Colorectal Carcinoma
AJCC 8th Edition Updates & Ancillary Theranostic Testing

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UPMC LIFE CHANGING MEDICINE

Session Objectives

AJCC 8th Edition / CAP Cancer Protocol Updates

- pT3 versus pT4a.
- Importance of identifying venous invasion.
- Tumor deposits vs. lymph node metastasis.
- Peritumoral tumor budding and malignant polyps.
- Isolated tumor cells & micrometastasis in lymph nodes.
- Treatment effect after neoadjuvant therapy.

Ancillary Biomarker Testing

- 2017 CAP/ASCO/ASCP/AMP guidelines on biomarker testing.
- Emerging biomarkers in colorectal carcinoma.

pT4a versus pT3

AJCC 8th Edition and CAP definition of pT4a:

- Tumor present at the serosal surface with inflammatory reaction, mesothelial hyperplasia, and/or erosion/ulceration.
- Free tumor cells on the serosal surface with underlying ulceration of the visceral peritoneum.
- ***Tumor with perforation in which the tumor cells are continuous with the serosal surface through inflammation.***

NCCN High risk factors for stage II (node-negative) colon cancers

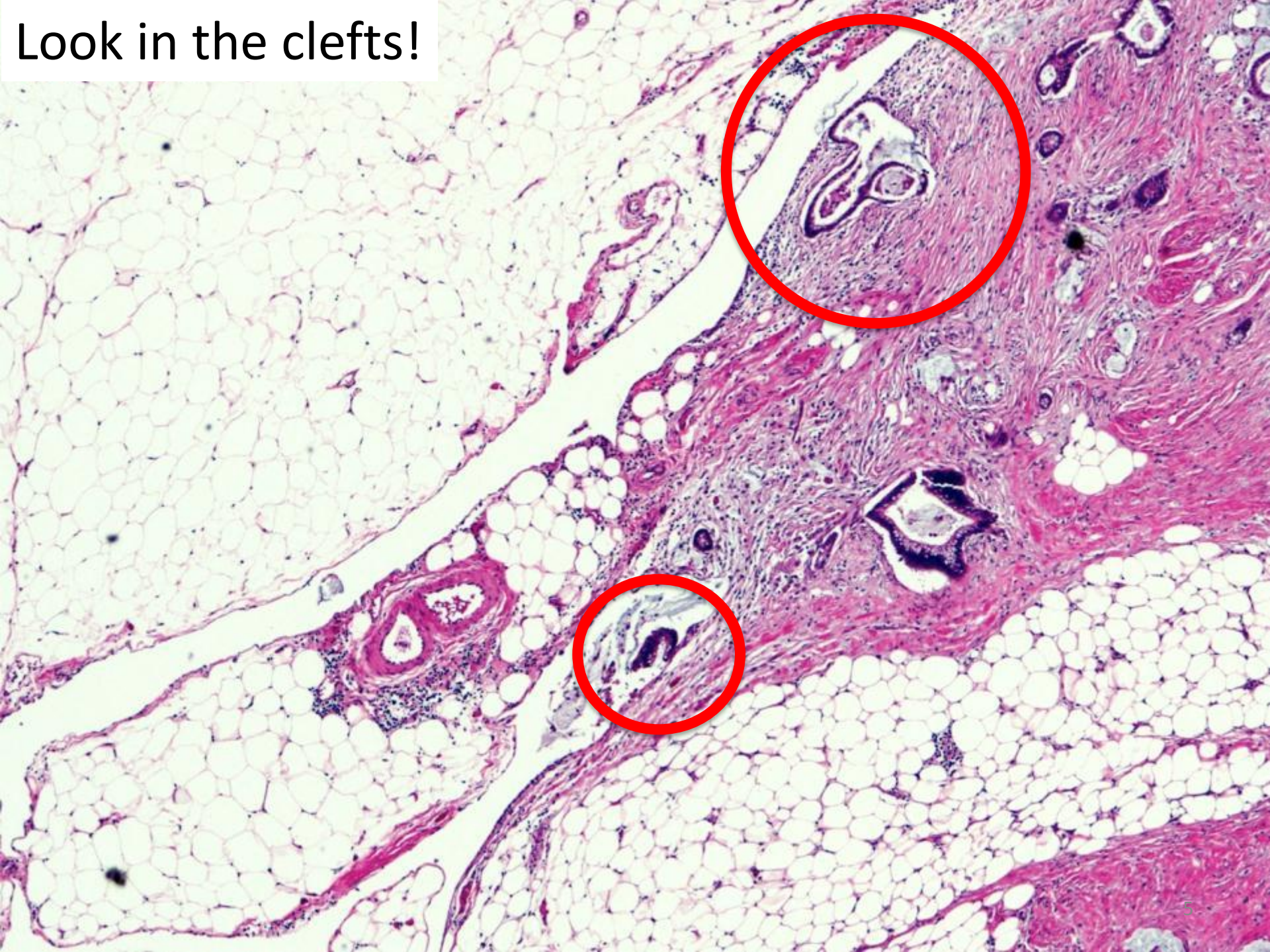
High risk factors

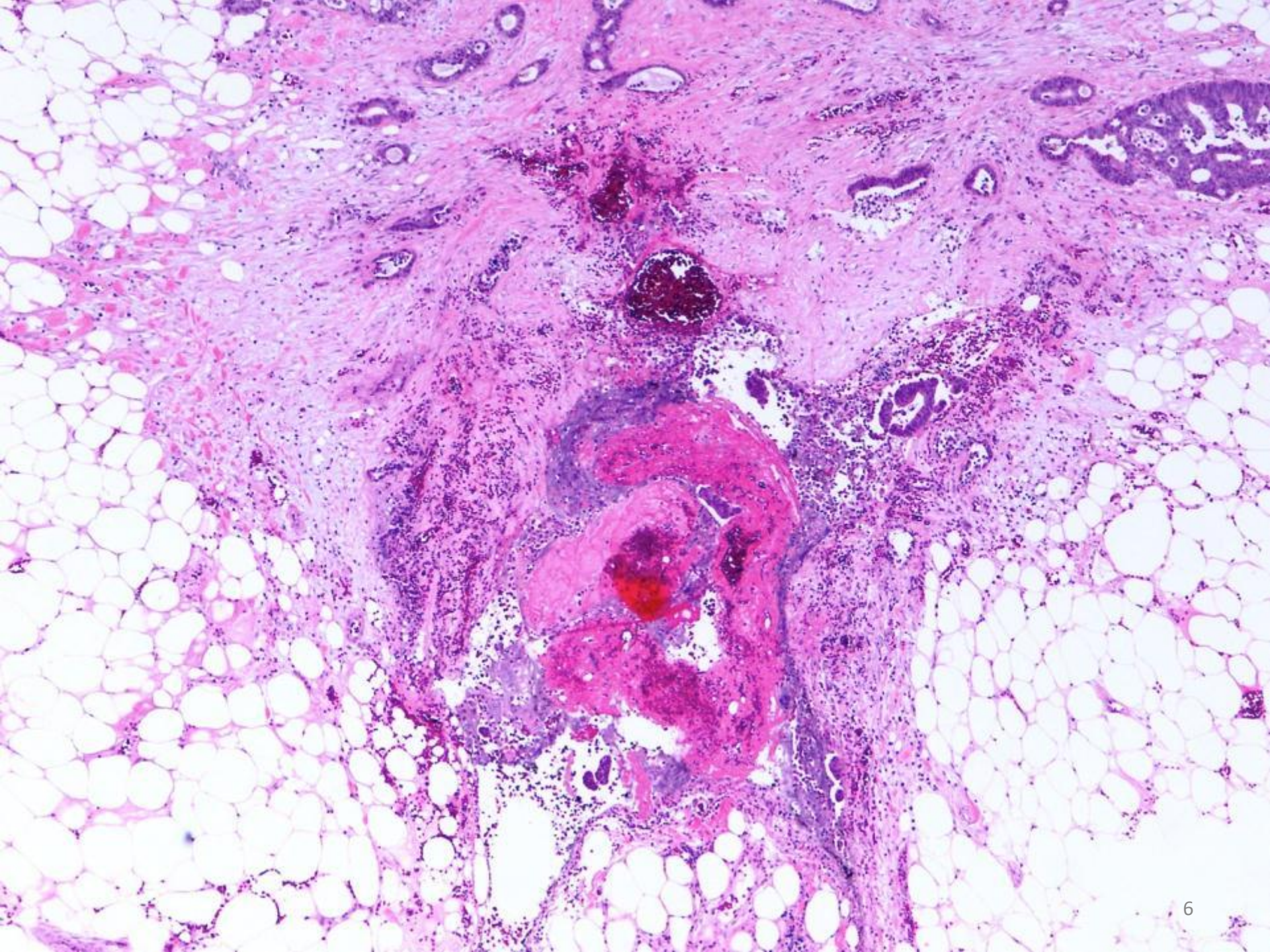
- **Poor differentiation (except MSI-high)**
- **Lymphovascular invasion**
- Bowel obstruction
- **<12 lymph nodes**
- **Perineural invasion**
- **Localized perforation (pT4)**
- **Close/indeterminate margins**

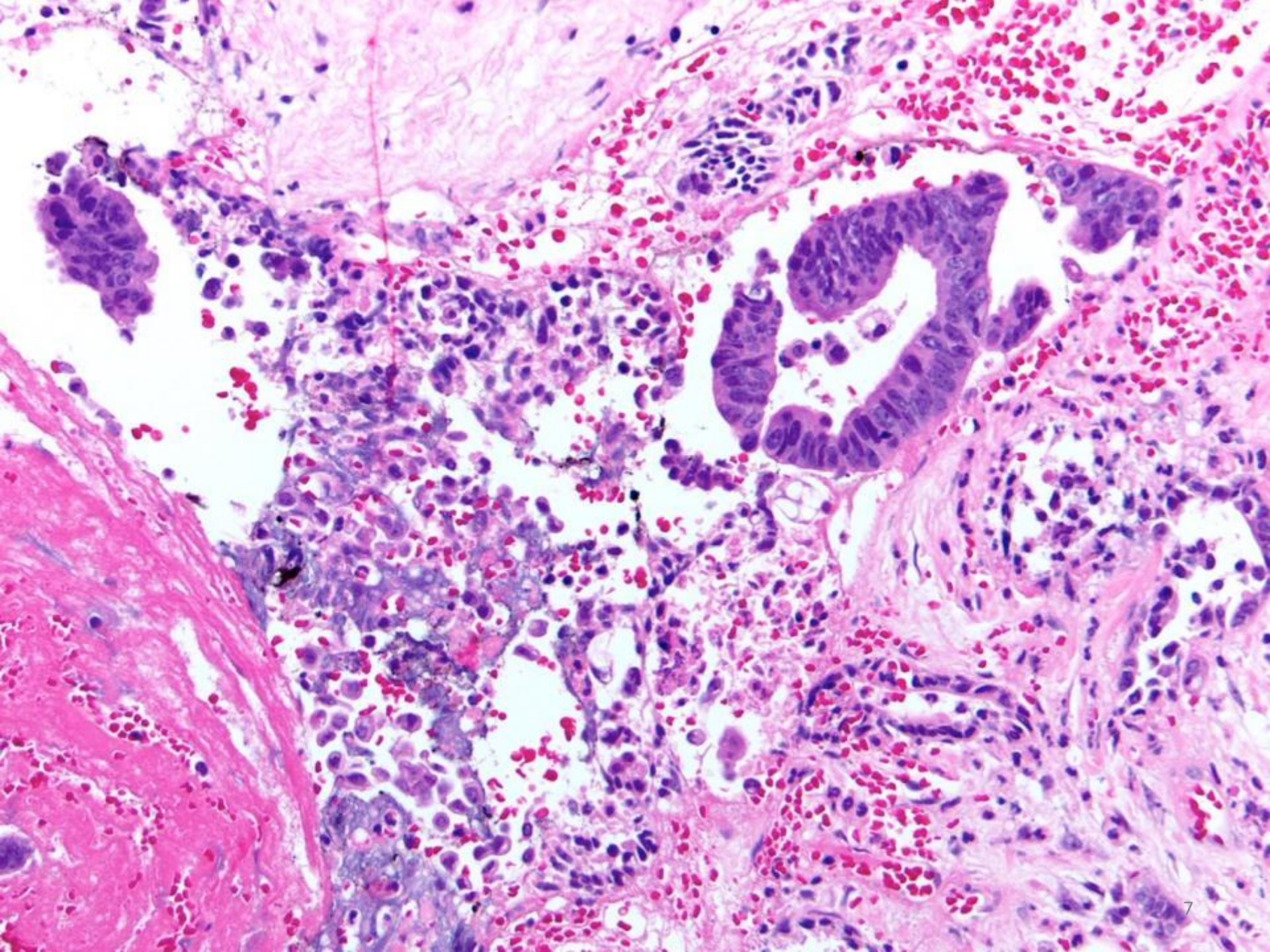
pT4a in stage II cancers

- Associated with decreased overall survival and risk of peritoneal dissemination.
- Patients with pT4a tumors will receive adjuvant chemotherapy.
- Likely underdiagnosed (~20% of pT3 are likely pT4a).
- In Europe, >20% pT4a is a quality metric (rate of pT4a is likely ~30-40% of resected colonic carcinomas).

Look in the clefts!

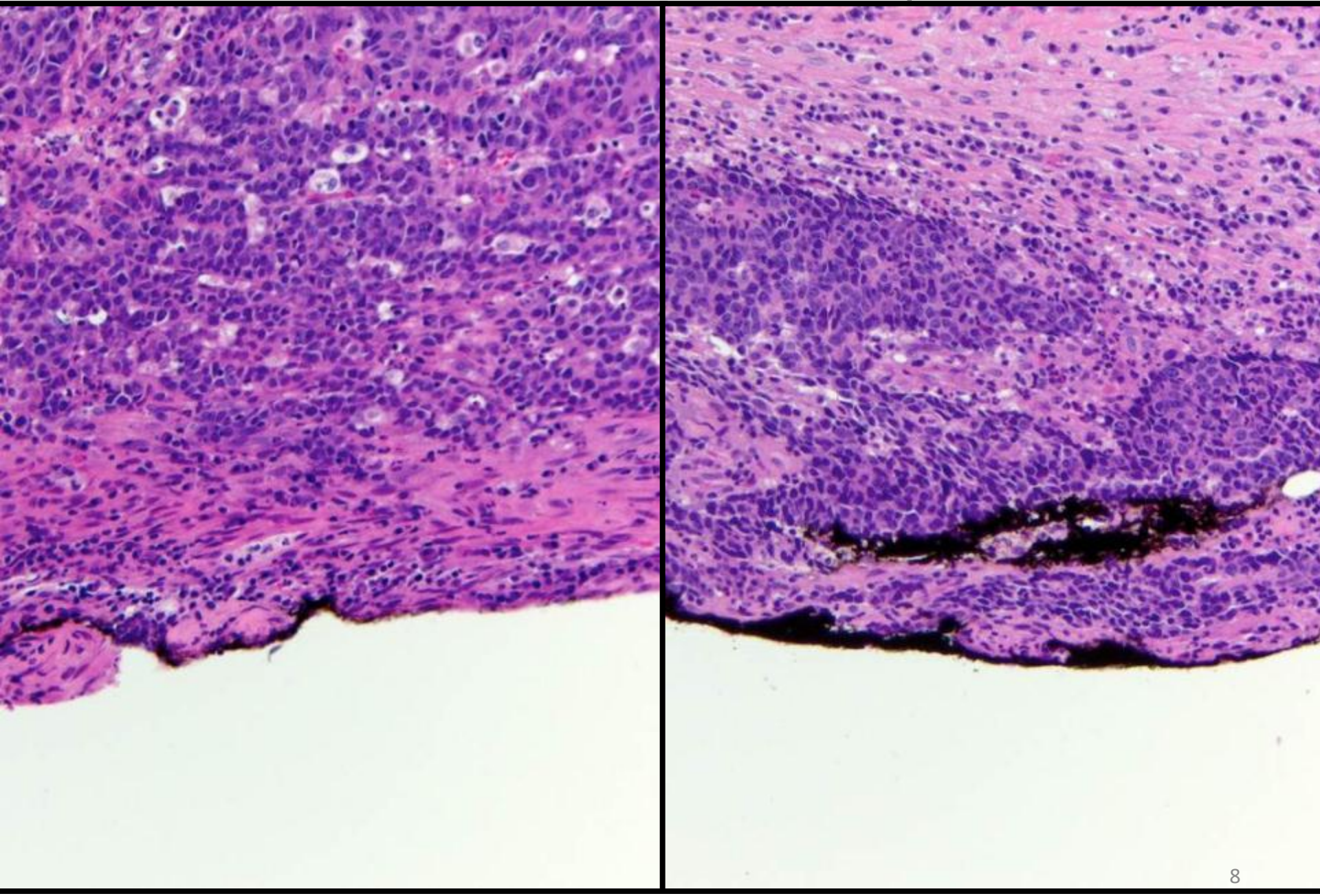


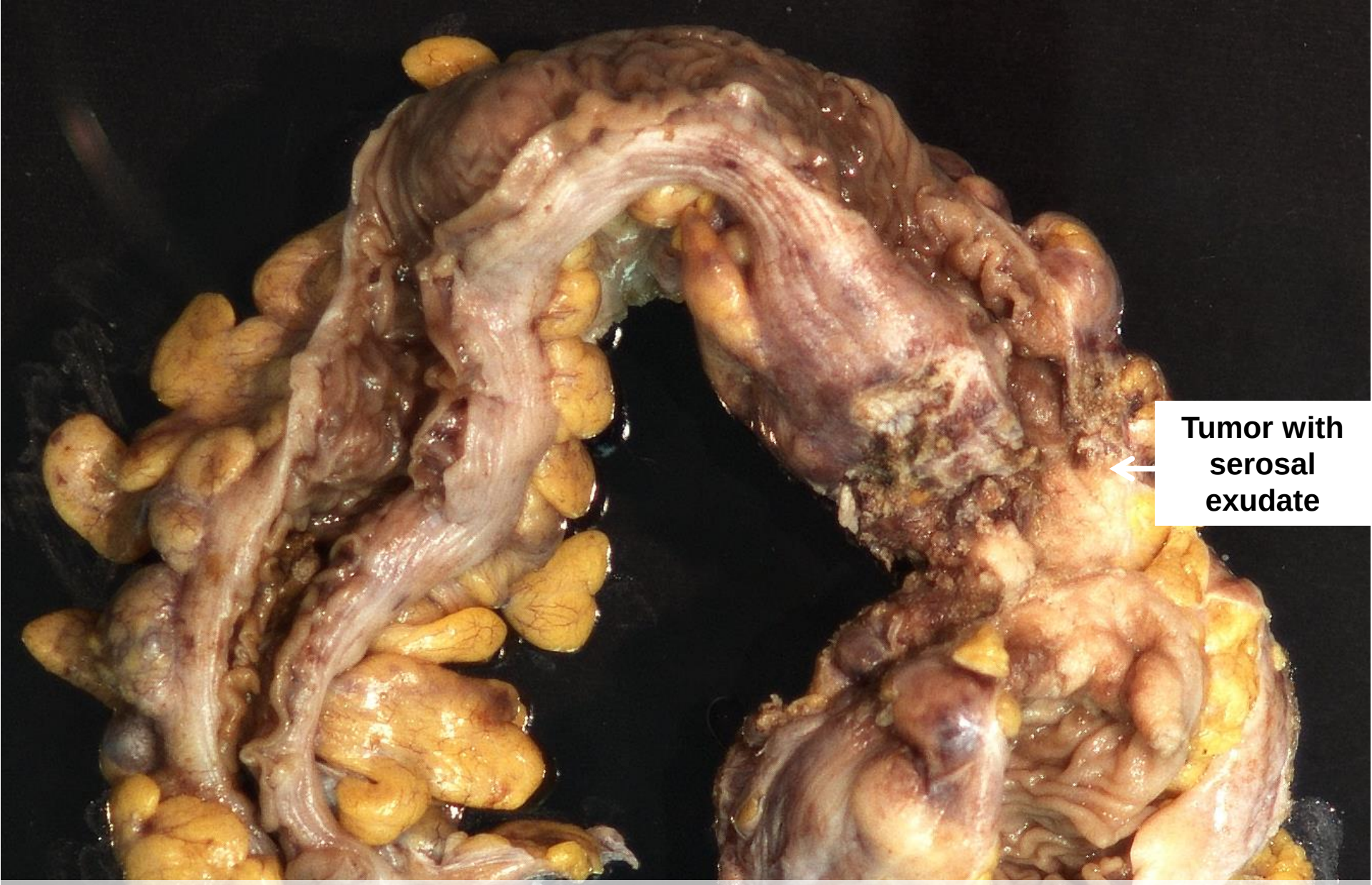




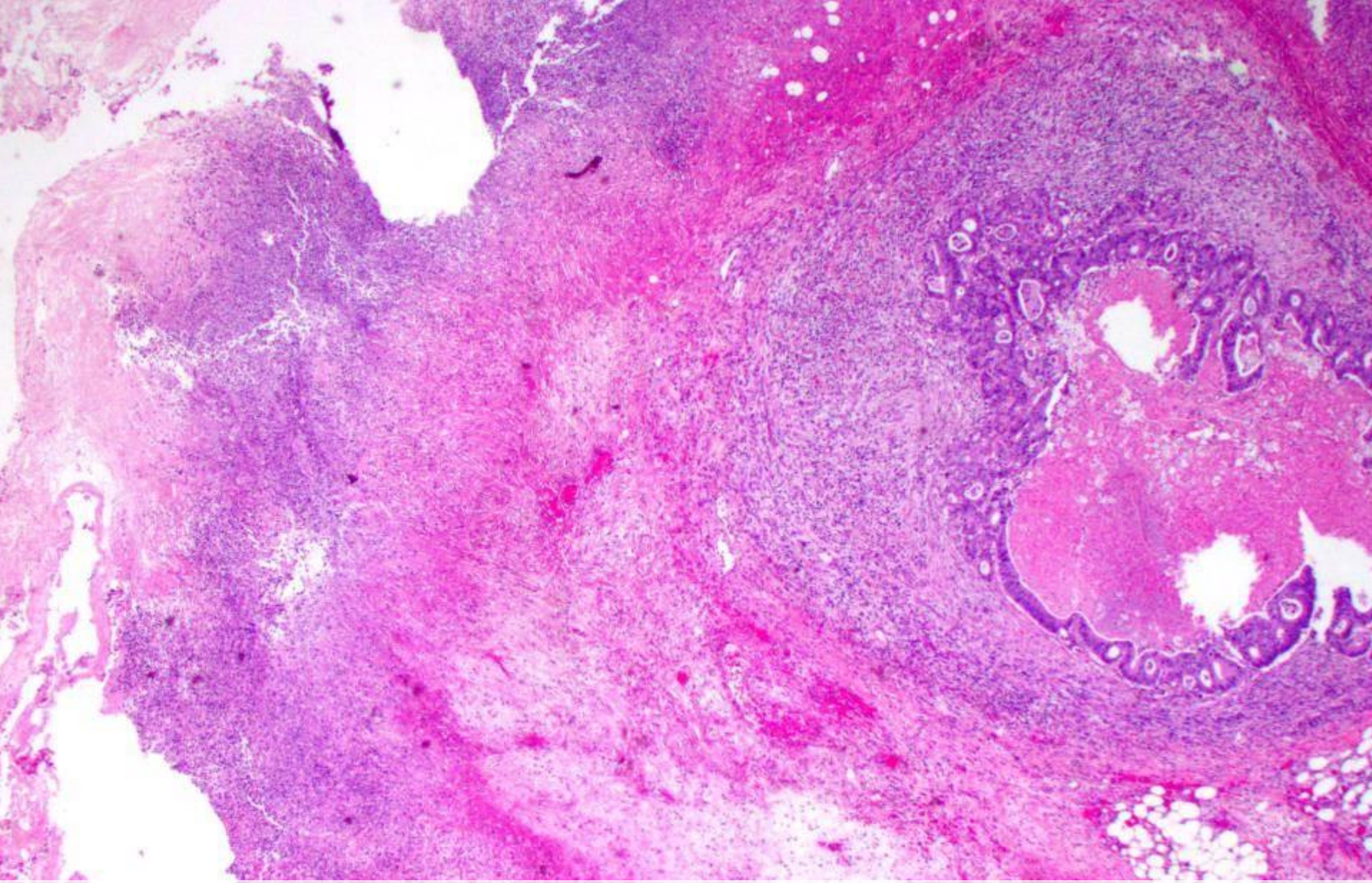
Original section

Deeper section





pT4a: Tumor w/ perforation where the tumor cells are continuous w/ the serosal surface through inflammation.



pT4a: Tumor w/ perforation where the tumor cells are continuous w/ the serosal surface through inflammation.

CAP template: pT4a versus pT3

- CAP definition of pT4a
  - Tumor present at the serosal surface with inflammatory reaction, mesothelial hyperplasia, and/or erosion/ulceration.
  - Free tumor cells on the serosal surface with underlying ulceration of the visceral peritoneum.
  - Tumor with perforation in which the tumor cells are continuous with the serosal surface through inflammation.

- ***What about tumors close to the serosal surface (< 1mm) with serosal reaction? pT3 or pT4a?***

Tumor ≤ 1mm with reaction

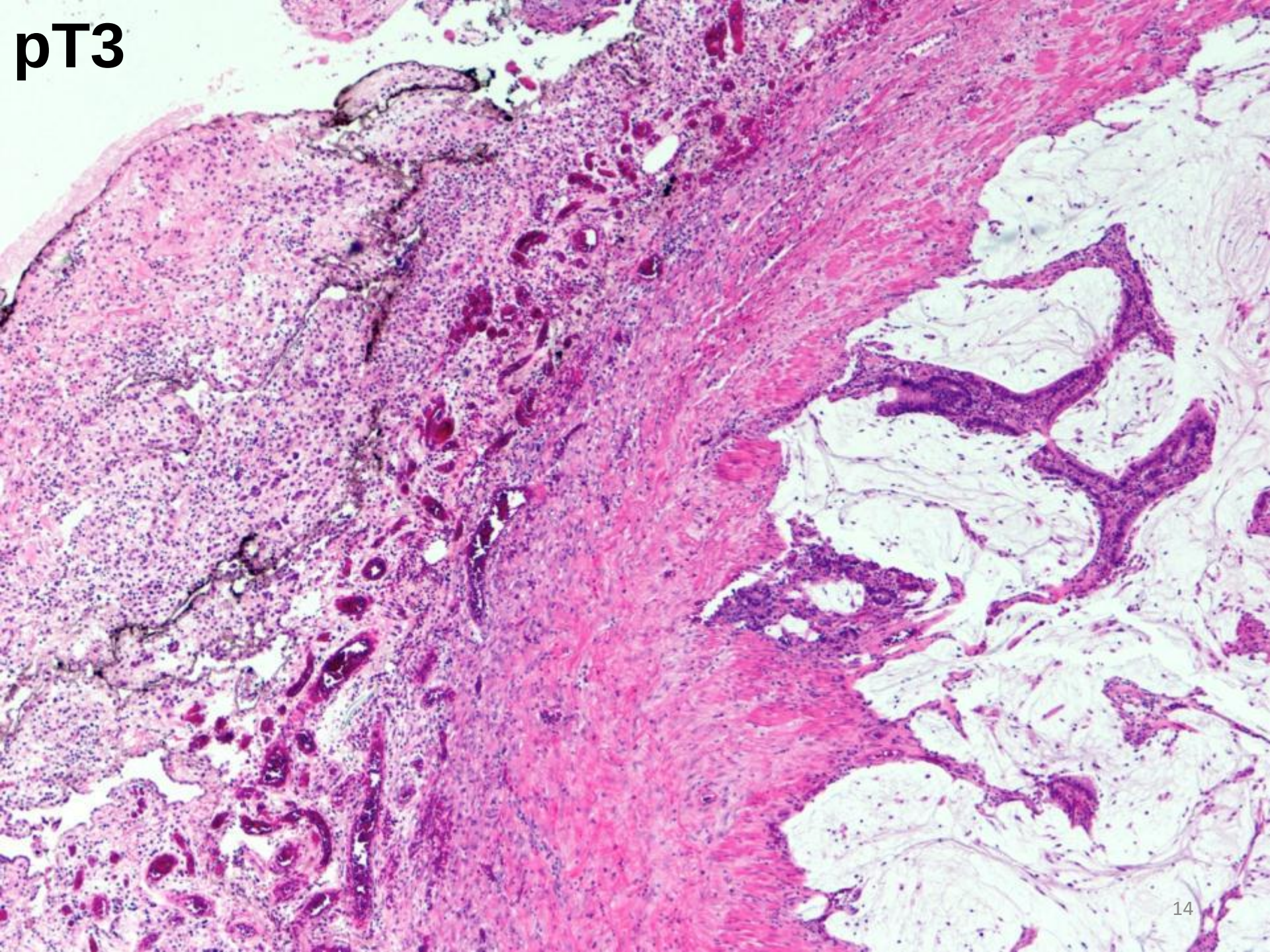
- pT3 > 1mm from serosal surface (n = 39)
  - None had serosal reaction.
  - None had tumor in serosal scrapings.
  - None had peritoneal recurrence.
- pT3 ≤ 1mm from serosal surface (n = 28)
  - 100% had some sort of serosal reaction
  - 13 (46%) had tumor in serosal scrapings
  - 11% had peritoneal recurrence
- pT4a based on CAP (n = 33)
  - 100% had serosal reaction
  - 15 (55%) had tumor in serosal scrapings.
  - 18% had peritoneal recurrence.

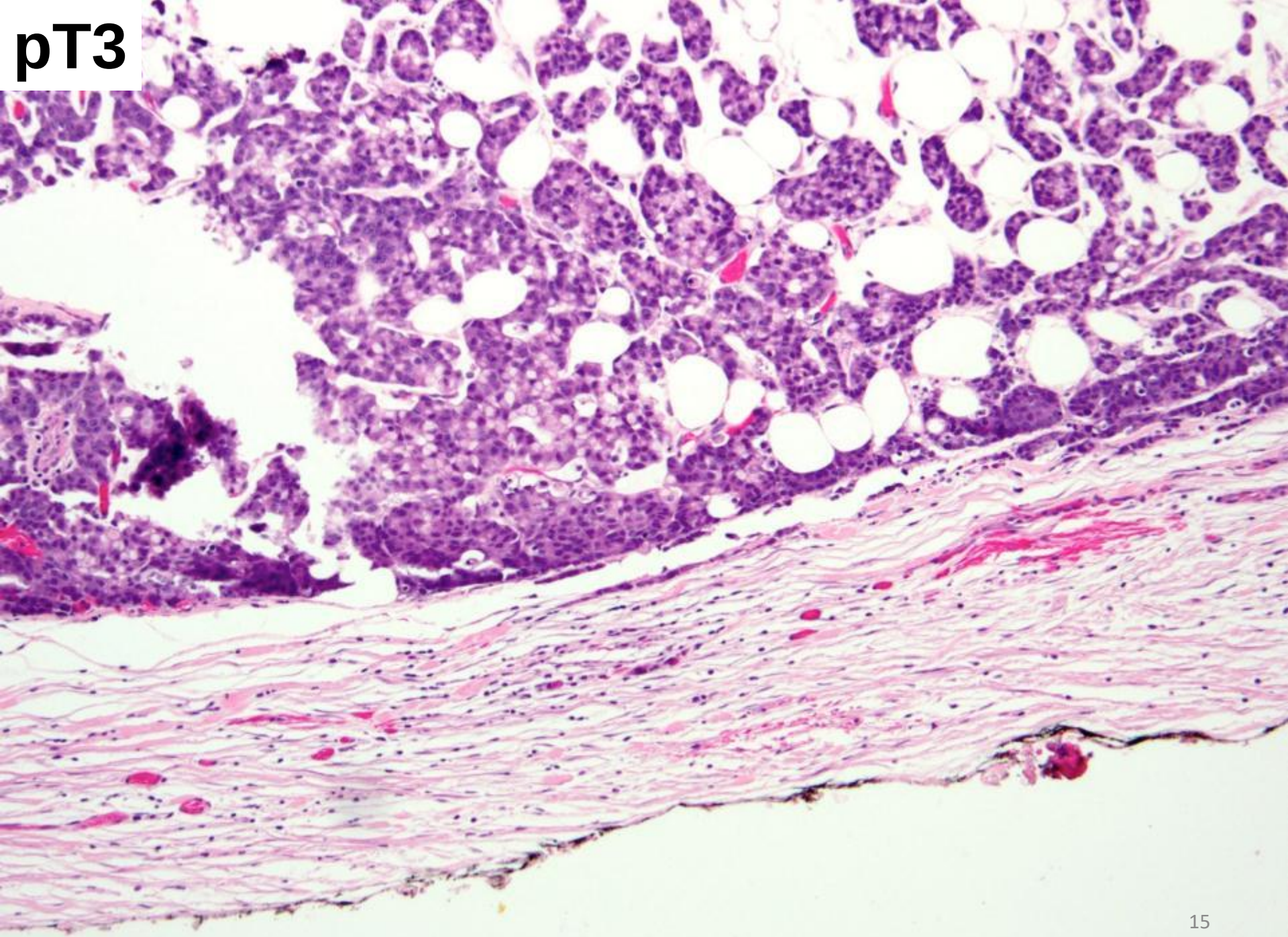
Panarelli, NC, et al. Am J Surg Pathol. 2013;37:1252-1258

Tumor ≤ 1mm with reaction: UPMC Approach

- If that reaction consists of a small rind of fibrosis, I still label as pT3.

- If only fibrin and inflammatory cells separates tumor from the serosal surface, I label as pT4a.


pT3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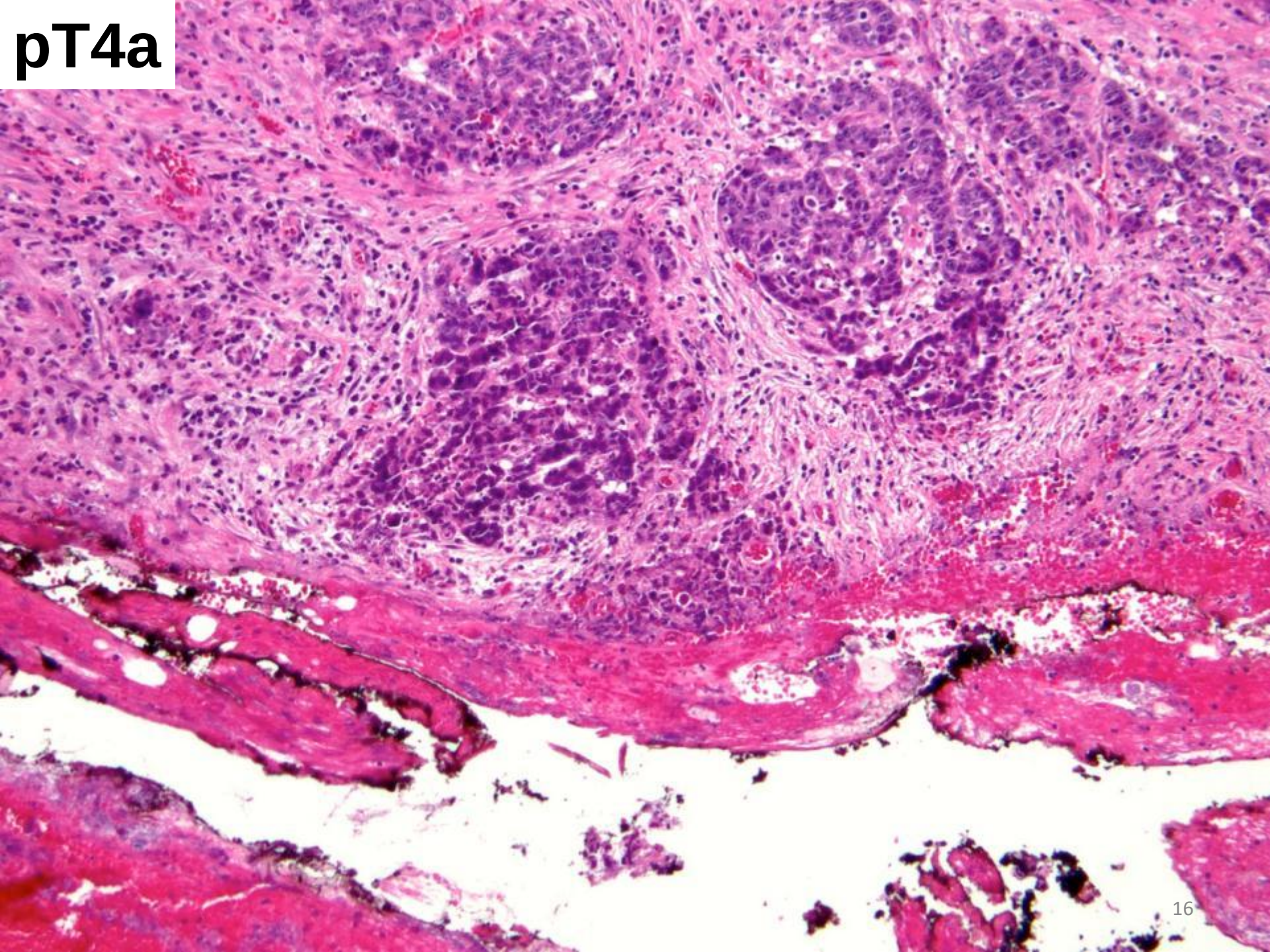
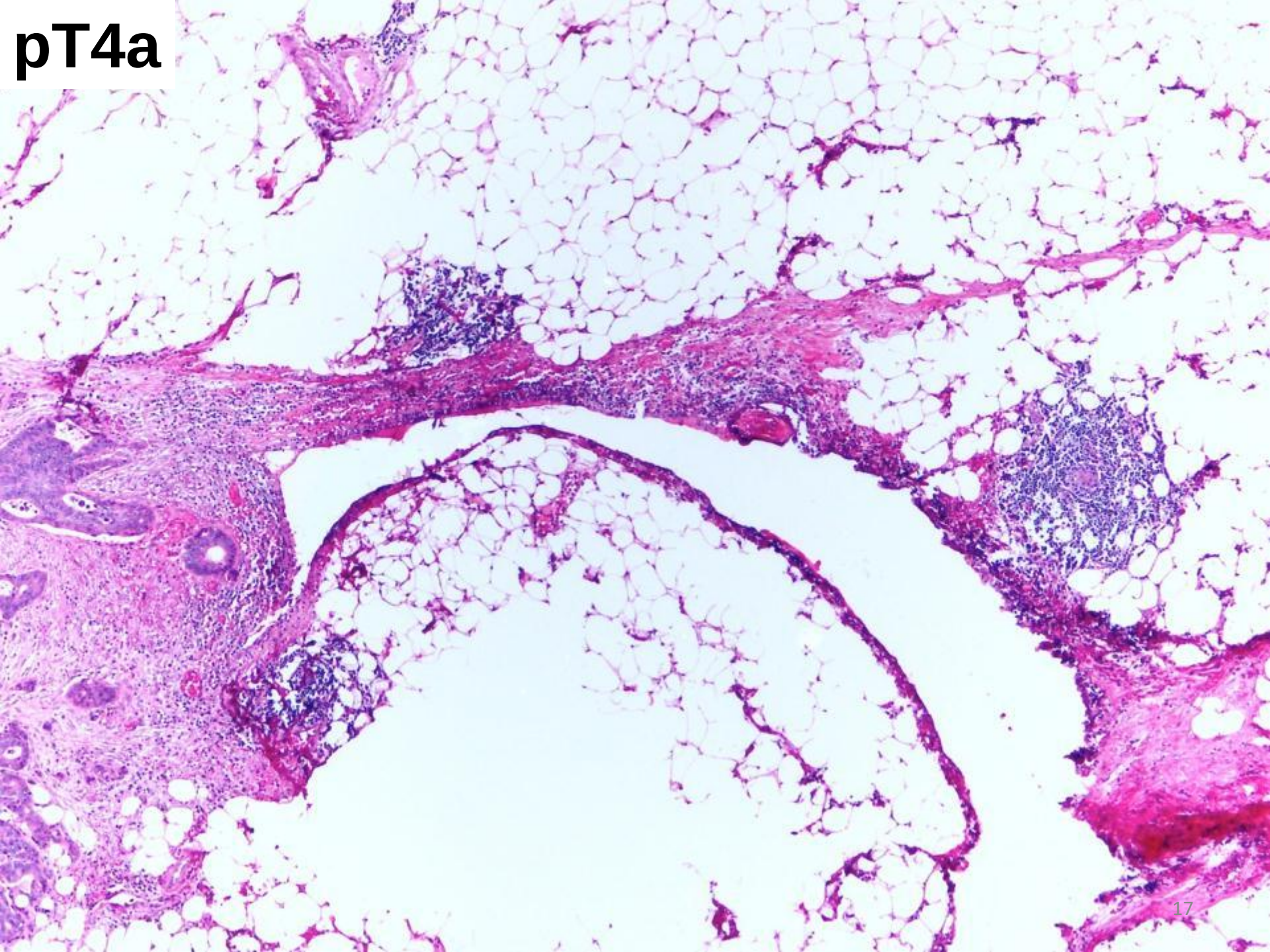
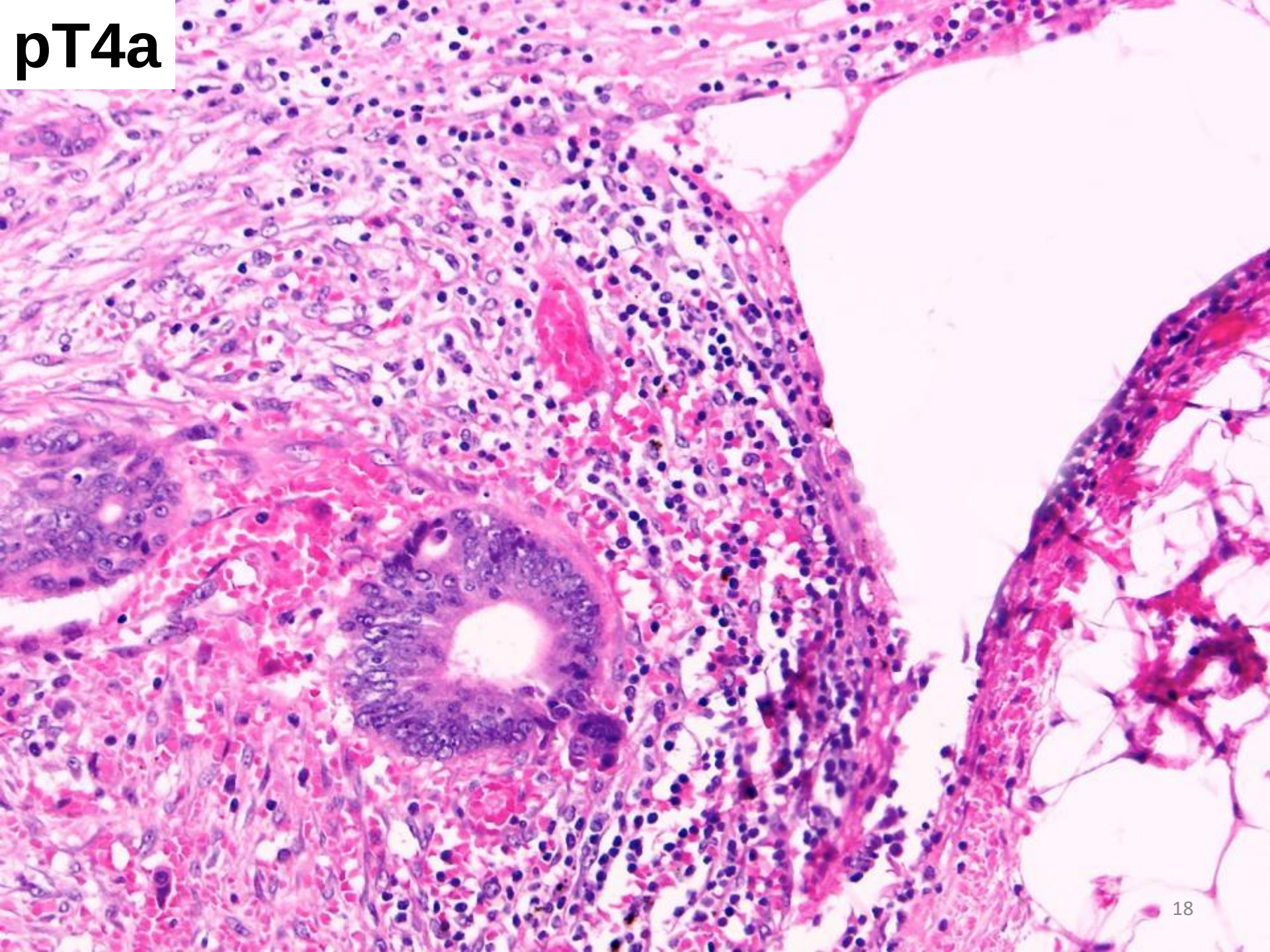

pT4a


pT4a


pT4a

CAP Protocol: Lymphovascular invasion

Lymphovascular Invasion (select all that apply) (Note E)

___ Not identified

___ Present

+ ___ Small vessel lymphovascular invasion

+ ___ Large vessel (venous) invasion

+ ___ Intramural

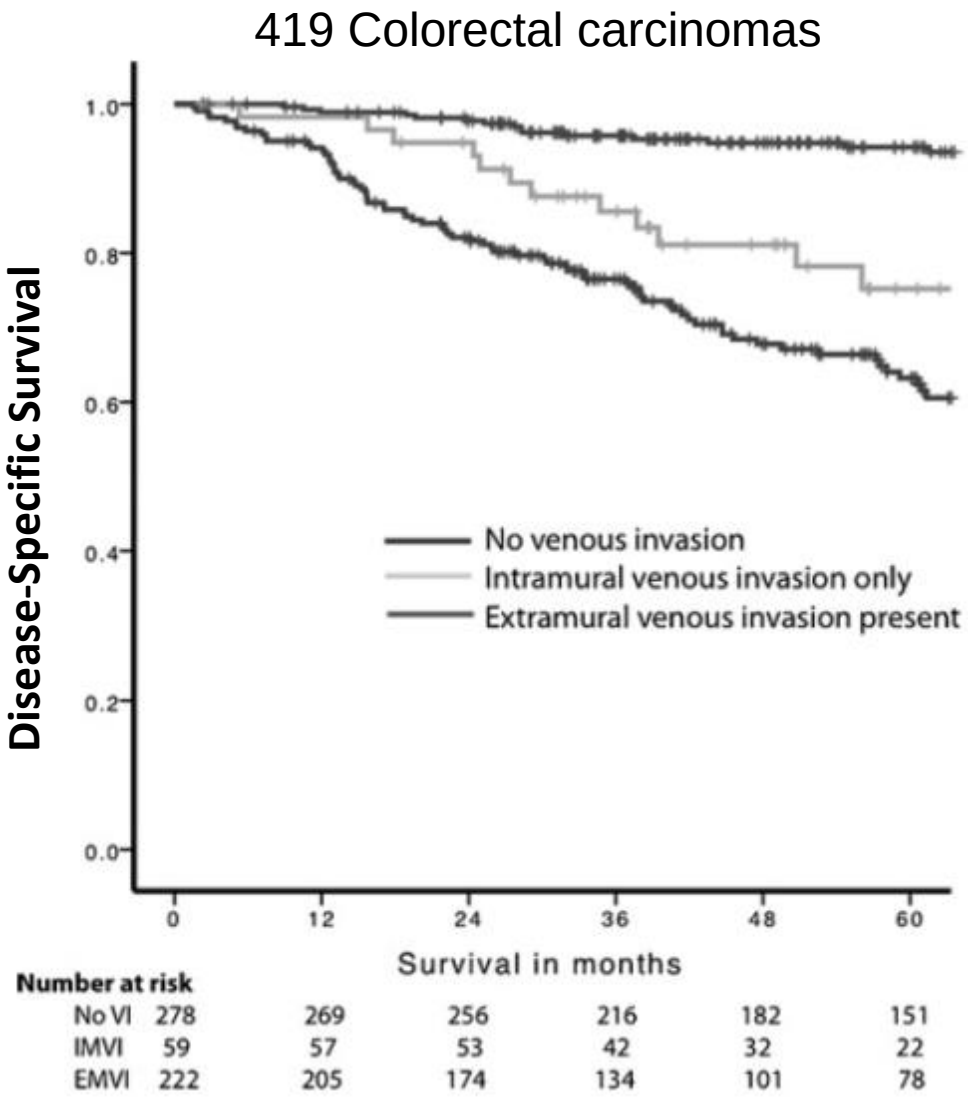
+ ___ Extramural

NOTE E: Small vessel invasion indicates tumor involvement of thin-walled structures lined by endothelium, without an identifiable smooth muscle layer or elastic lamina. Small vessels include lymphatics, capillaries, and postcapillary venules.

Tumor involving endothelium-lined spaces with an identifiable smooth muscle layer or elastic lamina is considered venous (large vessel) invasion. ***Circumscribed tumor nodules surrounded by an elastic lamina on hematoxylin-eosin (H&E) or elastic stain are also considered venous invasion.***

Extramural = beyond muscularis propria vs. **Intramural** = submucosa or muscularis propria.

Venous invasion: Prognostic Factor



Venous invasion absent		Venous invasion present	
pTN	5-yr survival	pTN	5-yr survival
pT4N0	91%	pT4N0	54%
pT4N1	71%	pT4N1	38%
pT4N2	67%	pT4N2	25%

Extramural venous invasion has been demonstrated to be an independent adverse prognostic factor in multiple studies and is a risk factor for liver metastasis.

Roxburgh CS, et al. Ann Surg. 2014 Jun;259(6):1156-65.

Venous invasion: Is H&E good enough?

Table 3 Detection of VI in comparative studies of standard H&E and elastic staining

Series	No. of patients	Vi detection rate (%)	
		H&E	Elastic stain (type)
Vass et al [40]	75	27	57 (Miller)
Inoue et al [39]	94	17	47 (EVG)
Kingston et al [36]	50	10	48 (EVG)
Howlett et al [35]	92	18	62 (Movat)
Sejben et al [41]	89	18	71 (Orcein)

Abbreviation: EVG, Elastic van Gieson.

Messenger DE, et al. Hum Pathol. 2012 Jul;43(7):965-73.

Only H&E to assess venous invasion		H&E and special stain (Elastin)	
Venous invasion	5-yr survival	Venous invasion	5-yr survival
Absent	**84%**	**Absent**	**96%**
Present	77%	Present	75%

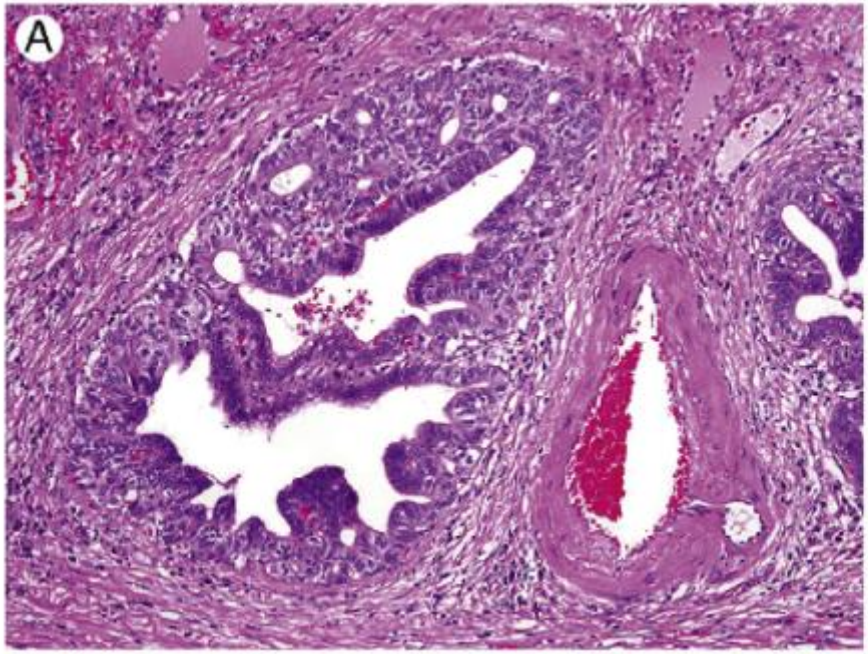
Roxburgh CS, et al. Ann Surg. 2010 Dec;252(6):989-97.

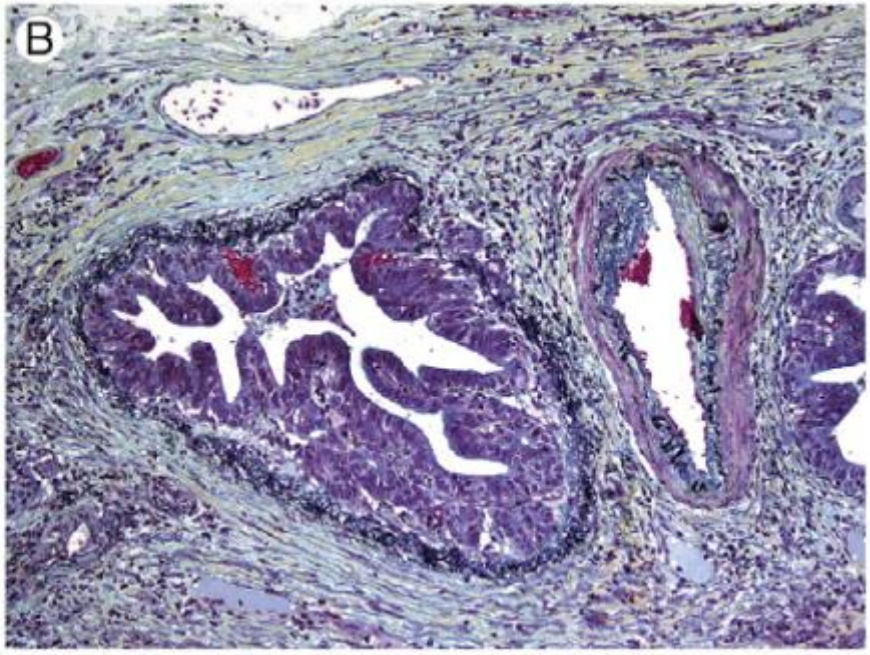
H&E

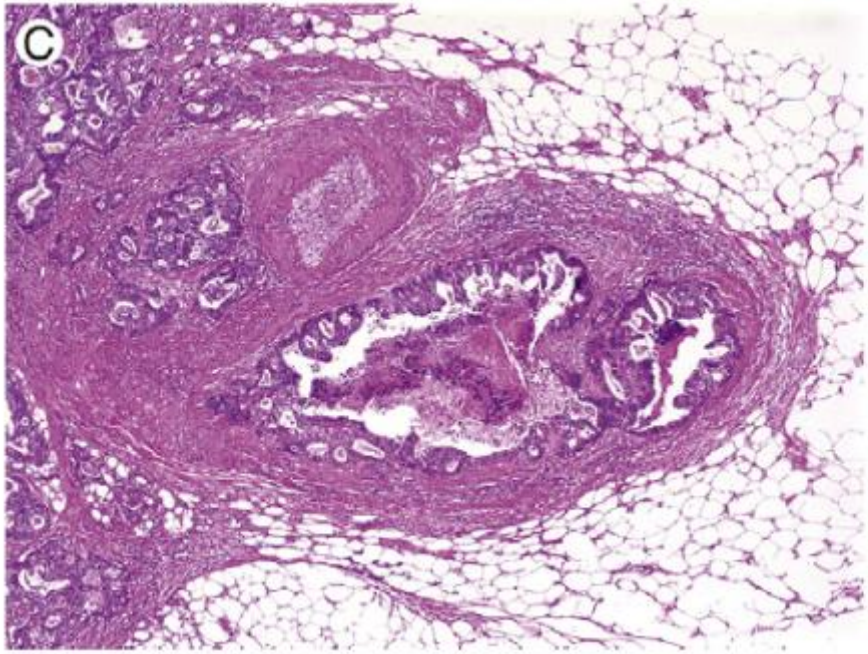
Movat or VVG

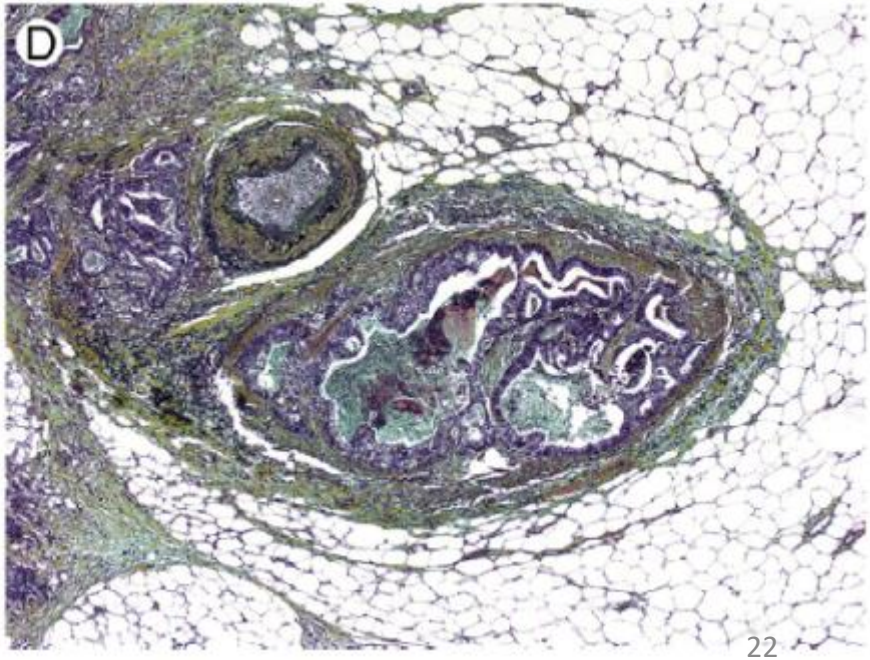
Orphan arteriole sign

Protruding tongue sign

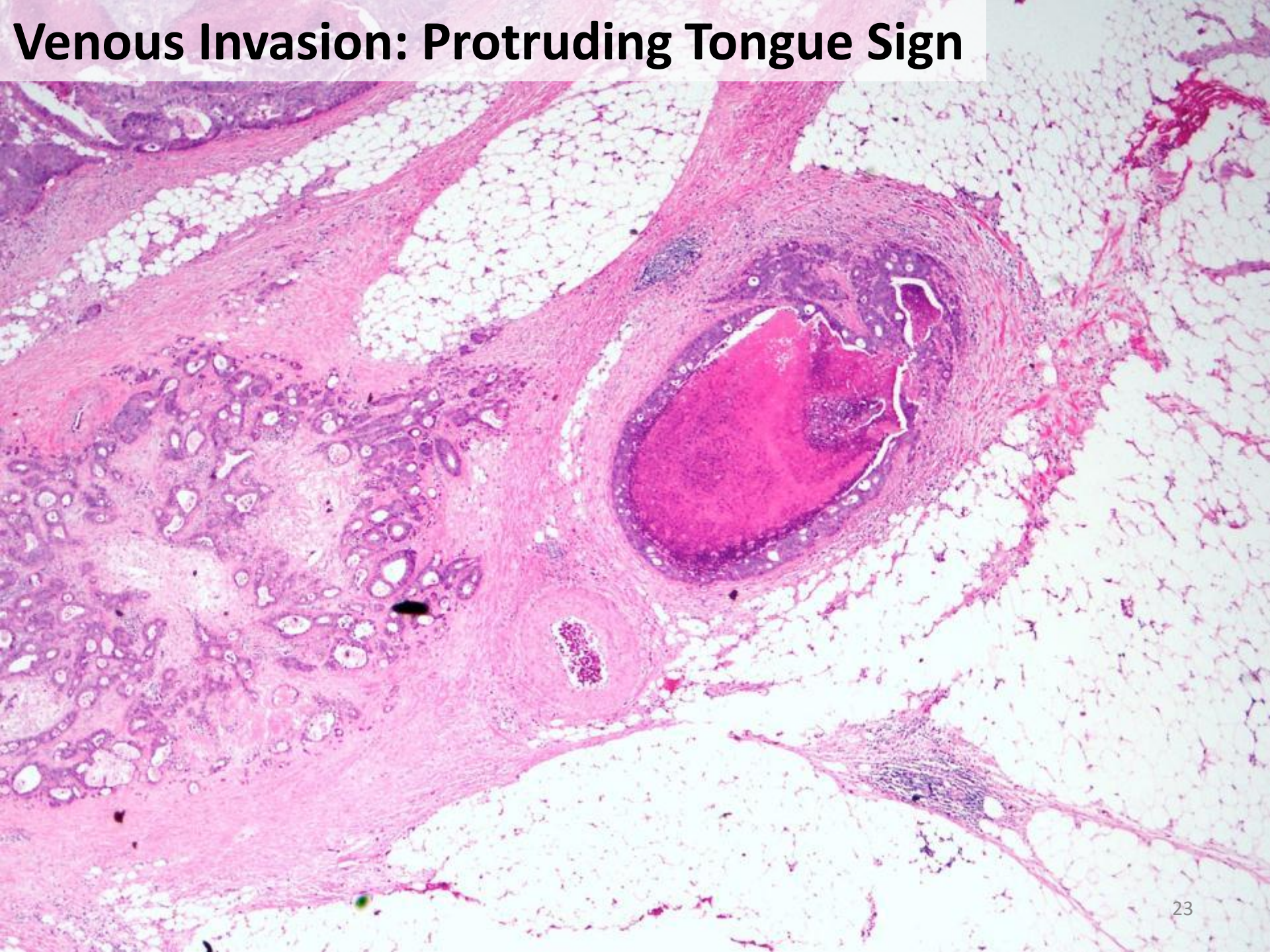




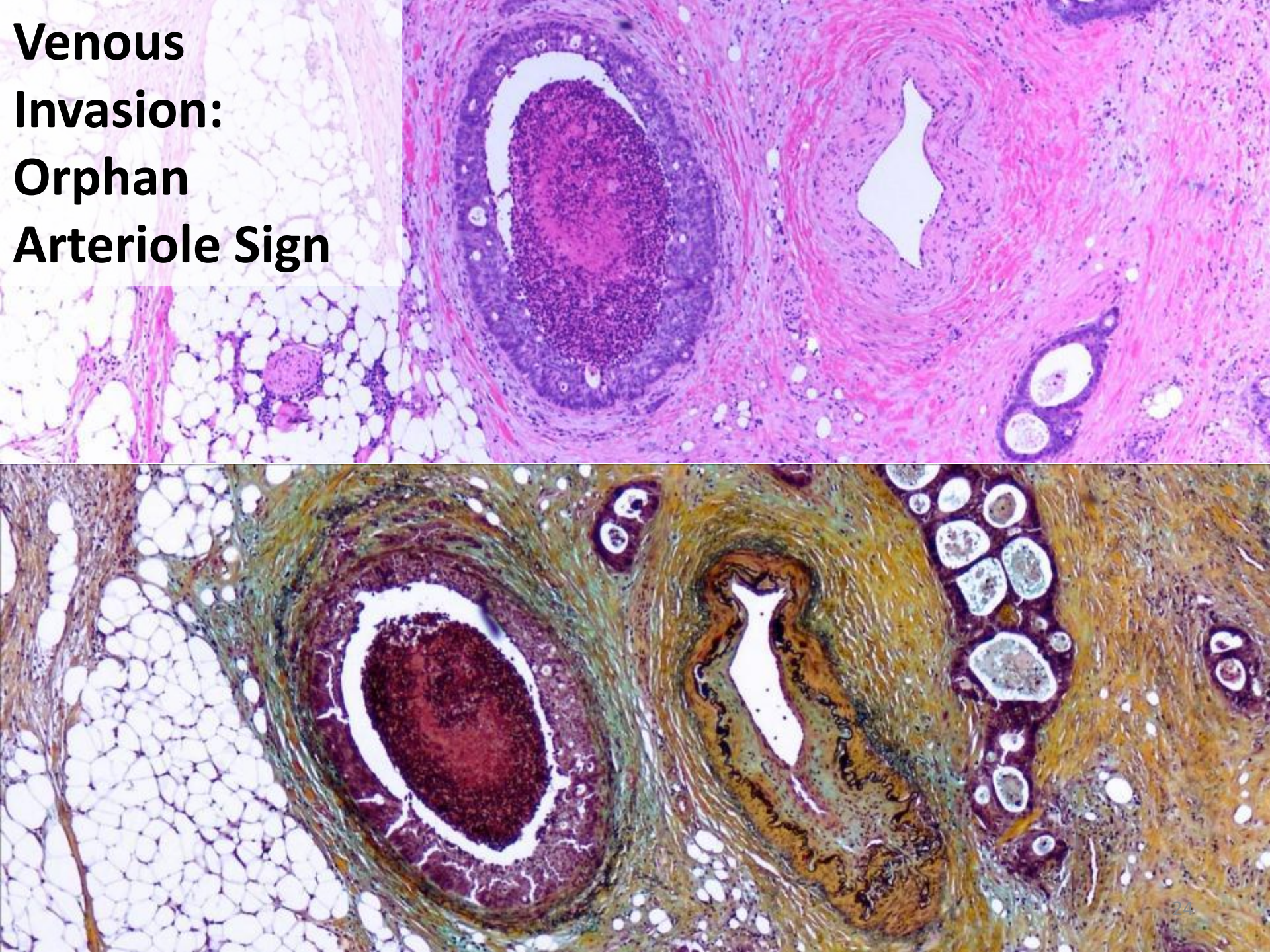


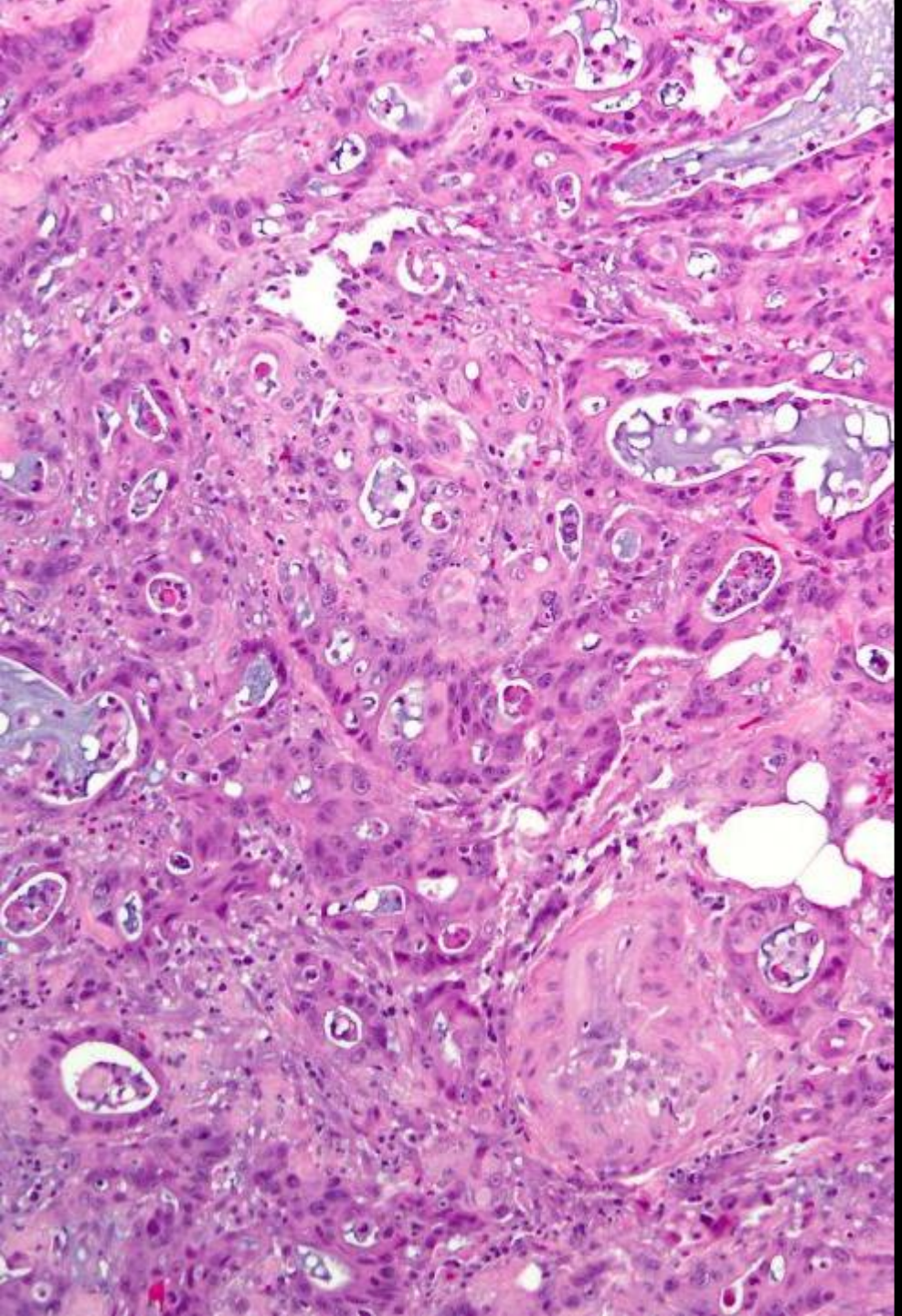


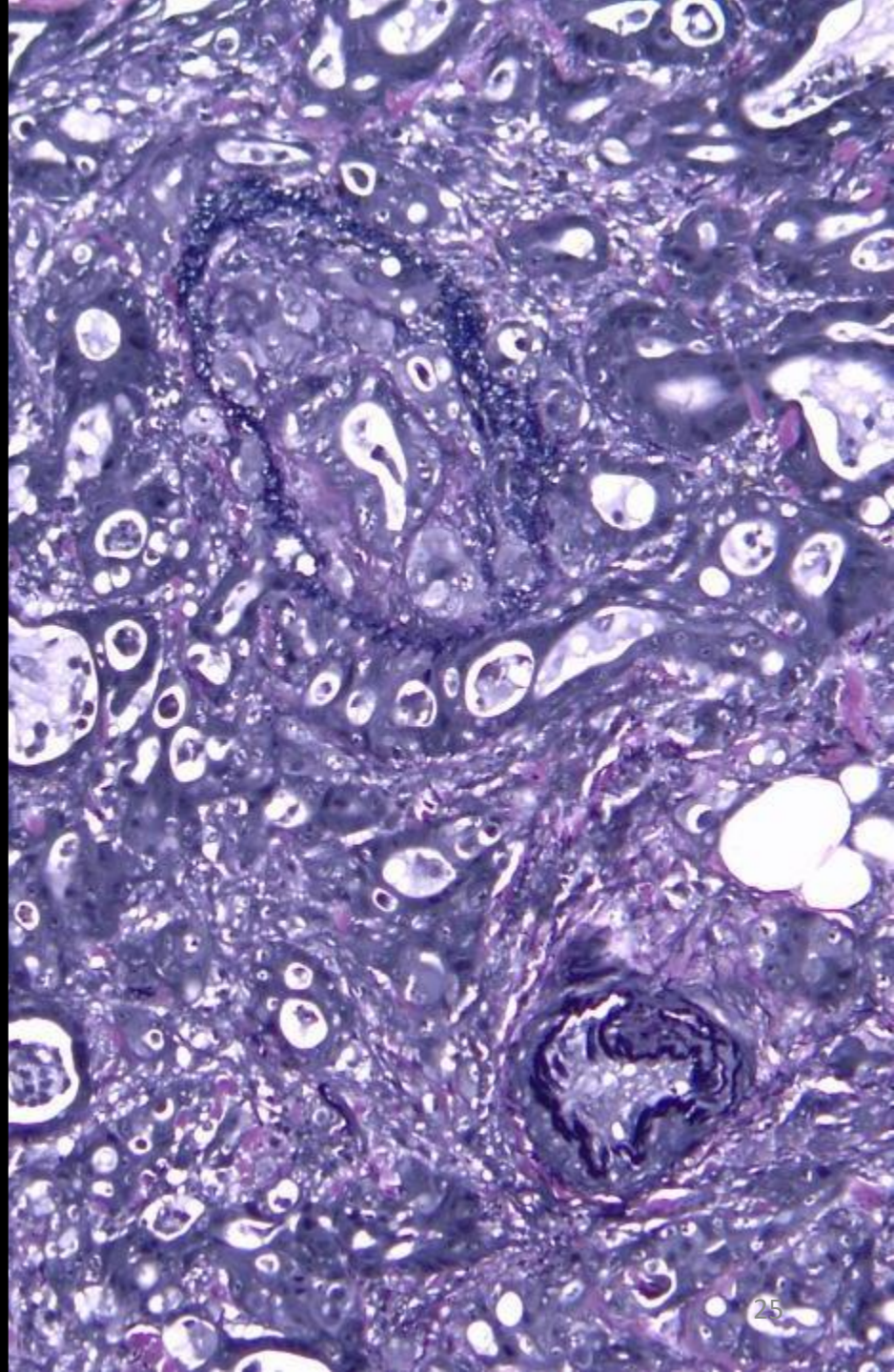
Venous Invasion: Protruding Tongue Sign



Venous Invasion: Orphan Arteriole Sign







Venous invasion: UPMC Approach

- Elastin stains are ordered “up-front” on all tumor sections for resected non-metastatic colorectal carcinoma (usually 5 elastin stains per case).

- The presence or absence of venous invasion is incorporated into every pathology report given its prognostic significance.

- If venous invasion is present, it is classified as either intramural or extramural.

Definition of Tumor Deposits

- **AJCC 6th Edition:**
  - Smooth contours = lymph node metastasis.
  - Irregular contours = tumor deposit.
- **AJCC 7th Edition:**
  - Discrete foci of tumor found in the pericolorectal fat away from the leading edge of the tumor and showing no evidence of residual lymph node tissue.
  - Classify as pN1c if no other lymph node metastases are seen.
- **AJCC 8th Edition**:
  - A tumor focus in the pericolorectal fat or in adjacent mesentery but ***without*** identifiable lymph node tissue or ***identifiable vascular or neural structure.***
  - Classify as pN1c if no other lymph node metastases are seen.

Lymph Node vs. Tumor Deposit

Lymph Node

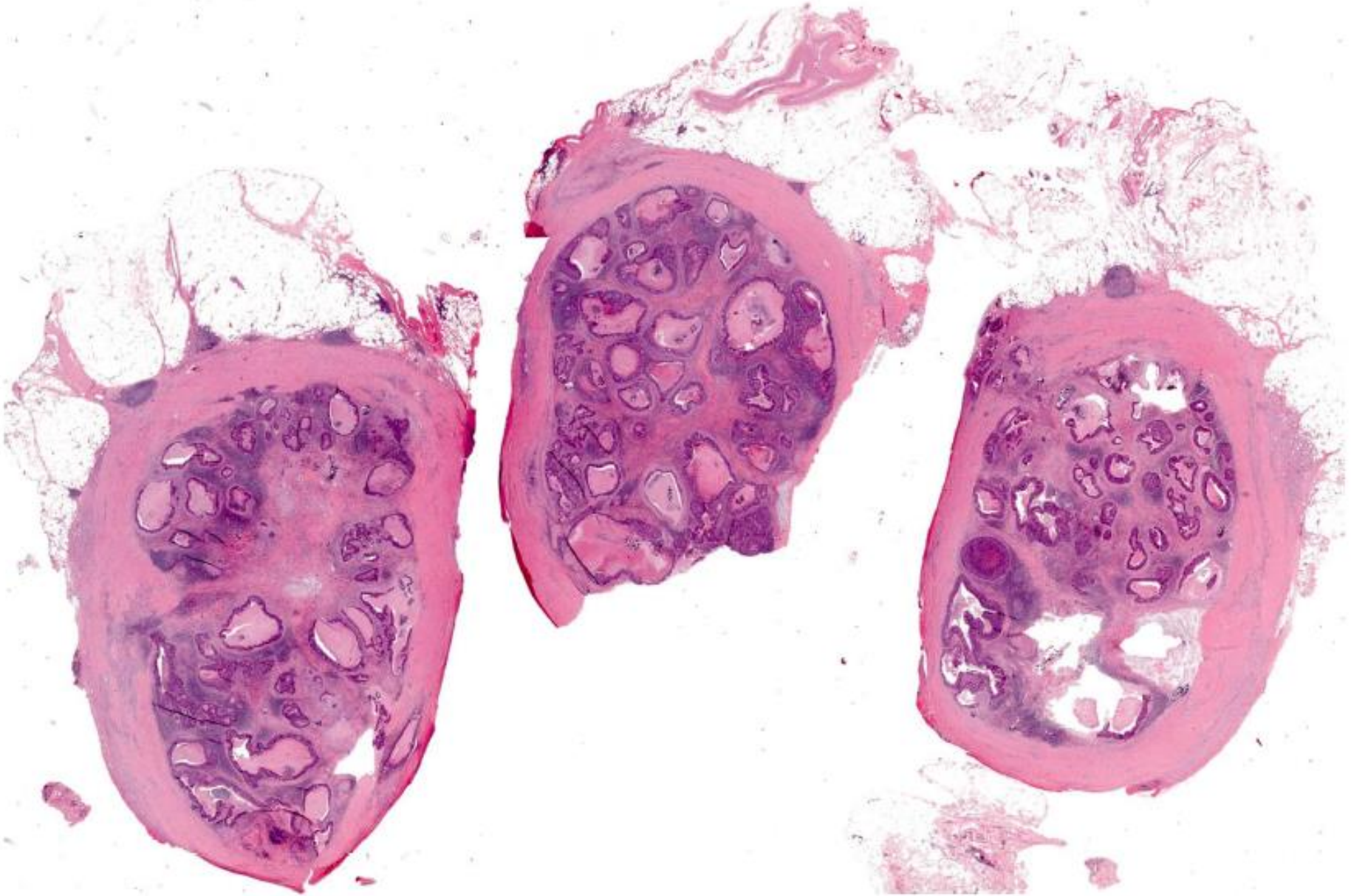
- Round Shape
- Thick capsule
- Peripheral lymphoid follicles
- Peripheral lymphocyte rim

Tumor Deposit

- Irregular shape
- No thick capsule
- No lymphocyte rim or peripheral lymphoid follicles

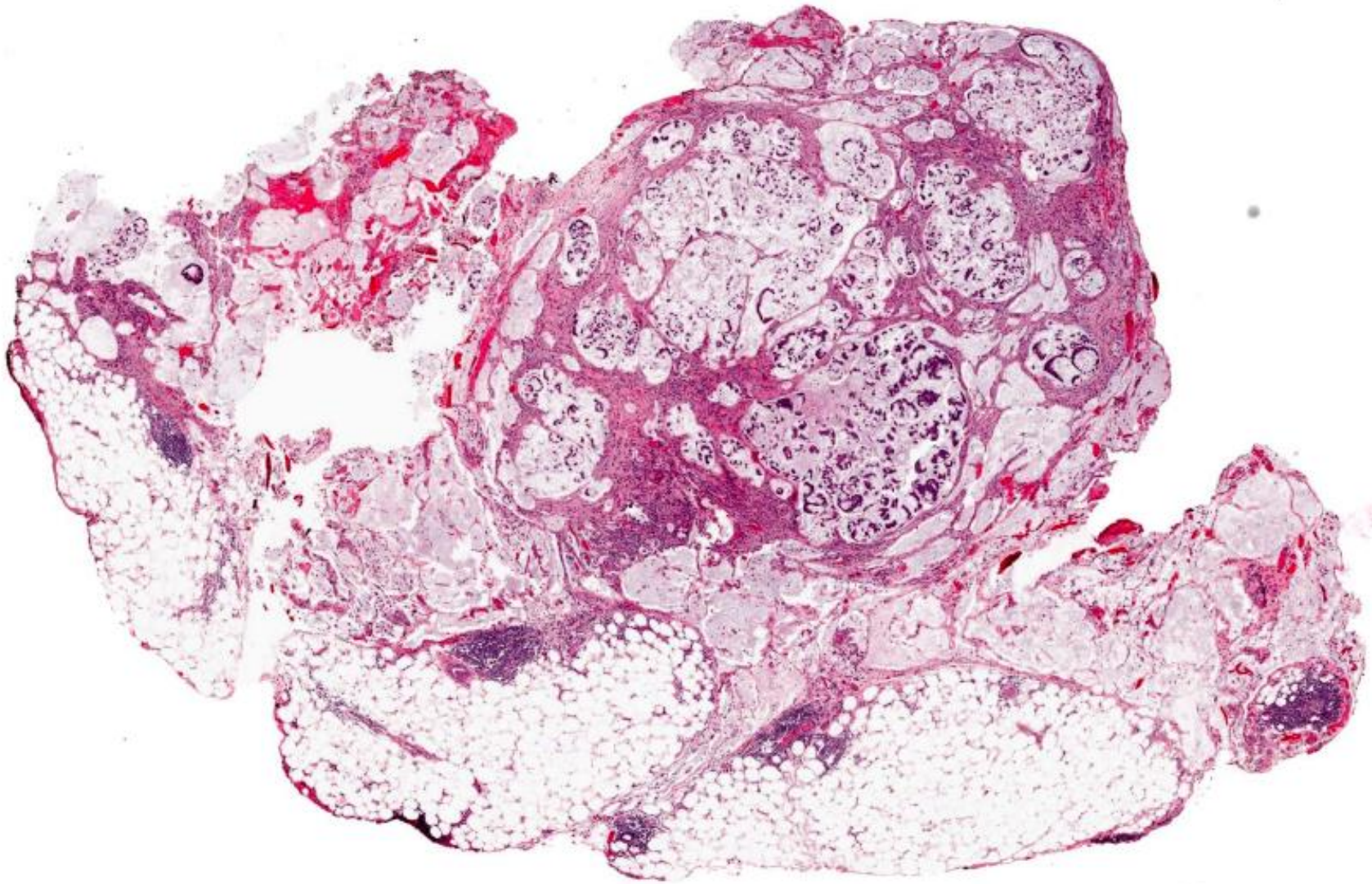
If any one of these features are present, I classify as a positive lymph node.

Lymph node vs Tumor deposit? Lymph node 7/7 reviewers



Rock JB, et al. Arch Pathol Lab Med. 2014 May;138(5):636-42.

Lymph node vs Tumor deposit? Tumor deposit 7/7 reviewers



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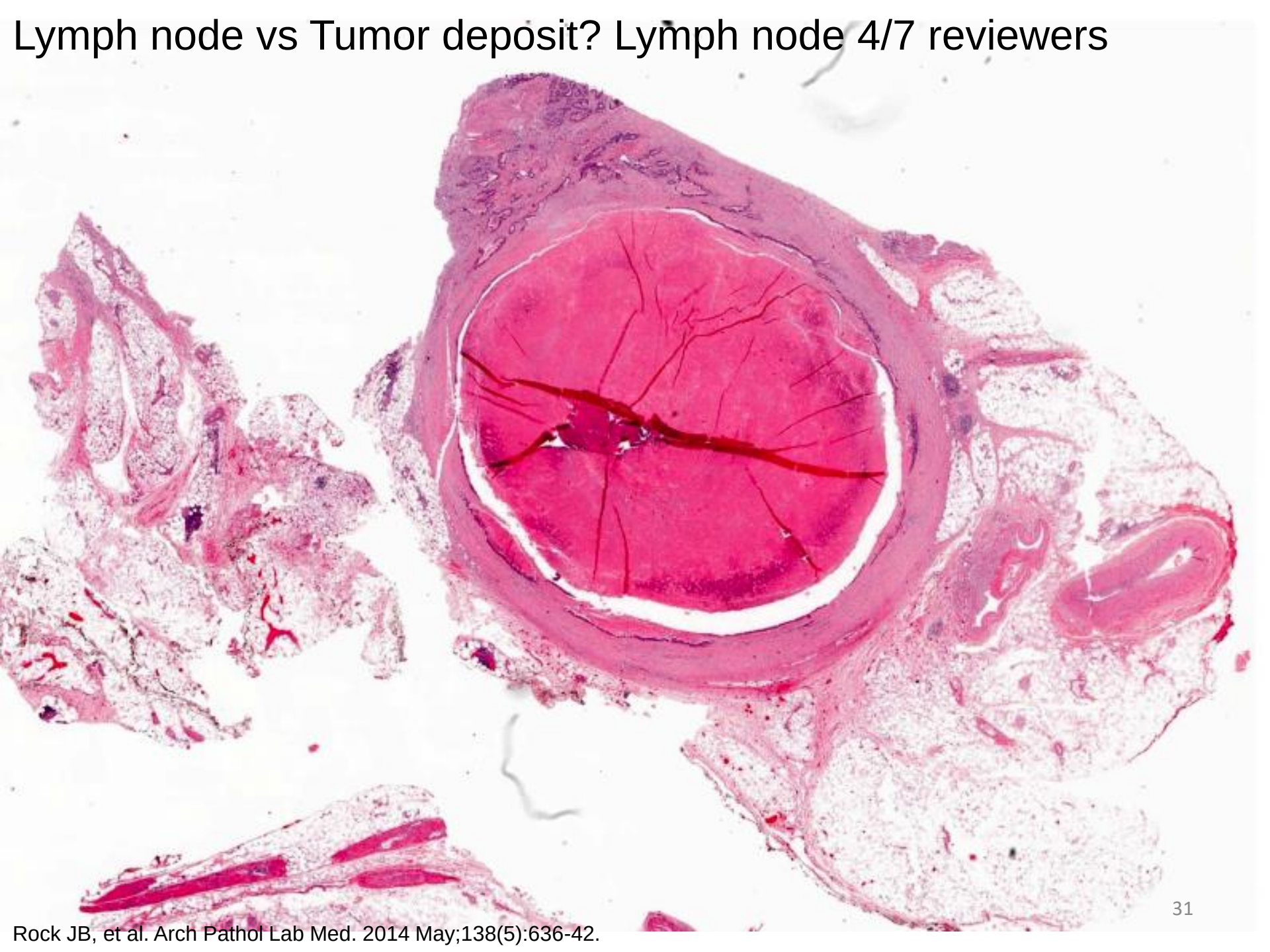
Lymph node vs Tumor deposit? Lymph node 4/7 reviewers



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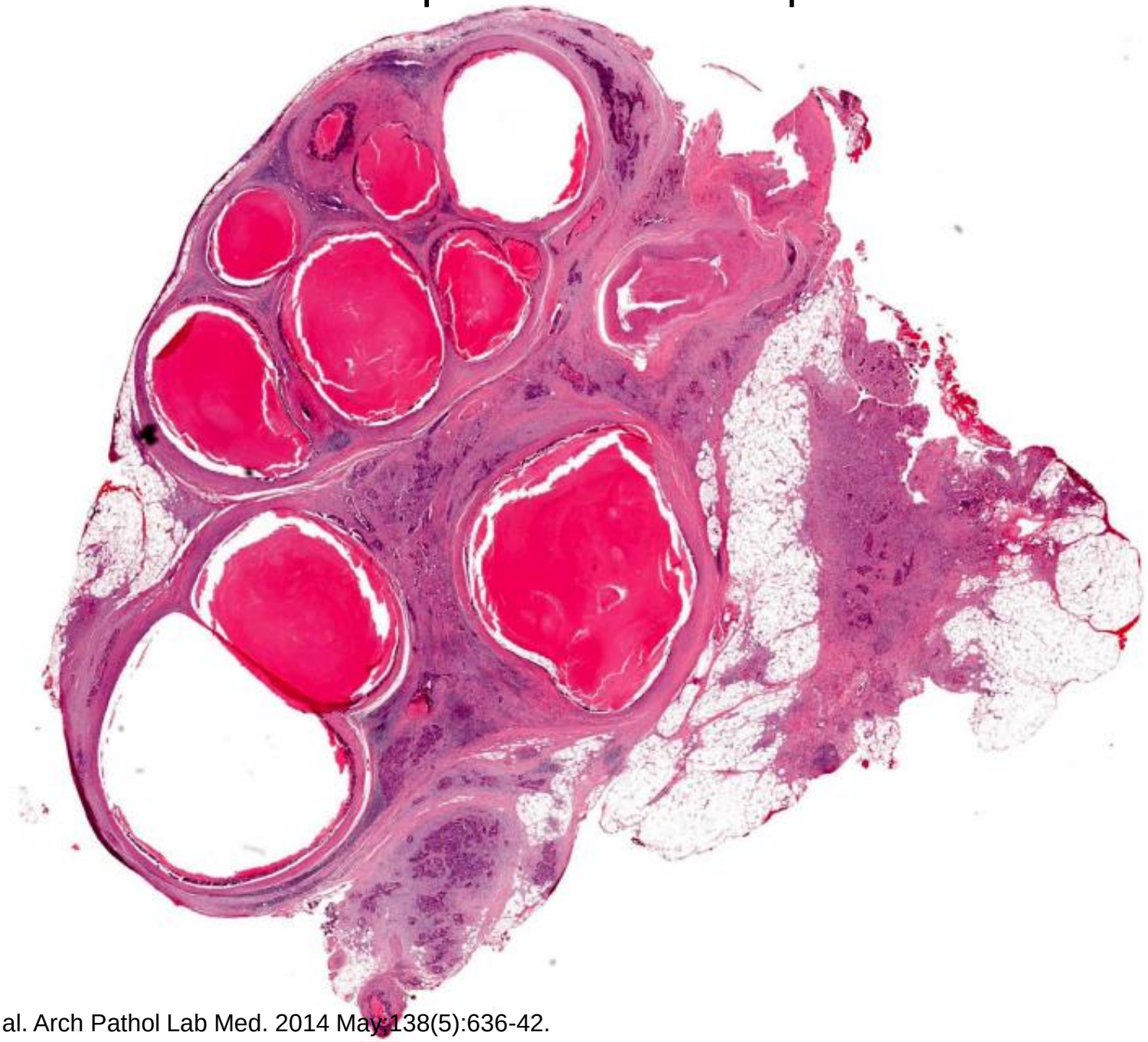
Lymph node vs Tumor deposit? Tumor deposit 4/7 reviewers



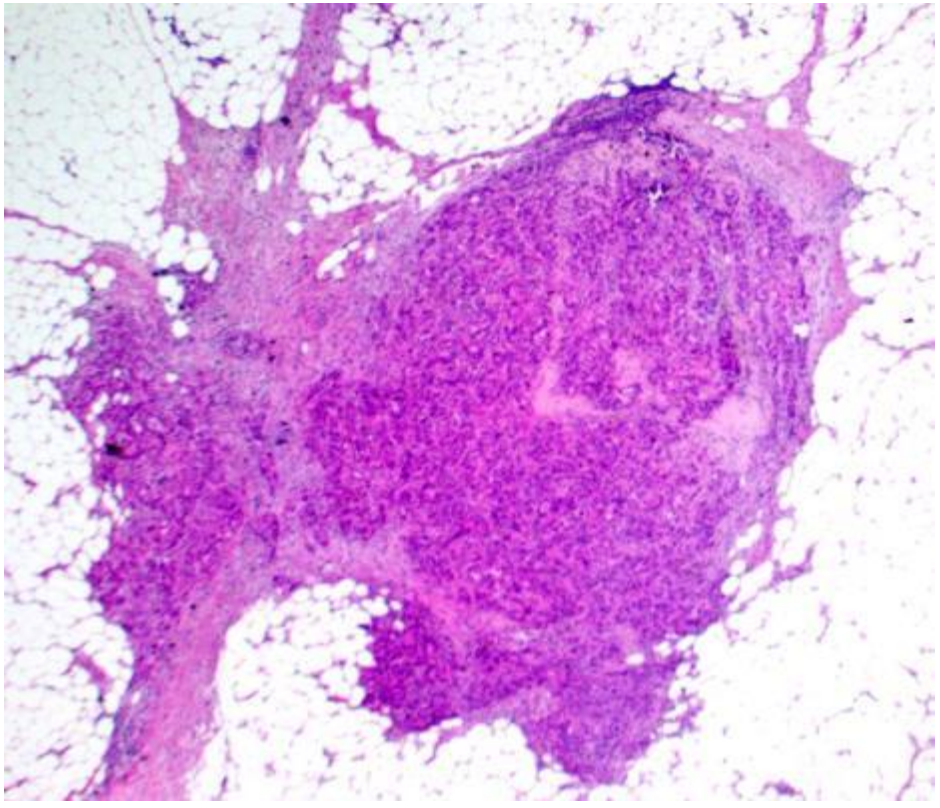
Rock JB, et al. Arch Pathol Lab Med. 2014 May;138(5):636-42.

Issues with AJCC 8th Edition of Tumor Deposits

- Minimum distance from invasive front of tumor & minimum size is not defined (these should be documented in gross description).
- ***If tumor is associated with an identifiable vascular structure or nerve, then it should NOT be classified as a tumor deposit.***
  - ***This AJCC 8th edition change influences tumor staging.***

Tumor Deposit (T3N1c) vs. Venous Invasion (T3N0)

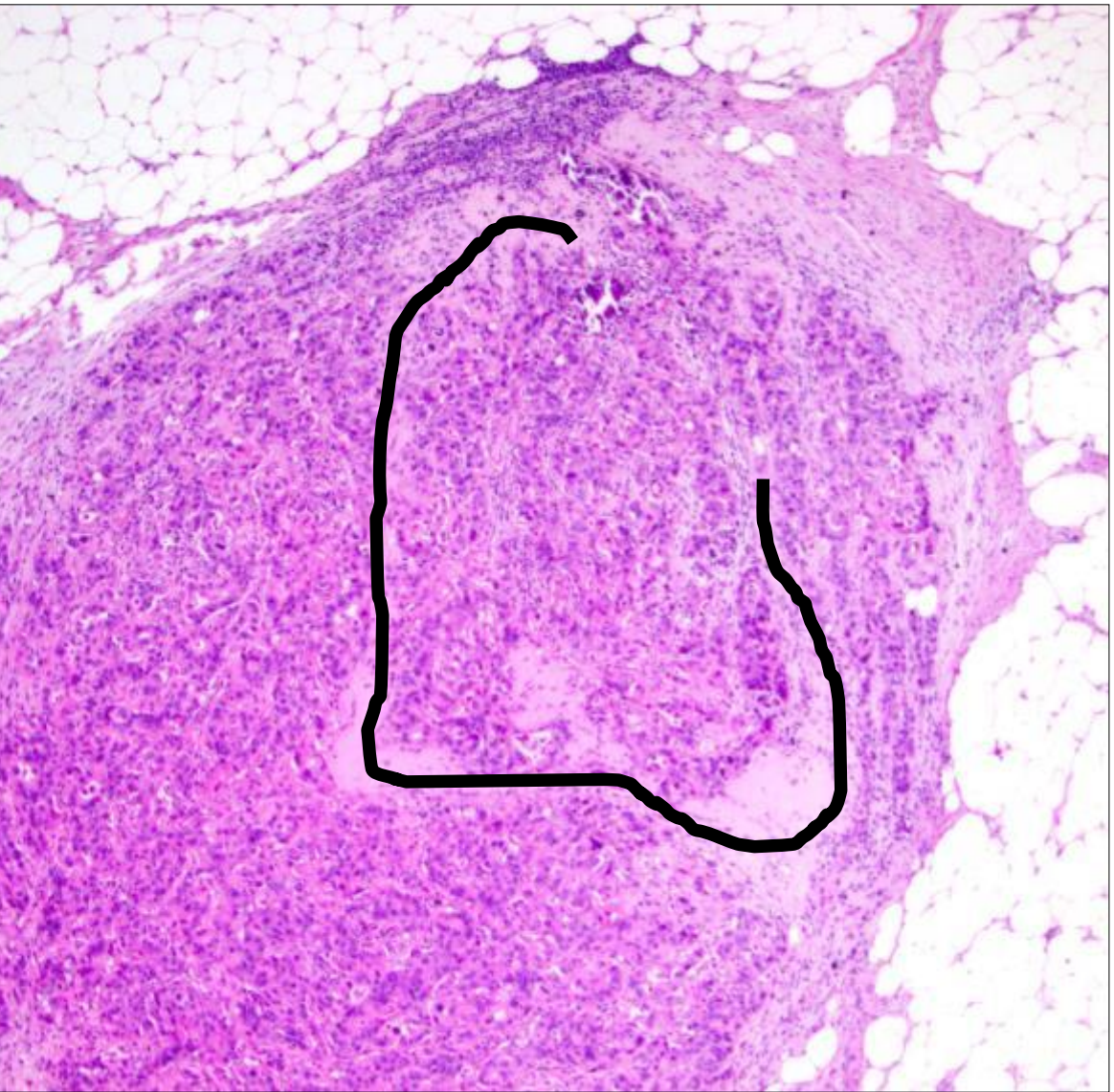
- T3 tumor with no lymph node metastases but with discrete focus of tumor within the pericolic fat away from the invasive front.



<u>AJCC 7th Edition</u>:
This would be classified as a tumor deposit.

The tumor would be staged as pT3 N1c (stage III).

Tumor Deposit (T3N1c) vs. Venous Invasion (T3N0)



<u>AJCC 8th Edition</u>:
Given the presence of remnant of large vein, this would be NOT be classified as a tumor deposit.

The tumor would be staged as pT3 N0 (stage II).

NCCN High risk factors for stage II (node-negative) colon cancers

High risk factors

- Poorly differentiation (except MSI-high)
- **Lymphovascular invasion**
- Bowel obstruction
- <12 lymph nodes
- Perineural invasion
- Localized perforation (pT4)
- Close/indeterminate margins

Stage II with venous invasion

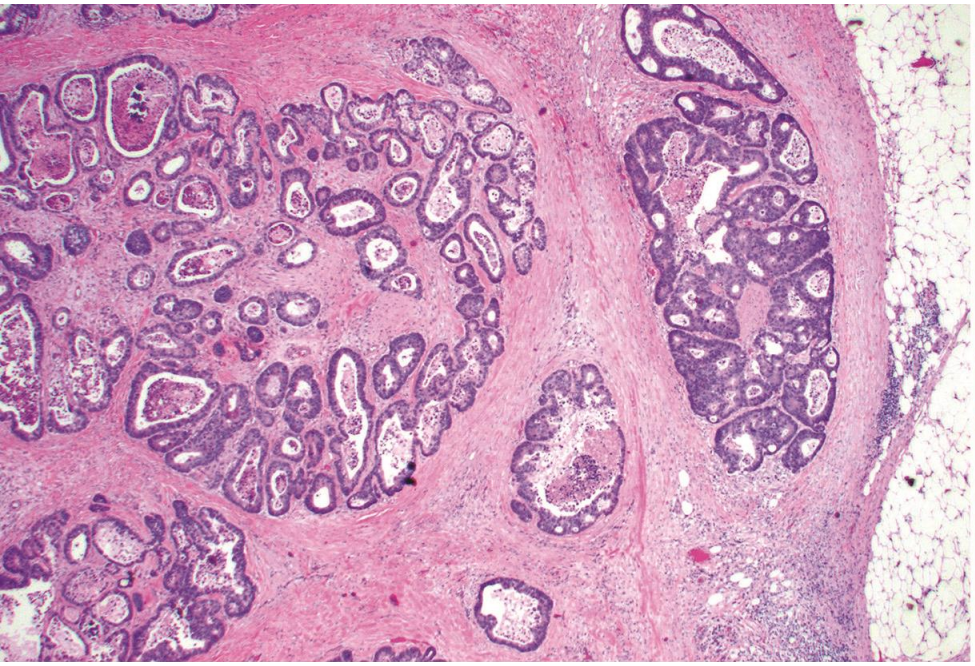
- Per NCCN guidelines, patients with stage II cancers with venous invasion should be offered adjuvant chemotherapy.

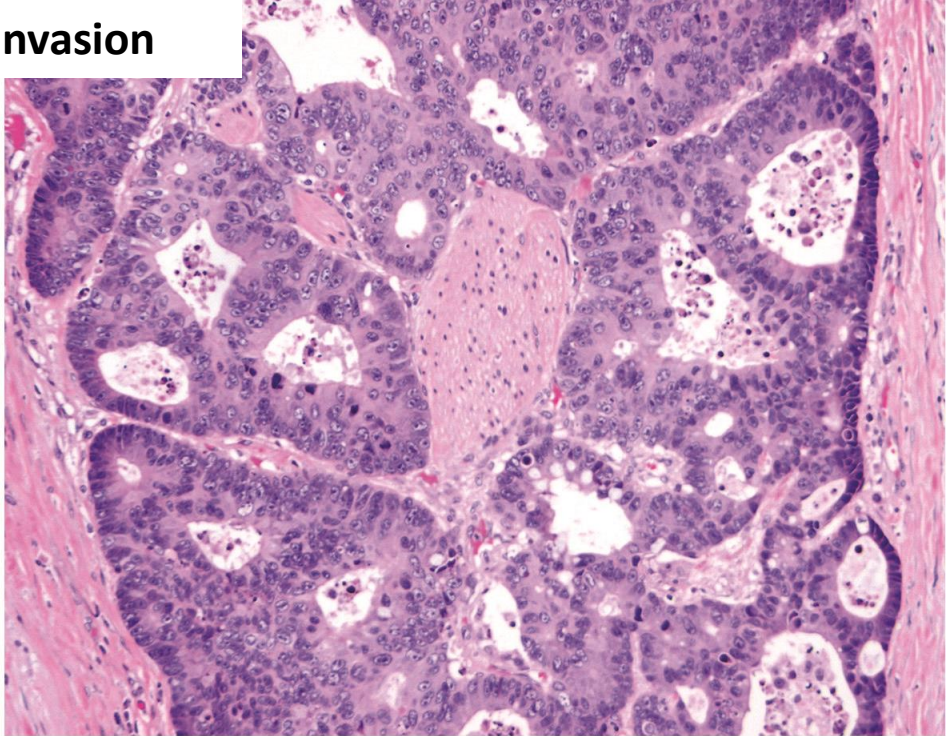
- Patients with stage III (T3 N1c) will be given adjuvant chemotherapy.

Issues with AJCC 8th Edition of Tumor Deposits

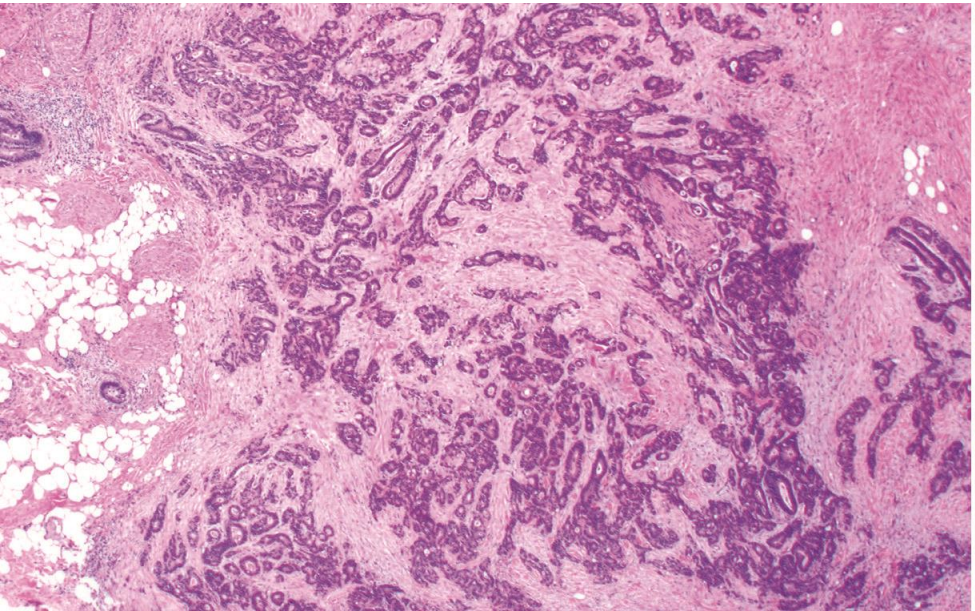
- Minimum distance from invasive front of tumor & minimum size is not defined (these should be documented in gross description).
- ***If tumor is associated with an identifiable vascular structure or nerve, then it should NOT be classified as a tumor deposit.***
  - *CAP protocol explanatory comment: not a tumor deposit if the focus is associated with a **"large"** nerve or vessel.*
  - *How big does the vessel or nerve have to be?*

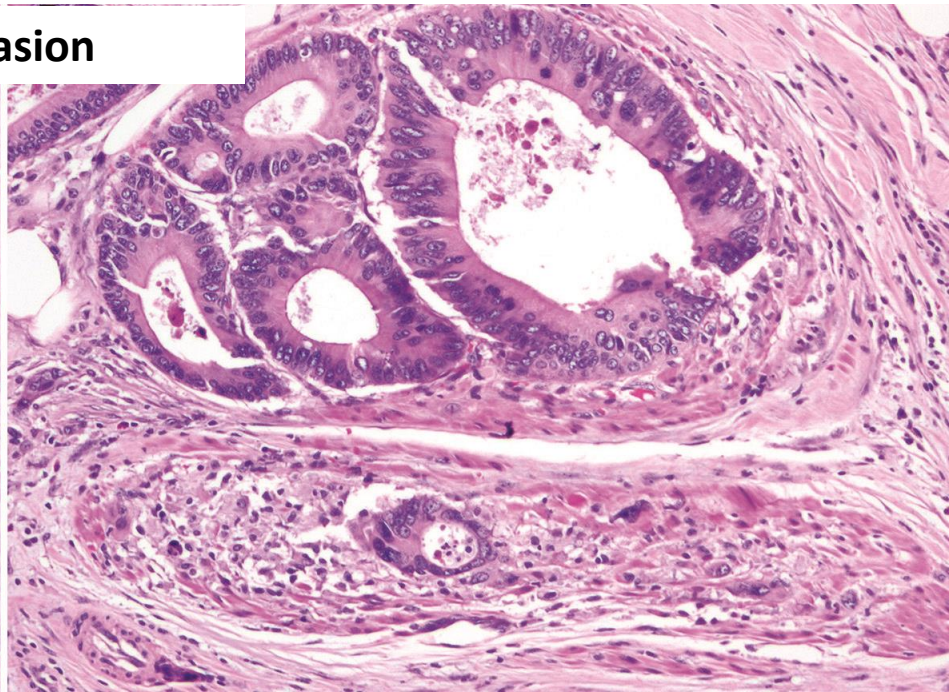
Tumor Deposit with “small” focus of perineural invasion





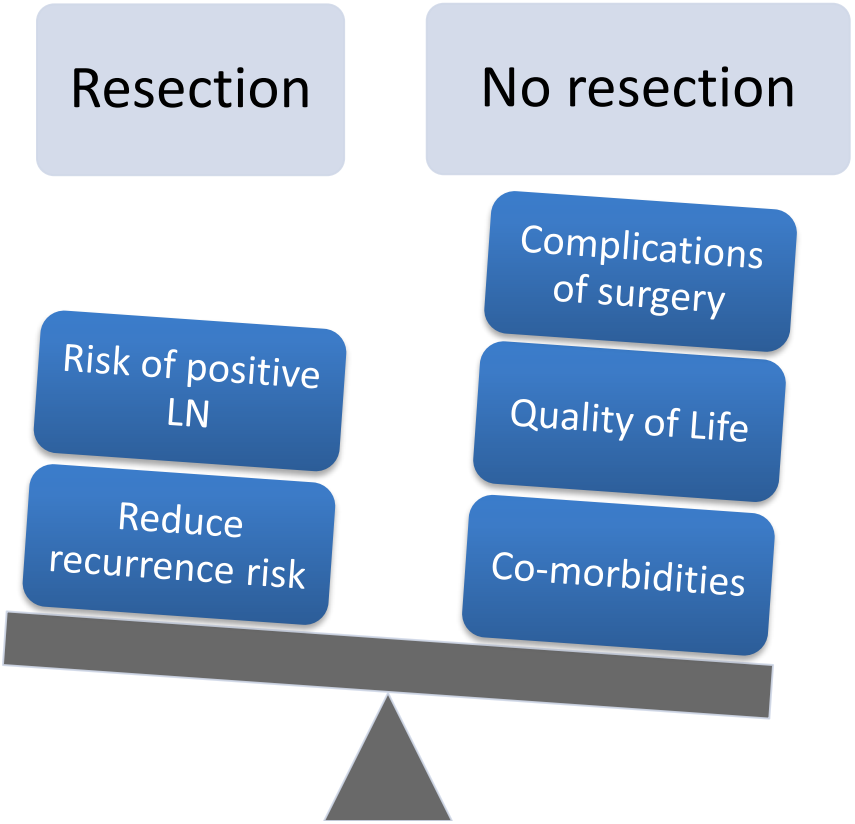
Tumor Deposit with “small” focus of venous invasion





Malignant Polyp on Polypectomy: To surgically resect or not?

Malignant polyp: polyp with carcinoma invading the submucosa (pT1)



Does the risk of surgery outweigh the risk of metastatic disease?

Malignant Polyp on Polypectomy: To surgically resect or not?

- NCCN (2018) Unfavorable Features:
  - Poorly differentiated tumor
  - Lymphovascular invasion
  - Margin status (positive, <1 or 2 mm, or cannot be assessed in piecemeal resection)
- **Newly Recognized Unfavorable Features**
  - **Tumor budding: NCCN (2018) states it is an adverse histologic feature associated with adverse outcome.**
  - **Depth of invasion: currently not incorporated by NCCN (2018).**

Peritumoral Tumor Budding: Should it be Included in Pathology Reports?

- Peritumoral budding is ***not*** mentioned in the AJCC 8th Edition.
- The CAP Cancer Protocol has added tumor budding as a ***recommended but not mandatory element*** in the following settings:
  - Malignant polyps (pT1) – helps to assess for risk of LN metastasis and need for surgery.
  - Stage II colorectal carcinoma – helps to select patients for adjuvant chemotherapy.

Ueno H, et al. Gastroenterology. 2004 ;127(2):385-94.
Petrelli F, et al. J Gastrointest Cancer. 2015;46(3):212-8.
Graham RP, et al. Am J Surg Pathol. 2015;39(10):1340-6.
Koelzer VH, et al. Hum Pathol. 2016;47(1):4-19.
Pai RK et al. Mod Pathol 2017;30:113-122.

Peritumoral Tumor Budding: How to assess?

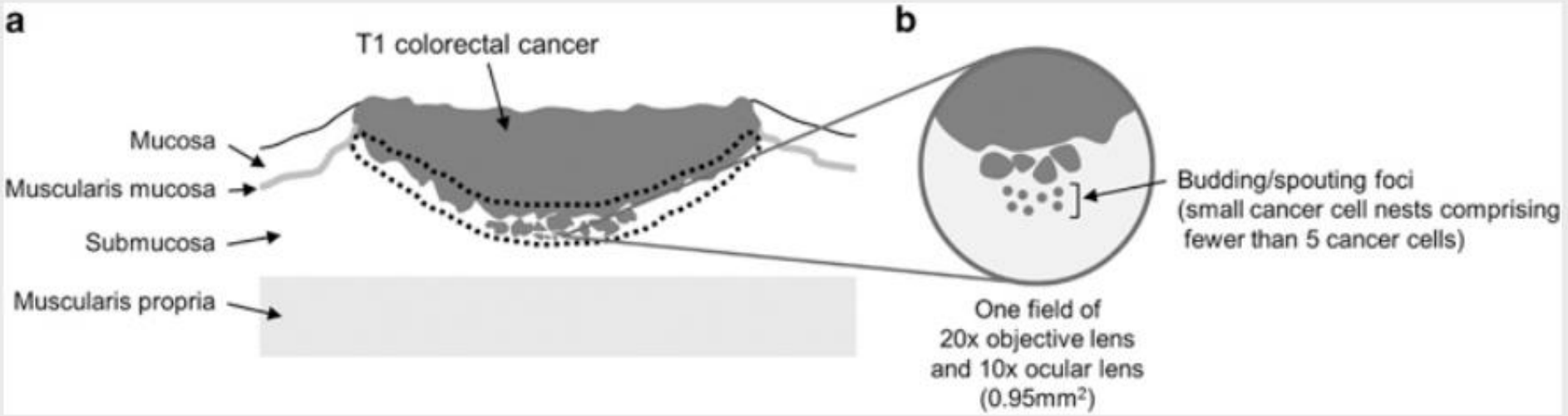
- **Tumor buds defined as**:
  - Isolated tumor cells.
  - Cluster of <5 tumor cells.
  - Present at the invasive front of the tumor.
- **Assess using the "hot spot" method**.
  - Use scanning (x10 objective magnification) to identify area of invasive front with maximal tumor budding.
  - In this area, count the number of tumor buds in one x20 objective field.

Peritumoral Tumor Budding: How to assess?

- The CAP cancer protocol recommends reporting the number of tumor buds per 0.785 mm^2.
  - My 20x objective has an area of 0.95 mm^2.
  - Must apply an appropriate correction factor based on your individual microscope (available in CAP protocol: for me, I divide by 1.21).

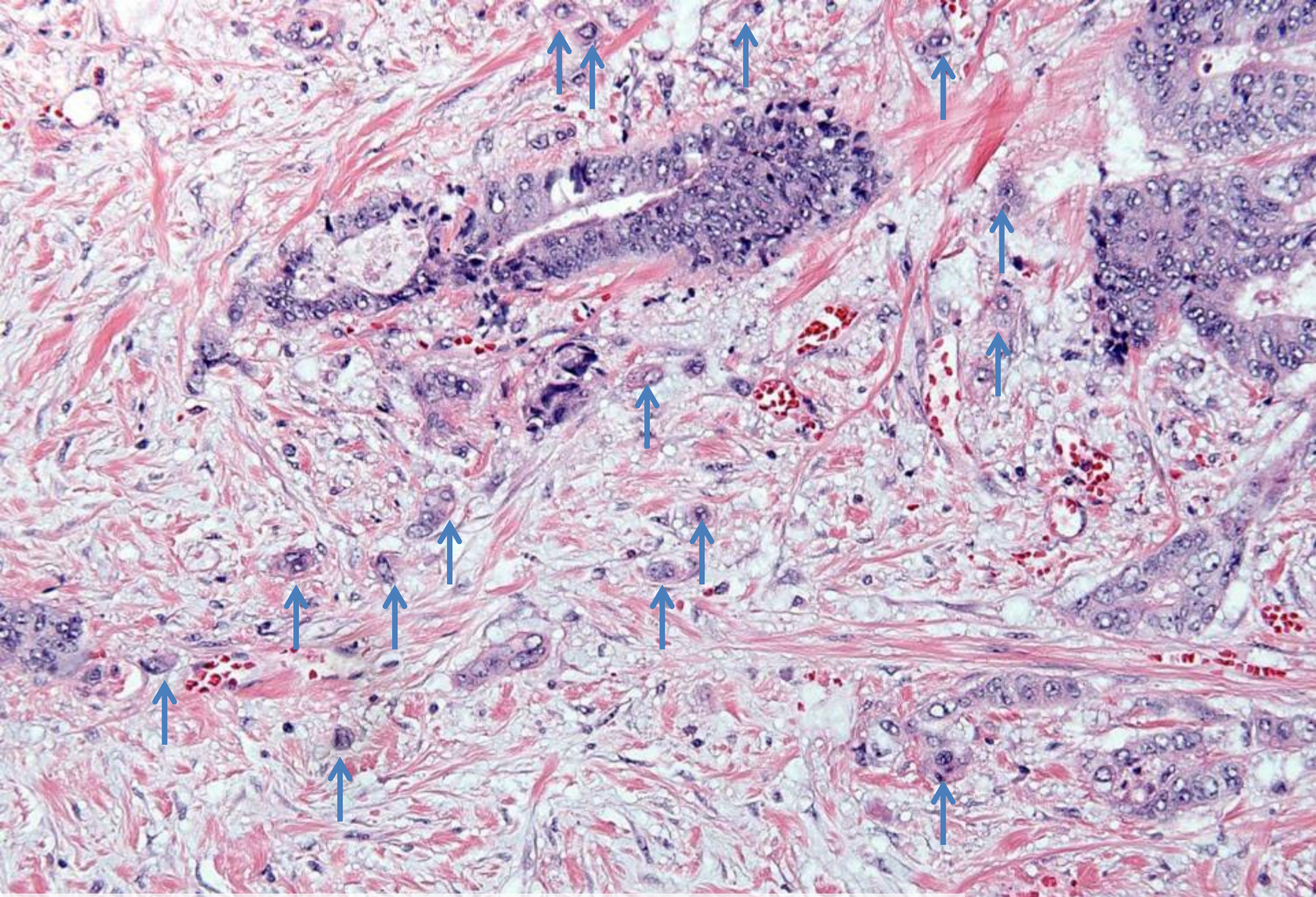
Tumor Budding Score	Tumor Budding (per 0.785 mm^2)
Low	<5
Intermediate	5 to 9
High	≥ 10

Peritumoral Tumor Budding: How to assess?



Must perform a correction to arrive at number of tumor buds per 0.785 mm^2

Kawachi H, et al. Mod Pathol. 2015 Jun;28(6):872-9.



High tumor budding score

15 tumor buds per 0.95 mm^2 = 12 tumor buds per 0.785 mm^2

pT1 Colorectal Carcinoma: Tumor Budding Associated with Increased Risk of LN metastasis

Study	Number of tumors analyzed (Number with lymph node metastasis)	Tumor budding % in node-negative vs. % in node-positive cases	P-value
Ueno, et al 2004	251 (33)	10% vs 49% **(≥5 per 0.785mm^2)**	**p<0.0001**
Nakadoi et al 2011	499 (41)	8.3% vs. 36.6% **(≥5 per 20X objective)**	**p<0.0001**
Tateishi et al 2010	322 (46)	28% vs. 61% **(≥5 per 0.785mm^2)**	**p<0.01**
Kawachi et al 2015	806 (97)	25% vs. 60.8% **(≥5 per 0.95mm^2)**	**p<0.0001**
Oka et al 2013	118 (13) Rectal only	11% vs. 54% **(≥5 per 20X objective)**	**p=0.0006**
Ueno, et 2014 (30 hospital consortium)	3556 (393)	14% vs. 37% **(≥5 per 20X objective)**	**p<0.0001**
Pai et al 2017	116 (28)	19% vs. 57% **(≥5 per 0.95mm^2)**	**p<0.001**

Peritumoral Tumor Budding in Malignant Polyps

These studies suggest that **≥ 5 tumor buds or at least an intermediate tumor budding score** in a malignant polyp indicates a higher risk of lymph node metastasis.

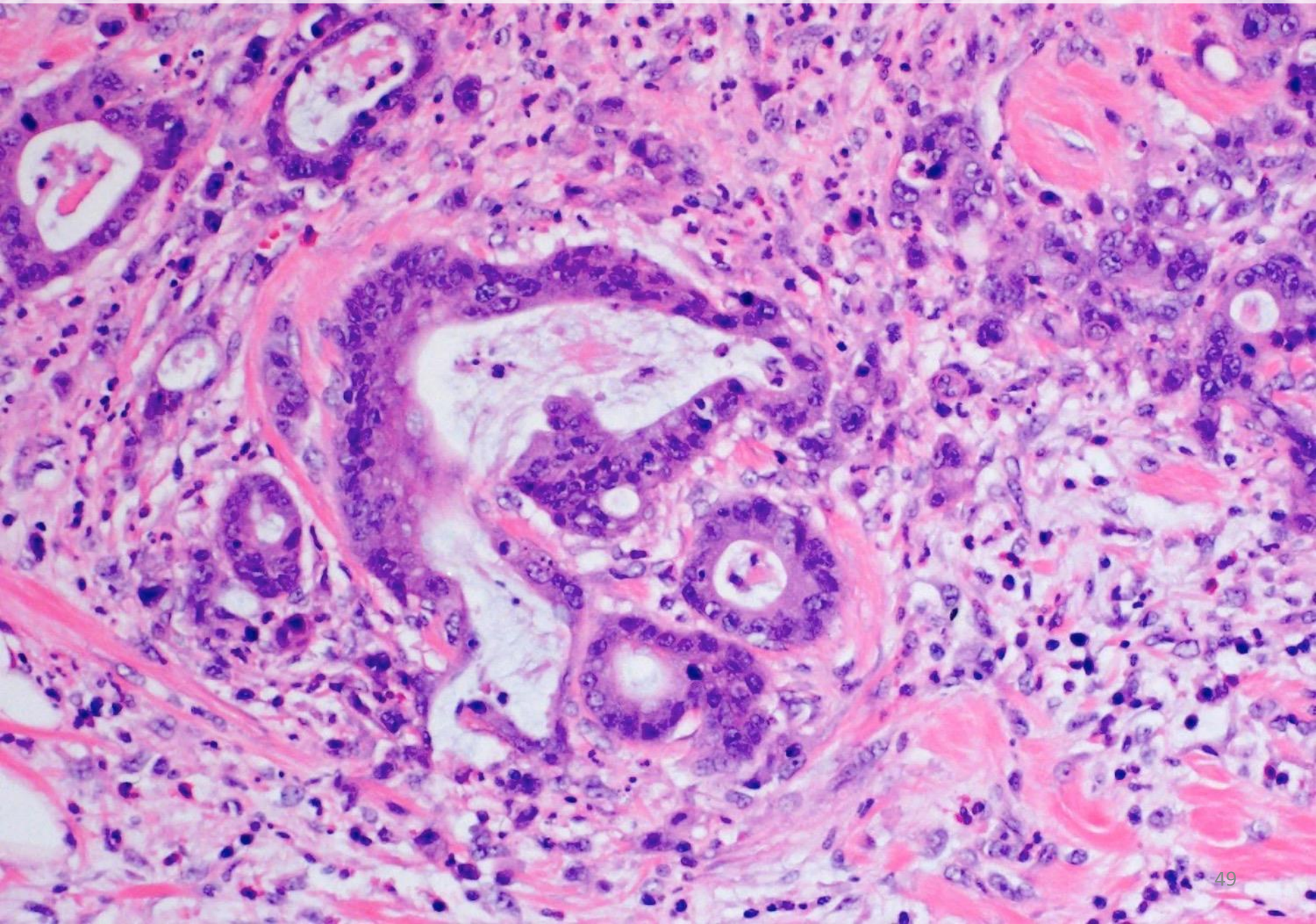
Tumor Budding Score	Tumor Budding (per 0.785 mm^2)
Low	<5
Intermediate	5 to 9
High	≥ 10

Tumor budding is different than grade

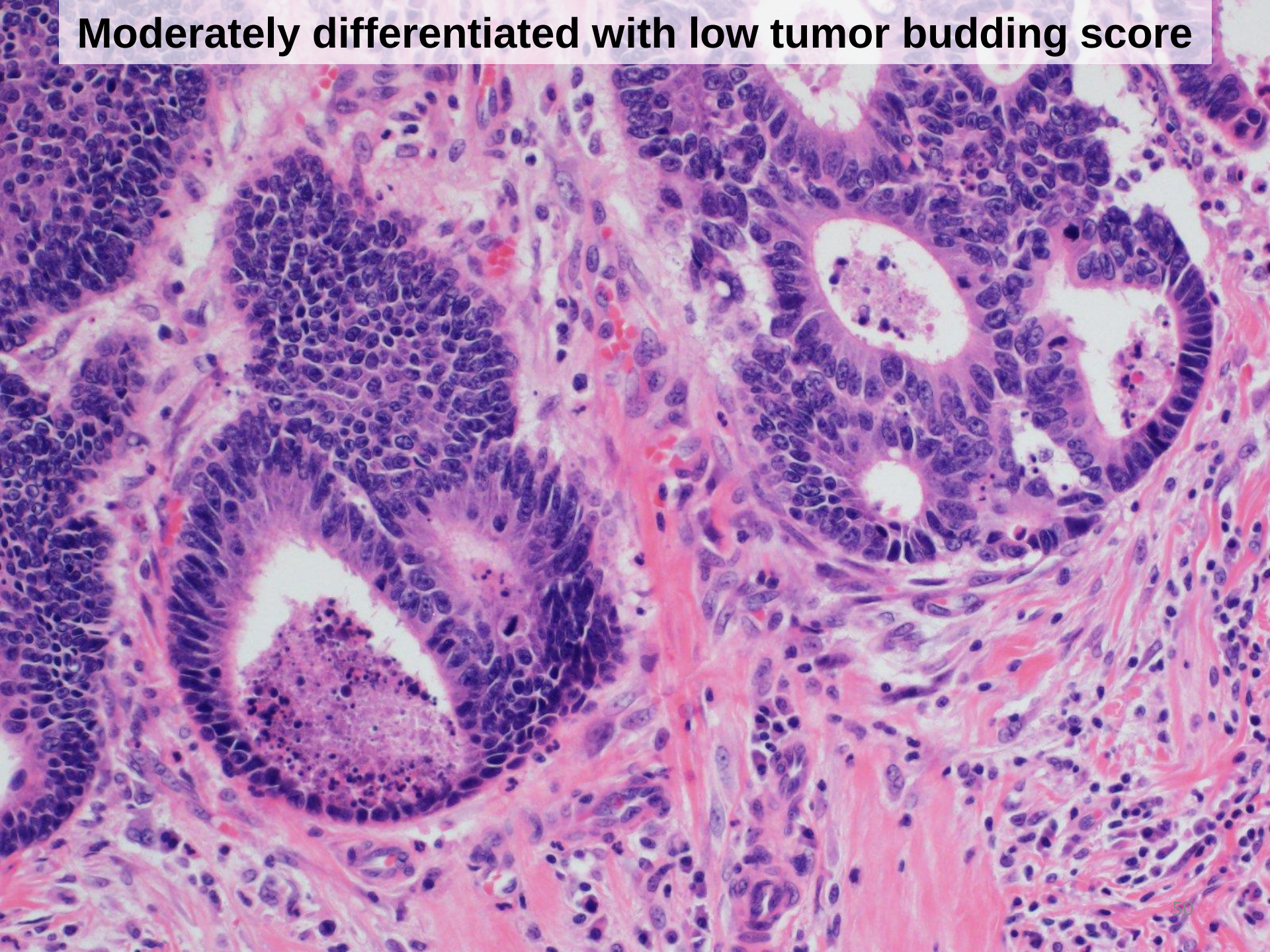
Grade	Definition
Well differentiated	>95% gland formation
Moderately differentiated	50-95% gland formation
Poorly differentiated	<50% gland formation
Undifferentiated	No gland formation or mucin; No squamous or neuroendocrine differentiation

The 2-tiered grading scheme (Low-Grade / High-Grade) is no longer advocated by the AJCC 8th edition and CAP protocol.

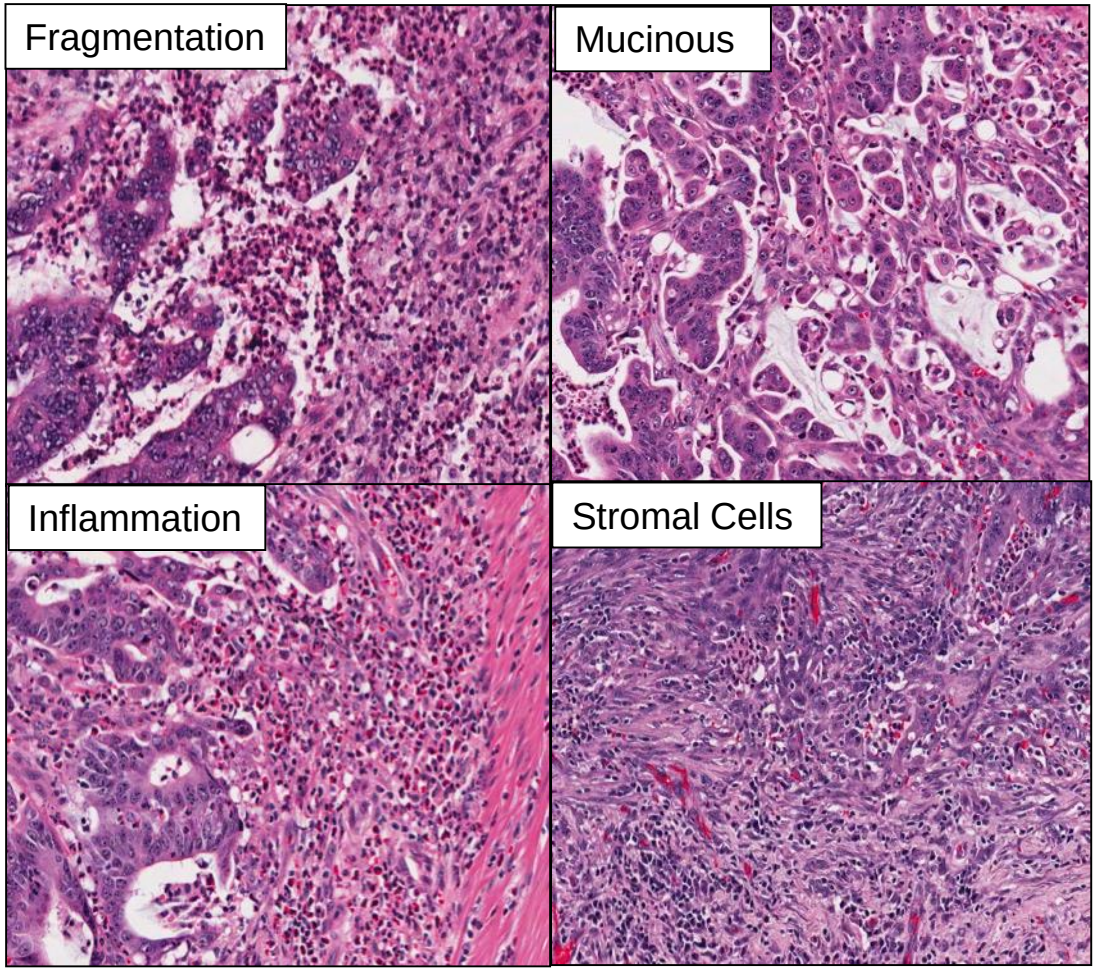
Moderately differentiated with high tumor budding score



Moderately differentiated with low tumor budding score

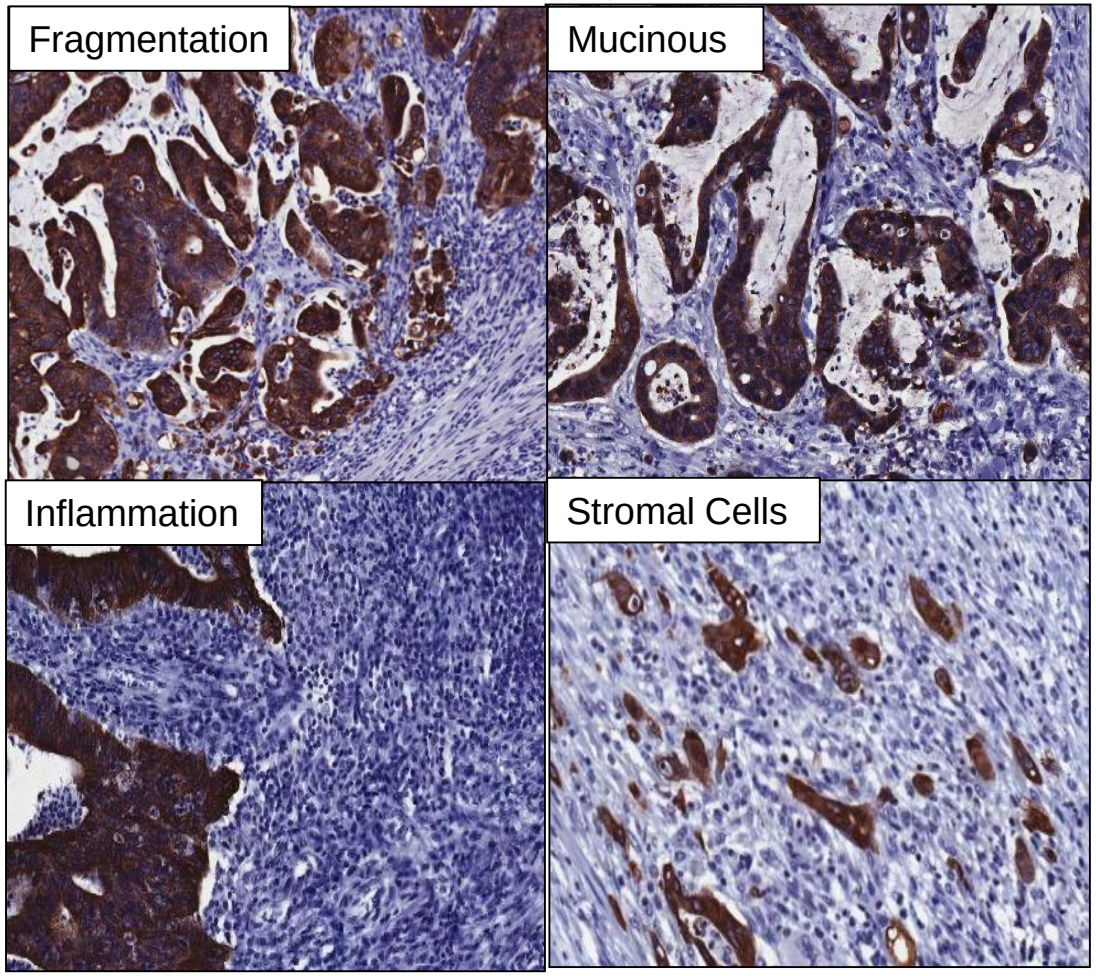


Tumor budding: *Challenging scenarios*



Features that can obscure tumor budding

Tumor budding: *Challenging scenarios*



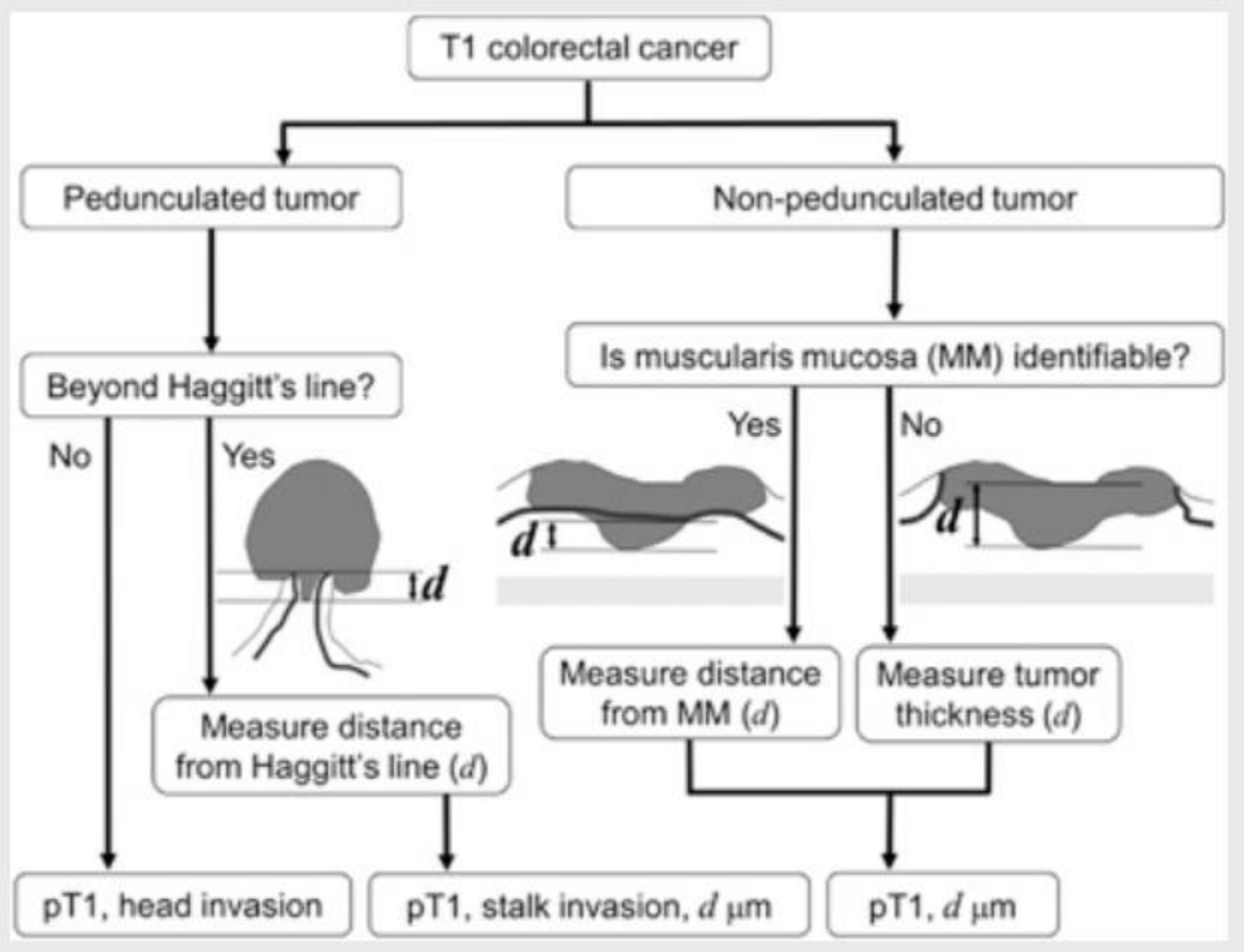
- **CAP protocol: cytokeratin immunohistochemistry can be used to identify buds at leading edge in challenging scenarios.**
- ***<u>However, the scoring should still be performed using H&E stained sections.</u>***

Malignant Polyp: Depth of invasion and risk of lymph node metastasis

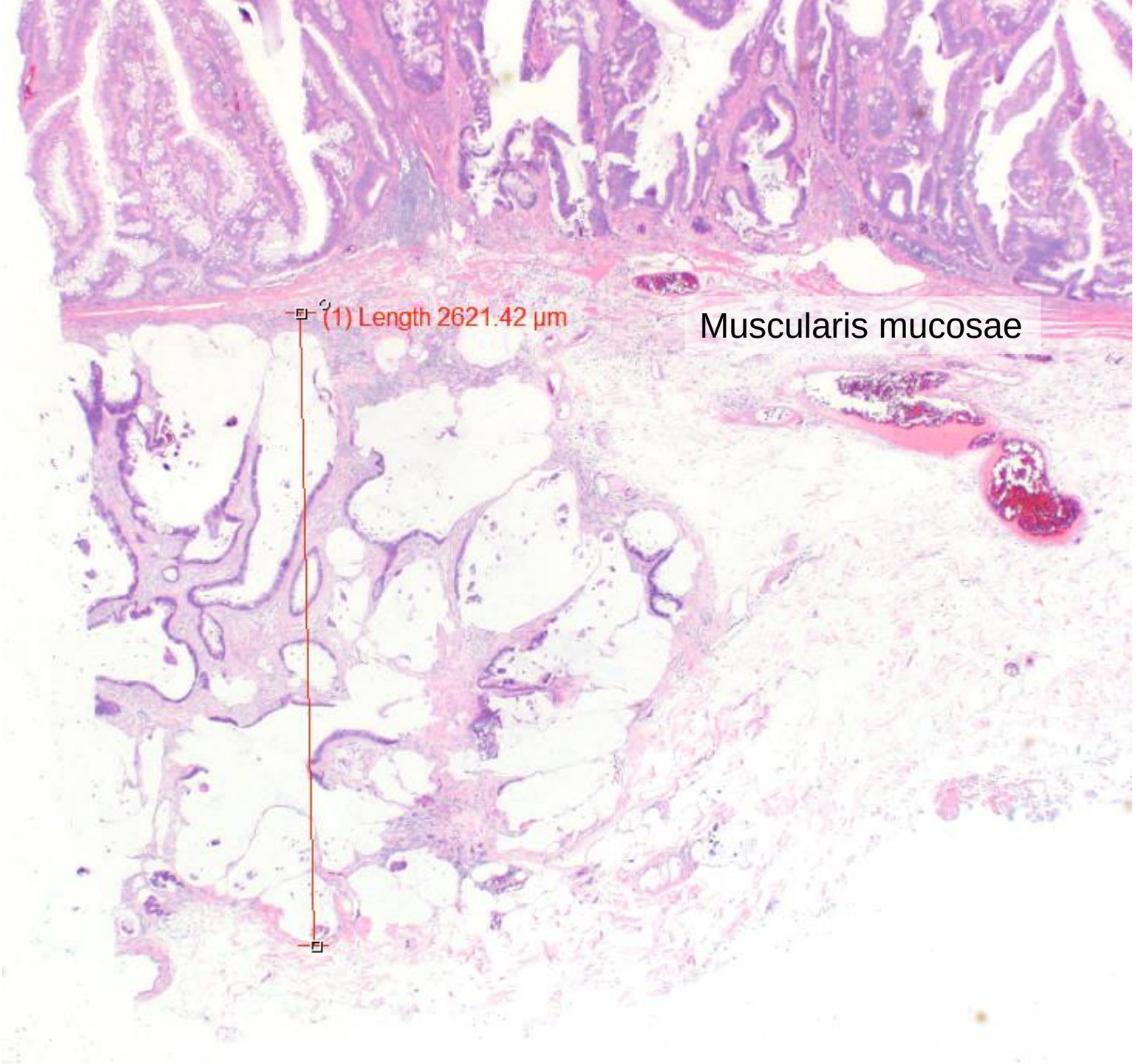
Study	Number of tumors analyzed (Number with lymph node metastasis)	Depth of submucosal invasion % in node-negative vs. % in node-positive cases
Ueno, et al 2004	251 (33)	Using ≥ 2000 µm 52% vs. 91% (p<0.0001)
Nakadoi et al 2011	499 (41)	Using ≥ 1800 µm 48% vs. 83% (p<0.0001)
Tateishi et al 2010	322 (46)	**Using ≥ 1000 µm** 88% vs. 98% (p=0.05)
Kawachi et al 2015	806 (97)	**Using ≥ 1000 µm** 76% vs. 96% (p<0.0001)
Oka et al 2013	118 (13) Rectal only	**Using ≥ 1000 µm** 73% vs. 92% (p=0.18)
Ueno, et 2014 (30 hospital consortium)	3556 (393)	**Using ≥ 1000 µm** 84% vs. 95% (p<0.0001)
Pai et al 2017	116 (28)	**Using ≥ 1000 µm** 60% vs. 81% (p=0.04)

Depth of submucosal invasion ≥ 1000 µm is associated with increased risk of nodal metastasis.

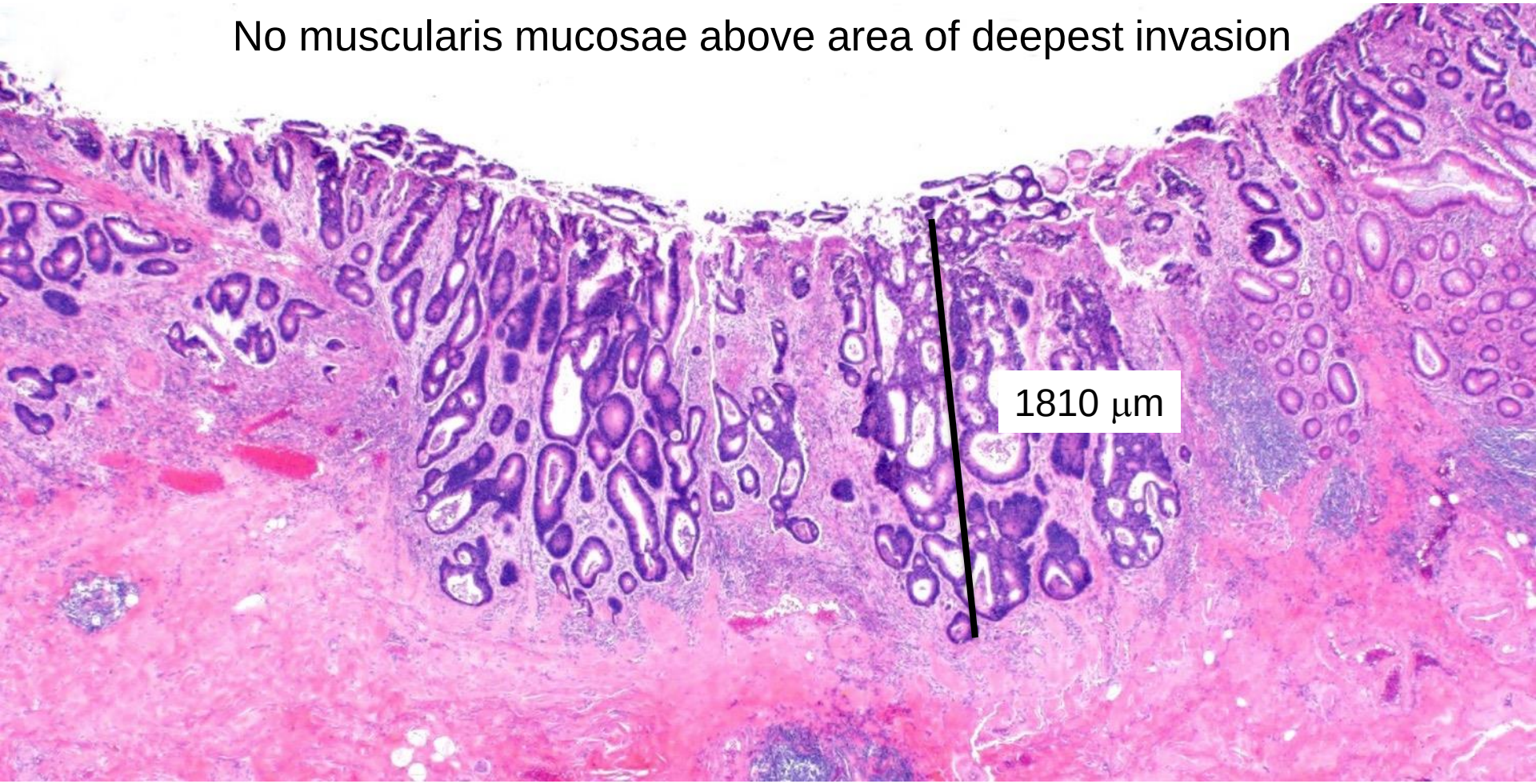
Malignant Polyp: Measuring depth of submucosal invasion



Kawachi H, et al. Mod Pathol. 2015 Jun;28(6):872-9.


(1) Length 2621.42 µm
Muscularis mucosae

No muscularis mucosae above area of deepest invasion

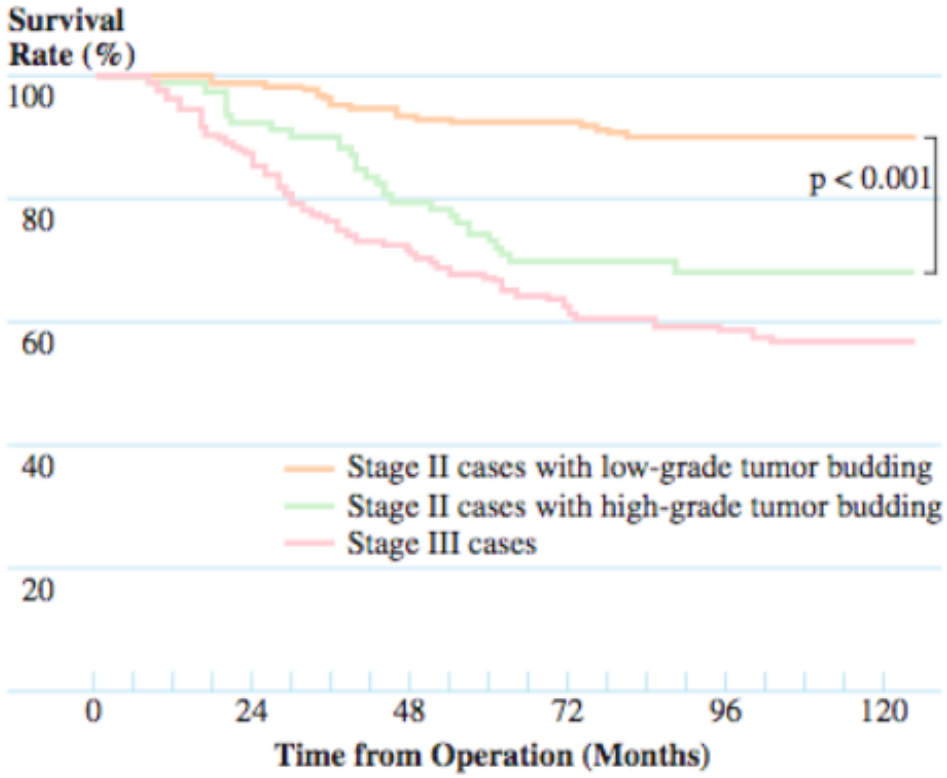


Malignant Polyp on Polypectomy: To surgically resect or not?

- NCCN (2018) Unfavorable Features:
  - **Poorly differentiated tumor**
  - **Lymphovascular invasion**
  - **Margin status (positive, <1 or 2 mm, or cannot be assessed in piecemeal resection)**
  - **Tumor budding**
    - Most studies suggest ≥5 buds per 0.785 mm^2 (intermediate or high tumor bud score) is associated with increased risk of lymph node metastasis.
  - **Depth of submucosal invasion (not yet included in CAP protocol or NCCN guidelines)**

Tumor Budding as a high-risk factor for stage II colon cancers?

FIGURE 3. Comparison of postoperative survival between patients who had Stage II colon cancer with low-grade tumor budding or high-grade tumor budding and patients Stage III colon cancer.



At least 10 studies have analyzed tumor budding in stage II colon cancer and demonstrate worse outcomes for **high tumor budding score (≥10 tumor buds per 0.785 mm^2)**.

Tumor Budding as a high-risk factor for stage II colon cancers?

High risk factors (NCCN)

- Poorly differentiation (except MSI-high)
- Lymphovascular invasion
- Bowel obstruction
- <12 lymph nodes
- Perineural invasion
- Localized perforation (pT4)
- Close/indeterminate margins
- ***High tumor budding?? Recommended to be included in pathology reports by CAP but currently not included in NCCN (2018)***

Stage II with high tumor budding

- Most studies have shown poor overall and disease-free survival for patients with stage II colorectal carcinoma and high tumor budding.

Isolated Tumor Cells in Lymph Nodes?

Isolated Tumor Cells (ITC): single tumor cells or small clusters of cells ≤0.2 mm in greatest dimension.

Micrometastases: tumor foci >0.2 mm but ≤2.0 mm.

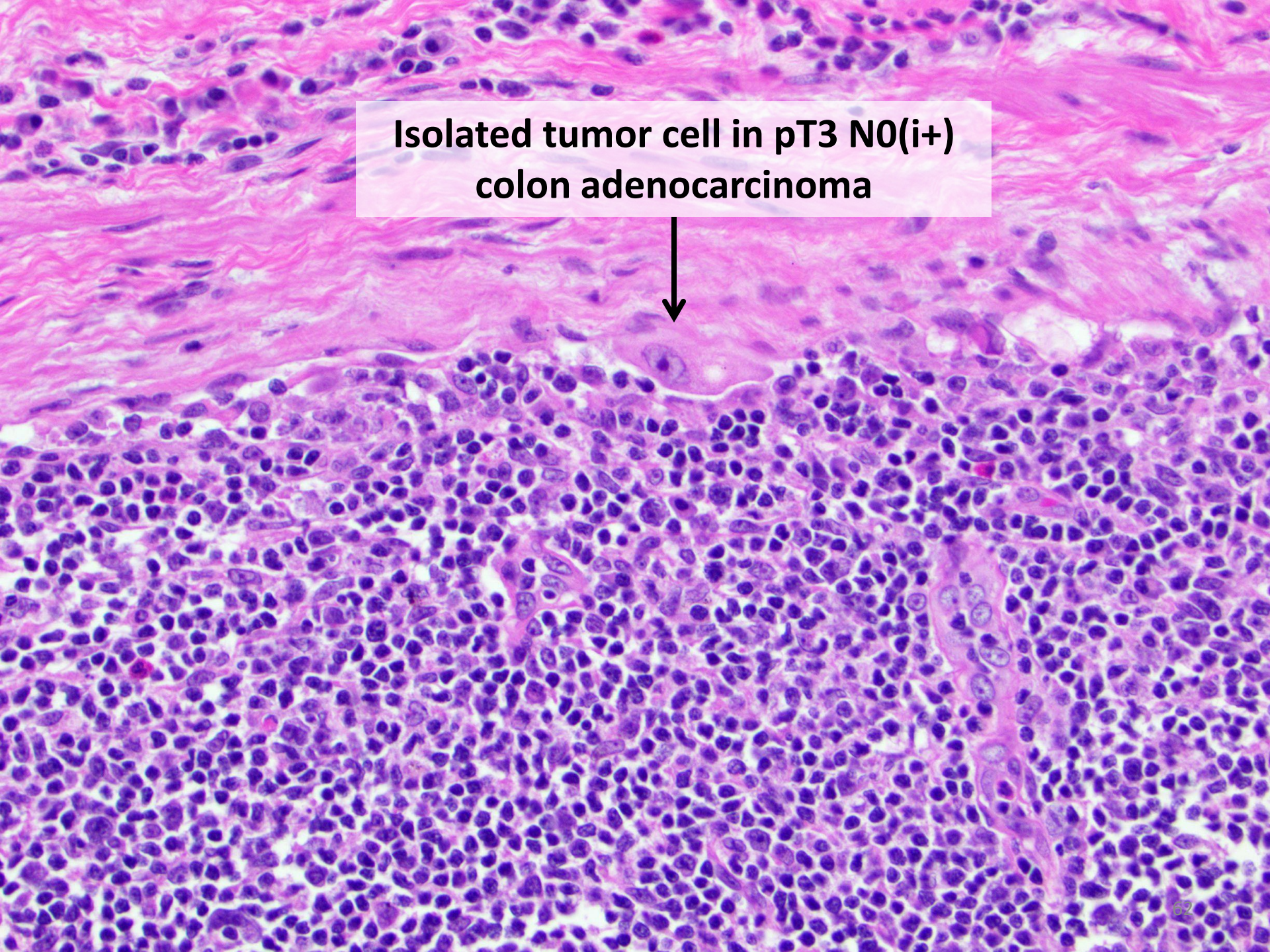
Reference	Study Design	Findings
Sloothak DA et al. Eur J Surg Oncol 2014;40:263-9.	Meta-analysis including 5 studies **(using H&E)**	• Increased risk of recurrence with micrometastasis • **No increased risk of recurrence with ITC**
Mescoli C et al. J Clin Oncol 2012;30:965-971.	312 patients **using CK IHC** in N0 tumors	• **Higher recurrence (14% vs. 4.7%) in patients with ITCs.**
Protic M, et al. J Am Coll Surg 2015;221:643-651.	203 patients **using CK IHC** in N0 tumors	• **Higher recurrence (16.7% vs. 2.6%) in patients with ITCs.** • Decreased in survival due to ITC seen in patients with T3/T4 but not T1/T2 tumors.

Isolated Tumor Cells in Lymph Nodes?

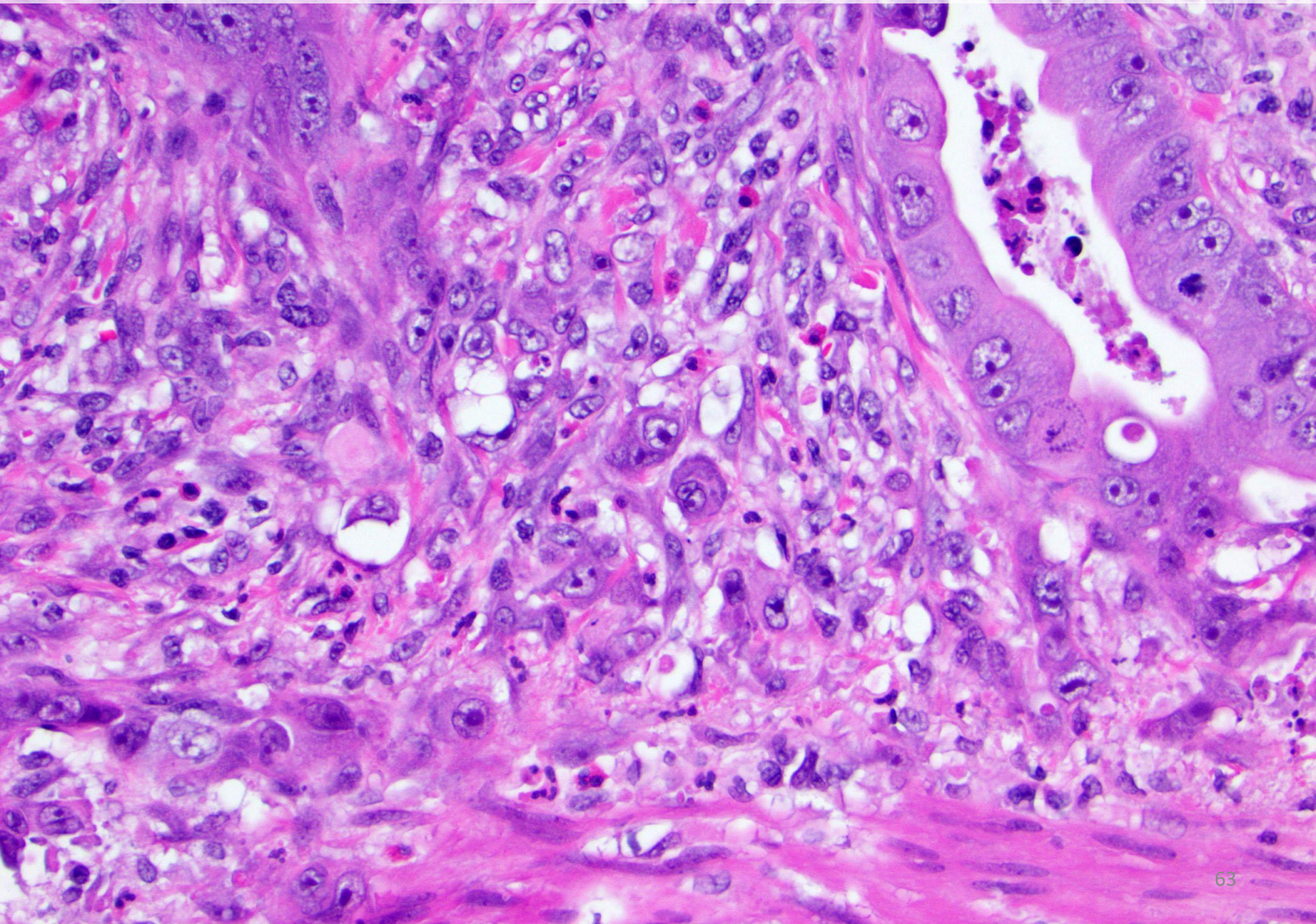
N1 category in AJCC 8th Edition: "One to three regional lymph nodes are positive (tumor in lymph nodes >0.2 mm)…"

- **Isolated tumor cells (ITCs) classified as pN0(i+).**
  - CAP recommendations:
    - Only use H&E stains to identify tumor cells in lymph nodes.
    - Cytokeratin immunohistochemistry is ***not*** recommended.
    - Provide an explanatory comment in the pathology report.

- **Micrometastases classified as pN1.**
  - There is no pN1*mi* designation for colorectal carcinoma.


Isolated tumor cell in pT3 N0(i+)
colon adenocarcinoma

Moderately differentiated with high tumor budding score

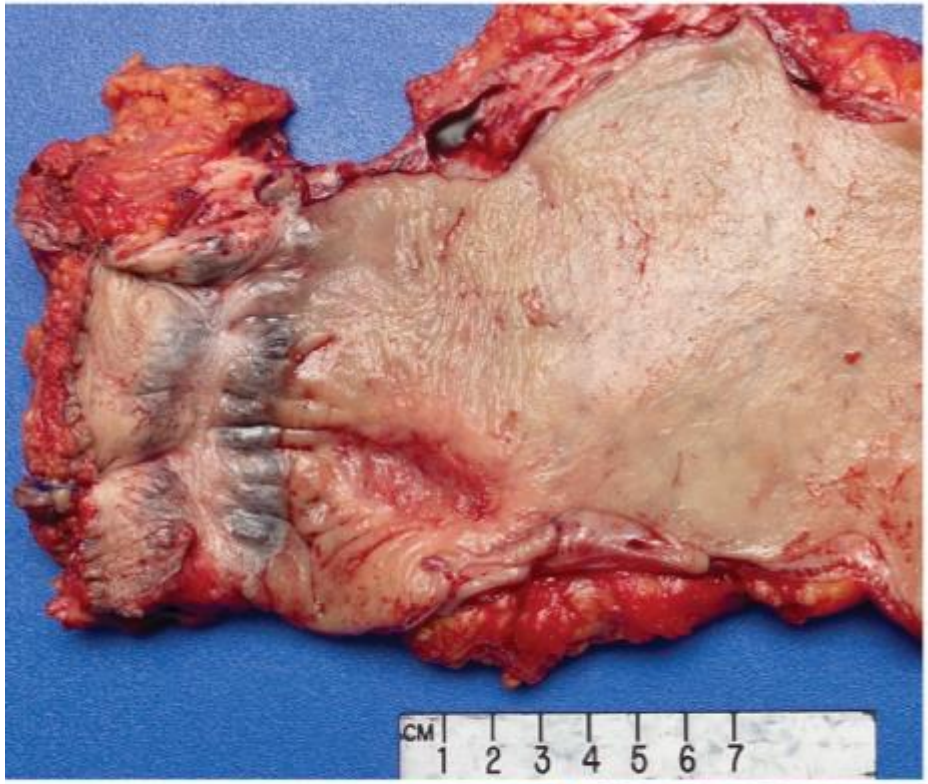


Assessing Treatment Response

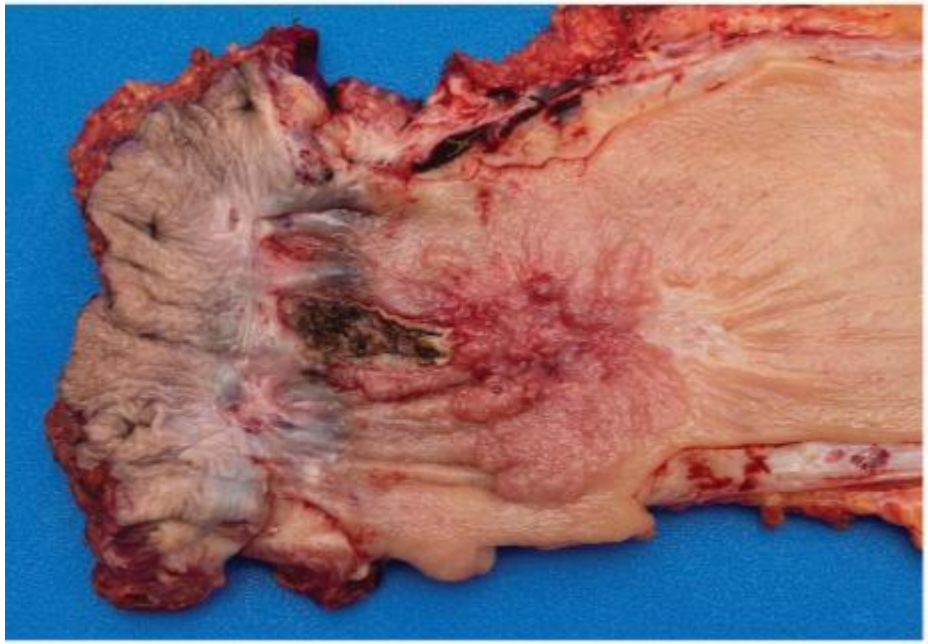
- CAP 2018 cancer protocol:

Treatment Effect (Note K)
___ No known presurgical therapy
___ Present
+ ___ No viable cancer cells (complete response, score 0)
+ ___ Single cells or rare small groups of cancer cells (near complete response, score 1)
+ ___ Residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells (partial response, score 2)
___ Absent
+ ___ Extensive residual cancer with no evident tumor regression (poor or no response, score 3)
___ Cannot be determined

- "+" means optional
- Should we do it? Is it useful?

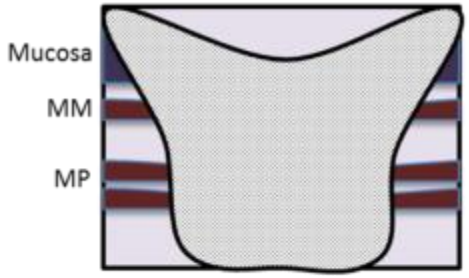
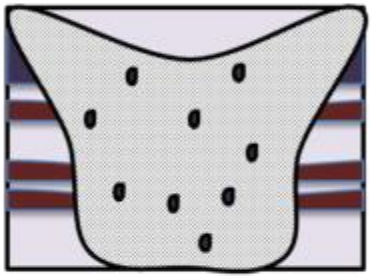
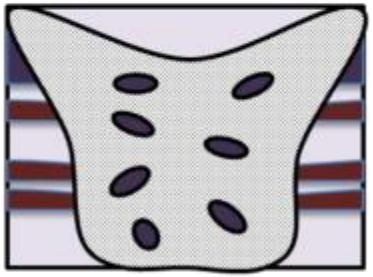
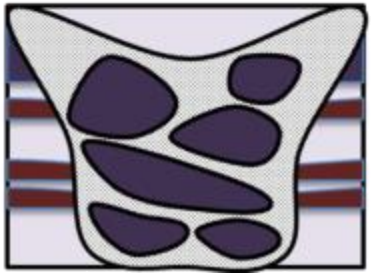


4.0 cm ulcer seen without obvious residual cancer on gross examination. The entire ulcer should be submitted for histologic examination.

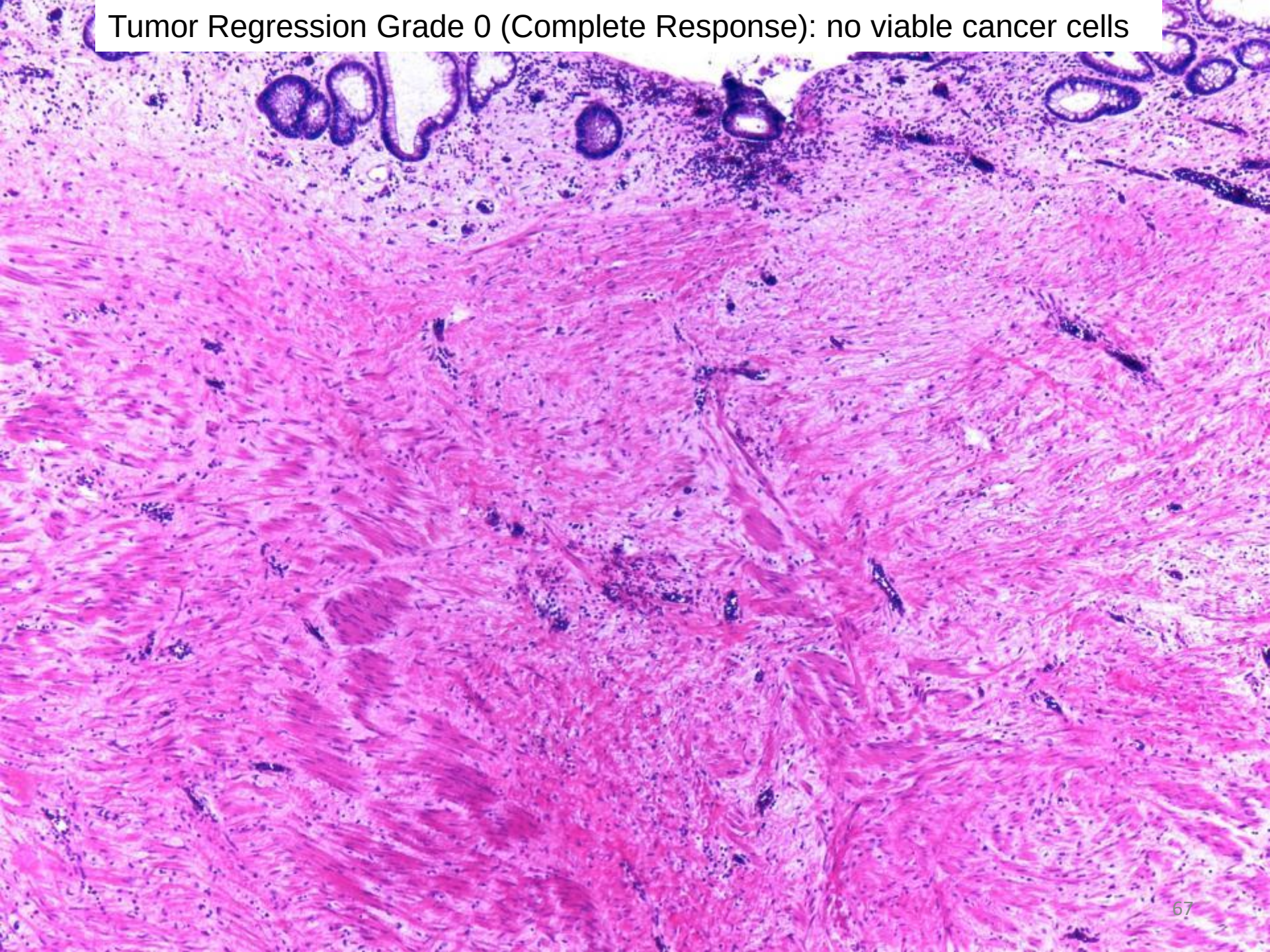


An obvious mass is seen in the rectum. Only representative sampling is needed.

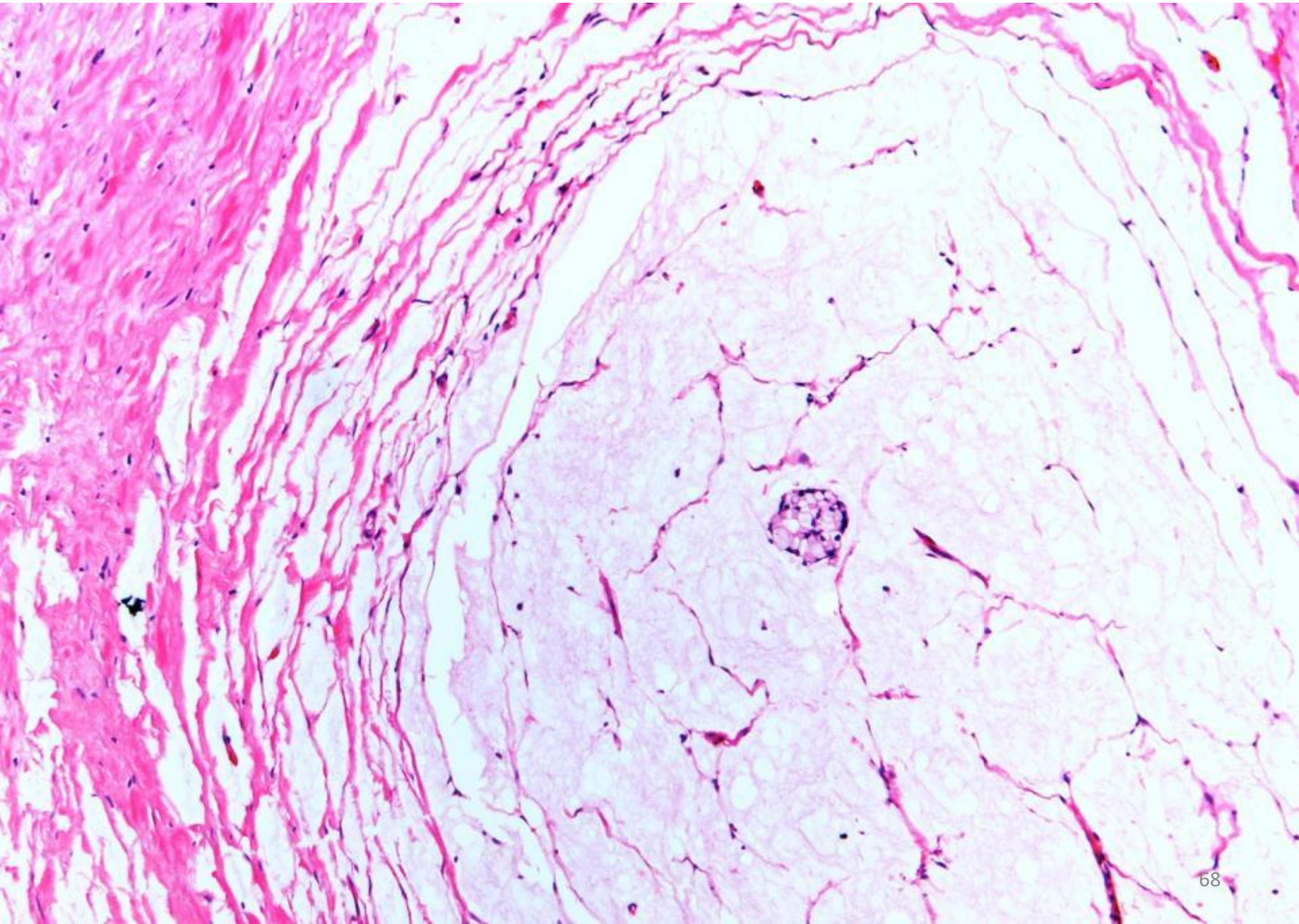
Tumor Regression Grade (TRG)

	TRG Score	CAP / Modified Ryan	% Viable Tumor
	0	Complete response; no viable cancer cells	0%
	1	Near complete response; Single cells or rare groups of cancer cells	1-10%
	2	Partial response; Residual cancer with evident tumor regression, but more than single cells or rare groups of cancer cells	11-50%
	3	Poor or no response; Extensive residual cancer with no evident tumor regression	>50%

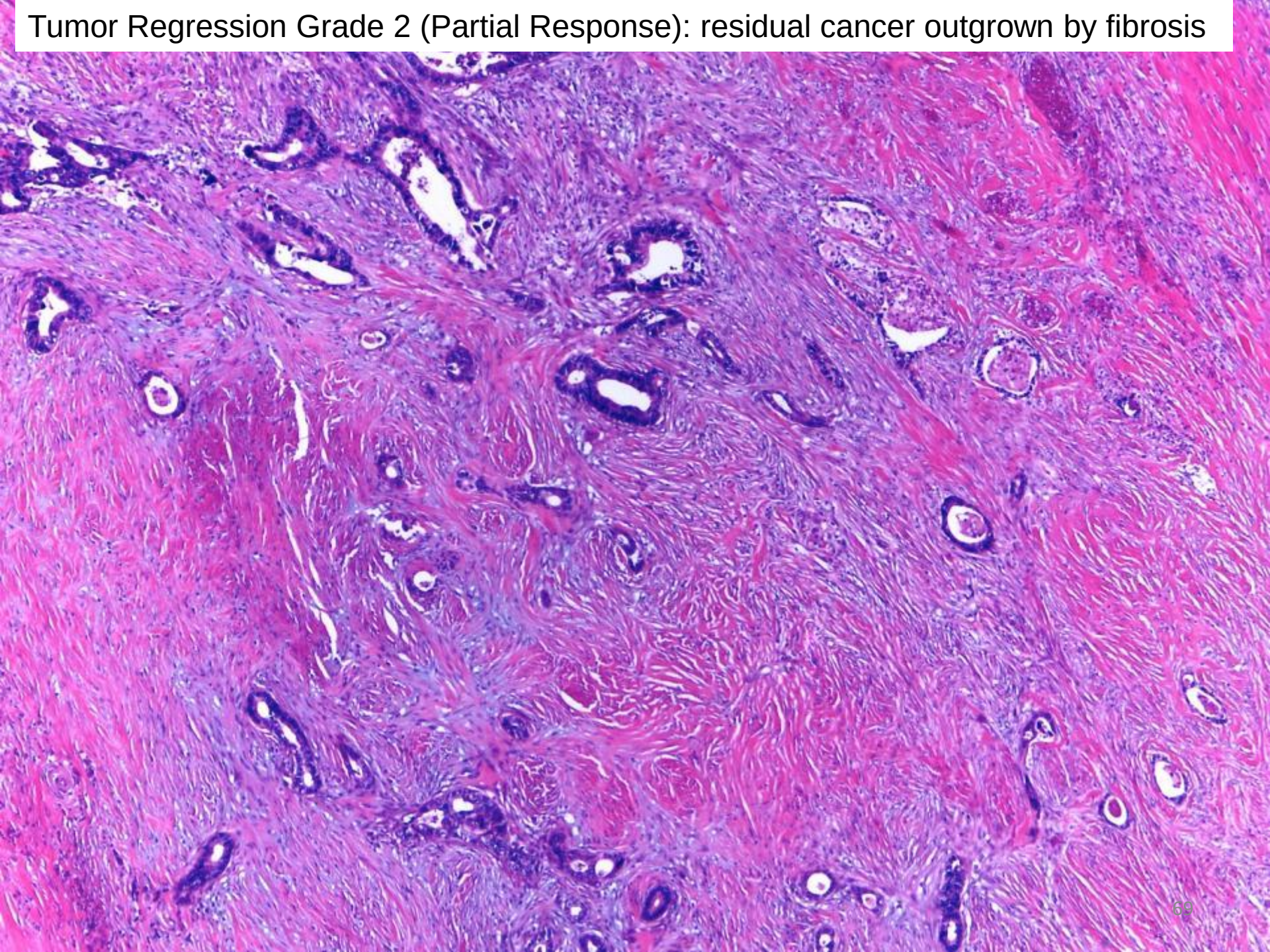
Tumor Regression Grade 0 (Complete Response): no viable cancer cells



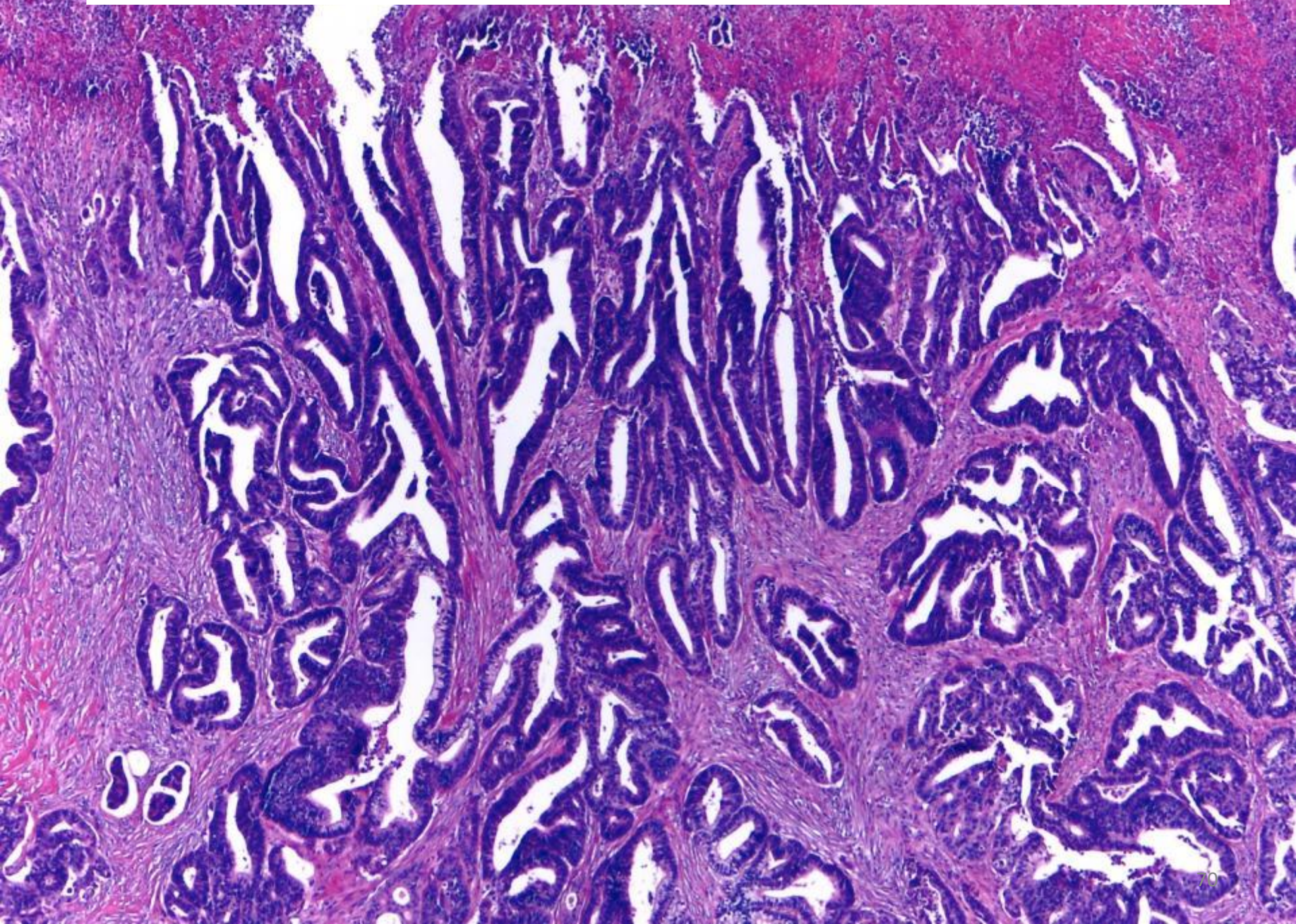
Tumor Regression Grade 1 (Near Complete Response): single or small groups of cancer cells



Tumor Regression Grade 2 (Partial Response): residual cancer outgrown by fibrosis



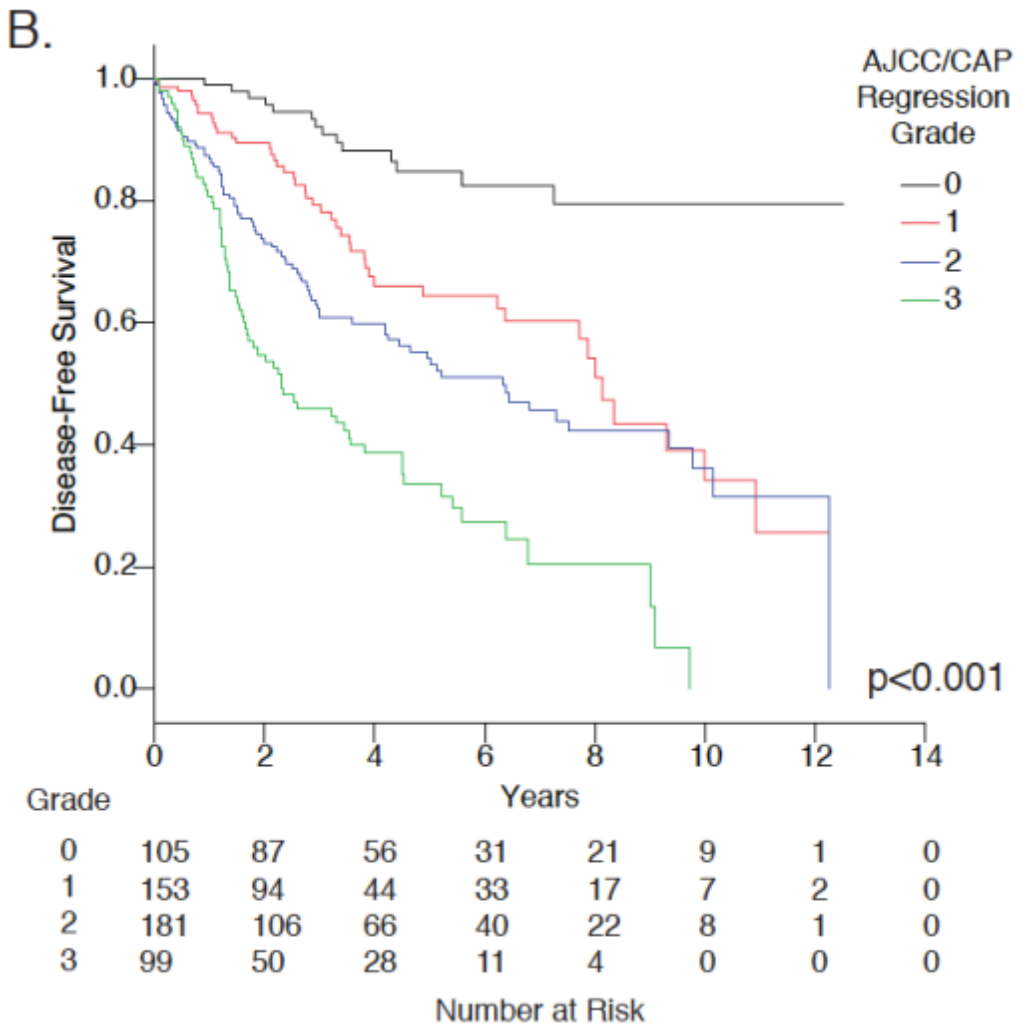
Tumor Regression Grade 3 (Poor or No Response): extensive residual cancer



Tumor Regression Grade & Survival

Study Design

- 538 rectal cancers with neoadjuvant therapy
- Graded according to AJCC/CAP regression grade.
- Assessed the ability of AJCC/CAP regression grade to predict survival and recurrence.
- Tumor regression grade is statistically significant even when controlling for pathologic stage (p<0.001).



Mace, A, et al. Dis Colon Rectum. 2015;58:32-44.

Take Home Points

AJCC 8th Edition / CAP Cancer Protocol Updates

- Importance of correctly identifying pT4a.
- Venous invasion – prognostic significance and utility of elastin stains.
- Tumor deposits vs. lymph node metastasis – changing AJCC8 definitions.
- Isolated tumor cells (N0(i+)) is now explicitly included in colorectal carcinoma staging.
- Peritumoral tumor budding – helpful in pT1 malignant polyps and stage II colon cancer.
- Assessing treatment response using CAP tumor regression score is prognostically relevant following neoadjuvant therapy.

Molecular Biomarkers for the Evaluation of Colorectal Cancer

Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology

Antonia R. Sepulveda, MD, PhD,[1] Stanley R. Hamilton, MD, PhD,[2] Carmen J. Allegra, MD,[5] Wayne Grody, MD, PhD,[6] Allison M. Cushman-Vokoun, MD, PhD,[7] William K. Funkhouser, MD, PhD,[8] Scott E. Kopetz, MD, PhD,[3] Christopher Lieu, MD,[9] Noralane M. Lindor, MD,[10] Bruce D. Minsky, MD,[4] Federico A. Monzon, MD,[11] Daniel J. Sargent, PhD,[12] Veena M. Singh, MD,[13] Joseph Willis, MD,[14] Jennifer Clark, SCT, MB(ASCP)[cm],[15] Carol Colasacco, MLIS,[16] R. Bryan Rumble, MSc,[17] Robyn Temple-Smolkin, PhD,[18] Christina B. Ventura, MT(ASCP),[16] and Jan A. Nowak, MD, PhD[19]

- **Joint effort by CAP, ASCP, AMP, ASCO to establish guidelines for molecular evaluation of colorectal cancer.**

- **Twenty-one (!) guideline statements published.**
  - Mismatch repair status evaluation.
  - Testing for anti-EGFR therapy.
  - Quality improvement and turnaround time metrics.

Arch Pathol Lab Med 2017;141:625-657.

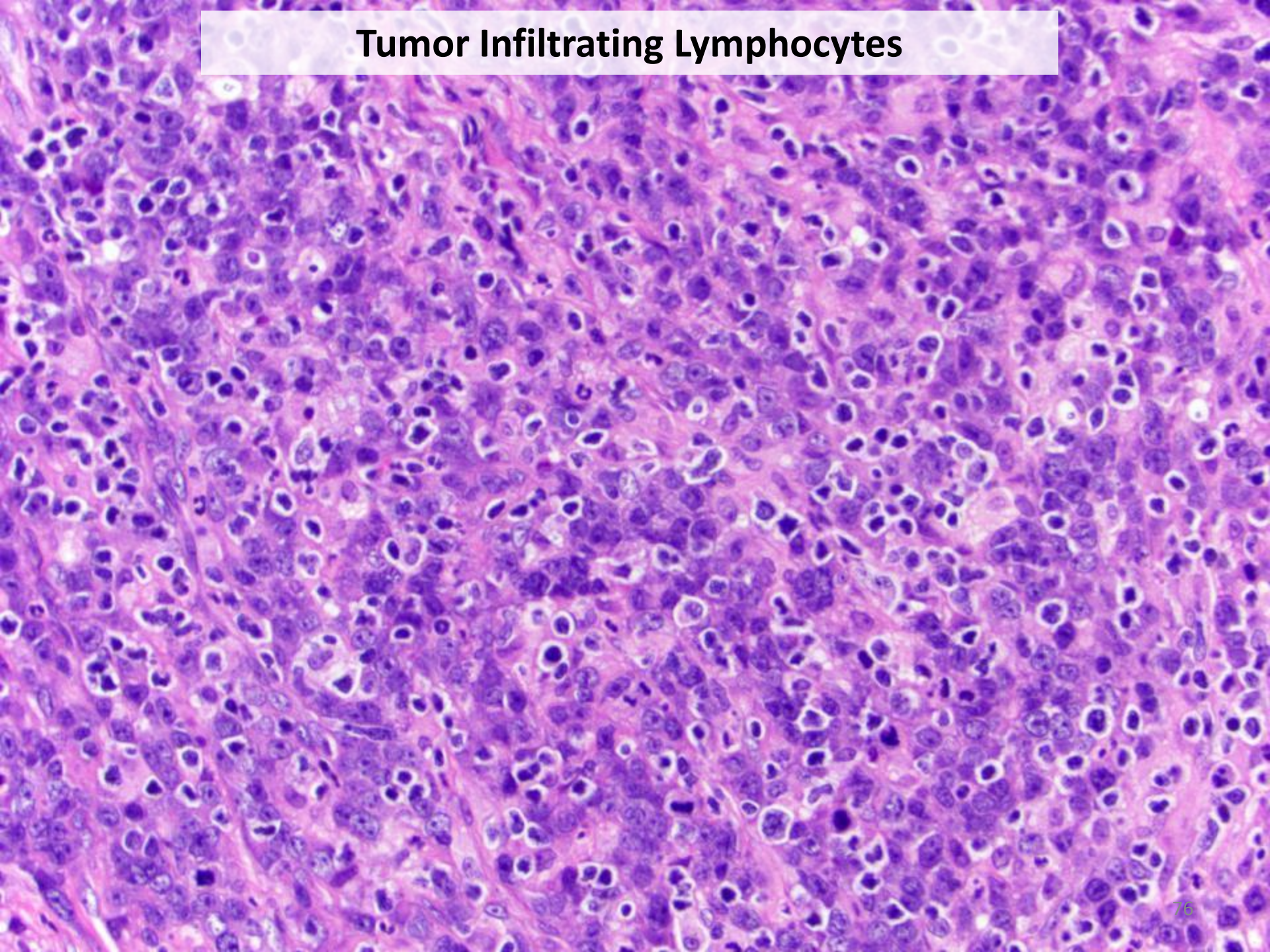
Lynch Syndrome Definition

- Germline mutations in DNA mismatch repair (MMR) genes:
  - *MLH1* (~35-40%)
  - *MSH2* (~40%)
  - *MSH6* (~10-15%)
  - *PMS2* (~5-10%)
- Deletions in *EPCAM/TACSTD1* (~2%)
  - Result epigenetic silencing of the *MSH2* gene by hypermethylation and loss of MSH2 and MSH6 expression.

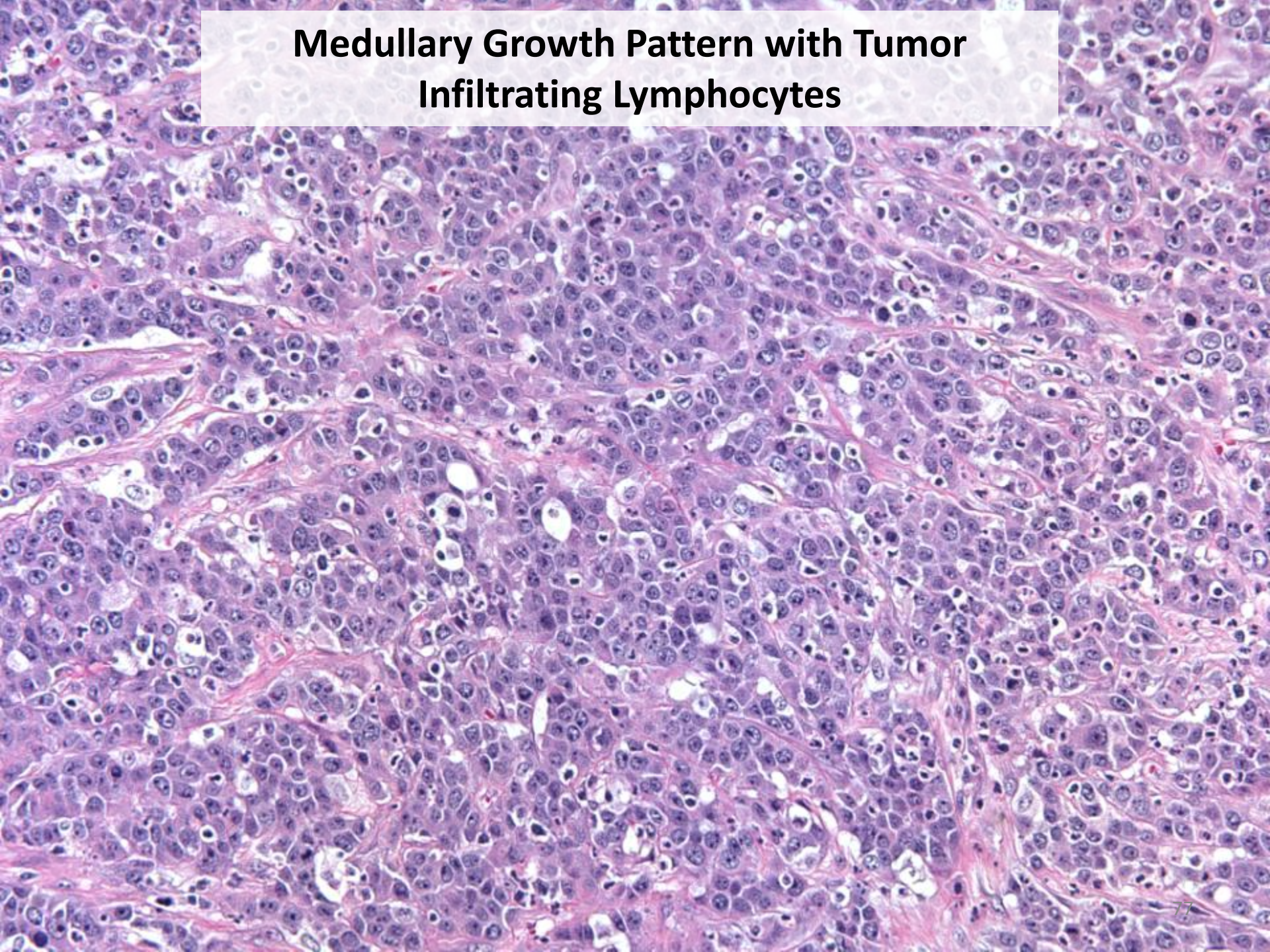
Who to screen for Lynch Syndrome?

- **Universal screening of all patients with CRC**
  - Endorsed by the following organizations:
    - National Comprehensive Cancer Network (NCCN), EGAPP (working group sponsored by the CDC), American Society of Medical Oncology (ASCO), US Multi-Society Task Force on Colorectal Cancer, American College of Gastroenterology (AGA)
- **Selective Screening of all patients <70 years of age & in patients >70 years fulfilling revised Bethesda guidelines** (misses up to 5% of patients with Lynch syndrome)
  - Endorsed as an option by the following organizations:
    - National Comprehensive Cancer Network (NCCN) and the American Society of Medical Oncology (ASCO)

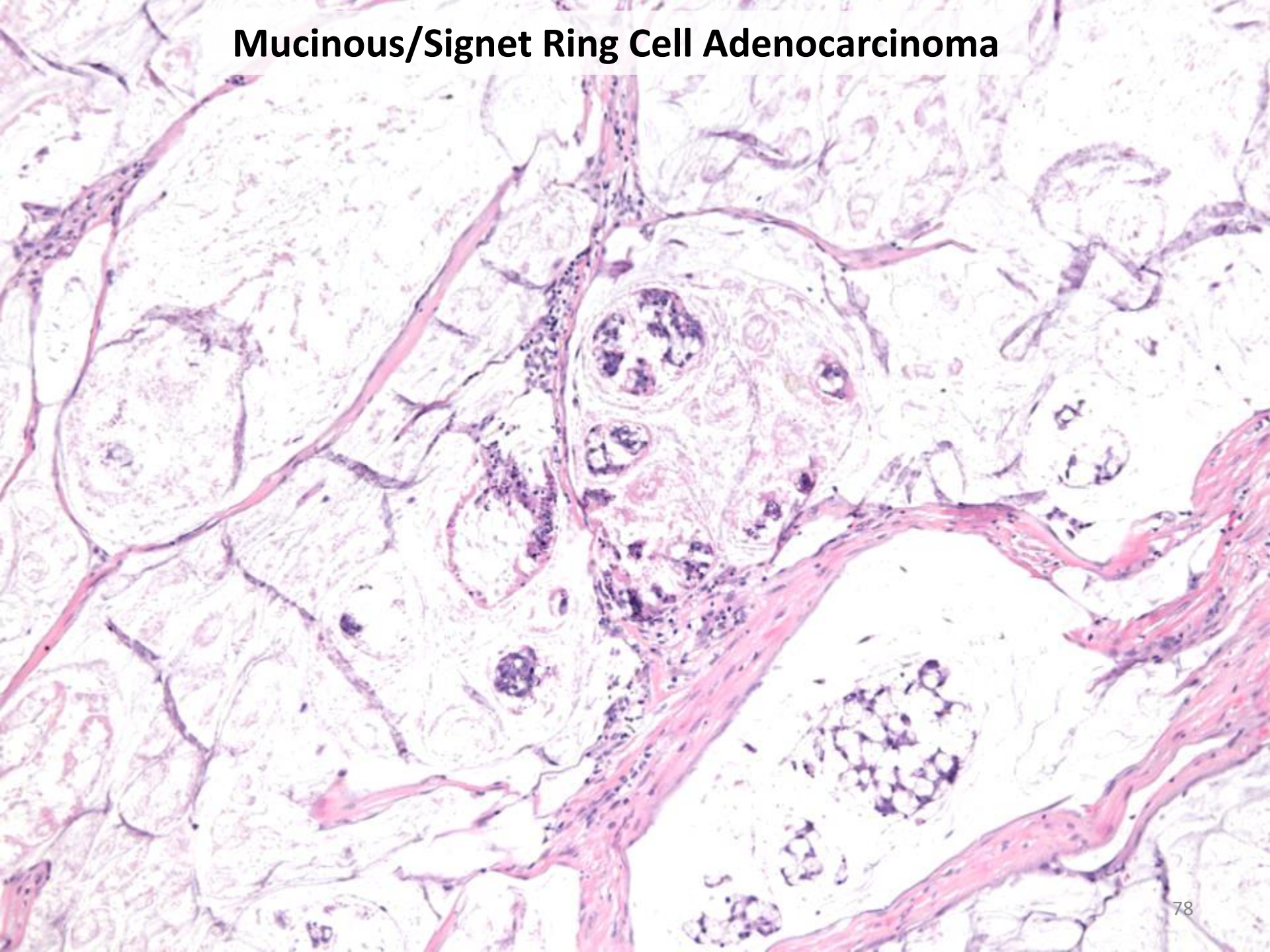
Tumor Infiltrating Lymphocytes



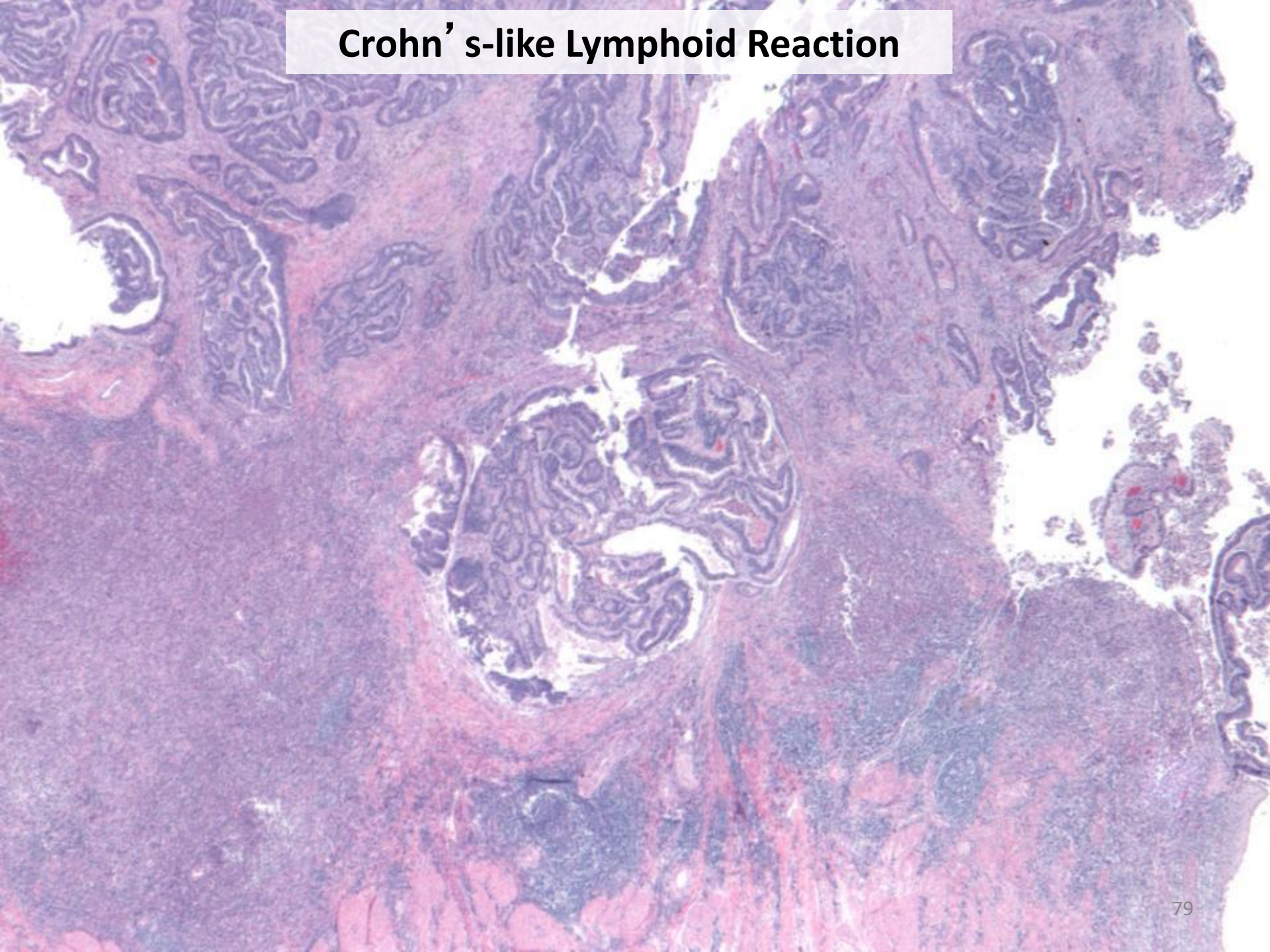
Medullary Growth Pattern with Tumor Infiltrating Lymphocytes



Mucinous/Signet Ring Cell Adenocarcinoma



Crohn's-like Lymphoid Reaction



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Recommendation

- Mismatch repair status testing in patients with colorectal cancers should be performed for the identification of patients at high-risk for Lynch syndrome and/or prognostic stratification.

- Testing can be performed by immunohistochemistry or by MSI DNA-based testing.

Arch Pathol Lab Med 2017;141:625-657.

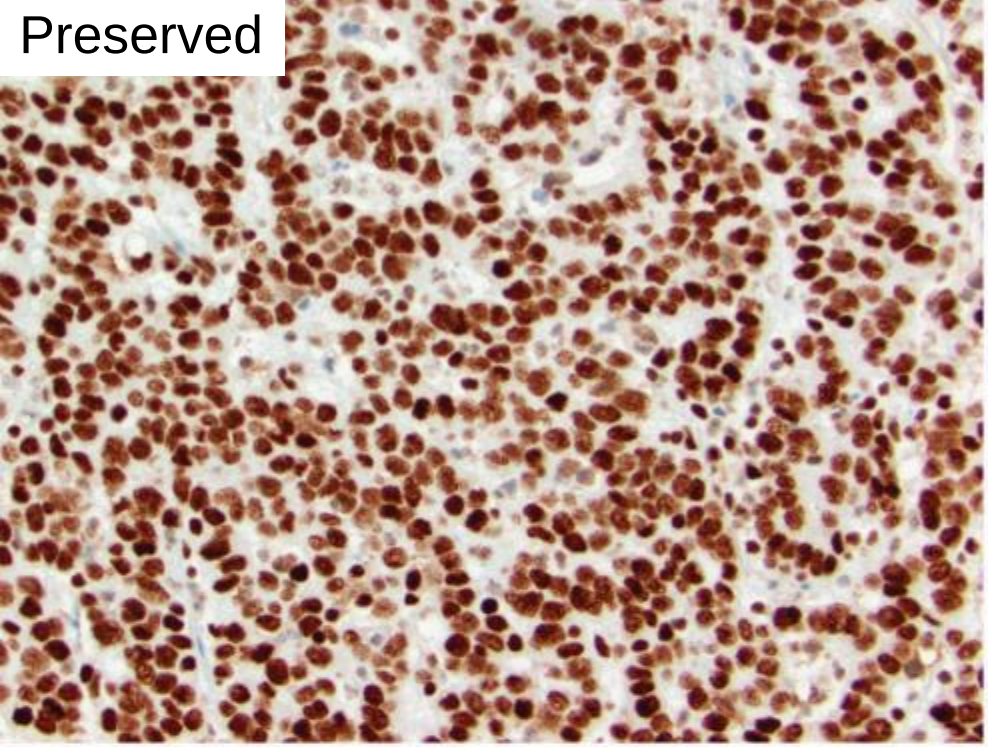
How to screen for Lynch Syndrome?

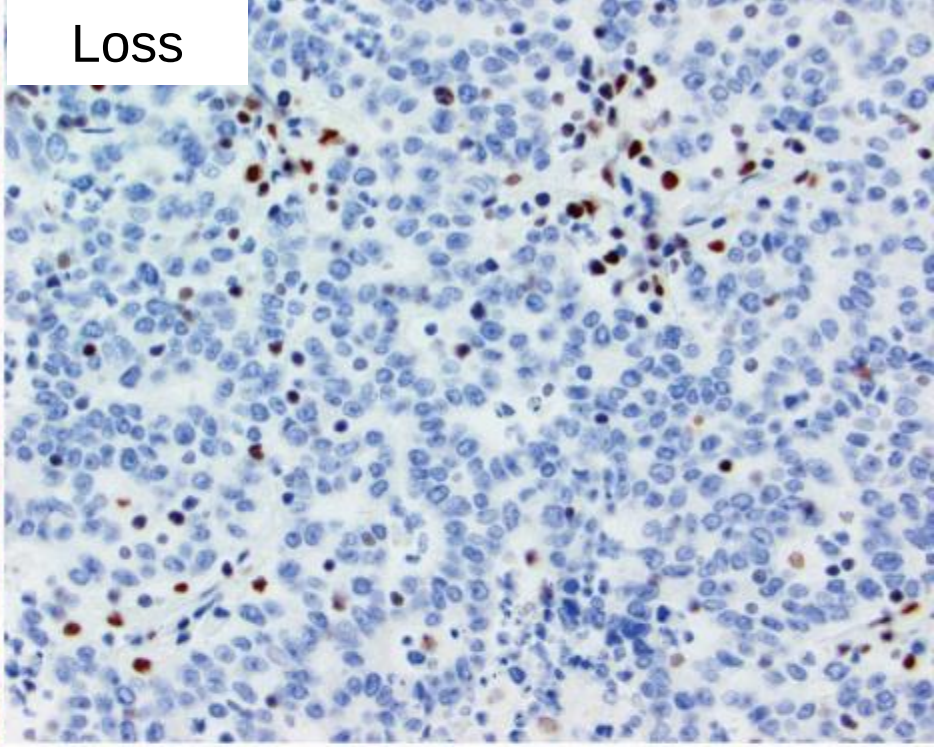
- **Mismatch repair (MMR) immunohistochemistry**
- **Microsatellite instability PCR**

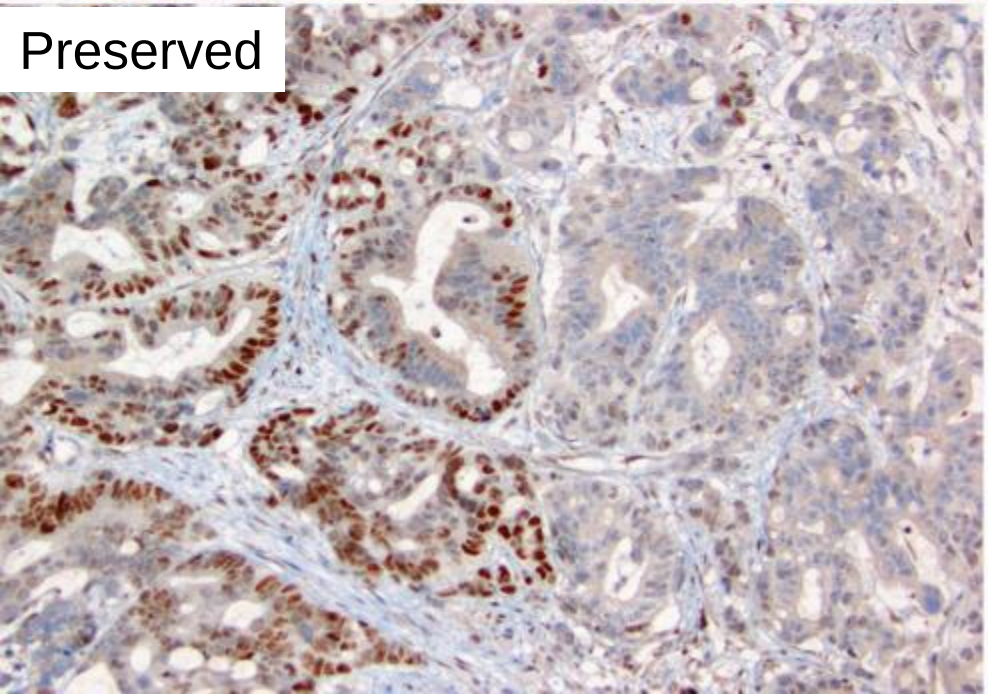
- Both MMR immunohistochemistry and MSI PCR have equivalent sensitivities (~95%).
- MMR immunohistochemistry has advantages:
  - Readily available in most pathology laboratories.
  - IHC results help to direct germline gene sequencing efforts.
  - In many cases, IHC allows for distinction between sporadic MMR protein deficiency and Lynch syndrome.

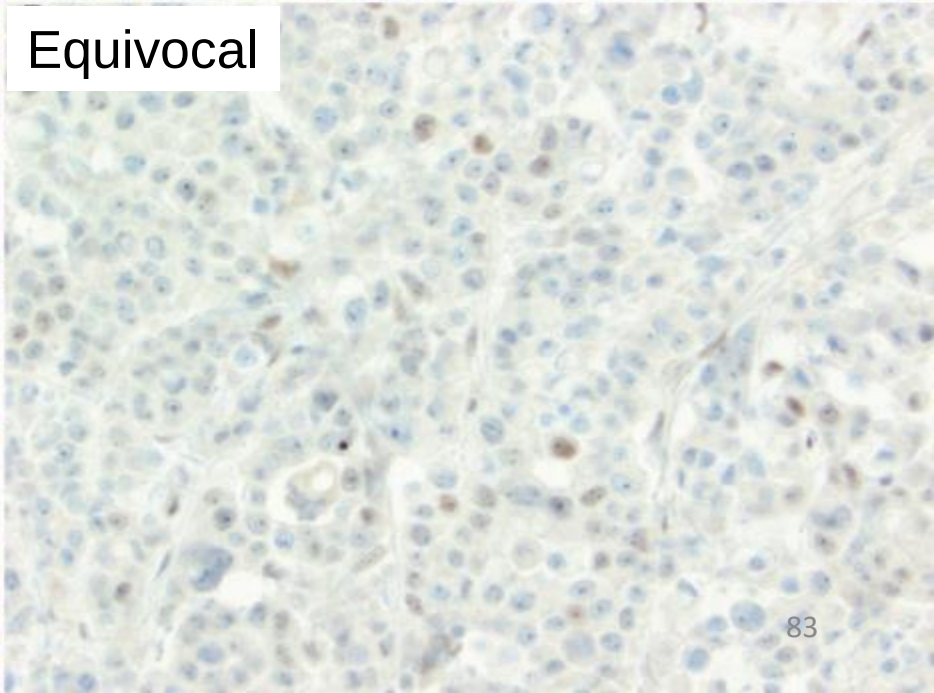
Mismatch Repair (MMR) Protein IHC

- Defective MMR genes results in ***loss of immunohistochemical expression***
- All 4 antibody testing (MLH1, PMS2, MSH2 and MSH6)
- If >10% of tumor nuclei demonstrate expression, then protein expression is preserved.
- If <10% of tumor nuclei demonstrate expression, then protein expression is equivocal. Repeat stain, or reflex to MSI PCR.
- **Must see <u>complete lack of staining</u> to call loss of expression.**


Preserved


Loss


Preserved


Equivocal

MMR IHC as a screening tool

IHC result	Most likely defective gene
Loss of MLH1 and PMS2	*MLH1*
Loss of MSH2 and MSH6	*MSH2* or *EPCAM*
Isolated loss of MSH6	*MSH6*
Isolated loss of PMS2	*PMS2* or *MLH1*

→ Seen in sporadic deficiency in DNA MMR protein expression and Lynch syndrome **(need to perform *BRAF* or *MLH1* promoter hypermethylation)**

→ Concerning for Lynch syndrome ***but not diagnostic***

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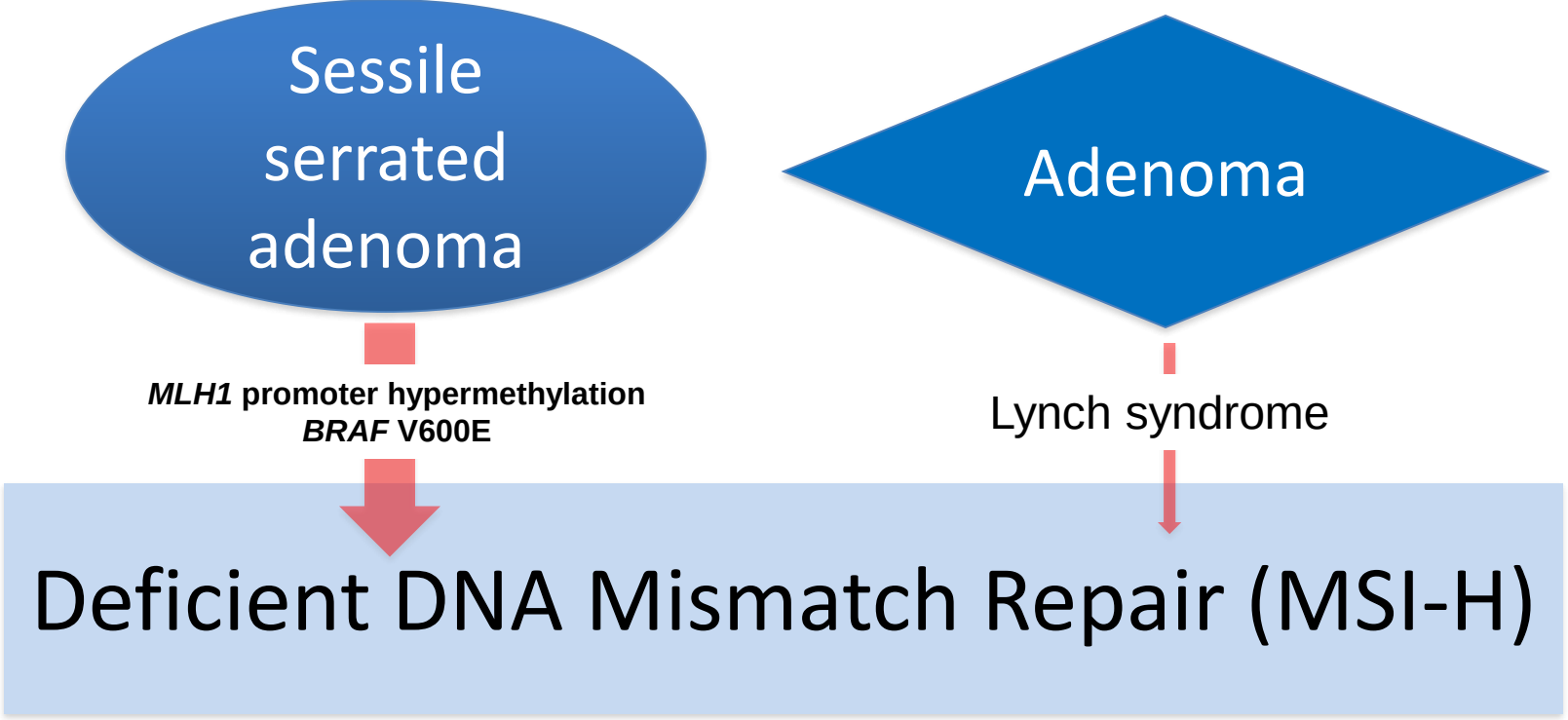
Recommendation

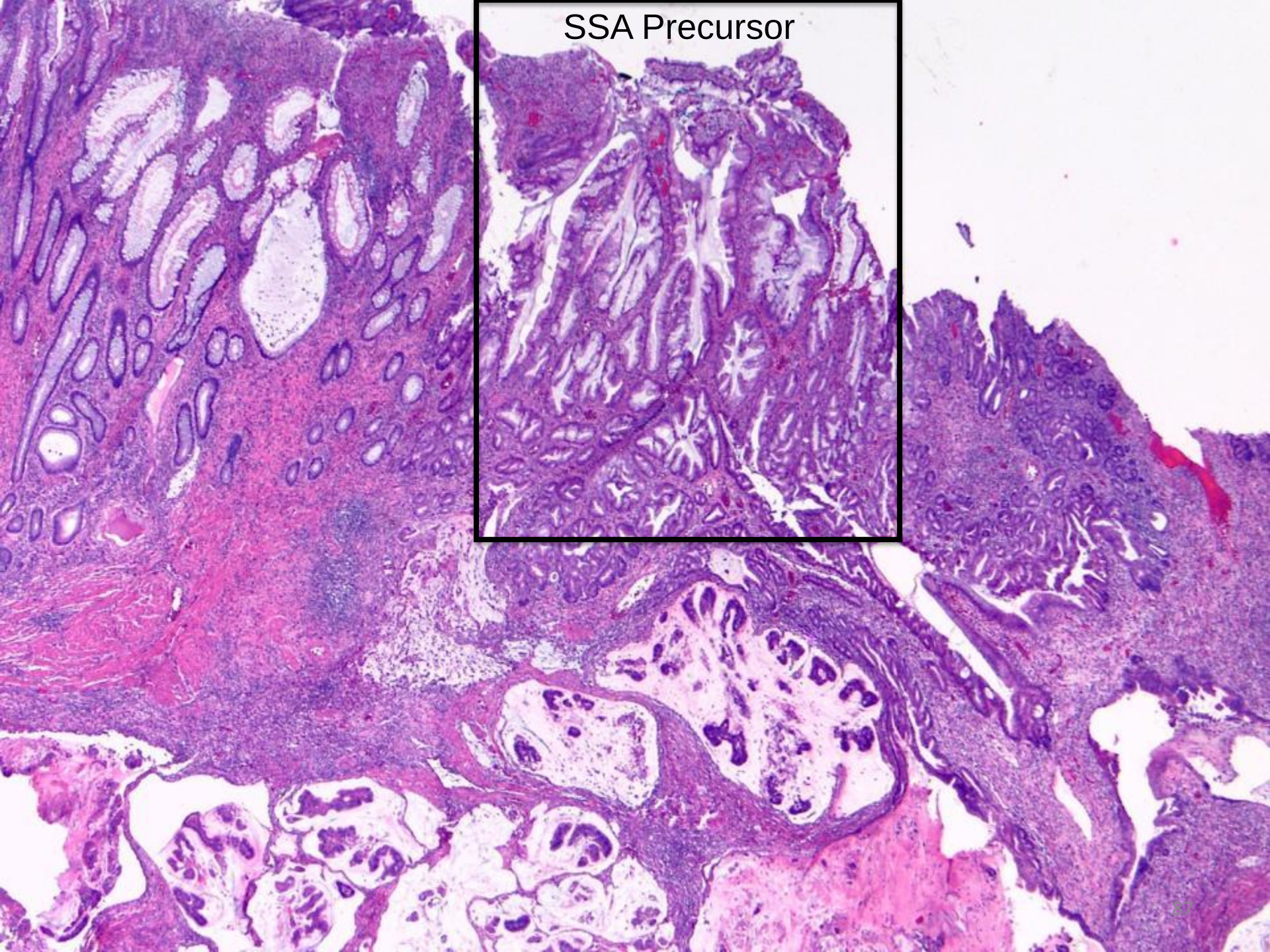
- *BRAF* p.V600E (BRAF c.1799) mutational analysis should be performed in MMR deficient tumors with loss of MLH1 to evaluate for Lynch syndrome risk.

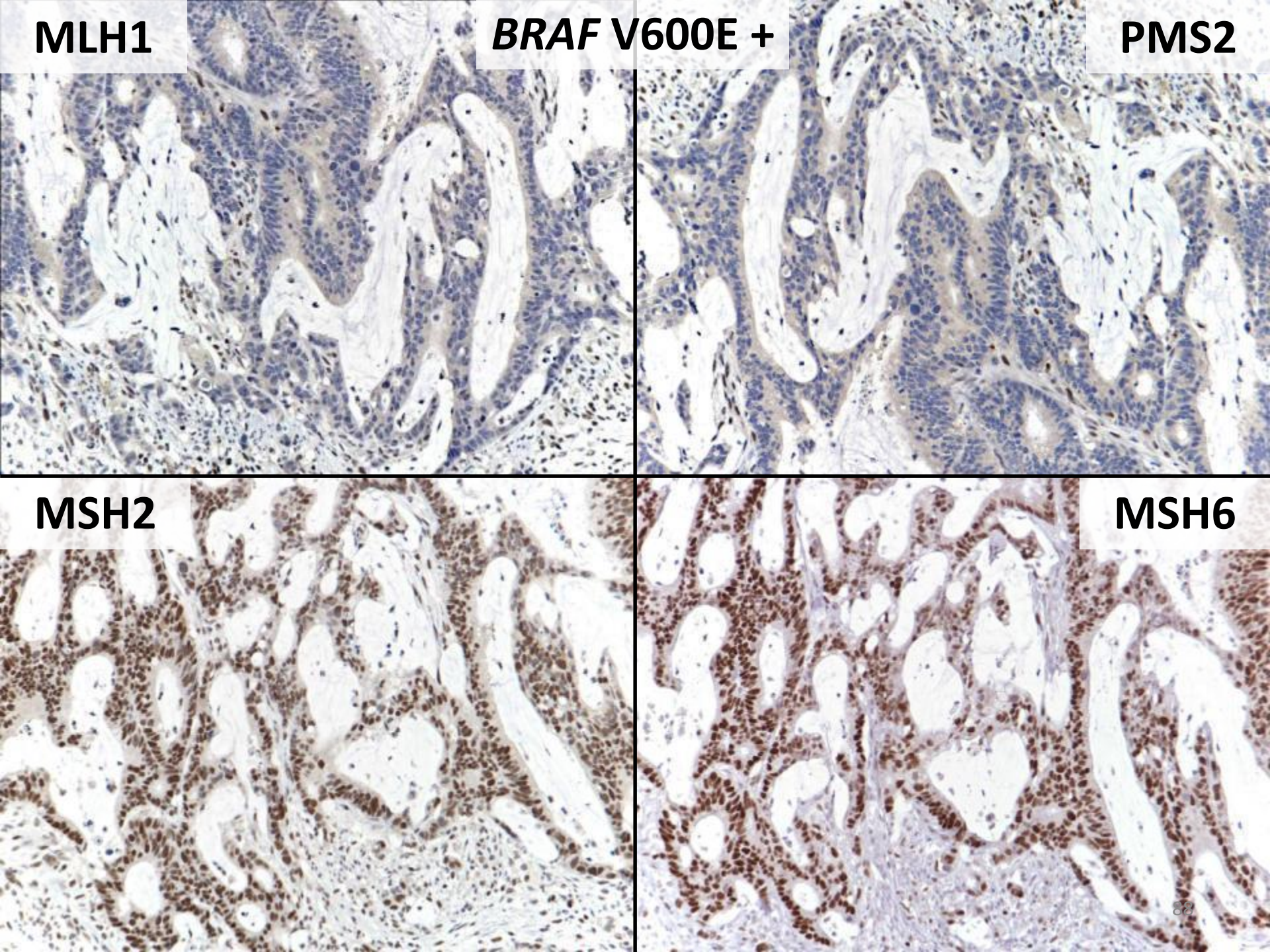
- The presence of a *BRAF* mutation strongly favors a sporadic pathogenesis. The absence of a *BRAF* mutation does not exclude a sporadic pathogenesis.

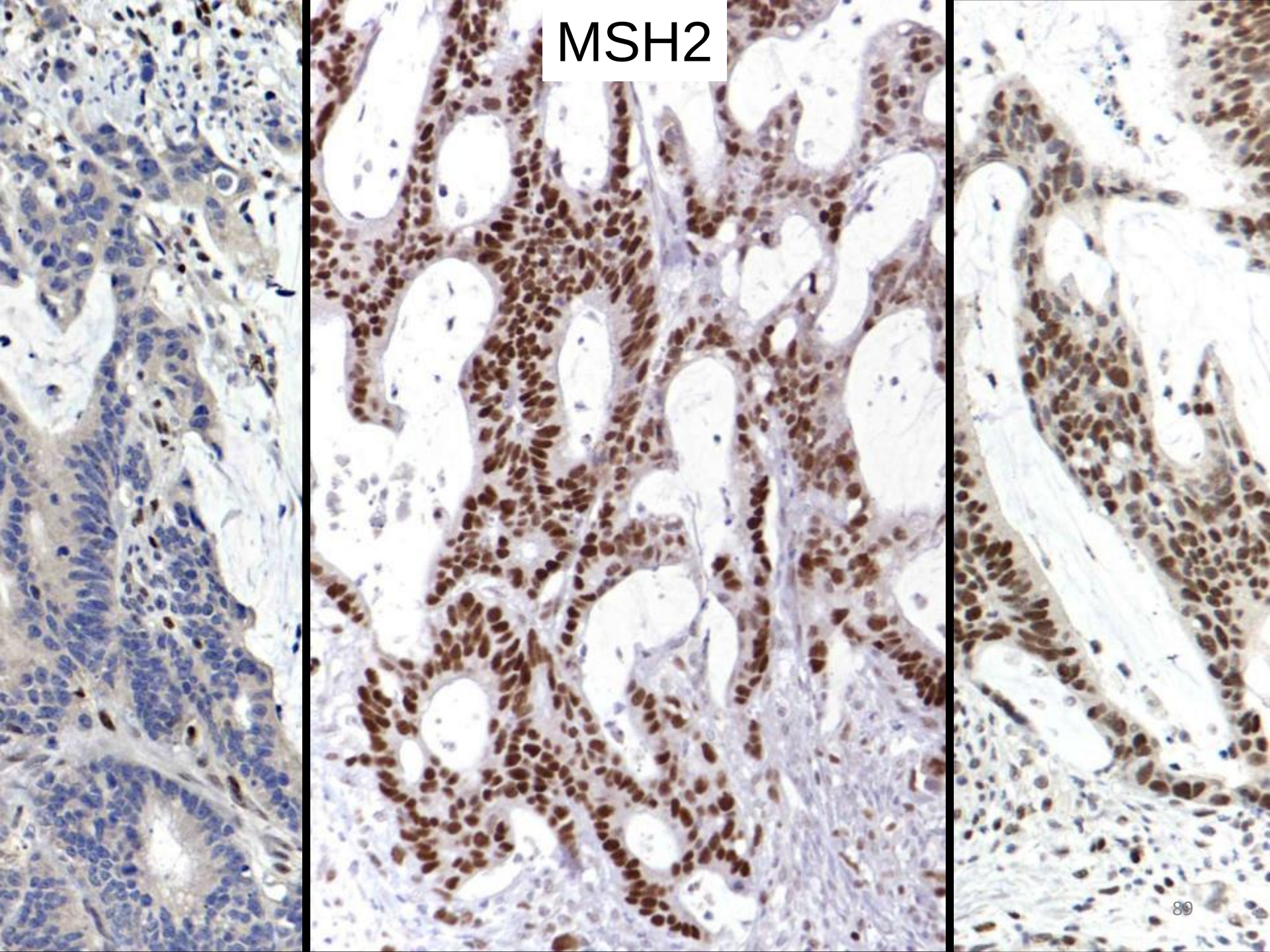
Arch Pathol Lab Med 2017;141:625-657.



- The *BRAF* V600E mutation is closely linked to *MLH1* promoter hypermethylation in colorectal carcinoma and indicates ***sporadic MMR deficiency***.
- However, the absence of the *BRAF* V600E mutation **does not** imply Lynch syndrome as only 50% to 70% of colorectal carcinomas with *MLH1* promoter hypermethylation harbor the *BRAF* V600E mutation.


SSA Precursor


MLH1
BRAF V600E +
PMS2
MSH2
MSH6


MSH2

MMR IHC as a screening tool

IHC result	Most likely defective gene
Loss of MLH1 and PMS2	*MLH1*
Loss of MSH2 and MSH6	*MSH2*
Isolated loss of MSH6	*MSH6*
Isolated loss of PMS2	*PMS2 or MLH1*

Suggestive of Lynch Syndrome

Reporting MMR protein IHC results

- Be clear in describing what you see. Avoid words like positive and negative. Use “preserved expression” and “loss of expression”.
- Use templates

Immunohistochemical studies for DNA mismatch repair proteins were performed on this tumor and demonstrate the following results:

MLH1: PRESERVED EXPRESSION
PMS2: PRESERVED EXPRESSION
MSH2: PRESERVED EXPRESSION
MSH6: PRESERVED EXPRESSION

These results indicate that this tumor is microsatellite stable (MSS).

General Background Information
Immunohistological staining for mismatch repair proteins is complementary to the PCR studies, and is also useful in directing gene sequencing efforts in MSI-H samples. Immunohistological staining is performed on paraffin embedded tissue sections, using standard protocols, using monoclonal antisera reacting with MLH1 (clone G168-728, Ventana), MSH2 (clone G219-1129, Ventana), MSH6 (clone 44, BD Transduction), and PMS2 (clone EPR3947, Cell Marque). Normal expression is defined as nuclear staining within tumor cells, using nuclei at the base of normal crypts (or infiltrating lymphocytes), as positive internal control. These results should be interpreted in the context of clinical findings, family history, and other laboratory data.

References:
1. Moreira L, et al. Identification of Lynch syndrome among patients with colorectal cancer. JAMA 2012; 308:1555-65.

2. Hampel H, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851-60.

Reporting IHC results

- More templates

Immunohistochemical studies for DNA mismatch repair proteins were performed on this tumor and demonstrate the following results:

MLH1: PRESERVED EXPRESSION
PMS2: PRESERVED EXPRESSION
MSH2: LOSS OF EXPRESSION
MSH6: LOSS OF EXPRESSION

These results support that this tumor has arisen through the microsatellite instability (MSI) pathway.

Tumors that demonstrate mismatch repair protein abnormalities account for 15% of all colon cancers and 90% of colon tumors from persons with Lynch syndrome. Lynch syndrome is a hereditary syndrome that causes increased risk for certain cancers. These results should be evaluated within the context of the patient's family history. Due to the finding of abnormal mismatch repair protein expression in this patient's tumor, genetic counseling is recommended.
Dr. _ was notified of these results on _ .

General Background Information
Immunohistological staining for mismatch repair proteins is complementary to the PCR studies, and is also useful in directing gene sequencing efforts in MSI-H samples. Immunohistological staining is performed on paraffin embedded tissue sections, using standard protocols, using monoclonal antisera reacting with MLH1 (clone G168-728, Ventana), MSH2 (clone G219-1129, Ventana), MSH6 (clone 44, BD Transduction), and PMS2 (clone EPR3947, Cell Marque). Normal expression is defined as nuclear staining within tumor cells, using nuclei at the base of normal crypts (or infiltrating lymphocytes), as positive internal control.

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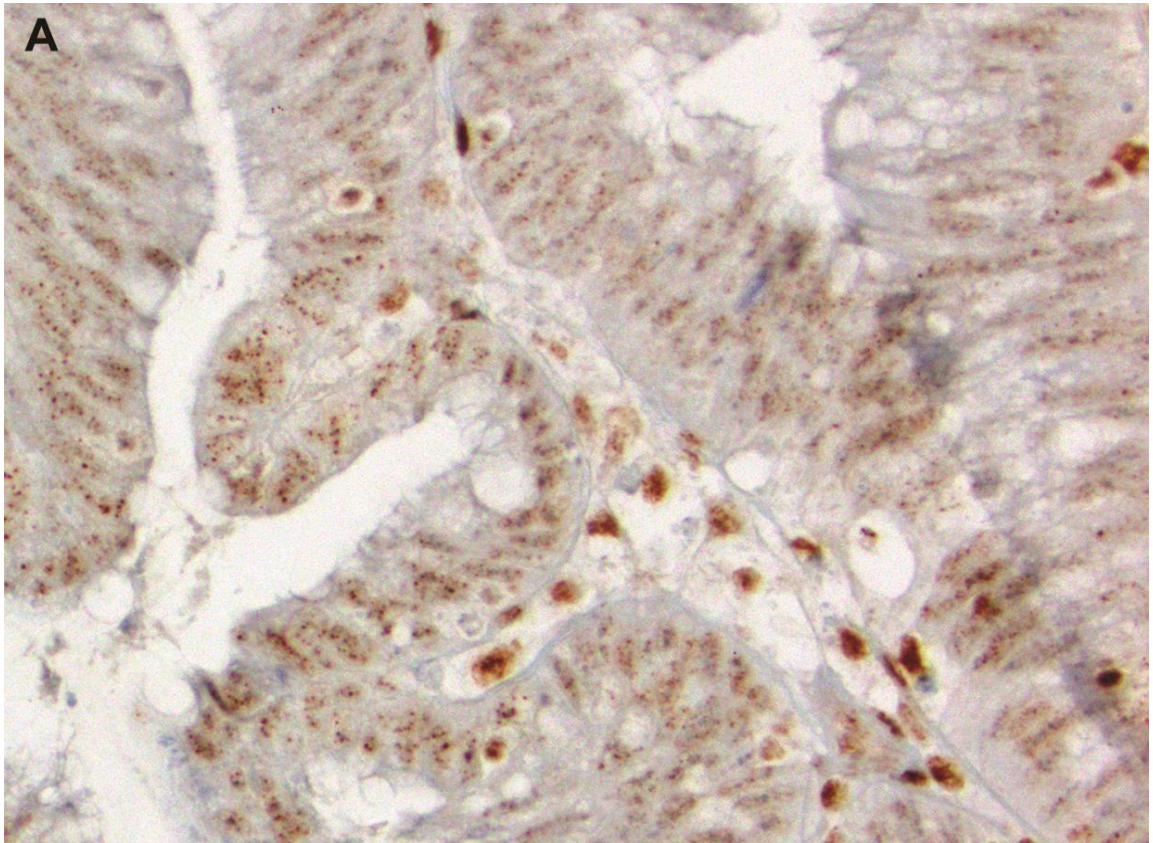
2. Hampel H, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851-60.

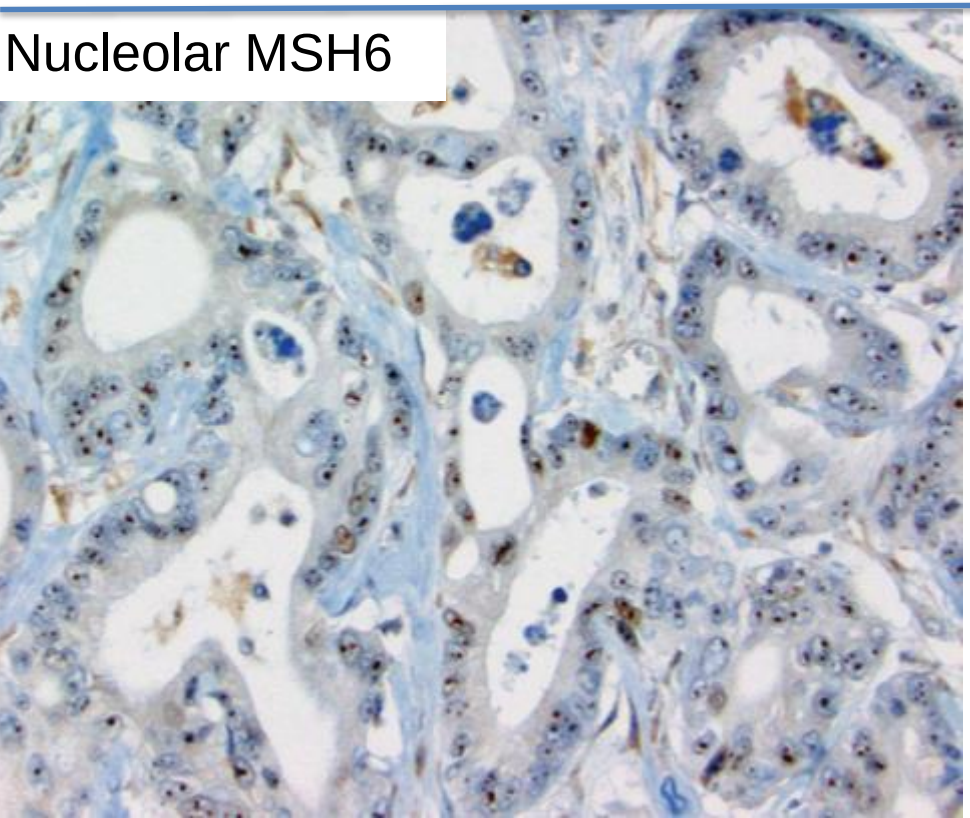
Unusual MMR IHC Patterns

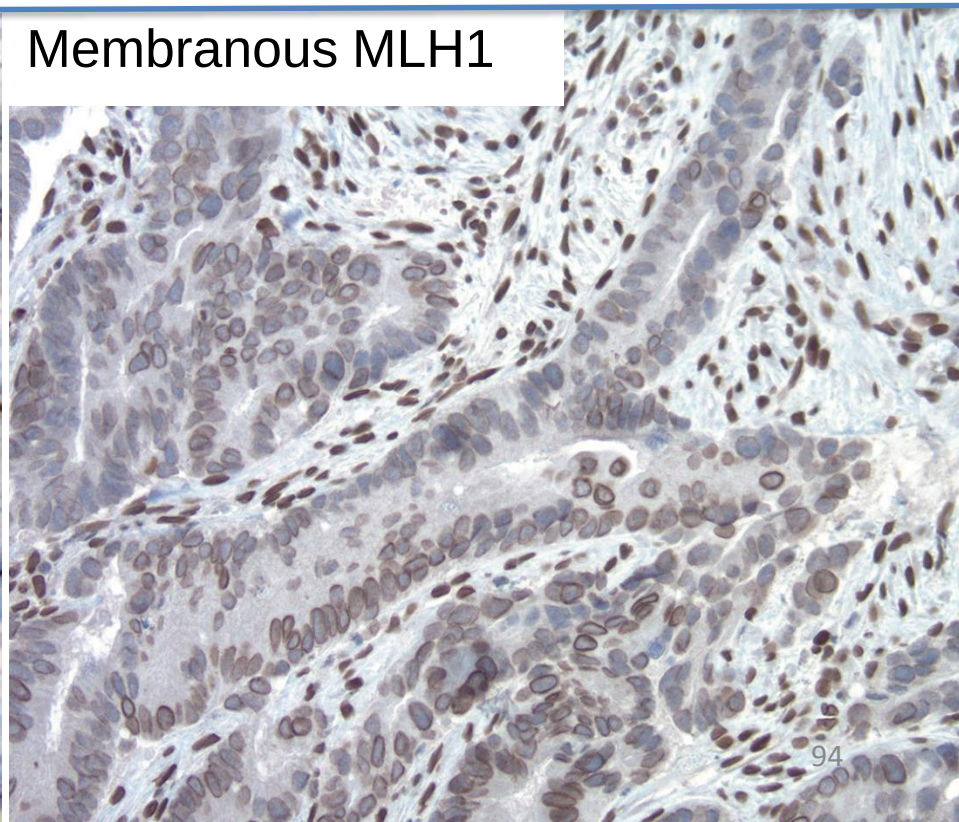
- **Punctate/speckled nuclear MLH1**
  - Typically seen with concurrent PMS2 loss and *BRAF* V600E mutation/*MLH1* promoter hypermethylation.
  - Likely a technical issue with staining protocol.



Unusual MMR IHC Patterns

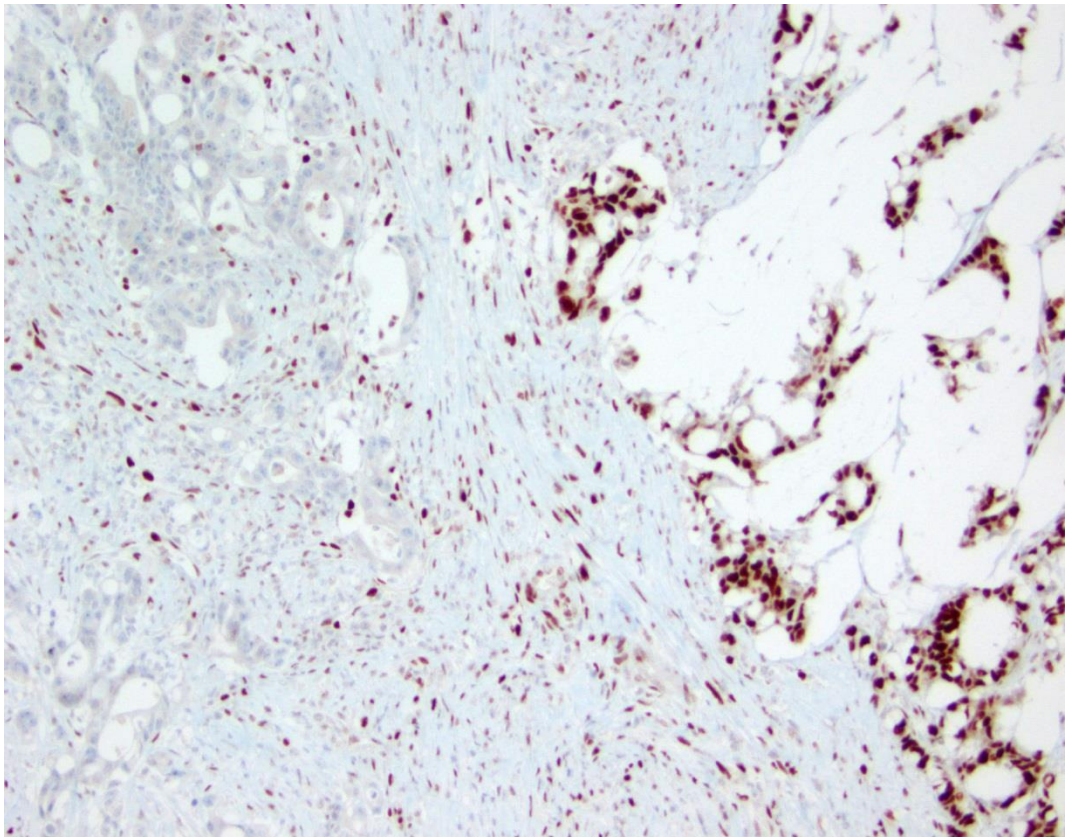
- **Nucleolar MSH6 *or* Membranous MLH1**
  - Should not be taken as evidence of preserved expression. MSI PCR should be performed.
  - Likely a technical issue with staining protocol.





Unusual MMR IHC Patterns

- **Clonal Loss of MLH1 and PMS2**
  - Large areas of tumor show abrupt loss of expression
  - Typically the result of *MLH1* promoter hypermethylation in a clonal population of the tumor.

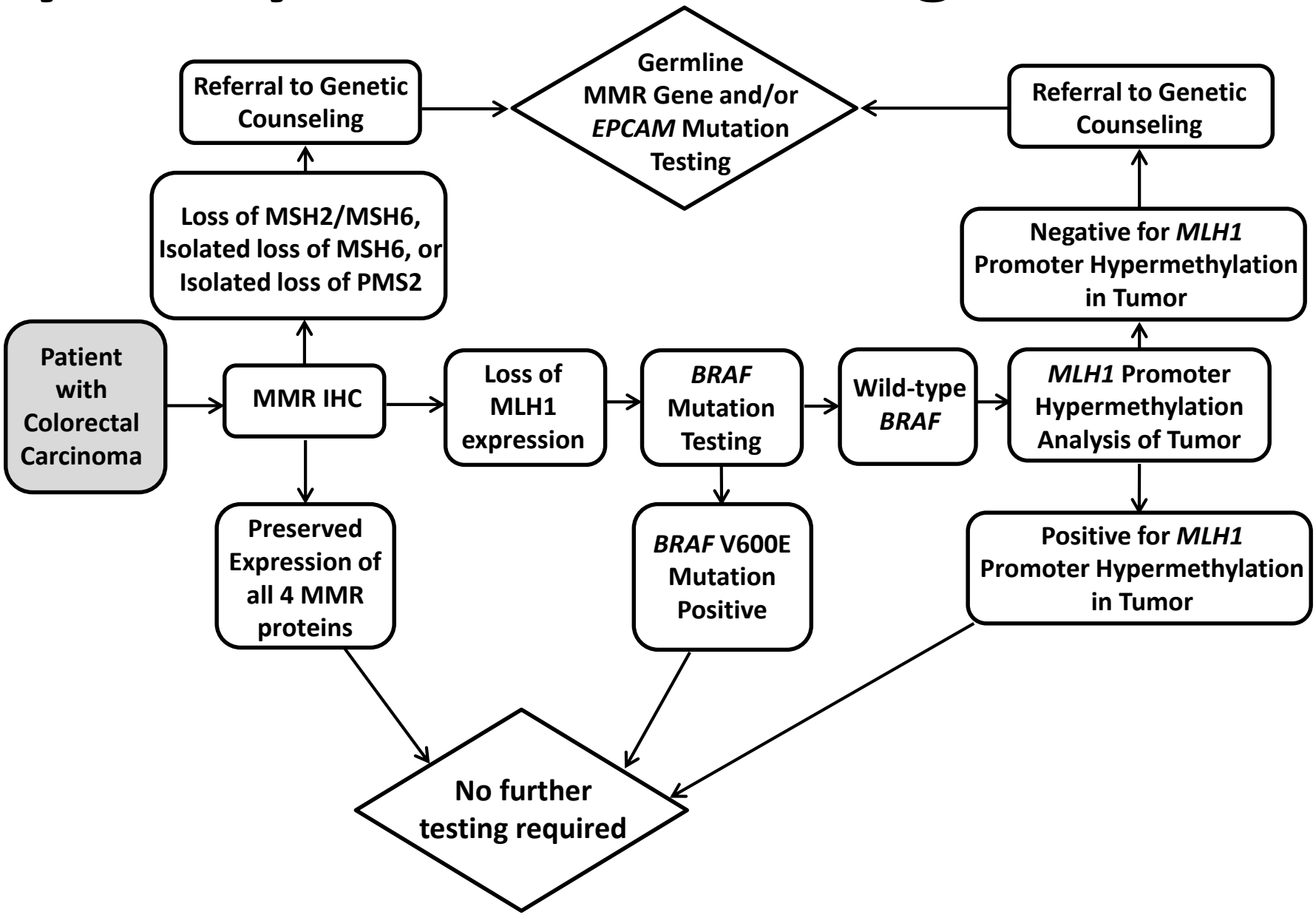


Unusual MMR IHC Patterns

- **Concurrent Loss of MLH1, PMS2, and MSH6**
  - MSH6 loss is most often due to secondary **somatic** mutation of coding mononucleotide tracts within the *MSH6* gene. Although germline mutation is unlikely, genetic counseling is still advised.

- **Loss of MSH6 post-therapy in rectal cancers**
  - Test the pre-treatment biopsy if available.

Lynch Syndrome Screening for CRC



Pai RK and Pai RK. Am J Surg Pathol 2016;40:e17-34.

MMR Status in Therapeutic Decision-Making

NCCN High risk factors for stage II colon cancers

- Poor differentiation **(except MMR protein deficient or MSI-high tumors)**
- Lymphovascular invasion
- Bowel obstruction
- <12 lymph nodes
- Perineural invasion
- Localized perforation (pT4)
- Close/indeterminate margins

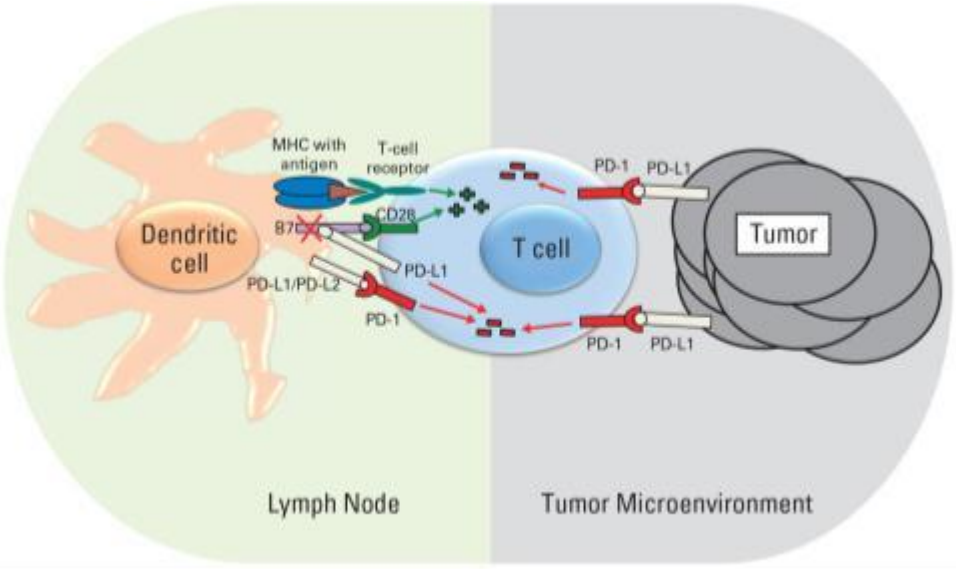
Most oncologists will not recommended adjuvant chemotherapy for patients with stage II tumors that are MMR protein deficient.

Patients with stage III or IV tumors benefit from chemotherapy regardless of MMR protein status.

MMR in Immune Checkpoint Blockade

- Activation of immune checkpoints allow for tumor cells to evade antitumor immunity.
- Therapeutic antibodies targeting immune checkpoint proteins have been developed (nivolumab, pembrolizumab, etc.).

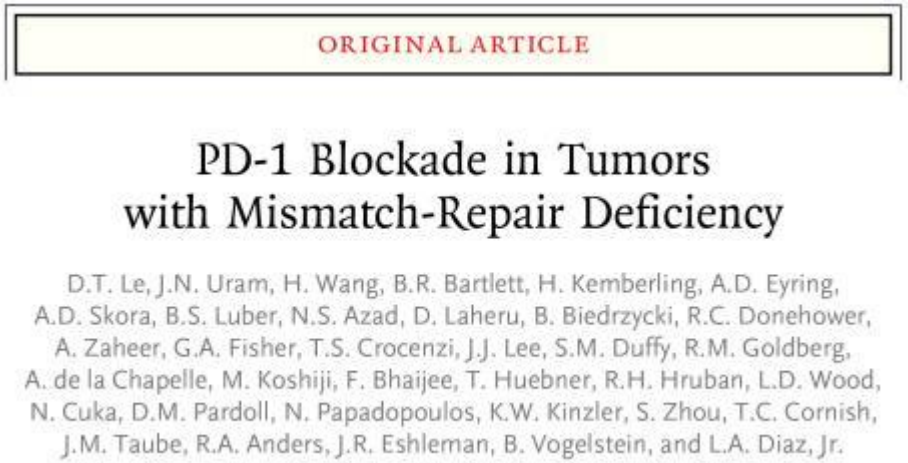
Programmed Cell Death Protein 1 (PD-1 / PD-L1 Pathway)



- PD-1 limits activity of T-cells when it interacts with its ligand PD-L1 allowing for tumor evasion of immunity.
- Antibodies targeting PD-1 (nivolumab, pembrolizumab) are effective in subsets of colorectal carcinoma.

J Clin Oncol 2015; 33:1974.

MMR & Immune Checkpoint Blockade



ORIGINAL ARTICLE

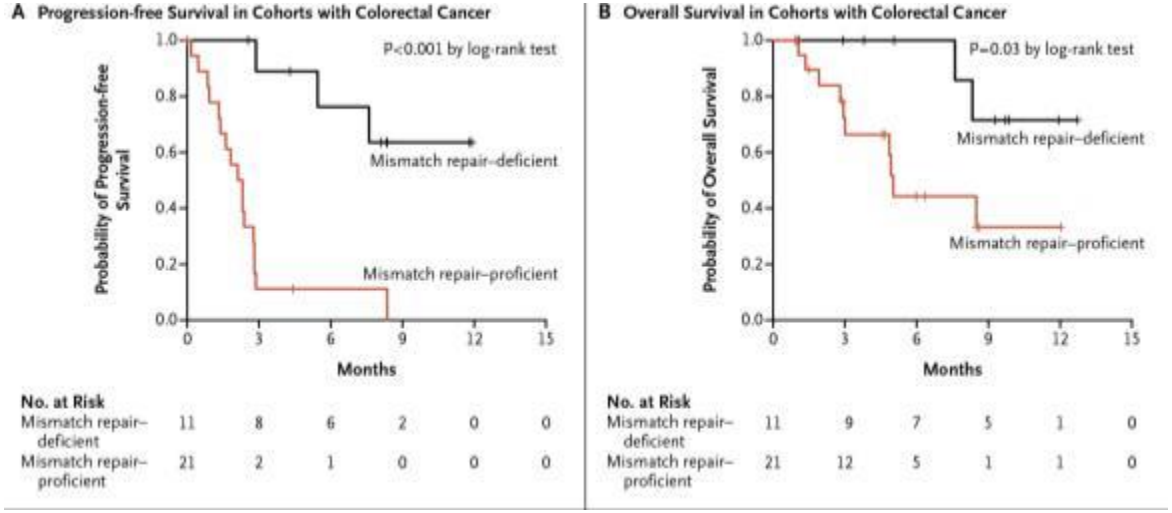
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, A.D. Skora, B.S. Luber, N.S. Azad, D. Laheru, B. Biedrzycki, R.C. Donehower, A. Zaheer, G.A. Fisher, T.S. Crocenzi, J.J. Lee, S.M. Duffy, R.M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R.H. Hruban, L.D. Wood, N. Cuka, D.M. Pardoll, N. Papadopoulos, K.W. Kinzler, S. Zhou, T.C. Cornish, J.M. Taube, R.A. Anders, J.R. Eshleman, B. Vogelstein, and L.A. Diaz, Jr.

- Phase II study evaluating pembrolizumab in patients with metastatic carcinoma (primarily colorectal)
- Patients were stratified by DNA mismatch repair protein status.
- Evaluated response rates, progression-free survival rate, and overall survival.

New Engl J Med June 2015; 372:2509.

MMR & Immune Checkpoint Blockade



- **In May 2017, the FDA approved pembrolizumab in any tumor with MMR deficiency (MSI-H).**

- **In July 2017, the FDA approved use of nivolumab in colorectal carcinoma with MMR deficiency (MSI-H).**

New Engl J Med June 2015; 372:2509.

MMR & Immune Checkpoint Blockade

- **High somatic mutational burden stratified by MMR status**:
  - MMR deficient carcinomas: mean of 1782 somatic mutations per tumor.
  - MMR proficient carcinomas: mean 73 somatic mutations per tumor.

- **These somatic mutations can give rise to amino acid changes in proteins resulting in mutation-associated neoantigens in MMR deficient carcinomas.**

- **Potential evolving predictive biomarker in immunotherapy: Assessment of tumor mutational burden (TMB).**
  - A higher tumor mutational burden identified by next generation sequencing analysis is associated with improved response and survival following PD-1 blockade therapy (but high cost for testing).

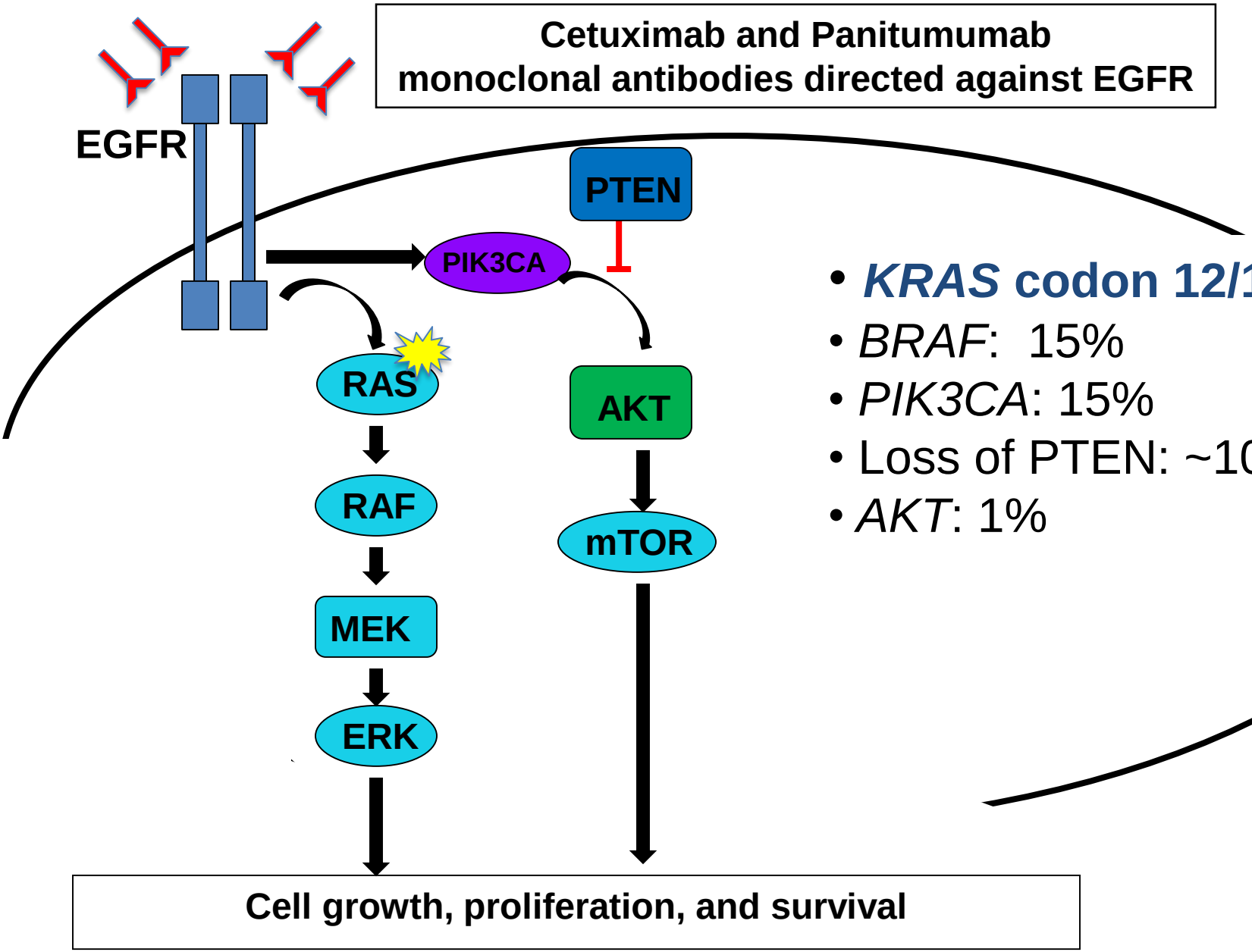
New Engl J Med June 2015; 372:2509.

Selecting Patients for Immune Checkpoint Blockade in Colorectal Carcinoma

- **Currently, MMR deficiency (MSI-H) is the biomarker used to select patients for immune checkpoint blockade using anti-PD1 therapy in colorectal carcinomas.**

- **In contrast to NSCLC, PD-L1 immunohistochemistry has not been advocated to select patients with colorectal carcinoma for anti-PD1 therapy outside of clinical trials.**
  - No published guidelines on what percent tumor staining should be considered “positive”.
  - Different PD-L1 antibody clones can yield different results.

Predictive Biomarkers in CRC: EGFR



- ***KRAS* codon 12/13: 40%**
- *BRAF*: 15%
- *PIK3CA*: 15%
- Loss of PTEN: ~10-20%
- *AKT*: 1%

Extended *RAS* Testing in mCRC

- **17% of patients with wild-type *KRAS* codon 12 or 13 have other *RAS* mutations**
  - *KRAS* codon 61 (4%) and codon 117 or 146 (6%)
  - *NRAS* codon 12/13 (3%), codon 61 (4%), codon 117 or 146 (0%)

- Patients with extended *RAS* mutation treated with anti-EGFR therapy have inferior progression-free and overall survival. Patients with extended wild-type *RAS* benefit from anti-EGFR therapy.

• **NCCN (2014)**: All patients with mCRC who are candidates for anti-EGFR therapy should have their tumor tested for ***KRAS* and *NRAS*** mutations to include **exons 2, 3, and 4**.

Douillard J, New Engl J Med 2013;369:1023.

Molecular Biomarkers for the Evaluation of Colorectal Cancer

Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology

Antonia R. Sepulveda, MD, PhD,[1] Stanley R. Hamilton, MD, PhD,[2] Carmen J. Allegra, MD,[5] Wayne Grody, MD, PhD,[6] Allison M. Cushman-Vokoun, MD, PhD,[7] William K. Funkhouser, MD, PhD,[8] Scott E. Kopetz, MD, PhD,[3] Christopher Lieu, MD,[9] Noralane M. Lindor, MD,[10] Bruce D. Minsky, MD,[4] Federico A. Monzon, MD,[11] Daniel J. Sargent, PhD,[12] Veena M. Singh, MD,[13] Joseph Willis, MD,[14] Jennifer Clark, SCT, MB(ASCP)^{cm},[15] Carol Colasacco, MLIS,[16] R. Bryan Rumble, MSc,[17] Robyn Temple-Smolkin, PhD,[18] Christina B. Ventura, MT(ASCP),[16] and Jan A. Nowak, MD, PhD[19]

Recommendation

- Patients with CRC being considered for anti-EGFR therapy must receive *RAS* mutational testing.

- Mutational analysis should include *KRAS* and *NRAS* codons 12 and 13 of exon 2, 59 and 61 of exon 3, and 117 and 146 of exon 4 (“expanded” or “extended” *RAS*).

Arch Pathol Lab Med 2017;141:625-657.

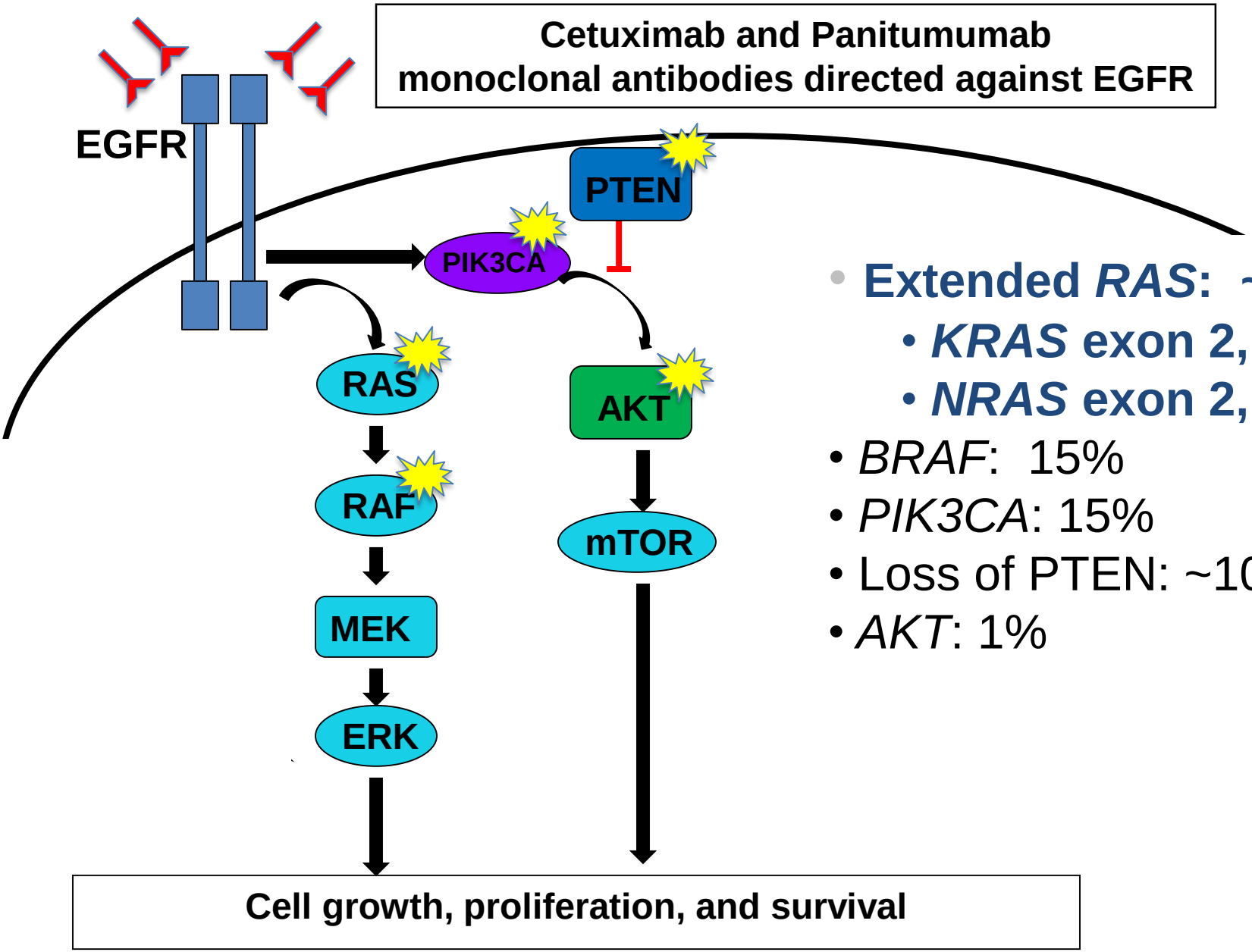
Extended *RAS* Testing in mCRC

- ***KRAS* and *NRAS* exon 2 (codons 12 & 13), exon 3 (codon 61), and exon 4 (codons 117 and 146)**
- What to test?
  - Primary
  - Metastasis
  - Post-treatment (rectal)

All are acceptable. The patient should ***not*** be subjected to an additional biopsy of metastatic lesions.

- How to test?
  - **Next generation sequencing platform (at our institution)**
  - Sanger sequencing: Less sensitive (requires higher density of tumor)
  - Pyrosequencing: Very sensitive

Predictive Biomarkers in CRC: EGFR



- **Extended *RAS*: ~50%**
  - ***KRAS* exon 2, 3, & 4**
  - ***NRAS* exon 2, 3, & 4**
- *BRAF*: 15%
- *PIK3CA*: 15%
- Loss of PTEN: ~10-20%
- *AKT*: 1%

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Statement

- There is insufficient evidence to recommend *BRAF, PIK3CA*, and *PTEN* analysis as predictive markers for therapy selection outside of clinical trials.

Arch Pathol Lab Med 2017;141:625-657.

Prognostic Biomarkers in CRC

Association Between Molecular Subtypes of Colorectal Cancer and Patient Survival

Amanda I. Phipps,[1,2] Paul J. Limburg,[3] John A. Baron,[4] Andrea N. Burnett-Hartman,[1,2] Daniel J. Weisenberger,[5] Peter W. Laird,[5] Frank A. Sinicrope,[3] Christophe Rosty,[6,7] Daniel D. Buchanan,[6] John D. Potter,[1,2,8] and Polly A. Newcomb[1,2]

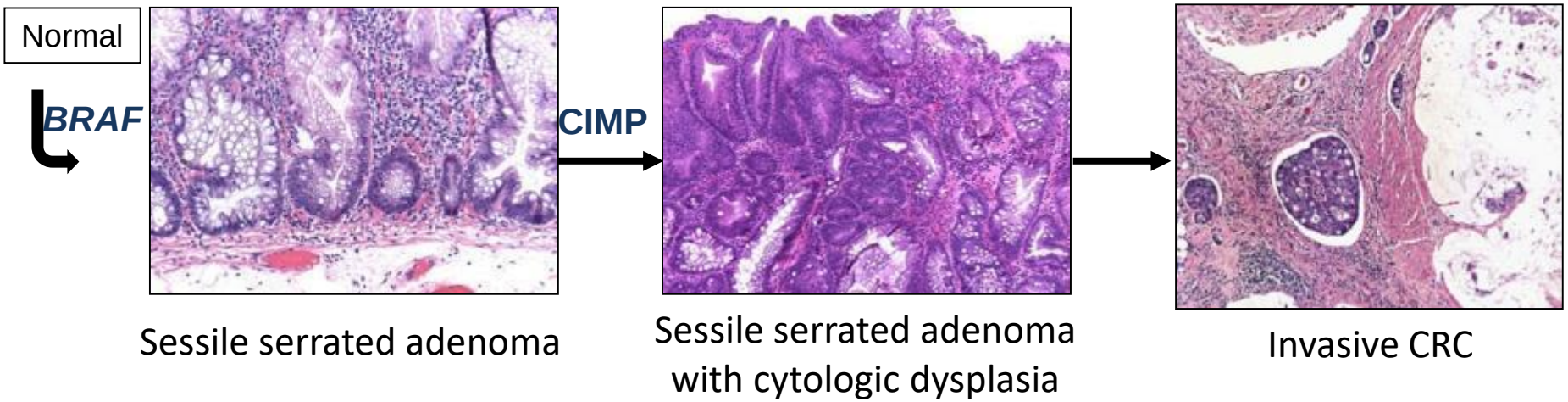
[1]Epidemiology Department, University of Washington, Seattle, Washington; [2]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; [3]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota; [4]Department of Medicine, University of North Carolina, Chapel Hill, North Carolina; [5]USC Epigenome Center, USC/Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California; [6]Cancer and Population Studies Group, Queensland Institute of Medical Research, Herston, QLD, Australia; [7]Department of Molecular and Cellular Pathology, University of Queensland School of Medicine, Herston, QLD, Australia; and [8]Centre for Public Health Research, Massey University, Wellington, New Zealand

Molecular Markers Identify Subtypes of Stage III Colon Cancer Associated With Patient Outcomes

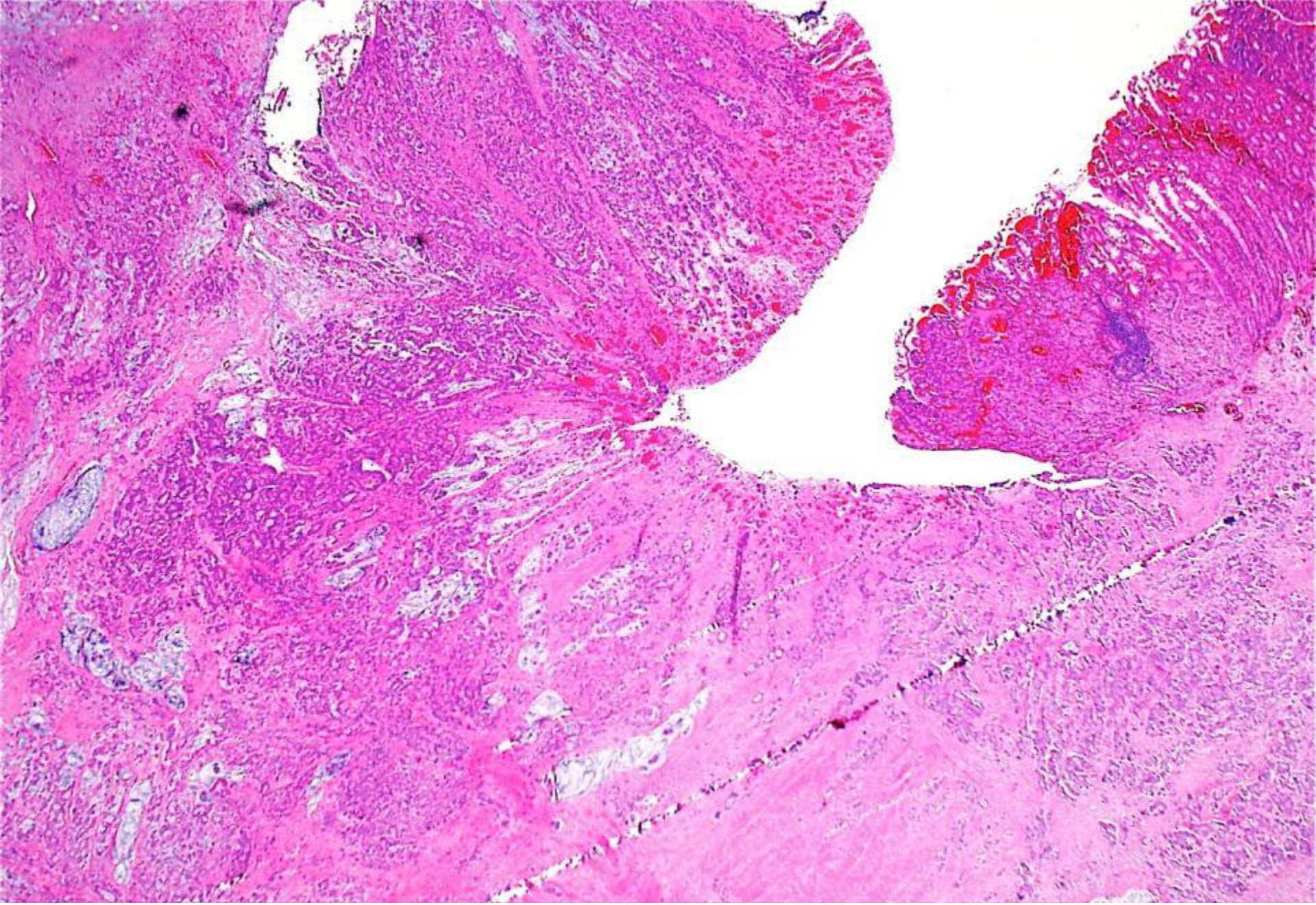
Frank A. Sinicrope,[1,2] Qian Shi,[3] Thomas C. Smyrk,[4] Stephen N. Thibodeau,[4] Rodrigo Dienstmann,[5] Justin Guinney,[5] Brian M. Bot,[5] Sabine Tejpar,[6] Mauro Delorenzi,[7] Richard M. Goldberg,[8] Michelle Mahoney,[3] Daniel J. Sargent,[3] and Steven R. Alberts[2]

[1]Department of Medicine; [2]Department of Oncology; [3]Alliance Statistics and Data Center; [4]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; [5]Sage Bionetworks, Seattle, Washington; [6]Molecular Digestive Oncology, KU Leuven, The Netherlands; [7]Swiss Institute of Bioinformatics, University Lausanne, Switzerland; and [8]Division of Medical Oncology, Ohio State University Comprehensive Cancer Center, Columbus, Ohio

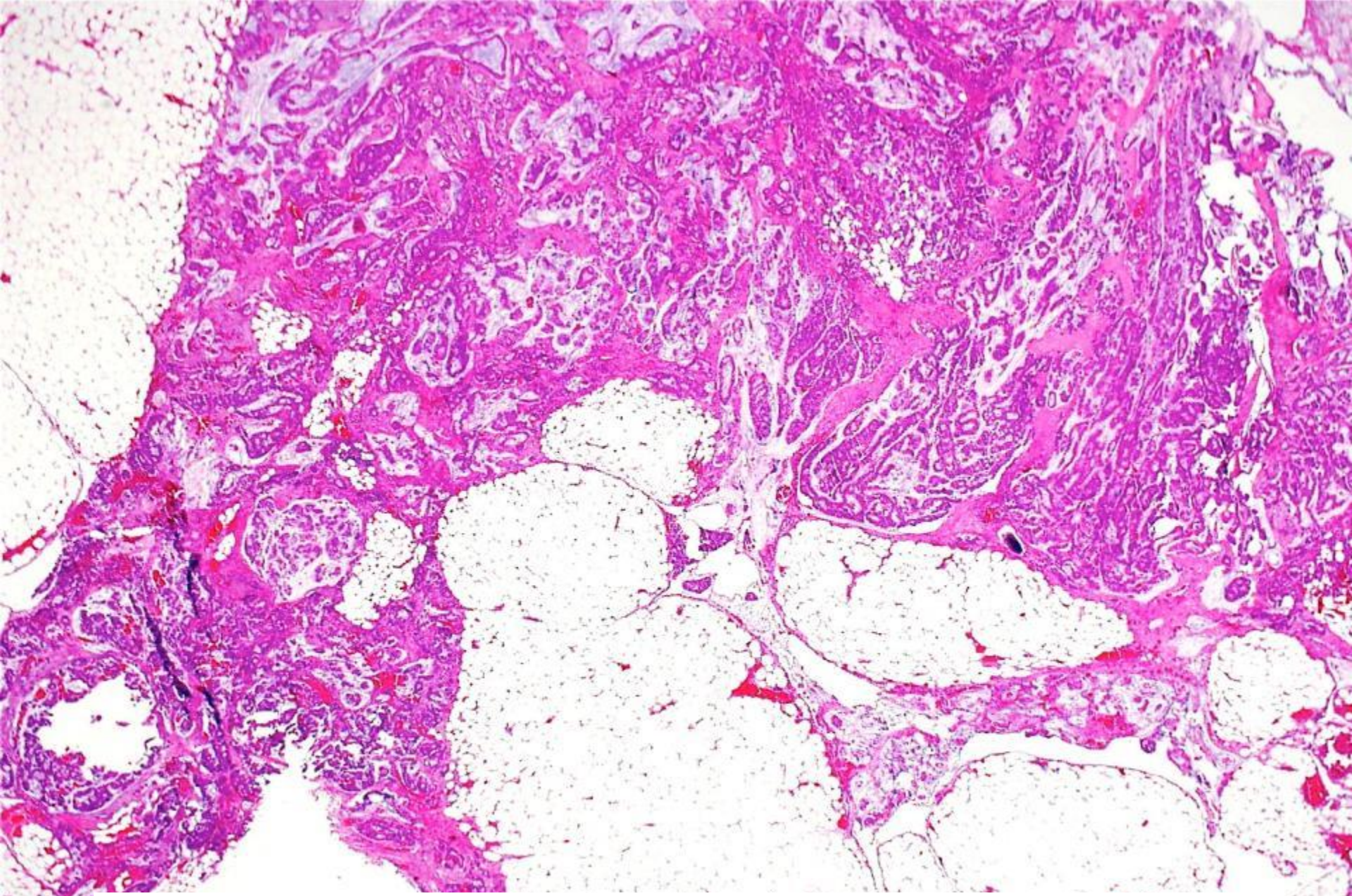
BRAF mutated, Microsatellite Stable Colorectal Carcinoma (~5%)



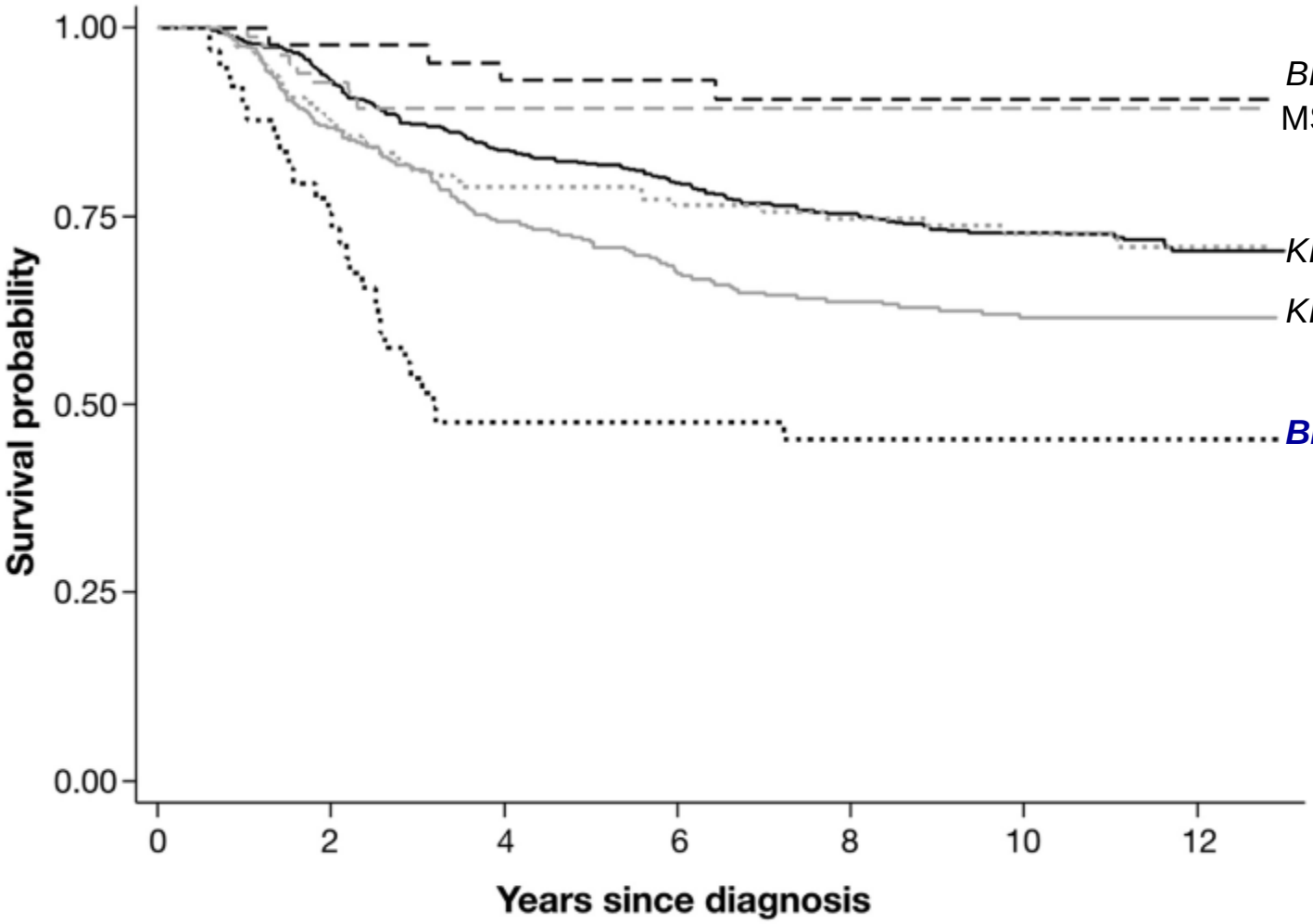
- *BRAF* mutation occurs early in sessile serrated adenoma.
- Hypermethylation of CpG islands (CIMP+) *without* MSI-H.
- Highly aggressive morphologic features:
  - Extensive lymphatic, venous, and perineural invasion.
  - Often with mucinous morphology, may have mixed high-grade neuroendocrine differentiation, right colon >> left colon/rectum.
  - Often presents with widely metastatic disease.
- Aberrant immunohistochemical staining profile: CK20 may be negative, CDX2 often negative, and CK7 may be positive.



70 yo male with with an ascending colon mass



- **Invasive mucinous adenocarcinoma, pT4a N2b with liver metastasis**
- **Microsatellite stable and *BRAF* mutated**



Number at risk

Type 1	100	78	71	66	56	43	14
Type 2	55	38	24	23	21	20	4
Type 3	353	268	223	191	160	130	37
Type 4	631	541	473	433	370	300	113
Type 5	50	43	39	37	32	26	10
Other	155	120	103	96	84	65	22

Figure 1. Kaplan-Meier survival curves for disease-specific survival.

Phipps AI, *Gastroenterology* 2015;148:77-87.

Molecular Biomarkers for the Evaluation of Colorectal Cancer

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Recommendation

- *BRAF* p.V600 (BRAF c.1799) mutational analysis should be performed in patients with metastatic colorectal carcinoma for prognostic stratification.

- **Patients with *BRAF* MSS colorectal carcinoma have aggressive disease that does not typically respond to conventional chemotherapy.**

Arch Pathol Lab Med 2017;141:625-657.

HER2 in Colorectal Carcinoma

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

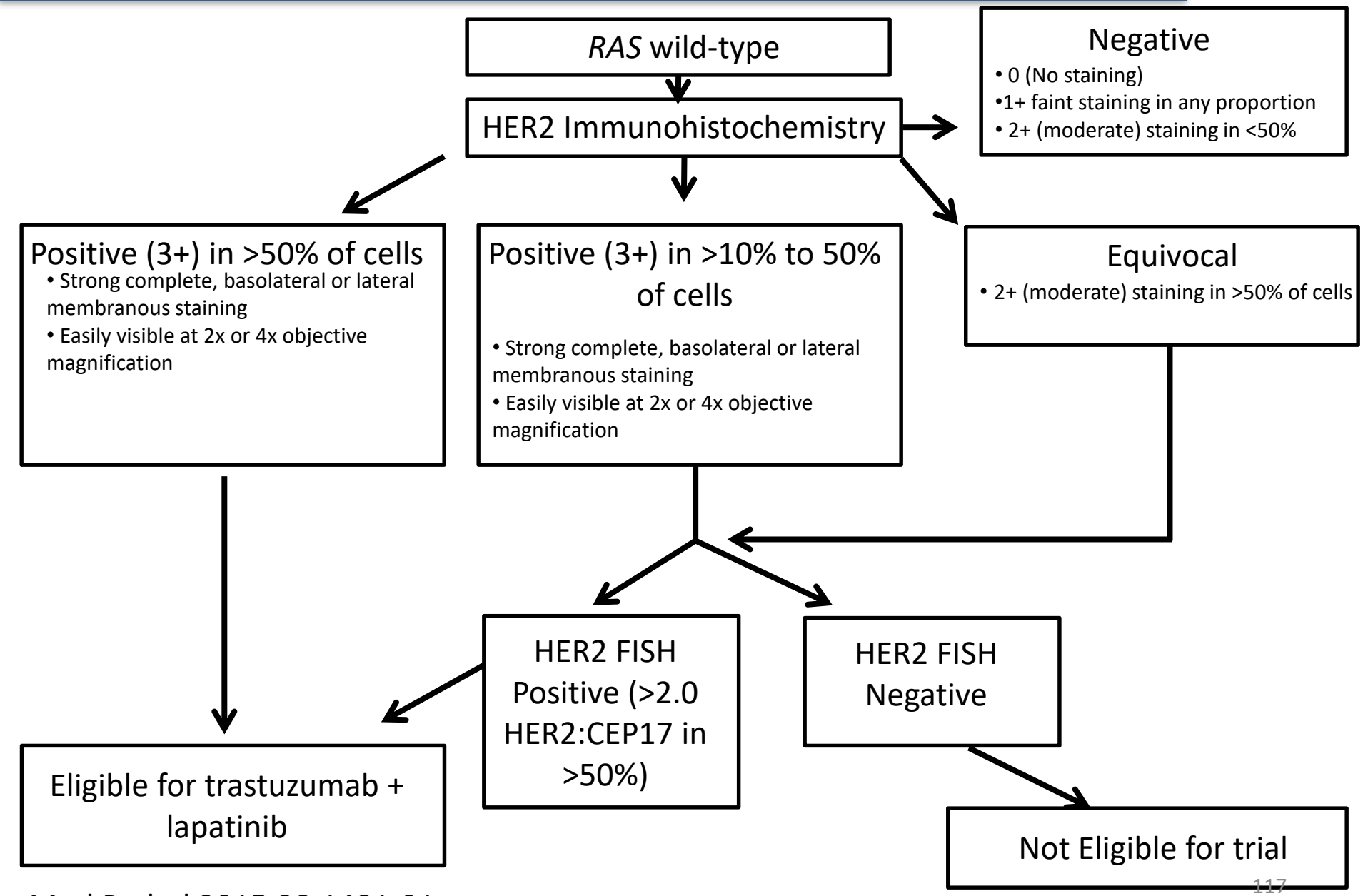
- **5% patients with *KRAS* wild-type tumors were HER2 positive.**

- **HER2 Positive defined as (different eligibility criteria compare to GEJ/gastric)**:
  - **3+ HER2 IHC in more than 50% of cells**
  - **2+ HER2 IHC AND *HER2:CEP17* ratio >2 in more than 50% of cells**

- **Phase 2 trial of Trastuzumab and Lapatinib (tyrosine kinase inhibitor that interrupts the HER2 and EGFR pathways)**
  - 59% achieved disease control (complete response, partial response, or stable disease).

Lancet Oncol 2016;17:738-746.

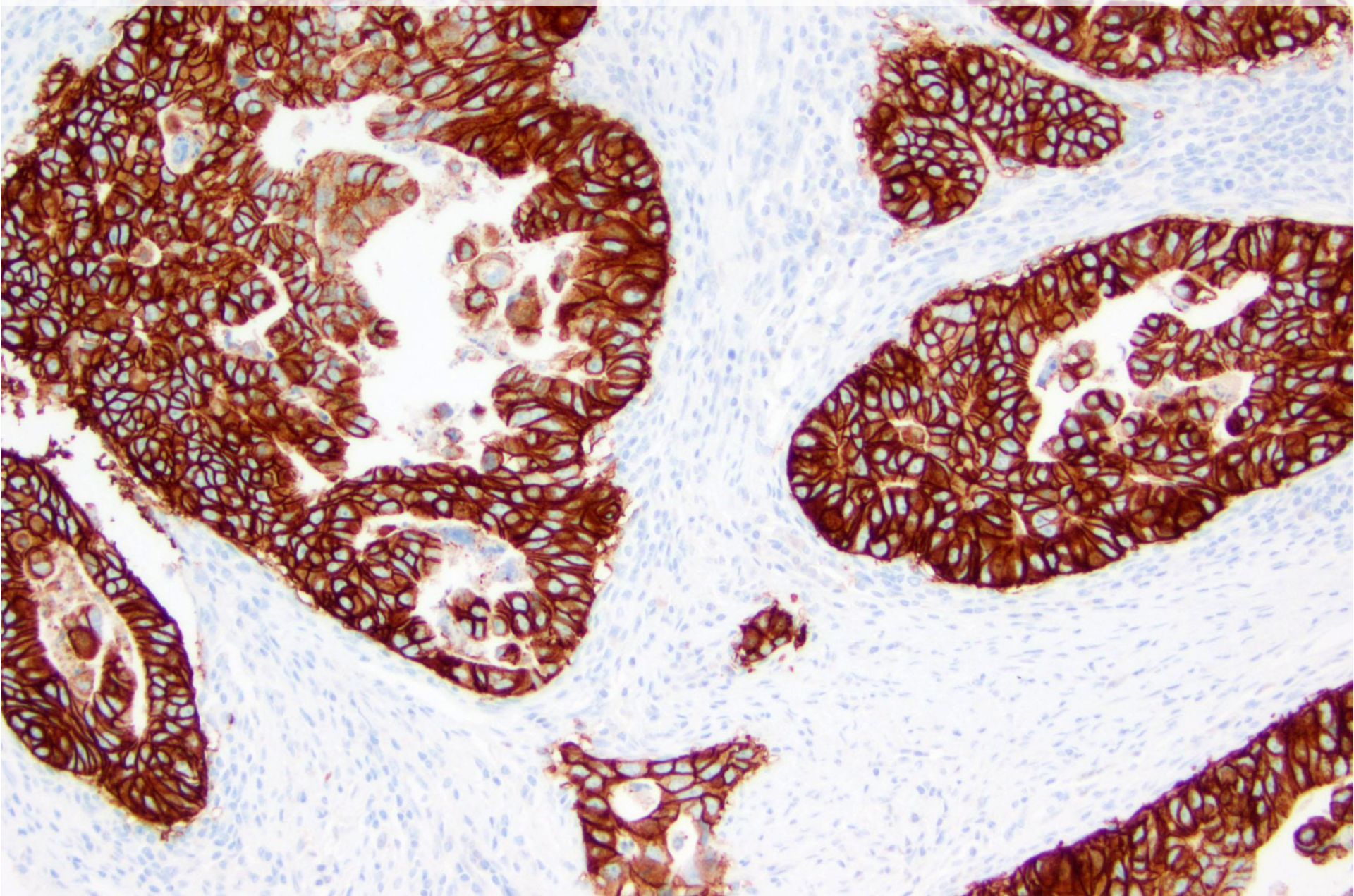
HER2 in Colorectal Carcinoma



Mod Pathol 2015;28:1481-91

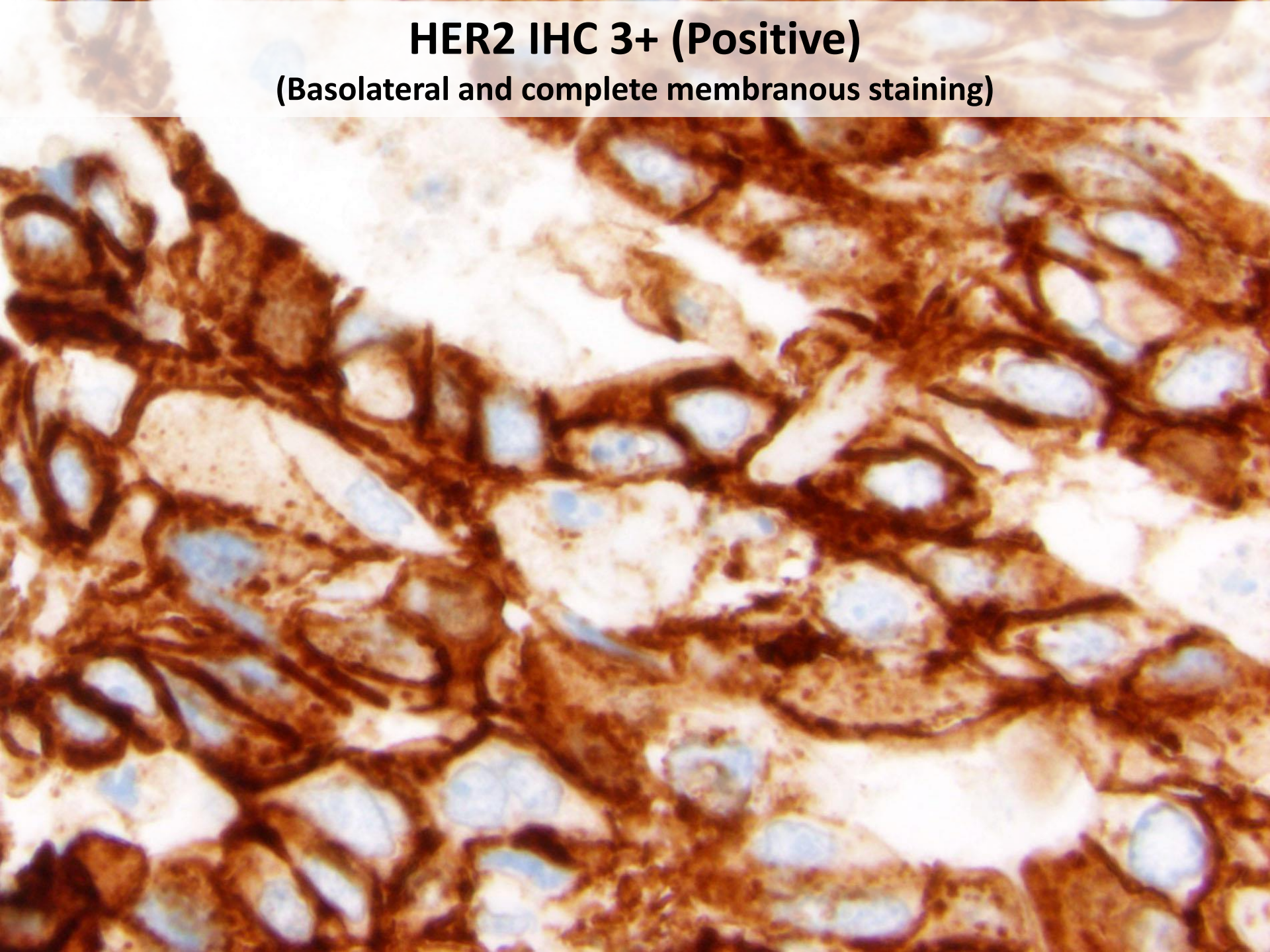
HER2 IHC 3+ (Positive)

(Strong membranous staining easily visible with 2x objective magnification)

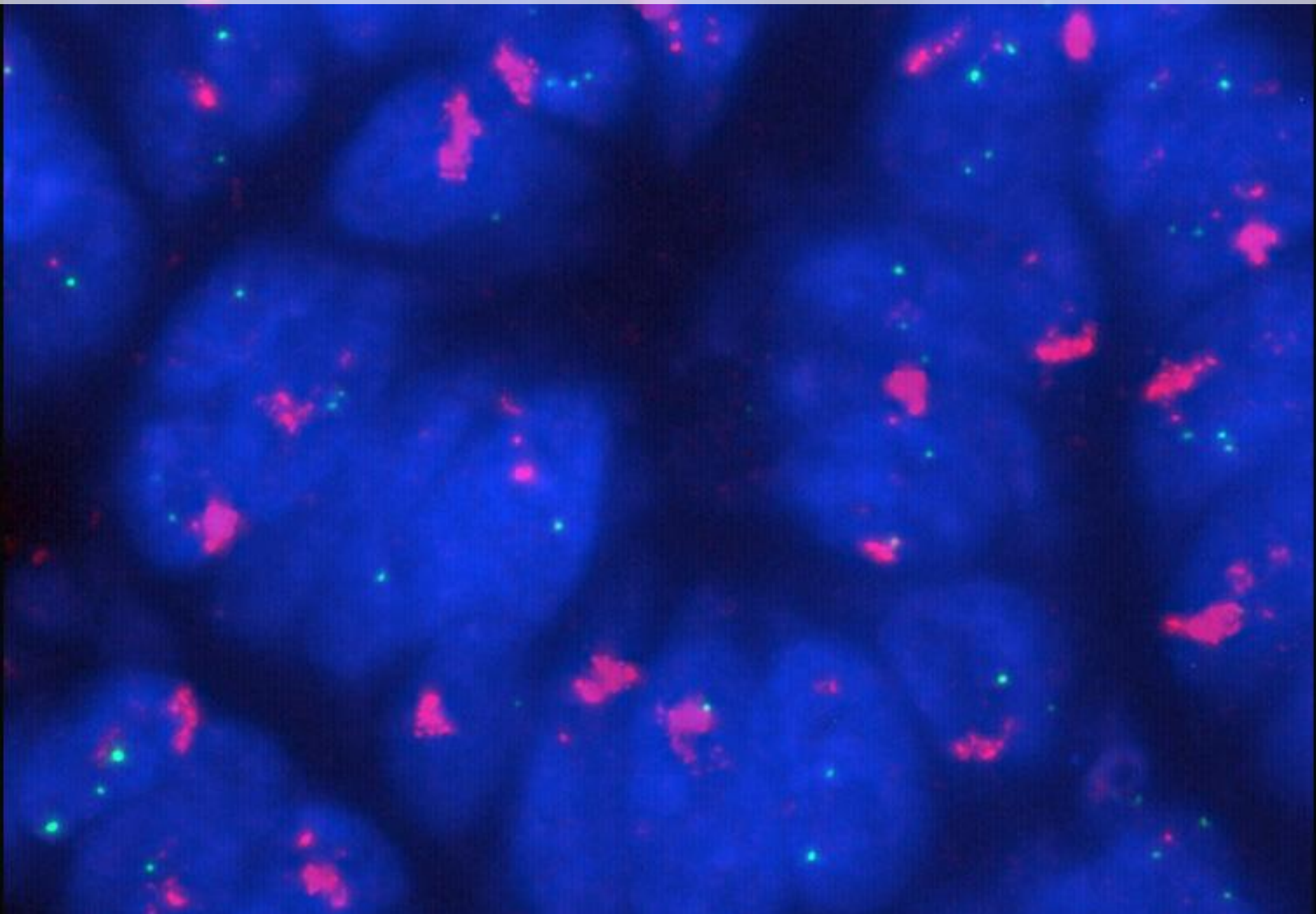


HER2 IHC 3+ (Positive)

(Basolateral and complete membranous staining)

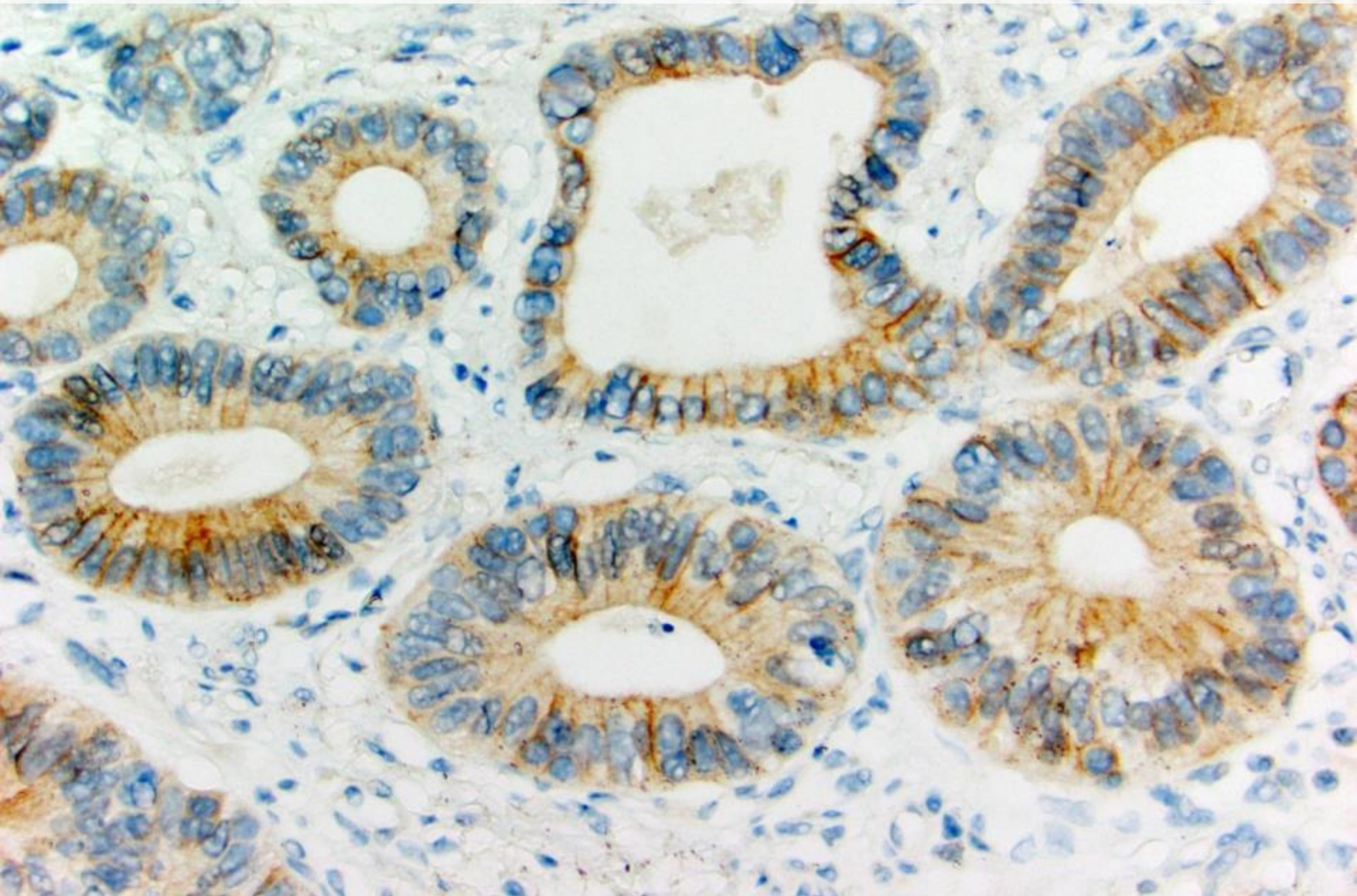


HER2 FISH (Positive *HER2*/*CEP17* >>2.0)



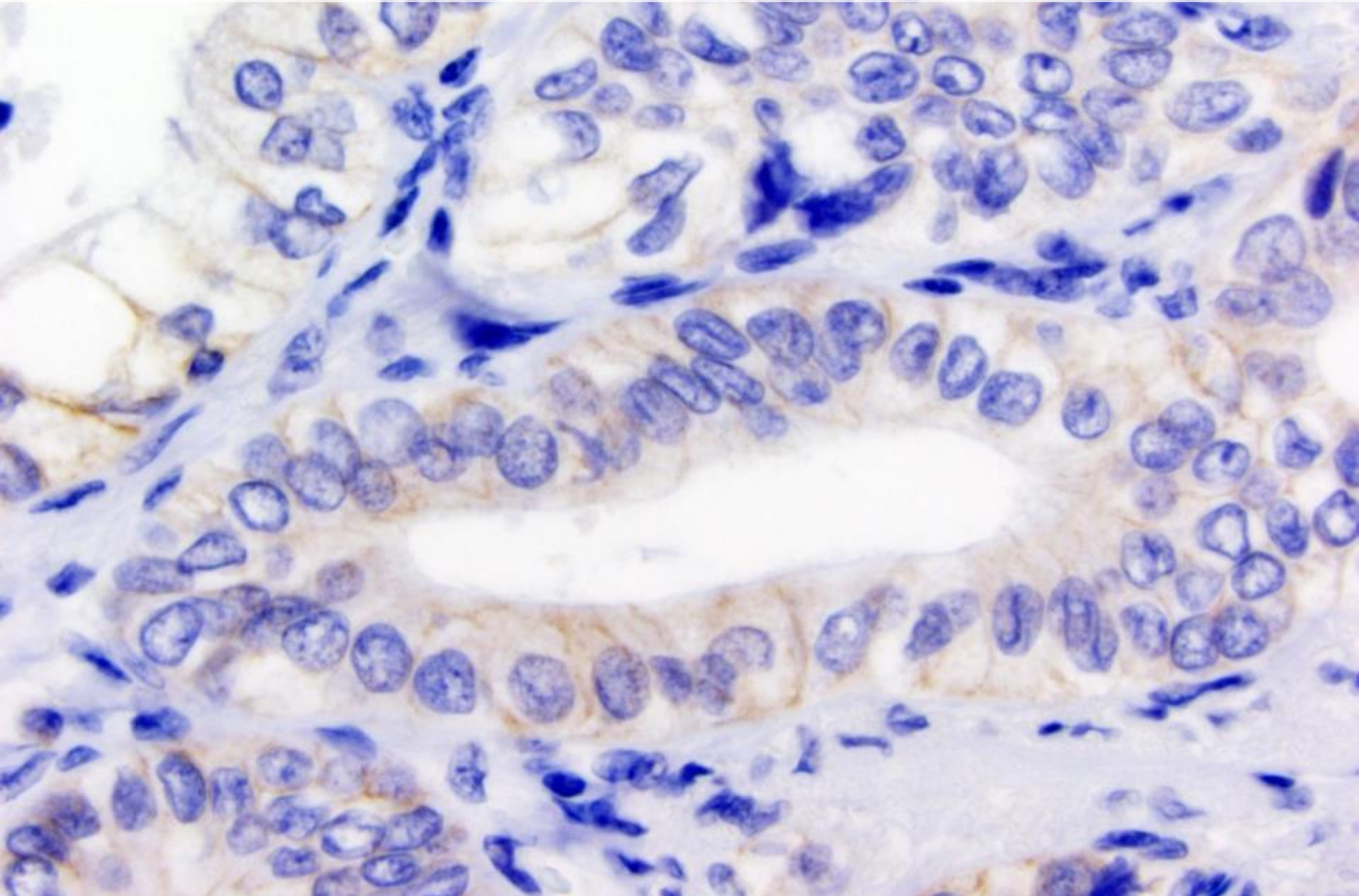
HER2 IHC 2+ (Equivocal, Reflex to HER2 ISH)

(Membranous staining only visible with 10-20x objective magnification)

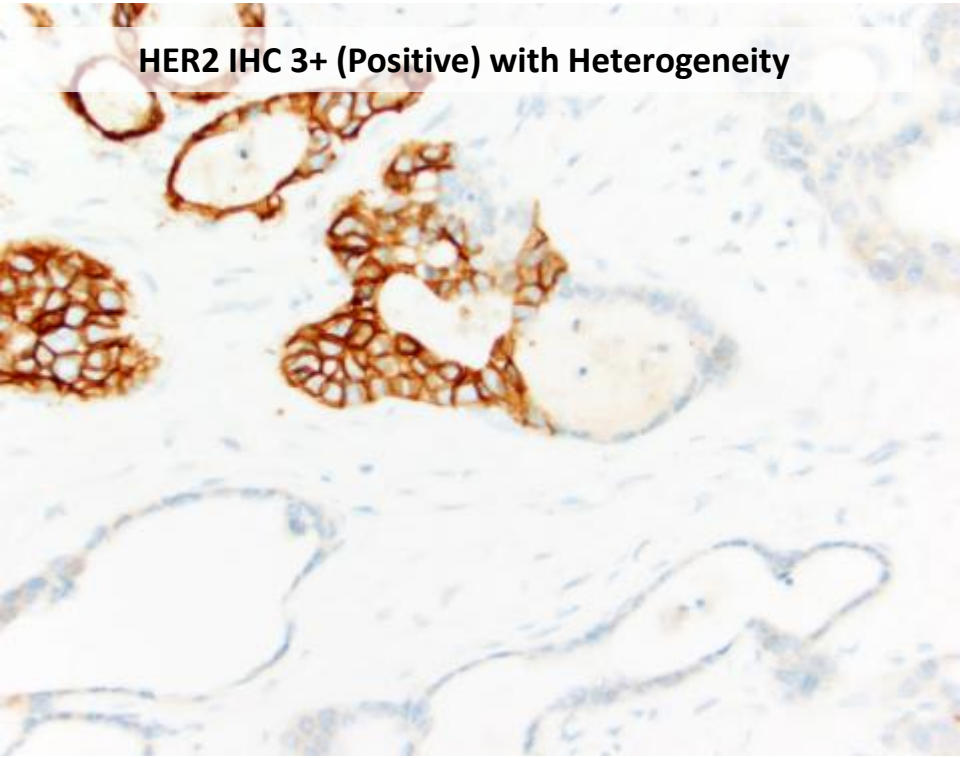


HER2 IHC 1+ (Negative)

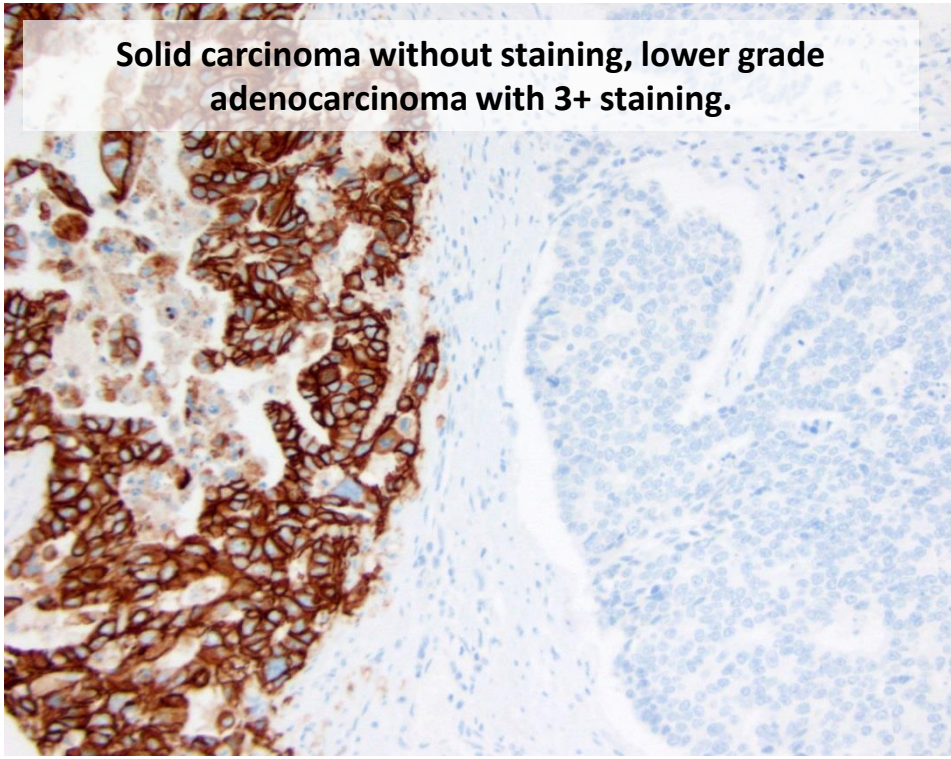
(Faint membranous staining only visible with 40x objective magnification)



HER2 in Colorectal Carcinoma: Issues with Heterogeneous HER2 Expression



HER2 IHC 3+ (Positive) with Heterogeneity



Solid carcinoma without staining, lower grade adenocarcinoma with 3+ staining.

HER2 IHC: Antibody Selection, Pre-Analytic Considerations & Reporting

- **FDA approved HER2 IHC tests**
  - CAP/ASCP/ASCO/AMP has no recommendations on HER2 testing in colorectal carcinoma.
  - Ventana Pathway clone (4B5) performed better than HercepTest (DAKO) when compared with ISH in the HERACLES trial of colorectal carcinoma.

- **Pre-analytic Considerations**
  - A minimum of 5 biopsy fragments (preferably 6 to 8) should be obtained to account for intratumoral heterogeneity.
  - Tissue should be placed in formalin within 1 hour (cold ischemic time).
  - Tissue should be fixed in 10% neutral buffered formalin for 6 to 72 hours.

- **Reporting (Gastric/Esophageal)**
  - Recommended benchmark of 90% of HER2 reports available within 10 working days.
  - If send out to reference laboratory, recommended benchmark of 90% of specimens sent to the reference lab within 3 working days.

Mod Pathol 2015;28:1481-91.
Arch Pathol Lab Med 2016;140:1345-1363.

Take Home Points

Ancillary Biomarker Testing

- Detection of MMR protein deficiency is advocated to screen for Lynch syndrome, is prognostically significant, and is a predictive biomarker for immunotherapy.
- Testing for mutations in *KRAS* and *NRAS* exons 2, 3, and 4 should be performed to determine eligibility for anti-EGFR therapy.
- *BRAF*-mutated microsatellite stable colorectal carcinoma is an aggressive subtype.
- HER2 testing in *RAS*-wild type colorectal carcinoma is increasingly requested; eligibility criteria are different than for gastric/esophageal tumors.

Colorectal Carcinoma
AJCC 8th Edition Updates & Ancillary Theranostic Testing

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