Predictive and Prognostic Immunohistochemistry in the Evaluation of Colorectal Cancer

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Inspired Medicine

No relevant disclosures or conflicts of interest

Objectives

- Discuss interpretation and clinical significance of immunohistochemical biomarkers in colorectal cancer
  - Mismatch repair (MMR) proficiency and deficiency
  - Checkpoint inhibitor therapy (PD-L1)
  - Her2neu amplification

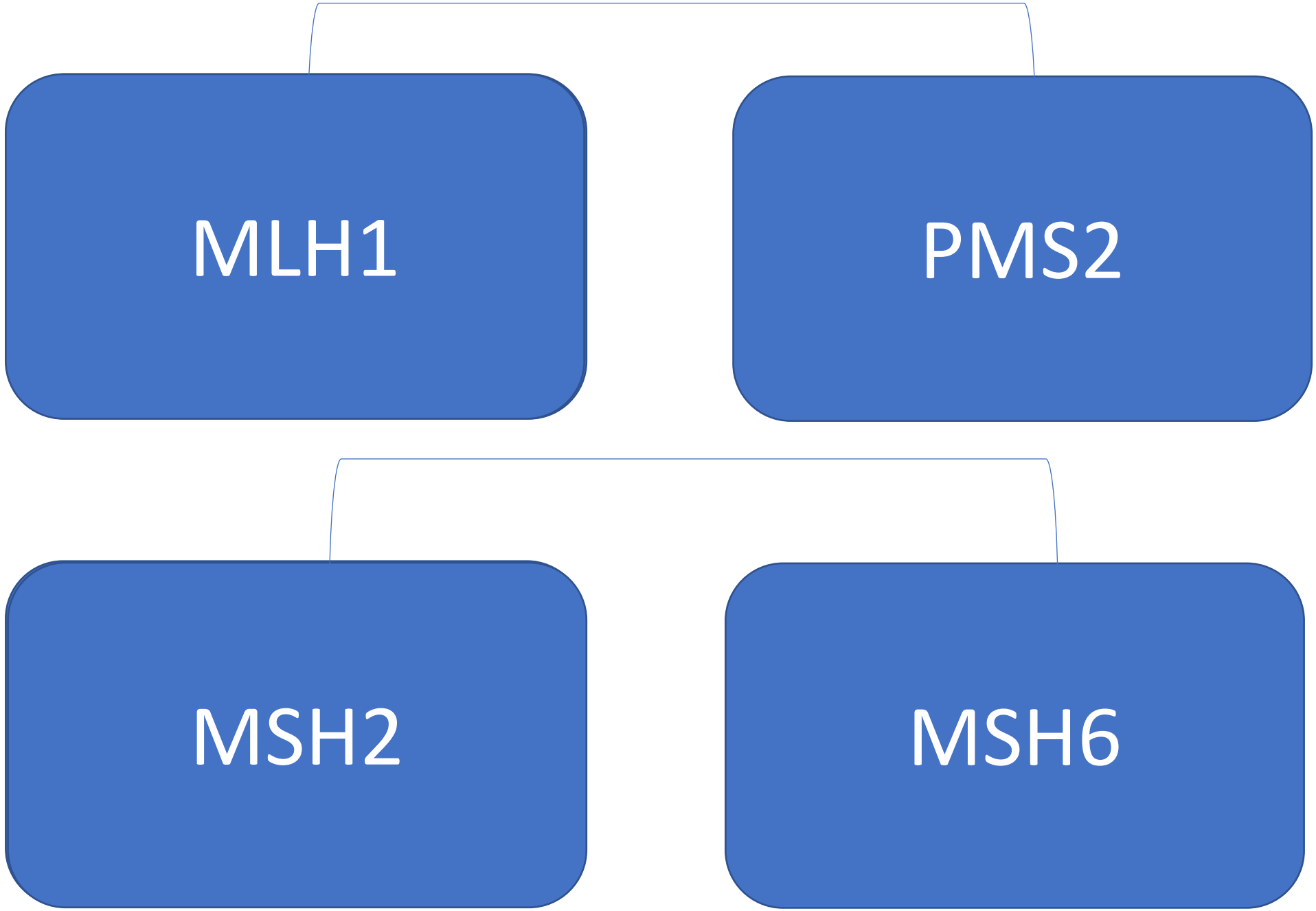
Immunohistochemical Biomarkers

- **Mismatch repair (MMR) proficiency and deficiency**
- PD-L1
- Her2neu amplification

Mismatch Repair Proteins

- Correct single base mismatches in DNA microsatellites
- MLH1, PMS2, MSH2, MSH6
- Presence of all 4 indicates microsatellite stable (mismatch repair proficient - pMMR)*
- Staining loss of 1 or more indicates microsatellite instability (mismatch repair deficient - dMMR)

* Like all rules, there are exceptions. Stay tuned.


MLH1
PMS2
MSH2
MSH6

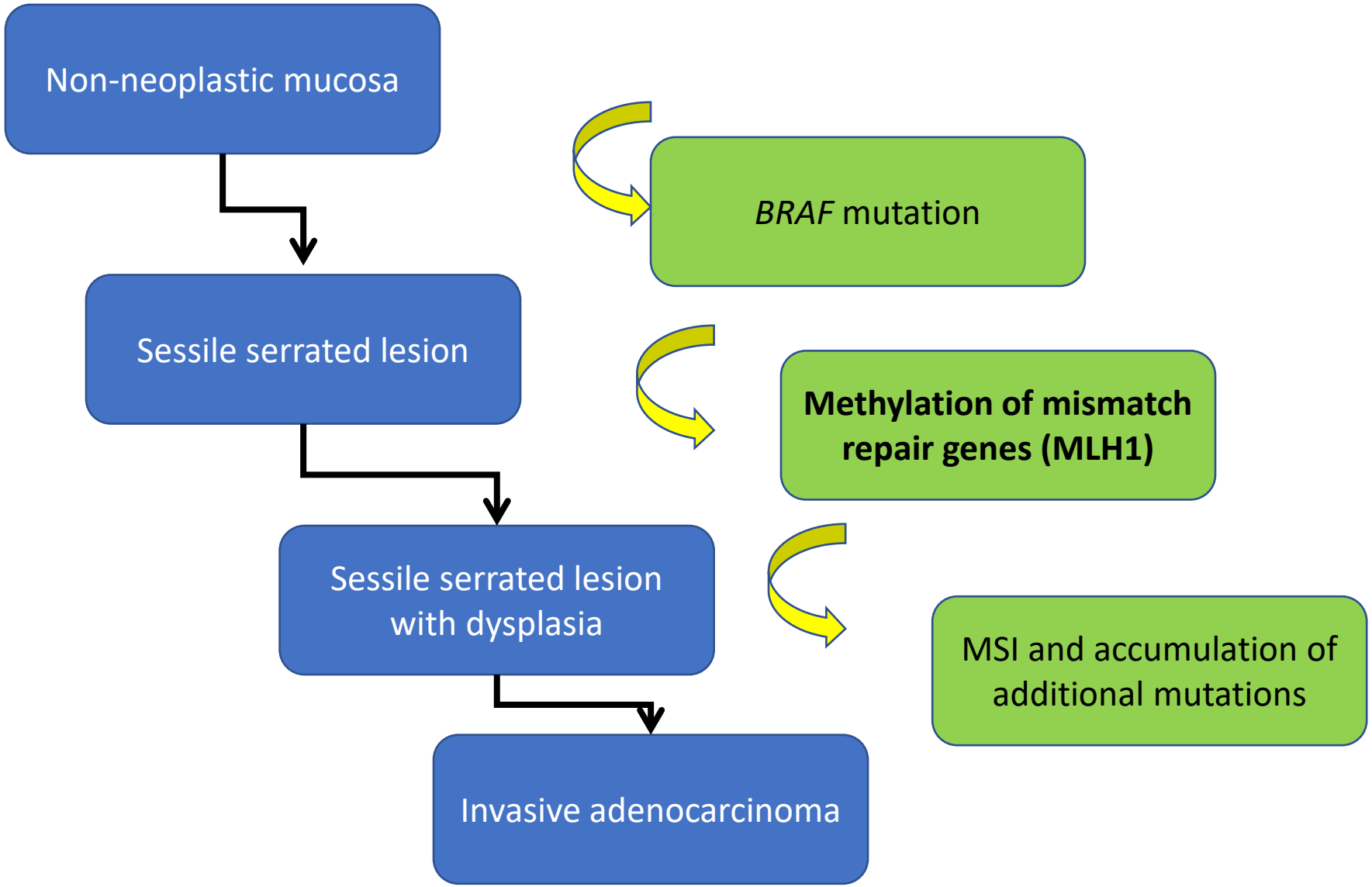
Lynch Syndrome

- Autosomal dominant
- Germline mutations in 1 of the 4 mismatch repair genes or in *EPCAM*
- Universal screening advocated by Centers for Disease Control and Prevention, National Comprehensive Cancer Network, American College of Gastroenterology, American Society of Clinical Oncology

Screening for Lynch Syndrome

- Why do we do it?
  - To enter patients and their families into monitoring programs
  - To plan appropriate surgery
    - Subtotal colectomy
    - Only possible when the biopsy is tested

Sporadic MSI Cancers and the Serrated Neoplastic Pathway



Identifying Sporadic MSI Colon Cancers

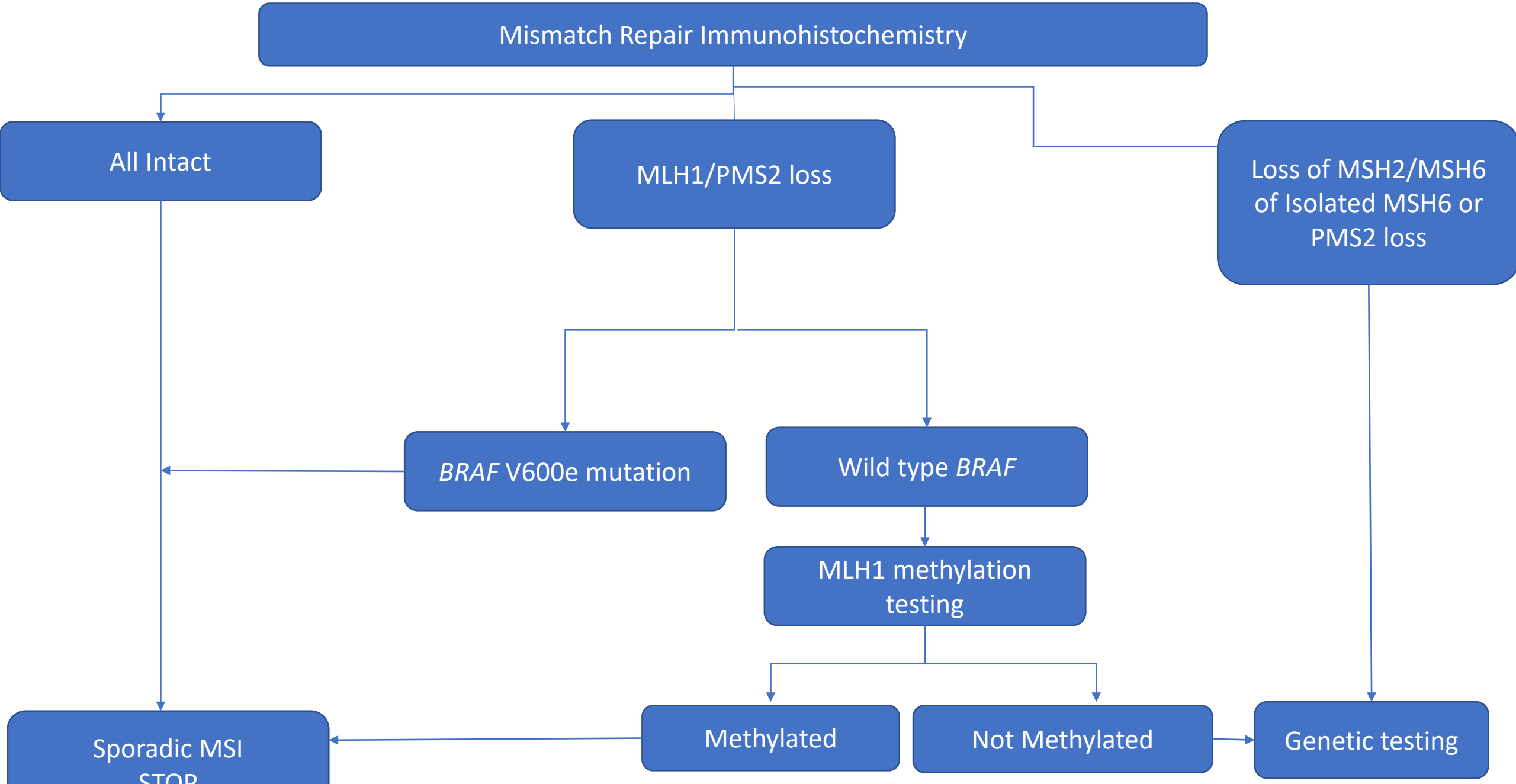
- Why do we do it?
  - Overall better prognosis
  - Poor response to Fluorouracil-based therapy
  - Benefit from immune checkpoint inhibitors

Screening for Mismatch Repair Deficiency: How Do We Do It?

- Most institutions perform immunohistochemistry (IHC) on biopsy samples
- Discordant results between IHC and PCR rare
- PCR is warranted when IHC results are equivocal
- MSI status is included in some next generation sequencing panels

Hissong E, et al. Mod Pathol. 2018

Hechtman JF, et al. Mod Pathol. 2020 May;33(5):871-879

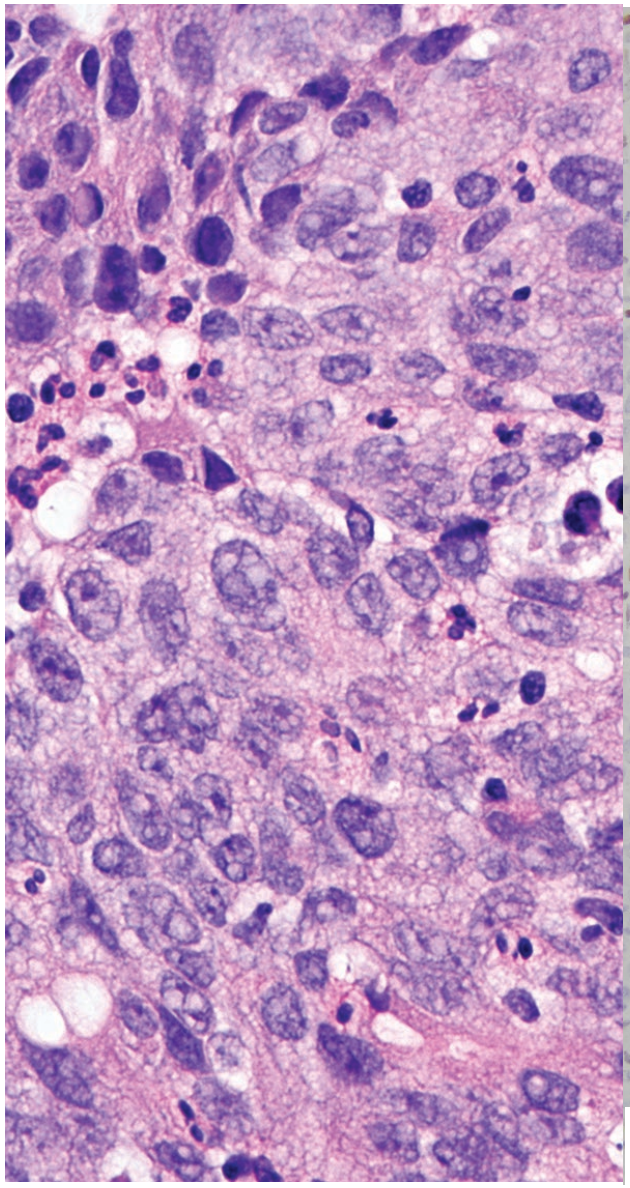


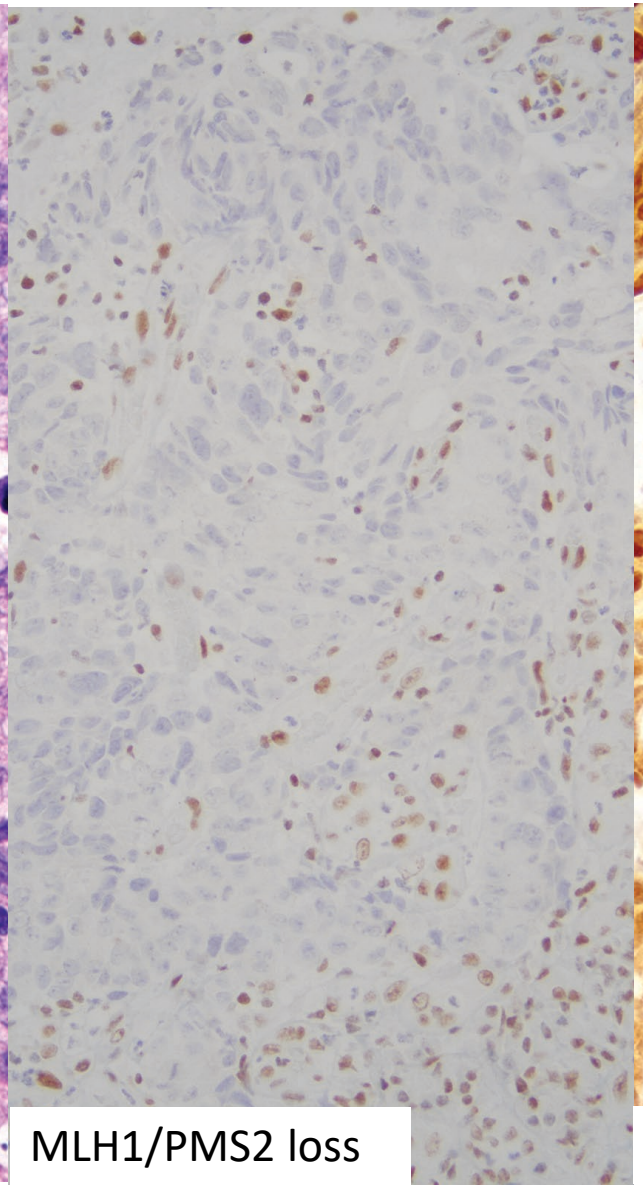
Adapted from Chen W, Frankel WL. Mod Pathol. 2019.

MMR Protein Immunohistochemistry

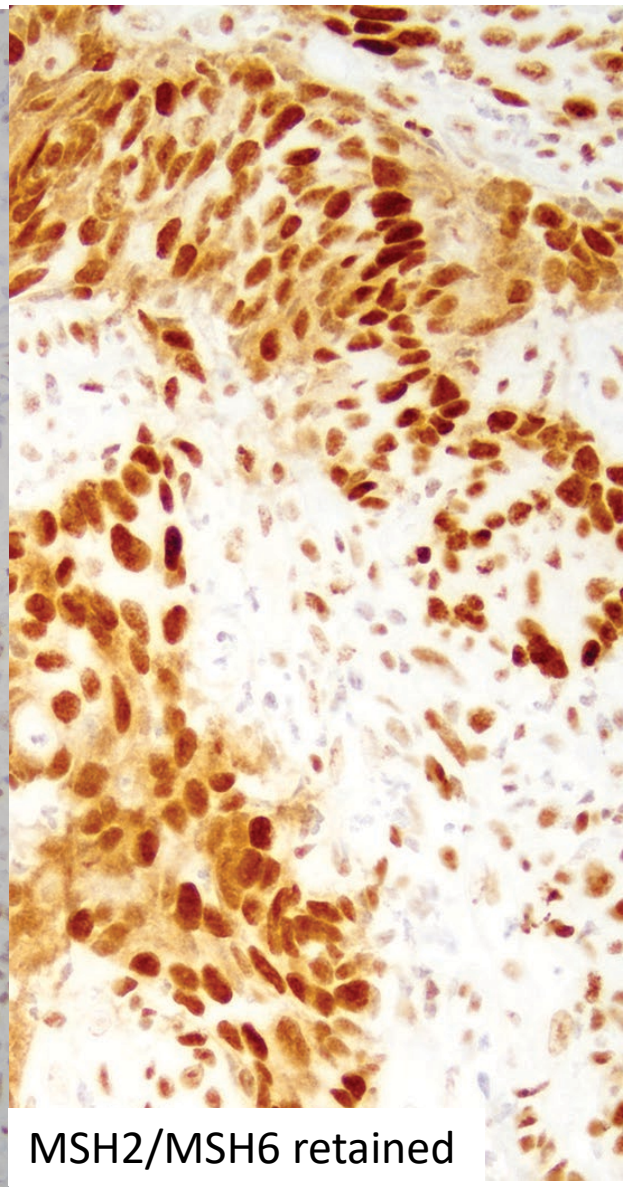
Interpretation and Pitfalls

Mismatch Repair Protein Immunohistochemistry





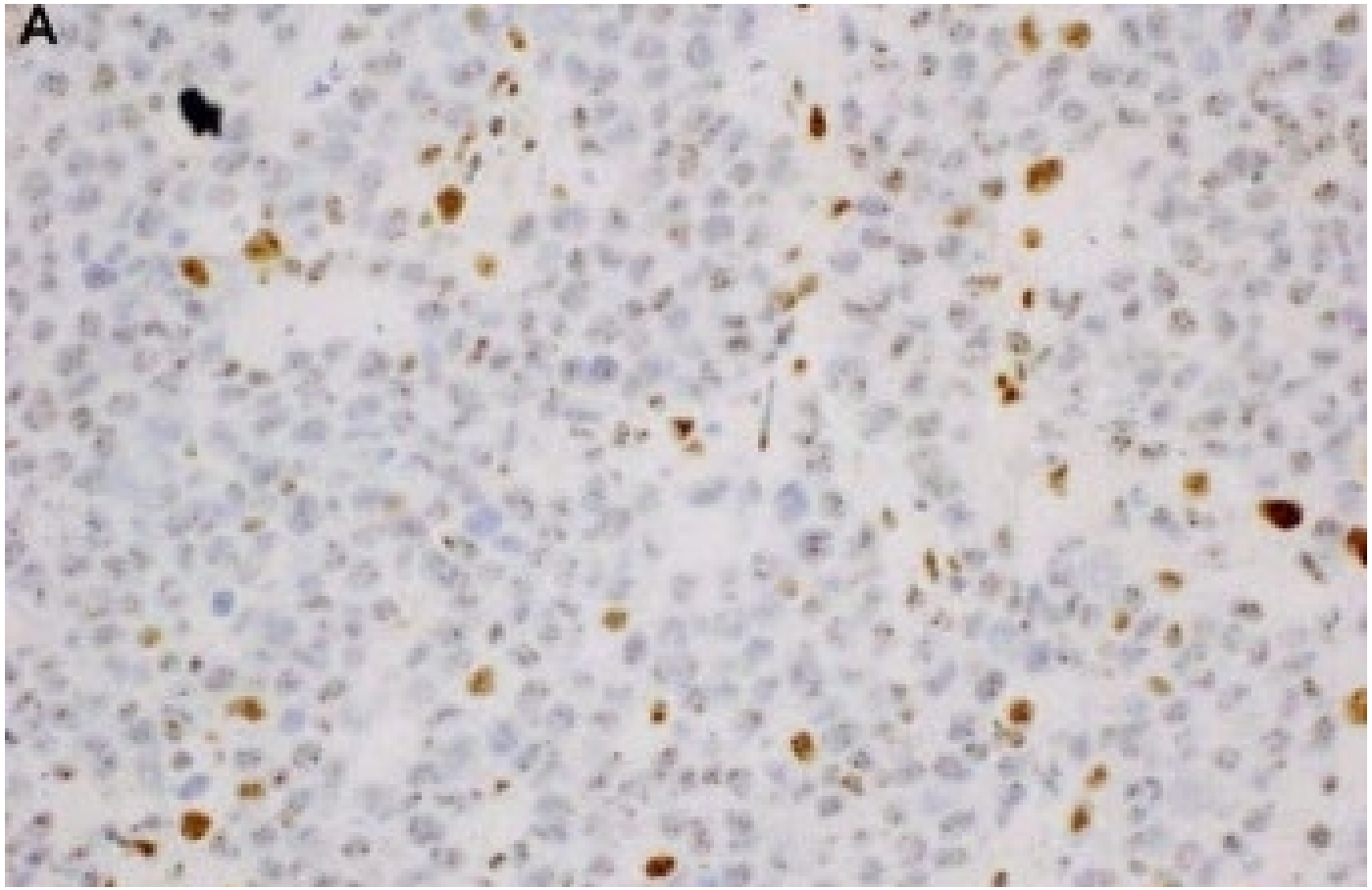
MLH1/PMS2 loss



MSH2/MSH6 retained

- Intact staining should be AT LEAST as strong as the internal control
  - Any proliferating cell
- No official threshold for proportion of cells staining
  - Some experts endorse ≥5%

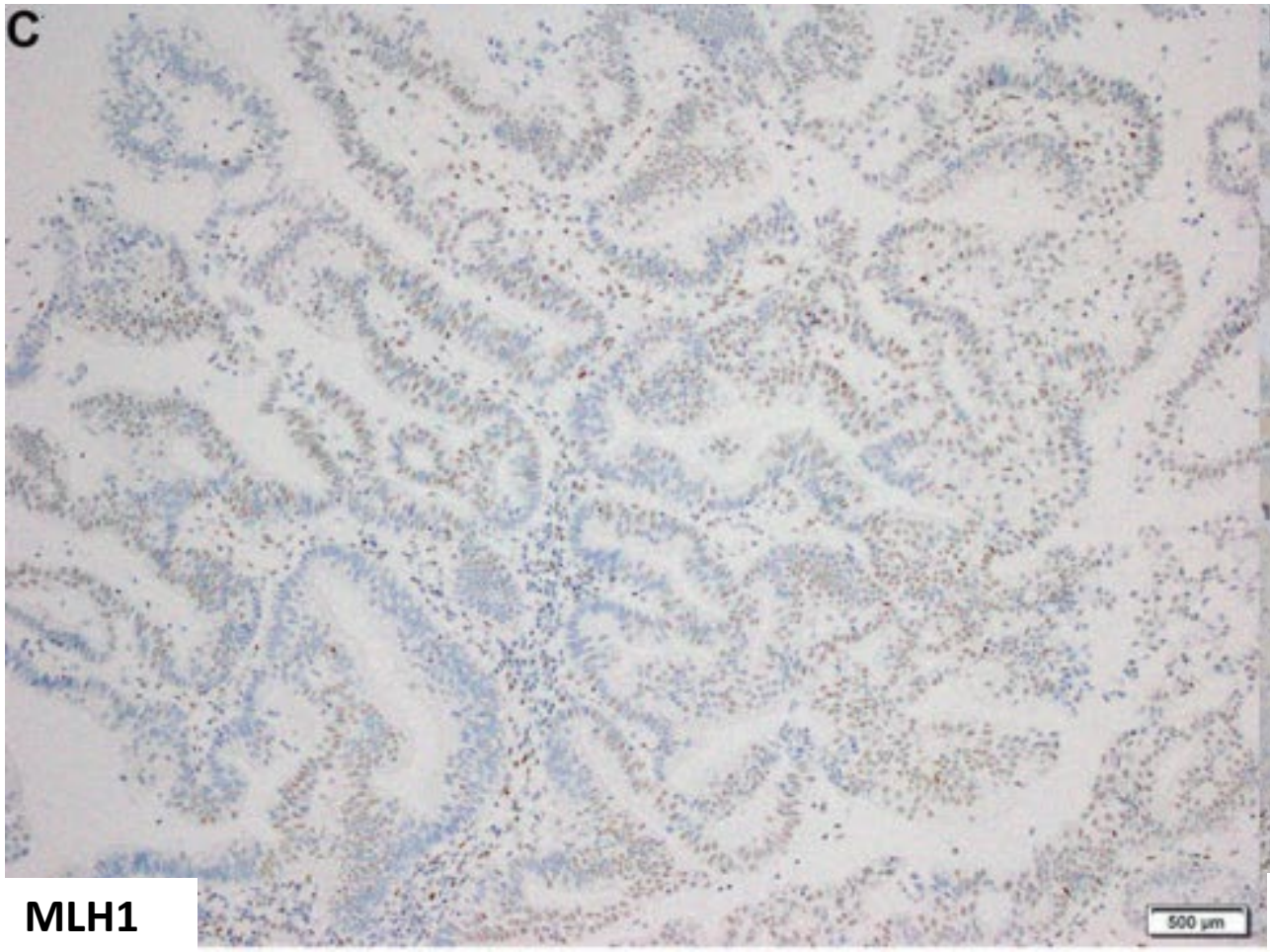
“Discordant” IHC



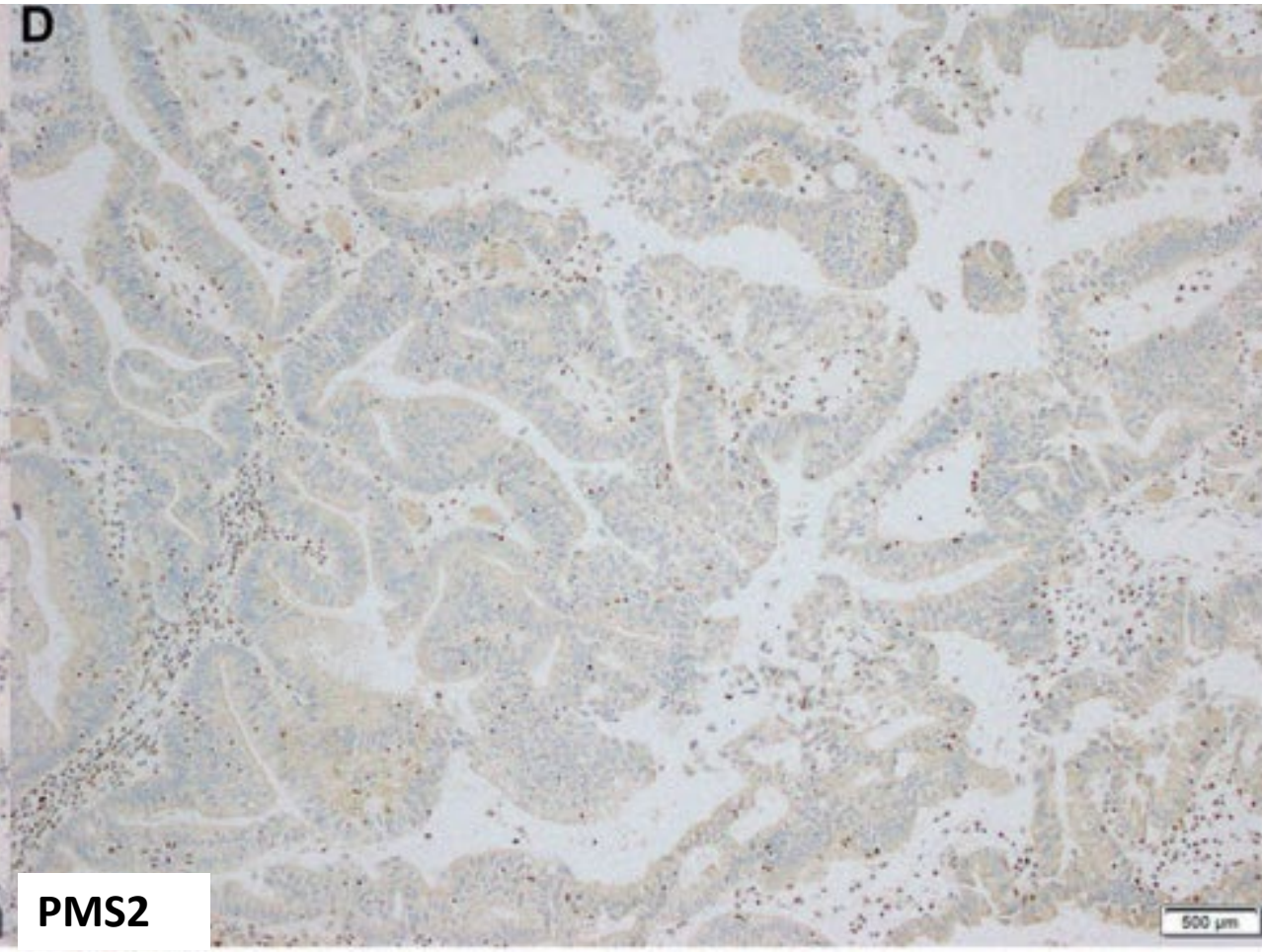
Dot-like MLH1 staining may be seen in cases with germline, somatic, or promotor hypermethylation

Hechtman JF, et al. Mod Pathol. 2020 May;33(5):871-879.
Zhang Q, et al. Int J Surg Pathol. 2020 Apr;28(2):146-152.

“Discordant” IHC



MLH1

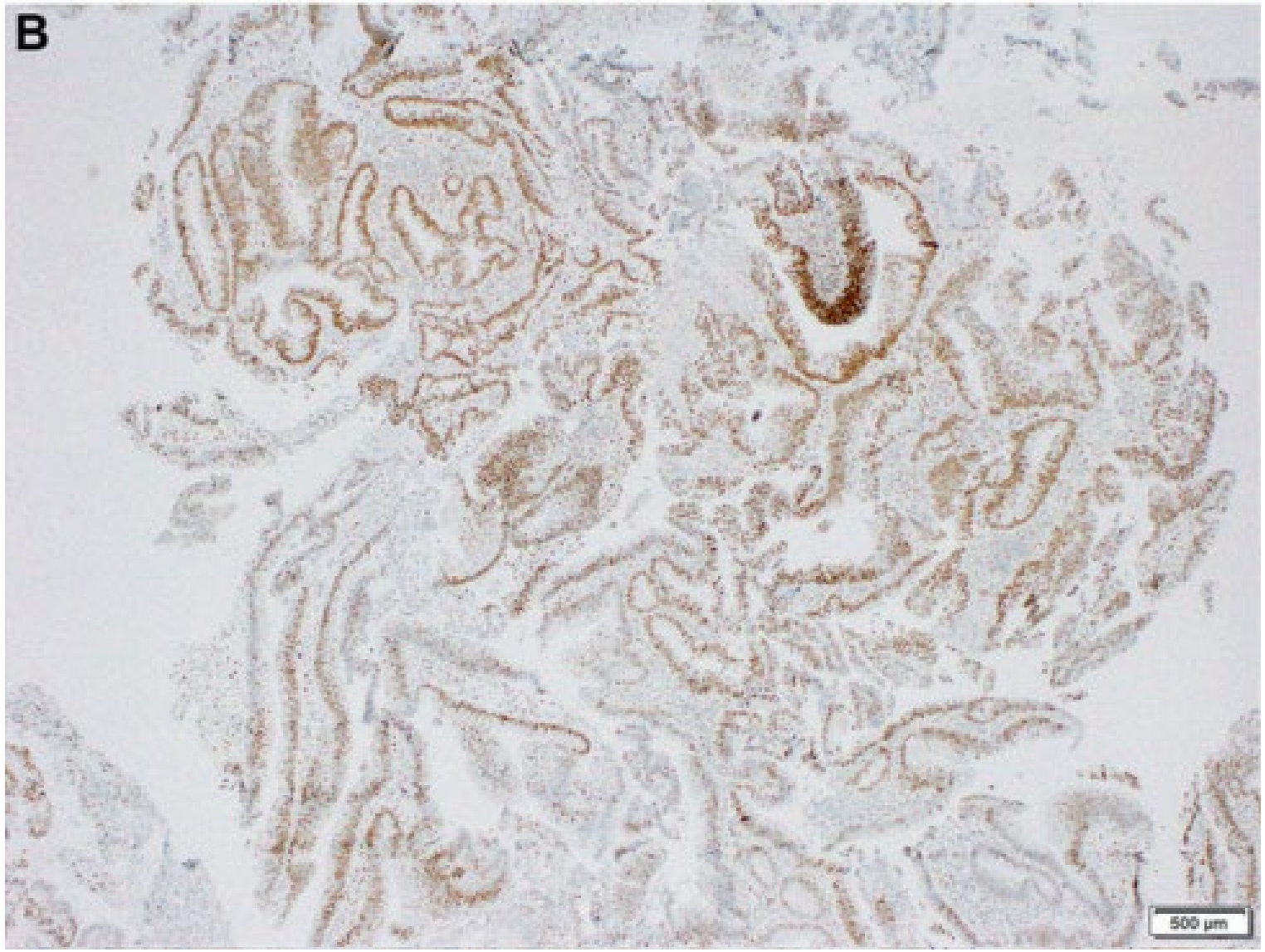


PMS2

Weak MLH1 staining and PMS2 loss in a case with MLH1 promotor hypermethylation

Hechtman JF, et al. Mod Pathol. 2020 May;33(5):871-879

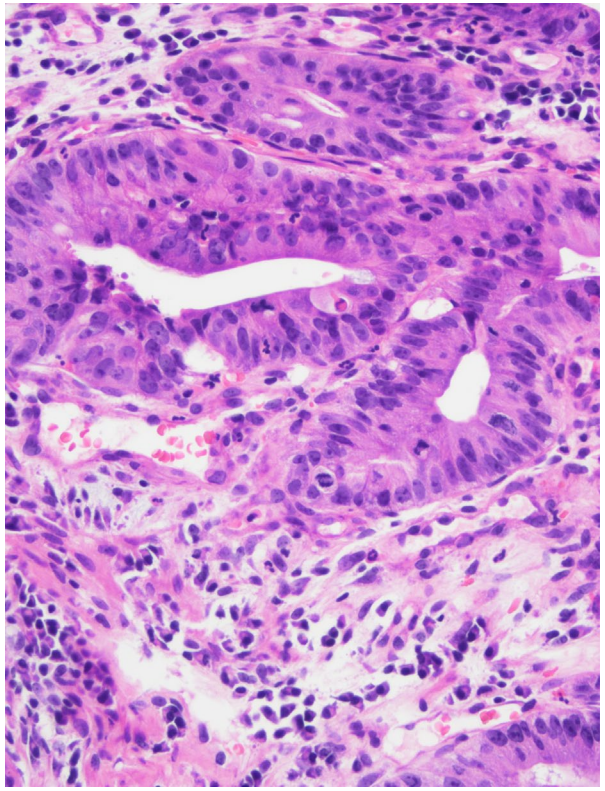
Discordant IHC

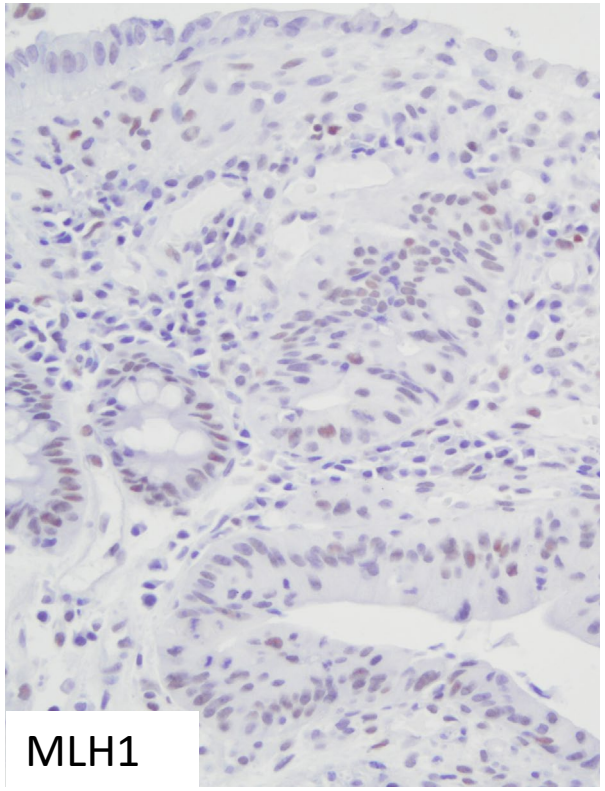


Retained MSH6 staining in a case with two somatic *MSH6* mutations

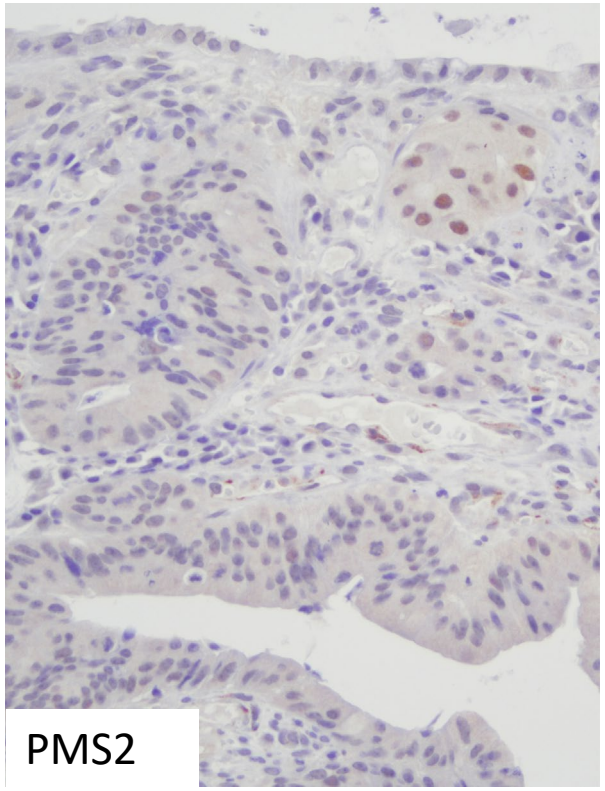
Hechtman JF, et al. Mod Pathol. 2020 May;33(5):871-879

A Case From My Signout…

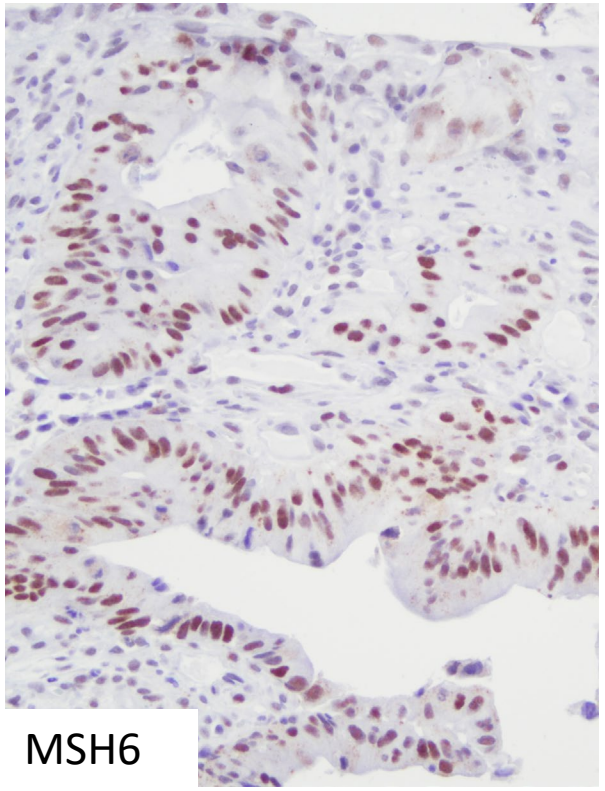




MLH1

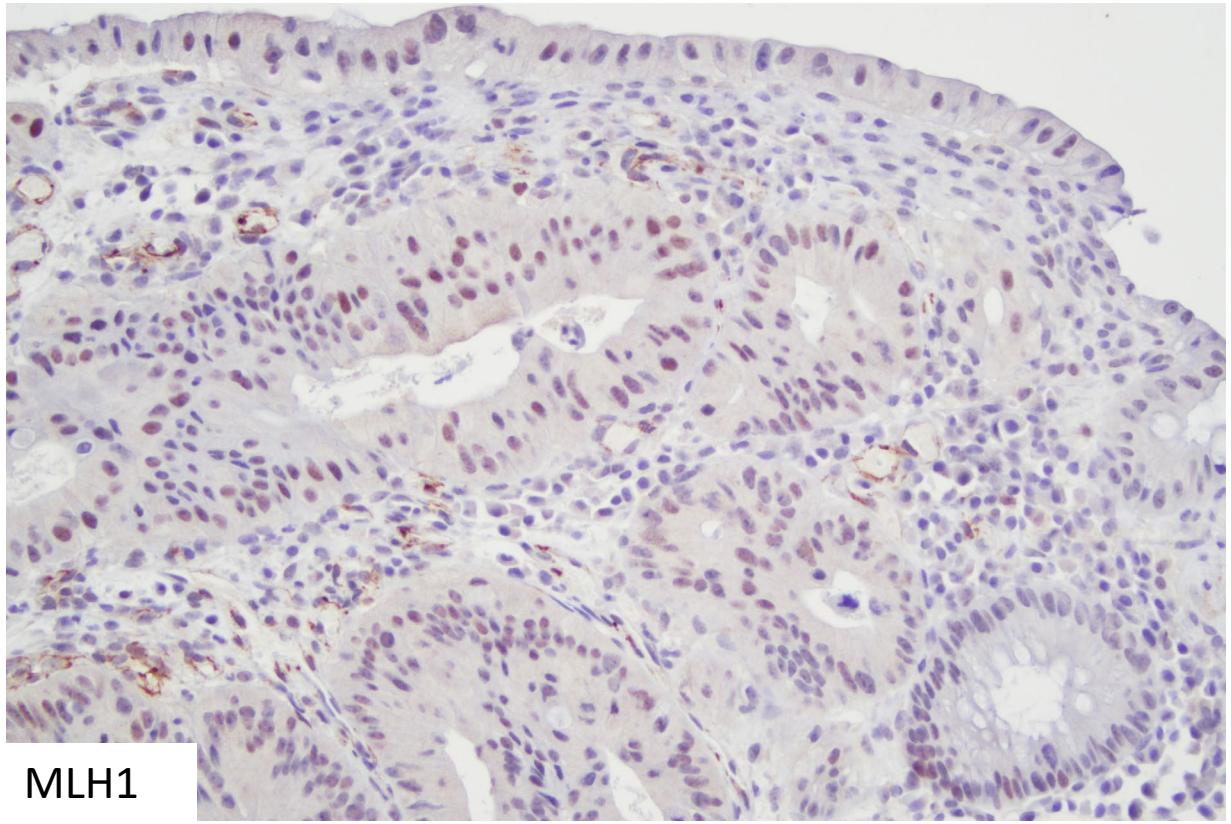


PMS2

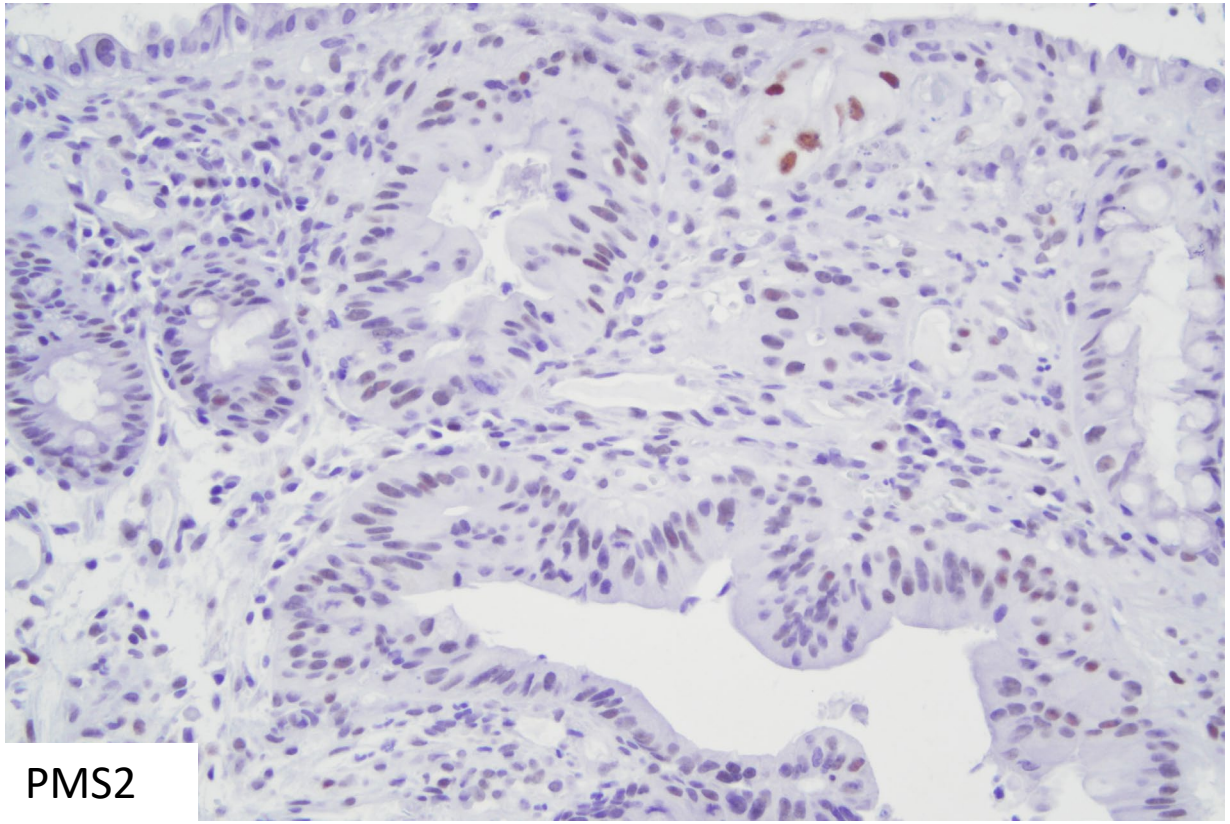


MSH6

Repeat Stains



MLH1



PMS2

We could probably convince ourselves of retained staining in the tumor cells, but the control is still pretty weak

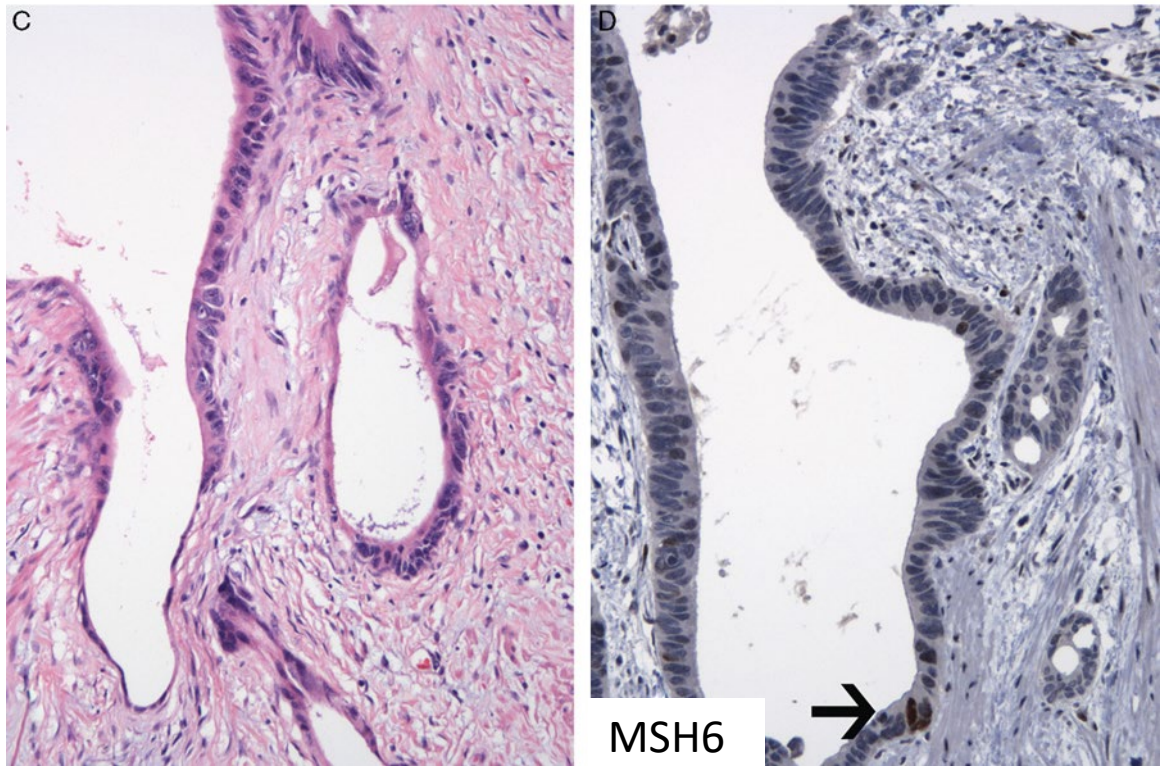
Referred for PCR → MSS

Summary

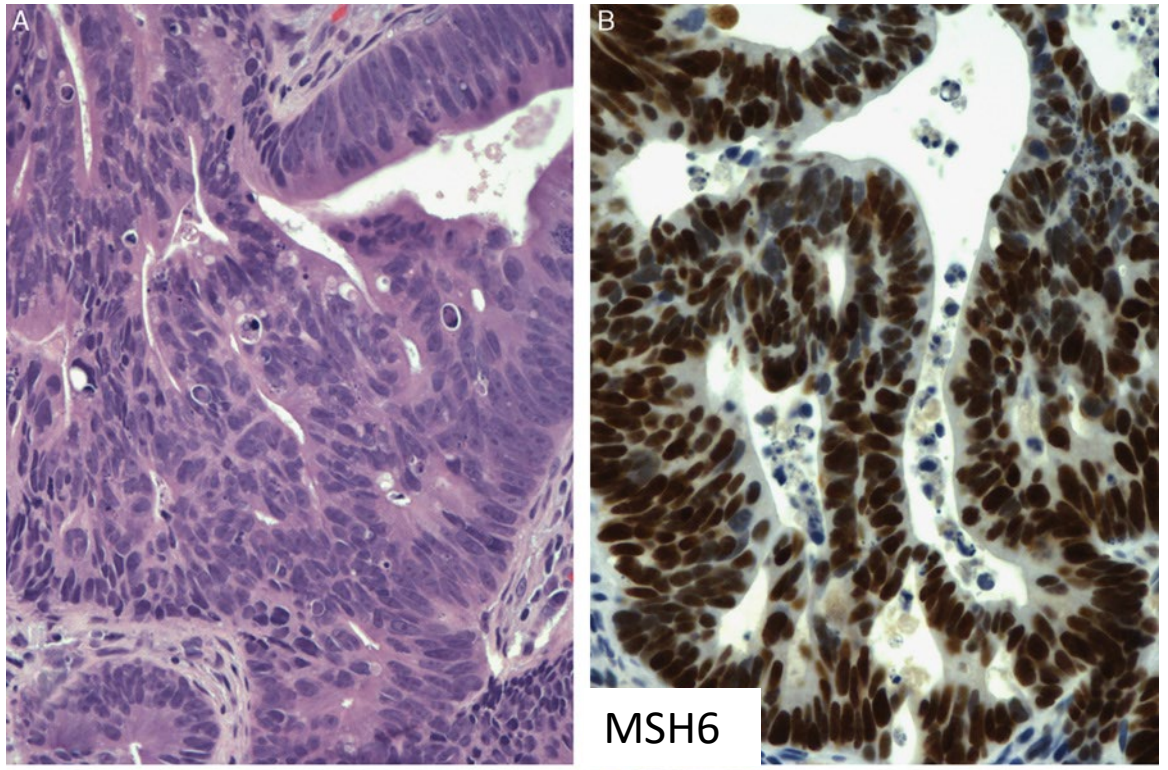
- Staining must be AT LEAST as strong as the internal control
- DO NOT interpret cases without a valid internal control
- Mutated mismatch repair proteins may be immunoreactive but this is rare
- Most discrepancies result from use of outdated criteria for interpreting IHC and can be solved by further testing (i.e. PCR)

One last pitfall…
Post-Neoadjuvant Therapy

Resection



Biopsy

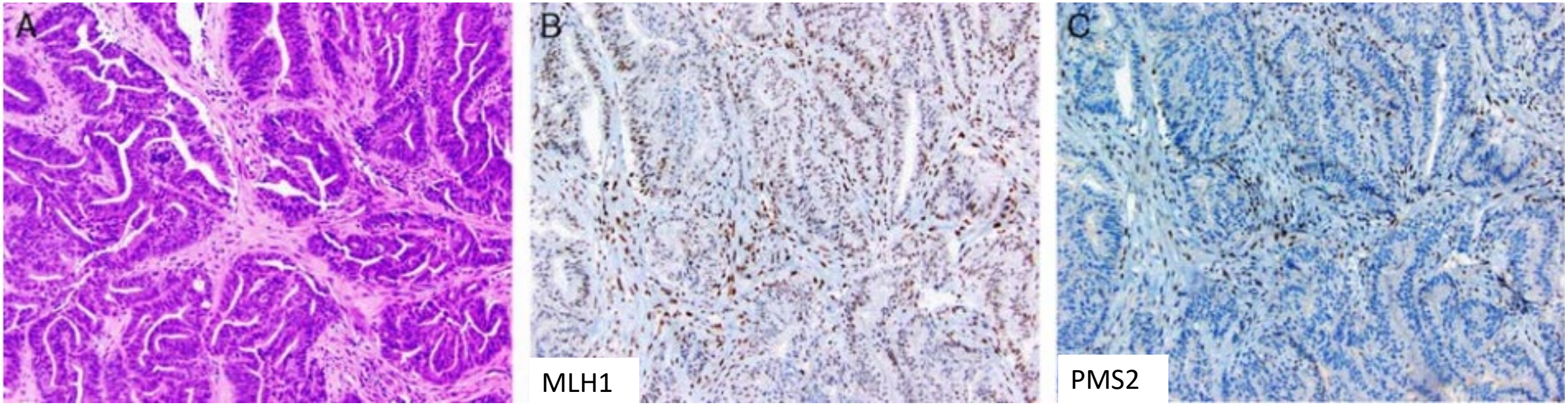


Bao F, et al. Am J Surg Pathol. 2010 Dec;34(12):1798-804.

MMR Protein Immunohistochemistry

Some unusual patterns to know about

Isolated PMS2 Loss Without a Germline *PMS2* Mutation

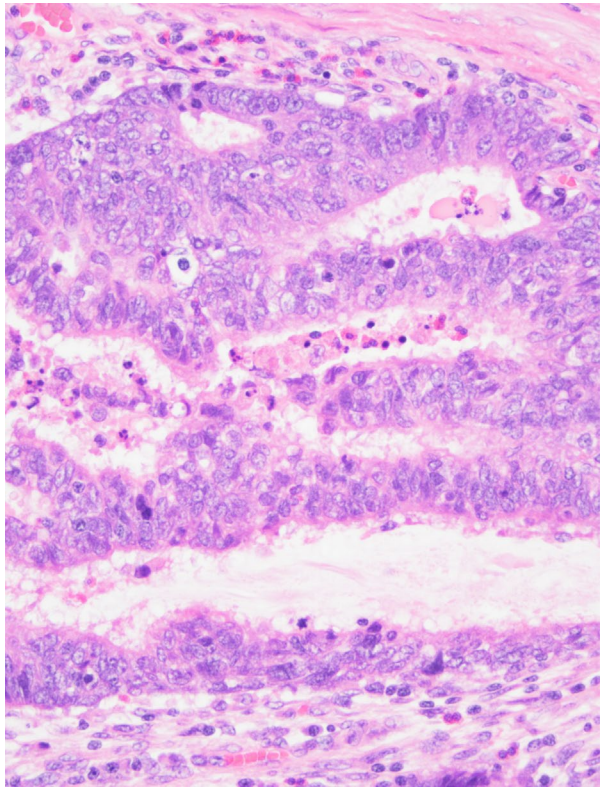


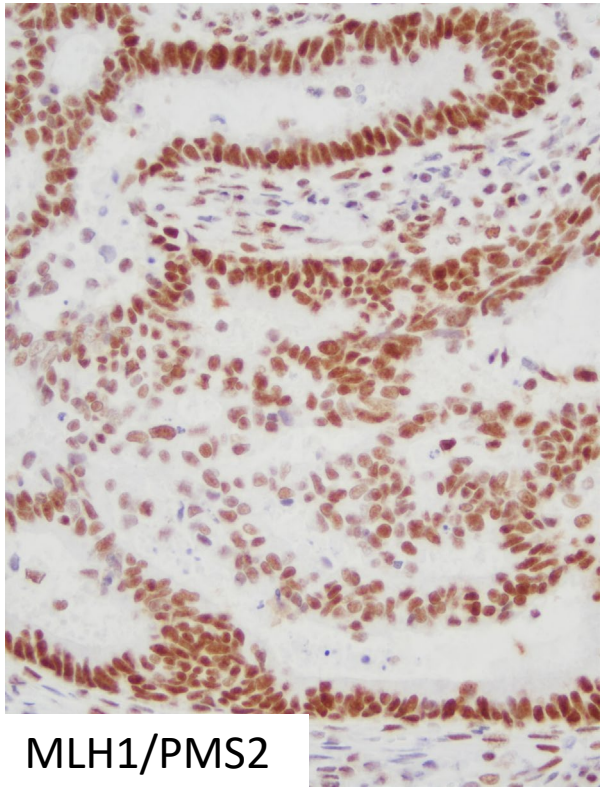
Dudley B, et al. Am J Surg Pathol. 2015 Aug;39(8):1114-20.

- Germline pathogenic variants in *MLH1*
- Bottom line: This pattern is dMMR
  - If *PMS2* is normal, *MLH1* should be tested

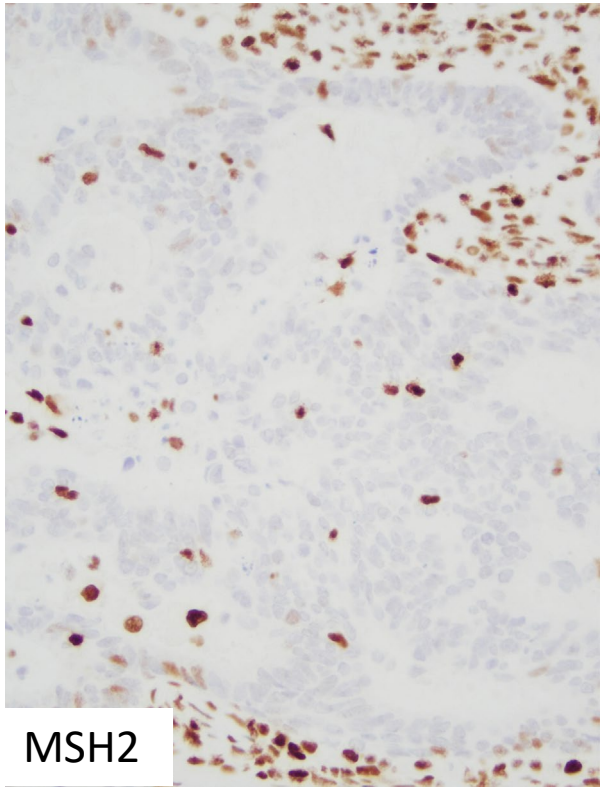
Dudley B, et al. Am J Surg Pathol. 2015 Aug;39(8):1114-20.
Rosty C, et al. BMJ Open. 2016 Feb 19;6(2):e010293.

Another Case From My Signout…

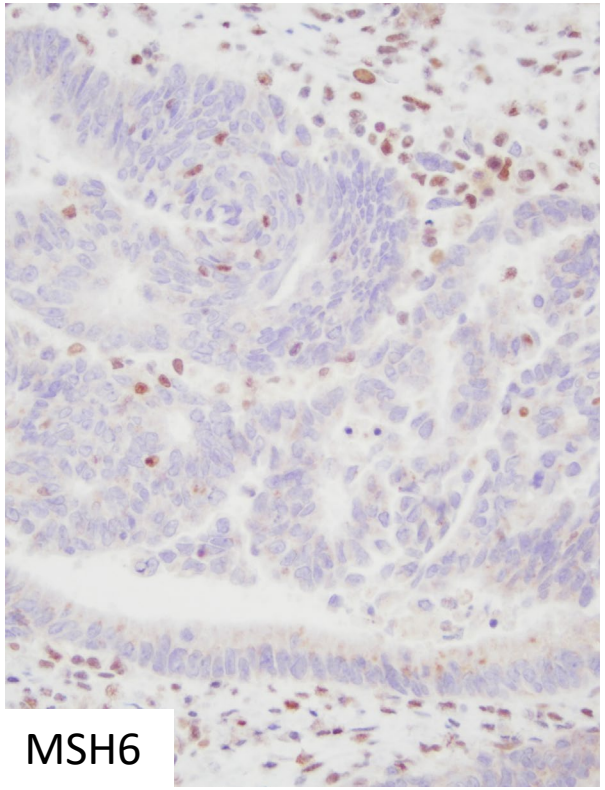




MLH1/PMS2

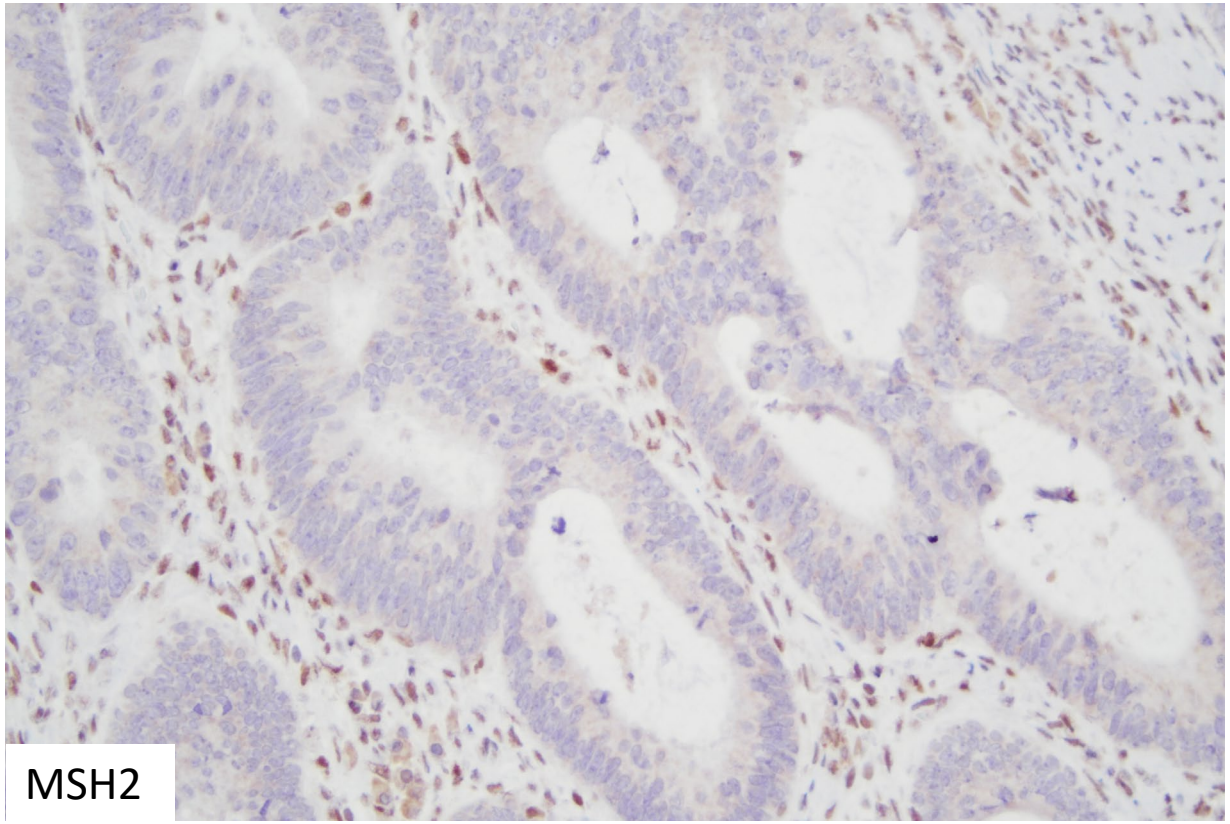


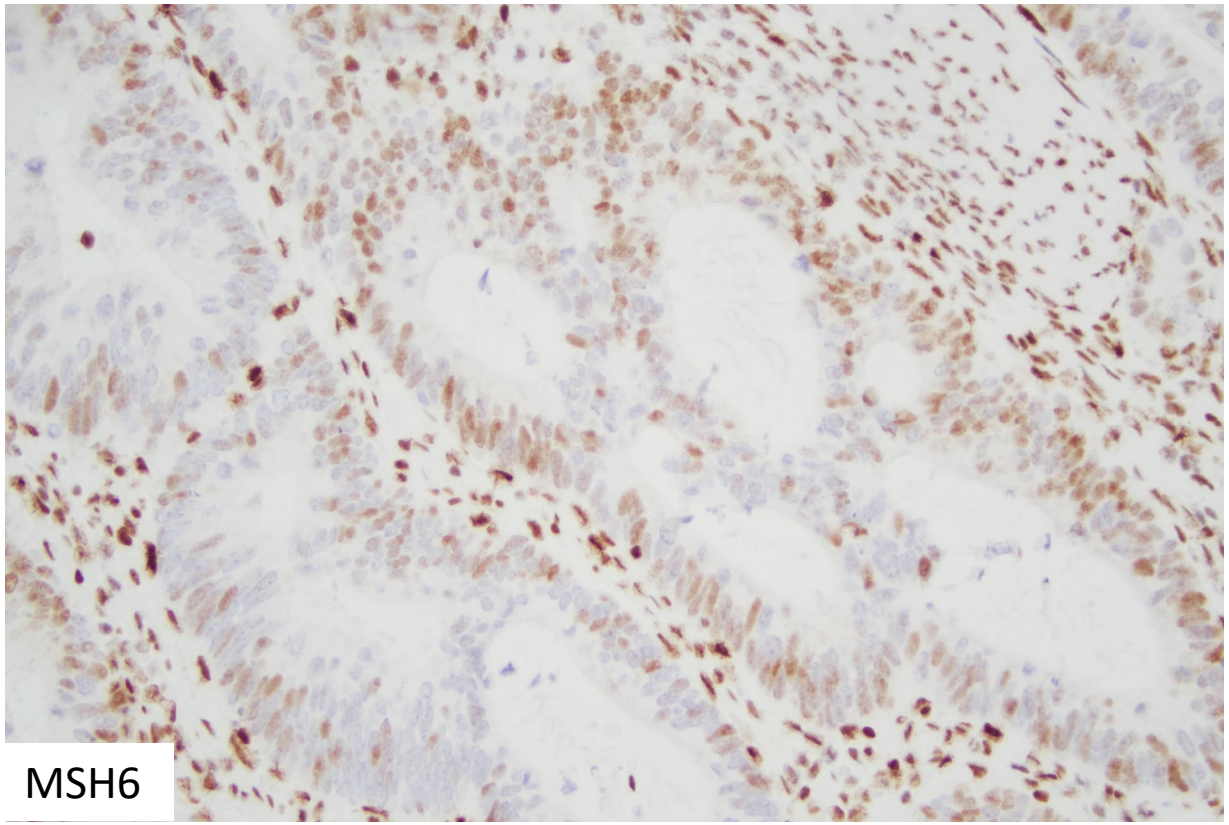
MSH2



MSH6

Same Case, Different Area



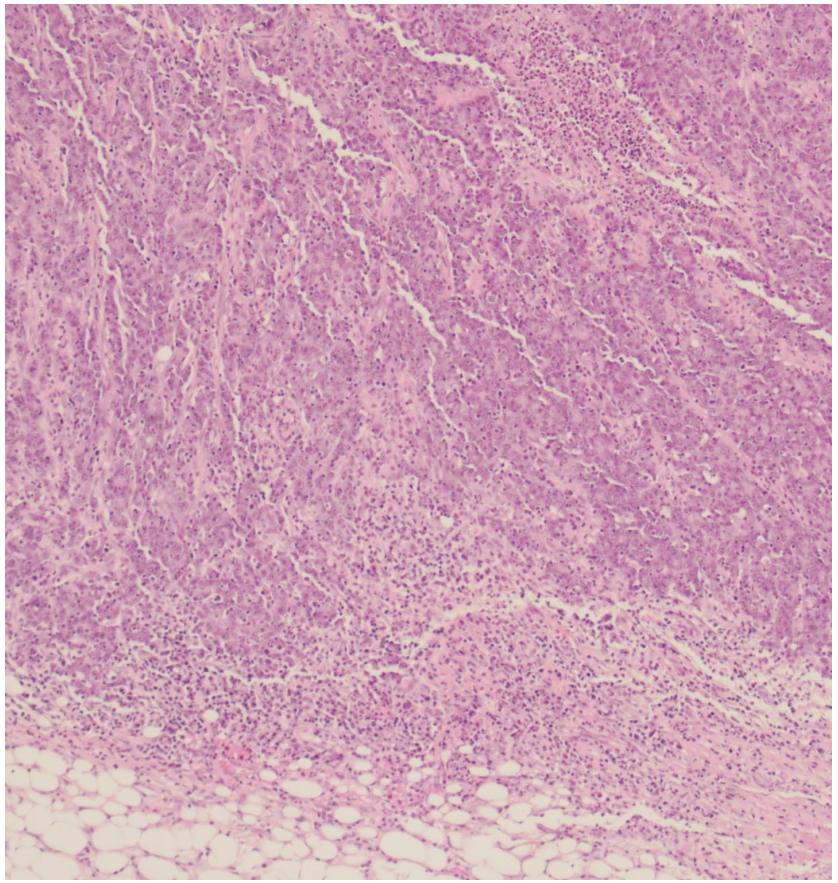


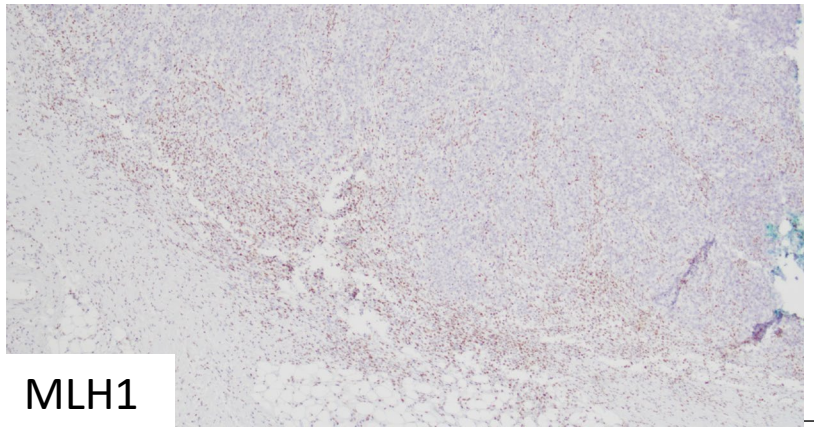
Isolated MSH2 Loss

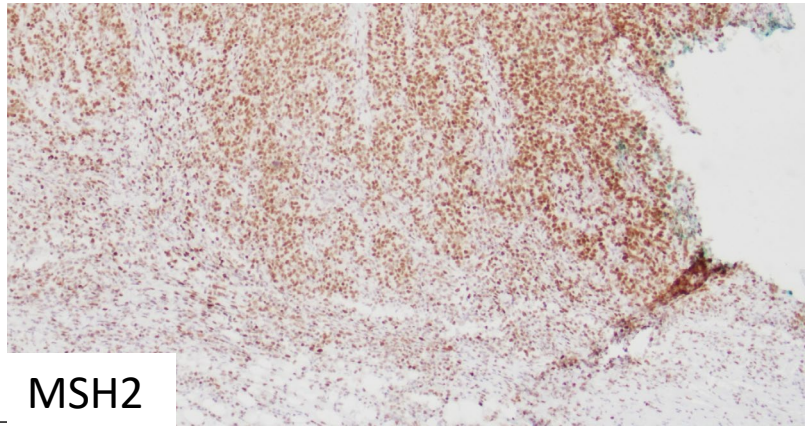
- These cases have been found to have germline or somatic mutations in *MSH2*
  - Mechanism of MSH6 retention unclear
  - Bottom line: This pattern is dMMR

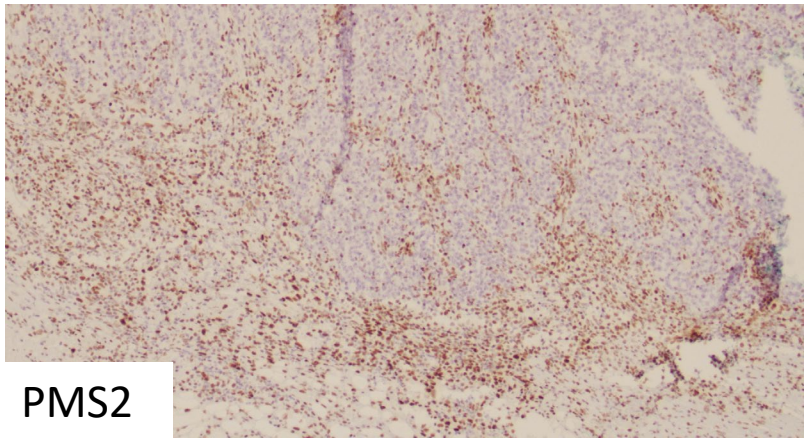
Pearlman R, et al. Mod Pathol. 2018 Dec;31(12):1891-1900.

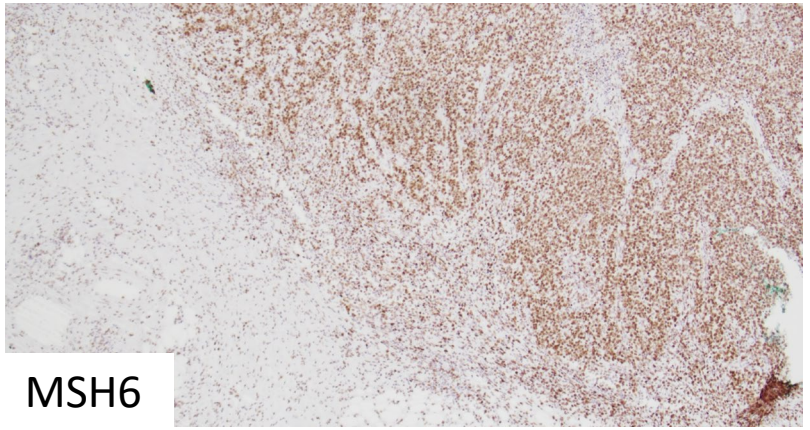
Another Case From My Signout
Tumor #1: Medullary carcinoma

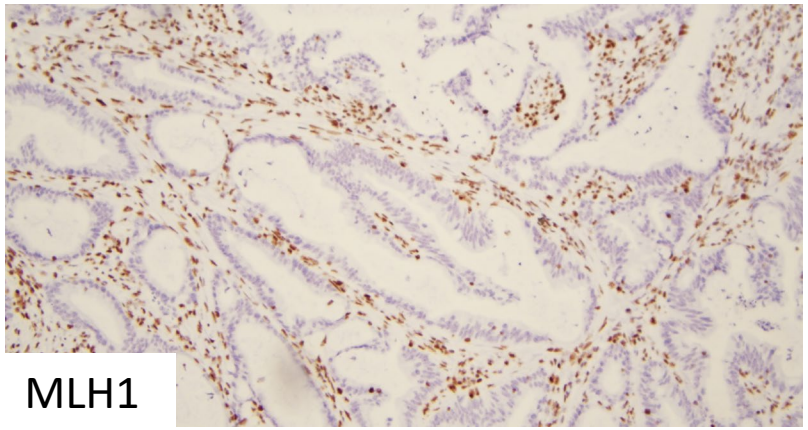


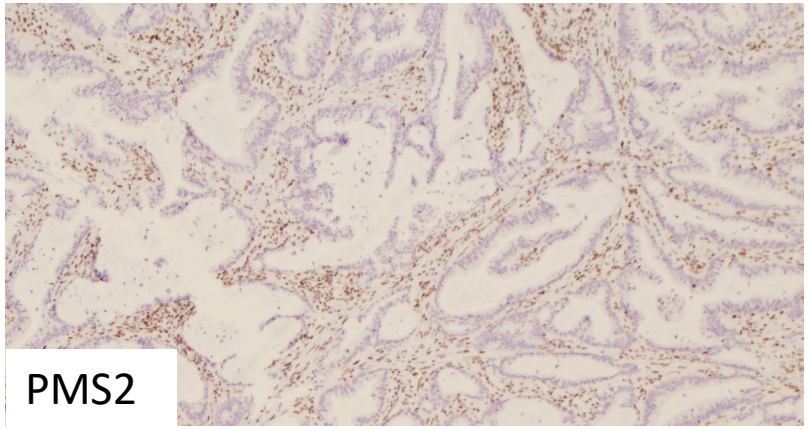


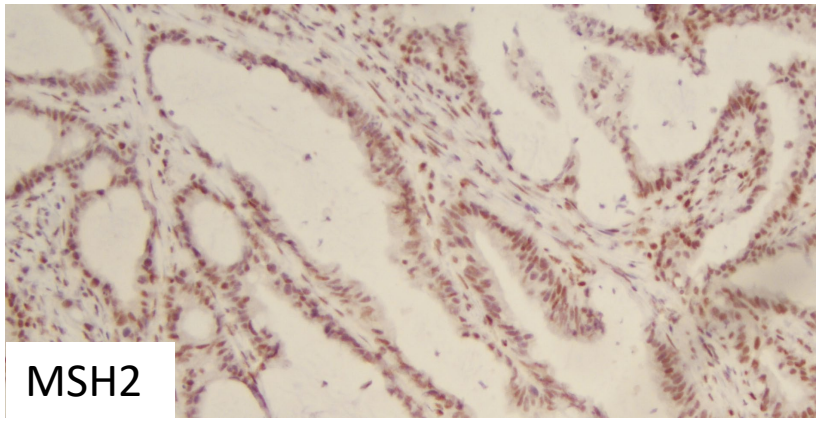


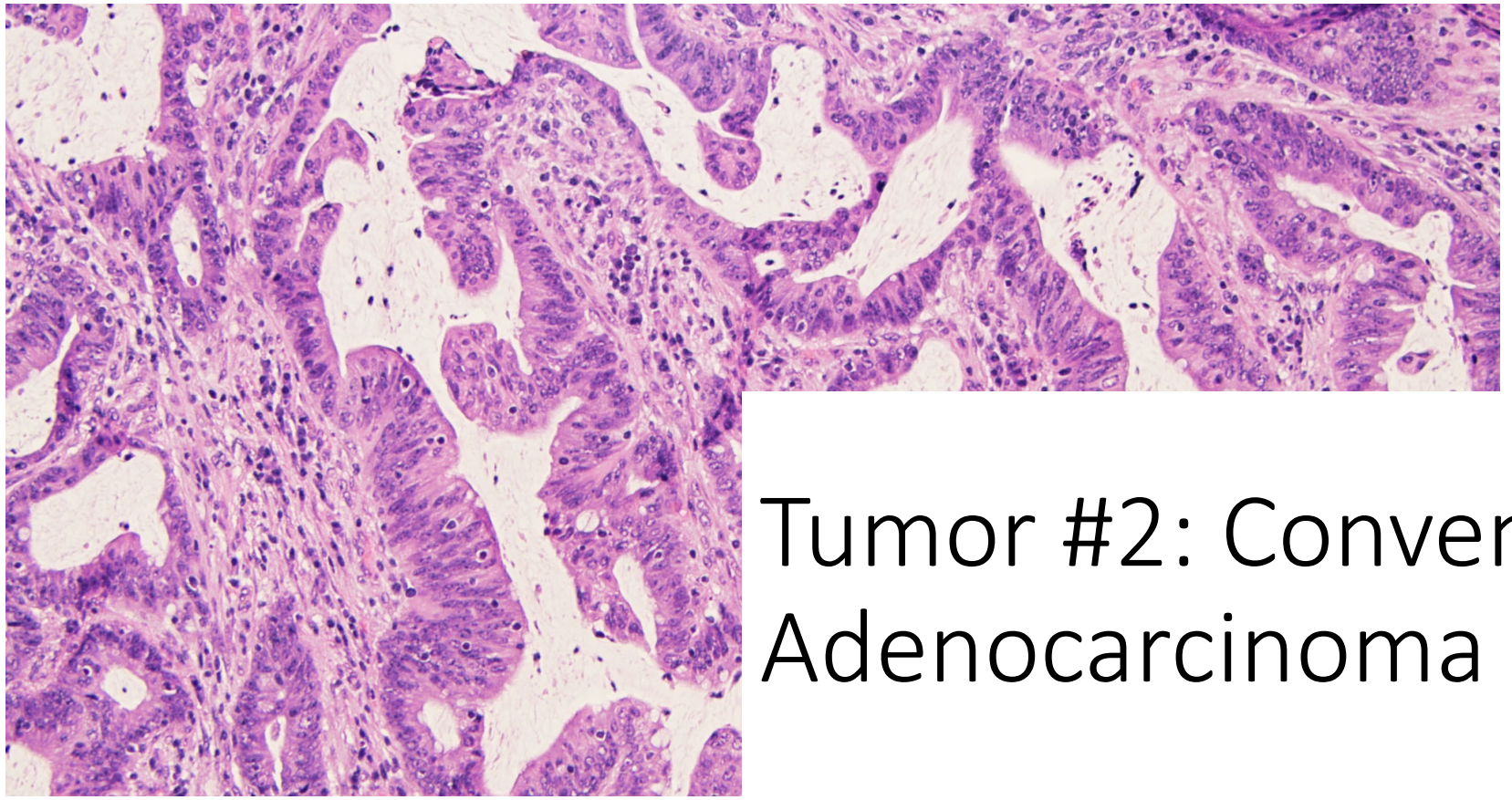


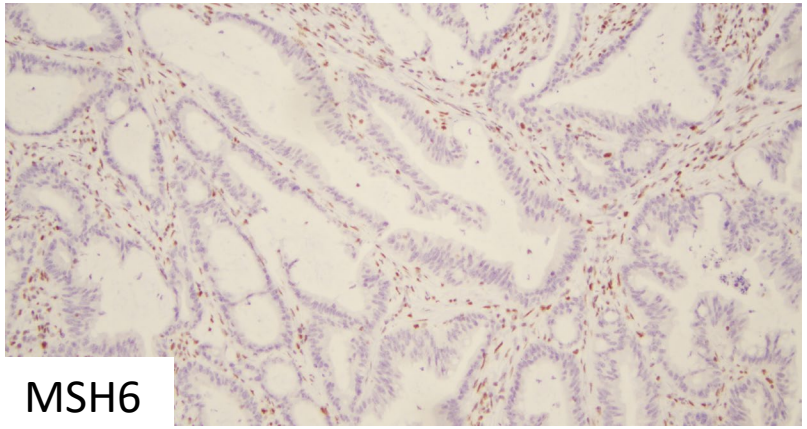












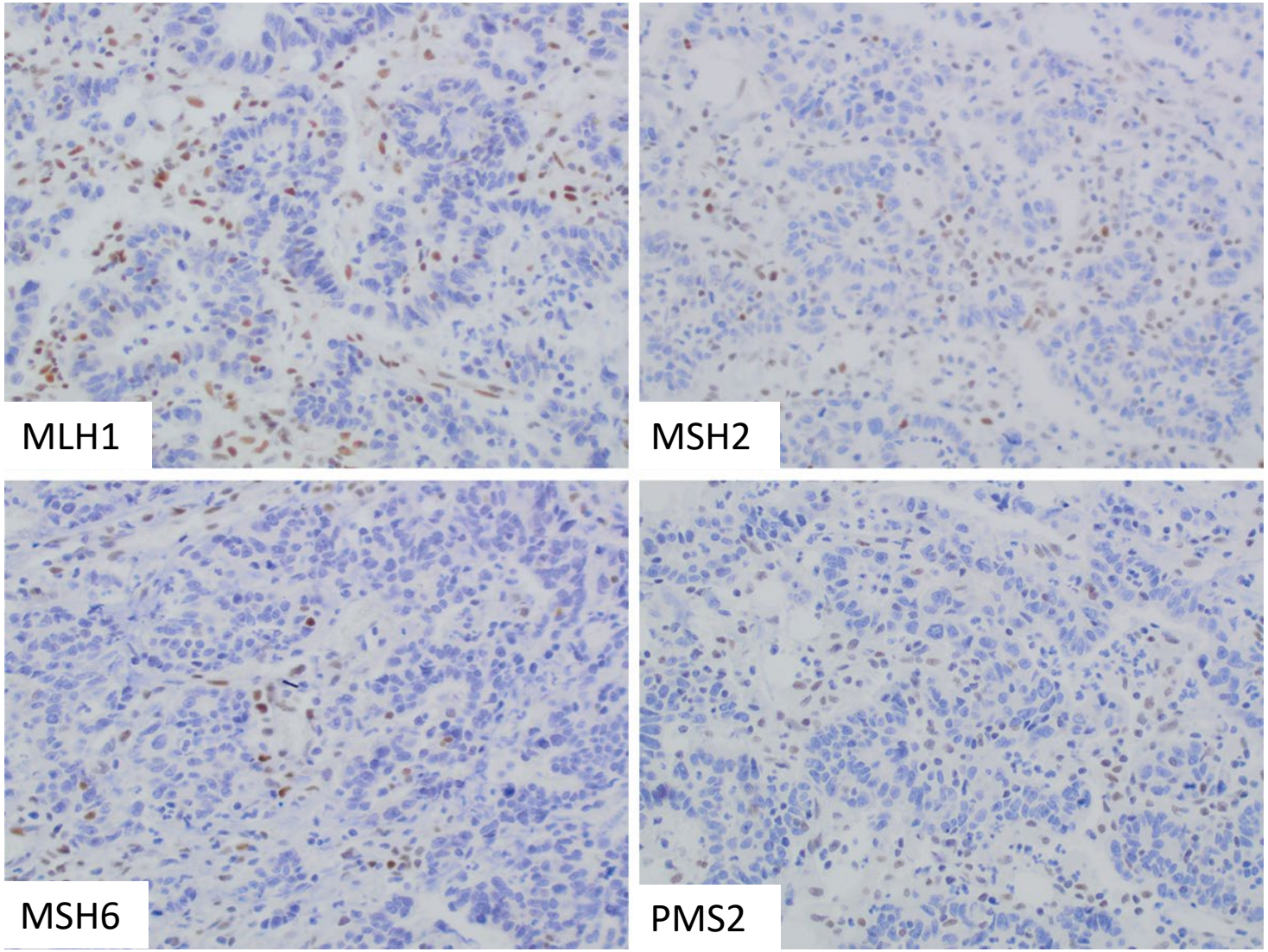
Tumor #2: Conventional Adenocarcinoma

Loss of MLH1 and/or PMS2, and minimal or absent MSH6

- Hypermethylation of *MLH1* causes MLH1/PMS2 loss
- Tumor subsequently develops somatic mutation in *MSH6*

Shia J, et al. Mod Pathol. 2013 Jan;26(1):131-8.

Null Pattern



- Germline mutation in *MSH2*
- Hypermethylation of *MLH1*

Hagen CE, et al. Am J Surg Pathol. 2011 Dec;35(12):1902-5.

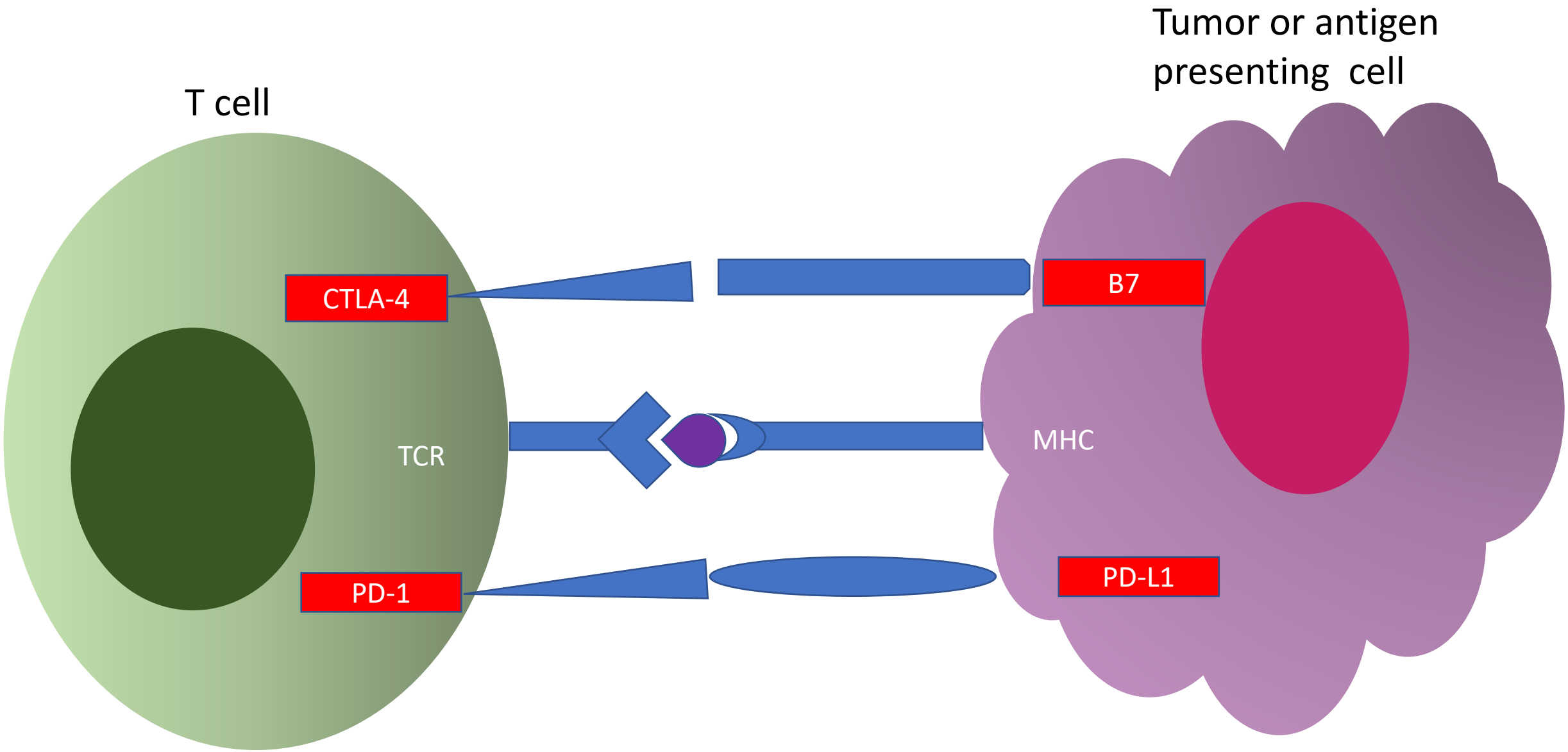
Take Home Points

- Universal Screening for MSI
  - Identifies families with Lynch syndrome
  - Is prognostic and predictive of response to some therapies for colorectal cancer patients
- Immunohistochemistry is sufficient in the vast majority of cases
  - It’s all about the internal control
- Have a low threshold for repeating stains when non-standard patterns occur
- PCR or NGS for confirmation or equivocal results

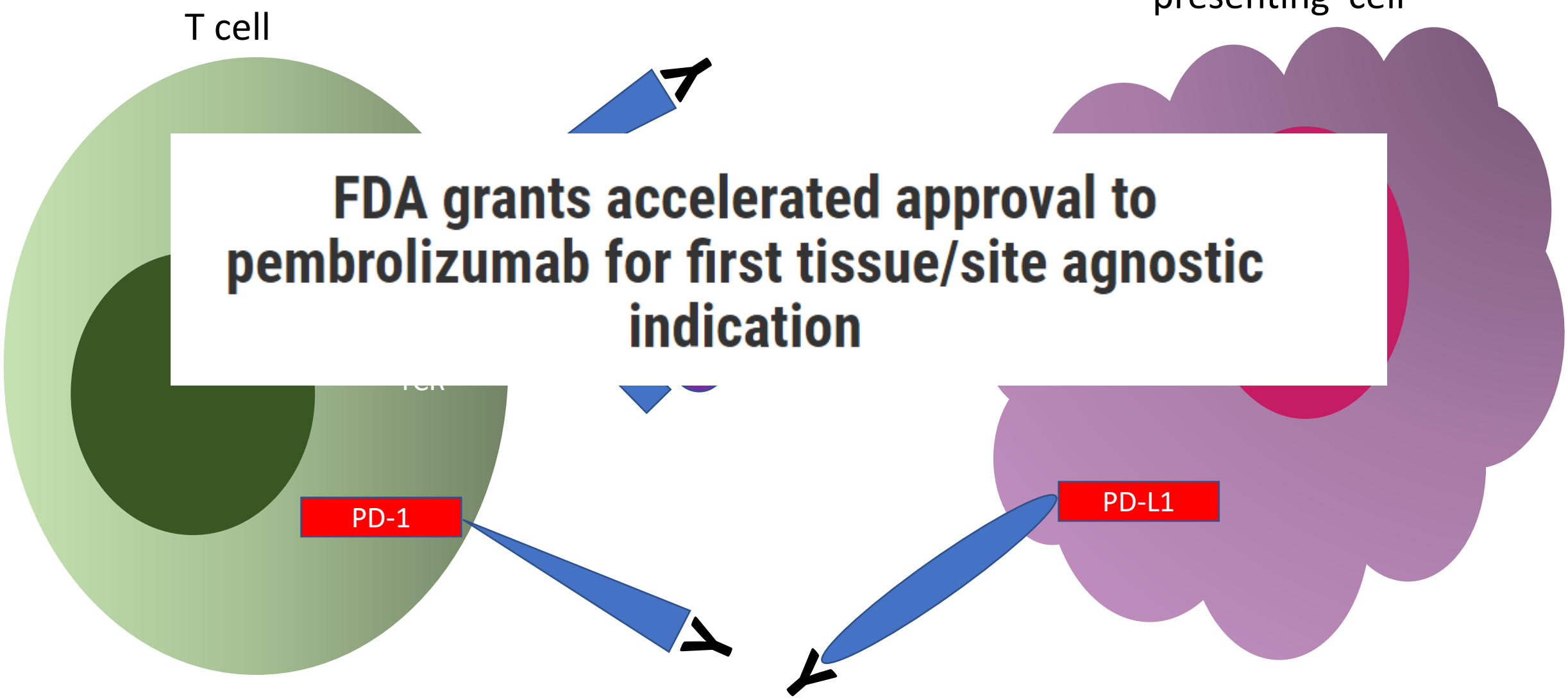
Immunohistochemical Biomarkers

- Mismatch repair (MMR) proficiency and deficiency
- **Checkpoint inhibitor therapy (PDL1)**
- Her2neu amplification

Immune Checkpoint Function



Immune Checkpoint Inhibition



FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication

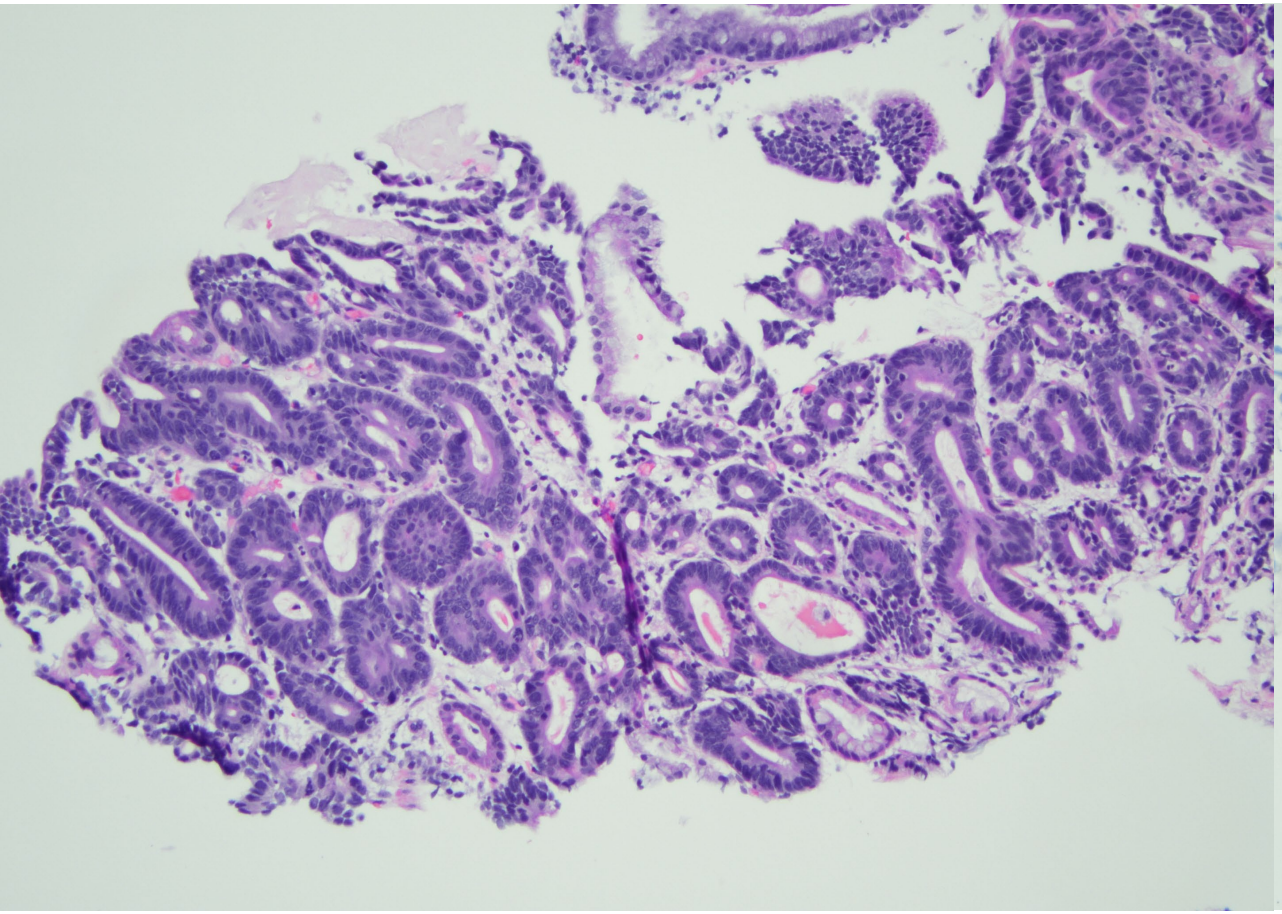
PD-L1 Immunohistochemistry: Gastric and Gastroesophageal Adenocarcinoma

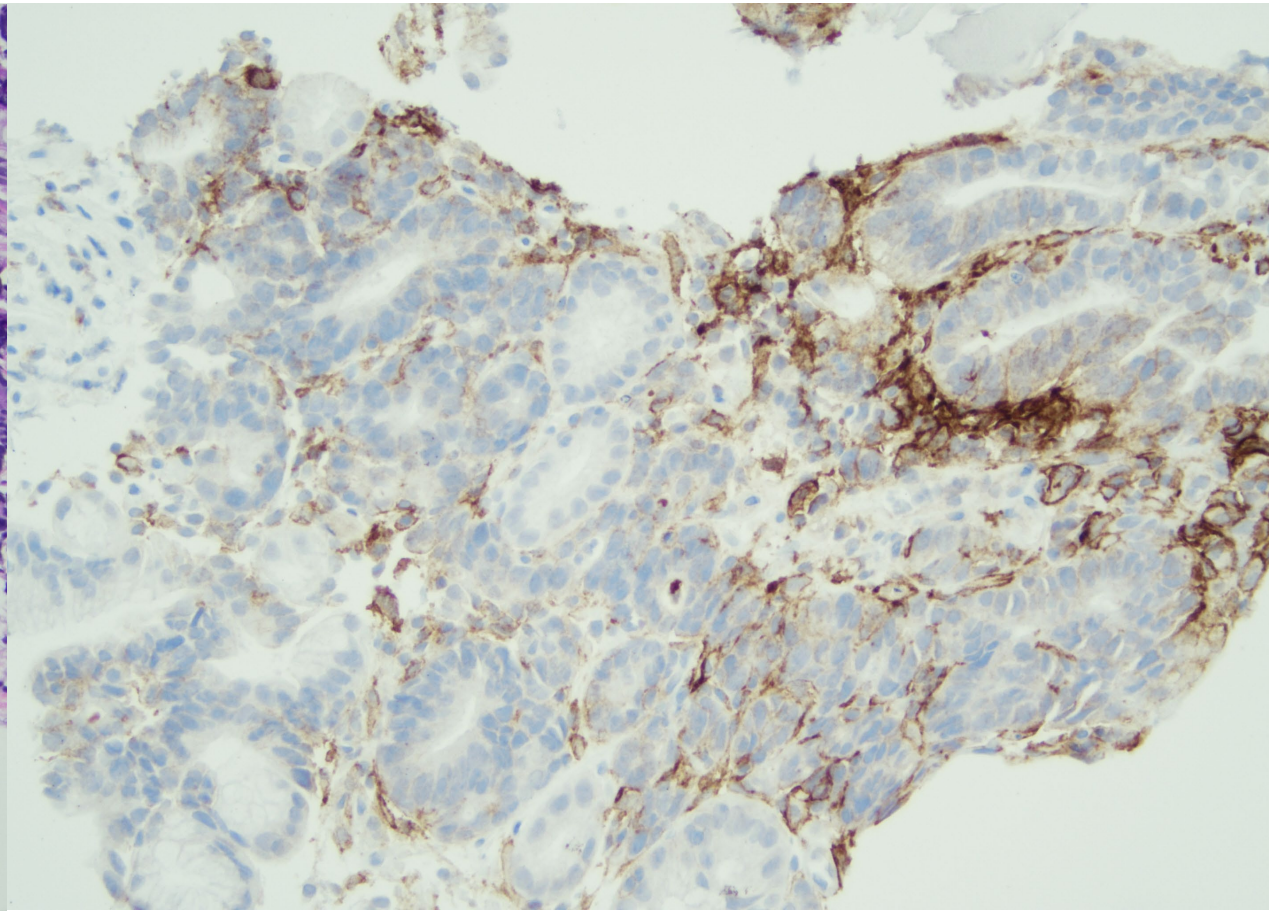
$$\text{Combined positive score (CPS)} = \frac{\text{PD-L1 positive tumor AND immune cells}}{\text{Total viable tumor cells}}$$

- Used for upper gastrointestinal cancers
- Cutoff >1 determines response to checkpoint inhibitor therapy
- Cutoff>10 recently proposed as more predictive of response to checkpoint inhibitor monotherapy

Kulangara K, et al. Arch Pathol Lab Med. 2019 Mar;143(3):330-337.
Wainberg ZA, et al. Clin Cancer Res. 2021 Apr 1;27(7):1923-1931.

CPS: PD-L1 Stain





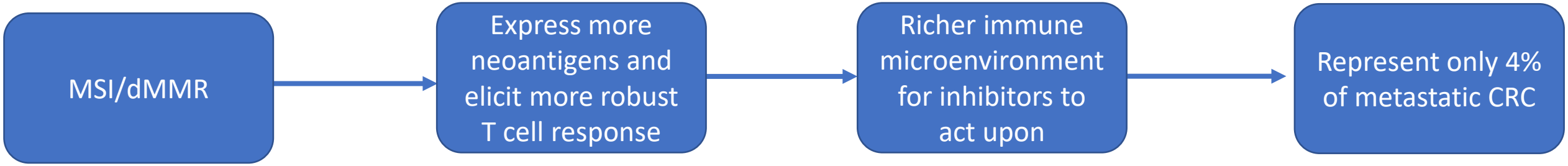
Checkpoint Inhibitor Therapy in Colon Cancer

		Response Rate		
Phase II Study	Agents	dMMR CRC	dMMR non-CRC	pMMR CRC
KEYNOTE 016	pembrolizumab	40%	78%	0%!!!!
CheckMate 142	nivolumab	31%	N/A	N/A
	Nivolumab + ipilimumab	55%	N/A	N/A

- Phase III trials ongoing

Le DT, et al. N Engl J Med. 2015;372:2509-2520.
Overman MJ, et al. Lancet Oncol. 2017 Sep;18(9):1182-1191.

Checkpoint Inhibitor Therapy in Colon Cancer



What about mismatch repair proficient (pMMR) CRC?

- Some may harbor mutations that stimulate T cell response (*TP53, KRAS*)
- Combination of traditional chemo and radiotherapy may expose tumor antigens that potentiates the effect of checkpoint inhibition
- Trials are ongoing

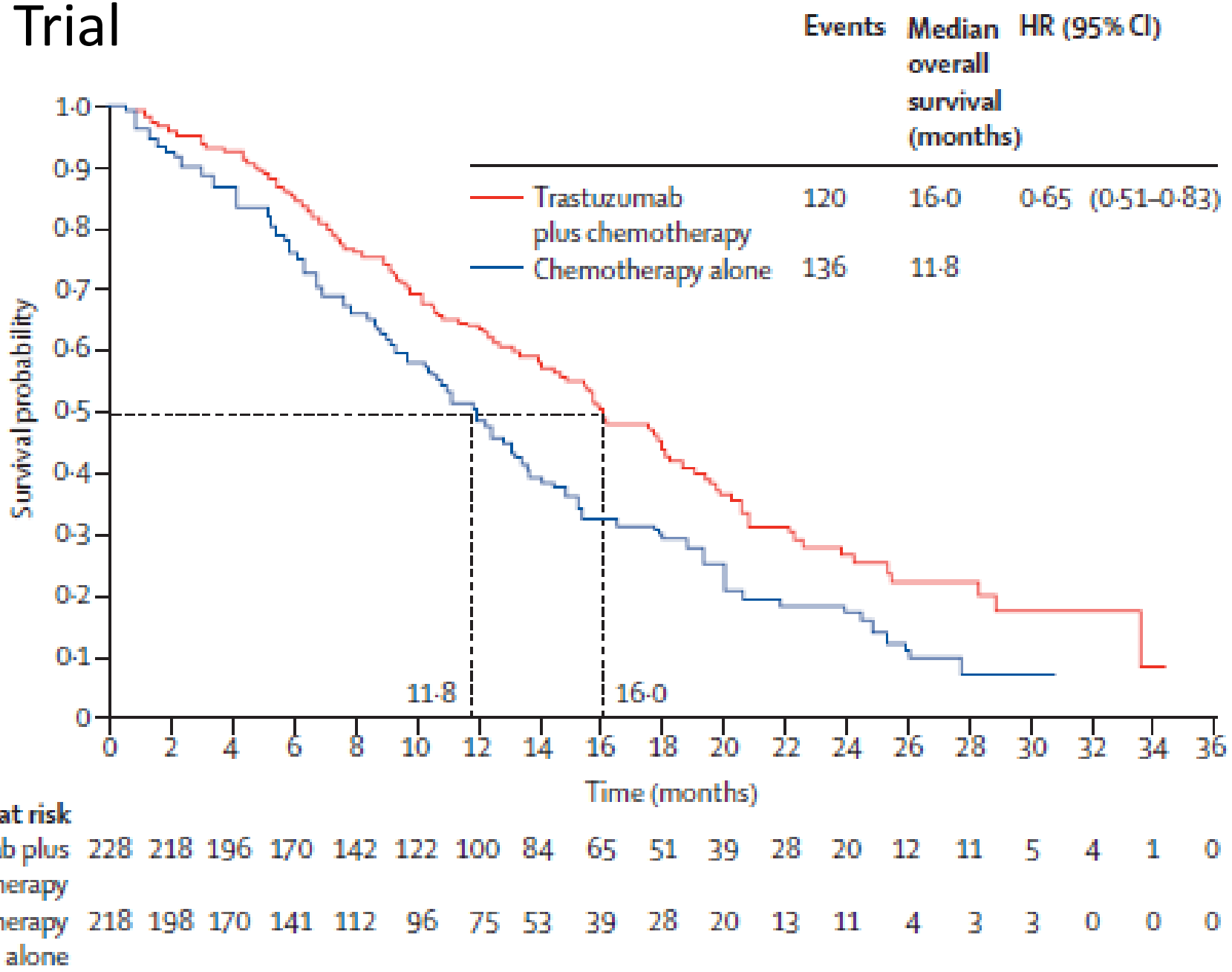
Take-Home Points

- Currently, the best marker of checkpoint inhibitor therapy response for colorectal cancer is dMMR

Immunohistochemical Biomarkers

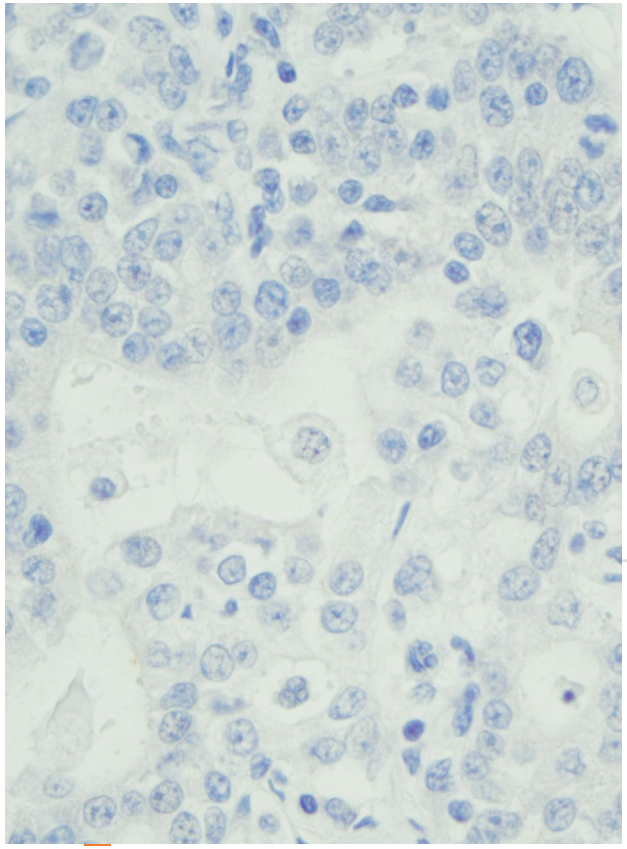
- Mismatch repair (MMR) proficiency and deficiency
- PDL1
- **Her2neu amplification**

ToGA Trial

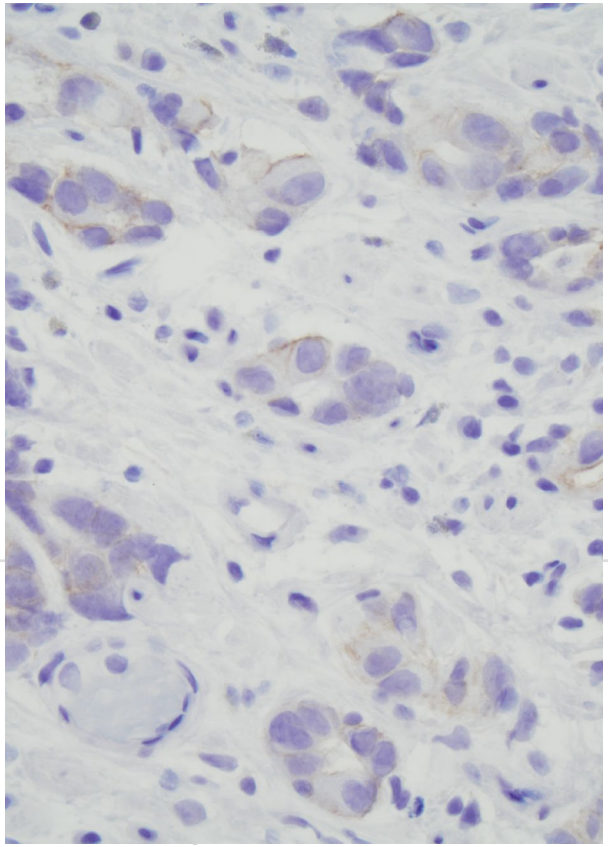


Bang YJ, et al Lancet. 2010 Aug 28;376(9742):687-97.

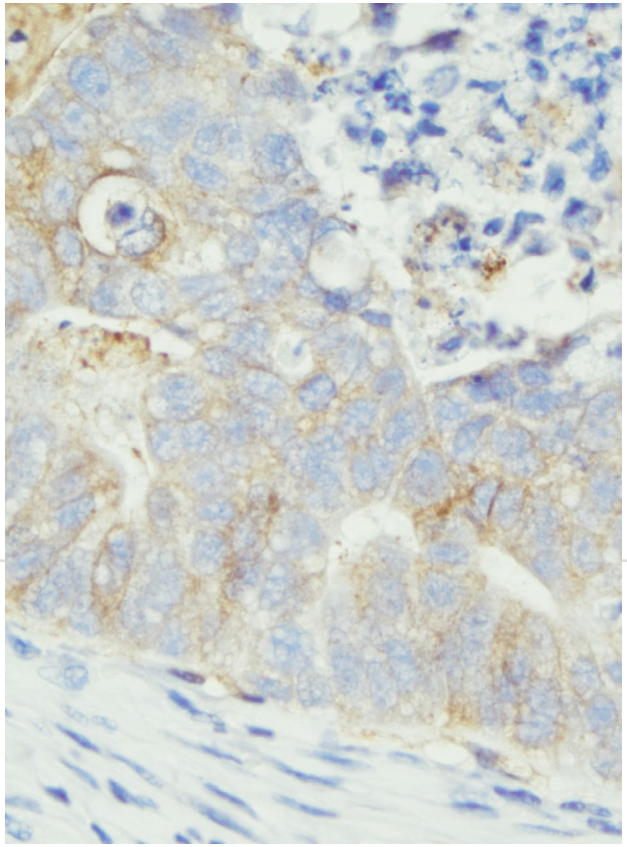
Gastric and Gastroesophageal Junction Cancer HER2 Scoring



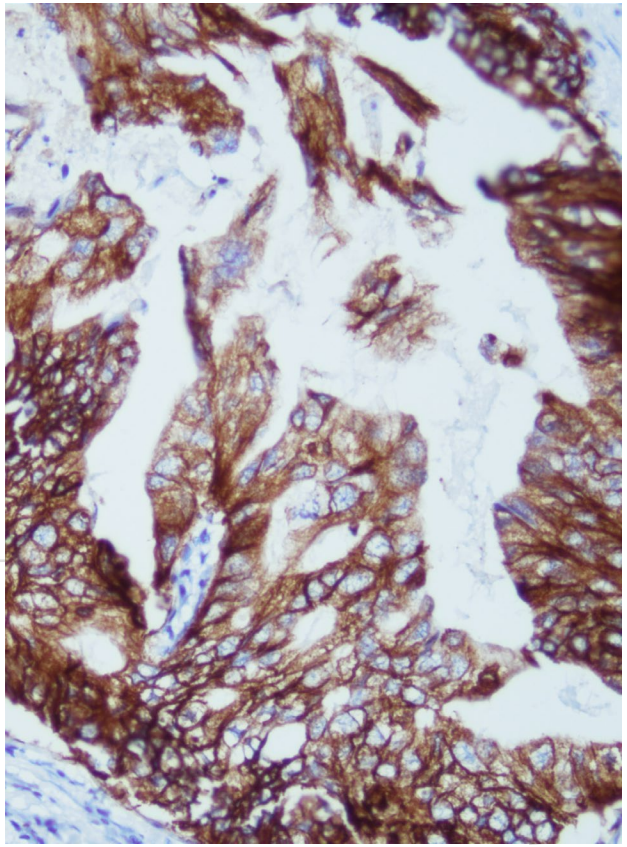
0: No staining



1+: Barely perceptible

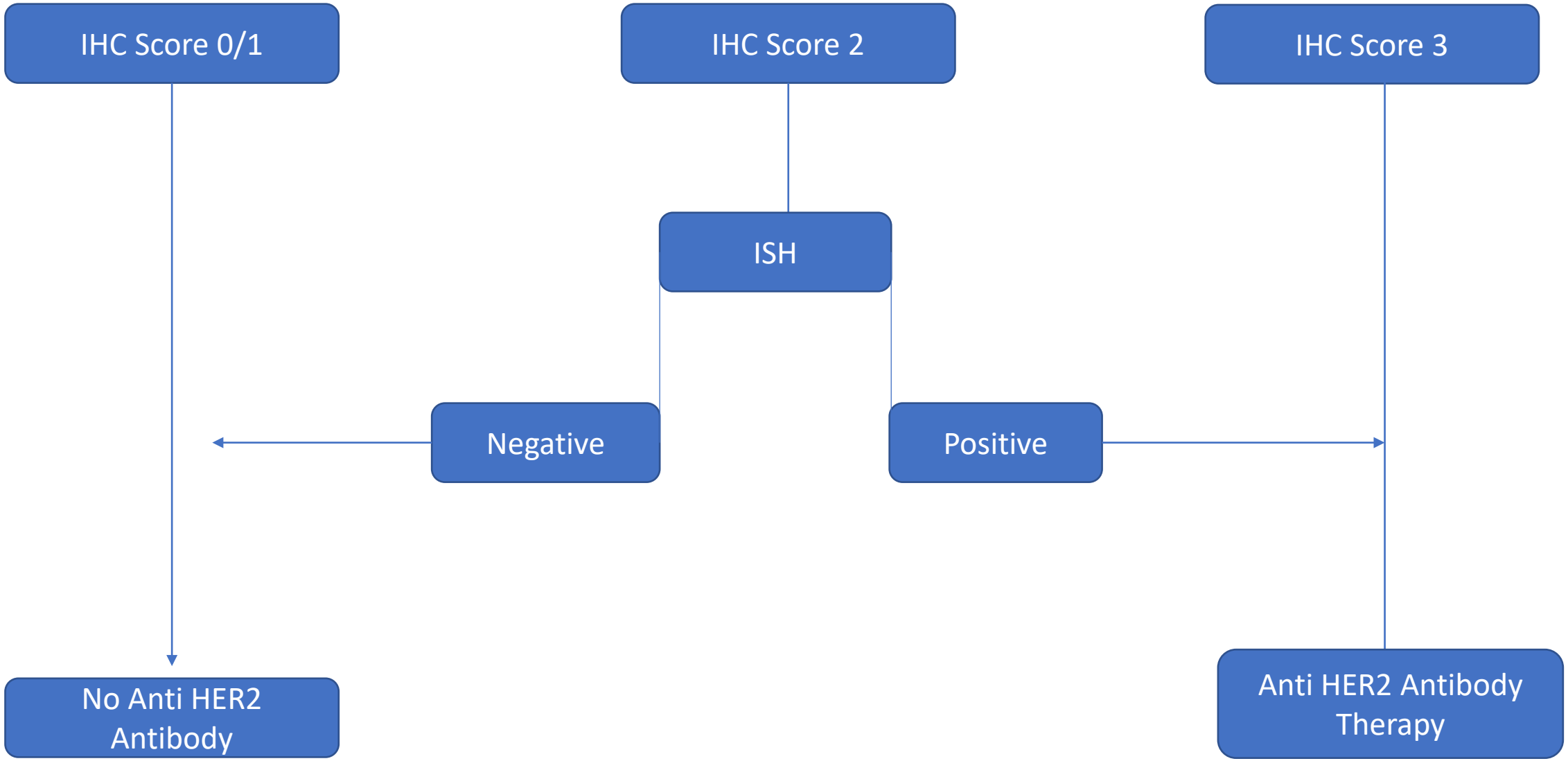


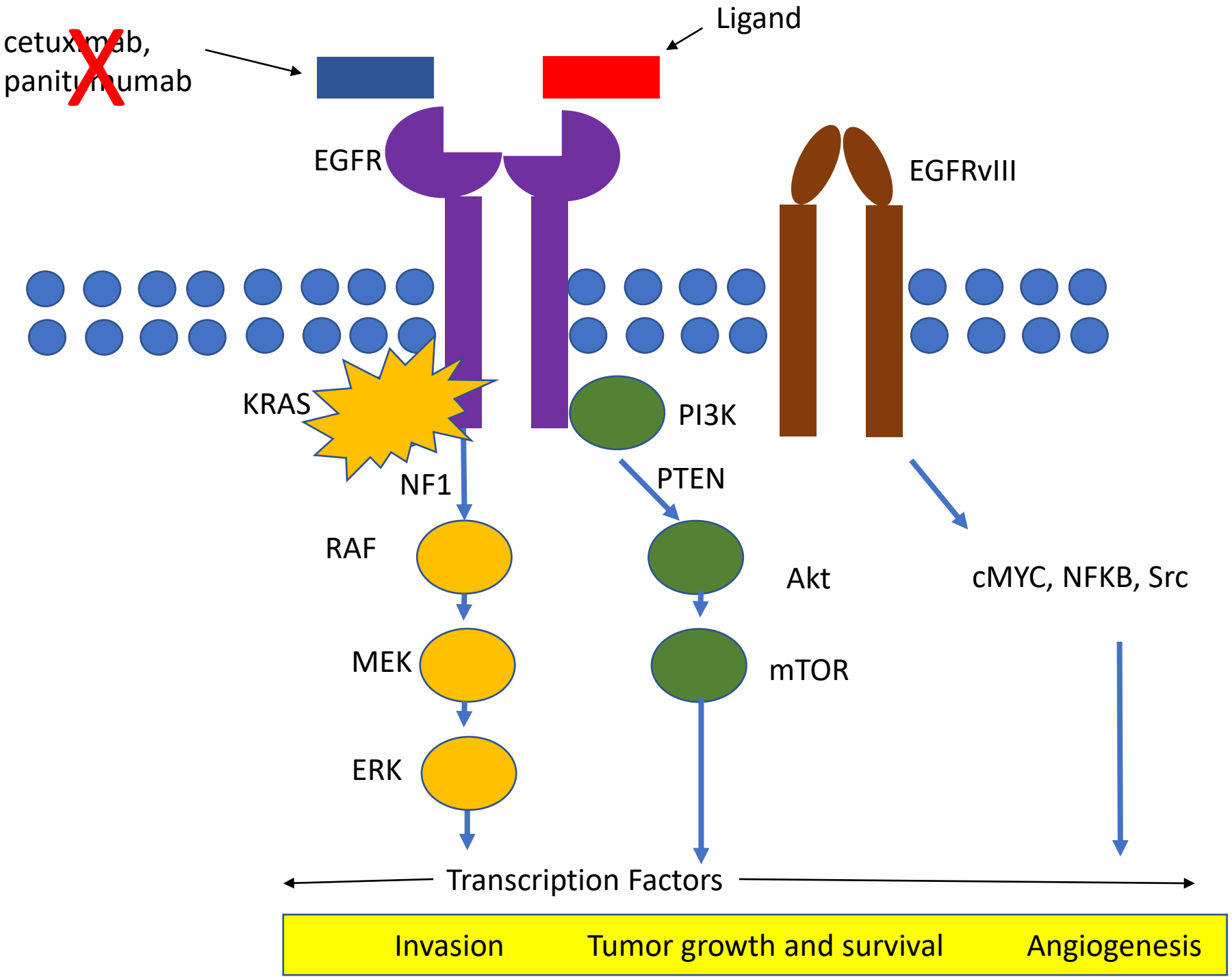
2+: Weak to moderate

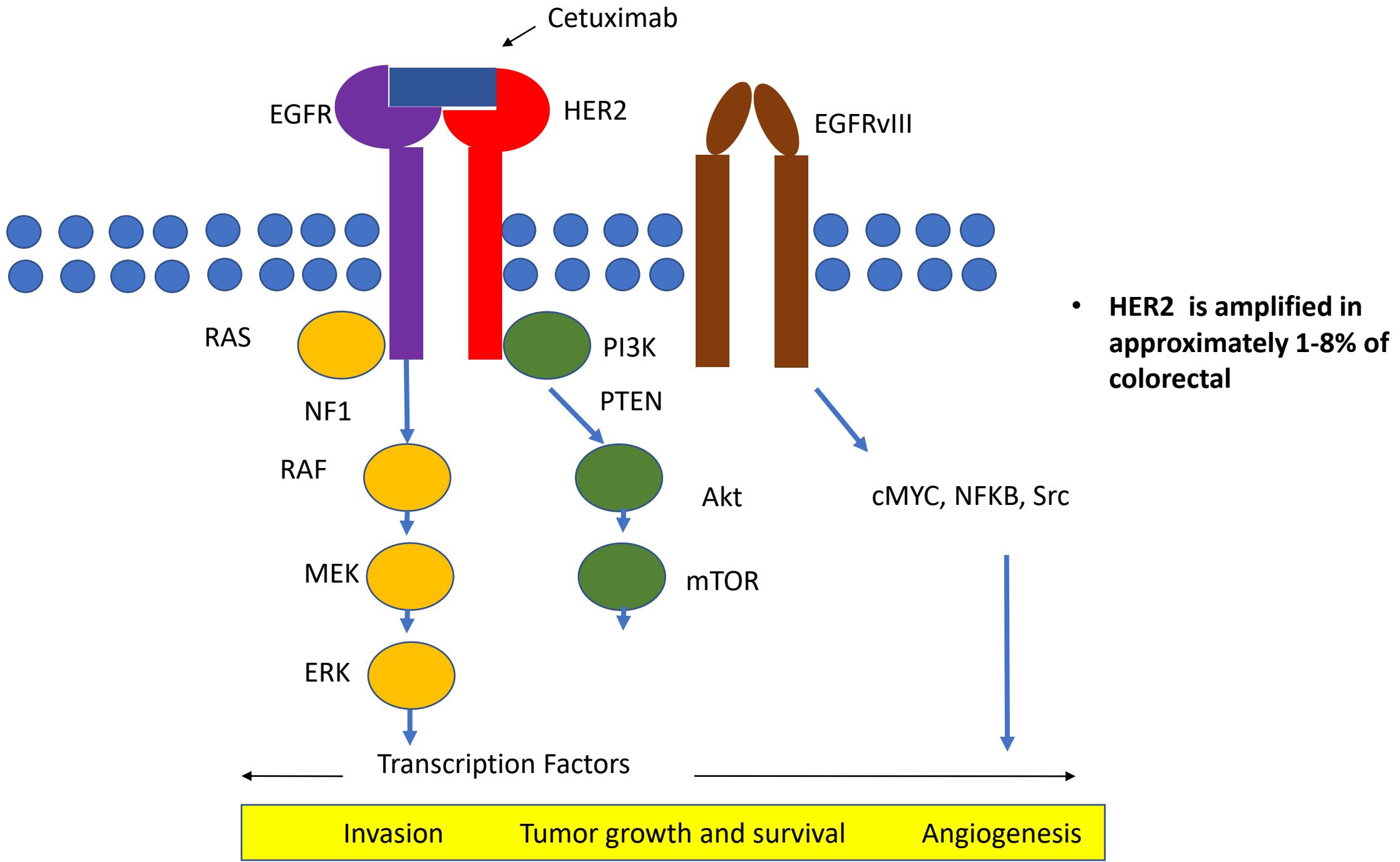


3+: Strong

Gastric and Gastroesophageal Junction Her2 Expression Testing Algorithm




cetuximab,
panitumumab
Ligand
EGFR
EGFRvIII
KRAS
PI3K
NF1
PTEN
RAF
Akt
cMYC, NFKB, Src
MEK
mTOR
ERK
Transcription Factors
Invasion
Tumor growth and survival
Angiogenesis



- **HER2 is amplified in approximately 1-8% of colorectal**

HERACLES (HER2 Amplification for Colo-rectaL cancer Enhanced Stratification)

- Patients with metastatic colorectal cancer
- Had progressed on standard therapies (inclusive of cetuximab and panitumumab)
- Trastuzumab – anti-Her2 antibody
- Lapitinib – dual tyrosine kinase inhibitor that blocks HER2/neu and EGFR

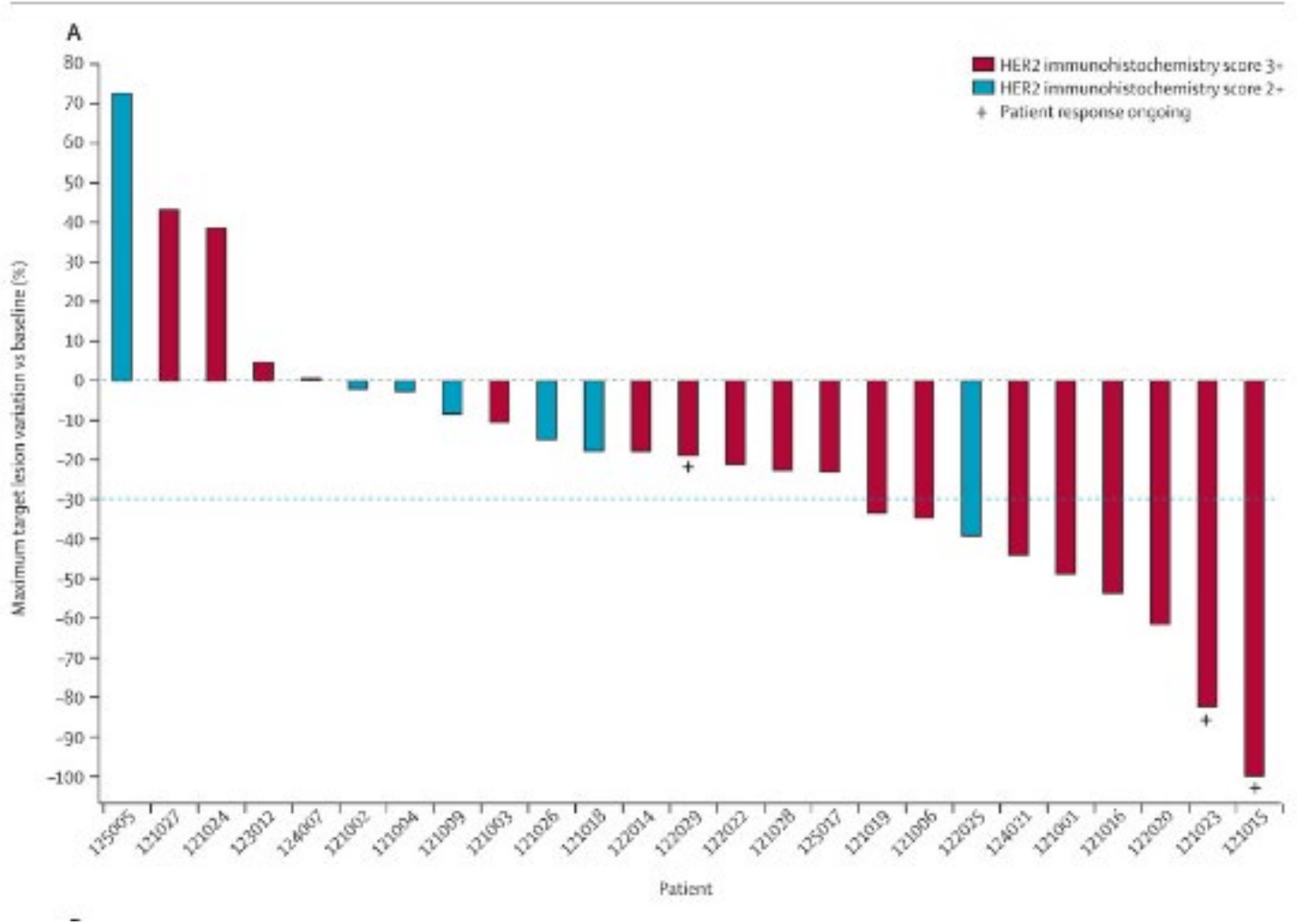
HERACLES (HER2 Amplification for Colo-rectaL cancer Enhanced Stratification)

Score	Staining Pattern
0	No staining
1+	Faint segmental or granular staining
2+	Moderate circumferential, basolateral, or lateral staining
3+	Intense circumferential, basolateral, or lateral staining

- HER2 positivity
  - 3+ immunostaining in >50% of cells
  - 3+ in ≥10, but <50% of cells and positive by FISH
  - 2+ in >50% of cells and positive by FISH

Valtorta E, et al. Mod Pathol. 2015 Nov;28(11):1481-91.

HERACLES Results



- 27 patients
  - 1 complete response
  - 7 partial response
  - 12 stable disease
  - Duration of response at least 16 weeks

Sartore-Bianchi A, et al. Lancet Oncol. 2016 Jun;17(6):738-746.

MyPathway

- HER2 positivity was defined as any of the following:
- HER2 IHC 3+ in >10 percent of cells
- *HER2*:CEP17 ratio ≥2.0 or *HER2* count >6 per cell
- Increased *HER2* gene copy number by molecular methods
- *HER2* activating mutations
- 57 HER2-positive advanced colorectal cancer patients
- Eighteen patients achieved an objective response, including one complete response and 17 partial responses; seven additional patients had stable disease for greater than four months

Meric-Bernstam F, et al. *Lancet Oncol.* 2019;20(4):518–530.

Take-Home Points

- Mismatch repair proteins
  - Control is the KEY
- Checkpoint inhibitor (PDL1)
  - Mismatch repair currently the best marker
- Her2neu amplification
  - Promising target
  - No uniformly agreed upon IHC criteria
  - Criteria may differ from upper gastrointestinal tract
  - May employ multiple modalities in conjunction with IHC

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