Integration of Multidisciplinary Team in Diagnosing Lynch Syndrome and Early Surveillance

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Objectives

- Review Lynch Syndrome mismatch repair gene variants
- Discuss the integration of hereditary cancer syndrome screening recommendations into clinical practice
- Discuss the utilization of clinical history and germline mutations in determining appropriate surveillance strategies

No disclosures or conflicts-of-interest

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54-year-old Female

- Surveillance with WVU Oncology for PMH of Stage IIA Colon Cancer
- PMH of endometrial adenocarcinoma
- Previously documentation and genetic testing consistent with a diagnosis of Lynch syndrome
- Family history is negative for malignancy. Patient's daughter has been diagnosed with Lynch Syndrome by a genetic counselor
- Social history is negative for tobacco or alcohol use

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April 2022 – Stage IA Grade I Endometrioid adenocarcinoma (pT1aN0)

Final Pathologic Diagnosis:

A. Endometrial Curettings: **Endometrioid Adenocarcinoma, FIGO Grade 1.**

B. Endocervical Curettings: **Minute Fragments Of Benign Appearing Endocervical Mucosa.**

bk/4/4/2022 ***Electronically Signed Out By*** Busaina Khalil, MD

Peer Review Pathologist(s):
Michael M Yousef, MD

March 2022

FINAL DIAGNOSIS
SEVEN REACTIVE LYMPH NODES, NEGATIVE FOR MALIGNANCY (0/7).

D. UTERUS, CERVIX, BILATERAL ADNEXA; TOTAL LAPAROSCOPIC HYSTERECTOMY AND BILATERAL SALPINGO-OOPHORECTOMY:
CHRONIC INACTIVE CERVICITIS.
NABOTHIAN CYSTS.
PROLIFERATIVE ENDOMETRIUM WITH FOCAL ATYPICAL COMPLEX HYPERPLASIA.
ADENOMYOSIS.
INTRAMURAL LEIOMYOMA.
BILATERAL OVARIES WITH NO SPECIFIC PATHOLOGIC CHANGES.
BILATERAL FALLOPIAN TUBES WITH BENIGN PARATUBAL CYSTS, SEE COMMENT.

COMMENT: The patient's history of endometrial curetting showing FIGO grade 1 endometrioid adenocarcinoma of the endometrium is noted (diagnosis made at Wheeling Hospital, Accession #: S22-3580). In this hysterectomy specimen, the endometrium is entirely submitted for histologic examination. No residual adenocarcinoma is seen. Outside slides will be reviewed; the results including a cancer case summary will be issued in an addendum report. Clinical correlation is recommended.

E. LYMPH NODES, LEFT PERIAORTIC, LYMPHADENECTOMY:
TWO REACTIVE LYMPH NODES, NEGATIVE FOR MALIGNANCY (0/2).

April 2022 – Stage IA Grade I Endometrioid adenocarcinoma (pT1aN0)

Addendum Report

Addendum Explanation
Summary of Results: Findings strongly suggestive of a germline related endometrial carcinoma associated with Lynch Syndrome (see comment).
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Comment: This tumor shows deficiency of PMS2 by Immunohistochemistry and microsatellite instability by PCR testing. Deficient PMS2 function is strongly suggestive of a germline-related mutation which is associated with Lynch Syndrome. Genetic counseling is recommended followed by a germline sequencing//analysis of PMS2 to assess for definitive diagnosis of Lynch Syndrome. Consider MLH1 mutation analysis if PMS2 is negative for mutations. Patient informed consent is required for genetic analysis. Germline mutation analysis is performed at Integrated Oncology and peripheral blood is required for testing.

See report signed by Suzanne Martin, M.D. at Accupath Diagnostic Laboratories, Inc.

DNA Mismatch Repair Protein Analysis by IHC (Integrated Oncology Reference #: BC22-001101).

DIAGNOSIS:

These results show absent expression of the mismatch repair protein PMS2 and are suggestive for a deficient DNA mismatch repair function, which is often associated with microsatellite instability. A MSI assay by PCR is currently pending (see case BMM22-000528). Deficient PMS2 function is strongly suggestive of a germline-related mutation which is associated with Lynch Syndrome. Genetic counseling is recommended followed by a germline sequencing/analysis of PMS2 to assess for definitive diagnosis of Lynch Syndrome. Consider MLH1 mutation analysis is PMS2 is negative for mutations. Patient informed consent is required for genetic analysis. Germline mutation analysis is performed at Integrated Oncology and peripheral blood is required for testing.

Immunohistochemistry:

Antibody:	Results:
MLH-1	Preserved (intact)
MSH-2	Preserved (intact)
MSH-6	Preserved (intact)
PMS-2	Complete loss

See report signed by Suzanne Martin, M.D. at Accupath Diagnostic Laboratories, Inc.

Detection of Microsatellite Instability by PCR (Integrated Oncology Reference #: BMM22-000528).

INTERPRETATION:
These results show high frequency microsatellite stability (MSI-H).

Microsatellite Marker:	Result:
BAT25	Unstable
BAT26	Unstable
MONO-27	Stable
NR-21	Unstable
NR-24	Stable

Instability is reported as either high (MSI-H) or low (MSI-L) or stable (MSS) dependent upon the number of markers analyzed that show instability. The reference range for instability is interpreted as follows:

	MSI-H	MSI-L	MSS
Markers show instability	≥ 2	1 of 5	0 of 5

Summary of Results
Findings strongly suggestive of a germline related endometrial carcinoma associated with Lynch Syndrome (see comment).

Summary Comment
This tumor shows deficiency of PMS2 by Immunohistochemistry and microsatellite instability by PCR testing. Deficient PMS2 function is strongly suggestive of a germline-related mutation which is associated with Lynch Syndrome. Genetic counseling is recommended followed by a germline sequencing /analysis of PMS2 to assess for definitive diagnosis of Lynch Syndrome. Consider MLH1 mutation analysis if PMS2 is negative for mutations. Patient informed consent is required for genetic analysis. Germline mutation analysis is performed at Integrated Oncology and peripheral blood is required for testing.

Pembrolizumab is indicated for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.

Electronically Signed by Suzanne Martin, M.D. at 0910 ET on 05/15/2022 at Accupath Diagnostic Laboratories, Inc.
Suzanne Martin, M.D.
Pathologist

October 2022 – Stage IIA Colon cancer (pT3pN0) of the ascending colon

Final Diagnosis

Right Colon And Appendix: Moderately Differentiated Adenocarcinoma Invading Through Muscularis Propria Focally Into Surrounding Adipose Tissue.
All margins are uninvolved by carcinoma.
Nine Lymph Nodes, Negative For Carcinoma (0/9).
Appendix With Mild Acute Appendicitis And Fibrofatty Obliteration Of The Tip.
See synoptic report for details.

TUMOR	
Tumor Site	Ascending colon
Histologic Type	Adenocarcinoma
Histologic Grade	G2, moderately differentiated
Tumor Size	Greatest dimension (Centimeters): 5.2 cm
Tumor Extent	Invades through muscularis propria into pericolorectal tissue
Macroscopic Tumor Perforation	Not identified
Lymphovascular Invasion	Not identified
Perineural Invasion	Not identified
Treatment Effect	No known presurgical therapy

MARGINS	
Margin Status for Invasive Carcinoma	All margins negative for invasive carcinoma
Closest Margin(s) to Invasive Carcinoma	Distal
Distance from Invasive Carcinoma to Closest Margin	5.6 cm

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-------------------------- FINAL DIAGNOSIS --------------------------
RIGHT COLON AND APPENDIX:
PMS2:       LOSS OF EXPRESSION
MLH1:  EXPRESSED
MSH2:       EXPRESSED
MSH6:       EXPRESSED
SEE COMMENTS

COMMENTS:
Abnormal loss of immunohistochemical expression of PMS2 observed.
This pattern is most commonly seen with PMS2 deficiency.
Immunohistochemical expression of MLH1, MSH2 and MSH6 was detected.
Control tissues showed adequate staining for all four markers.
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MICROSATELLITE INSTAB-MSI POSITIVE

Comment: The DNA demonstrated a high frequency of microsatellite instability (MSI-H): three of the five markers showed instability. Germline mutation analysis for the mismatched repair genes is recommended.

October 2022 – Stage IIA Colon cancer

→ *CARIS SEPT 2023*

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
Mismatch Repair Status	**IHC**	**Protein**	**Deficient (Loss)**	**BENEFIT**	**dostarlimab, pembrolizumab**	**Level 1**
					nivolumab, nivolumab/ipilimumab combination	**Level 2**
MSI	**Seq**	**DNA-Tumor**	**High**	**BENEFIT**	**nivolumab, nivolumab/ipilimumab combination, pembrolizumab**	**Level 2**
TMB	**Seq**	**DNA-Tumor**	**High, 20 mut/Mb**	**BENEFIT**	**pembrolizumab**	**Level 2**
KRAS	Seq	DNA-Tumor	Pathogenic Variant Exon 4 | p.K117N	LACK OF BENEFIT	cetuximab, panitumumab	Level 2

* Biomarker reporting classification: Level 1 – Companion diagnostic (CDx); Level 2 – Strong evidence of clinical significance or is endorsed by standard clinical guidelines; Level 3 – Potential clinical significance. Bolded benefit therapies, if present, highlight the most clinically significant findings.

Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
PMS2	Seq	DNA-Tumor	Pathogenic Variant	c.705+1G>A	6	c.705+1G>A	81
PPP2R1A	Seq	DNA-Tumor	Pathogenic Variant	p.R183W	5	c.547C>T	26
PTEN	Seq	DNA-Tumor	Pathogenic Variant	p.L57fs	3	c.170delT	12
	Seq	DNA-Tumor	Pathogenic Variant	p.K267fs	7	c.800delA	14

Unclassified alterations for DNA sequencing can be found in the MI Portal.
Formal nucleotide nomenclature and gene reference sequences can be found in the Appendix of this report.

Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
ERBB2 (Her2/Neu)	Negative | 0	MSH6	Intact nuclear expression
Mismatch Repair Status	Deficient (Loss)	PD-L1 (SP142)	Negative | 0%
MLH1	Intact nuclear expression	PMS2	Loss of nuclear expression
MSH2	Intact nuclear expression	PTEN	Positive | 1+, 60%

Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
PIK3CA	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 21 | p.H1047R**
		DNA-Tumor	Variant of Uncertain Significance Exon 8 | p.P458R
PTEN	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 3 | p.L57fs**
		DNA-Tumor	**Pathogenic Variant Exon 7 | p.K267fs**
	IHC	Protein	Positive | 1+, 60%
	CNA-Seq	DNA-Tumor	Deletion Not Detected
BRAF	Seq	DNA-Tumor	Mutation Not Detected
		RNA-Tumor	Fusion Not Detected
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected

Biomarker	Method	Analyte	Result
RET	Seq	RNA-Tumor	Fusion Not Detected
EGFR	CNA-Seq	DNA-Tumor	Amplification Not Detected
	Seq	DNA-Tumor	Mutation Not Detected
ERBB2 (Her2/Neu)	IHC	Protein	Negative | 0
	CNA-Seq	DNA-Tumor	Amplification Not Detected
KRAS	CNA-Seq	DNA-Tumor	Amplification Not Detected
NF1	Seq	DNA-Tumor	Variant of Uncertain Significance Exon 18 | p.M693T
NRAS	Seq	DNA-Tumor	Mutation Not Detected

Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	High
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	Low - 3% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

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INVITAE MULTI-CANCER PANEL/LYNCH SYNDROME PANEL RESULTS

RESULT: POSITIVE

One Likely Pathogenic variant identified in PMS2. PMS2 is associated with autosomal dominant Lynch syndrome and autosomal recessive constitutional mismatch repair deficiency syndrome.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
PMS2	c.705+1G>A (Splice donor)	heterozygous	Likely Pathogenic

About this test

This diagnostic test evaluates 5 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic geneti testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

A Likely Pathogenic variant, c.705+1G>A (Splice donor), was identified in PMS2.

- The PMS2 gene is associated with autosomal dominant Lynch syndrome (also called hereditary nonpolyposis colorectal cancer syndrome, or HNPCC) (MedGen UID: 325005) and autosomal recessive constitutional mismatch repair deficiency syndrome (CMMR-D) (MedGen UID: 78553).
- This result is consistent with a predisposition to, or diagnosis of, autosomal dominant PMS2-related conditions.
- PMS2-related Lynch syndrome is characterized by an increased risk of colorectal cancer as well as cancers of the endometrium, ovaries, urinary tract, bladder, biliary tract, brain, and possibly prostate, small bowel, and pancreas. Estimated lifetime cancer risks appear to be lower than those seen with other mismatch repair genes and include a 9-20% risk for colorectal cancer, up to a 3.7% risk of urinary tract cancer, up to a 2.4% risk of bladder cancer, up to a 1% risk of biliary tract cancer, and up to a 1% risk of brain/nervous system cancer. Affected females also have an increased risk of uterine/endometrial cancer (13–26%) and ovarian cancer (3%) (PMID: 18602922, 30161022, 31204389, 28754778, 26657901, 31337882).

  CMMR-D is a childhood-onset condition characterized by an increased risk of developing blood cancers, brain tumors, and very early-onset colorectal cancer. Nearly all affected individuals have clinical signs reminiscent of neurofibromatosis type 1 (NF1) such as cafe-au-lait spots (PMID: 18709565, 20442441, 17539897).
- Biological relatives have a chance of being at risk for autosomal dominant PMS2-related conditions and have a chance of being carriers for autosomal recessive PMS2-related conditions. Those at risk should consider testing.

December 2023 – Recurrent Grade 3 Endometrial Cancer

Final Diagnosis

PERIRECTAL MASS, ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE BIOPSY:

- **Endometrioid carcinoma with squamous differentiation, FIGO grade 3. See comment.**
- **MMR deficient (isolated loss of expression of PMS2). See comment.**

Synoptic Report

DNA Mismatch Repair Biomarker Testing (DNA MISMATCH REPAIR BIOMARKER TESTING FOR CHECKPOINT INHIBITOR IMMUNOTHERAPY - All Specimens) Protocol posted: 6/30/2021

DNA MISMATCH REPAIR TESTING	
Specimen Site	Perirectal mass
Testing Performed on Block Number(s)	A1
Immunohistochemistry (IHC) Results for Mismatch Repair (MMR) Proteins	
MLH1 Result	Intact nuclear expression
Immunohistochemistry (IHC) Results for Mismatch Repair (MMR) Proteins	
MSH2 Result	Intact nuclear expression
Immunohistochemistry (IHC) Results for Mismatch Repair (MMR) Proteins	
MSH6 Result	Intact nuclear expression
Immunohistochemistry (IHC) Results for Mismatch Repair (MMR) Proteins	
PMS2 Result	Loss of nuclear expression
Immunohistochemistry (IHC) Results for Mismatch Repair (MMR) Proteins	Background non-neoplastic tissue / internal control shows intact nuclear expression
Mismatch Repair (MMR) Interpretation	Loss of nuclear expression of one or more MMR proteins: deficient mismatch repair

The presence of MSI-H / deficient mismatch repair may also be an indication for additional testing for Lynch syndrome and genetic counselling.

Heterogeneous expression of MLH1 and PMS2 has been infrequently encountered in endometrial carcinomas (up to 3% of cases). The incidence of heterogeneous expression in other cancer types and its impact on predicting sensitivity to checkpoint inhibition is not currently known.

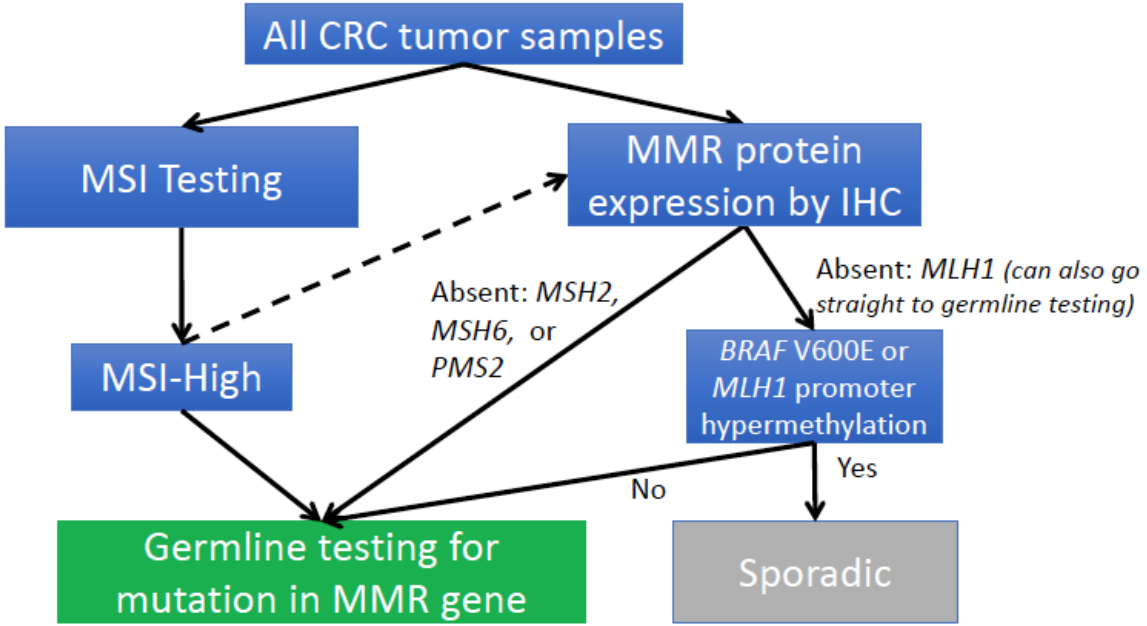
Timeline
54-year-old Female

- April 2022 – Stage IA Grade I Endometrioid adenocarcinoma (pT1aN0) [*Mon General*]
  - TLH, BSO, BPSLND and Laparoscopic staging
  - No adjuvant treatment
  - Microsatellite instability suggestive of Lynch Syndrome
  - Denied further genetic testing at that time
- October 2022 – Stage IIA Colon cancer (pT3pN0) of the ascending colon [*Wheeling Cancer Center*]
  - s/p robotic laparoscopic right colon resection
  - IHC revealed **MMR deficient (loss of PMS2)** and PCR showed **MSI-High**
  - Persistent CEA elevation 4 months post op and PET revealed an 18 mm right pelvic LN (SUV 7.7) → negative for malignancy later
  - Continues to have persistent CEA elevation between 5 – 7.5 and negative ct-DNA
- December 2023 – Recurrent Grade 3 Endometrial Cancer
  - PET 1.8 x 2.1 cm soft tissue lesion abutting to right sided rectosigmoid wall and 0.4 cm local LN (measured 8 mm on EUS)
  - Currently receiving carboplatin/taxol/pembrolizumab week, then re-evaluate

Lynch Syndrome Genetics

- Autosomal Dominant Inheritance
- Germline mutation in mismatch repair (MMR) genes
  - Majority (~80%) of cases are due to mutations in MLH1and MSH2 leading to classic variation
  - PMS2 <5% of cases → Attenuated, lower risks and later age of onset
- >90% of tumors have microsatellite instability (MSI-high) and/or lack expression of an MMR protein
  - IHC typically performed on colon and/or endometrial tumors
  - Can also be performed on colorectal adenomas and metastatic sites

Algorithm for LS Testing



Credited to MD Anderson Review Series 2023 – Cancer Genetic Risk Factors Claudine Issacs

PMS2 Associated Lynch Syndrome

- Multiple heterozygous PMS2 mutations have been documented
- Difficulty identifying PMS2 mutation carriers because of a markedly lower penetrance
- J Clin Oncol. 2019 Mar 20;37(9):761]. PMS2 mutation carriers were at increased risk for colorectal cancer *(cumulative risk to age 80 years of 13% [95% CI, 7.9% to 22%] for males and 12% [95% CI, 6.7% to 21%] for females)* and endometrial cancer *(13% [95% CI, 7.0%-24%]),* compared with the general population (*6.6%, 4.7%, and 2.4%, respectively)*
- There was no clear evidence of an increased risk of ovarian, gastric, hepatobiliary, bladder, renal, brain, breast, prostate, or small bowel cancer.

Ten Broeke SW, van der Klift HM, Tops CMJ, et al. Cancer Risks for PMS2-Associated Lynch Syndrome [published correction appears in J Clin Oncol. 2019 Mar 20;37(9):761]. J Clin Oncol. 2018;36(29):2961-2968. doi:10.1200/JCO.2018.78.4777

PMS2 Associated Lynch Syndrome

- Lynch Syndrome associated with PMS2 has a lower risk of malignancy compared to MLH1 and MSH2
- Multiple heterozygous PMS2 mutations with variable penetrance demonstrate the need for continued surveillance

	Colon Cancer	Endometrial Cancer
Carrier Screening Recommendations	Colonoscopy every 1-2 yrs beginning age 20-25 or 2-5 yrs prior to earliest CRC (if <25)	• Consider risk reducing hysterectomy and RRBSO once childbearing complete –timing individualized • Consider endometrial biopsy q 1-2yr starting age 30-35
Surveillance	• History and physical examination every 3–6 mo for 2 y, then every 6 mo for a total of 5 y • CEA every 3–6 mo for 2 y, then every 6 mo for a total of 5 y • C/A/P CT every 6–12 mo (category 2B for frequency <12 mo) from date of surgery for a total of 5 y	Physical exam (including pelvis) every 3–6 mo for 2–3 y, then every 6–12 mo for up to year 5, then annually

54-year-old Female

- Clinical decision making based on persistent elevation of CEA and germline testing consistent with Lynch Syndrome led to strict surveillance
- Early diagnosis of endometrial cancer in a high-risk patient
- Patient's clinical history can be a consideration when discussing surveillance and preventative interventions with the patient's family, although varied penetrance needs to be considered

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Integration of Multidisciplinary Team in Diagnosing Lynch Syndrome and Early Surveillance

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