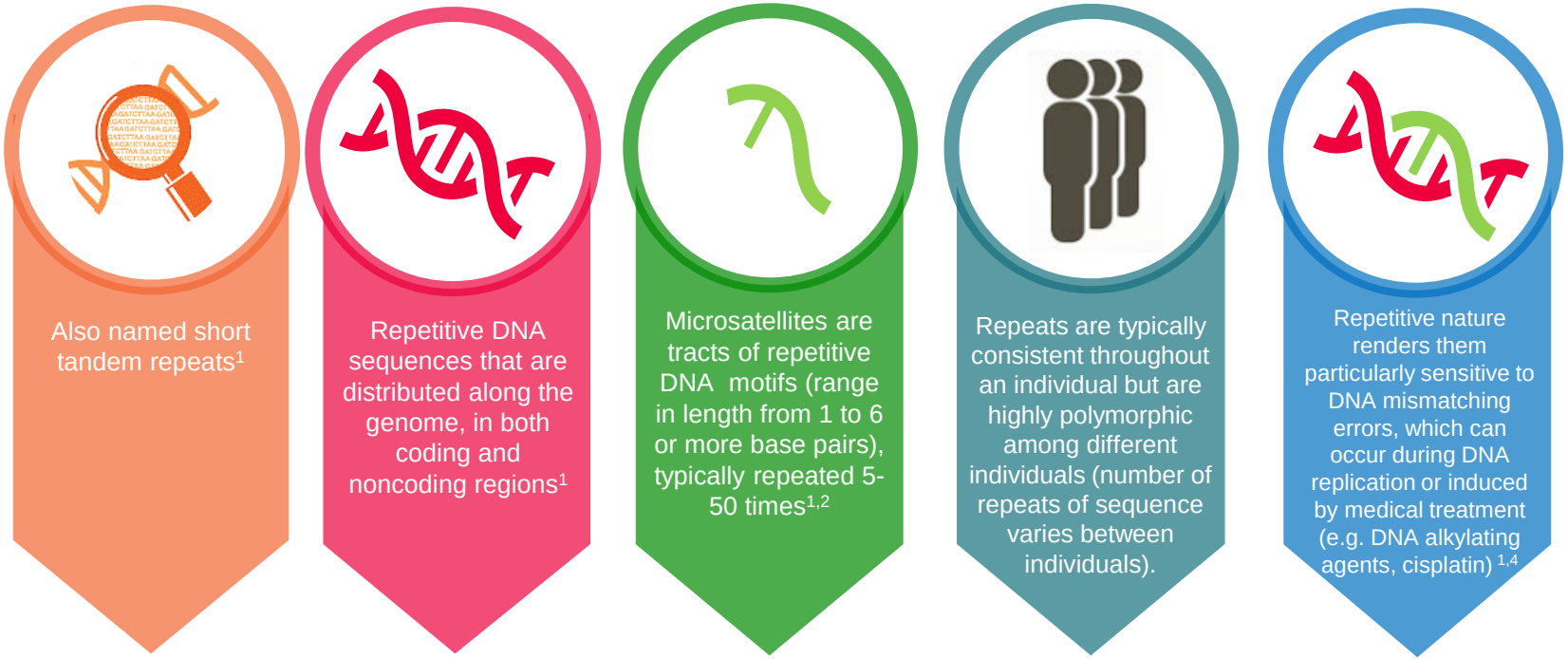


Adapted by permission from GSK: Springer, Stress-Induced Mutagenesis, Microsatellite repeats: canaries in the coalmine, Chatterjee N et al. New York, NY: Springer; 2013.

1. Martin A, Scharff MD. *Nat Rev Immunol.* 2002;2:605-614. 2. Chatterjee N et al. Microsatellite repeats: canaries in the coalmine. In: Mittelman D et al, eds. *Stress-Induced Mutagenesis.* New York, NY: Springer; 2013.

Microsatellites Are Repetitive DNA Sequences Within the Genome

Consensus definition[1]



1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243; 2. Kumar R. *Encyclopedia of Animal Cognition and Behavior*. Springer, Cham. https://doi.org/10.1007/978-3-319-47829-6_167-1; 3. OncologyPro. Factsheets on Biomarkers. Accessed October 13, 2020; 4. Li Z. *DNA Repair.* 2016; 38:94-101.

Microsatellite instability results from defective DNA repair mechanisms

Consensus definition[1]

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.

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1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243.

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]

1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243.

Identification of Defective Mismatch Repair and Microsatellite Instability

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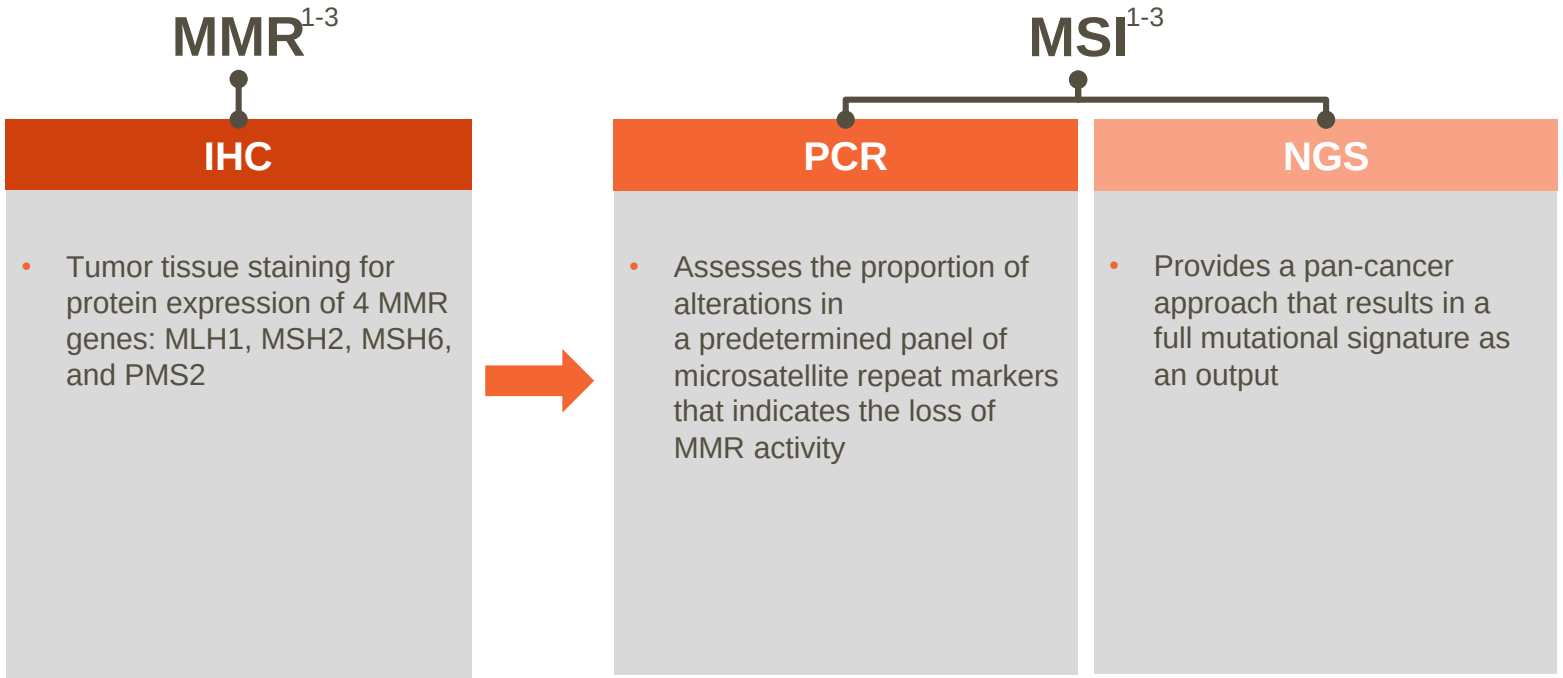
A number of tests are available to assess MMR/MSI status



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

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment Colorectal. V.1.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed [October 13, 2020]. To view the most recent and complete version of the guideline, go online to NCCN.org. ; 2. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243; 3. Roland R et al. *Cancer Res.* 1998;58:5248-57

Recommendations for dMMR detection

Immunohistochemistry (IHC, Recommendation A)[1]

- 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”

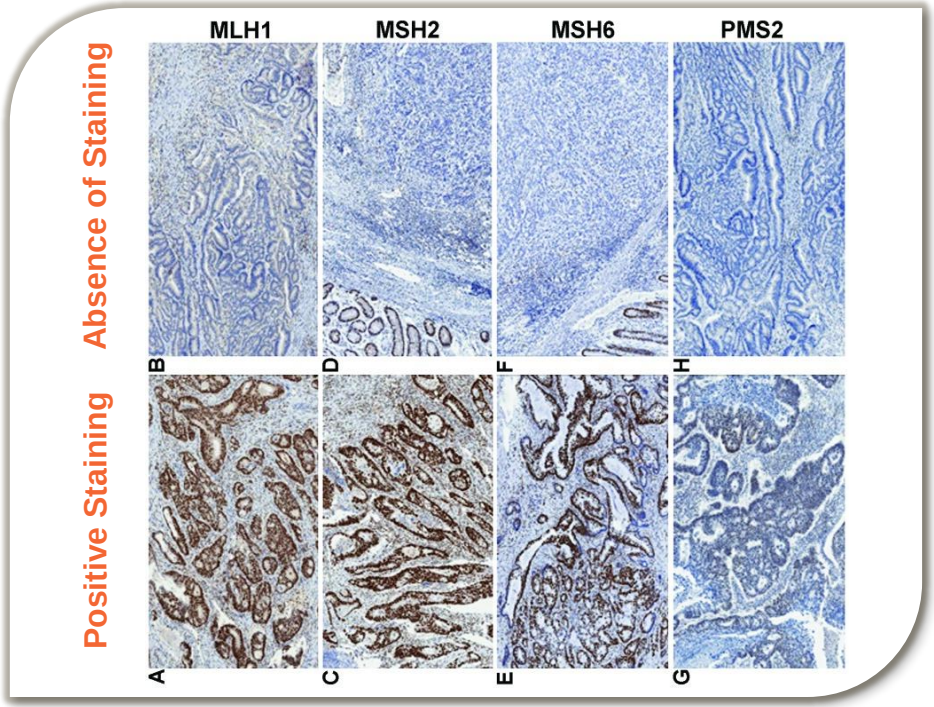


Image adapted from Richman S. *Int J Oncol.* 2015;4747(4):1189–1202
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Recommendations from the European Society of Medical Oncology (ESMO)

IHC, immunohistochemistry; MMR, mismatch repair

1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243.

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,[1,2] 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) [1,2]
      - 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 testing[1]

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

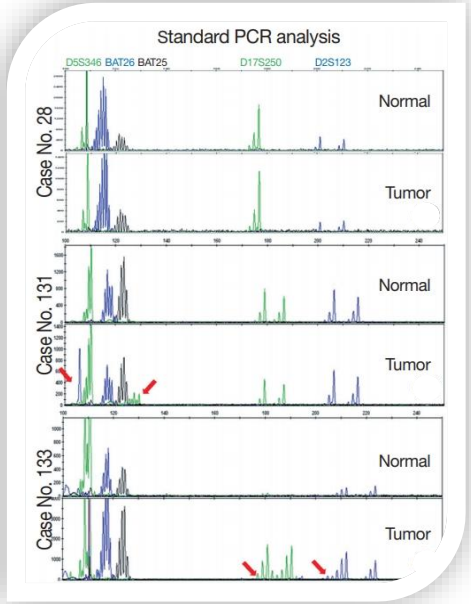

Image adapted from Lee M et al. *J Pathol Transl Med.* 2019;53:386-392
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Recommendations from the European Society of Medical Oncology (ESMO)
MSI, microsatellite instability; NCI, National Cancer Institute; PCR, polymerase chain reaction.

1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243; 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment Colorectal. V.1.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed [October 13, 2020]. To view the most recent and complete version of the guideline, go online to NCCN.org

Commonly Used Microsatellite Markers and Classifications

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

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

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

* 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.

MSI, microsatellite instability; NCI, National Cancer Institute

1. Zhang L. *J Mol Diagn.* 2008;10:301-307; 2. Roland R et al. *Cancer Res*. 1998;58:5248-57; 3. . Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment Colorectal. V.1.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed [October 13, 2020]. To view the most recent and complete version of the guideline, go online to NCCN.org

Recommendations for MSI Testing (cont.)

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]

NGS can identify MSI but also total tumor mutational burden

Image adapted from Lee M et al. *J Pathol Transl Med.* 2019;53:386-392
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MSI, microsatellite instability; NGS, next-generation sequencing

1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243; 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment Colorectal. V.1.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed [October 13, 2020]. To view the most recent and complete version of the guideline, go online to NCCN.org

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.

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1. Luchini C et al. *Ann Oncol.* 2019;30:1232-1243.

Endometrial Cancer and Defective Mismatch Repair/ Microsatellite Instability

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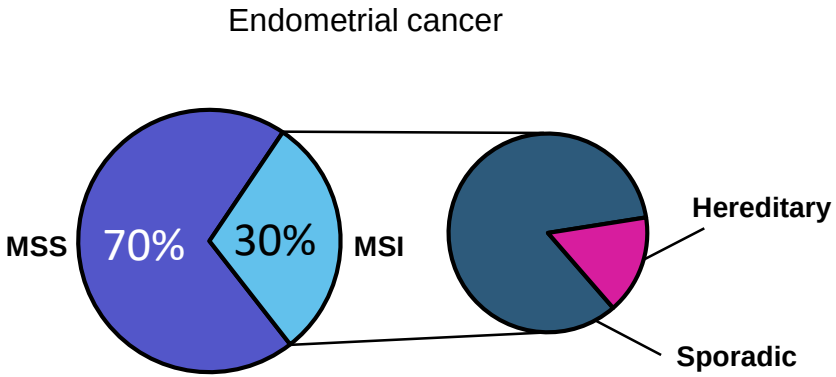
dMMR/MSI-H Occurs in Endometrial Cancer

- Approximately 30% of endometrial cancers harbor dMMR/MSI-H[1]
  - 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[2,3]
- 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[4]



Reprinted from Trends CancerKloor M et al. The Immune Biology of Microsatellite-Unstable Cancer, 2:121-133, Copyright (2016), with permission from Elsevier

DNA, deoxyribonucleic acid; IHC, immunohistochemistry; dMMR, deficient mismatch repair ; MSI, microsatellite instability; MSI-H, high microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction

1. Kloor M et al. Trends Cancer. 2016;2:121-133.; 2. Zhao P et al. J Hematology and Oncology. 2019;12:54; 3. Steello E et al. Ann Oncol. 2017;28:96-102, 4. Diaz-Padilla I et al. Crit Rev Oncol Hematol. 2013;88:154-167.

SGO Recommendations

SGO Clinical Practice Statement[1]
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.

1. SGO Clinical Practice Statement: Screening for Lynch Syndrome In Endometrial Cancer. Published March 2014. https://www.sgo.org/clinical-practice/guidelines/screening-for-lynch-syndrome-in-endometrial-cancer/. Accessed. October 13, 2020

Biomarker Selection in GARNET

- In GARNET, assignment of patients with endometrial cancer into different cohorts was determined by IHC testing for MMR status[1]
  - Screening for GARNET could be performed using local ICH, PCR, or next-generation sequencing[1]
  - If local IHC testing was not available, then MMR conducted by a central laboratory was allowed[1]

IHC, immunohistochemistry; MMR, mismatch repair ; MSI, microsatellite instability; MSI-H, high microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction

MSI-H = microsatellite instability-high; MSS = microsatellite stable; dMMR = deficient mismatch repair; IHC = immunohytochemistry; NGS = Next Generation DNA Sequencing; MMR = mismatch repair; PCR = polymerase chain reaction

1. https://clinicaltrials.gov/ct2/show/NCT02715284. Accessed October 13, 2020.

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]

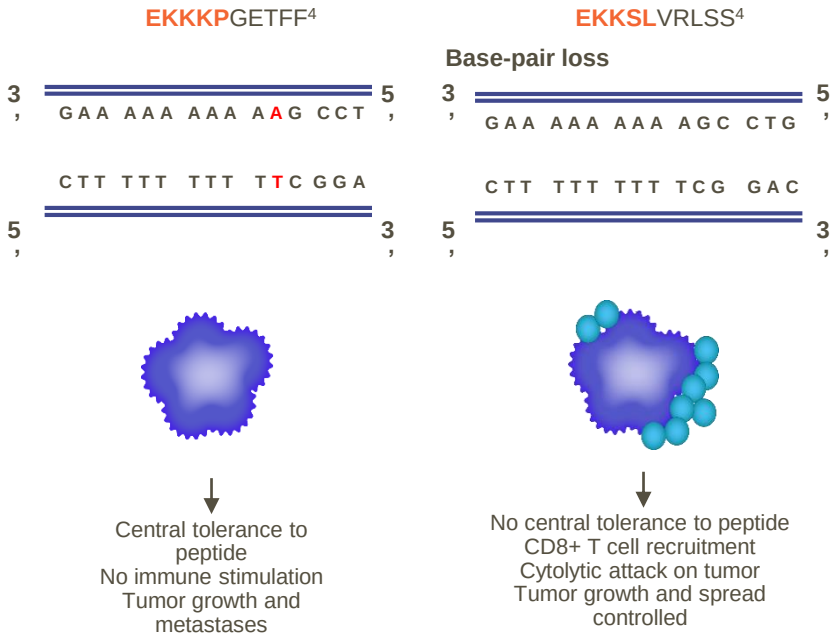


Image adapted from: Drescher KM et al. *Clin Dev Immunol.* 2010; 2010: 170432.

1. Le DT et al. N Engl J Med. 2015;372:2509-2520. 2. Eggink FA et al. Oncoimmunology. 2017;6:e126456. 3. Gargiulo P et al. Cancer Treat Rev. 2016;48:61-68. 4. Drescher KM et al. Clin Dev Immunol. 2010; 2010: 170432.

Proposed Checkpoint Blockade: Mechanism of Action in MSI-High Tumors[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

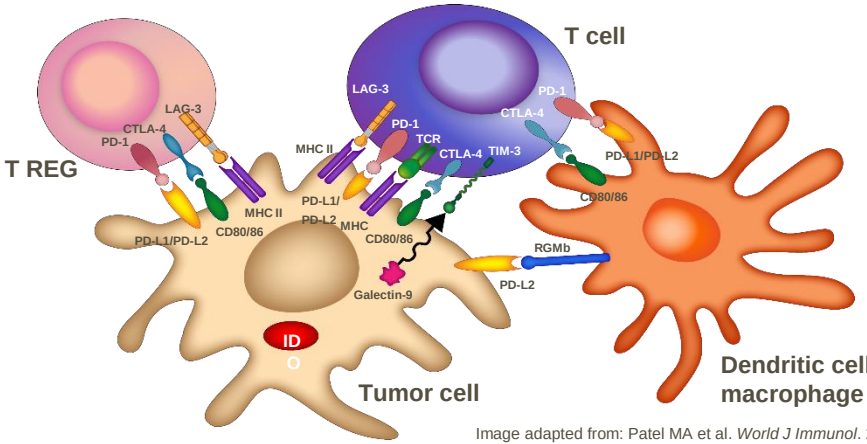


Image adapted from: Patel MA et al. *World J Immunol*. 2015;5(1):1-15.
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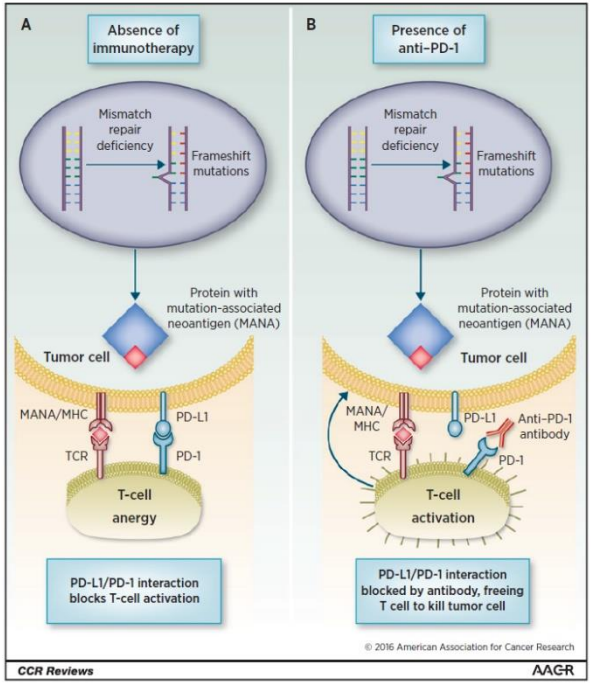

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1. Dudley JC et al. Clin Cancer Res. 2016;22:813-820. 2. Patel MA et al. World J Immunol. 2015;5:1-15.

Determining dMMR/MSI Status Can Identify Patients Who May Benefit From PD-1/L1 Inhibition

- 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