Colorectal Cancer Molecular Diagnostics

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Disclosures

Dr. Bronner has no disclosures pertaining To this presentation.

Molecular CRC Testing

NOW MANDATORY

- MSI, MMR IHC, Sporadic, Lynch
- KRAS
- BRAF

- Other genes and mutations in clinical trials

MSI Testing

- MSI-H Sporadic: 15% CRC
- MSI-H Lynch: 2-3% CRC
- MSS: 92% CRC

Lynch Syndrome Cancers

- Colorectal CA 80% & Endometrial CA 50%
- Other CA's: panc/bil, gastric, small bowel, sebac skin (Muir Torre), ovarian, GU, GBM (Turcot's)
- Screening: Age 25 or 10 yrs < youngest in family

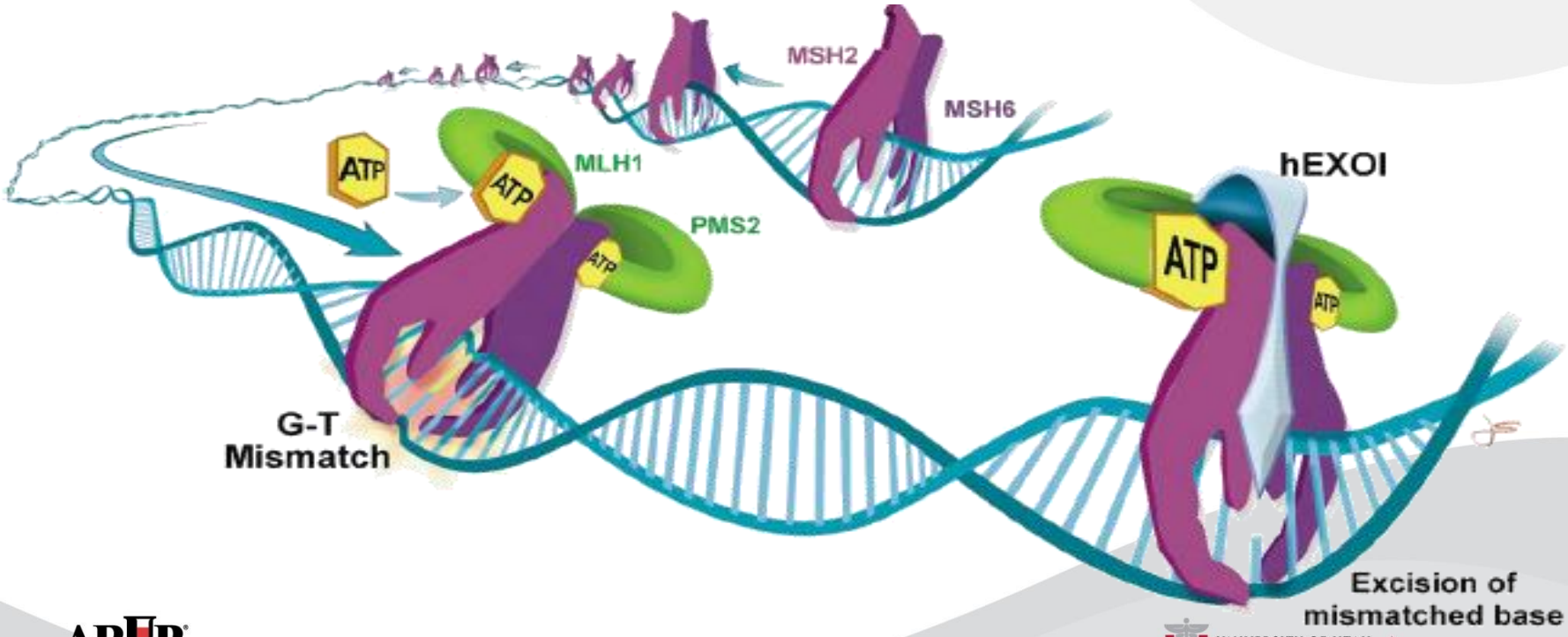
Annual colonoscopy & endometrial bx, periodic EGD, EUS of pancreas, pelvic exam, brain scans, urine cytology

HUGE & LIFELONG IMPACT ON LYNCH PATIENTS: DX IS CRITICAL

Microsatellite instability (MSI)

Microsatellites: Short, stably inherited repetitive DNA sequences prone to error during replication

Normally repaired by MMR proteins: proofreading complex for the DNA polymerase



Microsatellite instability (MSI)

- Lynch Syndrome: GERMLINE autosomal dominant mutation in a MMR gene
- MMR genes: MLH-1, MSH-2, MSH-6, PMS-2
- MSI: microsatellites of altered lengths accumulate throughout the genome due MMR deficiency
- Lynch phenotype not so obvious (nonpolyposis)
- Family history not always obvious or available
- MMR deficiency permits molecular diagnosis

MSI-High Colon Cancer

- Sporadic: Tumor limited, nearly all MLH1 methylation
  - Rare somatic MMR mutations described
- Lynch: Germline mutations
  - MLH1 60%, MSH2 35%, PMS2, MSH6, EPCAM, POL 5%
- Lynch & Sporadic Pathology: identical

Reasons to Diagnose MSI-H CRC

- Hereditary and syndromic components of Lynch
- Improved survival for sporadic CRC in randomized/stage-matched trials
- Chemo- and immuno-therapy selection
  - 5FU out
  - Immunotherapy in

Andre T, et al. N Engl J Med 383:2207;2020
Zaanan A, et al. Clin Cancer Res 17:7470;2011
Ribic CM, et al. NEJM 349:247;2003

MSI-H CRC: Clinicopathologic Features

Right-sided location

Age < 50 years (Lynch)

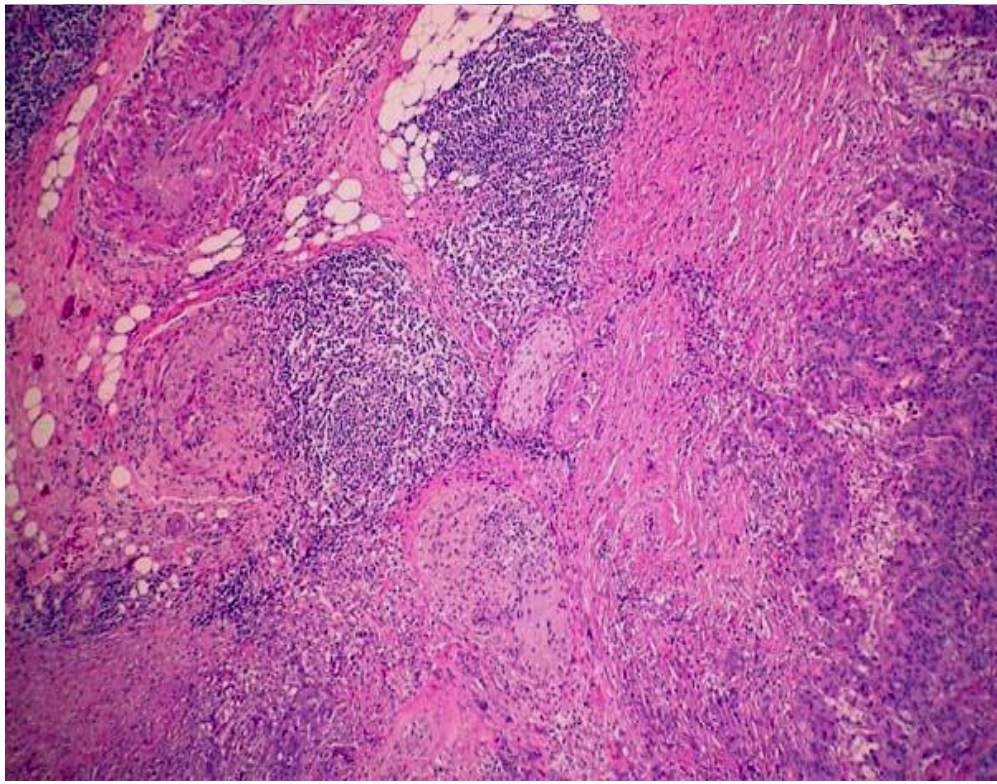
Poor differentiation

Absence of dirty necrosis

>2 tumor infiltrating lymphs/hpf

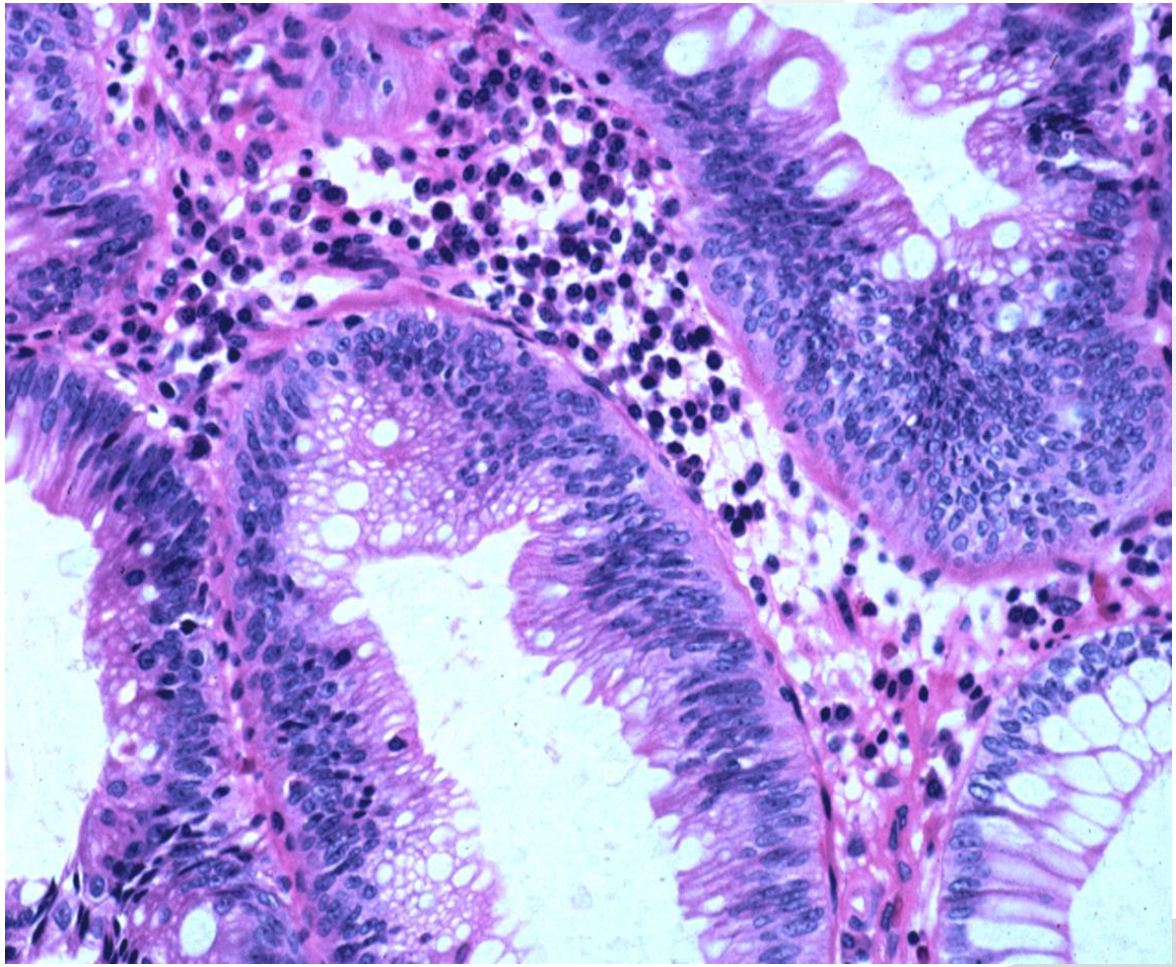
Mucinous change

Crohn's-like lymphoid reaction



Greenson JK, et al. *Am J Surg Pathol* 27:563-570, 2003.

Duodenal or Gastric Adenoma



Consider FAP and Lynch Syndrome

How do we work up Lynch syndrome?

- Determine mismatch repair deficiency
  - PCR for microsatellite instability
  - IHC for mismatch repair proteins
- Determine mismatch repair deficient tumor type
  - Sporadic: no germline testing
  - Possibly inherited: germline testing needed

Lynch Testing

Tumor screening assays (90% sens)

Detect affected **patients with tumor**

- MSI by PCR: paraffin works well
- MMR Immunohistochemistry:
  - MLH-1, MSH-2, MSH-6, PMS2 (work well with correct positive controls)

Blood germline mutation analysis

Detects **family members without tumor**

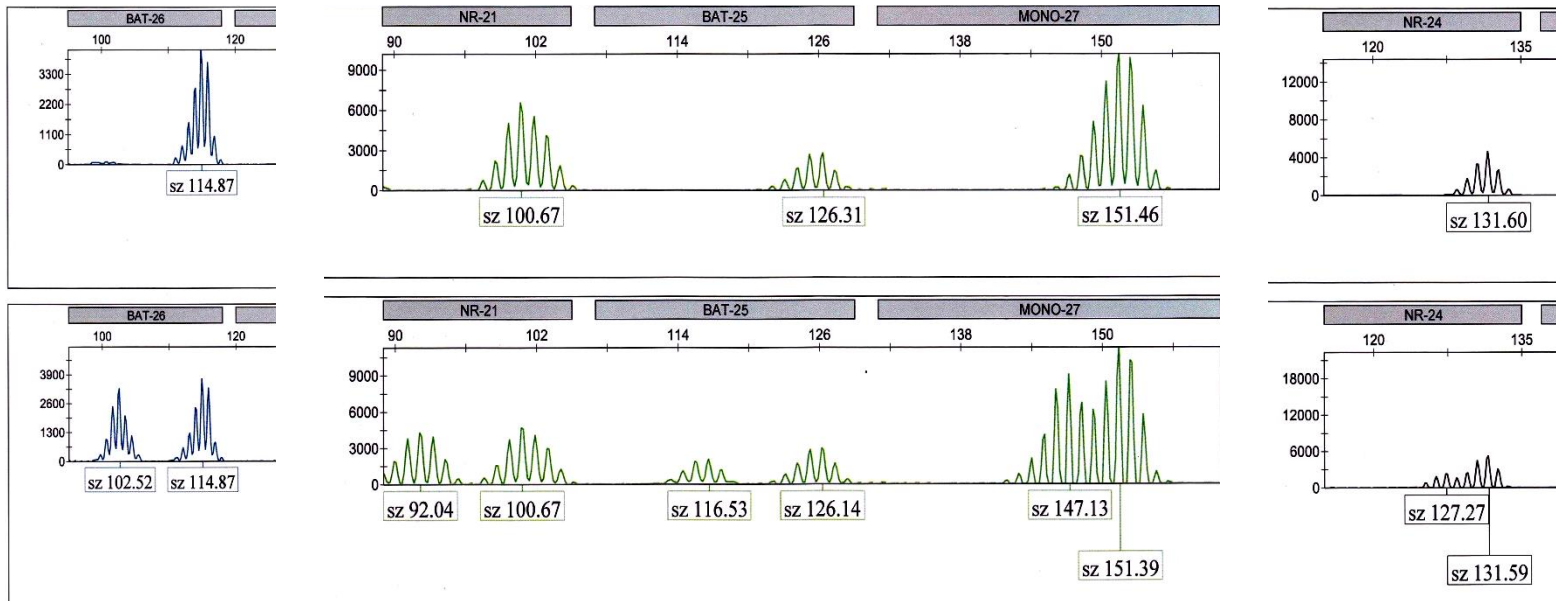
MSI Requirements

- ***Tumor***
- ***Normal DNA***
  - Non-tumor paraffin tissue
  - Blood
  - Buccal swab

Mononucleotide repeat panel

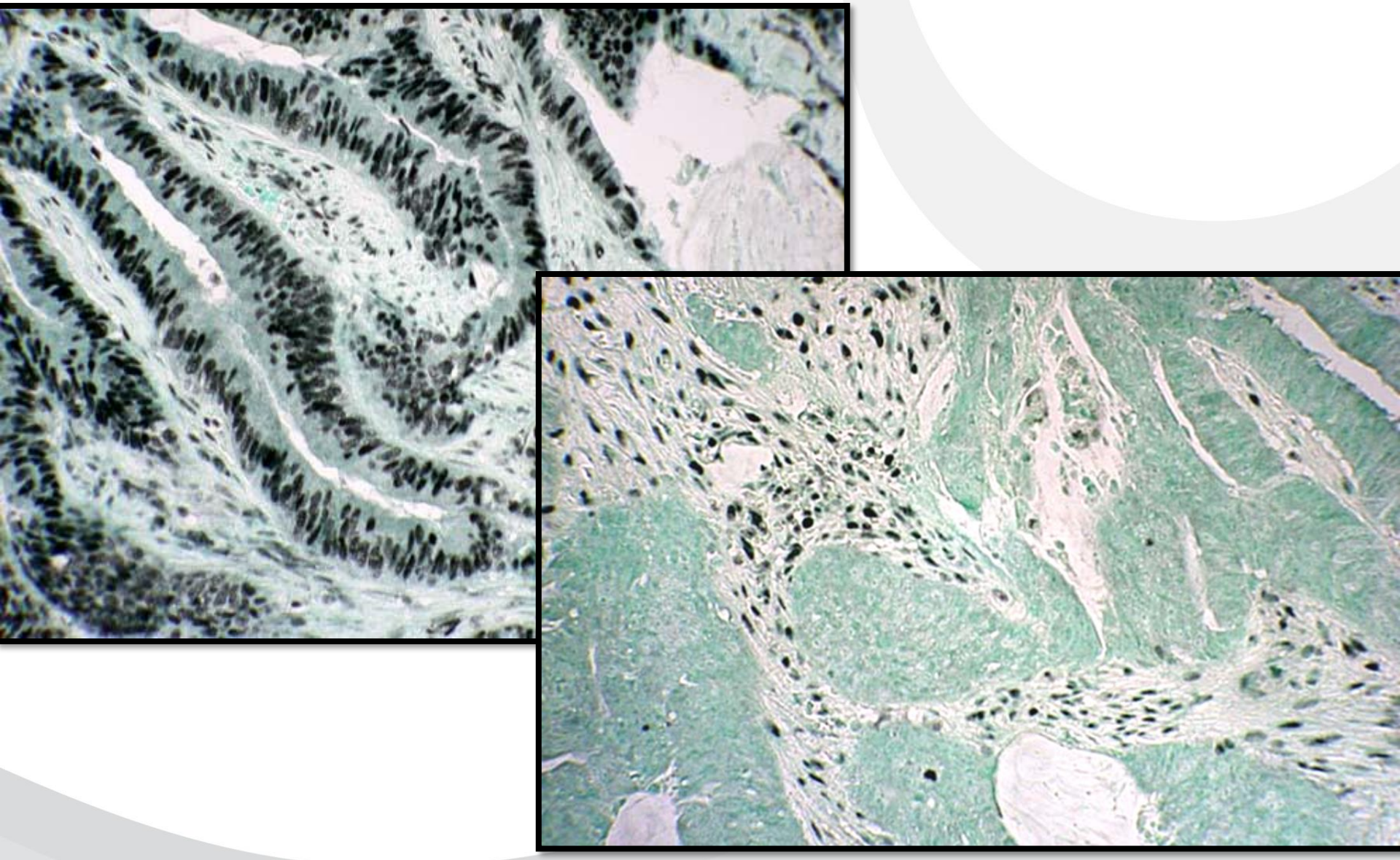
- Mononucleotide repeats are probably more sensitive and specific for MMR deficiency
- 5 mononucleotide repeat panel
  - MSI high: 2 or more unstable, although typically all (or almost all) repeats are unstable
  - Since instability in even one mononucleotide repeat may indicate MMR deficiency, instability in one repeat is termed “indeterminate” rather than MSI low

MSI Electropherogram Results



Microsatellite-High (MSI-H)	**Instability at ≥ 2 loci (usually all 5)**
Microsatellite-Low (Indeterminate)	**Instability at 1 locus**
Microsatellite stable (MSS)	**None of 5 loci unstable**

IHC in Lynch Syndrome

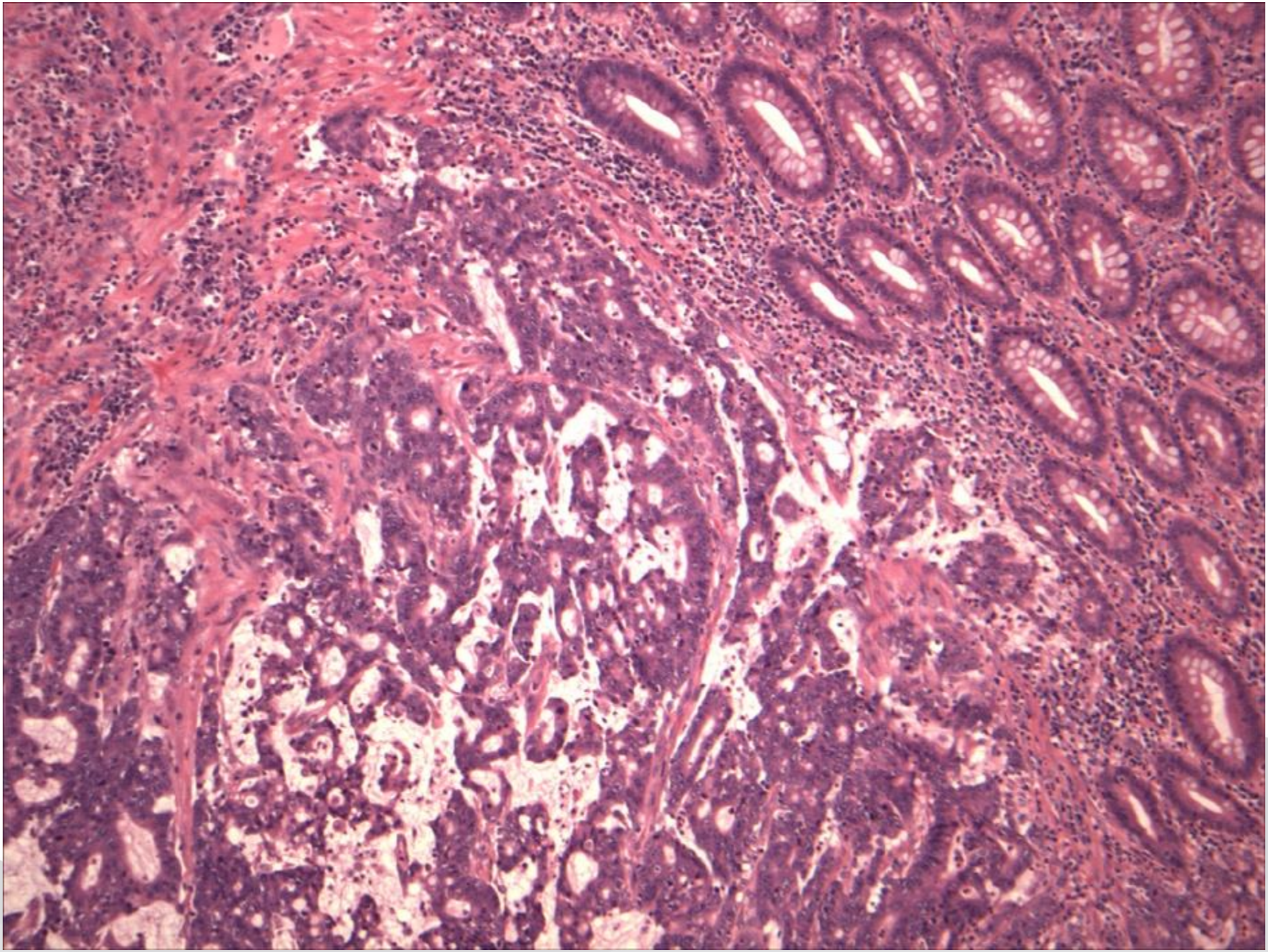


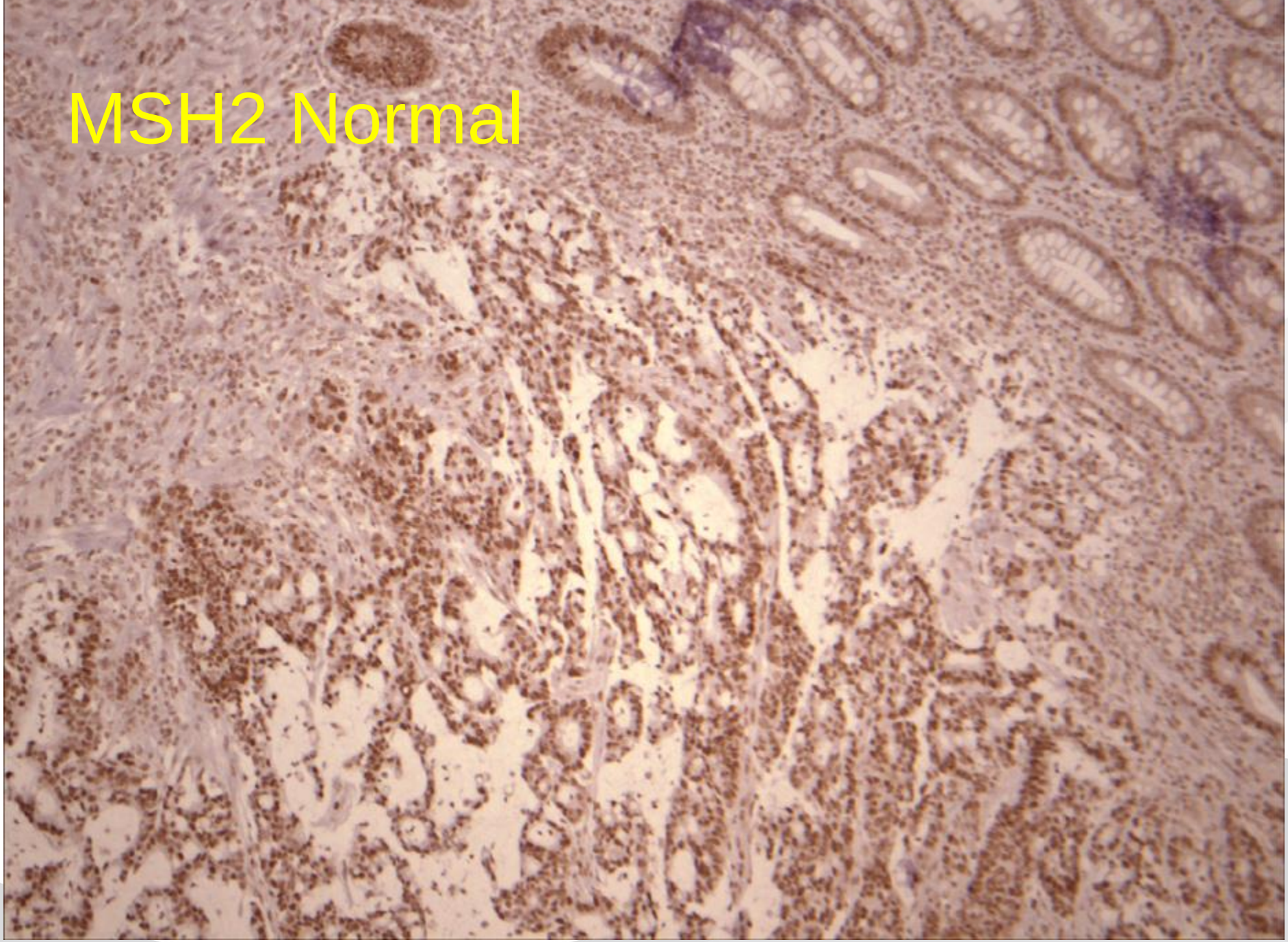
How do we interpret MMR IHC stains?

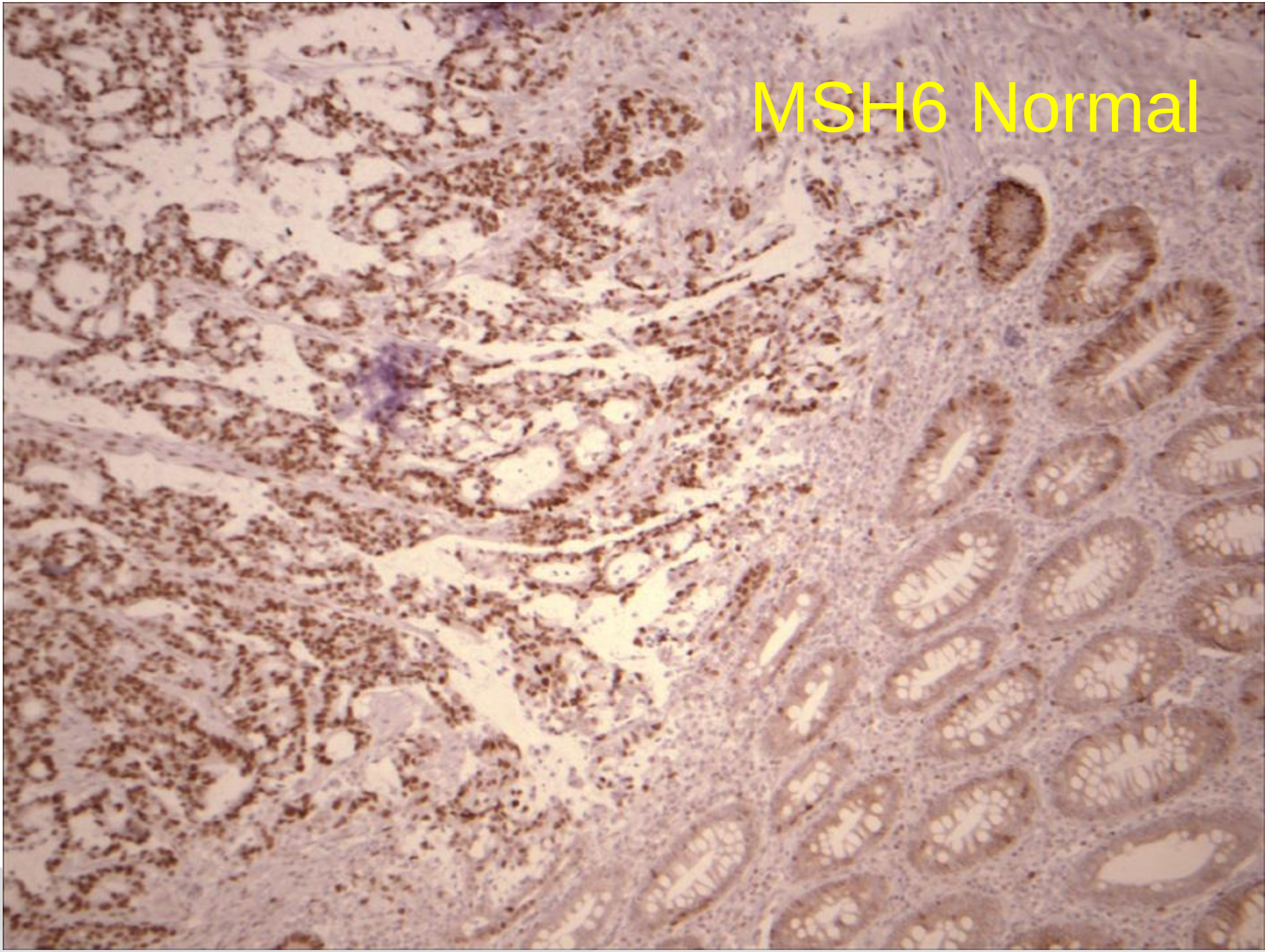
- Two MMR protein complexes:
  - MLH1/PMS2
  - MSH2/MSH6
- Stability of PMS2 and MSH6 depends upon these complexes
- Therefore, loss of staining of MLH1 leads to PMS2 loss
- Similarly, loss of staining of MSH2 leads to MSH6 loss
- MLH1 and MSH2 are stable without the complex; therefore, MSH6 or PMS2 mutations result in *solitary* IHC losses of either protein

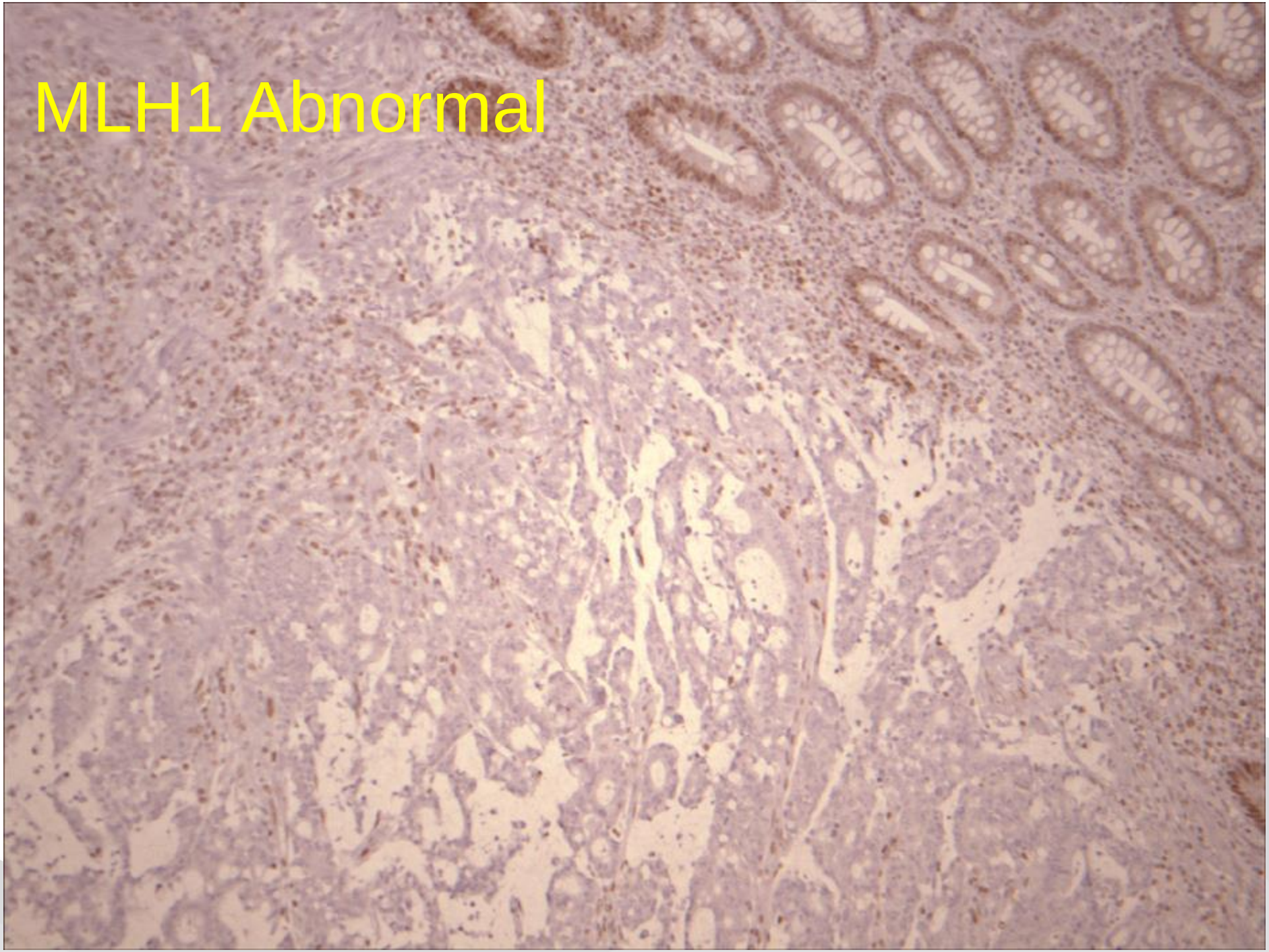
IHC interpretation

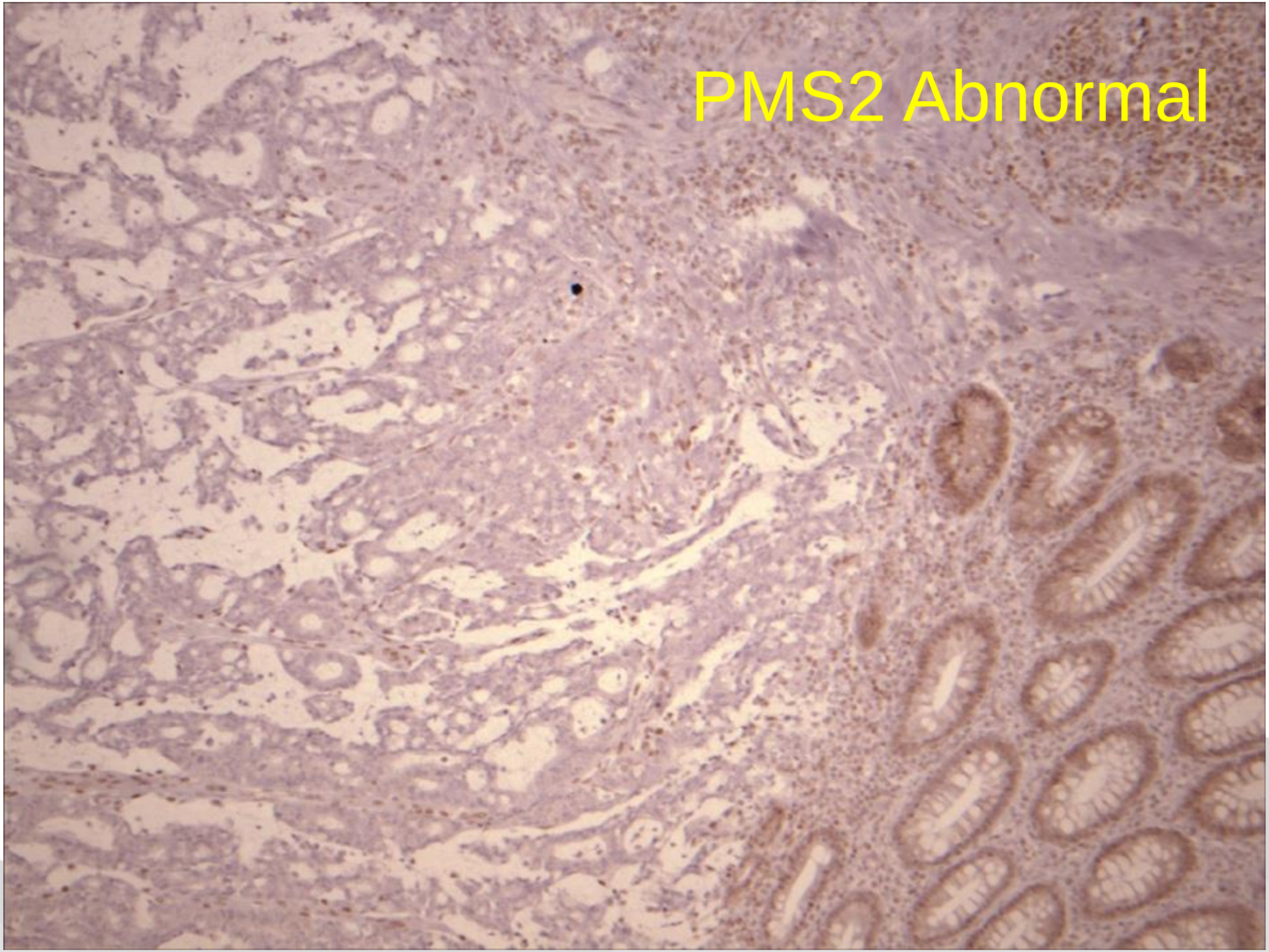
- Defect in MLH1: loss of MLH1/PMS2
- Defect in MSH2: loss of MSH2/MSH6
- Defect in MSH6: *isolated* loss of MSH6
- Defect in PMS2: *isolated* loss of PMS2
- There are exceptions
  - Isolated loss of PMS2 has been associated with MLH1 mutations
- Panel testing makes this less important




MSH2 Normal


MSH6 Normal

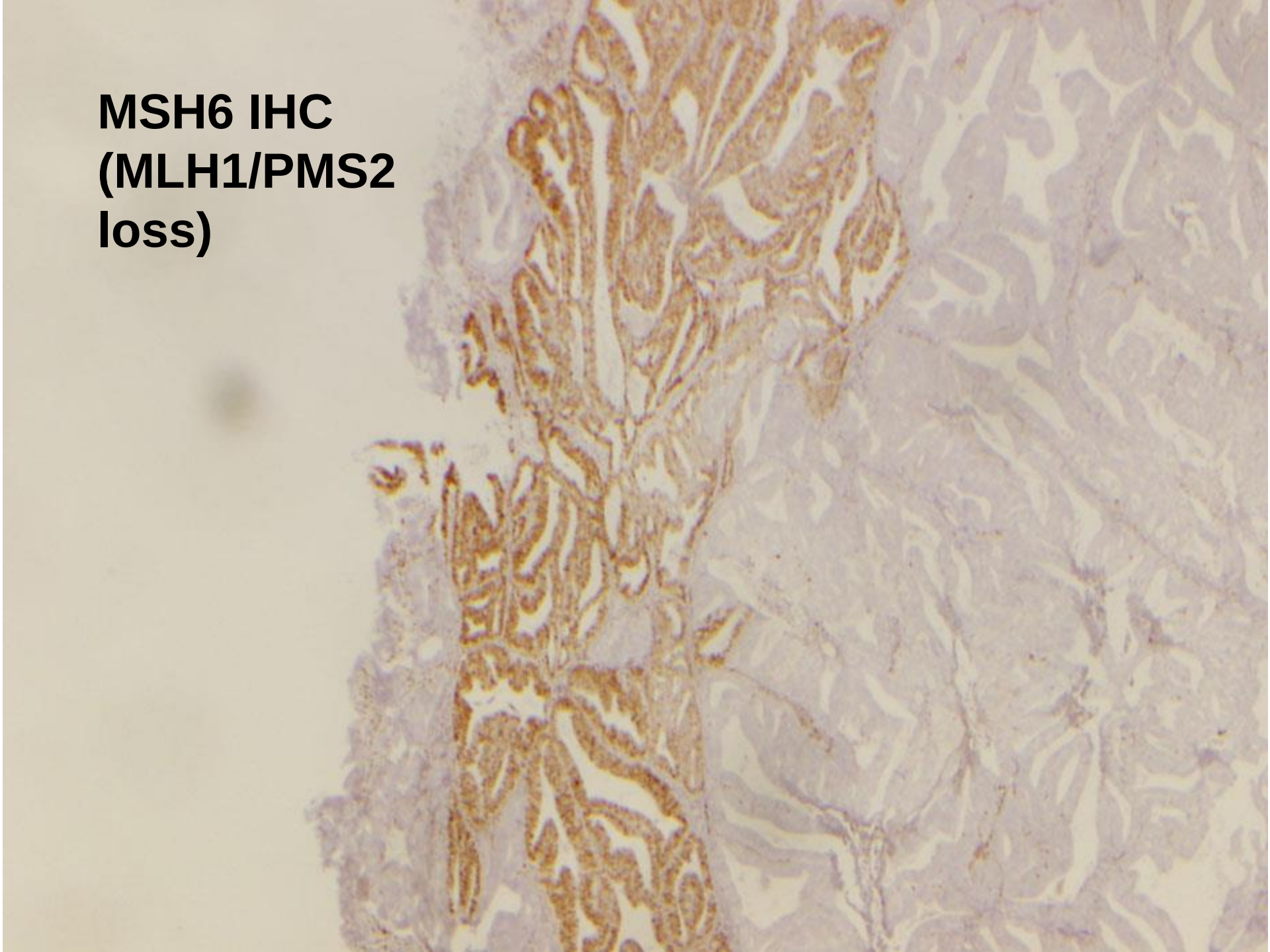

MLH1 Abnormal


PMS2 Abnormal

“Clonal” MSH6 loss

- Due to instability in a coding mononucleotide repeat in MSH6 (Shia, Modern Path 2013)
- Leads to focal (sometimes nearly complete/complete) MSH6 loss
- Primary cause of instability usually something else
  - MLH1 defect, either acquired methylation or germline
  - PMS2 defect

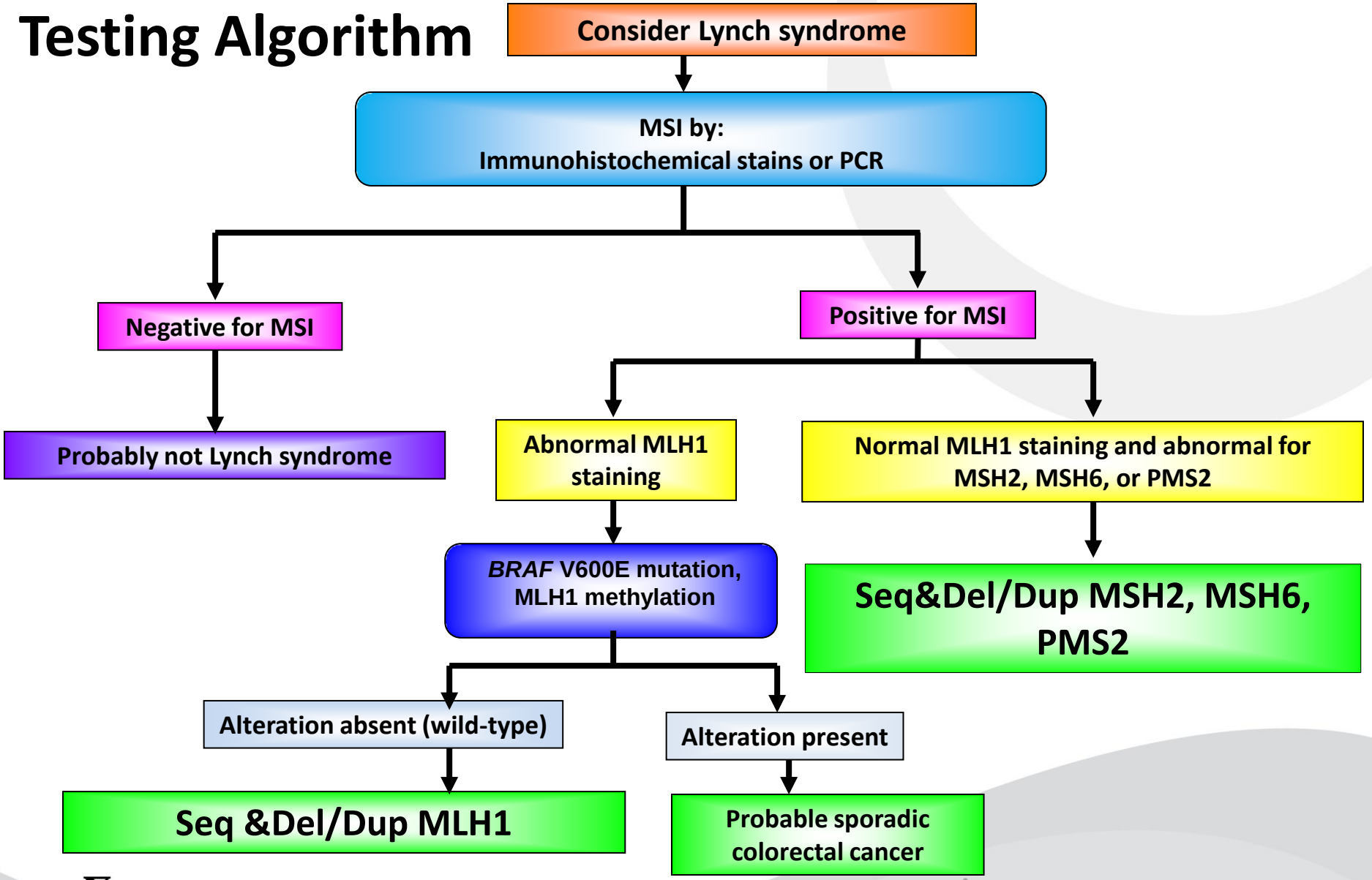
MSH6 IHC (MLH1/PMS2 loss)



Sporadic vs Lynch CRC

- Family history
- MSH2, MSH6, PMS2 IHC loss
- MLH1 promoter methylation (sporadic)
- BRAF point mutation V600E (sporadic)
  - NOTE: Not applicable to non-CRC tumors: endometrial
- *Germline* MMR gene mutation

Lynch Syndrome Testing Algorithm

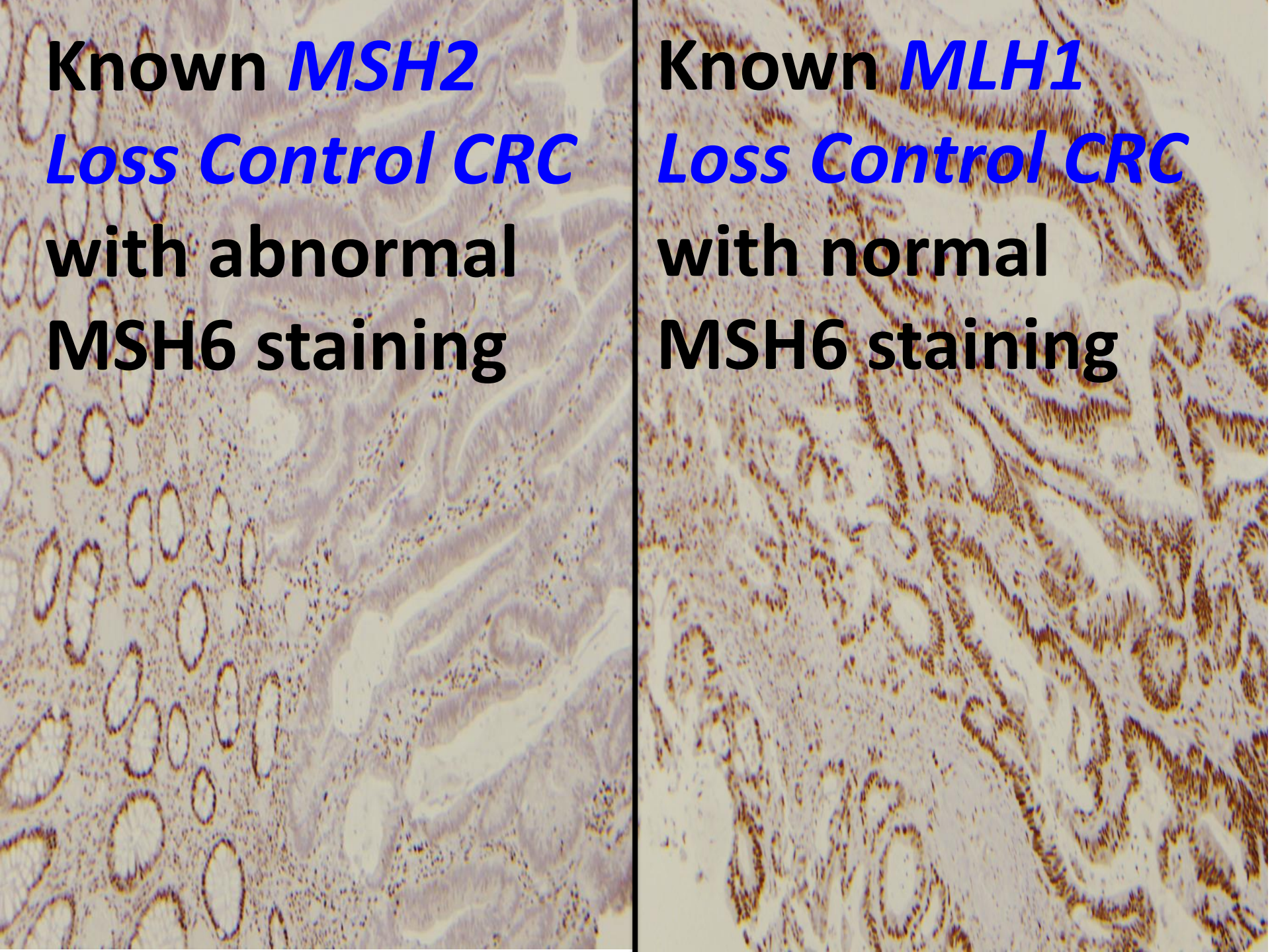


Mistake #1: IHC controls

- MMR IHC requires use of known positive and negative controls
  - Need 2 known control tumors: one with MSH2 loss, the other with MLH1 loss
  - Need two MMR stains: PMS2 & MSH6
  - 2 control slides per run with punches of both control tumors stained by PMS2 and MSH6
- Run these controls with *every* MMR IHC run
  - Need to see that antibodies stain tumors they should stain, and don't stain tumors they shouldn't
  - A tonsil doesn't show you this
  - Normal internal control cells surrounding a CRC (ex: lymphocytes) don't show you this


U149 PMS2 OV
CONTROL
ARUP
12/28/14
U176 MSH6 OV
CONTROL
ARUP
12/28/14
18
19


Known *MSH2*
Loss Control CRC
with normal
PMS2 staining
Known *MLH1*
Loss Control CRC
with abnormal
PMS2 staining

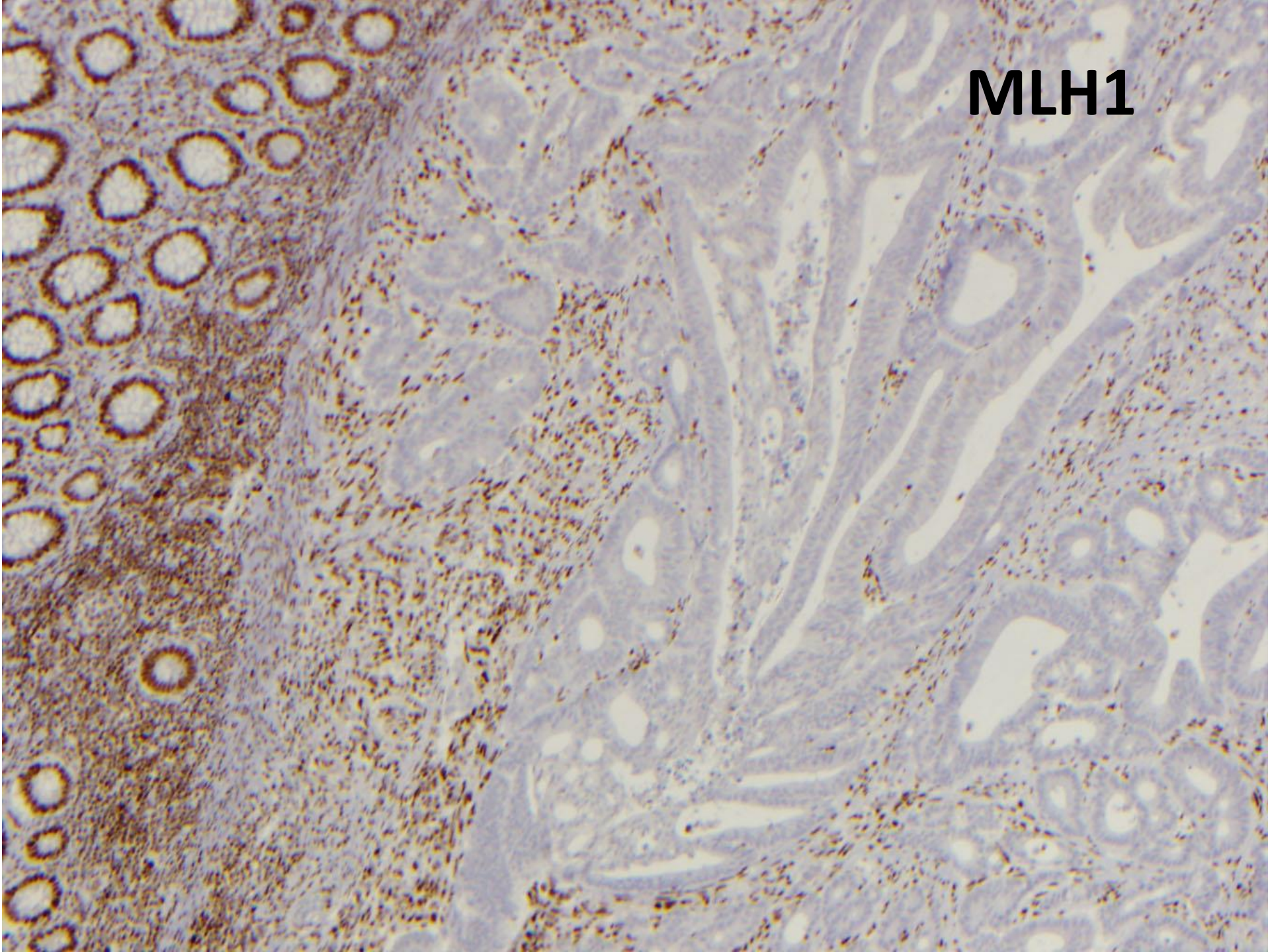

Known *MSH2*
Loss Control CRC
with abnormal
MSH6 staining
Known *MLH1*
Loss Control CRC
with normal
MSH6 staining

Mistake #2: Reporting IHC results

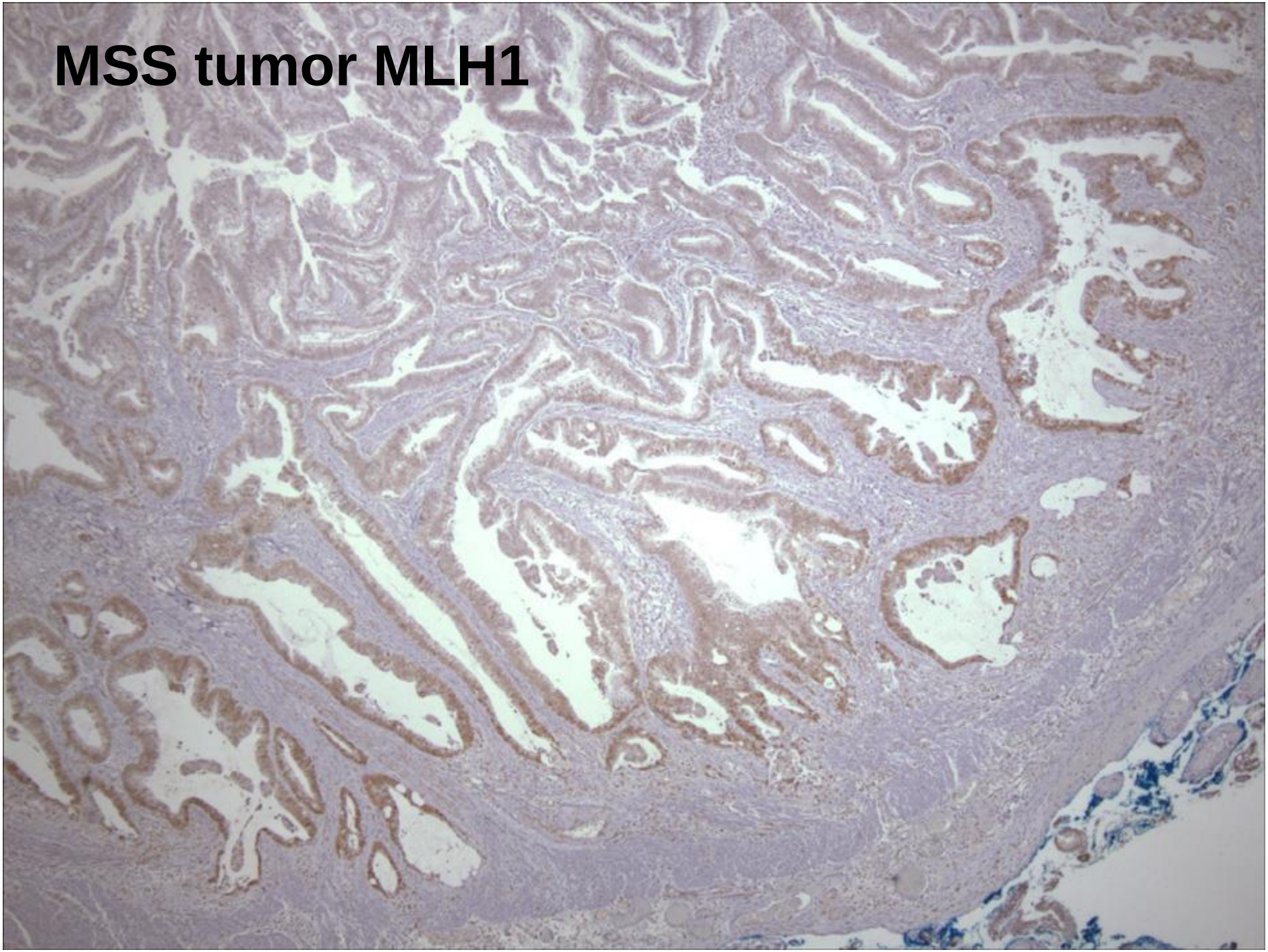
- Don’t describe IHC staining as “positive” or “negative”—confusing
- Report clearly; get feedback from clinicians (we say “normal” and “abnormal”)
- Don’t report results that no one sees or acts upon
    - Interact with colleagues who deal with results
    - Make sure your reports are comprehensible and clinicians are reacting appropriately (genetic counselors probably best)

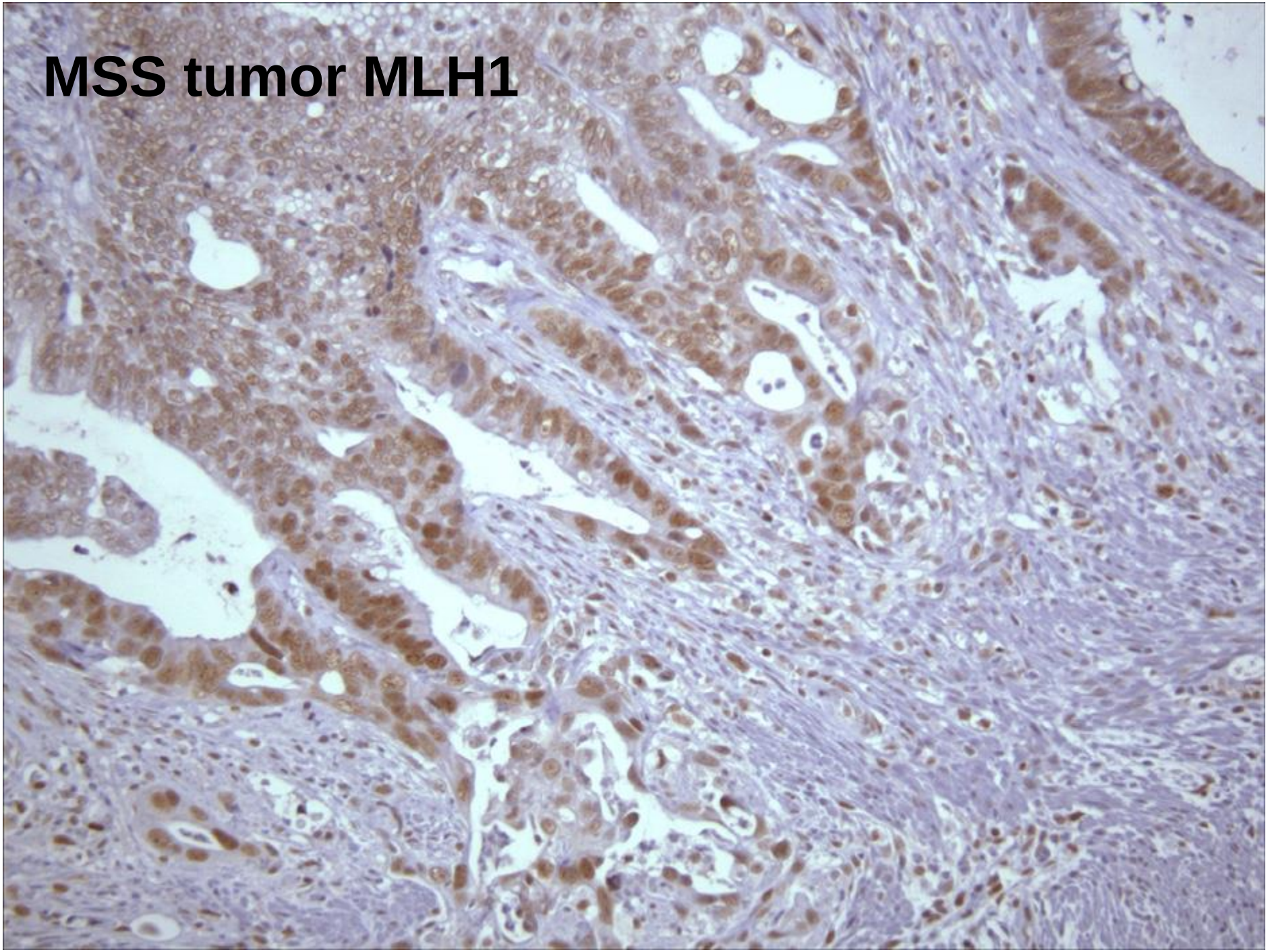
Mistake #3: IHC interpretation

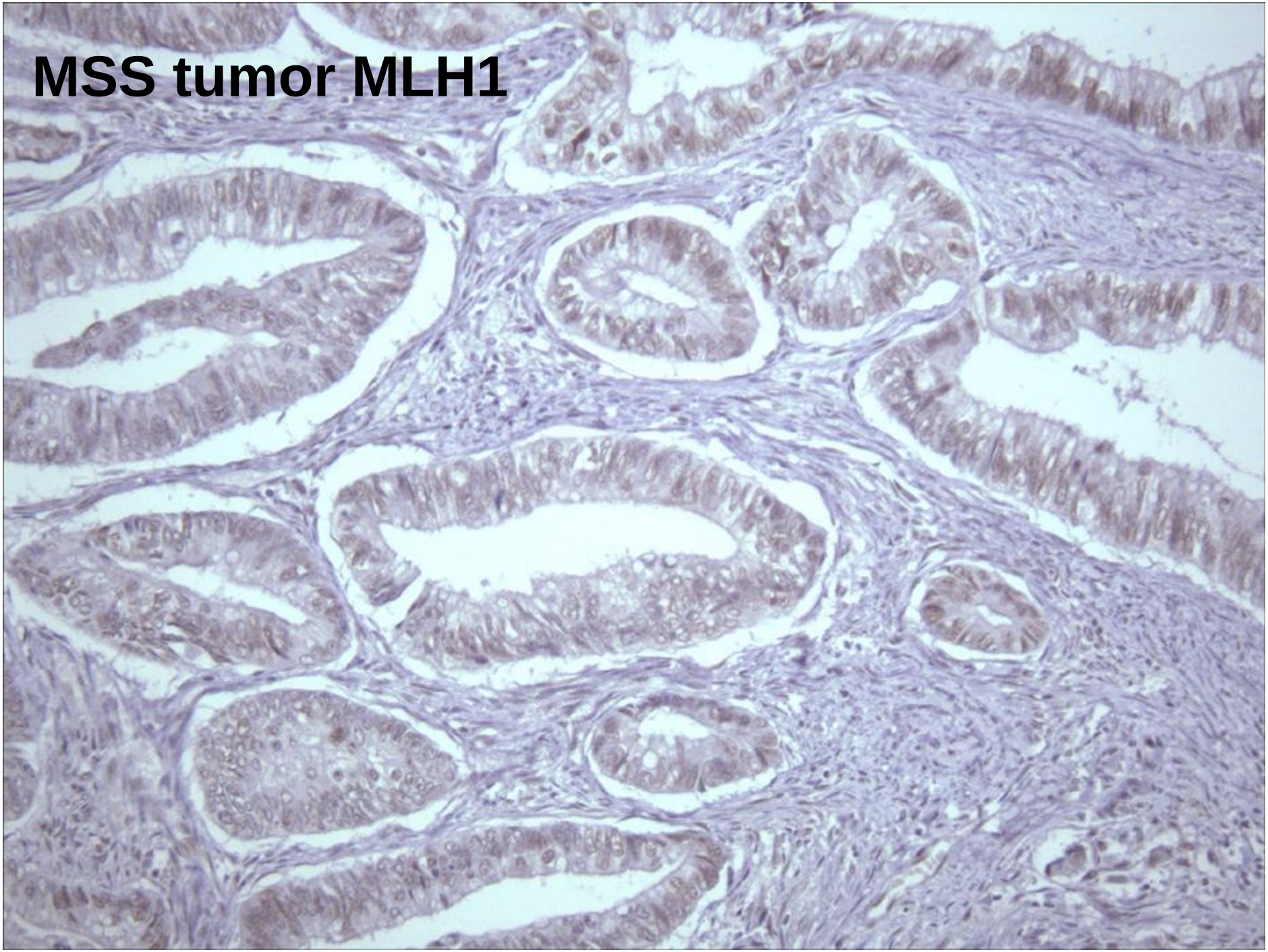
- Loss of tumor staining without contiguous *internal control* staining is uninterpretable: don't call this abnormal
- Decreased staining intensity, unless quite marked, probably doesn't mean anything: this is a qualitative test
    - If marked, suggest confirmatory MSI PCR testing


MLH1

MSS tumor MLH1




MSS tumor MLH1

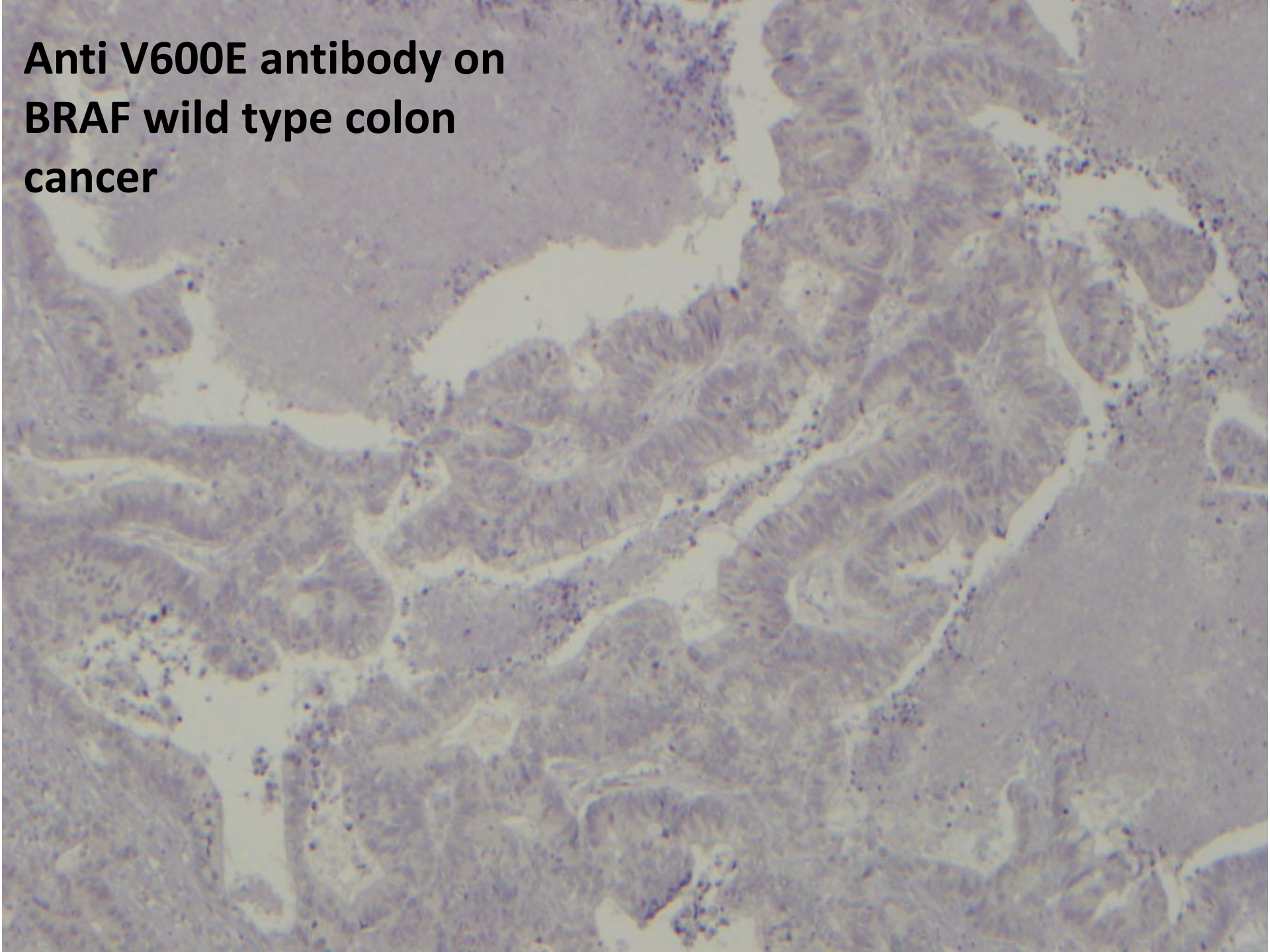

MSS tumor MLH1

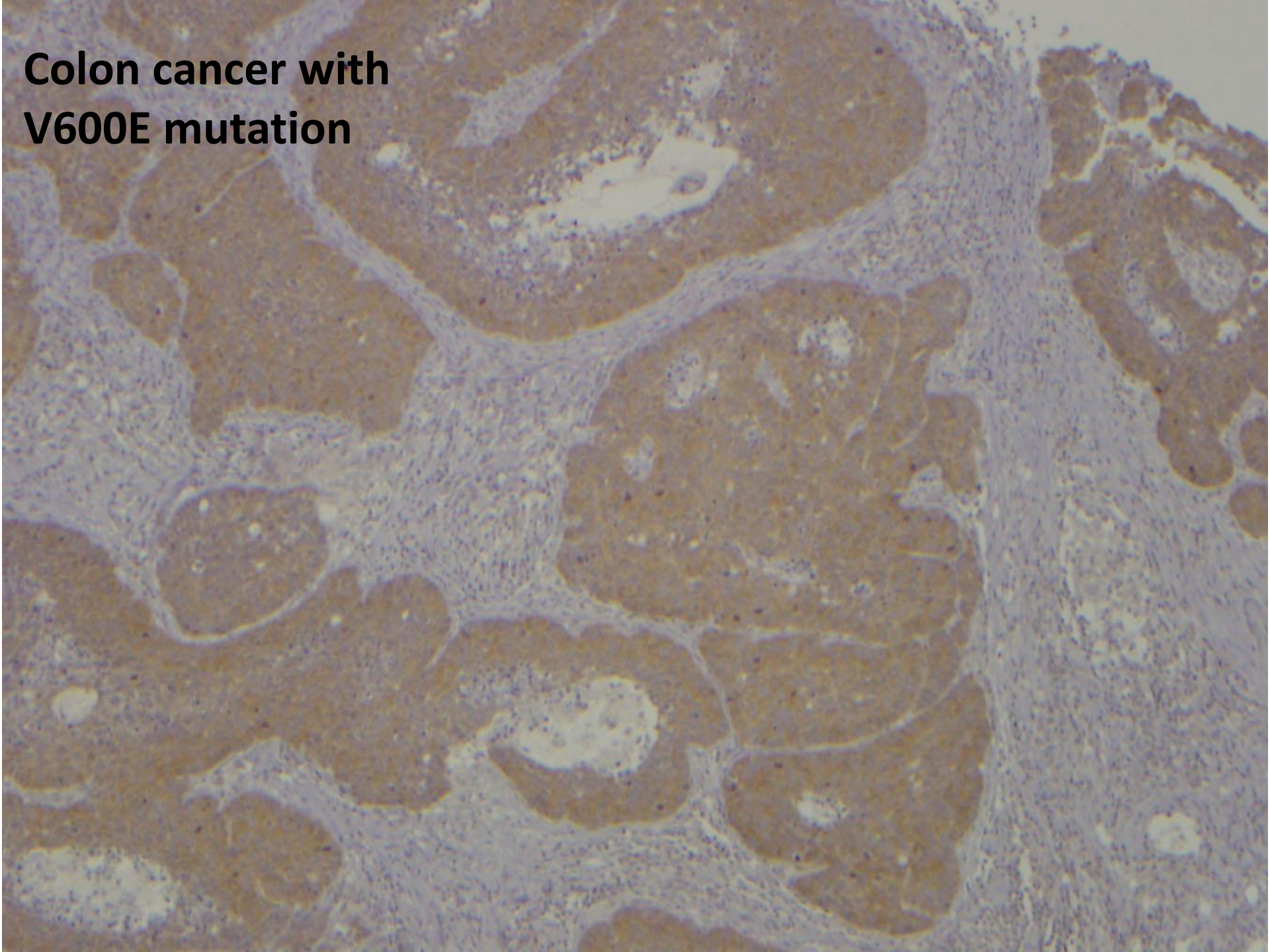
Mistake #4: Inappropriate BRAF testing

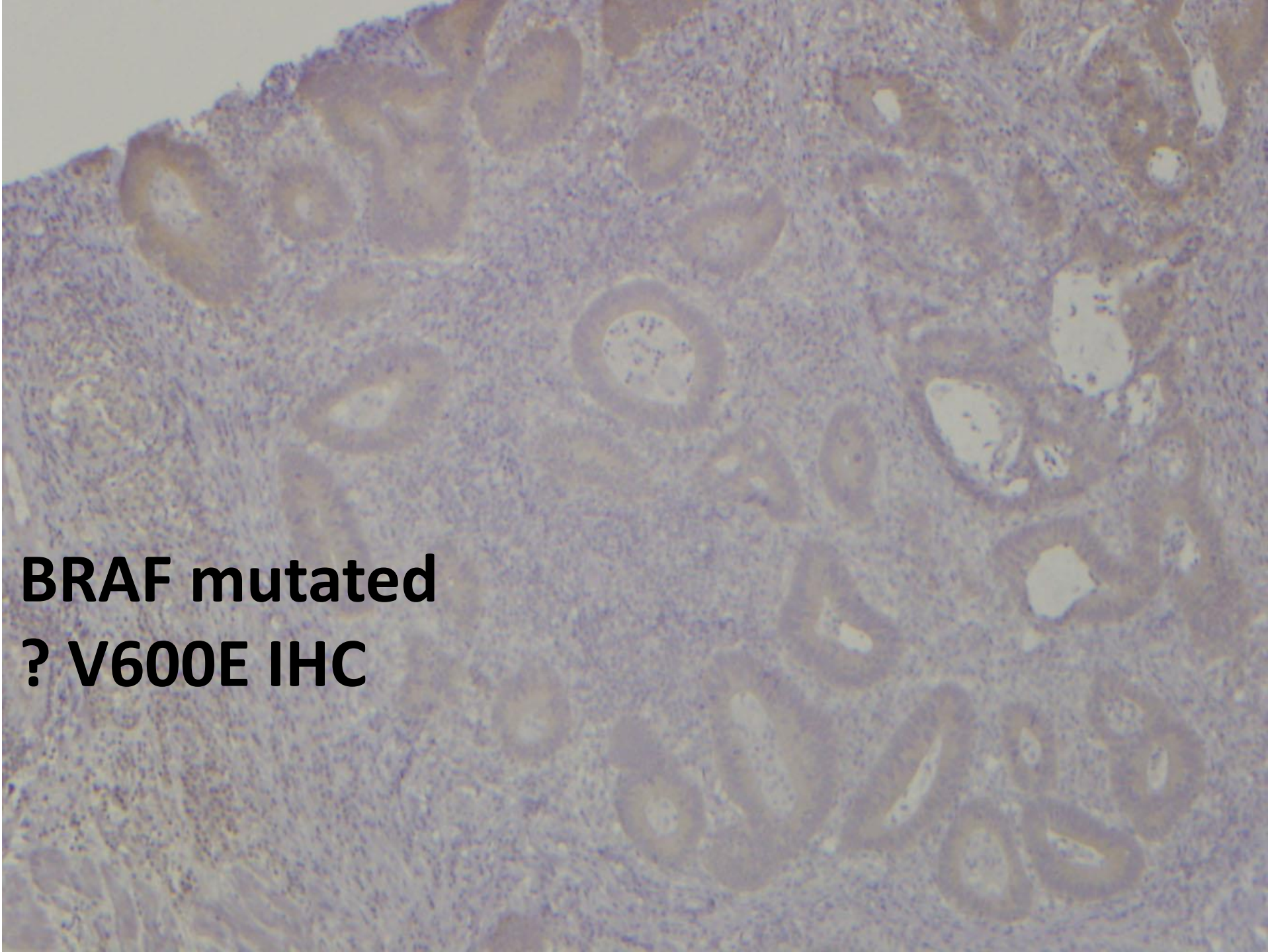
- Non-colorectal (e.g., endometrial) cancers rarely mutate BRAF, DON'T ORDER TEST
- Need to test MLH1 methylation for non-colorectal cancers
- Need to test MLH1 methylation for potentially sporadic colorectal cancers without BRAF mutations

Mistake #5: All IHC Lynch work-up

- BRAF antibody: detects BRAF V600E mutation (Affolter, Samowitz, Bronner; GCC 2013)
- Has same problems as all other IHC tests, including staining variability and difficult interpretation
- No internal controls for antibody staining
- Research vs. clinical test
    - Clinical test needs to be robust, easily interpretable


Anti V600E antibody on
BRAF wild type colon
cancer


Colon cancer with
V600E mutation


BRAF mutated
? V600E IHC

Mistake #6: Testing of serrated lesions

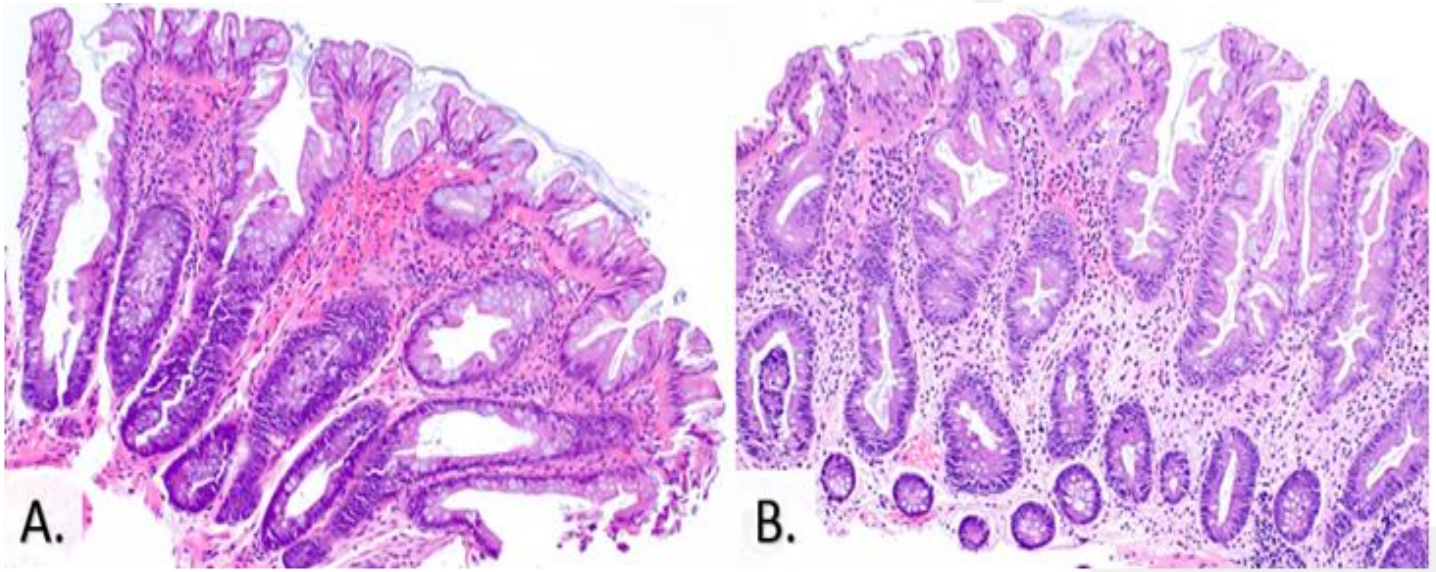
- Evaluating serrated lesions for *MMR deficiency: USELESS*
  - Based on incorrect notion that MSI will separate SSP's from HP's
- Evaluating serrated lesions for *BRAF mutations*: *USELESS*
  - Both SSP's and HP's commonly have BRAF mutations

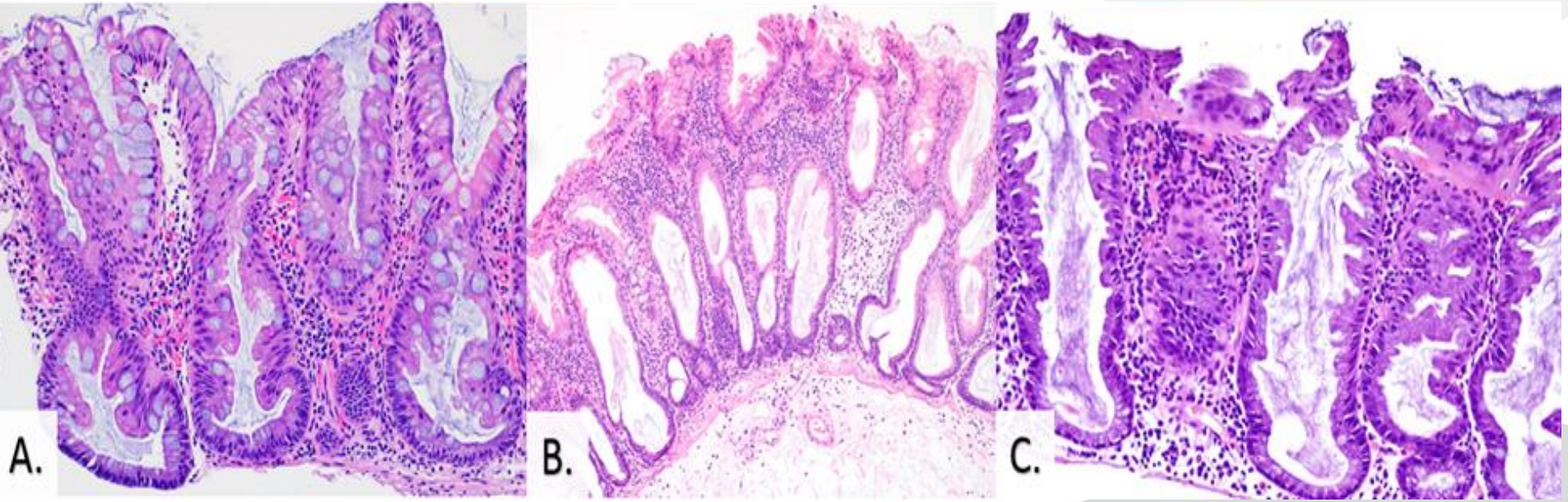
SSP vs. HP

- No molecular test reliably separates these lesions
- Use polyp site (R vs L), size (>2 biopsies), and histology to distinguish these lesions

Criteria to Distinguish HP from SSL

Criteria	ROC AUC
Morphology	69.3%
Size (cutoff bx number 2 or more)	**87.3%**
Endoscopic size	55.2%
Location	82.3%
Morphology, Size and Location	**93.7%**


A.
B.


A.
B.
C.

KRAS Testing

The Metastasis Problem

- 50-60% CRC patients present with or develop metastases
- 5-yr survival

  Stage I + II (N0) → 91%

  Stage III (N1,2) → 70%

  Stage IV (M1) → 11%

Search for Alternative Rx's

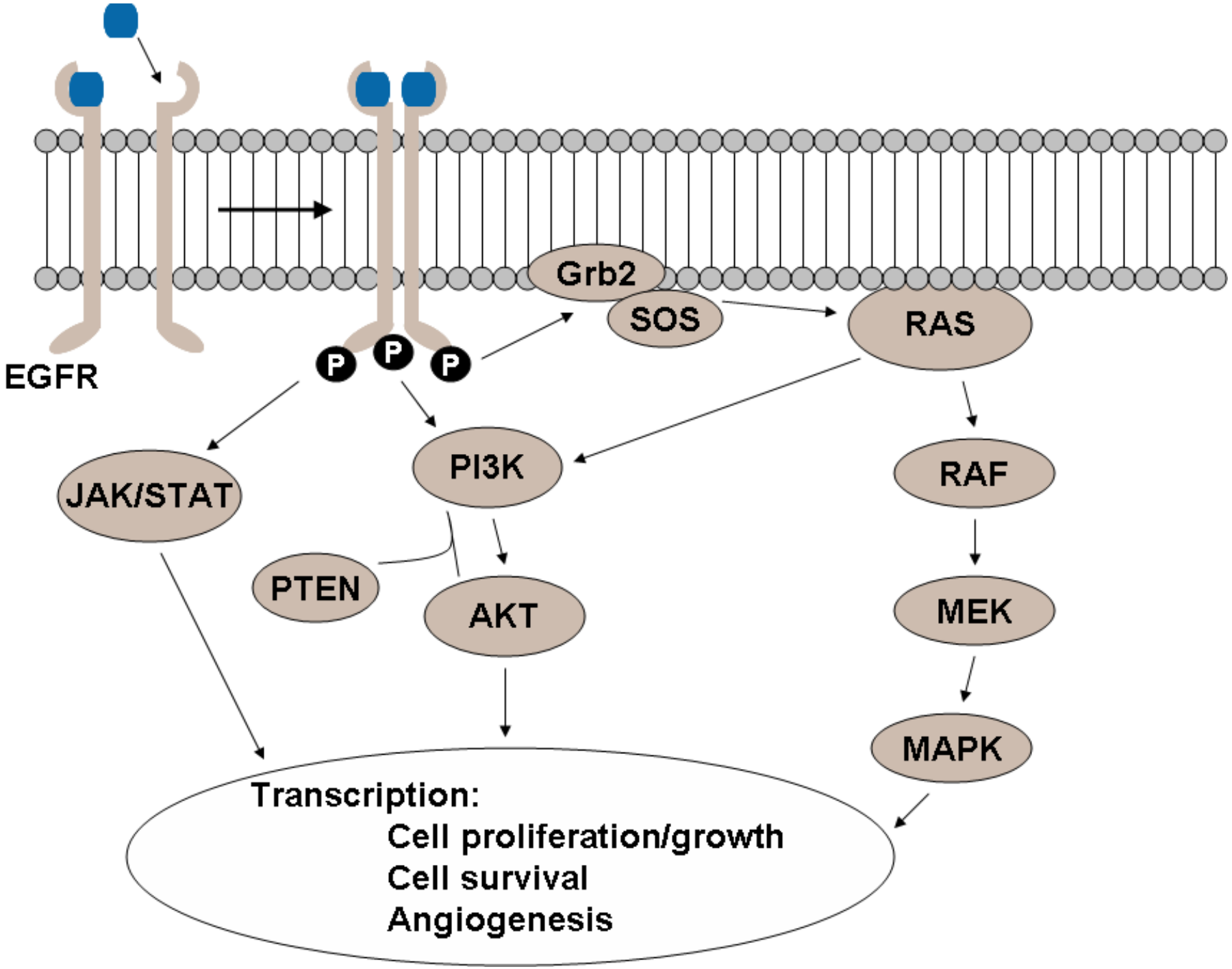
5 FU/Leucovorin mainstay for decades

After 2000 → New Therapies

- Oxaliplatin (FOLFOX)
- Irinotecan (FOLFIRI)
- Anti-VEGF (bevacizumab)
- Anti-EGFR (cetuximab, panitumumab)

EGFR inhibitor therapy for CRC

- EGFR pathway is activated (but EGFR is not mutated) in colorectal cancer
- Cetuximab is an antibody that binds to EGFR and turns off EGFR pathway
- Mutations downstream of EGFR (KRAS, BRAF, PIC3, PTEN) activate the pathway and make EGFR block irrelevant
- Bad to give a toxic and expensive drug if it won't work


EGFR
P
P
P
Grb2
SOS
RAS
JAK/STAT
PI3K
RAF
PTEN
AKT
MEK
MAPK
Transcription:
Cell proliferation/growth
Cell survival
Angiogenesis

KRAS mutation

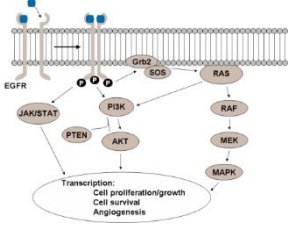
- <1% response rate to anti-EGFR Rx with codon 12 or 13 or 61 mutations (~40% of CRC)
- ~40% response rate with KRAS WT (~60% of CRC)
- But…. ~ 60% KRAS WT do not respond to EGFR inhibition
- Other markers play a role and in clinical trials

KRAS Testing: Cost Savings

- ~30,000 new *metastatic* CRC annually
- KRAS testing = $13 million ($452/pt)
- Cetuximab Rx= $2.1 *billion* ($71,120/pt)
- Mutated KRAS (~40%) excluded from cetuximab
- Cost savings: ~$750 million annually
- High toxicity; ~2 month added survival

No need for normal tissue to test for KRAS (unlike MSI)

Future



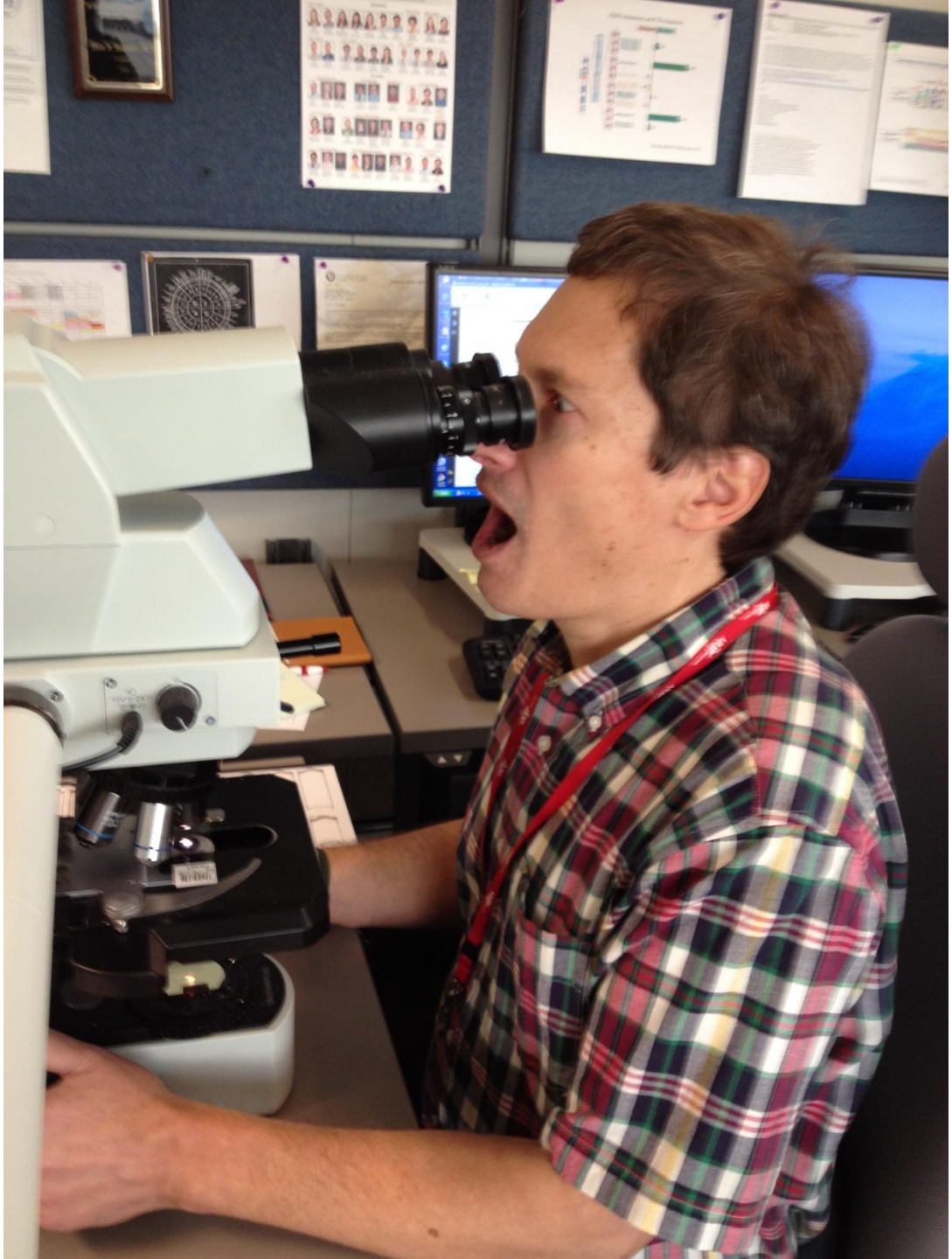
- Impact of *specific* KRAS 12/13 mutations?
  - Sotorasib targeted to KRAS G12C (~3-7% met CRC) in phase 2 trial did not meet primary endpoint (*Lancet Oncol* 2022;23:115), unlike in NSCLC
  - Combination trials of KRAS G12C with EGFR inhibitors or immunotherapy underway
- Other predictors of anti-EGFR response?
  - Other KRAS mutations: codon 61, others?
  - BRAF
  - EGFR copy no. (FISH,CISH,PCR,NGS), specific mutations
  - PTEN, PIK3CA mutations

KRAS Summary

- KRAS mutations occur in 30-40% CRC's
- Highly predictive of lack of response to anti-EGFR Rx (such as cetuximab)
- Pathologists play a key role in determining best Rx for stage III-IV CRC
- BRAF, PIK3CA, PTEN downstream markers may be useful in KRAS wild type tumors
- Additional biomarkers expected and many clinical trials underway

What is our role in this?

- Selecting block to test
- Circling tumor
- Maybe performing the test, interpreting results



Mistake #7: Choosing a bad block

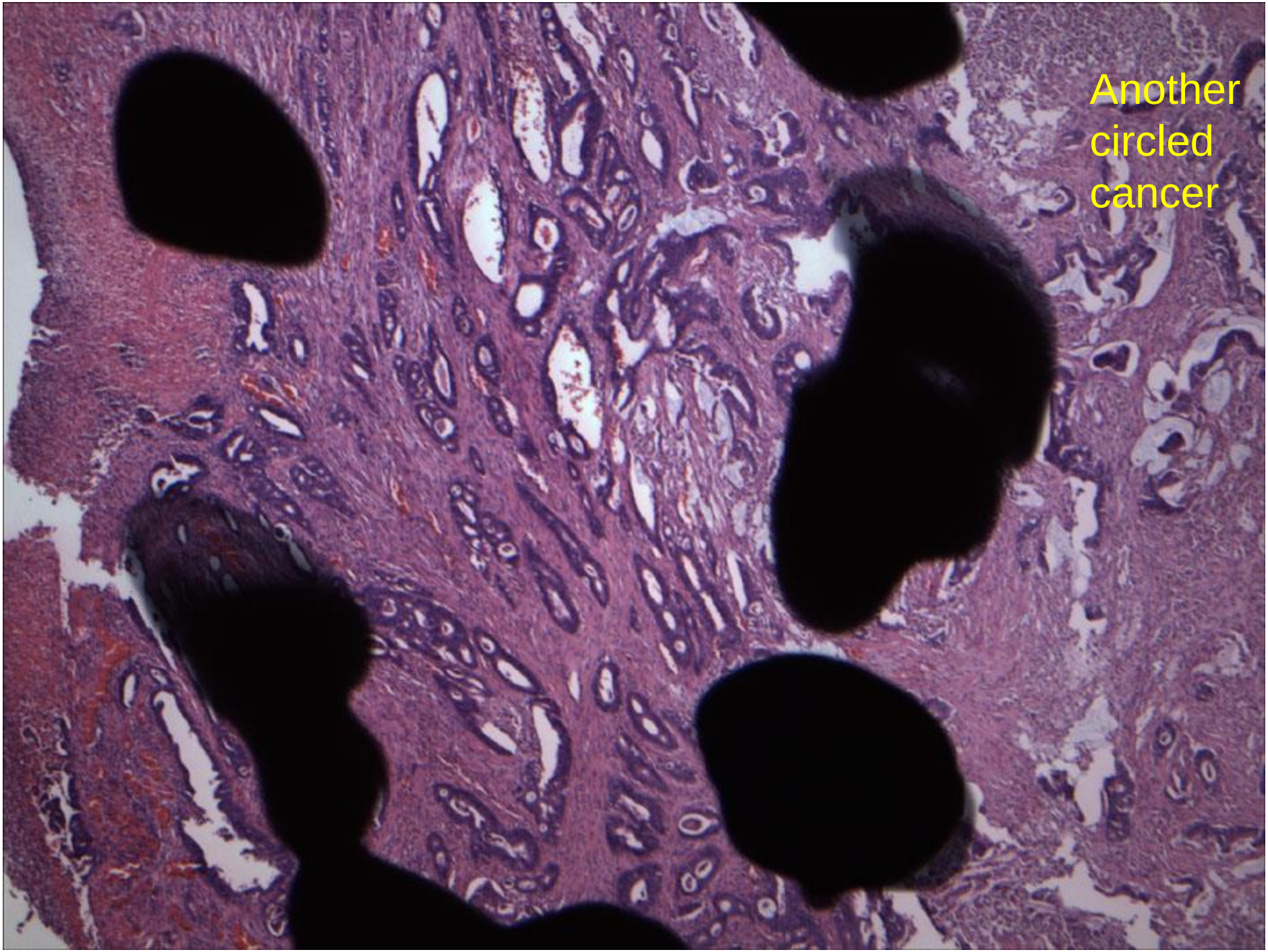
- PCR isn't magic; garbage in, garbage out still applies
- With colon cancer, finding a block with sufficient tumor usually isn't a problem
- Rectal cancers resected after chemoradiation may be hypocellular; often better to choose pre-treatment biopsy
- Don't use decalcified specimens, specimens fixed in unusual fixatives

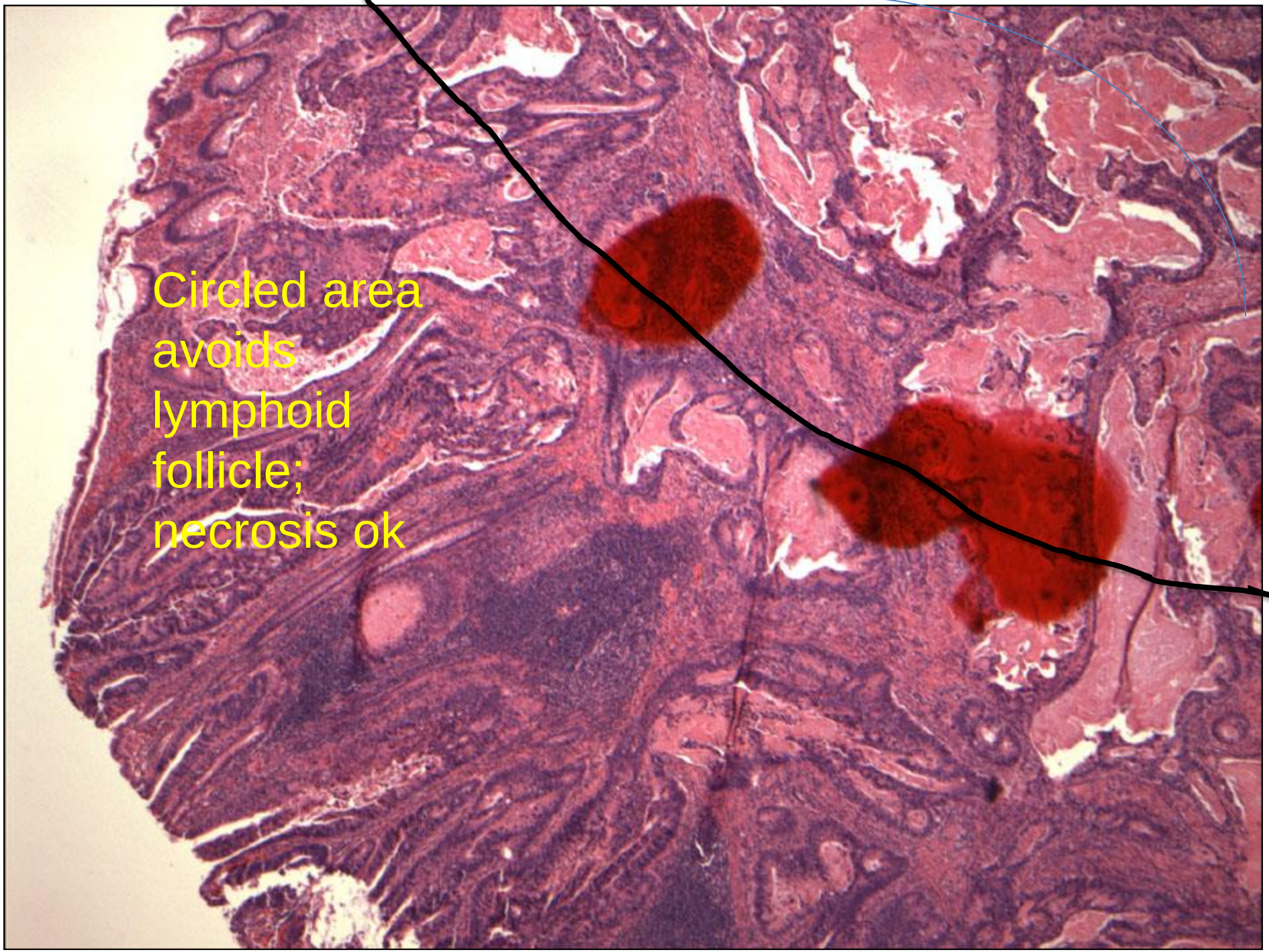
Mistake #8: Poor circling of tumor

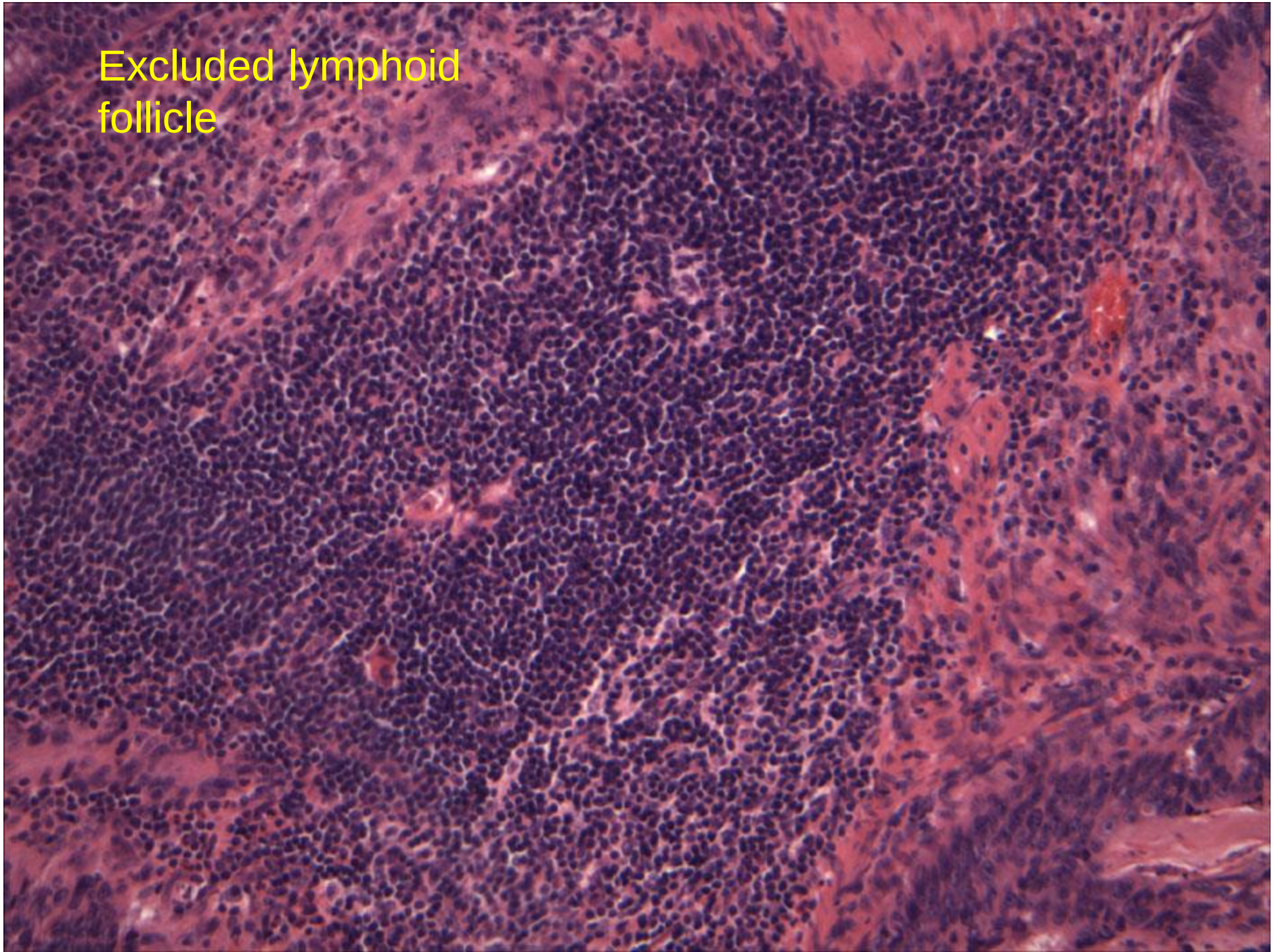
- Avoid (as much as possible) contaminating *normal cells* (lymphoid follicles, abscesses, muscle)
  - Don't be ridiculous about this, most tests will work with about 20% tumor, usually easily achievable with colon cancer
- Don't need all of the tumor
  - No need to "gerrymander" the circled area
    - Difficult to dissect, wastes everyone's time

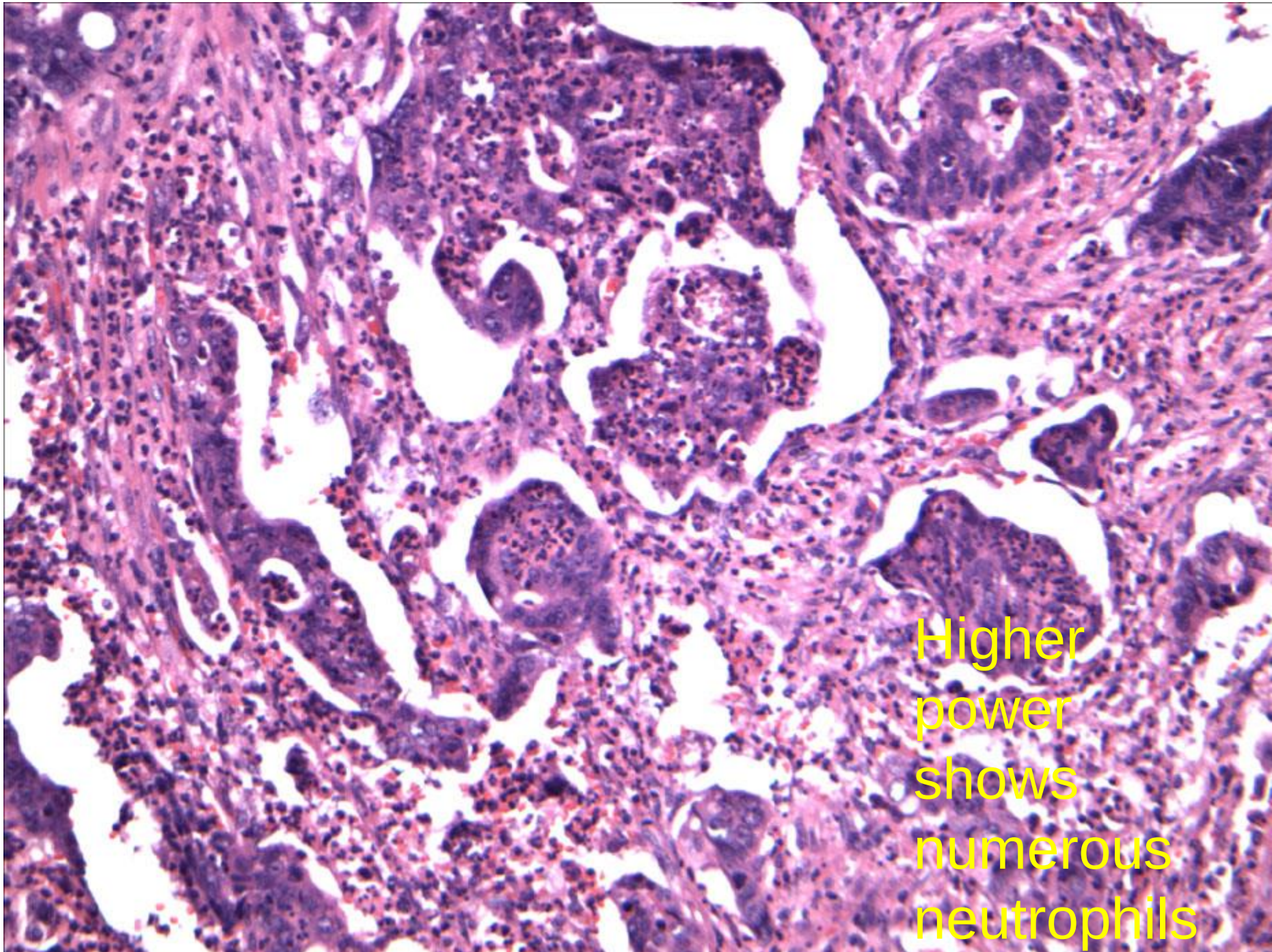

H&E
29


H&E
29


Another
circled
cancer


Circled area
avoids
lymphoid
follicle;
necrosis ok


Excluded lymphoid follicle

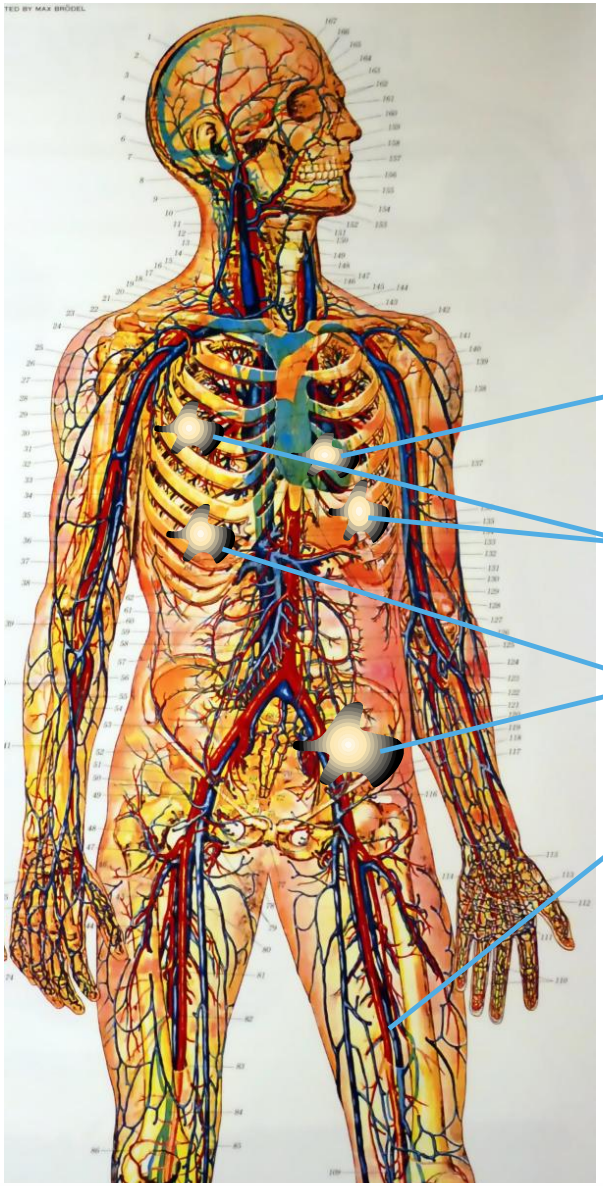

Higher power shows numerous neutrophils

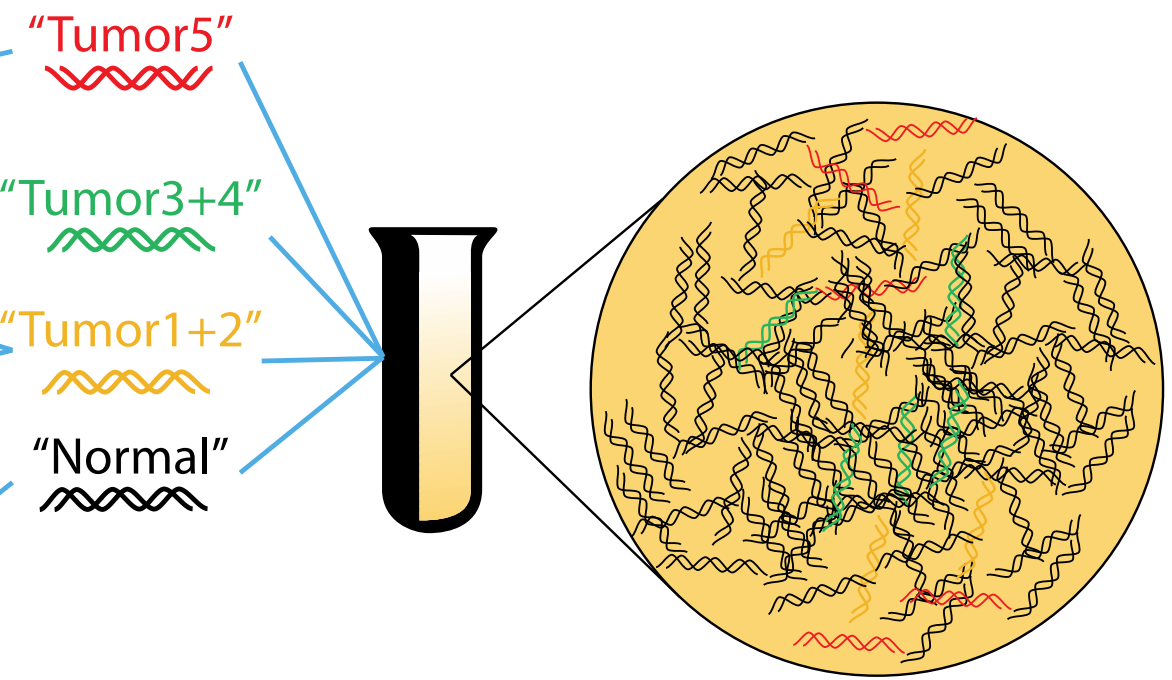
Mistake #9: Assuming tumor homogeneity

- Different areas of a tumor, different metastases may have different mutations
- We unfortunately ignore this by evaluating one part of a primary, or one of many metastases
- Evaluation of circulating tumor DNA may be a way to get a mutational evaluation of the entire tumor burden (for review see Heitzer, Clinical Chemistry, 2015)

ctDNA reduces sampling error and allows analysis of entire tumor burden: primary/mets/heterogeneous clones

Blood is the "window to the body"





Summary: CRC Molecular Dx

- Sporadic MSI-H CRC (15%): MMR IHC, MSI, MLH1 Methylation, BRAF
- Lynch MSI-H CRC (2-3%): MMR IHC, MSI, MLH1 Methylation, BRAF
- Metastatic CRC KRAS WT (50-60%) selects for Anti-EGFR therapy
- KRAS G12C specific inhibitor therapy
- KRAS WT Non-responders to Anti-EGFR (60%): work continues

What about EPCAM?

- EPCAM is just five prime of MSH2
- Three prime EPCAM deletions lead to transcriptional read through, MSH2 methylation and Lynch syndrome
- EPCAM deletions associated with similar colon cancer risk as MSH2 mutations, but less of an endometrial cancer risk

Does EPCAM IHC help in Lynch work-up?

- Standard MMR IHC won't miss Lynch due to EPCAM deletions
  - IHC profile will be MSH2/MSH6 loss
- Standard germline genetic analysis for MSH2 will detect EPCAM deletions
  - Already includes probes for EPCAM deletions