

Molecular testing in GI cancer

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Disclosures

- Potential royalties in the future related to the Ventana BRAF V600E antibody



THE TEN COMMANDMENTS

- I Thou shalt have no other gods before me.
- II Thou shalt not make unto thee any graven image, or any likeness of anything that is in heaven above, in earth beneath, or in the water under the earth.
- III Thou shalt not take the name of the Lord thy God in vain, for the Lord will not hold him guiltless that taketh his name in vain.
- IV Remember the Sabbath day, to keep it holy.
- V Honour thy father and thy mother; that thy days may be long upon the land.
- VI Thou shalt not kill.
- VII Thou shalt not commit adultery.
- VIII Thou shalt not steal.
- IX Thou shalt not bear false witness against thy neighbour.
- X Thou shalt not covet thy neighbour's house, wife, manservant, maidservant, ox, ass, nor anything that is thy neighbour's.

Exodus 20:7-17



Traditional Anatomic Pathology



COLON CANCER
A type of cancer that starts in the large intestine or rectum. It is the second leading cause of cancer death in the United States.

POLYPS
Small growths on the lining of the colon or rectum. Some polyps are precancerous and can turn into cancer if not removed. Some are benign.

Why do you need to know this

- A lot of ignorance out there
- Clinicians will bother you about this
- Even if your lab doesn't perform these tests
 - Need to know what to order
 - Need to know what it means

Topics

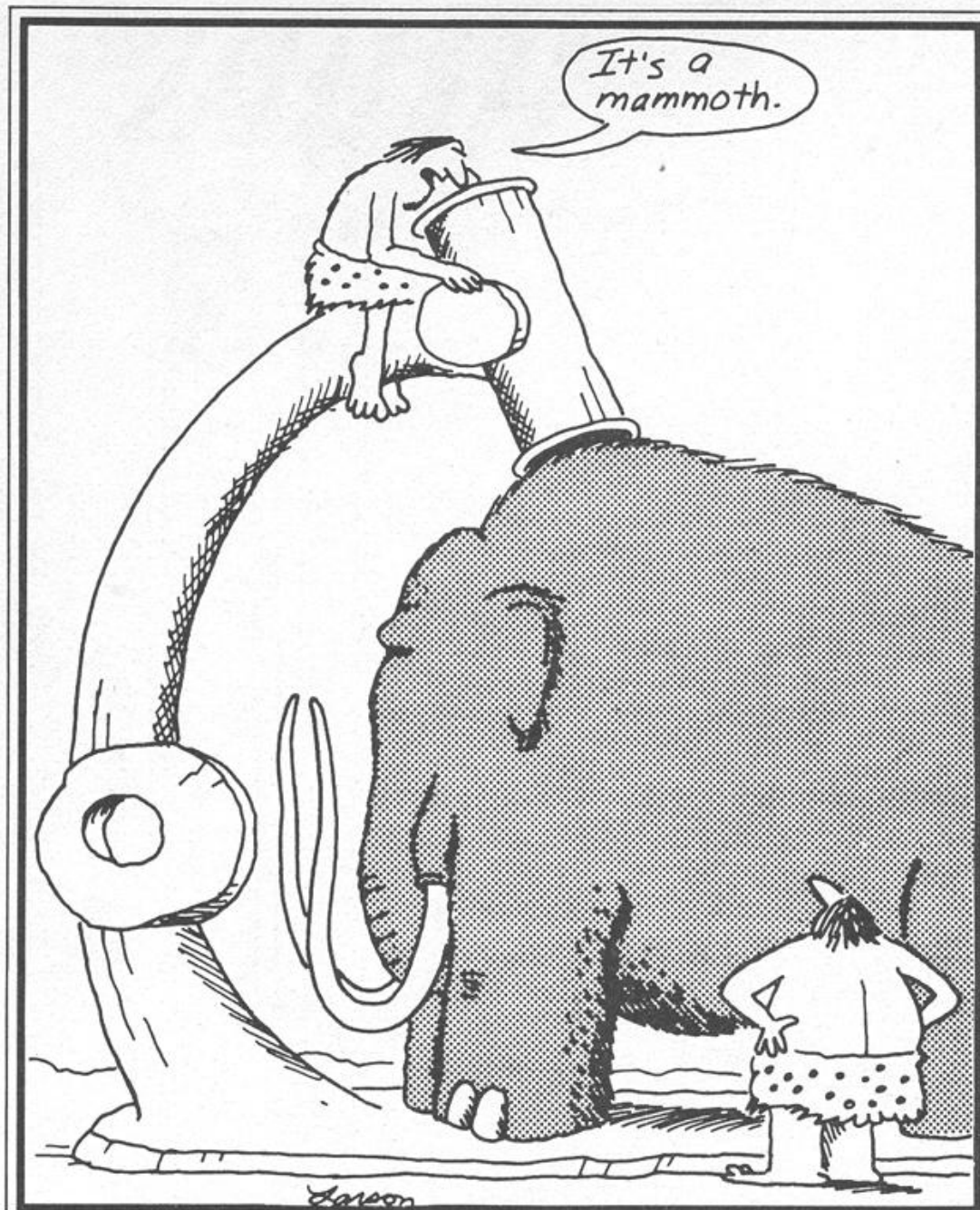
- Mutation detection in solid tumors: general considerations
- Lynch syndrome
- Therapy, especially EGFR pathway
- Future

Mutation detection in fixed tissue: General Considerations

- Solid tumors are different than germline DNA (or even most hematolymphoid samples)
 - Consist of heterogeneous cell types
 - Requires some form of microdissection
 - Need AP/CP coordination
- Garbage in, garbage out
 - Choose best tumor block (highest concentration of tumor)



Slide of a colon cancer with a circled area of colon cancer which will be microdissected

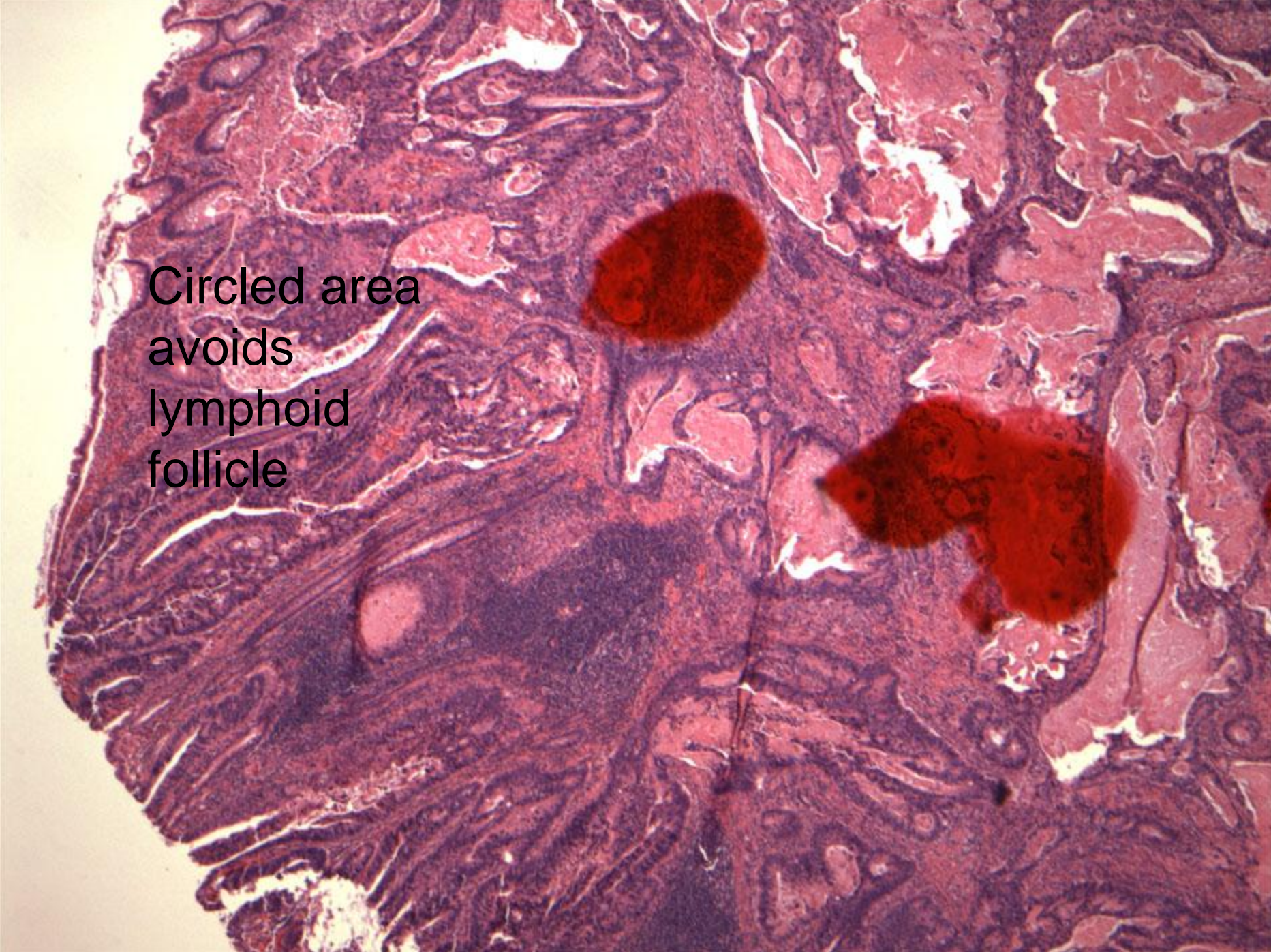


Early microscope

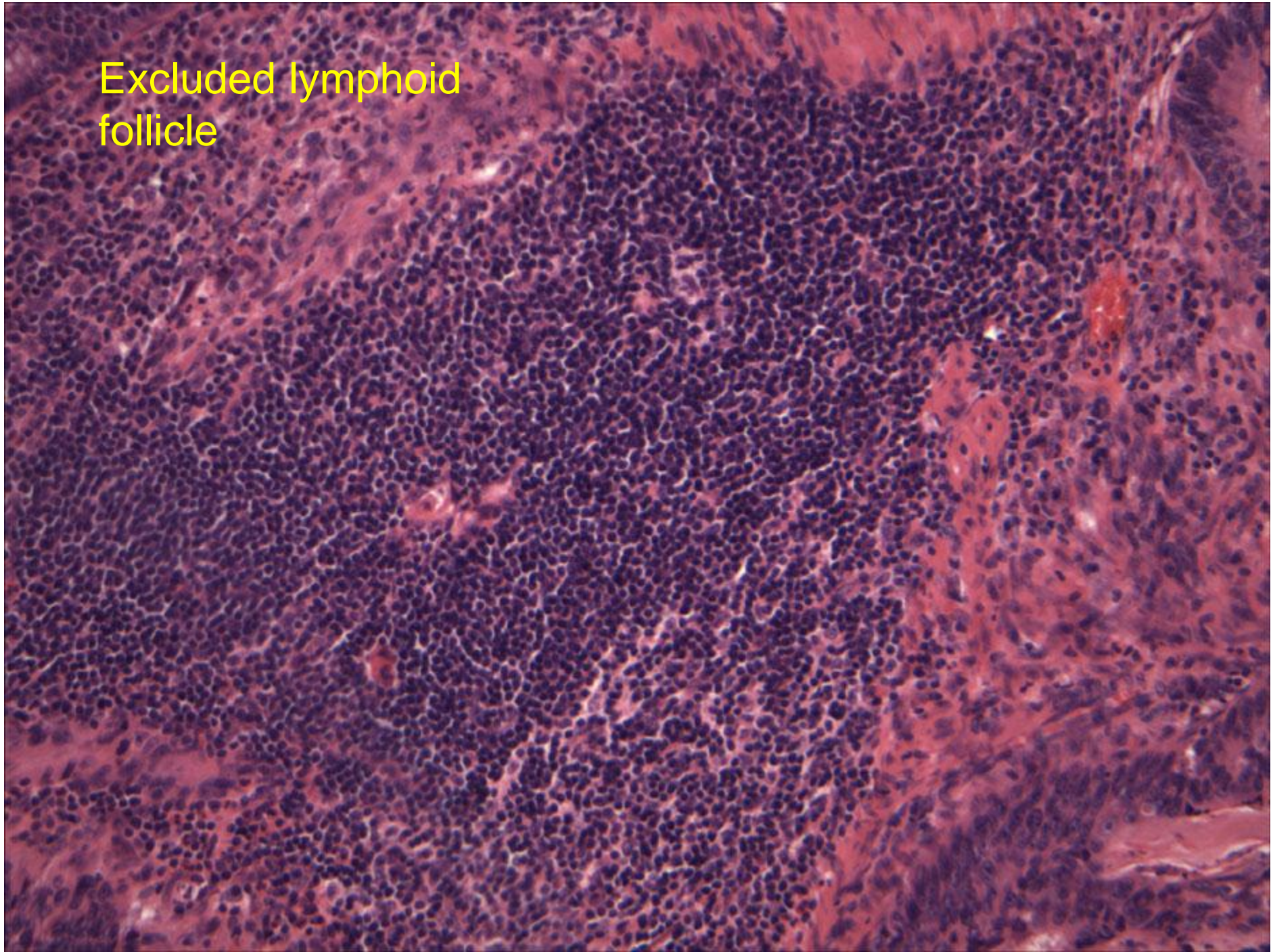
A histological section of endometrial tissue stained with hematoxylin and eosin (H&E). The image shows a complex arrangement of glandular structures and stromal components. A central area is circled in red, indicating a region of interest for higher magnification. The glands are lined by a simple columnar epithelium, and the stroma is composed of spindle-shaped cells. The overall appearance is characteristic of the secretory phase of the menstrual cycle.

Higher power
of circled area

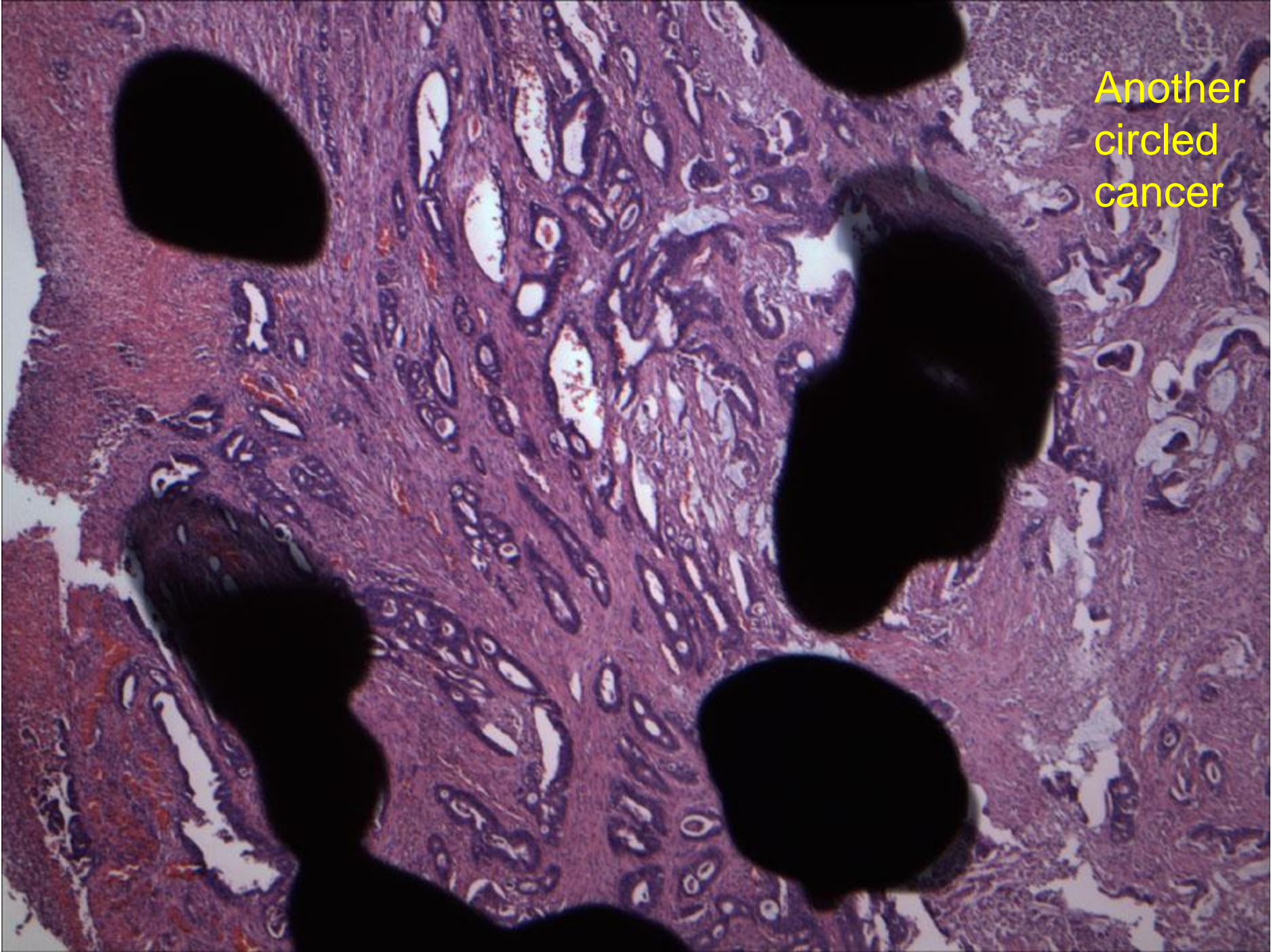
Circled area
avoids
lymphoid
follicle

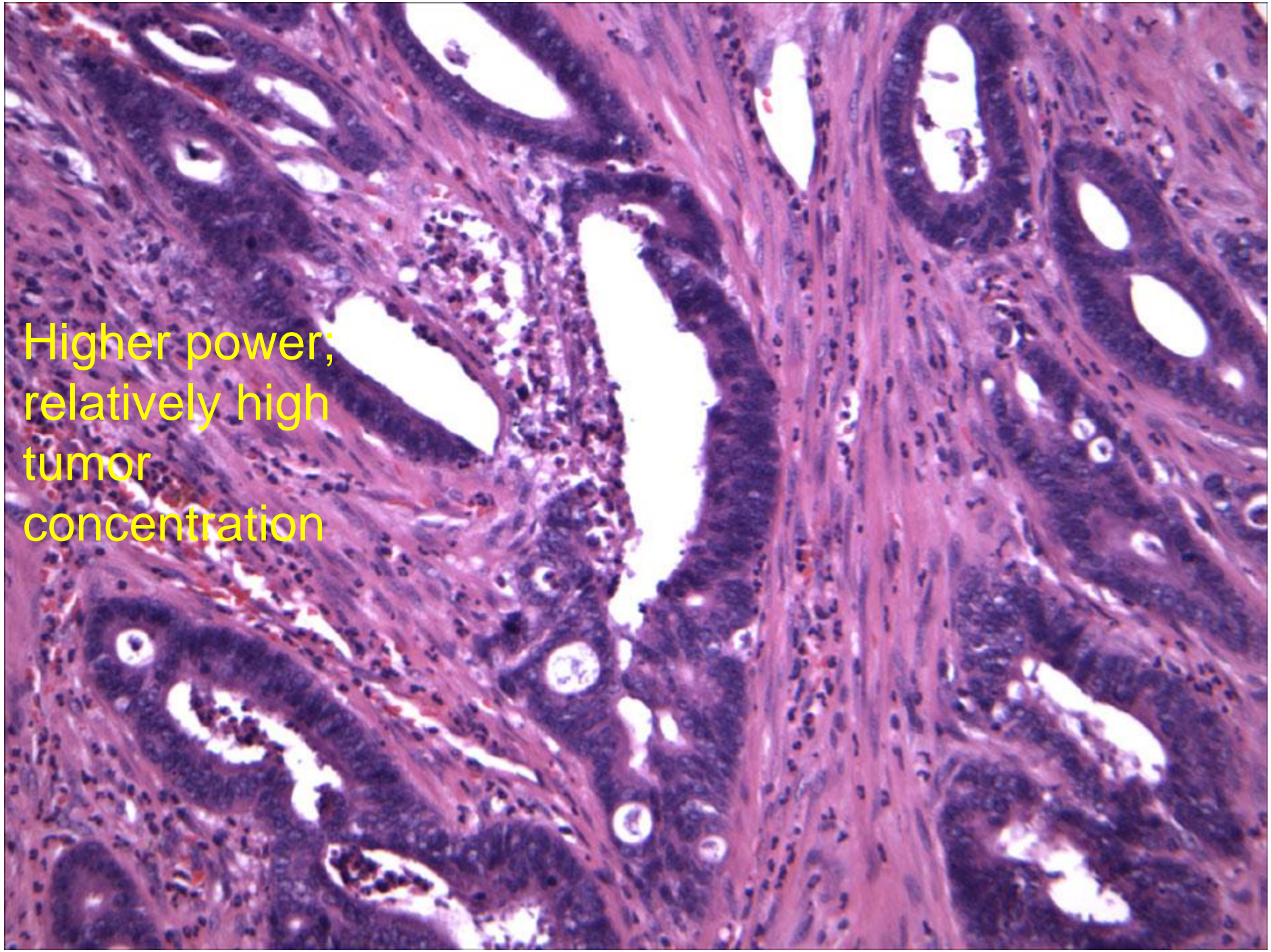


Excluded lymphoid
follicle



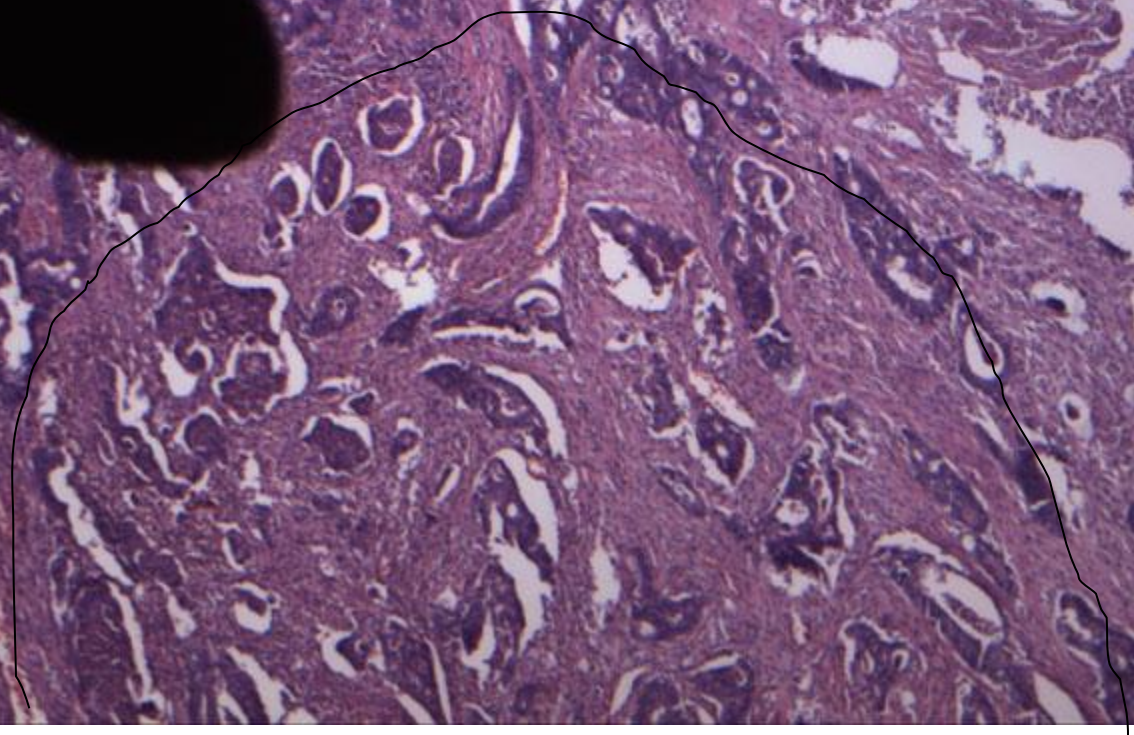
Another
circled
cancer

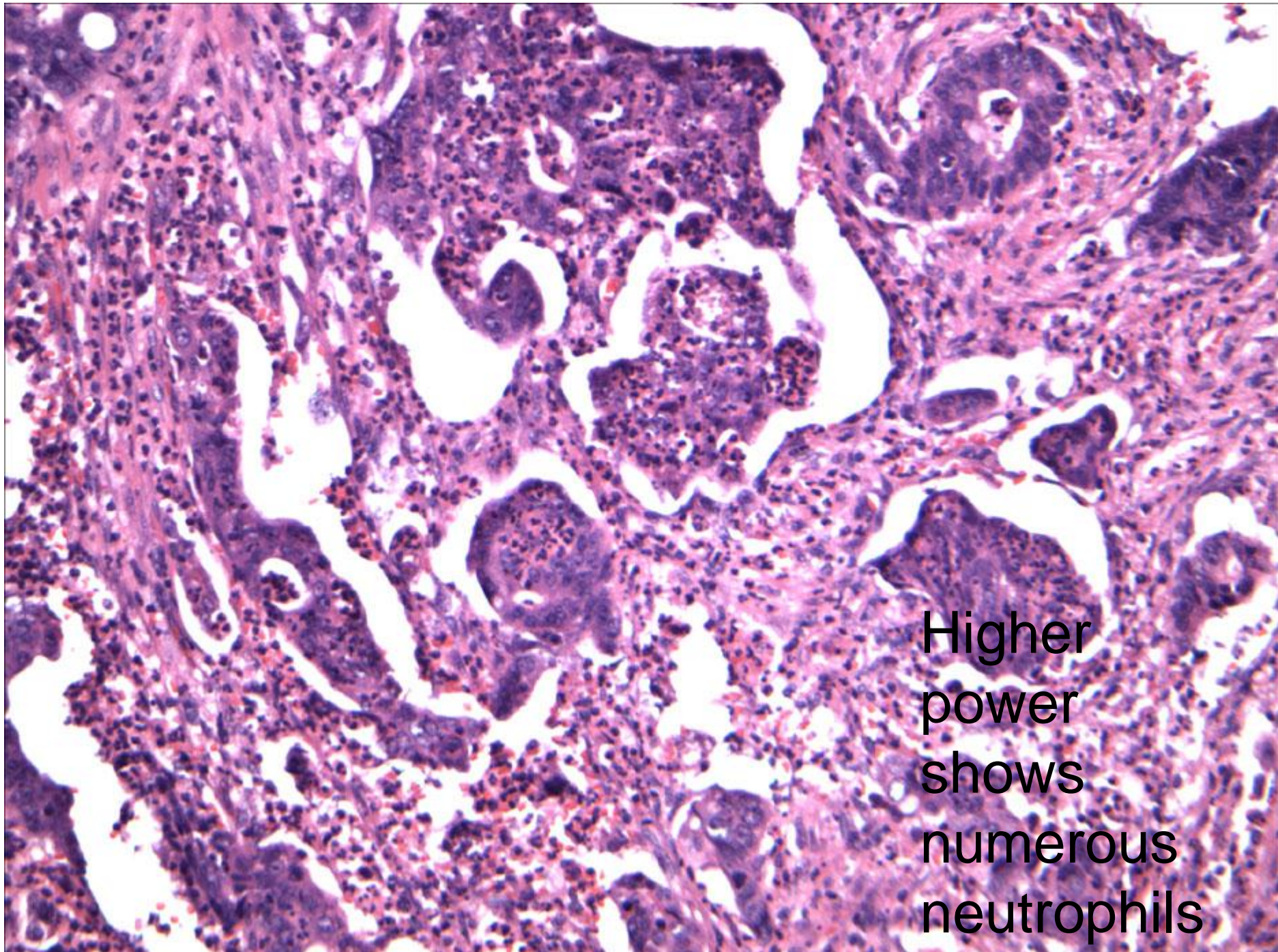




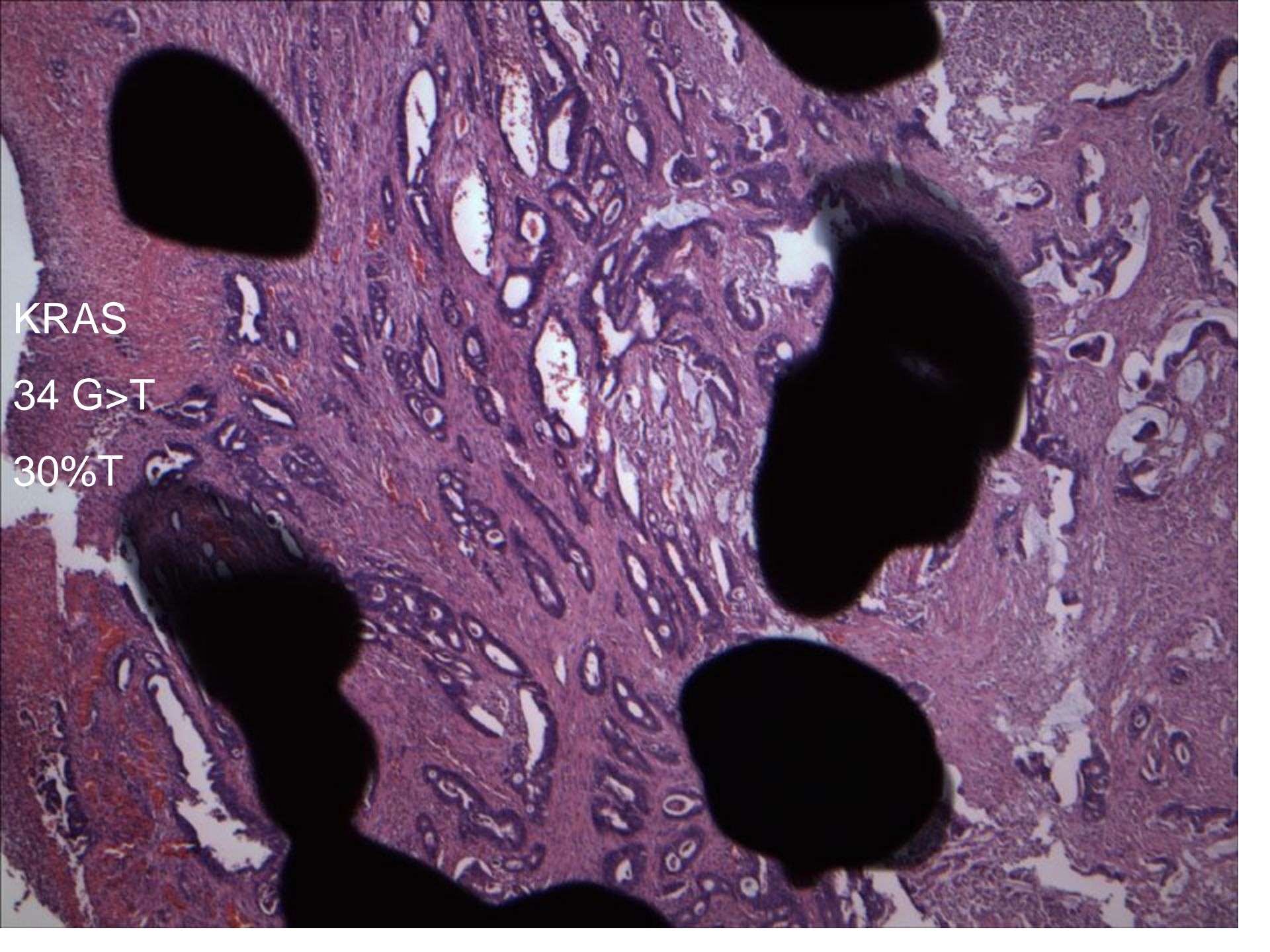
Higher power;
relatively high
tumor
concentration

Another
circled area
thin line

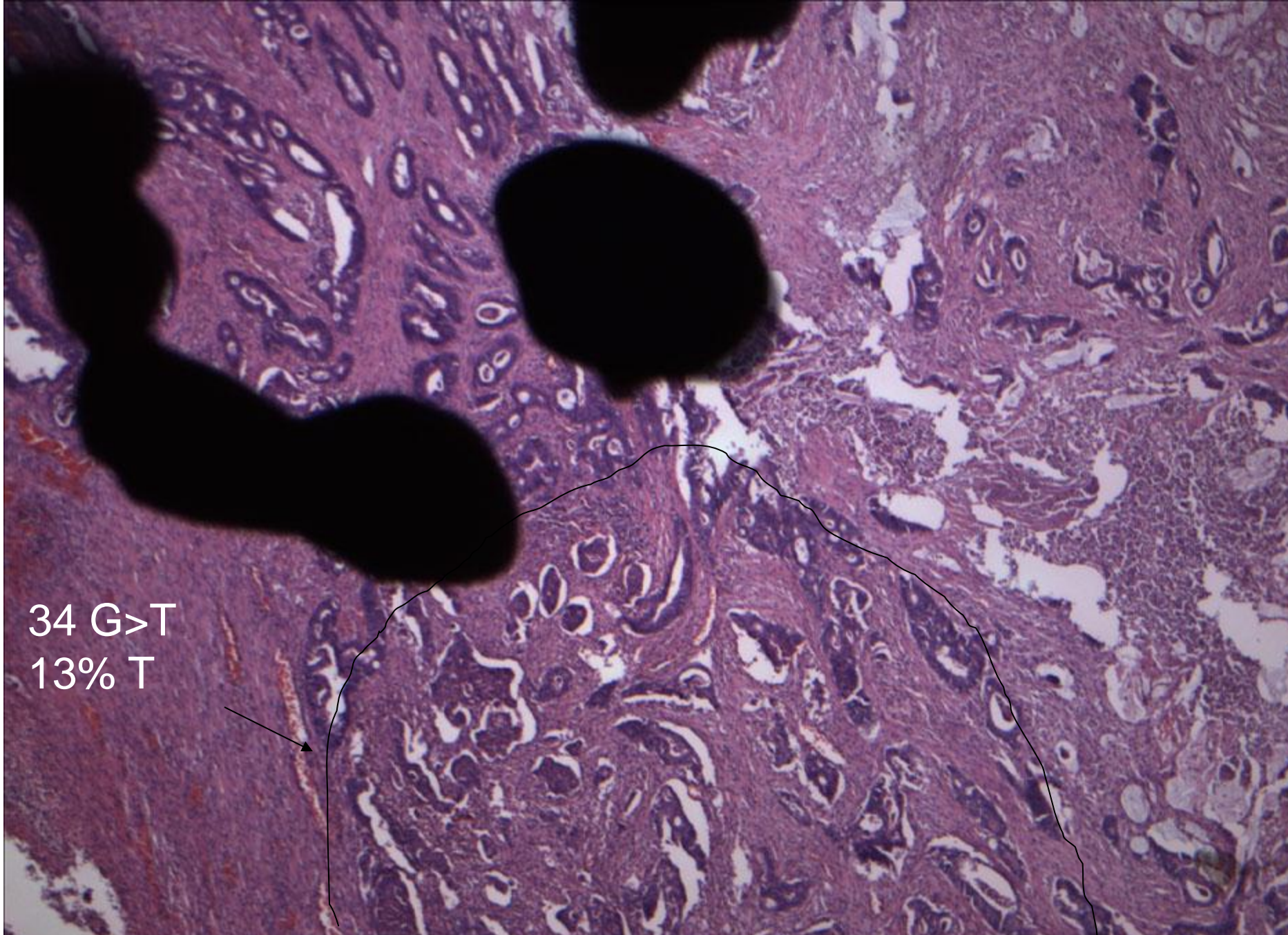




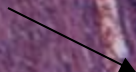
Higher
power
shows
numerous
neutrophils



KRAS
34 G>T
30%T



34 G>T
13% T



Topics

- Mutation detection in solid tumors: general considerations
- Lynch syndrome
- Therapy, especially EGFR pathway
- Future

Lynch syndrome (HNPCC)

- Early onset colon cancer
- Right-sided
- Extra-colonic cancers: endometrium, ovary, renal pelvis, ureter, small intestine, stomach, hepatobiliary tract, pancreas
- Muir-Torre: Lynch + sebaceous neoplasms
- Turcot's: Lynch + brain tumor (GBM)
(Hamilton, NEJM, 1995)

Lynch syndrome

- Germline mutations in mismatch repair genes: *MLH1, MSH2, MSH6* or *PMS2*
- Autosomal dominant
- Phenotype not so obvious (unlike FAP, for example)
- Family history not always obvious or available
- Fortunately, we can use the molecular features of the tumor (mismatch repair deficiency) to help in work-up

How do we assess mismatch repair deficiency?

- 1. Microsatellite instability
- 2. IHC for mismatch repair proteins

Microsatellite repeats

- Type of repetitive DNA in which repeat unit is short (1-6 nucleotides)
 - Mononucleotide: AAAAAAAAAAAA
 - Dinucleotide: CACACACACACA
- Most in non-coding regions
 - Some exceptions: Mononucleotide repeats such as in TGFBR11
- Often slippage during DNA replication of these repeats
 - Leads to changes in number of repeats
- Usually fixed by mismatch repair apparatus

Microsatellite instability

- Expansion or contraction of microsatellite repeats
 - For example, 10 CA's to 14 CA's
- Requires a mistake in replication plus deficiency in mismatch repair



Chromosome
5 Maternal



10 CA

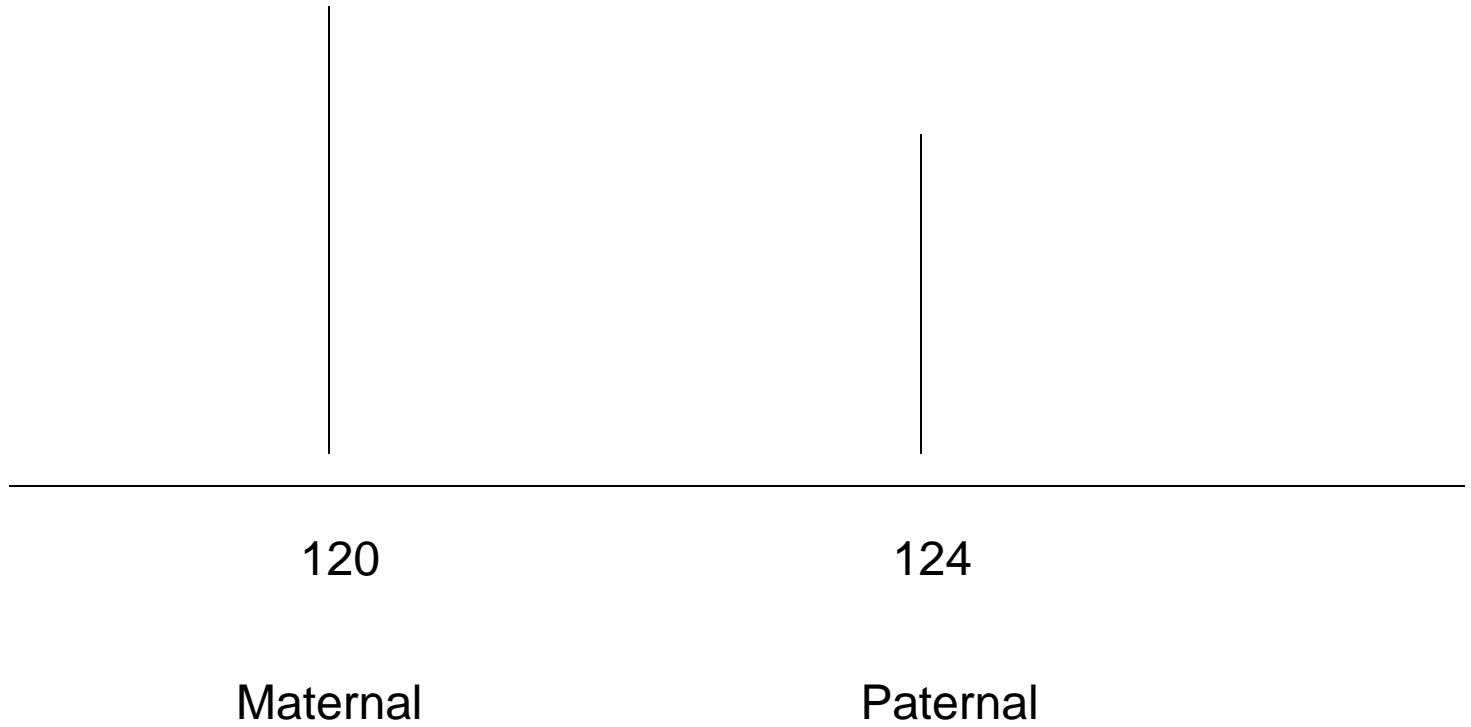


Chromosome
5 Paternal

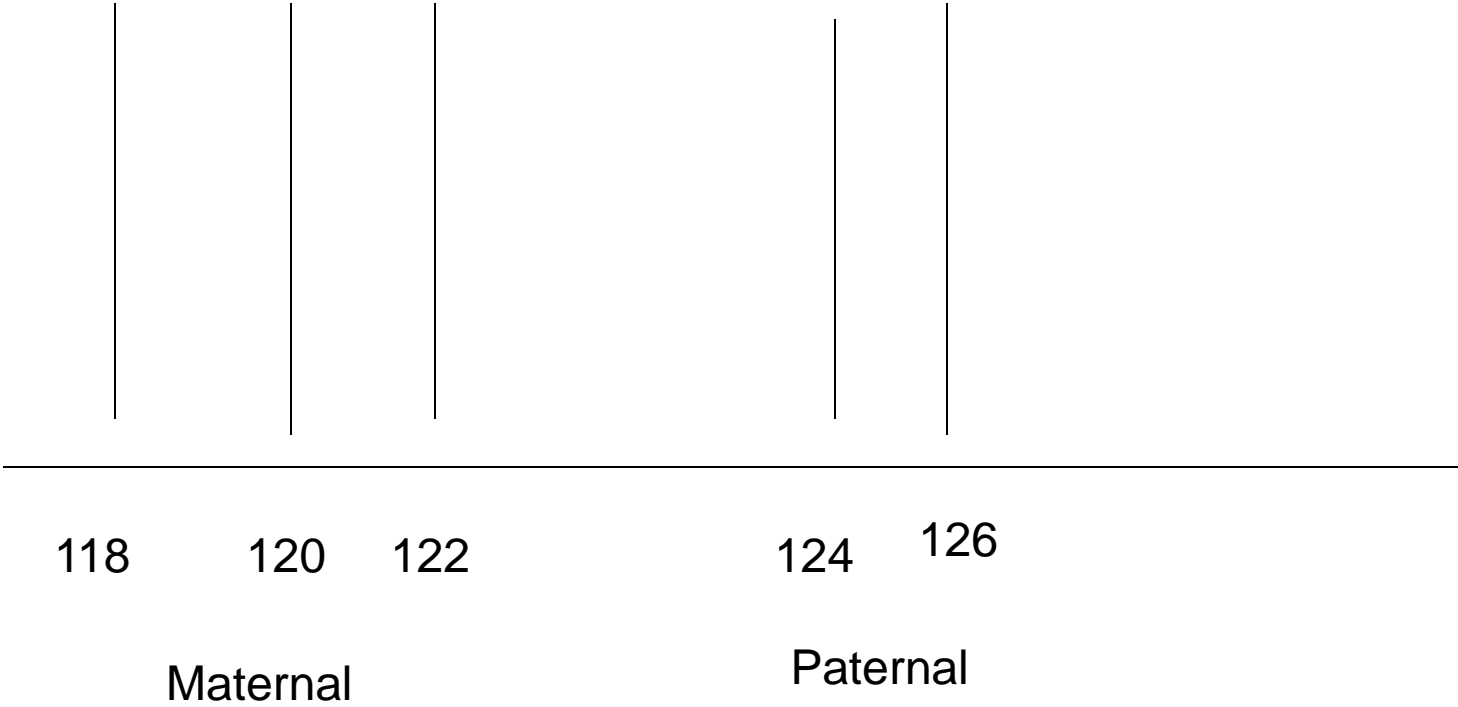


12 CA

PCR of Normal



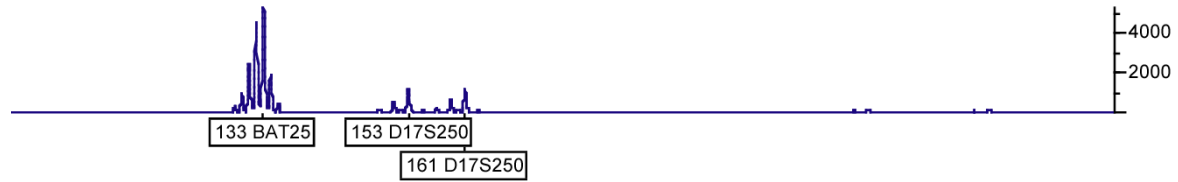
PCR of MSI



100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250

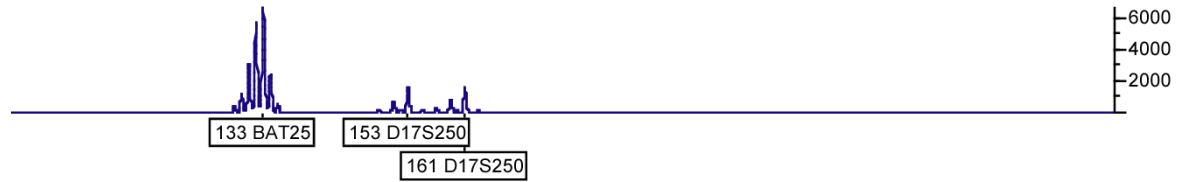
1_extracted_20N_C03_05.fsa 5 Blue

NI



1_extracted_20T_D03_07.fsa 7 Blue

T



1_extracted_20N_C03_05.fsa 5 Green

NI



1_extracted_20T_D03_07.fsa 7 Green

T



1_extracted_20N_C03_05.fsa 5 Yellow

NI



1_extracted_20T_D03_07.fsa 7 Yellow

T



100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250

NI

1_extracted_22N_G03_13.fsa 13 Blue



T

1_extracted_22T_H03_15.fsa 15 Blue



NI

1_extracted_22N_G03_13.fsa 13 Green



T

1_extracted_22T_H03_15.fsa 15 Green



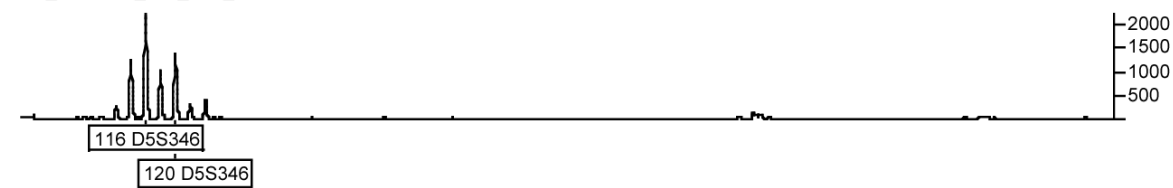
NI

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T

1_extracted_22T_H03_15.fsa 15 Yellow

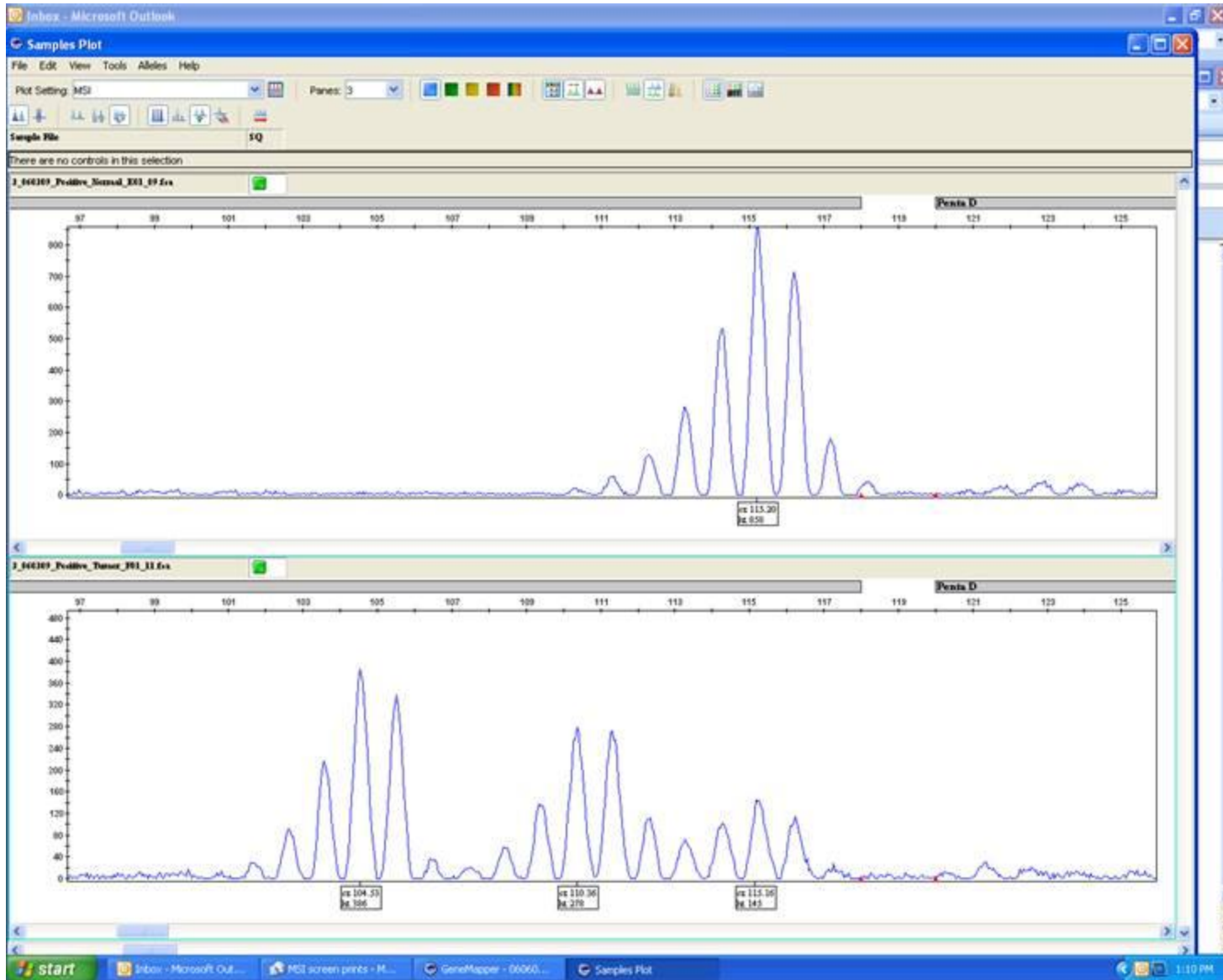


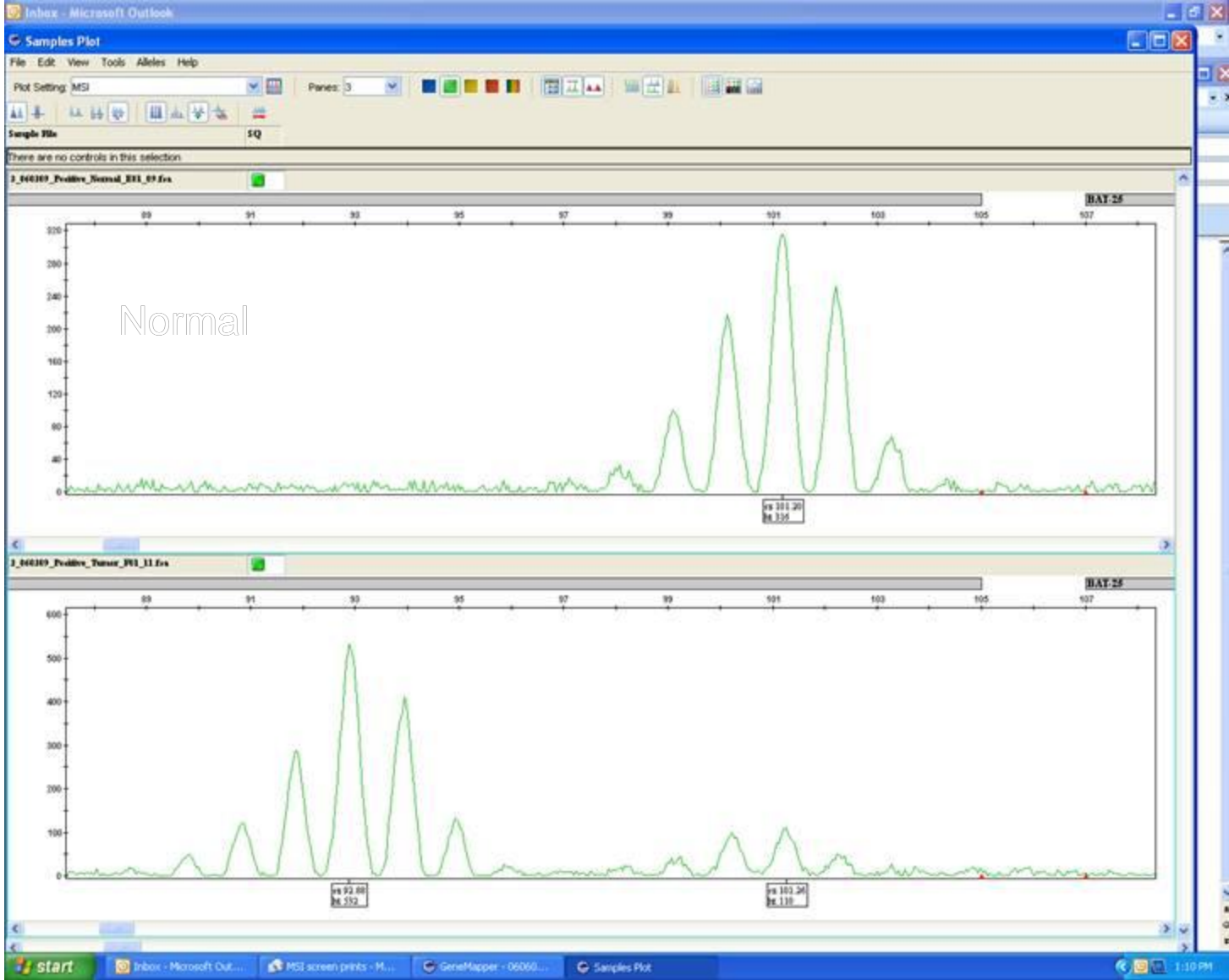
Bethesda Consensus Panel

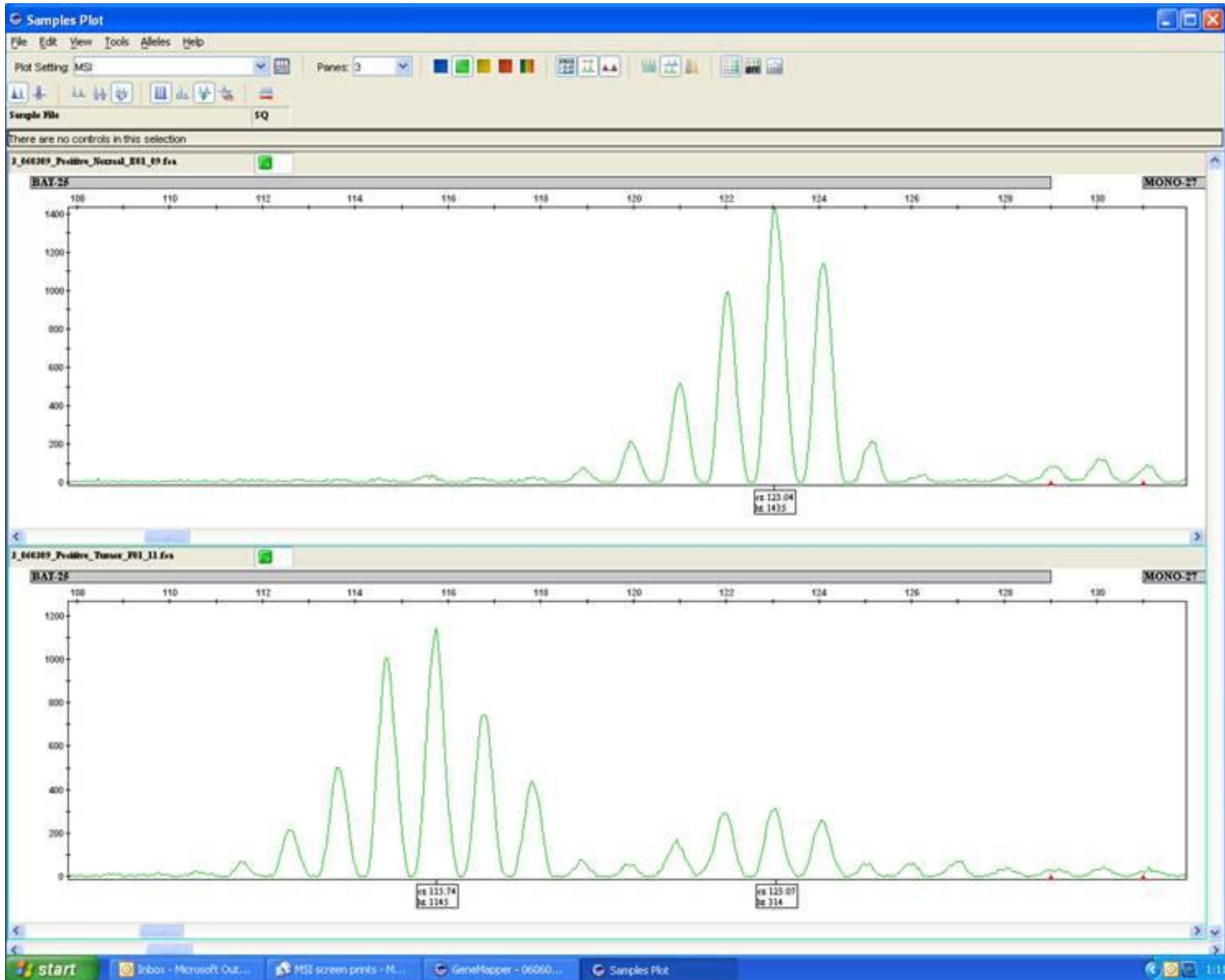
- Two mononucleotide repeats, three dinucleotide repeats
- MSI high: Instability in two or more repeats
- Microsatellite stable (MSS): No instability
- MSI low: Instability in one repeat
 - Controversial
 - Lynch-associated cancers show MSI high, not low

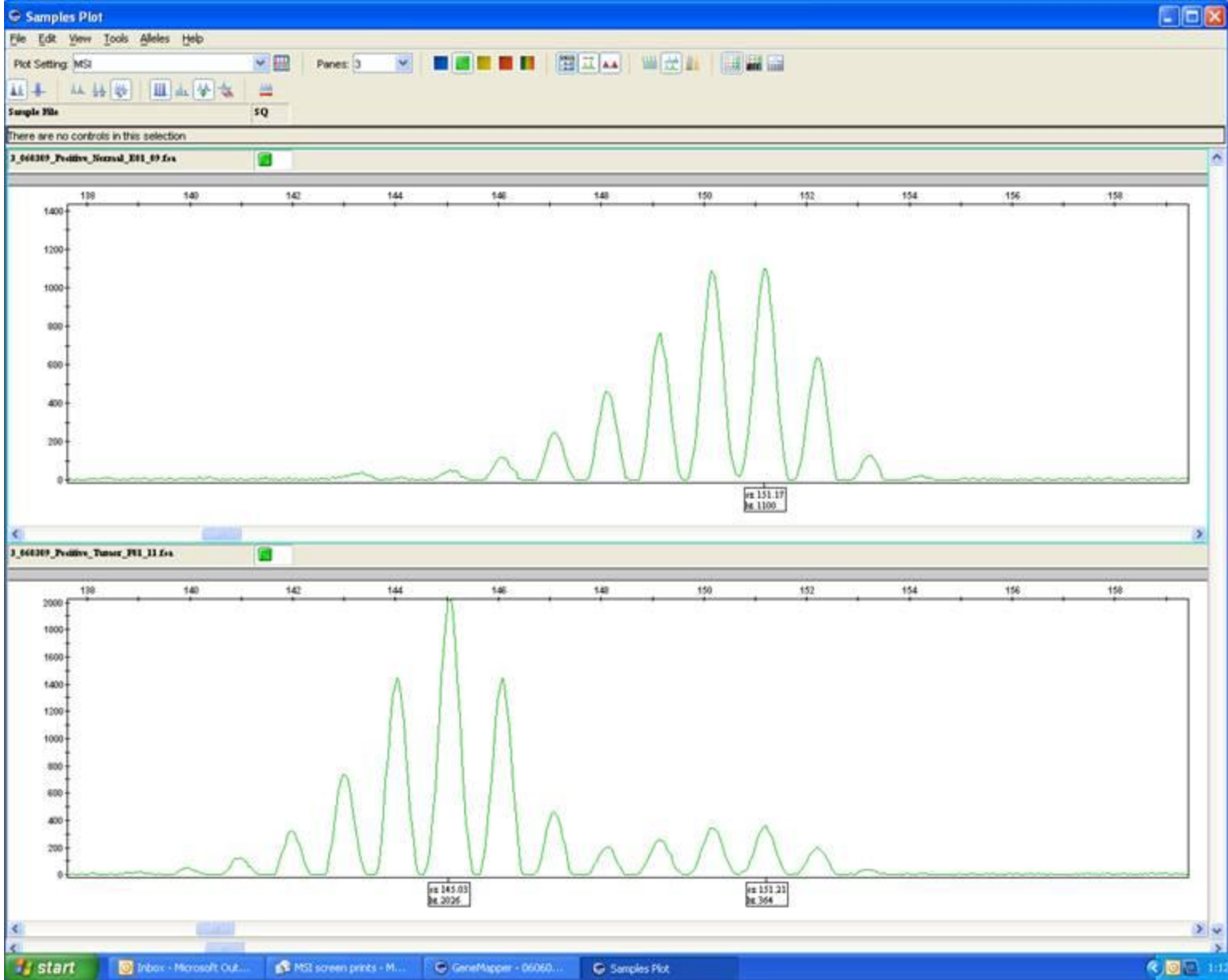
Mononucleotide repeat panel

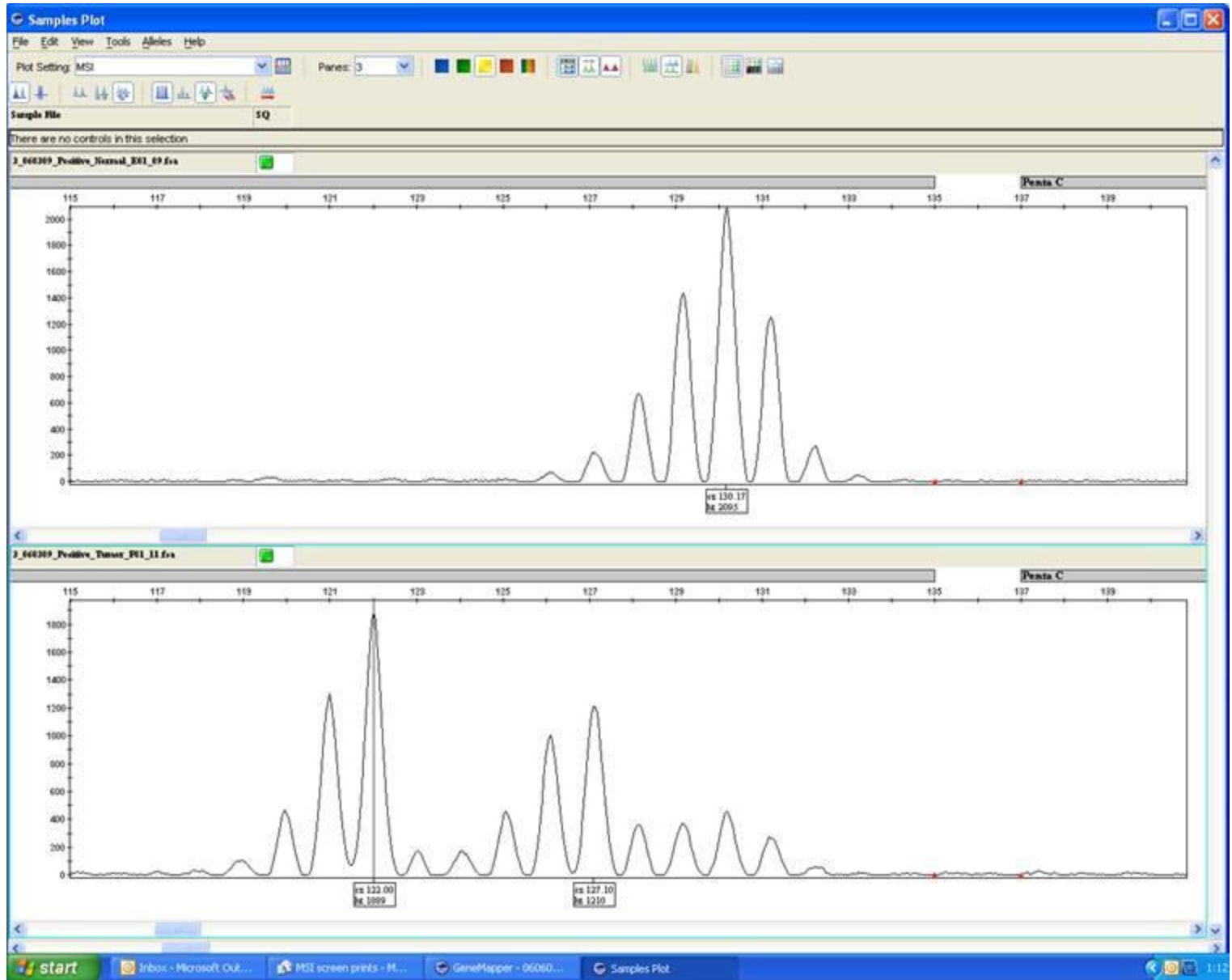
- Mononucleotide repeats are probably more sensitive and specific for MMR deficiency
- New panel(s) of 5 mononucleotide repeats
 - MSI high: two or more unstable, although typically all (or almost all) repeats are unstable
 - Since instability in even one mononucleotide repeat may indicate MMR deficiency,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





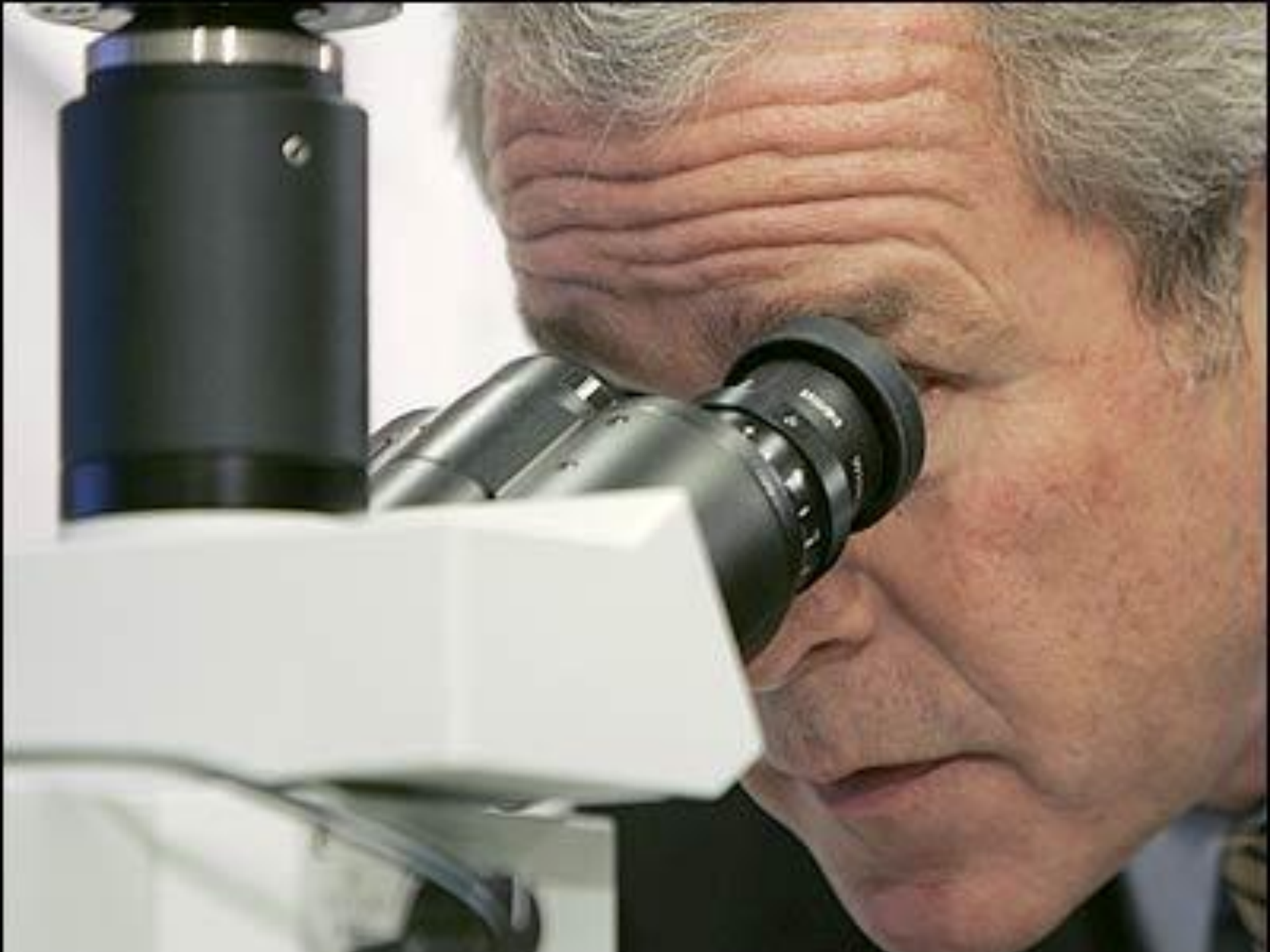


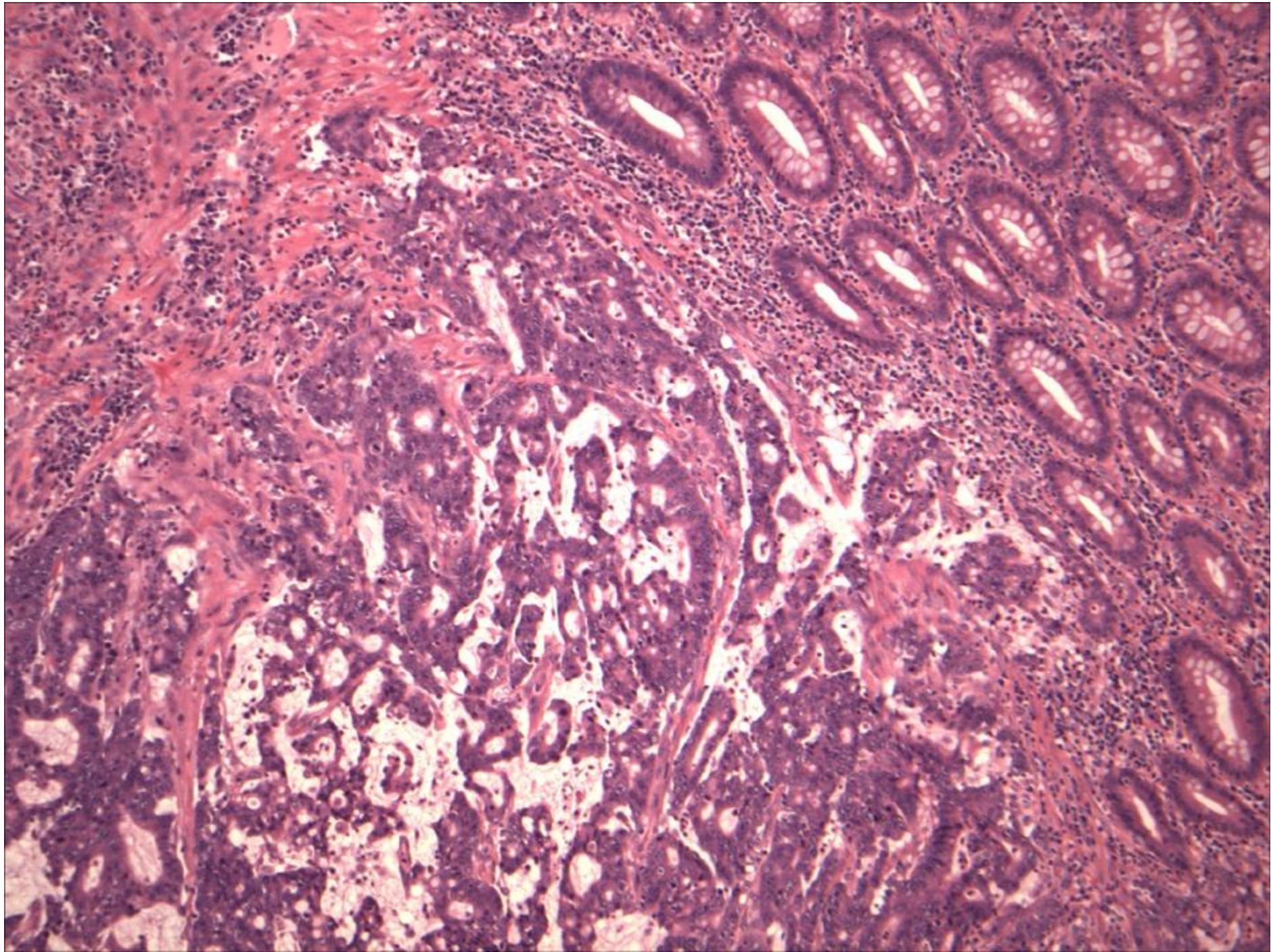




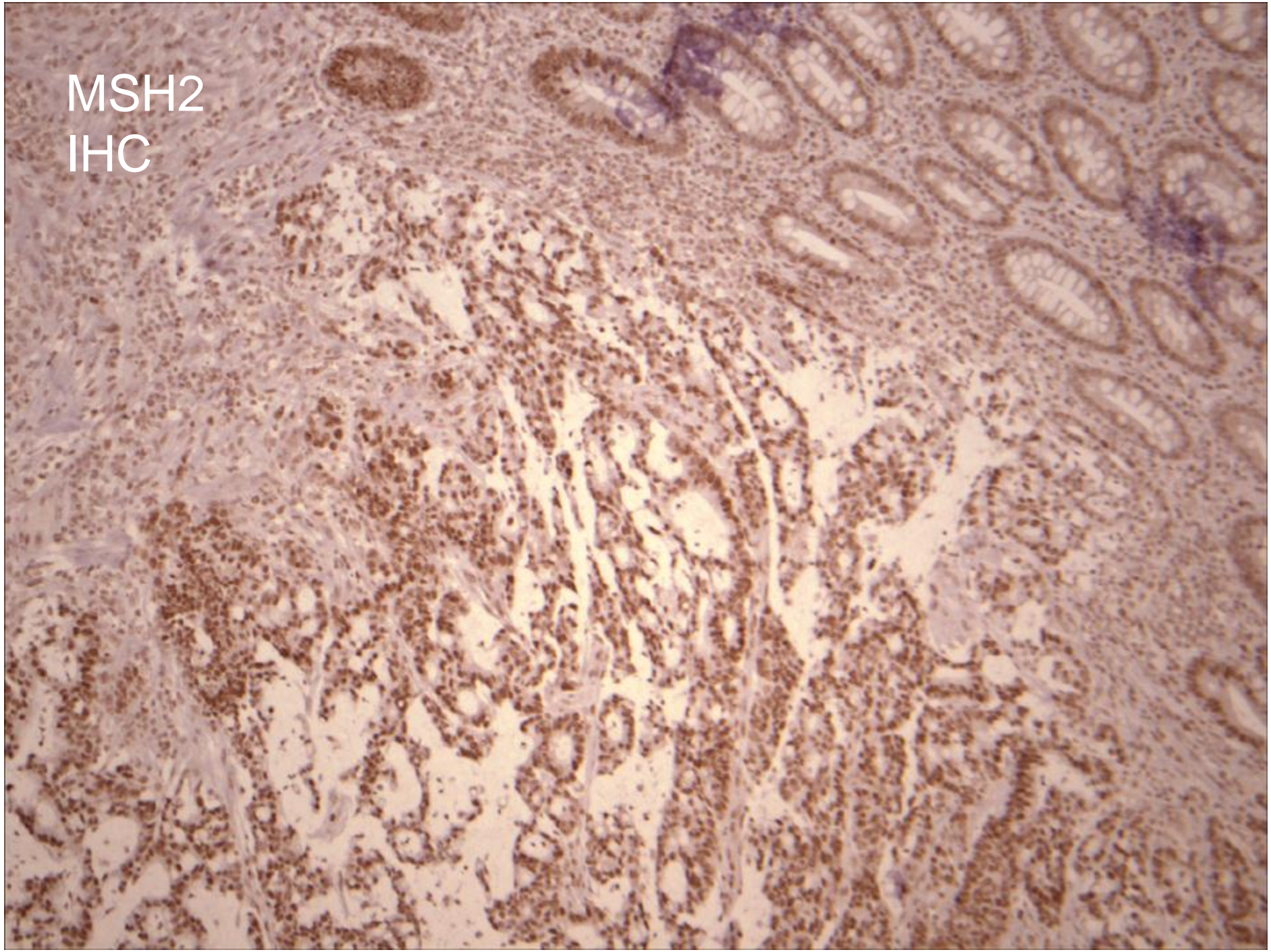
How do we assess mismatch repair deficiency?

- 1. Microsatellite instability
- 2. IHC for mismatch repair proteins

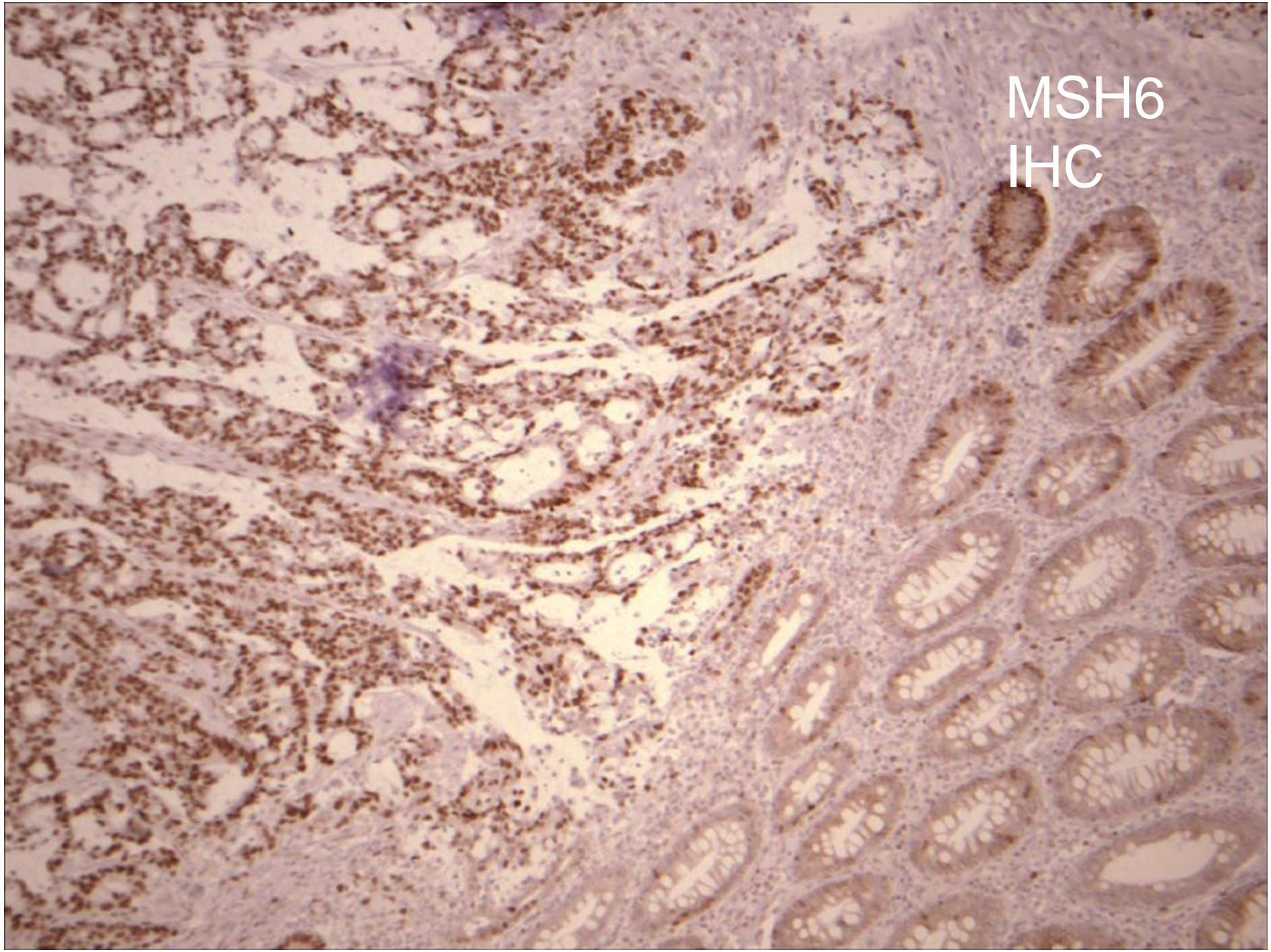




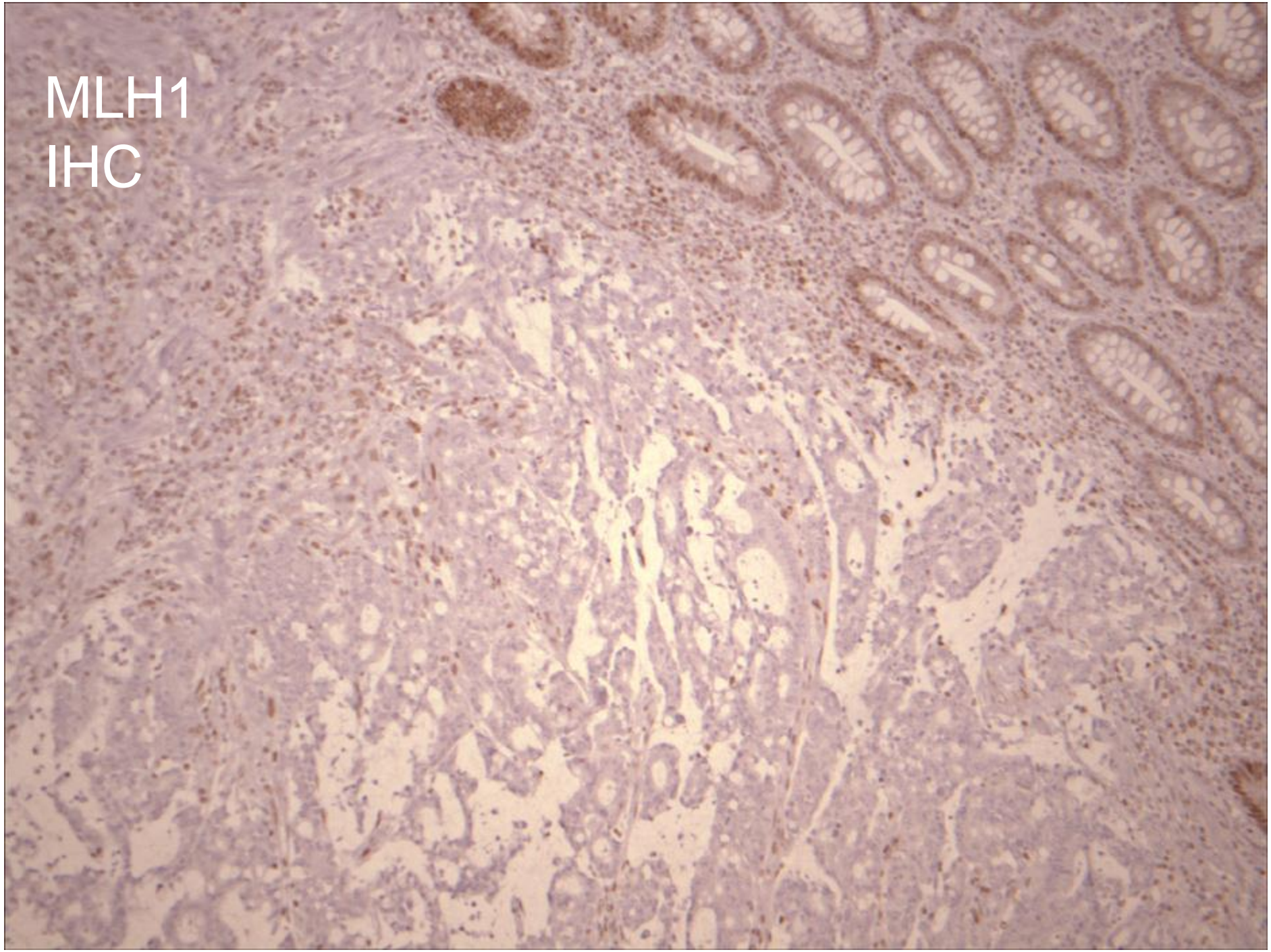
MSH2
IHC



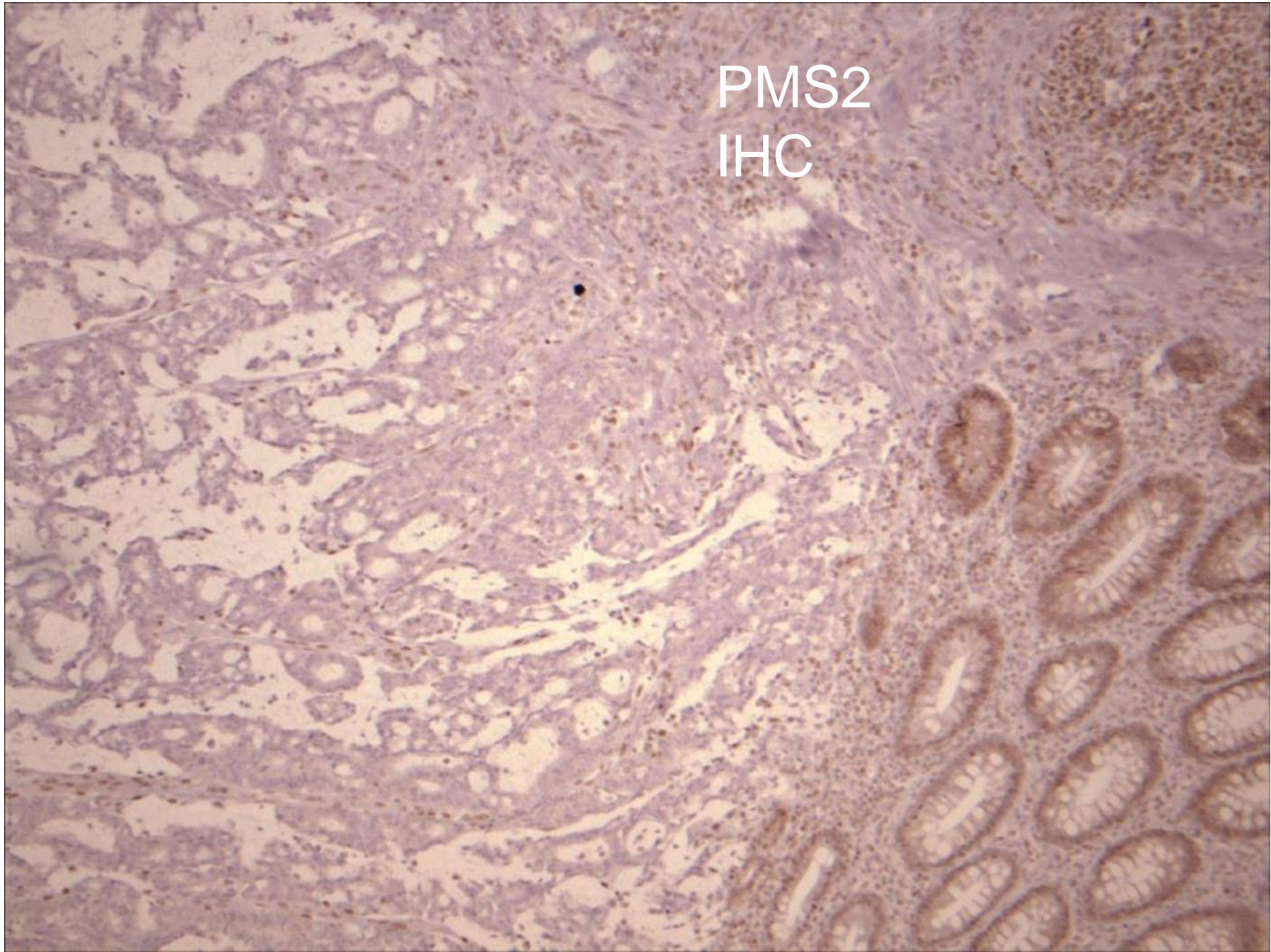
MSH6
IHC



MLH1
IHC



PMS2
IHC



IHC interpretation

- MLH1 complexes with PMS2
- MSH2 complexes with MSH6
- The stability of PMS2 and MSH6 depends upon these complexes
- Therefore, if MLH1 is lost, PMS2 is usually lost; if MSH2 is lost, MSH6 is lost.
- Corollary usually not true (MLH1 and MSH2 bind to other proteins as well)

IHC Result	Likely Defective Gene
Loss of MLH1, PMS2	MLH1
Loss of MSH2, MSH6	MSH2
Isolated Loss of MSH6	MSH6
Isolated Loss of PMS2	PMS2*

*Germline MLH1 mutations associated with isolated loss of PMS2 have been reported

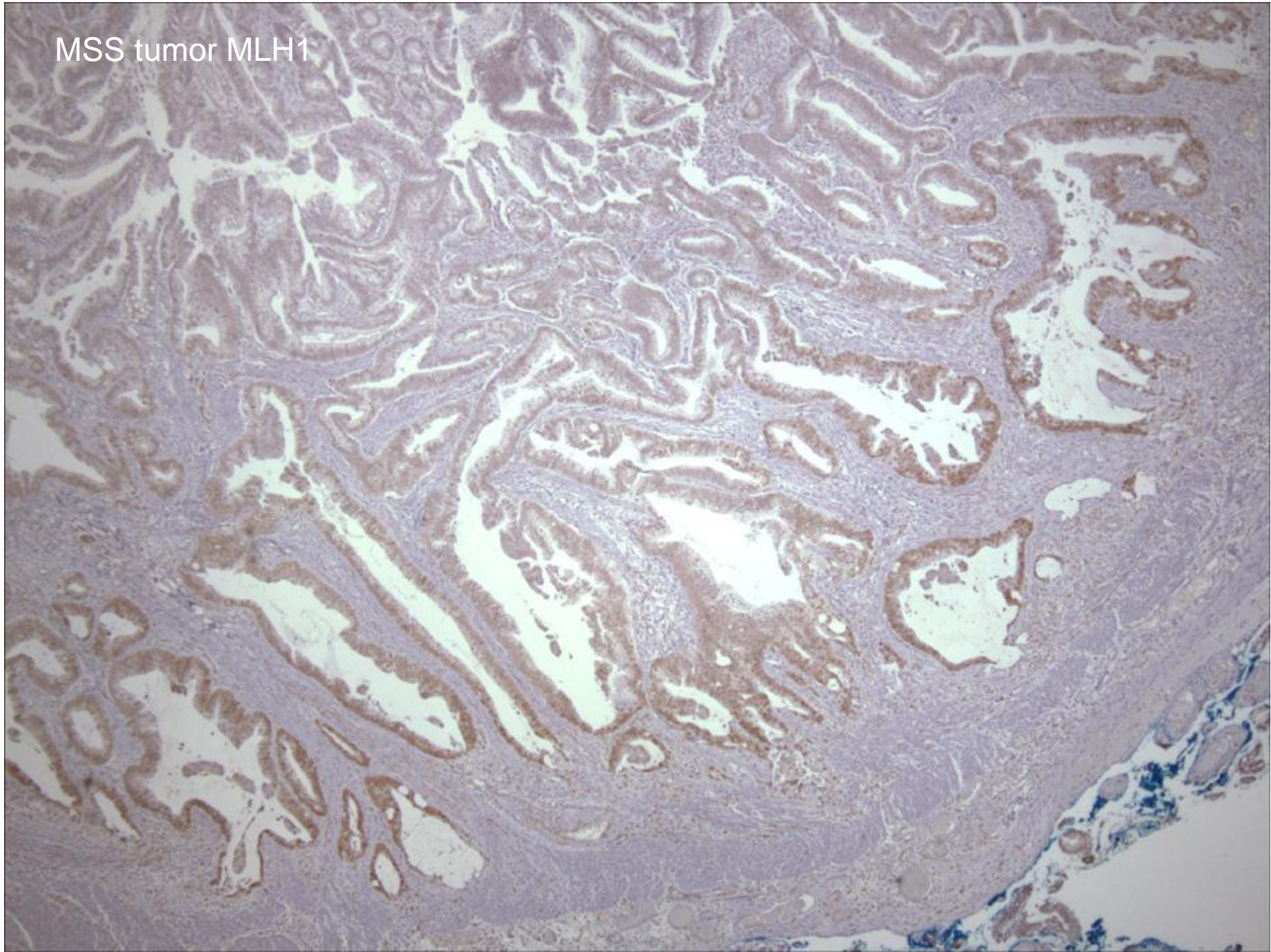
IHC vs. PCR

- Complementary (both miss some mmr def)
- MSH6 negative tumors can be stable by PCR (not as much of an issue with mononucleotide repeat panels)
- Missense mutations may be normal by IHC
- IHC can be hard to interpret
- IHC can guide subsequent mismatch repair gene testing

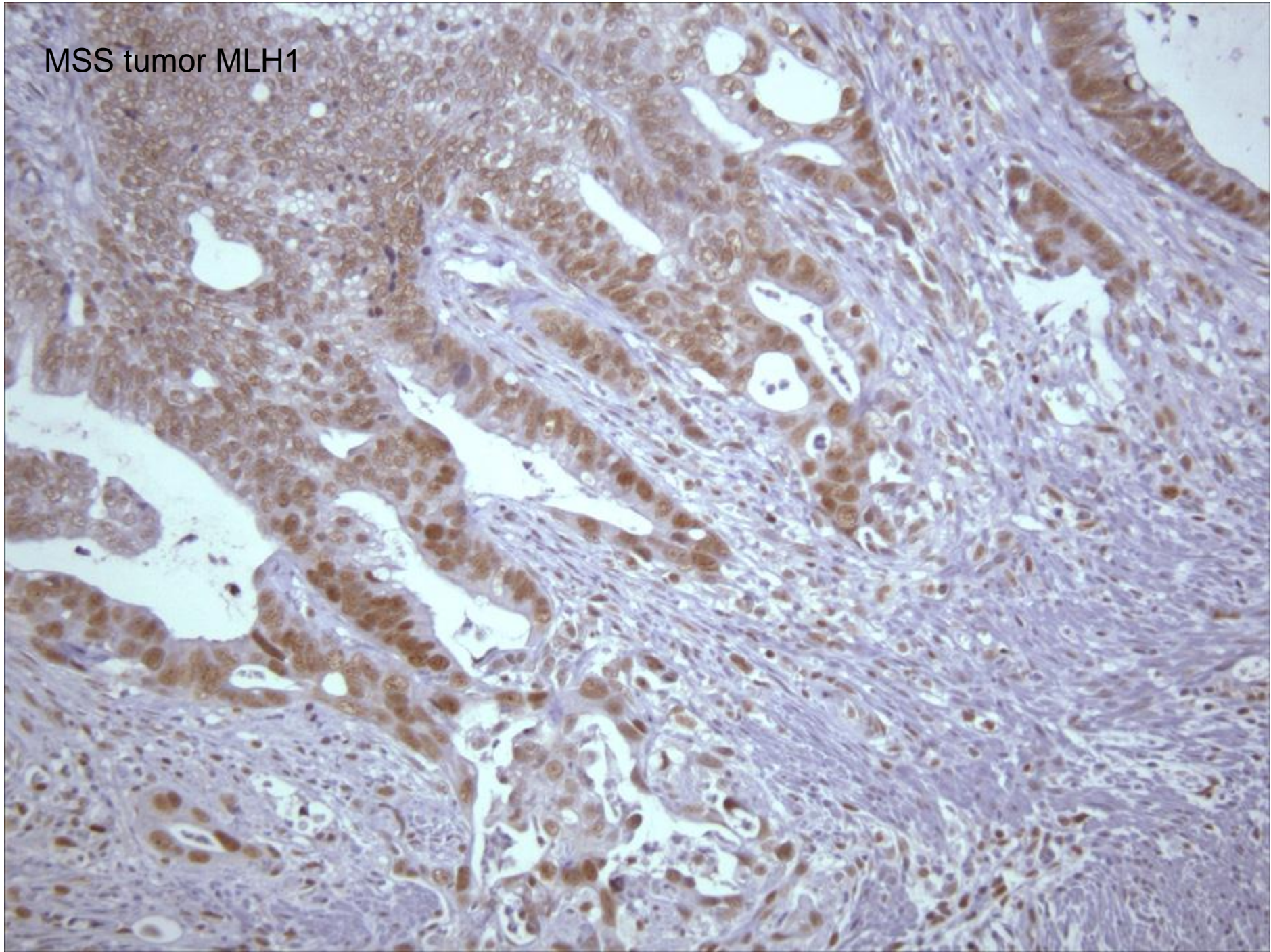
MMR IHC: Problems in interpretation

- Staining variability (use internal controls)
- “Clonal” MSH6 loss due to MSH6 coding mononucleotide repeat (Shia, Modern Path 2013)
- Decreased MSH6 staining after chemoradiation (Bao, Am J Surg Pathol, 2010)
- Decreased staining intensity rather than complete loss
 - If marked suggest MSI by PCR

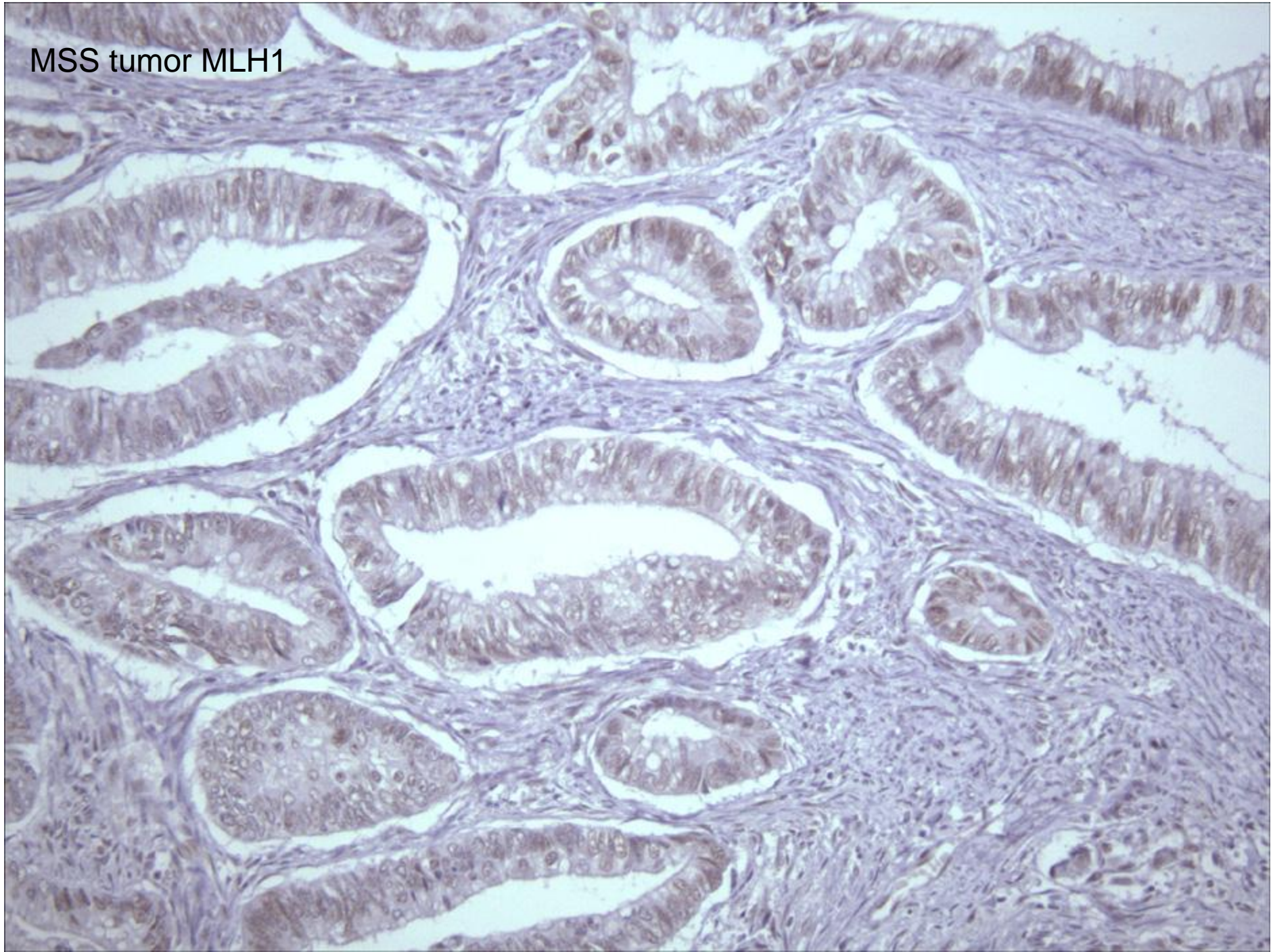
MSS tumor MLH1

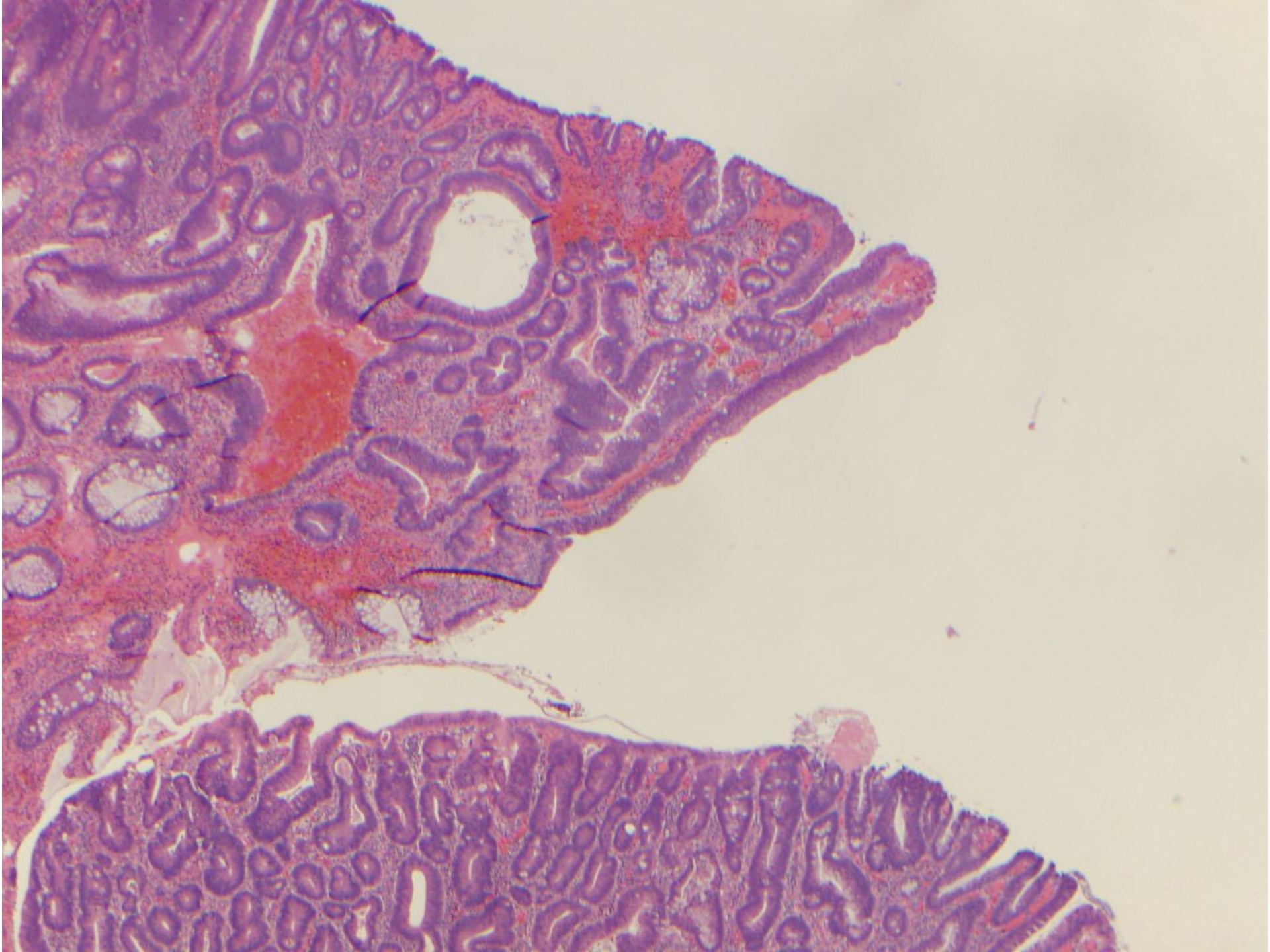


MSS tumor MLH1

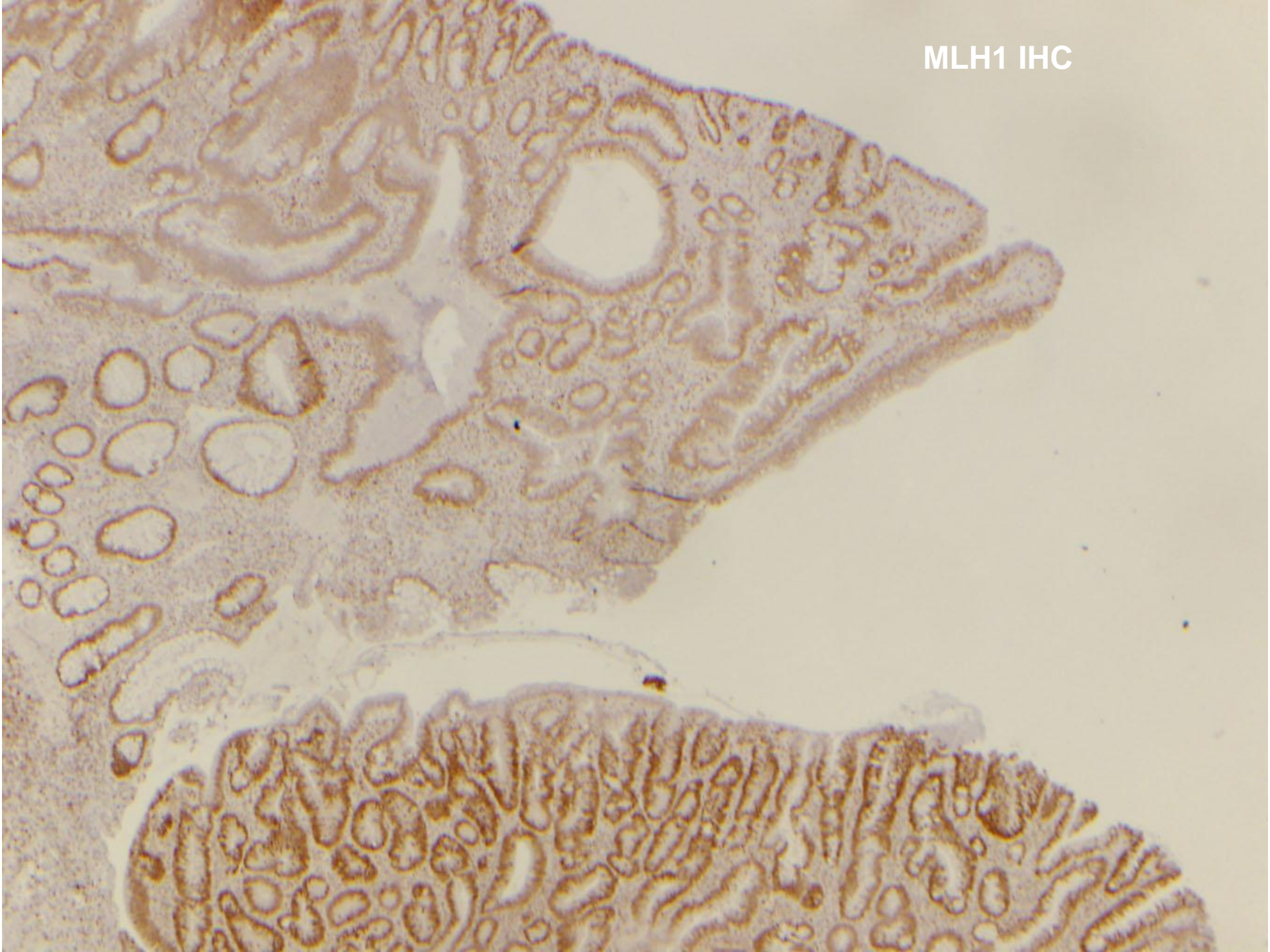


MSS tumor MLH1

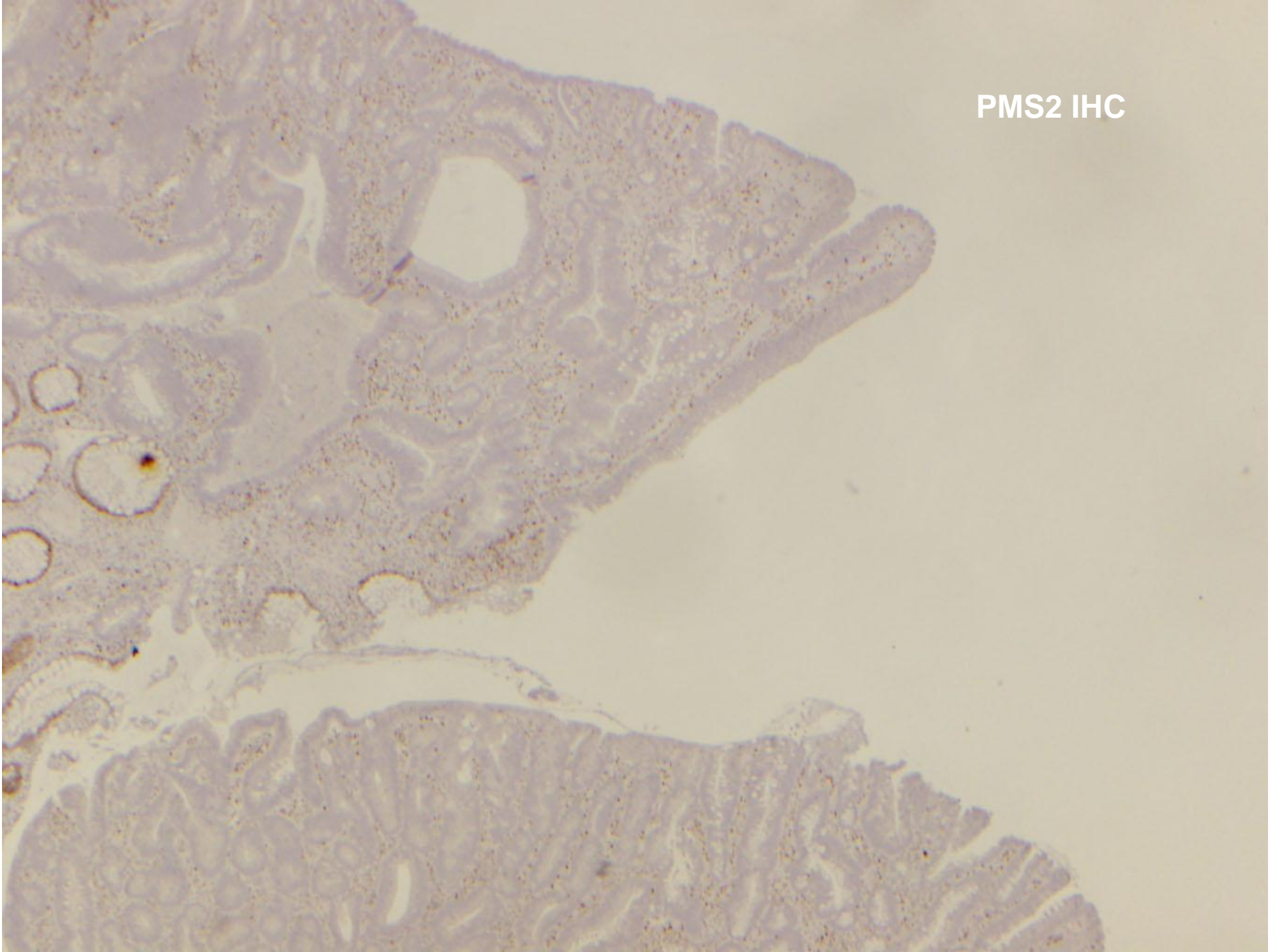


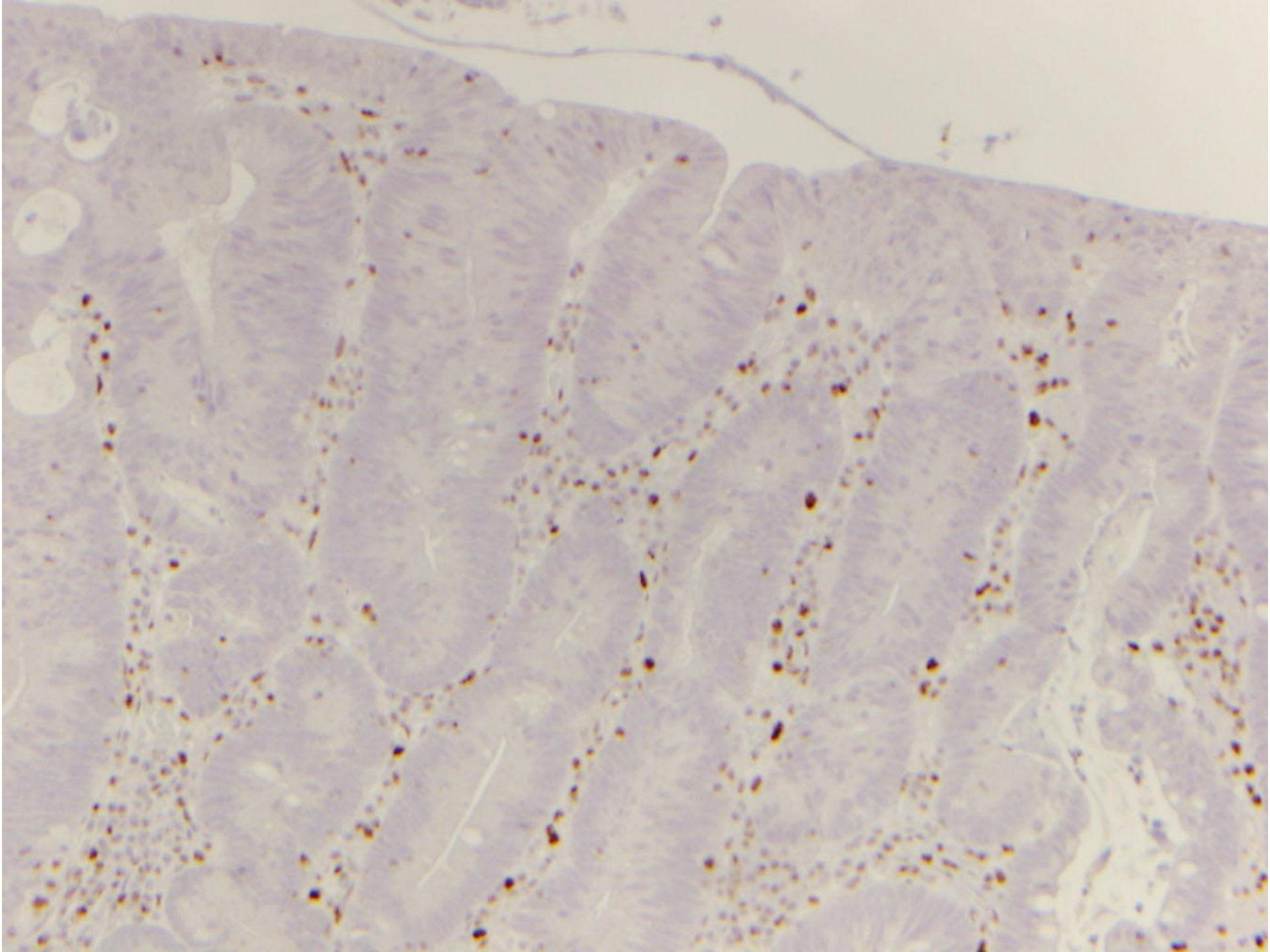


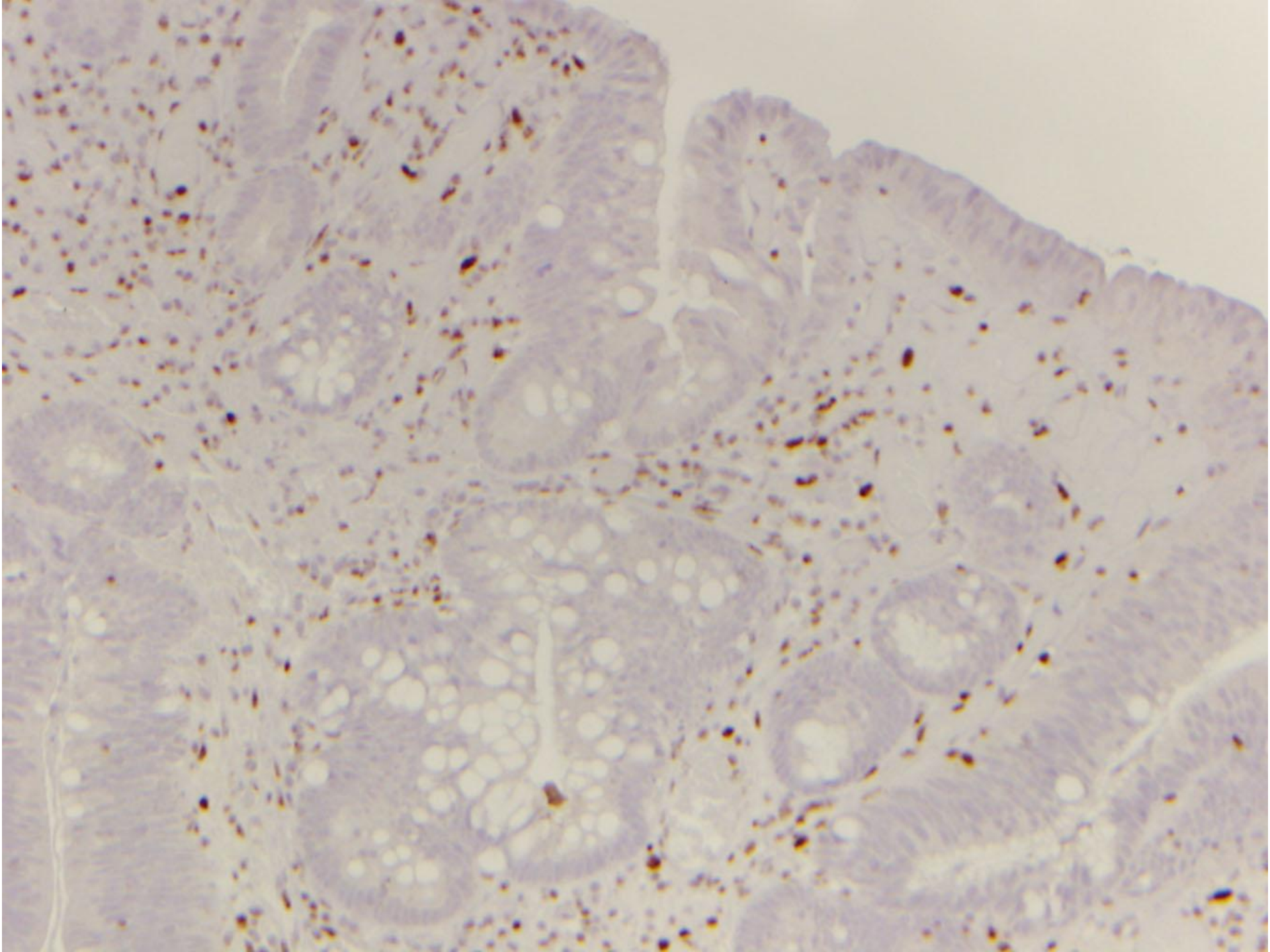
MLH1 IHC



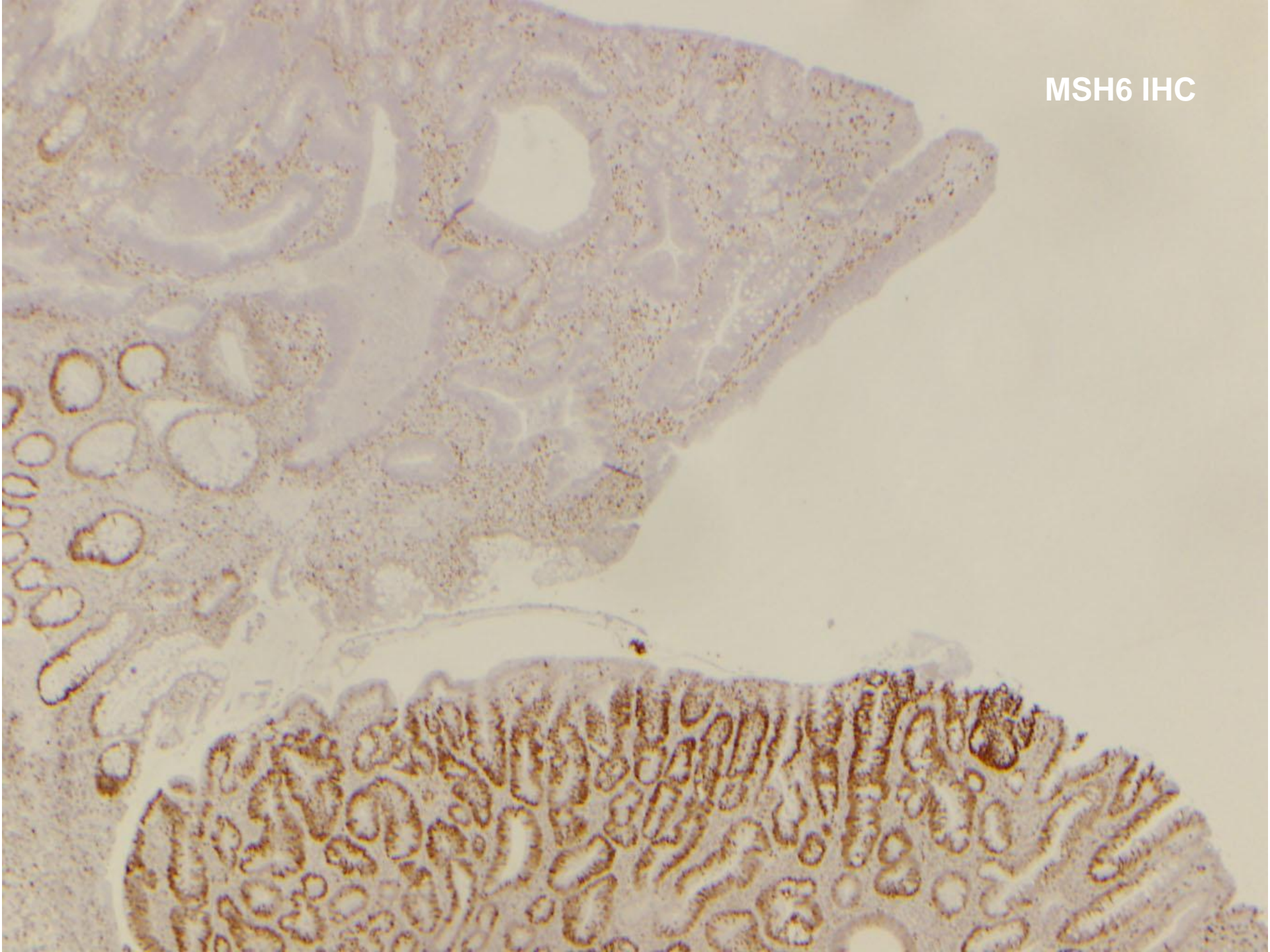
PMS2 IHC

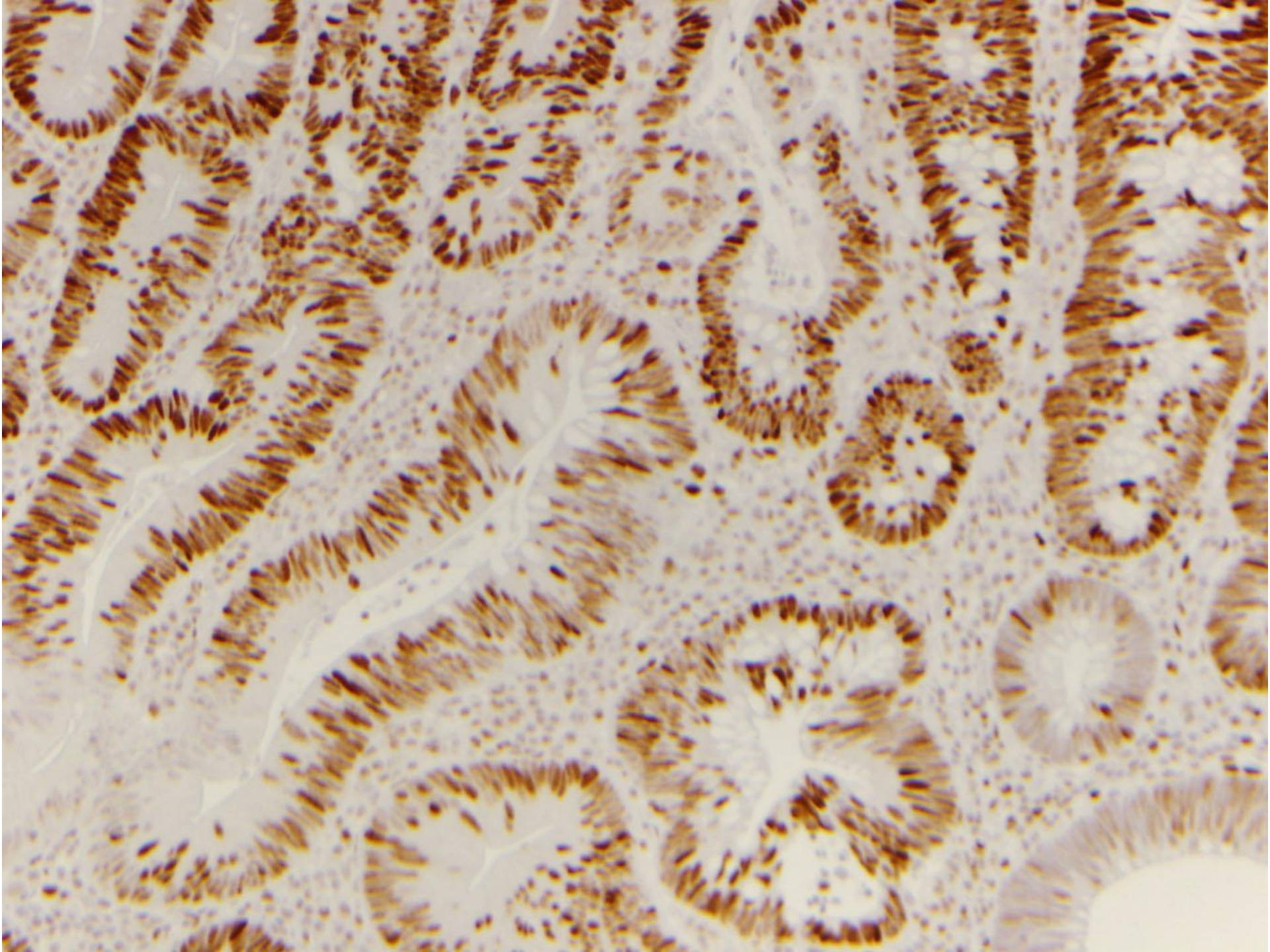


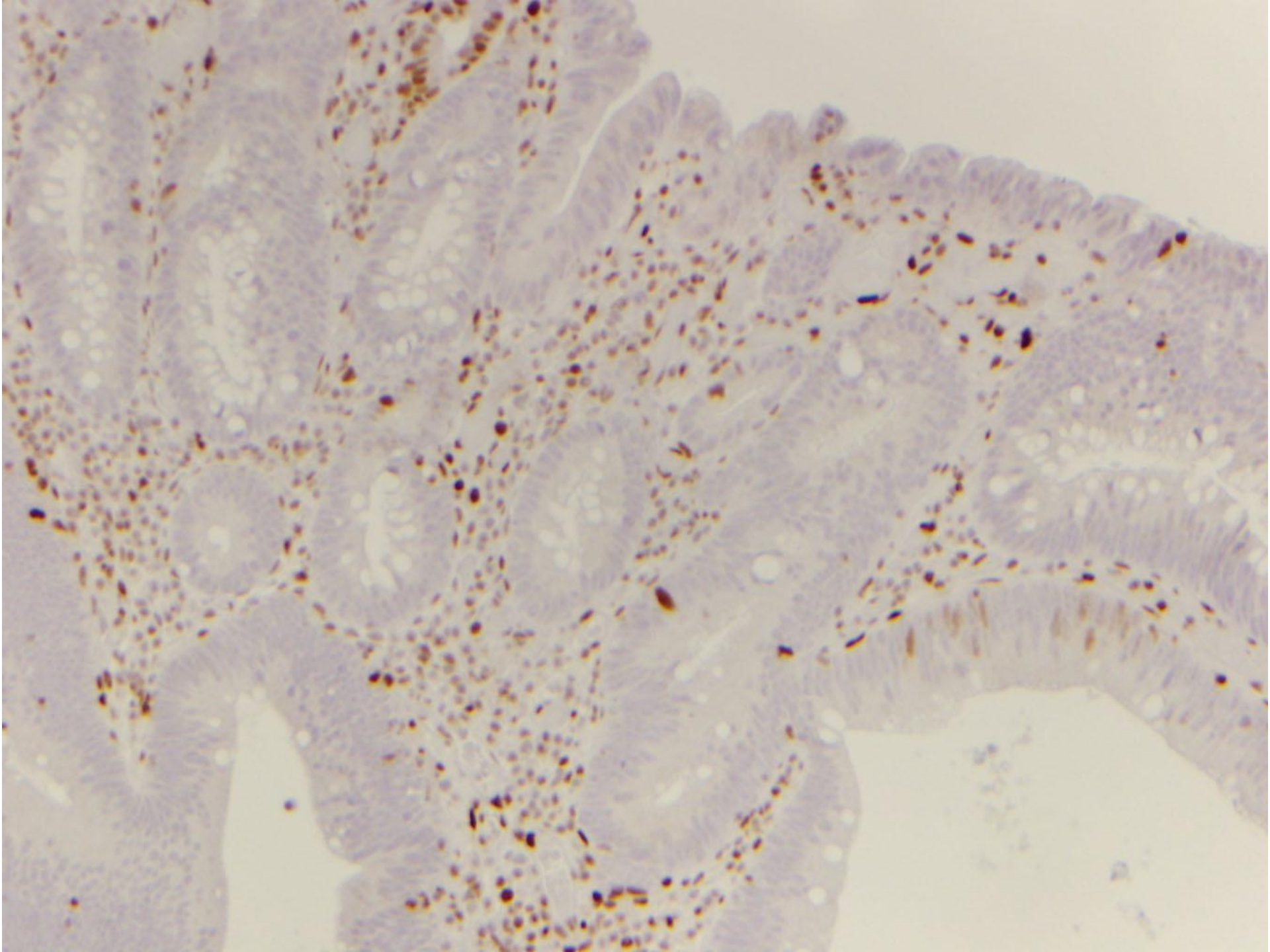




MSH6 IHC







Clonal MSH6 loss

- Caused by instability in the MSH6 coding mononucleotide repeat in certain parts of the tumor (but not in others)
- Typically occurs in a tumor which is mismatch repair deficient due to an alteration besides MSH6
 - Sporadic mmr deficiency
 - Germline mutation in another gene

MMR deficient colorectal cancer: two clinical contexts

- Lynch/HNPCC
 - Germline mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6, or PMS2)
- 10-15% Sporadic colorectal cancer
 - Acquired hypermethylation of MLH1 promoter
 - IHC: MLH1/PMS2 loss (same as Lynch syndrome due to germline MLH1 mutation)

Lynch syndrome work-up

- Is tumor mismatch repair deficient?
- Is mismatch repair deficient tumor sporadic or Lynch syndrome?

IHC loss of MLH1/PMS2: Sporadic or Lynch? Why care?

- Sporadic mmr deficient tumors are more common than Lynch; MLH1/PMS2 loss is the most common abnormal IHC result
- If it is sporadic
 - Don't need to sequence MLH1 in the germline
 - Don't need to follow-up patient as Lynch syndrome or evaluate family members

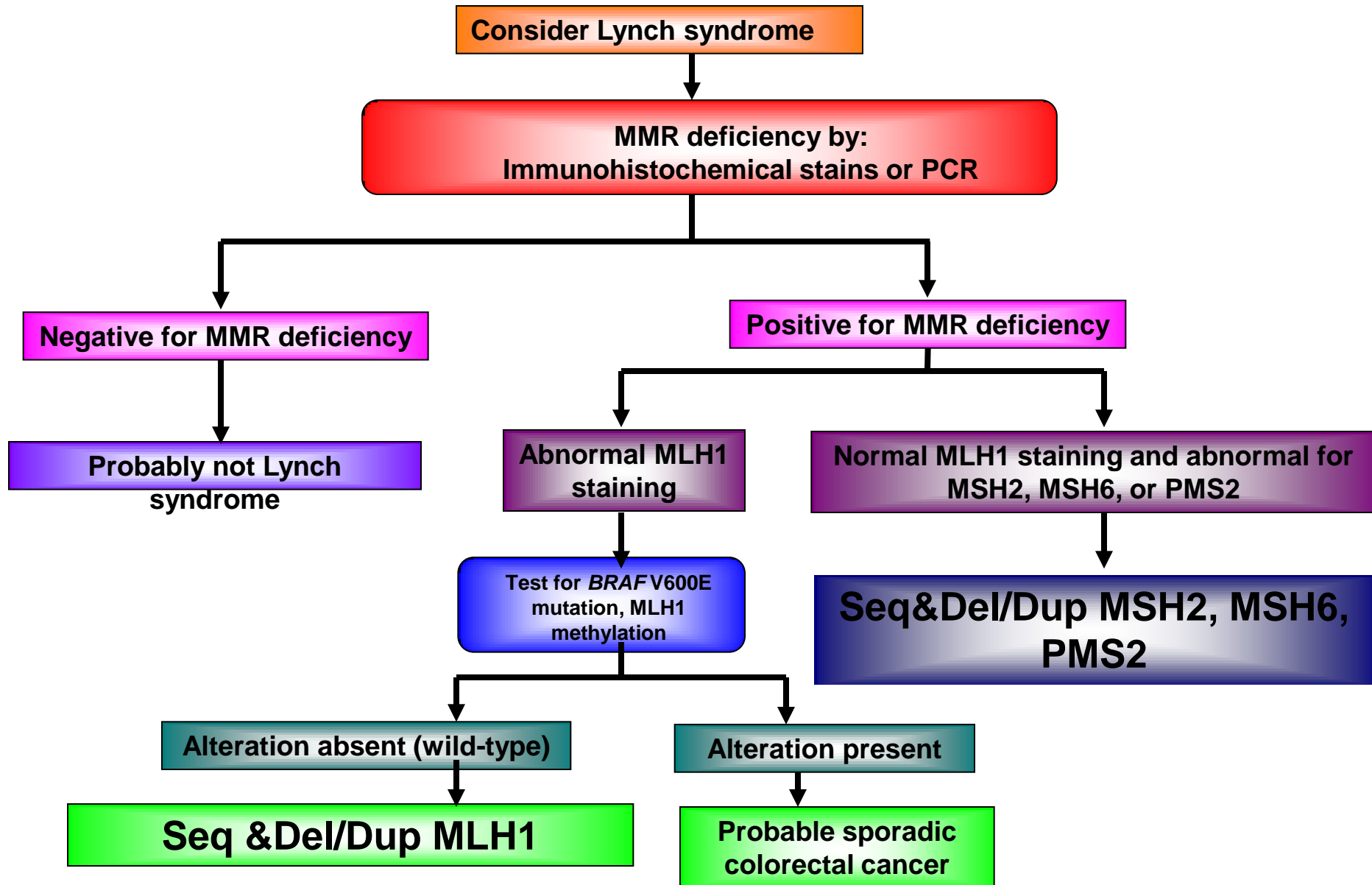
IHC loss of MLH1/PMS2: Sporadic or Lynch?

- Approximately 50% of sporadic mmr deficient *colorectal* cancers have BRAF V600E mutation; extremely rare in Lynch syndrome
 - Molecular test or antibody specific to V600E
 - Potentially a mostly IHC work-up (Toon et al, AJSP 2013)
- BRAF mutations are uncommon in extracolonic sporadic mmr deficient tumors (e.g. endometrial cancers)
- Most sporadic mmr deficient tumors of any site have MLH1 methylation; rarely seen in Lynch syndrome

What about the BRAF antibody?

- Molecular test is still the gold standard: most objective and definitive
- Still need molecular testing (MLH1 methylation) for Lynch work-up of many tumors
 - BRAF mutations are not common in extra-colonic mismatch repair deficient tumors (like endometrium)
 - 50% of sporadic mmr def colorectal cancers are BRAF wild type, and many of these are MLH1 methylated

Colorectal cancer Lynch Syndrome Test Algorithm



Do acquired mutations in mismatch repair genes occur?

- Previous literature suggested any abnormal IHC profile besides loss of MLH1/PMS2 was Lynch
- Also, MLH1/PMS2 loss without BRAF mutation or MLH1 methylation was Lynch
- New reports suggest substantial proportion of these are due to acquired mutations in MMR genes (Haroldsdottir et al, Gastroenterology, 2014).
- Implications for reporting of IHC results

Which tumors should be tested?

- Revised Bethesda guidelines
 - More than just age and histology, e.g. personal and family history of Lynch syndrome tumors
 - Estimated to miss nearly 30% of Lynch
- Colon cancer under 70 (Jerusalem criteria)
 - Misses 10% of Lynch
- Universal screening

Work with other clinicians!

- Make sure your findings are seen, understood and acted upon
- Work with genetic counselors
 - Appropriate follow-up and genetic testing are more likely (Heald et al, 2013)
 - Depending upon how likely they think Lynch is, might do MSI by PCR if IHC is normal, might still sequence genes even if BRAF is mutated or MLH1 is methylated

Should you test adenomas?

- Not all Lynch-associated adenomas are MMR deficient
 - 50% in recent study were not MMR deficient (Yurgelun, Cancer Prev Res, 2012)
 - Incidence of mmr deficiency related to size
 - Large polyps (8-10 mm or more) much more likely to be mmr deficient
- Therefore, the lack of mmr deficiency in an adenoma is not as strong a criterion to exclude Lynch syndrome as the lack of instability in a cancer
- However, the presence of mmr deficiency in an adenoma is probably more specific for Lynch

What about next generation sequencing?

- Someday cost of germline sequencing of all four genes (and many other inherited colon cancer genes) may be less than the cost of tissue testing, but
 - IHC alone is very cheap and will exclude most cases
 - IHC profile may help with interpretation of variants of unknown significance (VUS)

Topics

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Therapy

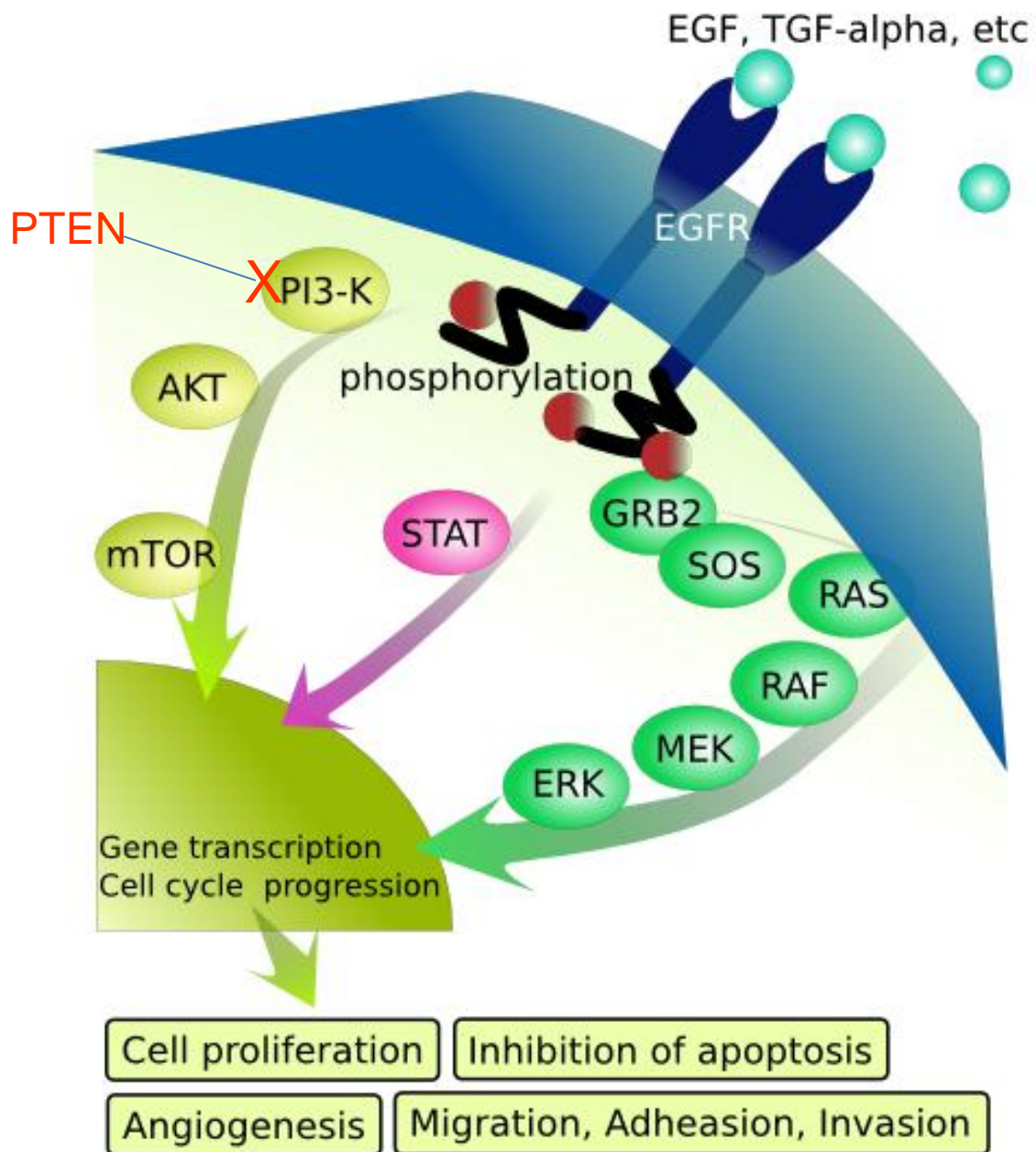
- MSI
 - 5FU may not be effective (may even be harmful) if tumor is unstable (Ribic, NEJM, 2003)
 - May not be relevant to new therapies which include oxaliplatin
 - MSI status sometimes used as part of decision on whether to treat Stage II disease (in addition to gene expression profiling, which predicts recurrence in Stage II and III)

Personalized (precision) medicine

- Not one size fits all, but targeted therapy based upon mutational profile of each tumor
- Need to evaluate molecular targets in each tumor type

Precision medicine for colorectal cancer

- EGFR pathway is activated (but EGFR is not mutated) in colorectal cancer
- Cetuximab is an antibody that binds to EGFR, blocking activation by ligands like epidermal growth factor
- A mutation downstream of EGFR that activates the pathway makes this blocking irrelevant
- Bad to give a toxic and expensive drug if it won't work



Wikimedia

EGFR pathway inhibition

- EGFR inhibitors used in Stage IV cancers
- Original studies: EGFR inhibition ineffective if mutation in codon 12 or 13 of KRAS
- Subsequently extended to codons 12, 13, 61, 117 or 146 of KRAS and NRAS
- Codon 1047 PIK3CA mutations, loss of PTEN
- BRAF may be prognostic marker (bad) rather than predictive of therapy response

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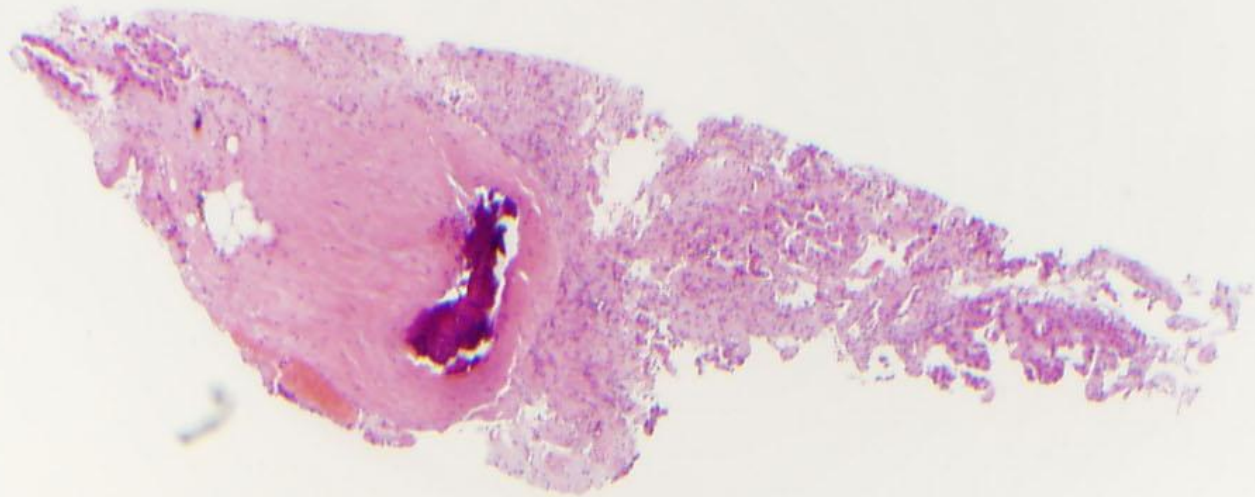
Next generation sequencing

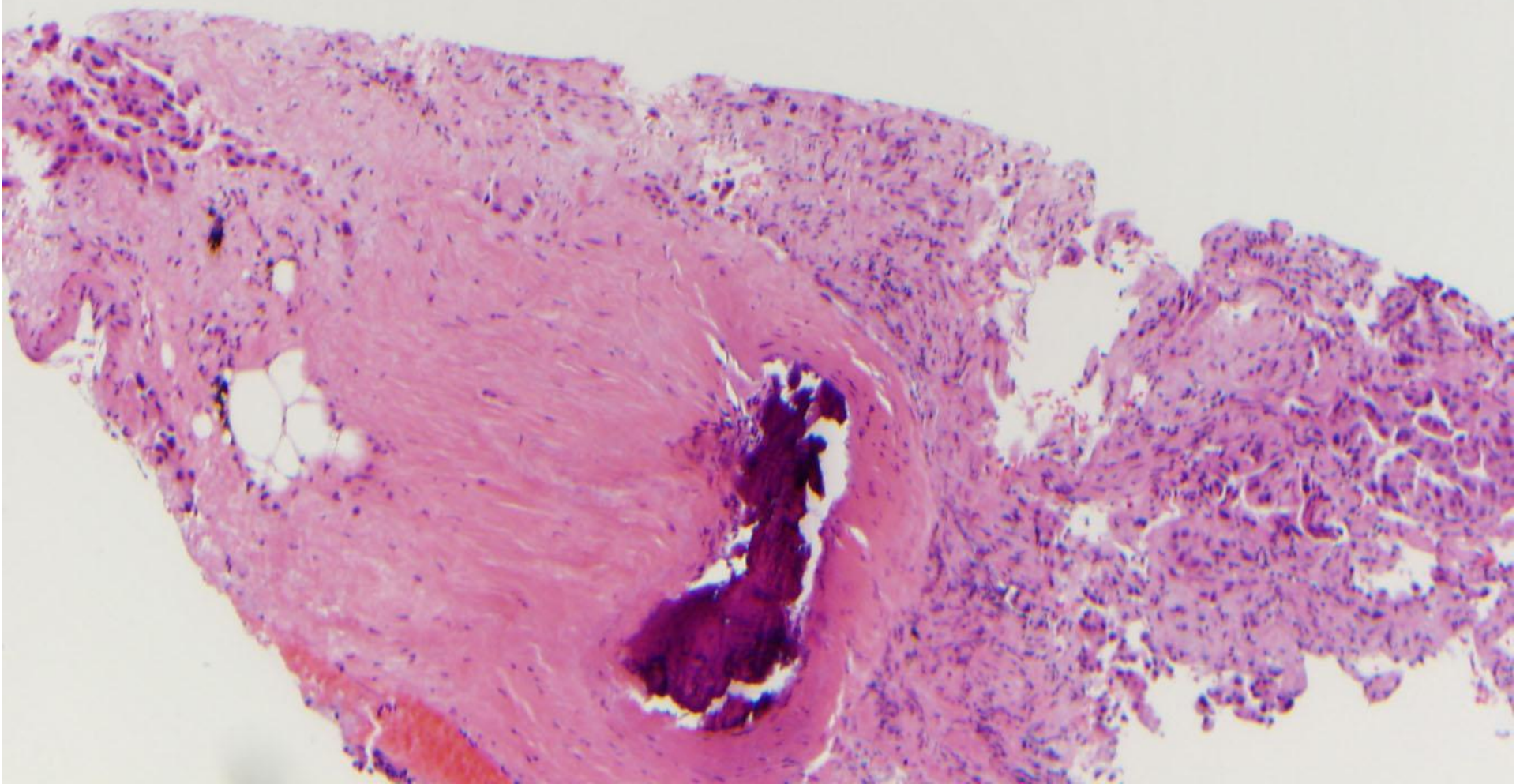
- Cost effective way to test multiple genes
 - And relevant genes (which code for targetable protein products) will continue to grow, making multiple single gene tests even more expensive
- Targeted NGS approach requires very little input DNA (10 ng) and provides fast turnaround time

ARUP

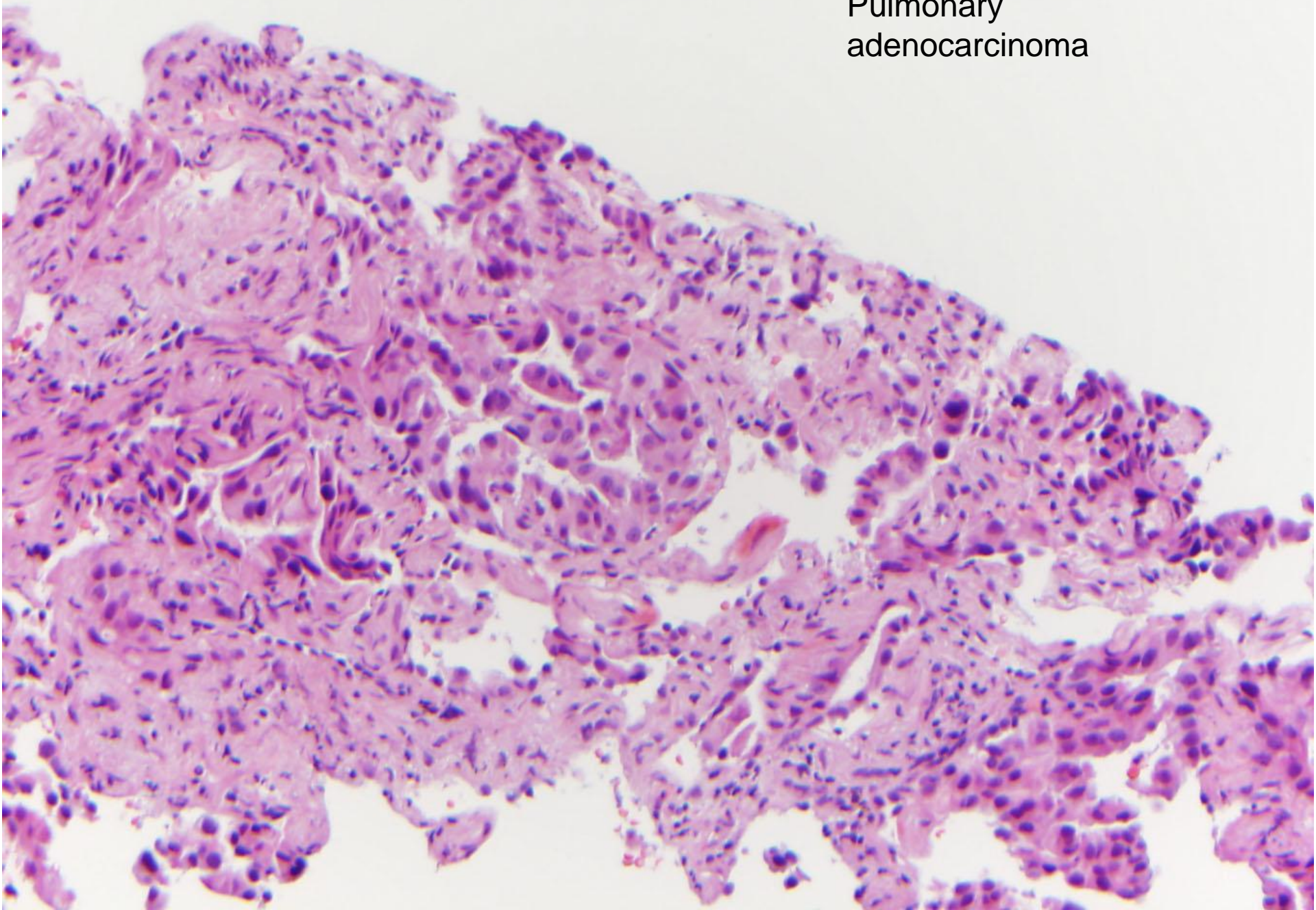
14







Pulmonary
adenocarcinoma



EGFR Exon 19: c.2236_2250del, p.E746_750del

