NCCN 2021 Virtual Congress:
Biomarkers in Solid Tumors

Friday, November 12, 2021
3:50 PM – 4:35 PM EST

Clinical Scenario #5: Applying Biomarkers in Colorectal Cancer

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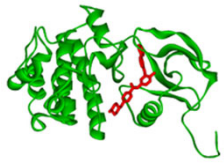
Case 1

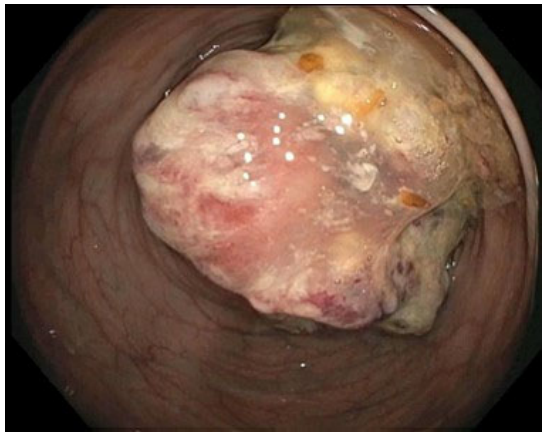
42 year-old male who is referred for a colonoscopy to evaluate iron deficiency anemia

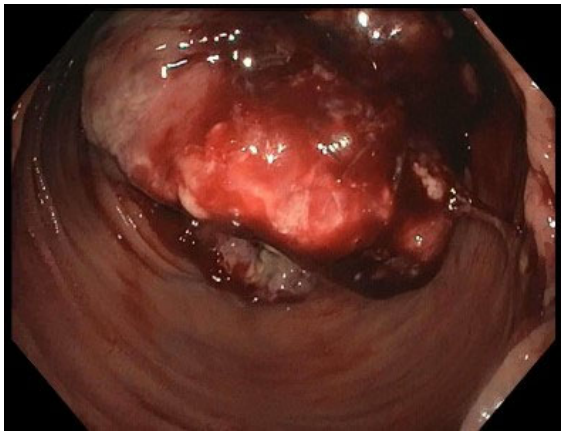
- No past medical history
- Family history: maternal aunt with uterine cancer at age 55



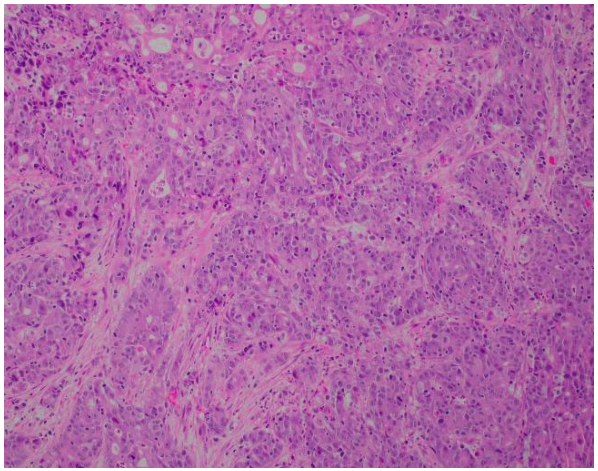
Colonoscopy

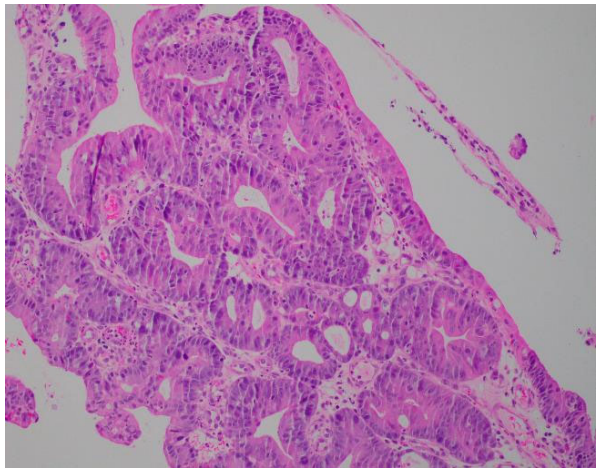
- Fungating mass located in the ascending colon – bled after biopsy
- No other mucosal lesions identified





Case 1: Pathology – Biopsy





Diagnosis: Invasive adenocarcinoma of the colon arising from a tubulovillous adenoma

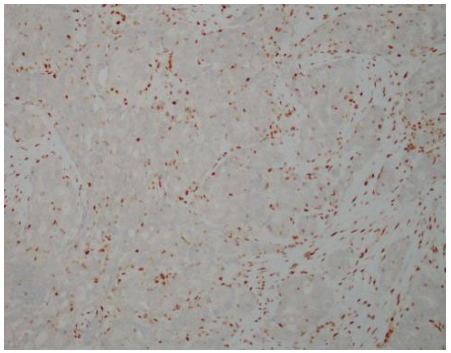
Polling Question

What biomarker(s) are recommended to perform on this patient sample?

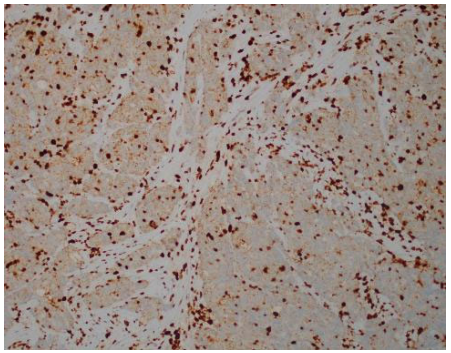
- Estrogen receptor immunohistochemistry
- APC mutation status
- Mismatch repair protein/Microsatellite Instability
- PD-L1 immunohistochemistry

MMR Immunohistochemistry

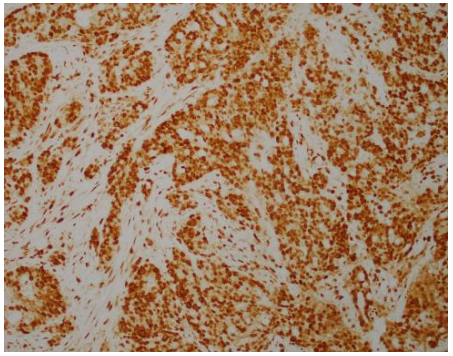
MLH1



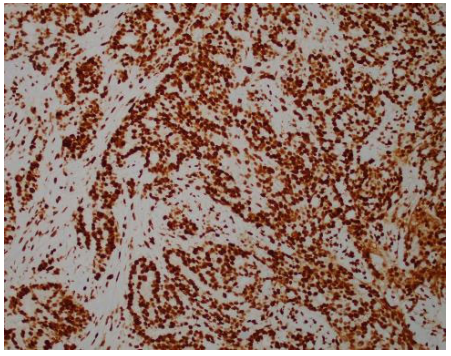
PMS2



MSH2



MSH6



MLH1/PMS2 Mismatch Repair (MMR) Deficient Tumor

Polling Question

What testing should be performed next?

- BRAF V600E
- P53
- HER2
- SDH1

BRAF V600E Testing → WT

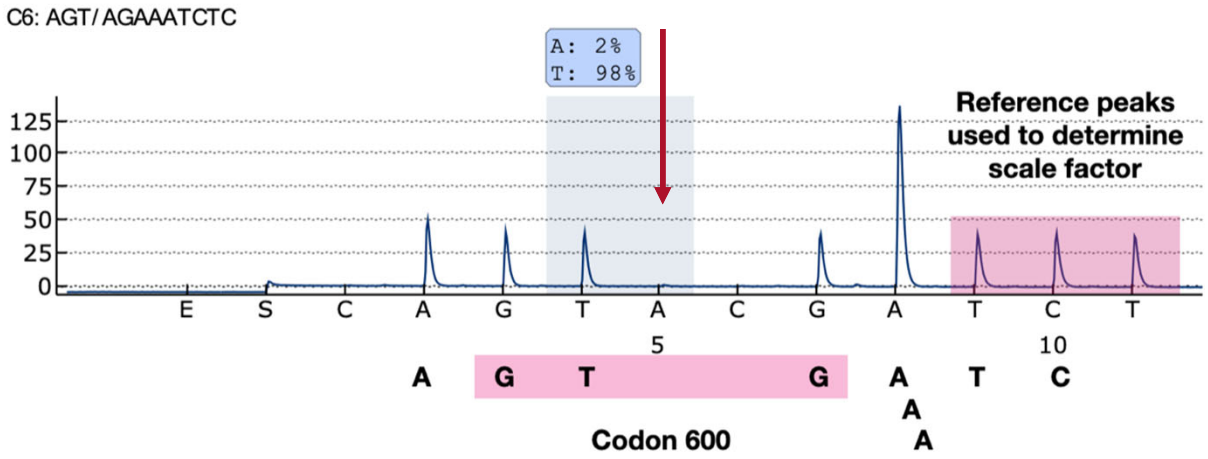
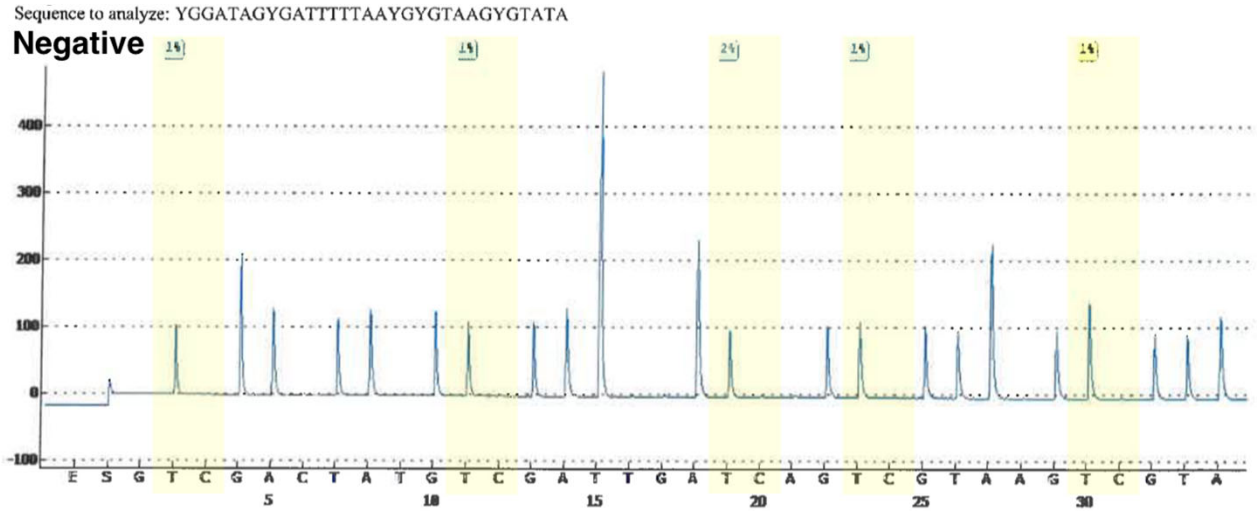


Image courtesy of Dr. Jesse Cox, UNMC

Negative MLH1 Promoter Test



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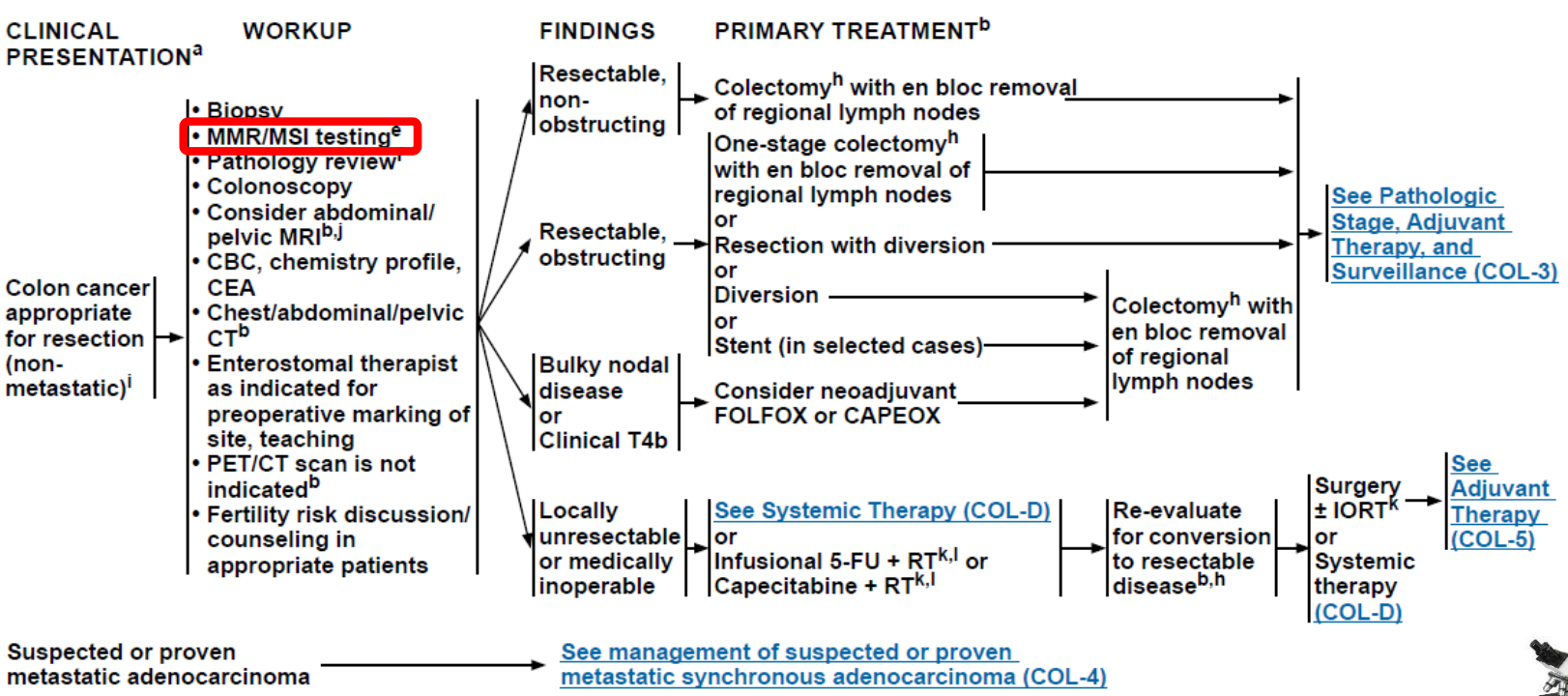
Colon Cancer



Mismatch Repair (MMR)

- MMR- proteins involved in DNA mismatch repair (MLH1, MSH2, MSH6, PMS2 and EpCAM)
  - Proteins form heterodimers in-vivo (MLH1/PMS2 & MSH2/MSH6)
  - Immunostaining is used on biopsy or resection specimens to look for loss/absence of proteins
- Should not be reported as positive/negative
- Common causes of protein expression loss:
  - MLH1 promoter hypermethylation
  - Germline genetic mutation of MMR genes
- Confirm internal positive controls are present (background normal epithelium or lymphocytes)

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PRINCIPLES OF dMMR TESTING FOR LYNCH SYNDROME

Pros and Cons of Universal Tumor Screening with IHC and/or MSI for LS Using Colonoscopy-Based Biopsy Versus Surgical Resection Specimen[19,20]

Pre-Surgical Testing Considerations

- Pros
  - Informs surgical decision-making (subtotal vs. segmental resection)
  - For rectal tumors requiring neoadjuvant chemotherapy and RT, IHC is more reliable when done on pre-radiation therapy specimens[21,22]
  - Allows for Lynch syndrome screening of patients with rectal cancer who elect for neoadjuvant therapy or nonoperative management
- Cons
  - Possibility of insufficient tissue for analysis
  - Screening could be done twice (once on biopsy and once on surgical resection), thereby decreasing cost-effectiveness

Post-Surgical Testing Considerations

- Pros
  - Larger specimen allows for higher chance of informative dMMR testing
  - Ensures test is only done once
- Cons
  - Cannot inform surgical decision-making
  - In rectal tumors exposed to neoadjuvant chemotherapy and RT, IHC may be less reliable, with the potential for false-negative result (particularly *MSH6*)

Pros and Cons of Universal Tumor Screening with IHC and/or MSI for LS Using Endometrial Biopsy Versus Surgical Resection

Pre-surgical Testing Considerations

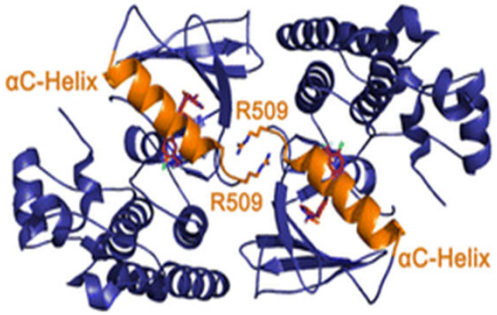
- Pros
  - Informs surgical decision-making (salpingo-oophorectomy vs. salpingectomy)
  - For endometrial tumors treated with progestin therapy, there may not be residual tumor at hysterectomy
  - Some patients may not undergo hysterectomy
- Cons
  - Possibility of insufficient tissue for analysis

Post-surgical Testing Considerations

- Pros
  - Larger specimen allows for higher chance of informative dMMR testing
- Cons
  - Possibility of insufficient tissue for diagnosis due to treatment response or complete resection at endometrial sampling. In these cases, the preoperative biopsy specimen may be tested for evidence of dMMR

BRAF V600E Testing

- 69% of MLH1 methylated colorectal cancers (CRCs) have substitution of the valine with glutamic acid at amino acid position 600 of BRAF
- BRAF V600E almost never found in Lynch syndrome
- Testing for BRAF V600E is a cost effective way to distinguish sporadic CRC from LS when MLH1/PMS2 loss/absent



Grasso et al. "ACS Chem Biol." 2016 Oct 21;11(10):2876-2888

BRAF PCR

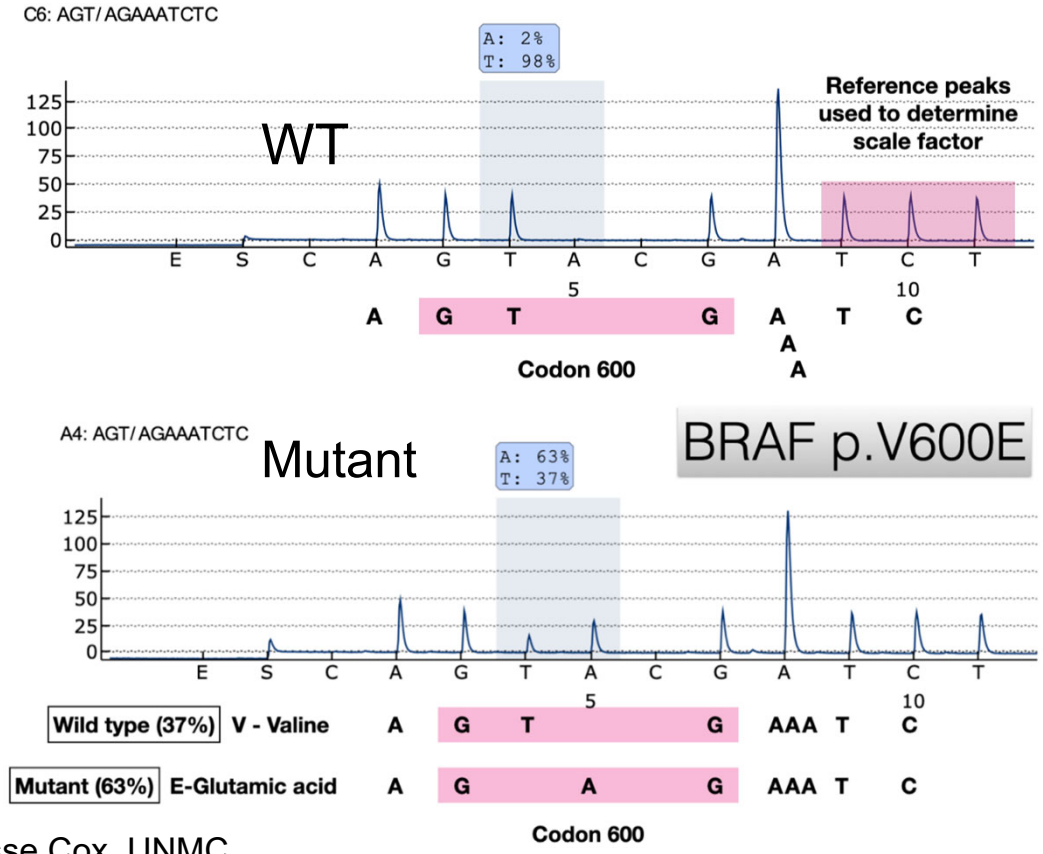


Image courtesy of Dr. Jesse Cox, UNMC

MLH1 Promoter Hypermethylation

If BRAF testing is done by itself and is normal → consider MLH1 promoter methylation next prior to germline testing or move straight to paired germline MMR/somatic tumor testing (which often includes MLH1 methylation testing)

###The goal is to decrease referral to genetic counseling

Adar et al. "A tailored approach to BRAF and MLH1 methylation testing in a universal screening program for Lynch syndrome." Modern Pathology Volume 30, pages 440–447 (2017)

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Lynch Syndrome

PRINCIPLES OF dMMR TESTING FOR LYNCH SYNDROME

- The panel recommends universal screening of all CRCs and endometrial cancers to maximize sensitivity for identifying individuals with Lynch syndrome (LS) and to simplify care processes. The panel also recommends considering tumor screening for MMR deficiency for sebaceous neoplasms as well as the following adenocarcinomas: small bowel, gastric, pancreas, biliary tract, brain, bladder, urothelial, and adrenocortical cancers regardless of age at diagnosis. Counseling by an individual with expertise in genetics is not required prior to routine tumor testing. An infrastructure needs to be in place to handle the screening results.

General

- IHC and MSI analyses are screening tests (either by themselves or in conjunction) that are typically performed on colorectal and endometrial cancer tissue to identify individuals at higher risk for having LS. Greater than 90% of LS tumors are MSI-H and/or lack expression of at least one of the MMR proteins by IHC. Ten percent to 15% of sporadic colon cancers exhibit abnormal IHC and are MSI-H most often due to abnormal methylation of the *MLH1* gene promoter, rather than due to LS. Mutant *BRAF* V600E is found in many sporadic MSI-H CRCs and is rarely found in LS-related CRCs. There are some tumors that will have *MLH1* methylation but lack a *BRAF* pathogenic variant. Thus, the presence of an abnormal *MLH1* IHC test increases the possibility of LS but does not make a definitive diagnosis. Confirmed diagnosis of LS is based on germline testing, when tumor-based testing scenarios or other factors raise suspicion for the diagnosis (see See LS-A 6 of 8). Also, sporadic endometrial cancers may exhibit abnormal MSI/IHC due to abnormal methylation of the *MLH1* promoter. Somatic MMR genetic testing of the corresponding gene(s) (see "Plausible Etiologies" for possibilities on LS-A 6 of 8) could be performed on tumor DNA to assess for pathogenic variants that might explain the abnormal IHC and/or MSI-H results.
- The panel recommends a universal screening strategy be the primary approach to identify CRC patients with LS. However, in other lower resource settings, other historic criteria for selecting patients for testing may be relevant. The Bethesda criteria (See Discussion) are intended to help identify CRC patients whose tumors should be tested for MMR defects, by MSI and/or IHC analysis, thereby identifying patients with a greater chance of having LS.

IHC

- IHC refers to staining tumor tissue for protein expression of the 4 MMR genes known to be mutated in LS: *MLH1*, *MSH2*, *MSH6*, and *PMS2*. A normal IHC test implies all 4 MMR proteins are normally expressed, and thus it is unlikely that an underlying MMR gene pathogenic variant is present. An abnormal test means that at least one of the proteins is "not detected," and an inherited pathogenic variant may be present in the related gene. Loss of protein expression by IHC in any one of the MMR genes guides further genetic testing (pathogenic variant detection) to the gene(s) where protein expression is not observed or to the corresponding protein dimer. Absent expression of one or more of the 4 DNA MMR proteins is often reported as abnormal or "positive" IHC. When "positive" IHC is reported, caution should be taken in making sure that positive refers to absence of MMR protein expression, and not to presence of expression.
- Abnormal *MLH1* IHC should be followed by either germline genetic testing or tumor testing for *MLH1* methylation for colorectal or endometrial cancers. Alternatively for colorectal cancers with loss of *MLH1* on IHC, the tumor can be tested for a *BRAF* V600E pathogenic variant. Testing for *BRAF* pathogenic variants using IHC is not sufficiently sensitive in general but it may be an option for situations with insufficient tumor material for molecular testing since it only requires one slide. Presence of *MLH1* hypermethylation, *BRAF* V600E pathogenic variant, or abnormal *BRAF* V600E protein by IHC is consistent with sporadic cancer. If *MLH1* promoter methylation or *BRAF* testing is normal, or negative, germline genetic testing is indicated (See LS-A 6 of 8). Those with a germline pathogenic variant are then identified as LS patients. *BRAF* V600E pathogenic variants are found in 69% of methylated colorectal cancers, so the absence of a *BRAF* V600E pathogenic variant does not rule out methylation. As a result, there may be a role for methylation testing to rule out LS in MSI-H tumors in which no *BRAF* pathogenic variant is found either prior to genetic testing or in the event genetic testing is negative. If abnormal IHC is followed by germline testing and no LS-causing pathogenic variants are identified, the panel strongly recommends proceeding with *MLH1* methylation analysis of the tumor. Patients who have normal germline testing and *MLH1* hypermethylation are likely to have sporadic cancer and should be treated as such taking into account their family history.
- If clinical suspicion for LS is high despite a normal IHC screening result, consider genetic evaluation and testing.
- There is a 5%–10% false-negative rate with IHC testing.

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PRINCIPLES OF dMMR TESTING FOR LYNCH SYNDROME

MSI

- MSI-H in tumors refers to the tumor having a proportion of alterations in a predetermined panel of microsatellite repeat markers that indicates the loss of MMR activity. Its significance, use, and implications are similar to that of IHC, although the tests are slightly complementary.
- Laboratories vary in their approach in testing MSI. Dinucleotide markers may be less specific than mononucleotide markers of MSI.[11]
- There is a 5%–15% false-negative rate with MSI testing.

General Principles of MSI Detection by PCR[12,13]

- In this method, MSI is identified by PCR amplification of microsatellite repeats, followed by either electrophoresis or liquid chromatography.
- Various panels exist that range from testing five (Bethesda/NCI) to seven (Promega) unique microsatellite loci.
- The Bethesda/NCI panel consists of two mononucleotide loci (BAT-25 and BAT-26) and three dinucleotide loci (D2S123, D5S346, and D17S250).
- The Promega panel consists of five mononucleotide loci (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) as well as two pentanucleotide loci (used for specimen identification).
- MSI is identified when a microsatellite in the tumor has changed in size compared to the patient's normal control.
- Using the Bethesda/NCI method, tumors are classified as microsatellite stable (MSS) (zero loci show a change in size/are unstable), microsatellite instability-low (MSI-L) (one locus shows a change in size/are unstable), or MSI-H (two or greater loci show a change in size/are unstable)
- Using the Promega method, tumors are classified as MSS (zero or one loci show a change in size/are unstable) or MSI-H (two or greater loci show a change in size/are unstable).
- The estimated specificity of the detection of LS by PCR-based methods for MSI is 90.2% (95% CI, 87.7%–92.7%).
- The estimated sensitivity of the detection of LS by PCR-based methods for MSI is 85% (95% CI, 75%–92%).

Microsatellite Instability (MSI)

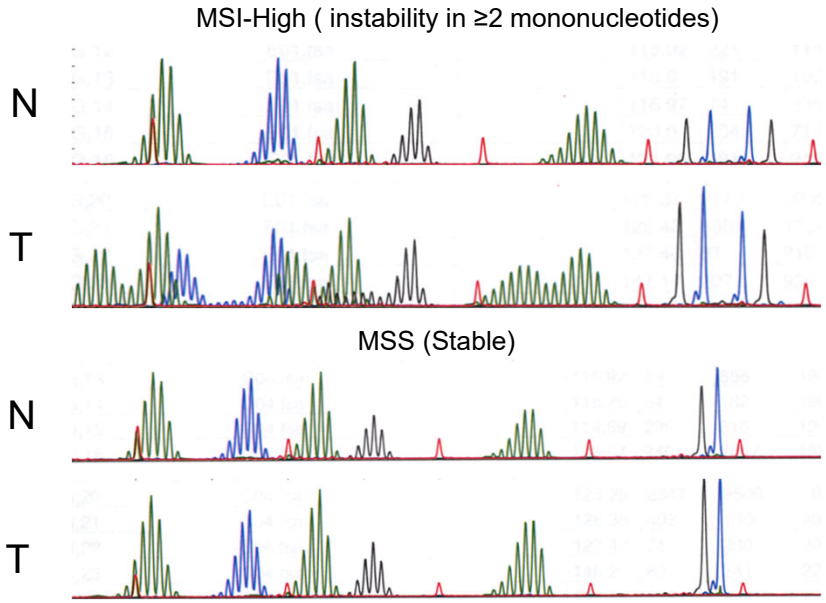

Image courtesy of Dr. Jesse Cox, UNMC

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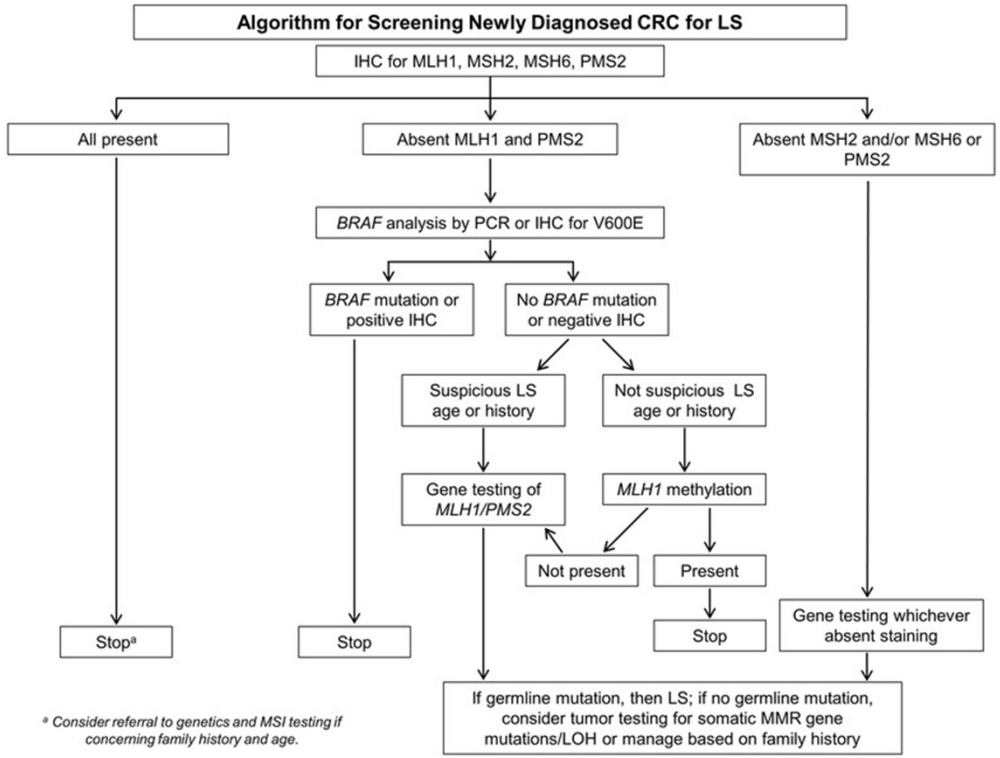
PRINCIPLES OF dMMR TESTING FOR LYNCH SYNDROME

General Principles of Next-Generation Sequencing (NGS) Testing for MSI[13-18]

- MSI can be detected through bioinformatic analysis of NGS.
- Rather than 5–8 microsatellite foci analyzed (as performed in MSI by PCR), NGS can analyze anywhere from dozens to hundreds of microsatellites.
- MSI is determined by comparing the length distribution and variation of a selection of microsatellite loci within a tumor and determining a differential as compared to the read counts of all normal alleles within a distribution.
- The size of microsatellite loci can include pentamers, tetramers, trimers, dimers, and monomers.
- Various comparative methods exist to identify MSI: tumor vs. paired normal or tumor vs. baseline normal
- Sophisticated bioinformatics protocols are necessary to use NGS as a method for MSI.
- Depending on the bioinformatic program used, analysis may be of whole exome sequencing data, whole genome sequencing data, or targeted genomic sequencing data.
- Tumor mutational burden (TMB) can be used as a surrogate to some degree for MSI, but there are causes of increased TMB other than dMMR.
- Further studies are needed to determine the sensitivity and specificity compared to MMR IHC and MSI by PCR.
- Any patient with a tumor that demonstrates MSI-H by NGS should be referred to a cancer geneticist for germline MMR testing.
- MSI by NGS does not require confirmation by more traditional measurement of MSI by PCR or IHC if the laboratory has validated the assay for use in the cancer in which it is being used.

Many Algorithms Exist for LS Workup



From Chen, Swanson and Frankel. "Molecular genetics of microsatellite-unstable colorectal cancer for pathologists."Diagn Pathol. 2017; 12: 24

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Lynch Syndrome

TUMOR TESTING RESULTS AND ADDITIONAL TESTING STRATEGIES[i]

Tumor Testing[a]							Plausible Etiologies	Additional Testing[e,f] NOTE: If younger than age 50 regardless of LS test results, consider genetic evaluation
IHC				MSI[b]	*BRAF* V600E[c]	*MLH1* Promoter Methylation		
MLH1	*MSH2*	*MSH6*	*PMS2*					
NL	NL	NL	NL	MSS	N/A	N/A	1) Sporadic cancer 2) Other (not LS hereditary CRC syndrome)	1) None[d]
NL	NL	NL	NL	MSI-H	N/A	N/A	1) Sporadic cancer 2) Germline pathogenic variant in any of the LS genes	1) Germline MMR testing or paired germline MMR/somatic MMR tumor testing;[g] 2) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[i]
N/A	N/A	N/A	N/A	MSI-H	N/A	N/A	1) Sporadic cancer 2) Germline pathogenic variant in any of the LS genes	1) Consider IHC analysis and additional testing depending on IHC results 2) If IHC not performed, consider germline MMR testing or paired germline MMR/somatic MMR tumor testing 3) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[i]
AB	NL	NL	AB	N/A	N/A	N/A	1) Sporadic cancer 2) Germline *MLH1* pathogenic variant or rarely *PMS2*	1) *BRAF* pathogenic variant testing[c]/*MLH1* promoter methylation testing first[j] 2) If *BRAF*/*MLH1* methylation testing normal, germline MMR testing or paired germline MMR/somatic MMR tumor testing;[g] 3) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[i]
AB	NL	NL	AB	N/A	Positive	N/A	1) Sporadic cancer 2) Rarely germline *MLH1* pathogenic variant or constitutional *MLH1* epimutation	1) None, unless young age of onset or significant family history; then consider constitutional *MLH1* epimutation testing[h] and/or germline MMR testing[g]
AB	NL	NL	AB	N/A	Negative	Positive	1) Sporadic cancer 2) Rarely germline *MLH1* pathogenic variant or constitutional *MLH1* epimutation	

N/A = Either testing was not done or results may not influence testing strategy; NL = Normal presence of positive protein staining; AB = Abnormal/Absence (negative) protein staining

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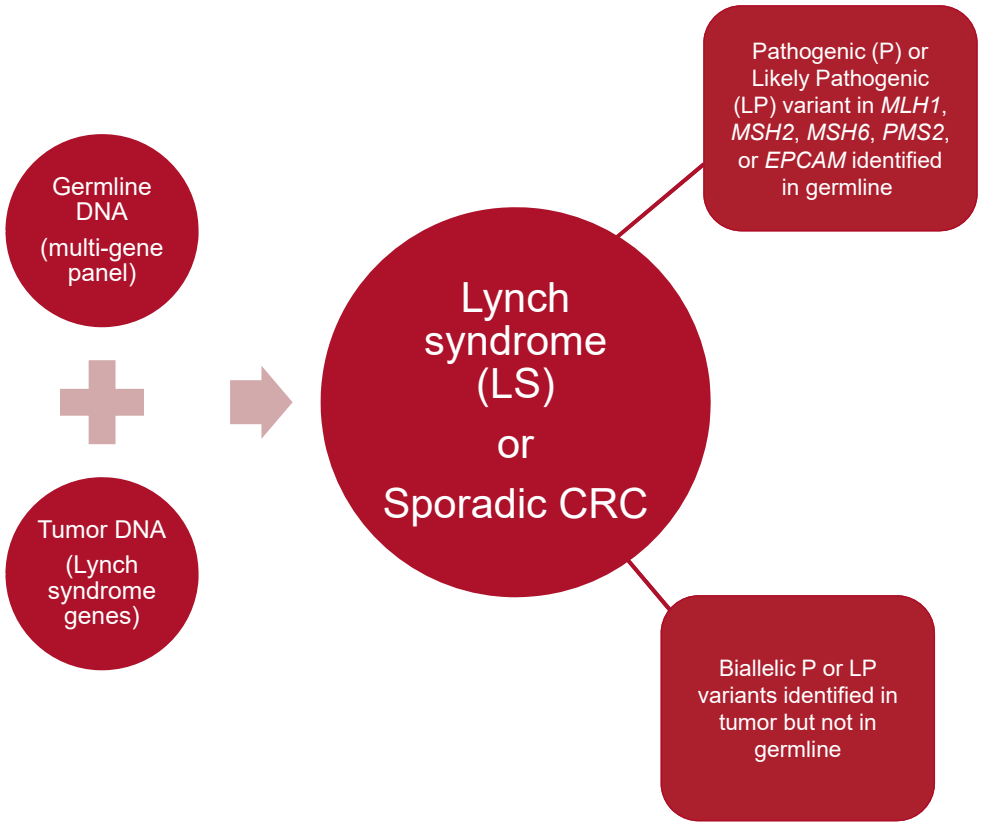
TUMOR TESTING RESULTS AND ADDITIONAL TESTING STRATEGIES[l]

Tumor Testing[a]							Plausible Etiologies	Additional Testing[e,f]	NOTE: If younger than age 50 regardless of LS test results, consider genetic evaluation
IHC				MSI	*BRAF* V600E[c]	*MLH1* Promoter Methylation			
MLH1	*MSH2*	*MSH6*	*PMS2*						
AB	NL	NL	AB	N/A	Negative	Negative	1) Germline *MLH1* pathogenic variant or rarely *PMS2* 2) Sporadic cancer	1) Germline MMR testing or paired germline MMR/somatic MMR tumor testing;[g] 2) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[l]	
NL	AB	AB	NL	N/A	N/A	N/A	1) Germline *MSH2/EPCAM* pathogenic variant; or rarely germline *MSH6* pathogenic variant 2) Sporadic cancer		
NL	NL	NL	AB	N/A	N/A	N/A	1) Germline *PMS2* pathogenic variant 2) Germline *MLH1* pathogenic variant 3) Sporadic cancer		
NL	AB	NL	NL	N/A	N/A	N/A	1) Germline *MSH2/EPCAM* pathogenic variant 2) Sporadic cancer		
NL	NL	AB	NL	N/A	N/A	N/A	1) Germline *MSH6* pathogenic variant 2) Germline *MSH2* pathogenic variant 3) Sporadic cancer/Treatment effect[k]	1) Germline MMR testing or paired germline MMR/somatic MMR tumor testing;[g] 2) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[l] 3) If applicable, consider MSI analysis or repeat IHC testing on nontreated tumor[k]	
AB	NL	NL	NL	N/A	N/A	N/A	1) Sporadic cancer; 2) Germline *MLH1* pathogenic variant; 3) Germline *PMS2* pathogenic variant; 4) Somatic *MLH1* or *PMS2* pathogenic variant	1) *BRAF* pathogenic variant testing[c]/*MLH1* promoter methylation;[j] 2) If *BRAF/MLH1* methylation testing normal, germline MMR testing or paired germline MMR/somatic MMR tumor testing;[g] 3) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[l]	
AB	AB	AB	AB	N/A	N/A	N/A	1) Germline pathogenic variant in *any* LS gene 2) Sporadic cancer	1) *BRAF* pathogenic variant testing[c]/*MLH1* promoter methylation AND Germline MMR testing or paired germline MMR/somatic MMR tumor testing (which often include *MLH1* methylation testing);[g] 2) If germline testing negative and paired somatic MMR genetic testing not done, consider somatic MMR genetic testing[l]	

N/A = Either testing was not done or results may not influence testing strategy; NL = Normal presence of positive protein staining; AB = Abnormal/Absence (negative) protein staining

Referral to Genetic Counseling

Paired Tumor/Germline Analysis



Case 1: Genetic Test Result

Germline *MLH1* pathogenic variant detected, consistent with Lynch syndrome

OVERALL SUMMARY

This individual's germline results are consistent with a diagnosis of Lynch syndrome. See below for additional information.

SEQUENCING AND DELETION/DUPLICATION RESULTS

GERMLINE ORIGIN

Gene	Variant	Classification/Effect
MLH1	p.G67E	Pathogenic Mutation

Germline Genes Analyzed: *MLH1, MSH2, MSH6, PMS2, APC, ATM, BARD1, BMPR1A, BRIP1, CDH1, CDKN2A, CHEK2, DICER1, MUTYH, NBN, PALB2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53, CDK4, NF1, BRCA1, BRCA2, MSH3, NTHL1, RECQL, SMARCA4, AXIN2* (sequencing and deletion/duplication); *POLD1, POLE, HOXB13* (sequencing only); *EPCAM, GREM1* (deletion/duplication only) .

SOMATIC ORIGIN

Gene	Variant	Classification/Effect
MLH1	p.L292M	Variant of Unknown Significance

Somatic Genes Analyzed: *MLH1, MSH2, MSH6, PMS2* (sequencing and deletion/duplication); *EPCAM* (deletion/duplication only) .

Genetic Counseling for Lynch Syndrome

Cancer Risk

- Increased risk for colon, uterine, ovarian, small bowel, renal pelvis/ureter, and others

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MLH1 Variant Cancer Risks

***MLH1* LYNCH SYNDROME: CANCER RISKS[a]**

Site	Estimated Average Age of Presentation	Cumulative Risk for Diagnosis Through Age 80 y[b]	Cumulative Risk for Diagnosis Through Lifetime for General Population[c]	Comments and References
Colorectal	44 years	46%–61%[d]	4.2%	See footnote f References 1, 2, 3
Endometrial	49 years	34%–54%	3.1%	References 1, 4
Ovarian	46 years	4%–20%	1.3%	References 1, 5
Renal pelvis and/or ureter	59–60 years	0.2%–5%	—[e]	See footnote g References 1, 2, 5, 6, 7
Bladder	59 years	2%–7%	2.4%	References 2, 5, 6, 7
Gastric	52 years	5%–7%	0.9%	References 2, 5, 8
Small bowel	47 years	0.4%–11%	0.3%	References 1, 5
Pancreas	No data	6.2%	1.6%	Reference 2
Biliary tract	50 years	1.9%–3.7%	0.2%	References 1, 2
Prostate	63 years	4.4%–13.8%	11.6%	See footnote h Reference 6
Breast (female)	No data	10.6%–18.6%	12.8%	See footnote i References 5, 9, 10, 11
Brain	No data	0.7%–1.7%	0.6%	References 6, 12
Skin	See footnote j References 13, 14			

Genetic Counseling for Lynch Syndrome

Cancer Risk

- Increased risk for colon, uterine, ovarian, small bowel, renal pelvis/ureter, and others

Medical Management

- Increased surveillance
- Risk reducing surgery
- Possible treatment implications

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Lynch Syndrome

MLH1 Medical Management Recommendations

***MLH1* LYNCH SYNDROME: SURVEILLANCE/PREVENTION STRATEGIES[k,l]**

Site	
Colon cancer	• High-quality colonoscopy at age 20–25 y or 2–5 y prior to the earliest colon cancer if it is diagnosed before age 25 y[m] and repeat every 1–2 y.[n] See Follow-up of Surveillance Findings (LS-F) • The panel recommends that all individuals with LS who have a risk for future colorectal cancer (ie, excluding those with prior total proctocolectomy) consider using daily aspirin to reduce their future risk of colorectal cancer.[o] The decision to use aspirin for reduction of colorectal cancer risk in LS and the dose chosen should be made on an individual basis, including discussion of individual risks, benefits, adverse effects, and childbearing plans.[p] In determining whether an individual with LS should take aspirin and in deciding on the appropriate dosing, the panel recommends that providers carefully review patient-specific factors that may increase the risk of aspirin therapy—including but not limited to increased age, prior allergy, concurrent use of antiplatelets/anticoagulants, and untreated H. pylori or unconfirmed H. pylori eradication—as well as patient-specific factors that indicate a comparably low future cumulative risk of colorectal cancer (ie, increased age, PMS2-associated Lynch syndrome, history of prior colectomy) and who may thus be less likely to experience significant benefit.

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Lynch Syndrome
MLH1 Medical Management Recommendations

***MLH1* LYNCH SYNDROME: SURVEILLANCE/PREVENTION STRATEGIES[k,l]**

Site	
Endometrial cancer	• Because endometrial cancer can often be detected early based on symptoms, women should be educated regarding the importance of prompt reporting and evaluation of any abnormal uterine bleeding or postmenopausal bleeding. The evaluation of these symptoms should include endometrial biopsy. • Total hysterectomy has not been shown to reduce endometrial cancer mortality, but can reduce the incidence of endometrial cancer. Therefore, hysterectomy is a risk-reducing option that can be considered. • Timing of total hysterectomy can be individualized based on whether childbearing is complete, comorbidities, family history, and LS gene, as risks for endometrial cancer vary by pathogenic variant. • Endometrial cancer screening does not have proven benefit in women with LS. However, endometrial biopsy is both highly sensitive and highly specific as a diagnostic procedure. Screening via endometrial biopsy every 1–2 y starting at age 30–35 y can be considered. • Transvaginal ultrasound to screen for endometrial cancer in postmenopausal women has not been shown to be sufficiently sensitive or specific as to support a positive recommendation, but may be considered at the clinician's discretion. Transvaginal ultrasound is not recommended as a screening tool in premenopausal women due to the wide range of endometrial stripe thickness throughout the normal menstrual cycle.
Ovarian cancer	• Bilateral salpingo-oophorectomy (BSO) may reduce the incidence of ovarian cancer. The decision to have a BSO as a risk-reducing option should be individualized. Timing of BSO should be individualized based on whether childbearing is complete, menopause status, comorbidities, family history, and LS gene, as risks for ovarian cancer vary by pathogenic variant. Estrogen replacement after premenopausal oophorectomy may be considered. • Since there is no effective screening for ovarian cancer, women should be educated on the symptoms that might be associated with the development of ovarian cancer, such as pelvic or abdominal pain, bloating, increased abdominal girth, difficulty eating, early satiety, or urinary frequency or urgency. Symptoms that persist for several weeks and are a change from a woman's baseline should prompt evaluation by her physician. • Data do not support routine ovarian cancer screening for LS. Transvaginal ultrasound for ovarian cancer screening has not been shown to be sufficiently sensitive or specific to support a routine recommendation, but may be considered at the clinician's discretion. Serum CA-125 is an additional ovarian screening test with caveats similar to transvaginal ultrasound. • Consider risk-reduction agents for endometrial and ovarian cancers, including discussing risks and benefits (See Discussion for details).
Urothelial cancer (Renal pelvis, ureter, and/or bladder)	• There is no clear evidence to support surveillance for urothelial cancers in LS. Surveillance may be considered in selected individuals such as those with a family history of urothelial cancer. Surveillance options may include annual urinalysis starting at age 30–35 y. However, there is insufficient evidence to recommend a particular surveillance strategy.
Gastric and small bowel cancer	• No clear data exist to support surveillance for gastric, duodenal, and more distal small bowel cancer for LS. Individuals with a family history of these tumors may have increased risk but the benefit of surveillance is unknown. Regarding gastric cancer, risk factors include male sex, older age, *MLH1* or *MSH2* pathogenic variants, a first-degree relative with gastric cancer, Asian ethnicity, residing in or immigrant from countries with high background incidence of gastric cancer, chronic autoimmune gastritis, gastric intestinal metaplasia, and gastric adenomas. Consider baseline EGD with random biopsy of the proximal and distal stomach to evaluate for H. pylori, autoimmune gastritis, and intestinal metaplasia beginning at age 40 y and surveillance EGD every 3–5 y in those with above risk factors. (Vasen HF, et al. Gut 2013;62:812-823; Kim J, et al. Clin Gastroenterol Hepatol 2020;18:830-837.) • Consider H. pylori testing. Treat H. pylori, if detected.

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Lynch Syndrome

MLH1 Medical Management Recommendations

***MLH1* LYNCH SYNDROME: SURVEILLANCE/PREVENTION STRATEGIES[k,l]**

Site	
Pancreatic cancer	• Consider pancreatic cancer screening beginning at age 50 years (or 10 years younger than the earliest exocrine pancreatic cancer diagnosis in the family, whichever is earlier) for individuals with exocrine pancreatic cancer in ≥1 first- or second-degree relatives from the same side of (or presumed to be from the same side of) the family as the identified pathogenic/likely pathogenic germline variant (Abe T, et al. J Clin Oncol 2019;37:1070-1080). • For individuals considering pancreatic cancer screening, the panel recommends that screening be performed in experienced high-volume centers, ideally under research conditions. The panel recommends that such screening only take place after an in-depth discussion about the potential limitations to screening, including cost, the high incidence of benign or indeterminate pancreatic abnormalities, and uncertainties about the potential benefits of pancreatic cancer screening. • The panel recommends that screening be considered using annual contrast-enhanced MRI/MRCP and/or EUS, with consideration of shorter screening intervals for individuals found to have potentially concerning abnormalities on screening. The panel emphasizes that most small cystic lesions found on screening will not warrant biopsy, surgical resection, or any other intervention. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic for additional details on pancreatic cancer screening.
Prostate cancer	• Men with LS should consider their risk based on the LS gene and family history of prostate cancer. The NCCN Guidelines for Prostate Cancer Early Detection recommend that it is reasonable for men with LS to consider beginning shared decision-making about prostate cancer screening at age 40 years and to consider screening at annual intervals rather than every other year.
Breast cancer	• There have been suggestions that there is an increased risk for breast cancer in LS patients; however, there is not enough evidence to support increased screening above average-risk breast cancer screening recommendations or those based on personal/family history of breast cancer. See NCCN Guidelines for Breast Cancer Screening and Diagnosis.
Brain cancer	• Patients should be educated regarding signs and symptoms of neurologic cancer and the importance of prompt reporting of abnormal symptoms to their physicians.
Skin manifestations	• Frequency of malignant and benign skin tumors such as sebaceous adenocarcinomas, sebaceous adenomas, and keratoacanthomas has been reported to be increased among patients with Lynch syndrome, but cumulative lifetime risk and median age of presentation are uncertain. • Consider skin exam every 1–2 years with a health care provider skilled in identifying Lynch syndrome-associated skin manifestations. Age to start surveillance is uncertain and can be individualized.
Reproductive options	• For patients of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including pre-implantation genetic testing. Discussion should include known risks, limitations, and benefits of these technologies. • For patients of reproductive age, advise about the risk of a rare recessive syndrome called constitutional MMR deficiency (CMMRD) syndrome (Wimmer K, et al. J Med Genet 2014;51:355-365). If both partners are a carrier of a pathogenic variant/s in the same MMR gene, then their future offspring will be at risk of having CMMRD syndrome.
Risk to relatives	• Advise patients to tell their relatives about possible inherited cancer risk, options for risk assessment, and management. • Recommend genetic counseling and consideration of genetic testing for at-risk relatives.

Genetic Counseling for Lynch Syndrome

Cancer Risk

- Increased risk for colon, uterine, ovarian, small bowel, renal pelvis/ureter, and others

Medical Management

- Increased surveillance
- Risk reducing surgery
- Possible treatment implications

Implications for Family Members

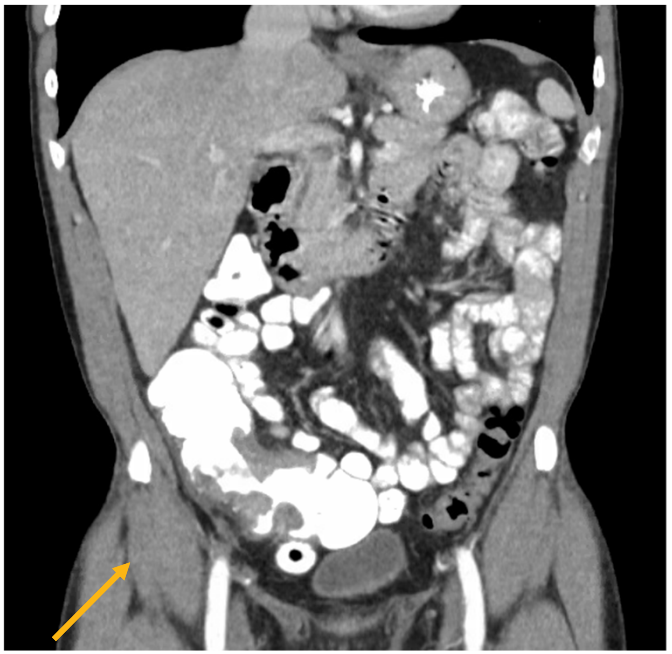
- 50% risk to siblings, children, parents
- Increased risk for distant relatives
- Reproductive risk for constitutional mismatch repair deficiency (CMMRD)
- Cascade testing

Case 1: Staging Scans

CT chest/abdomen/pelvis with IV and oral contrast

- Circumferential bowel wall thickening in ascending colon consistent with known colon cancer
- No evidence of metastatic disease

Consistent with early-stage colon cancer – plan for resection



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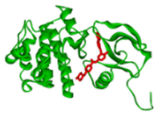
Clinical Management of Colon Cancer in Lynch Syndrome

PRINCIPLES OF SURGERY

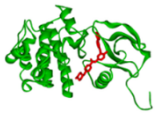
Colectomy

- Lymphadenectomy
  - Lymph nodes at the origin of feeding vessel(s) should be identified for pathologic exam.
  - Clinically positive lymph nodes outside the field of resection that are considered suspicious should be biopsied or removed, if possible.
  - Positive nodes left behind indicate an incomplete (R2) resection.
  - A minimum of 12 lymph nodes need to be examined to establish N stage.[1]
- Minimally invasive approaches may be considered based on the following criteria:[2]
  - The surgeon has experience performing laparoscopically assisted colorectal operations.[3,4]
  - Minimally invasive approaches are generally not indicated for locally advanced cancer or acute bowel obstruction or perforation from cancer.
  - Thorough abdominal exploration is required.[5]
  - Consider preoperative marking of lesion(s).
- Management of patients with carrier status of known or clinically suspected LS.
  - Consider more extensive colectomy for patients with a strong family history of colon cancer or young age (<50 y).
    See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal
- Resection needs to be complete to be considered curative.



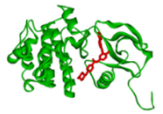
Clinical management of Colon Cancer in Lynch Syndrome

Operation	Total abdominal colectomy with ileorectal anastomosis	Segmental Colectomy
Who	• Younger patients (<50) • Unable/unlikely to comply with frequent colonoscopy	• Older patients (>60-65) • Underlying sphincter dysfunction • Likely to comply with frequent colonoscopy
Pro	• Risk-reducing procedure – reduces risk of metachronous cancer • Annual surveillance of retained rectum	• Less impact on frequency of bowel movements, continence
Con	• QOL – continence, frequency of bowel movements • Unknown survival benefit	• Surveillance: colonoscopy 1-2 years • Risk of metachronous cancer, adenoma



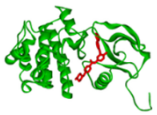
Case 1: Final Pathology and Adjuvant Therapy

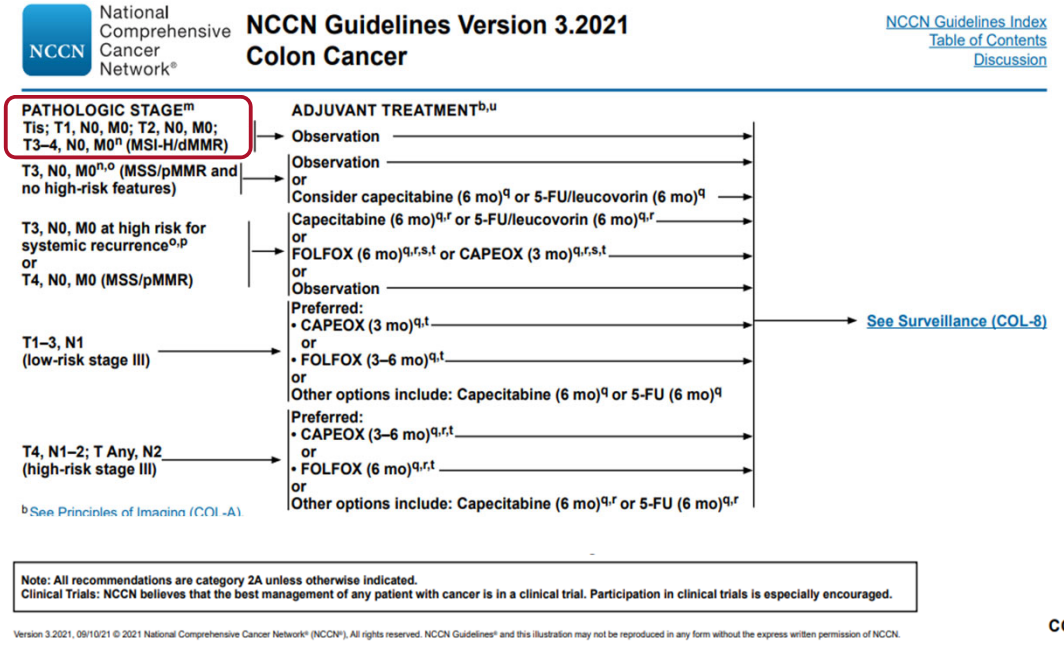
- Final pathology T4N0, surgical resection margins negative
- Medical oncologist recommends surveillance, no adjuvant chemotherapy



Clinical Management of dMMR/MSI-H Colon Cancer

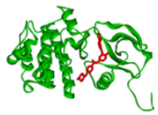
The presence of dMMR/MSI-H can influence the decision to offer adjuvant therapy.

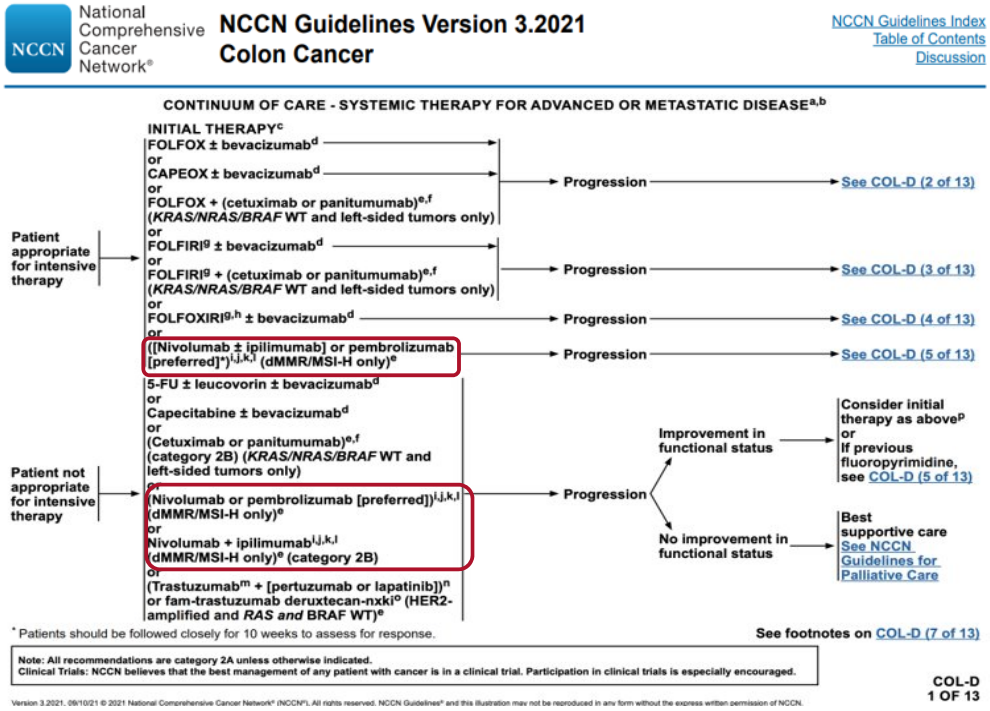




Clinical Management of dMMR/MSI-H Colon Cancer

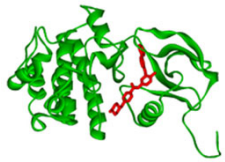
Immunotherapy with checkpoint inhibitor(s) is an important option to consider in patients with advanced colorectal cancer which is dMMR/MSI-H





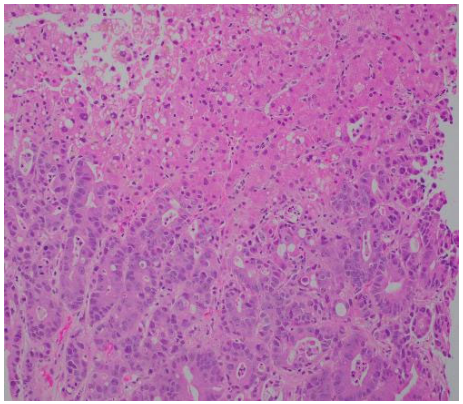
Case 2

- 58 year-old woman with a history of stage 3 sigmoid colon adenocarcinoma (MMR proficient)
- Now presents with right upper quadrant pain, CT scan shows multiple liver masses (largest: 4cm)
- Undergoes ultrasound-guided percutaneous biopsy

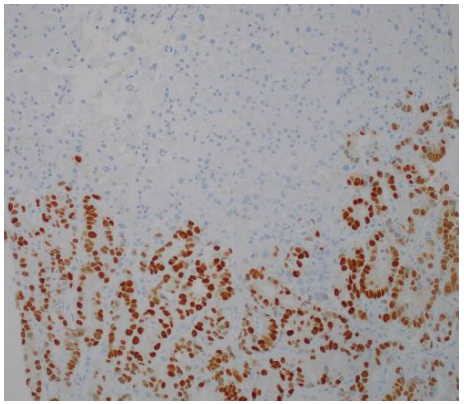


Liver Mass Biopsy

H&E



CDX2



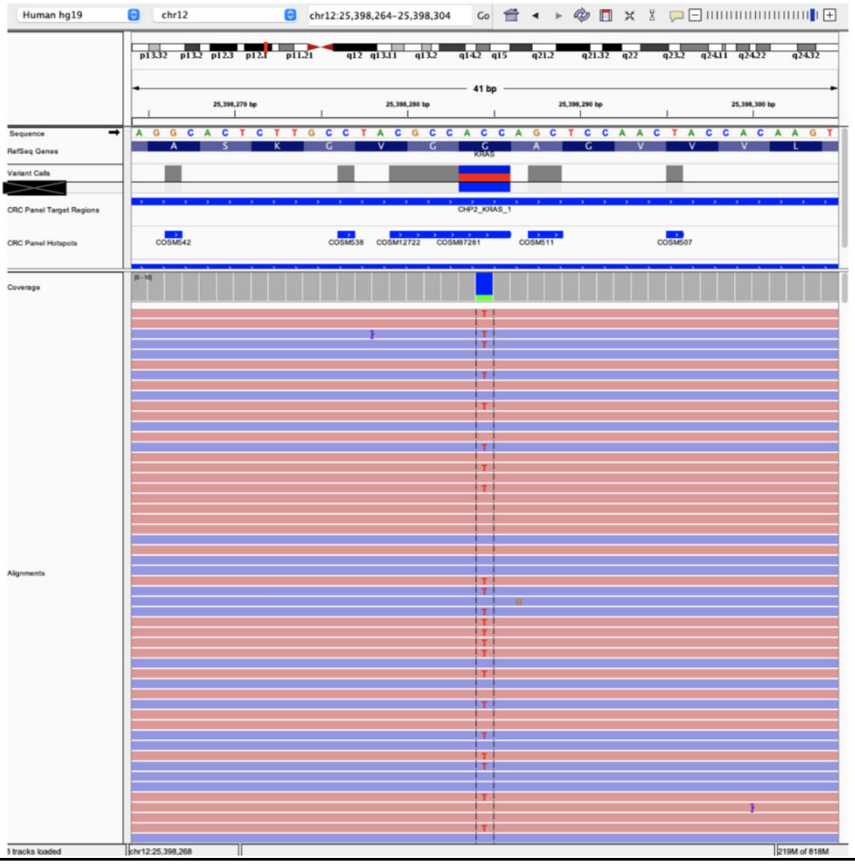
Diagnosis: Metastatic adenocarcinoma consistent with colorectal primary

Polling Question

Next-generation sequencing is performed on the liver biopsy, which of the following biomarkers should not be tested?

- HER2
- KRAS
- NRAS
- VHL

NGS Results



KRAS G12D
mutation detected

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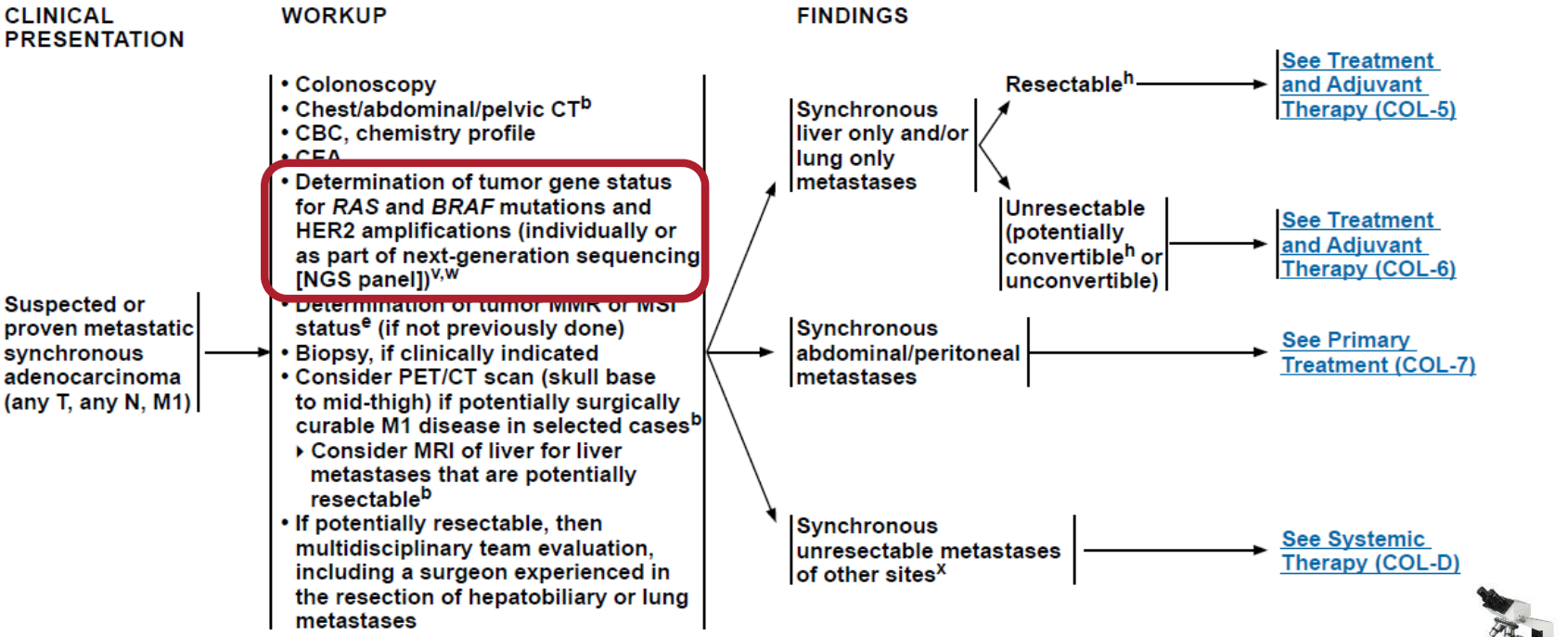

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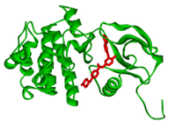
RAS Testing in Metastatic Colorectal Cancer

PRINCIPLES OF PATHOLOGIC REVIEW

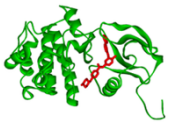
KRAS, *NRAS*, and *BRAF* Mutation Testing

- All patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of an NGS panel. Patients with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.[53-55] *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a *BRAF* inhibitor.[56-58]
- *BRAF* V600E mutation testing via immunohistochemistry is also an option.
- Testing for *KRAS*, *NRAS*, and *BRAF* mutations should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA-88) as qualified to perform *high-complexity* clinical laboratory (molecular pathology) testing. No specific methodology is recommended (eg, sequencing, hybridization).
- The testing can be performed on formalin-fixed paraffin-embedded tissue. The testing can be performed on the primary colorectal cancers and/or the metastasis, as literature has shown that the *KRAS*, *NRAS*, and *BRAF* mutations are similar in both specimen types.[59]



Case 2: Treatment

- Stage IV colon adenocarcinoma with biopsy proven liver metastases – mutation in KRAS G12D detected
- Starts first-line therapy with FOLFOX-bevacizumab

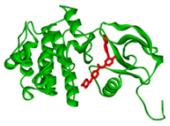


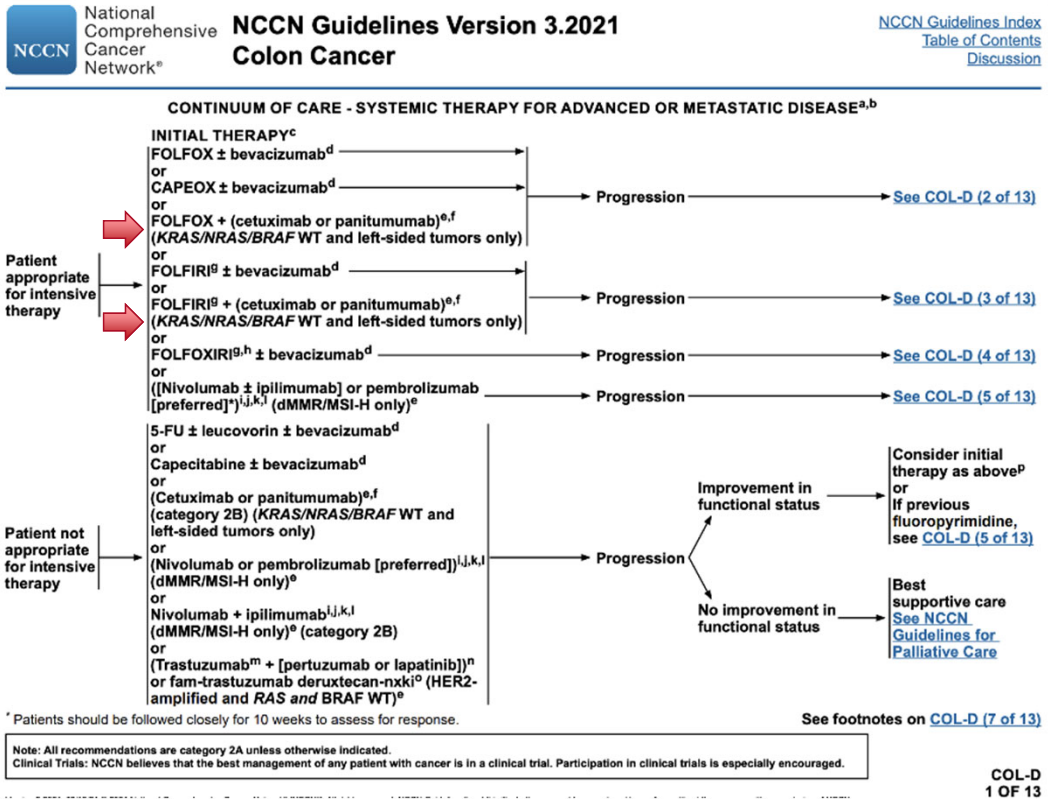
RAS Testing in Metastatic Colorectal Cancer

The presence of RAS alterations predicts lack of benefit from cetuximab, panitumumab

KRAS is a potential therapeutic target

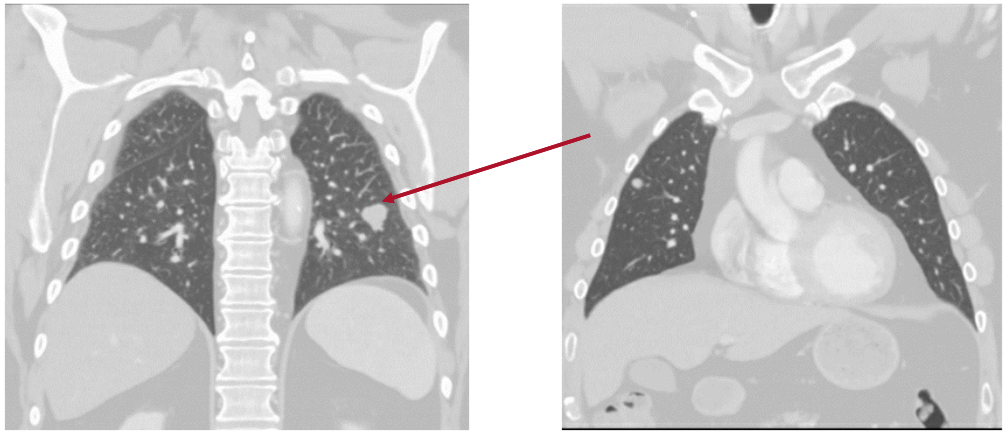
- Sotorasib - G12C; FDA approved in NSCLC





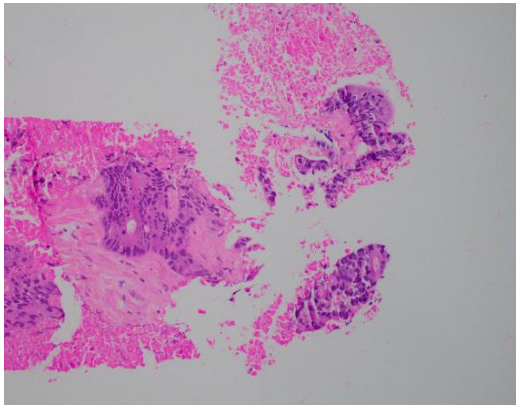
Case 3

- 65 y/o with a history of stage III colon cancer treated with adjuvant FOLFOX
- Surveillance scans show multiple new and enlarging pulmonary nodules
- Interventional radiology (IR) biopsy performed on lung nodule

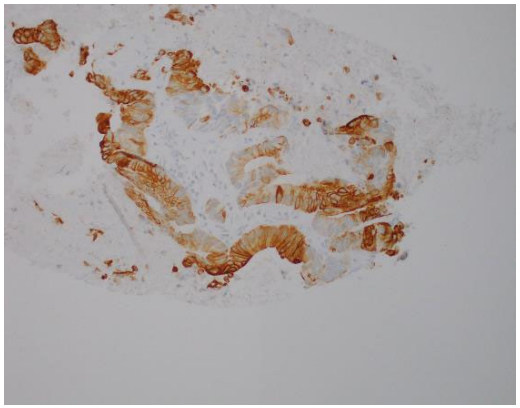


Lung Biopsy

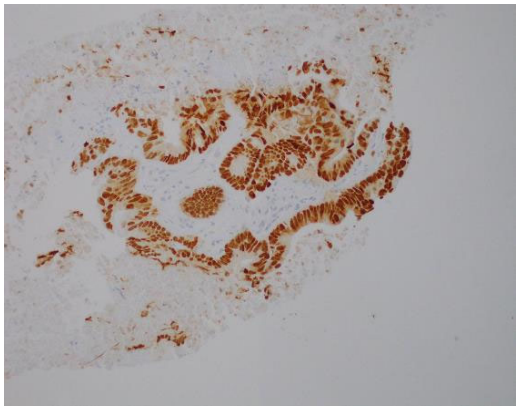
H&E



CK20

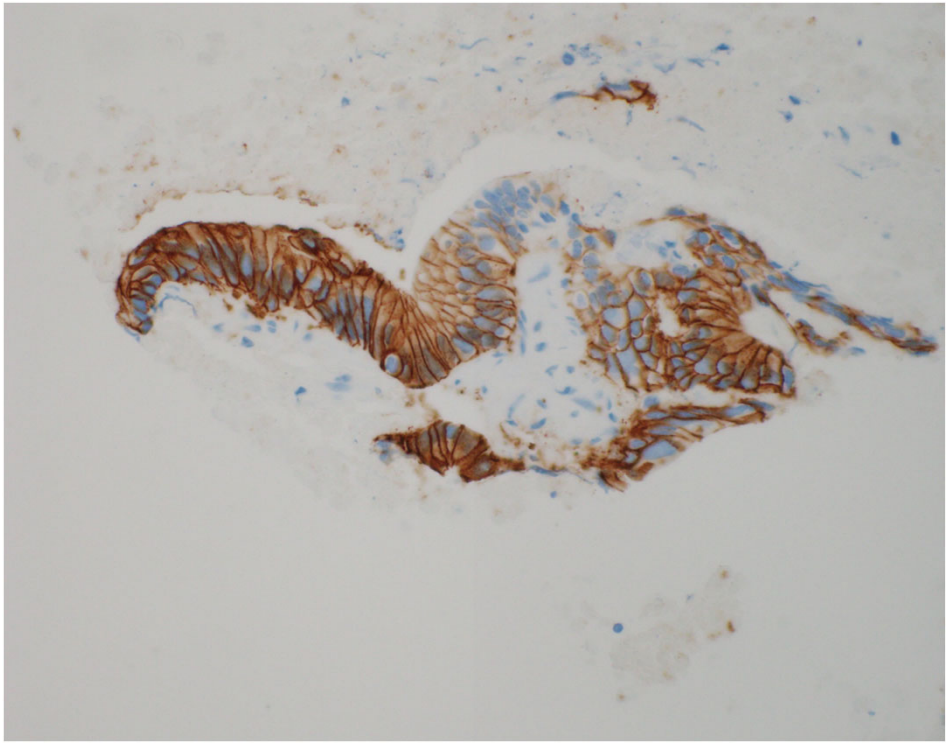


CDX2



Diagnosis: Metastatic adenocarcinoma consistent with colorectal primary

Her2 Immunohistochemistry



Polling Question

Which immunohistochemical scoring system should be used to assess HER2 overamplification?

- Tumor proportion score
- HERACLES
- Manual semi quantitation
- Combined positive score

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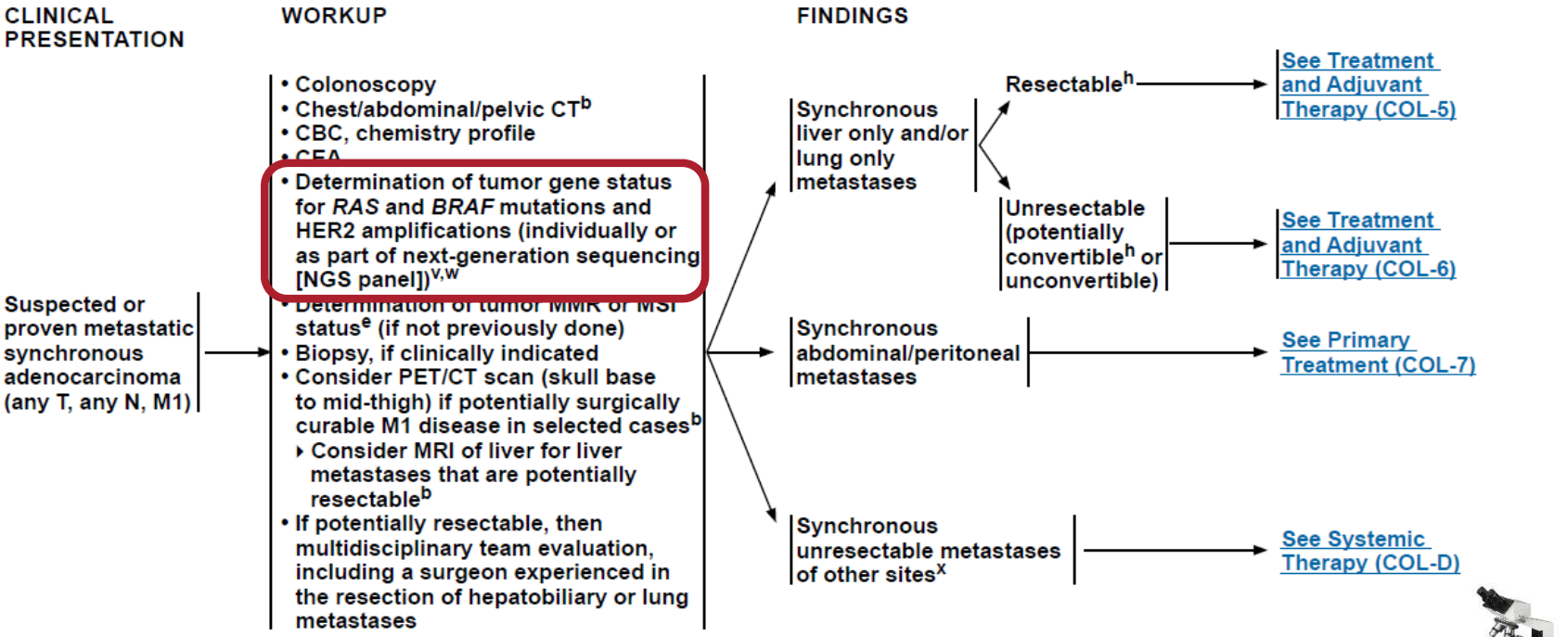
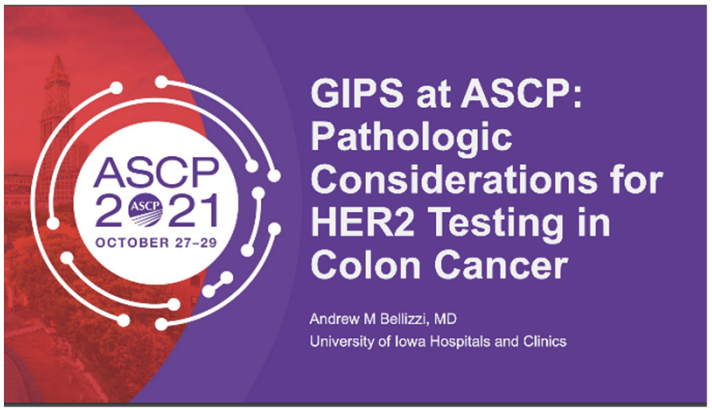


Heracles-Greek (Hercules-Roman)

Key Reference: Sartore-Bianchi et al. Sartore-Bianchi et al. "Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial."Lancet Oncol. 2016 Jun;17(6):738-746. doi: 10.1016/S1470-2045(16)00150-9.



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PRINCIPLES OF PATHOLOGIC REVIEW

HER2 Testing

- Diagnostic testing is via immunohistochemistry, fluorescence in situ hybridization (FISH), or NGS.
- Positive by immunohistochemistry is defined as: 3+ staining in more than 50% of tumor cells. 3+ staining is defined as an intense membrane staining that can be circumferential, basolateral, or lateral. Those that have a HER2 score of 2+ should be reflexed to FISH testing.[62-64] HER2 amplification by FISH is considered positive when the HER2:CEP17 ratio is ≥2 in more than 50% of the cells.[62-64] NGS is another methodology for testing for HER2 amplification.[65]
- Anti-HER2 therapy is only indicated in HER2-amplified tumors that are also *RAS* and *BRAF* wild type.

***NTRK* Fusions**

- *NTRK* fusions are extremely rare in colorectal carcinomas.[66] The overall incidence is approximately 0.35% in a cohort of 2314 colorectal carcinomas, with *NTRK* fusions confined to those tumors that are pan-wild type *KRAS, NRAS*, and *BRAF*. In one study of 8 colorectal cancers harboring *NTRK* fusions, 7 were found in the small subset that were dMMR (MLH-1)/MSI-H.[67] These data support limiting the subpopulation of colorectal cancers that should be tested for *NTRK* fusions to those with wild type *KRAS, NRAS, BRAF*, and arguably to those that are MMR deficient (dMMR)/MSI-H.[67]
- *NTRK* inhibitors have been shown to have activity ONLY in those cases with *NTRK* fusions, and NOT with *NTRK* point mutations.
- Methodologies for detecting *NTRK* fusions are IHC,[68] FISH, DNA-based NGS, and RNA-based NGS.[66,69] In one study, DNA-based sequencing showed an overall sensitivity and specificity of 81.1% and 99.9%, respectively, for detection of *NTRK* fusions when compared to RNA-based sequencing and immunohistochemistry showed an overall sensitivity of 87.9% and specificity of 81.1%. Since approximately 1 in 5 tumors identified as having an *NTRK* fusion by IHC will be a false positive, tumors that test positive by IHC should be confirmed by RNA NGS. That same study commented that RNA-based sequencing appears to be the optimal way to approach *NTRK* fusions, because the splicing out of introns simplifies the technical requirements of adequate coverage and because detection of RNA-level fusions provides direct evidence of functional transcription.[69] However, selection of the appropriate assay for *NTRK* fusion detection depends on tumor type and genes involved, as well as consideration of other factors such as available material, accessibility of various clinical assays, and whether comprehensive genomic testing is needed concurrently.[69]

Table 1. Comparison of CAP/ASCP/ASCO gastroesophageal adenocarcinoma and HERACLES colorectal cancer HER2 immunohistochemistry criteria

HER2 IHC Result	CAP/ASCP/ASCO Gastroesophageal Adenocarcinoma Guideline Interpretation (for resections)	Consequence	HERACLES Diagnostic Criteria Interpretation	Consequence
No reactivity or membranous reactivity in <10% of tumor cells	Negative (0)	No further testing required; not eligible for therapy	Negative	No further testing required; not eligible for therapy
Faint/barely perceptible reactivity in ≥10% of tumor cells	Negative (1+)	No further testing required; not eligible for therapy	Negative	No further testing required; not eligible for therapy
Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% but <50% of tumor cells	**Equivocal (2+)**	Perform ISH testing	**Negative**	No further testing required; not eligible for therapy
Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥ 50% of tumor cells	Equivocal (2+)	Perform ISH testing	Equivocal	**Mandatory IHC retesting to confirm staining in ≥50% of cells**; ISH testing required; eligible for therapy if ISH positive
Strong complete, basolateral, or lateral membrane staining in 10–50% of tumor cells	**Positive (3+)**	Eligible for therapy; no further testing required	**Conditionally positive**	**Mandatory IHC retesting to confirm staining in ≥10% of cells**; ISH testing required; eligible for therapy if ISH positive
Strong complete, basolateral, or lateral membrane staining in >50% of tumor cells	Positive (3+)	Eligible for therapy; no further testing required	Positive	Eligible for therapy; no further testing required

Reference:
www.captodayonline.com/qa-column-0220/

Table 2. Comparison of CAP/ASCP/ASCO gastroesophageal adenocarcinoma and HERACLES colorectal cancer HER2 in situ hybridization criteria for a positive result

CAP/ASCP/ASCO	HERACLES
■ *HER2*:CEP17 ratio ≥2.0 in >10% of cells ■ *HER2* count >6 per cell in >10% of cells (if *HER2*:CEP17 ratio is <2.0 and HER2 count is 4–6, count another 20 cells)	*HER2*:CEP17 ratio ≥2.0 in ≥50% of cells

Reference:
www.captodayonline.com/qa-column-0220/

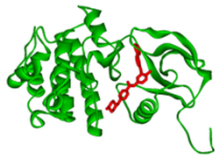
Treatment of HER2 Amplified Metastatic CRC

HER2 directed therapies are recommend in the following settings:

- First line therapy for metastatic disease in patients not appropriate for intensive therapy
- 2nd line and later

Options:

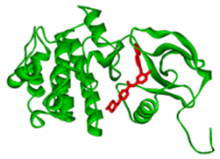
- Trastuzumab + pertuzumab
- Trastuzumab + lapatinib
- Fam-trastuzumab deruxtecan-nxki



The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Colon Cancer (Version 3.2021). © 2021 National Comprehensive Cancer Network, Inc. Available at: NCCN.org. Accessed October 27, 2021.

Case 3

- Stage IV colon cancer with biopsy-proven lung metastases
- 1st line treatment: FOLFIRI-bevacizumab x 4 months followed by 5FU+bevacizumab maintenance
- After 10 months, she presents with dyspnea
  - Scans show significant increase in pulmonary nodules
- Starts 2nd line therapy with trastuzumab-pertuzumab (mAb's targeting HER2) with rapid decrease in size of lung nodules and improvement in dyspnea



Summary

- Universal screening for MMR/MSI/NGS should be performed on all colorectal cancers as well as subsequent algorithmic testing when appropriate
  - Identification of a patient with Lynch syndrome informs genetic counseling
  - Lynch syndrome/MSI CRC have tailored oncologic treatments
- Metastatic CRC should be tested for mutations in RAS, BRAF and amplification of Her2
  - HERACLES scoring should be used for interpretation of IHC results

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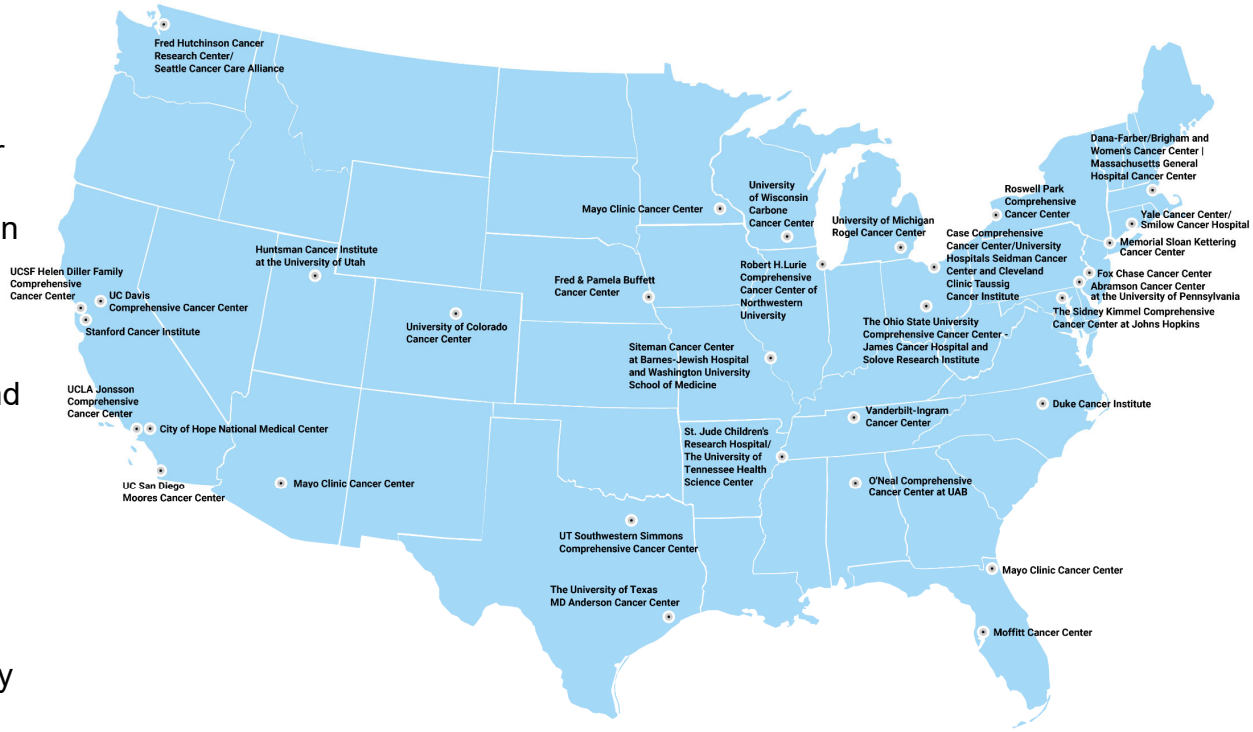

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