Gastrointestinal Pathology New Approaches to Old Problems

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Disclosures

- Salaried employee of AmeriPath Inc.
  - Wholly owned subsidiary of Quest Diagnostics Inc.
- Stock holder – Quest Diagnostics Inc.
- Advisory Board – Genomic Health Inc.
- Research collaborator – CSA Medical Inc.

Gastrointestinal Pathology New Approaches to Old Problems

- Colorectal cancer, MSI and serrated polyps
- Polyposis syndromes
- Gastrointestinal stromal tumors
- Neuroendocrine proliferations
- Barrett's esophagus

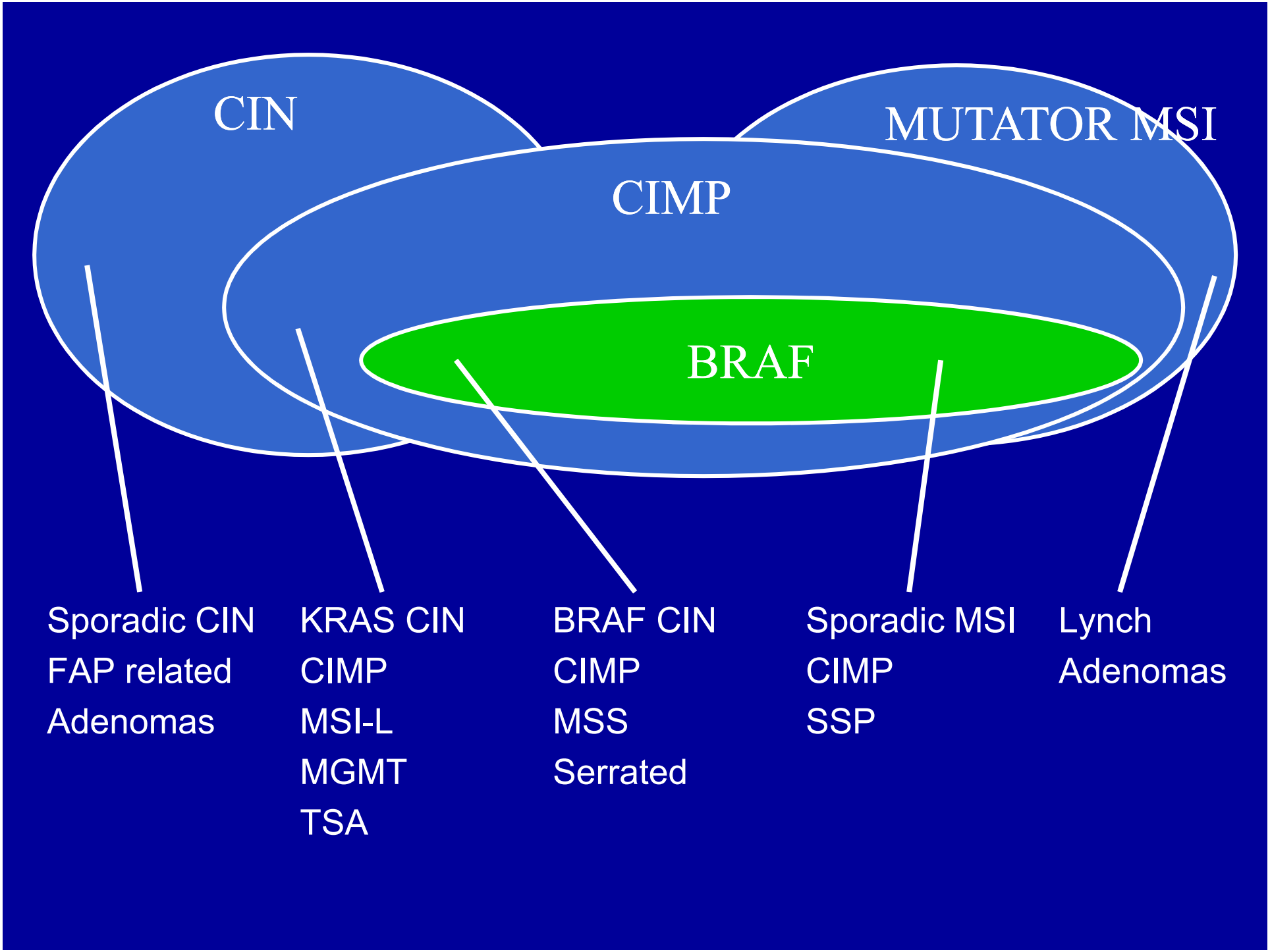
Kaiyo Takubo, Robert Petras, Jeremy Jass
IAP Montreal, 2006

Colorectal Cancer in a Molecular World

- Review known molecular pathways to colorectal carcinoma based on CIN, MSI, BRAF and CIMP
- Identify precursor lesions
- Discuss available testing
- Consider possible clinical implications

The Genetics of Colorectal Cancer

- Approximately 80 somatic mutations described
  - Limited number mutated in large proportion of cancers – “gene mountains”
  - 15 genes seen as critical drivers (e.g., APC KRAS, TP53, BRAF, PTEN, DCC, CMYC, etc.)
- More than 100 genes silenced by epigenetic mechanisms (e.g., hypermethylation)


CIN
MUTATOR MSI
CIMP
BRAF
Sporadic CIN
FAP related
Adenomas
KRAS CIN
CIMP
MSI-L
MGMT
TSA
BRAF CIN
CIMP
MSS
Serrated
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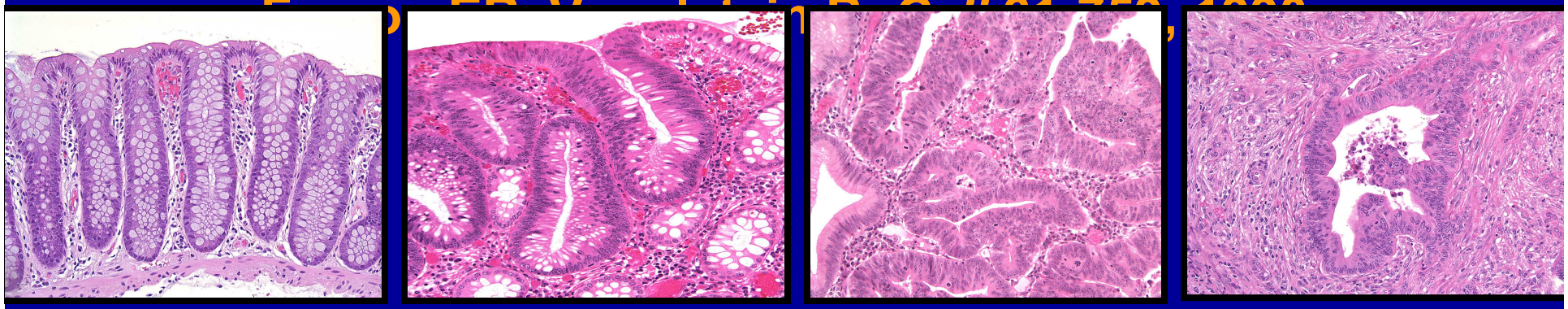
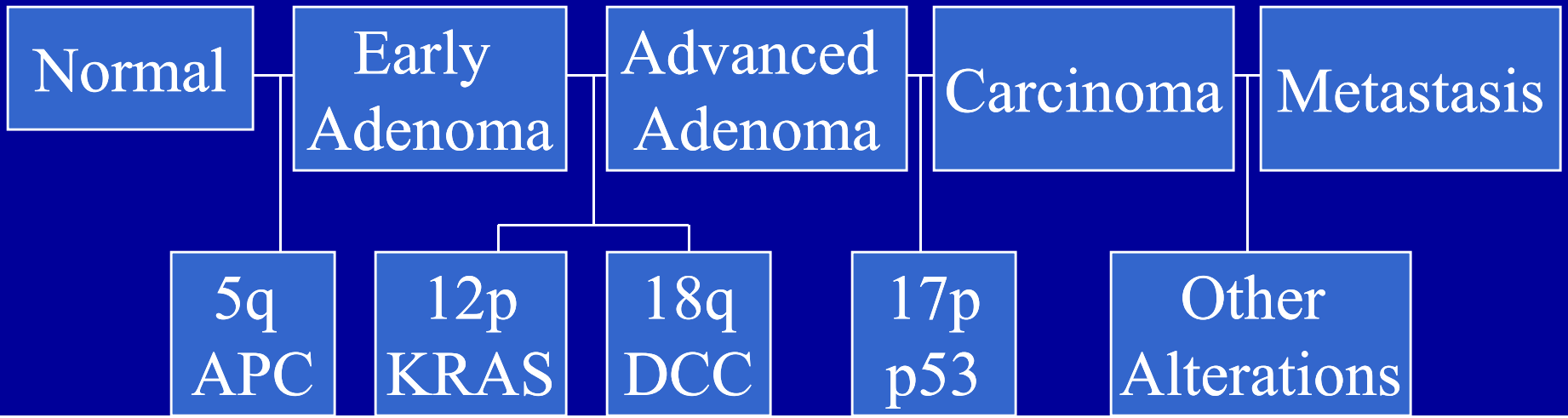
SSP
Lynch
Adenomas

Chromosomal Instability
The Adenoma Carcinoma Sequence
The Fearon Vogelstein Model

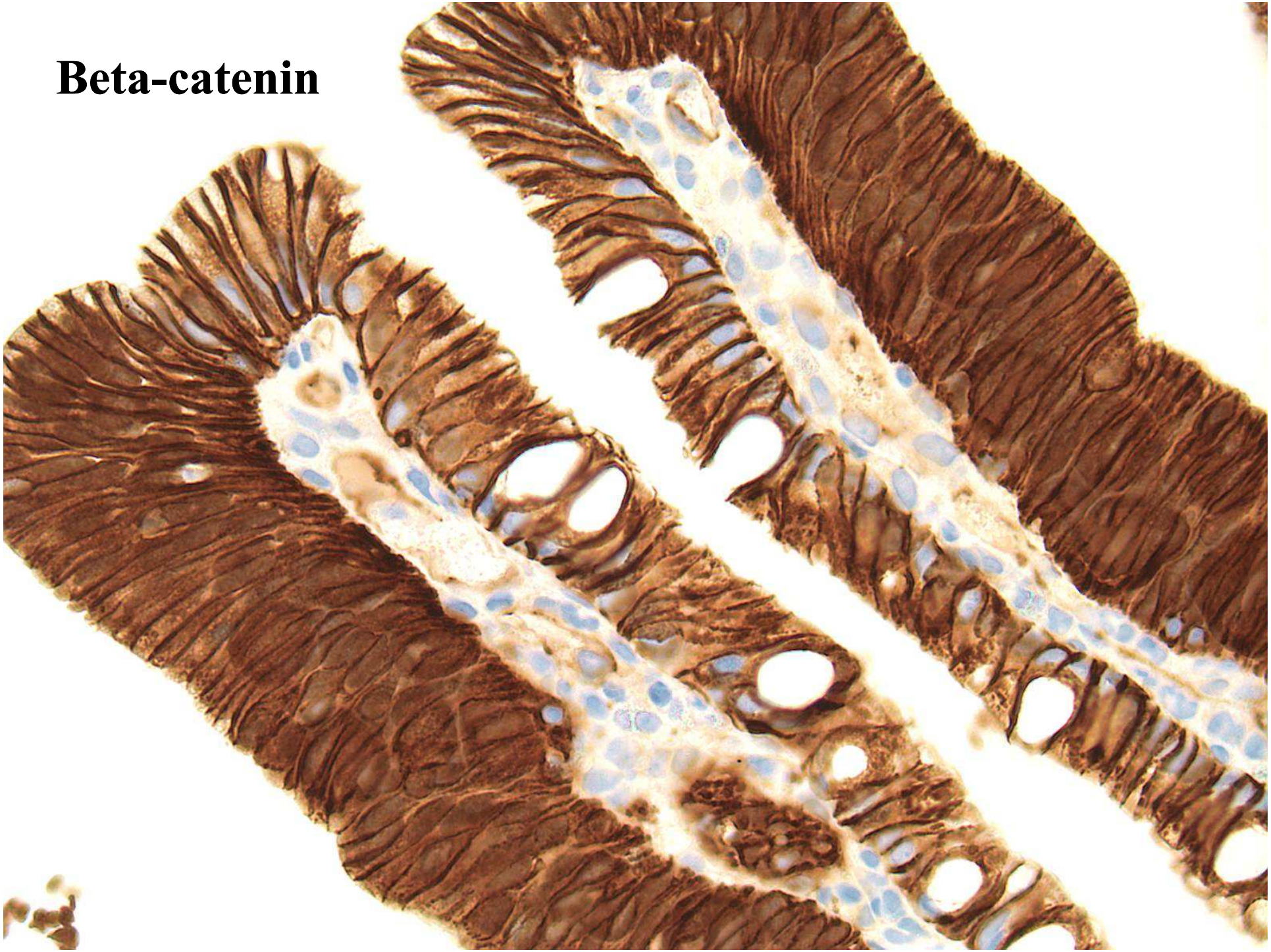




The APC Gene

- 70%-80% of colorectal cancers harbor somatic mutations that inactivate APC
  - Thought to be an early event; proportion of mutations high and similar small adenoma vs. advanced adenoma
- APC protein is a major regulator of beta-catenin in the Wnt signaling pathway
  - Without APC protein, destruction of beta-catenin disrupted
  - Accumulated beta-catenin translocates to nucleus and functions as a transcriptional coactivator for many genes (e.g., CMYC, cyclin D1)

Beta-catenin



The Other Genes

- KRAS
  - Part of the MAPK pathway
  - Involved in cellular proliferation and progression
  - Seen in 10% of small adenomas, 50% of those >1 cm and 30% of cancers
- DCC (SMAD-4)
  - Regulate gene transcription downstream of TGF-beta
  - Seen in 10% of small adenomas, 50% of advanced adenomas and 70% of cancers
- TP53
  - Stops cell cycle and induces apoptosis in cells that have irreversible DNA damage; rare in adenomas, seen in 70% of cancers

Chromosomal Instability
The Adenoma Carcinoma Sequence
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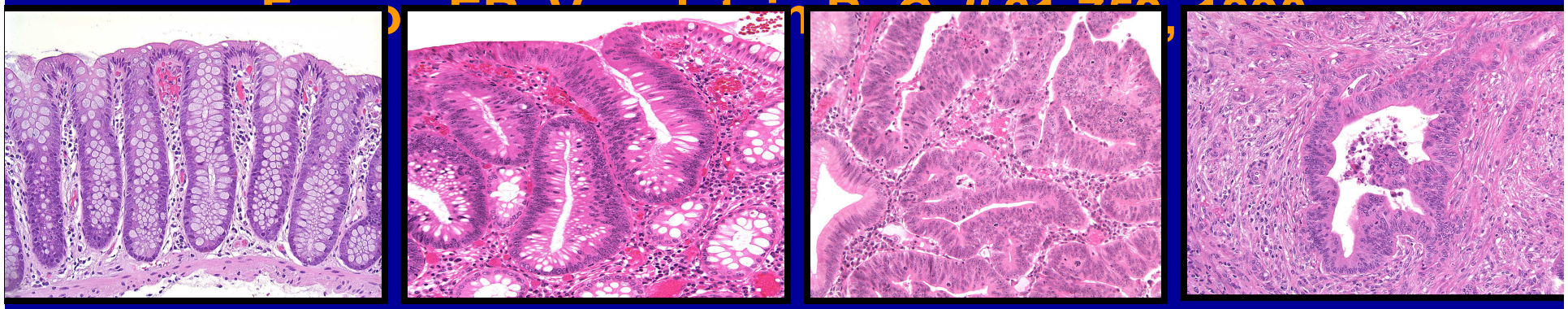
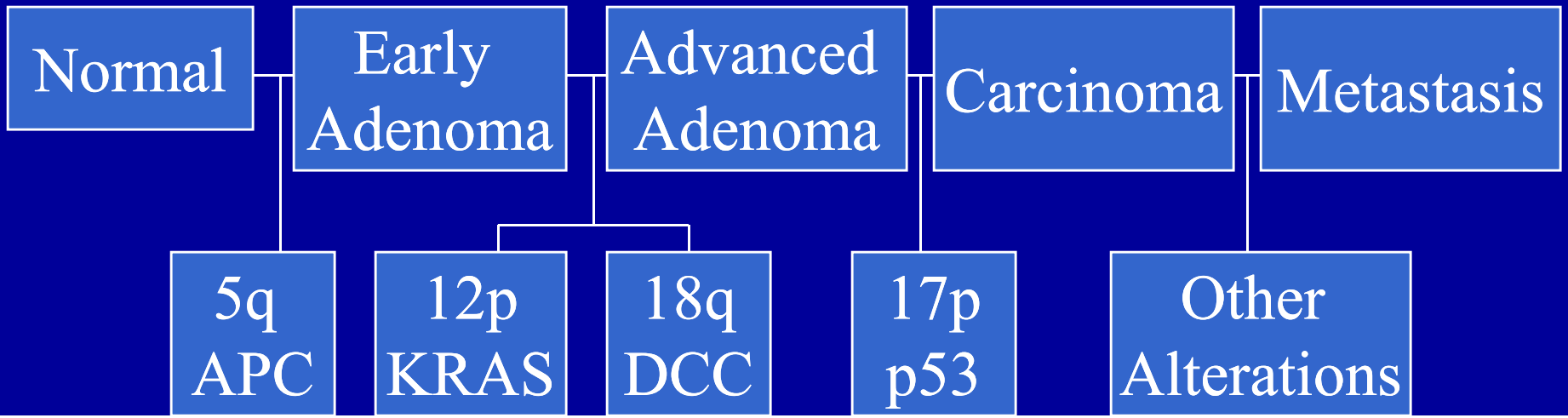


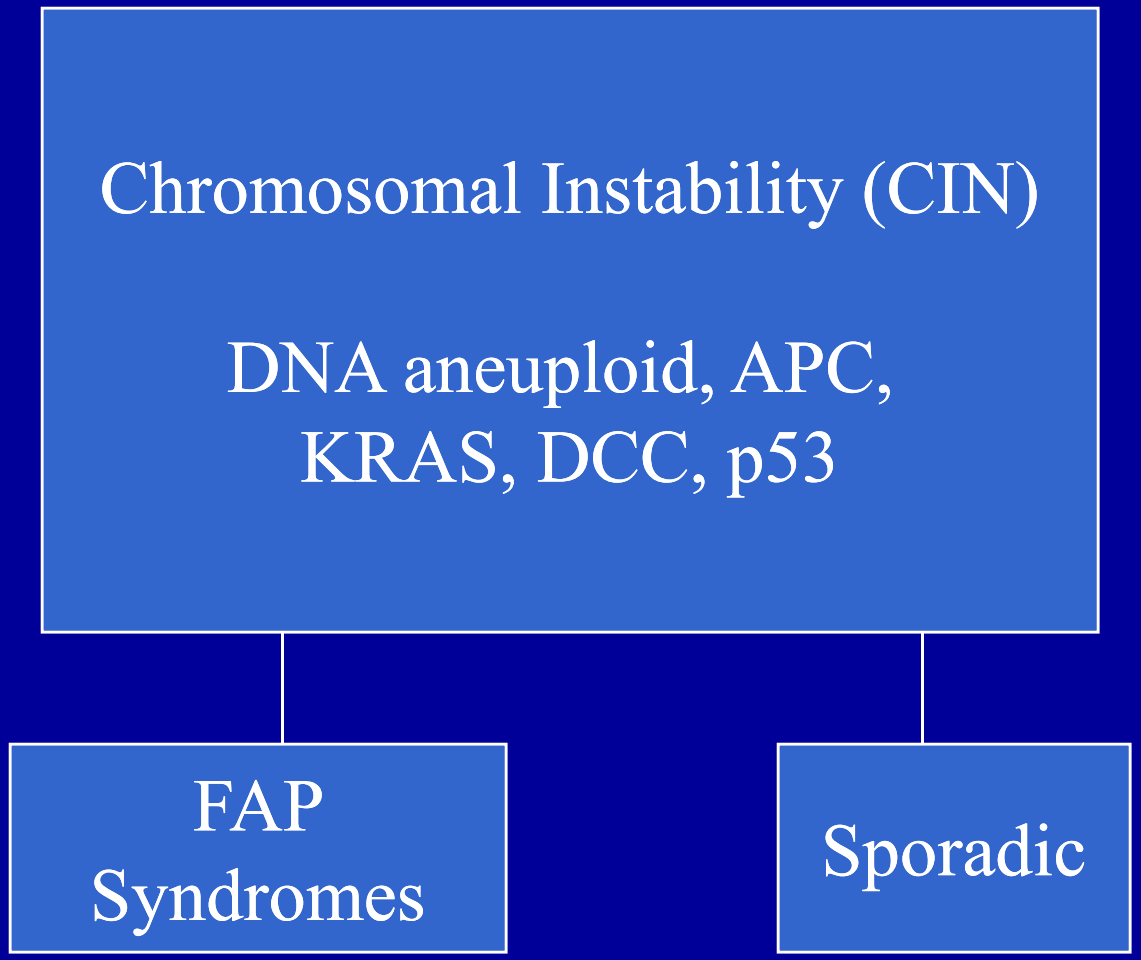


The Adenoma – Carcinoma Sequence

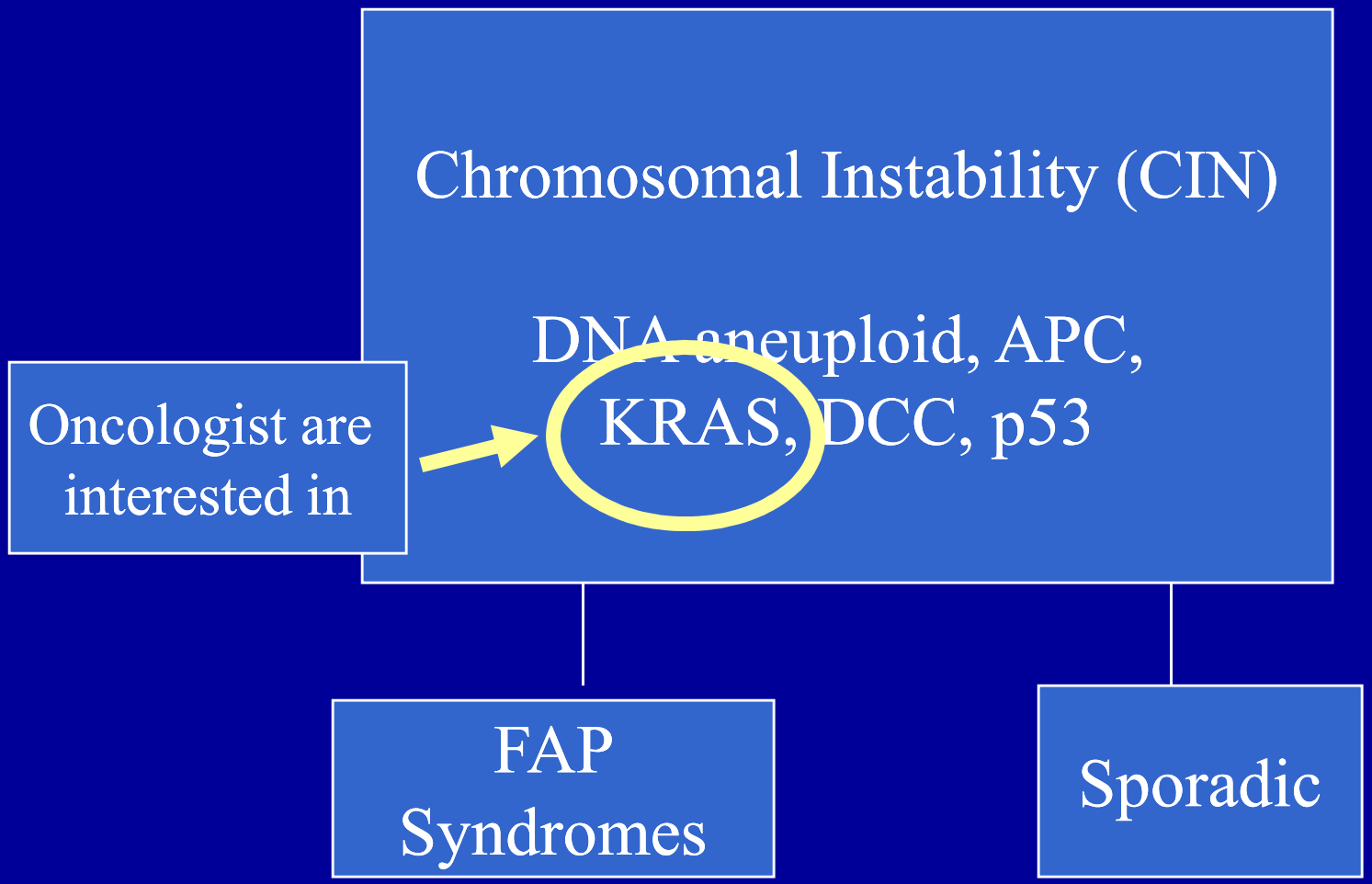
Fallacies of Thought

- Colorectal cancer is homogeneous
- Evolution of lesion follows the Fearon Vogelstein model and is uniform and linear
- FAP was the hereditary model for the vast majority or colorectal

Colorectal Cancer in the Molecular Era



Colorectal Cancer in the Molecular Era



Exploiting EGFR in Treatment

- Monoclonal antibodies that compete with the ligands for binding
  - Cetuximab, panitumumab
- Low molecular weight tyrosine kinase inhibitors
  - Gefitinib, erlotinib
  - The mutation in the catalytic domain of EGFR (predicts response) is rare (0.4%) in colorectal cancer

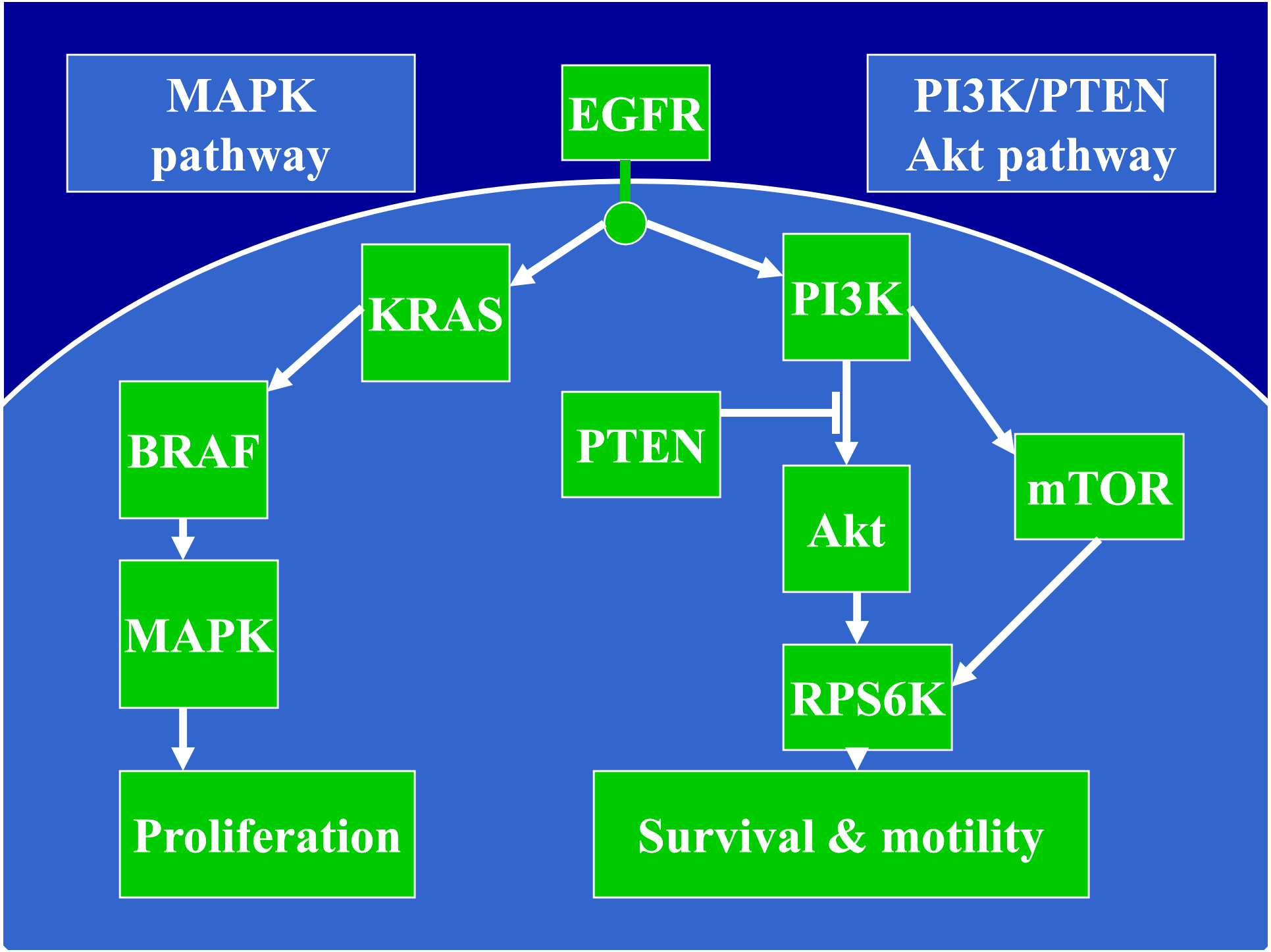
EGFR Targeted Monoclonal Antibodies

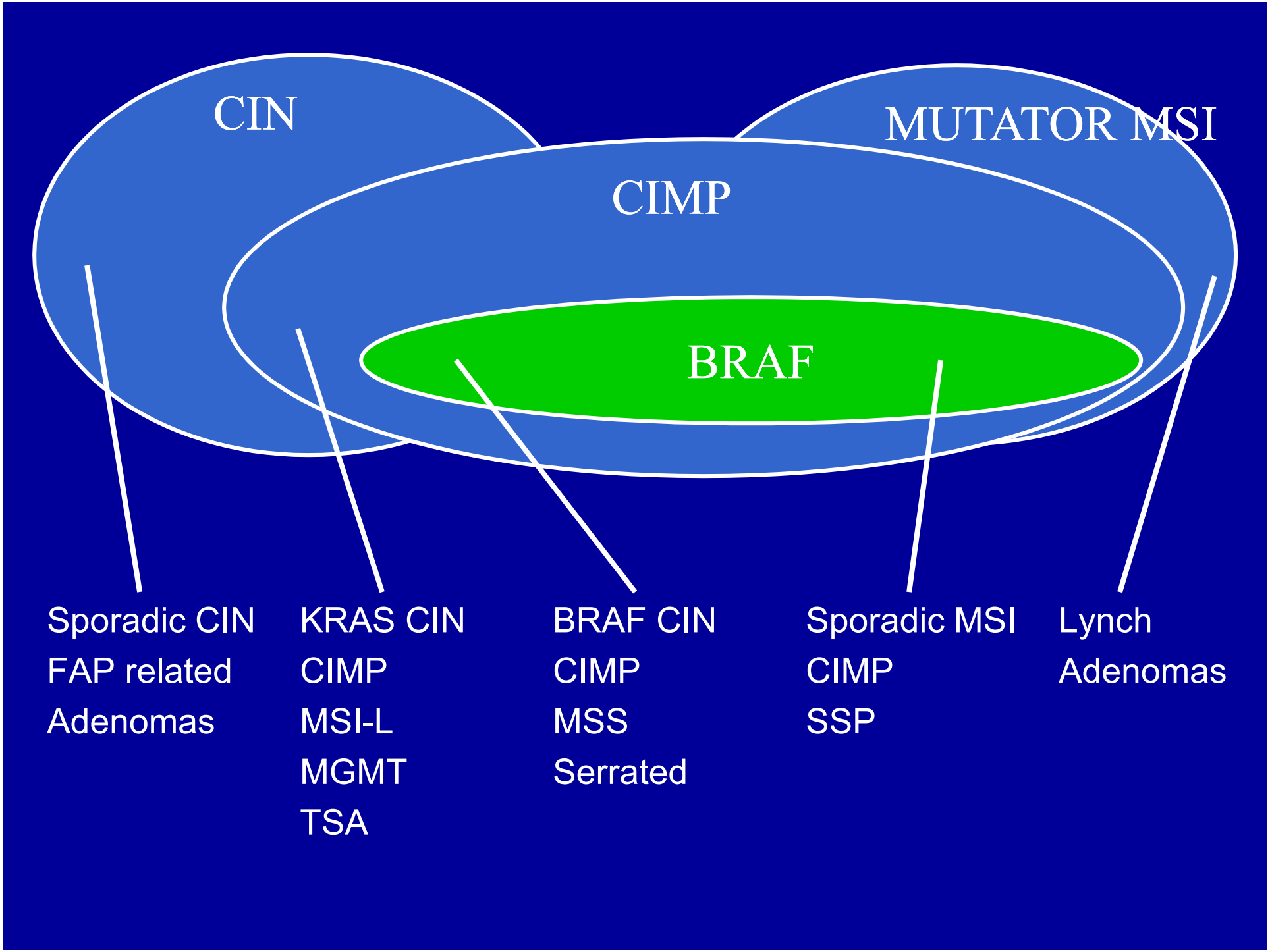
- Certuximab (Erbitux) - Chimeric monoclonal antibody
- Panitumumab (Vectibix) – fully humanized monoclonal antibody
- Trials show clinically significant activity against advanced colorectal cancer either alone or in combination with traditional chemotherapy

K-ras Mutation and Cetuximab

- Wild-type K-ras – Improved survival (median 9.5 vs. 4.8 mo.) $p<0.001$
- Mutated K-ras – No difference treatment vs. supportive care alone

Karapetis CS, et al. *NEJM* 359:1757, 2008.


MAPK pathway
EGFR
PI3K/PTEN Akt pathway
KRAS
PI3K
BRAF
PTEN
Akt
mTOR
MAPK
RPS6K
Proliferation
Survival & motility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Lynch
Adenomas

The Serrated Polyp Family: Its Relationship to Colorectal Cancer

- Review colorectal cancer genetics with reference to MSI and Lynch Syndrome (HNPCC)
- Describe morphology of serrated polyp family
- Present evidence linking a subset of “hyperplastic polyps” to MSI-H cancer
- Provide a pragmatic approach to these lesions

Colorectal Carcinoma
Molecular Classification

- 85% Chromosomal instability
  - DNA aneuploidy
  - Abnormalities of Chromosomes 5, 17, 18
  - < 1% FAP
- 15% Mutator phenotype
  - DNA diploid
  - Microsatellite instability
  - 1-3% Lynch syndrome

Microsatellites

- Genomic regions with repetitive sequences of 1-6 bases (e.g., CACACA etc.)
- Prone to replication error due to misalignment of repeat units
- Errors usually corrected by mismatch repair gene proteins
- Key genes that contain microsatellites in coding regions
  - *BAX* – apoptotic pathway *Bcl-2*
  - *TGF-BII* - disrupt TGF-beta tumor suppression

Microsatellite Instability

- MSI – expansion or contraction of short repeat DNA sequences (microsatellites)
  - Marker for abnormalities of MMR
- Phenomenon found in tumor DNA when compared to non-neoplastic mucosa
  - Somatic inactivation of mismatch repair genes (e.g., hypermethylation)
  - Detected in persons with germline mismatch repair gene mutation (Lynch)
- MSI detected in >95% of Lynch syndrome tumors and 15% of sporadic

International Guidelines for Evaluation of MSI

Consensus Markers

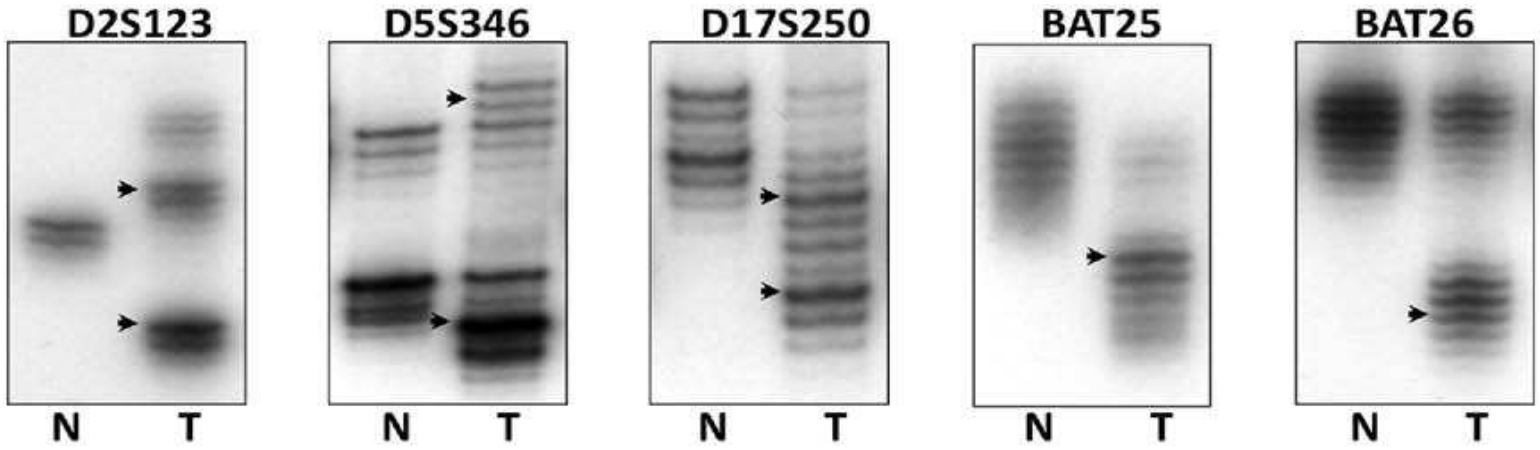
BAT 25

BAT 26

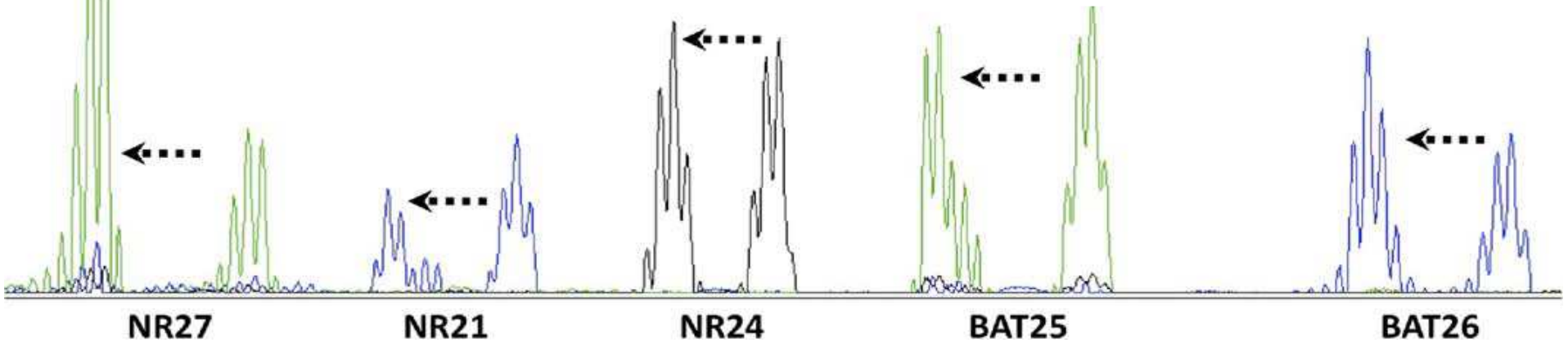
D5S346

D2S123

D17S250



Autoradiography of PCR products separated by gel electrophoresis



PCR products analyzed by automated DNA sequencing with fluorescent primers; mutant alleles (arrows)

Boland RC, Goel A. *Gastroenterol* 138:2073, 2010

International Guidelines for Evaluation of MSI

	5 loci	>5 loci
MSI – high	≥ 2	≥ 30-40%
MSI – low	1	< 30-40%
MSS	0	0

DNA Mismatch Repair Genes

- Maintain fidelity of DNA during replication
- Correct nucleotide base mispairs and small insertions and deletions generated by misincorporation or slippage of DNA-polymerase during DNA replication

Mismatch Repair Genes

Frequency in Lynch Syndrome

• *hMLH1*	49%	3p21
• *hMSH2*	45%	2p15
• *hPMS2*	4%	7p22
• *hMSH6*	1%	2p15
• *hPMS1*	1%	2p32
• *hMSH3*	0%	5q11-13

Hereditary Nonpolyposis Colon Cancer Syndrome

- Increased lifetime risk of colorectal cancer
  - 80% vs. 5% general population
- Cancer develops at early age
  - Mean 45 vs. 64 general and 39 in FAP
- Increased risk of other cancers
  - Endometrial (40%), ovary, gastric, urinary tract, renal pelvis, biliary tract, CNS (glioblastoma), small

HNPCC

Identifying Patients and Families

- Patient and family medical history
- Pathologic findings in tumors
- Special testing

Diagnosis of HNPCC

Amsterdam Criteria I

- Three or more relatives with colorectal carcinoma, at least one a first degree relative
- Colorectal carcinoma in 2 generations
- One or more colorectal carcinomas in a person younger than 50 years

Diagnosis of HNPCC
Amsterdam Criteria II

- Three or more relatives with any HNPCC related carcinoma
  - Colorectal, endometrial, ovarian, small bowel, urinary, biliary, gastric, kidney
- Colorectal carcinoma in 2 generations
- One or more HNPCC related carcinoma in a person younger than 50

Diagnosis of HNPCC

Limitations of Amsterdam

- Small families
- Family history unknown/incomplete
- Physician not thorough
- Up to 8% of Amsterdam negative people have mutation of mismatch repair gene
- Only 60 % of Amsterdam positive have detectable mutation of known genes
  - Familial colorectal cancer syndrome type X

Familial Colorectal Cancer Type X

- More left sided
- Less extracolonic manifestations
- Later age for cancer
- Different molecular mechanisms
  - Abnormalities of EPCAM found in some
    - Exon 8 and 9 deletion on chromosome 2
    - Hypermethylation of MSH2 with incomplete silencing

HNPCC

Identifying Patients and Families

- Patient and family medical history
- Pathologic findings in tumors
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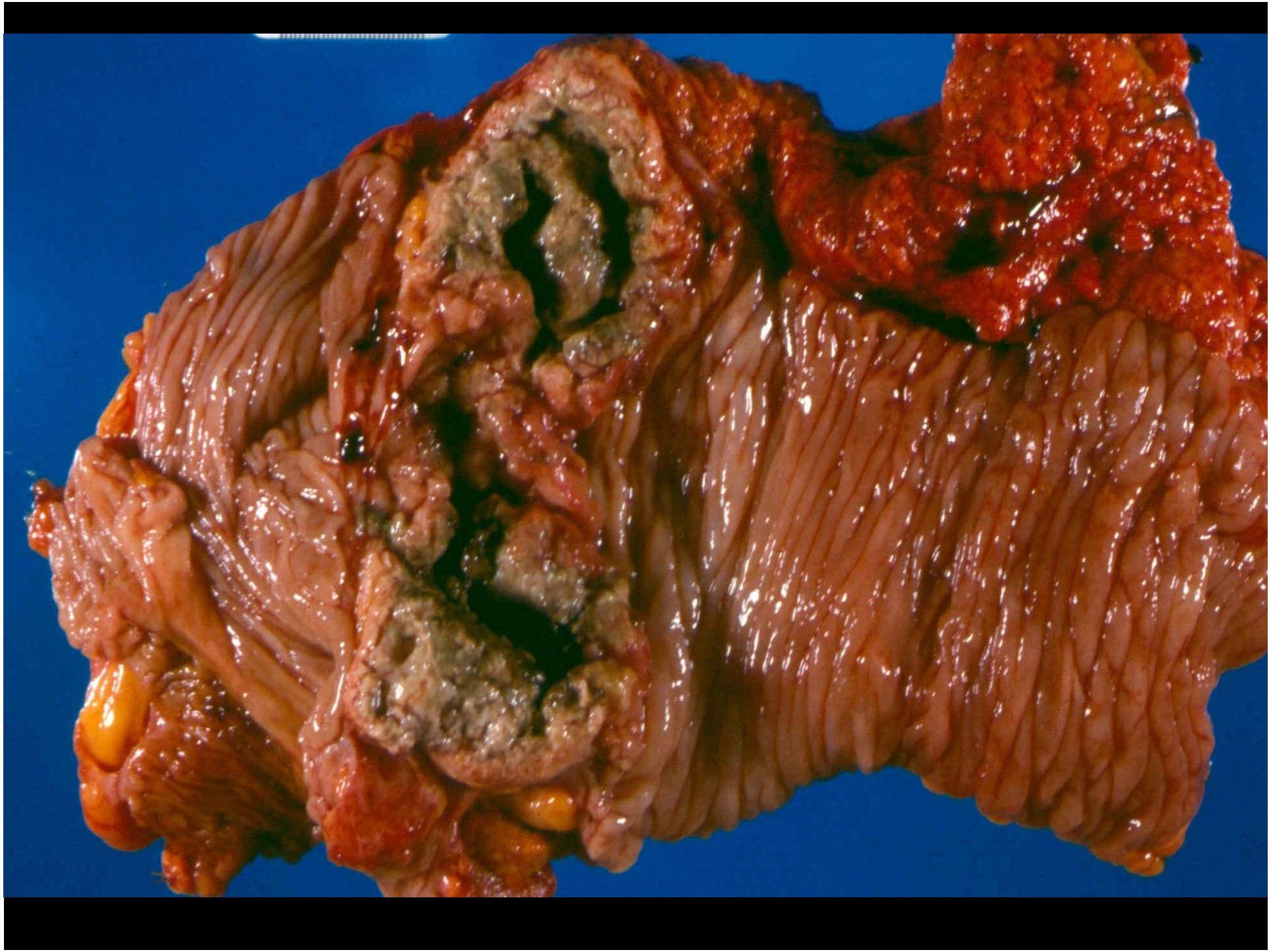
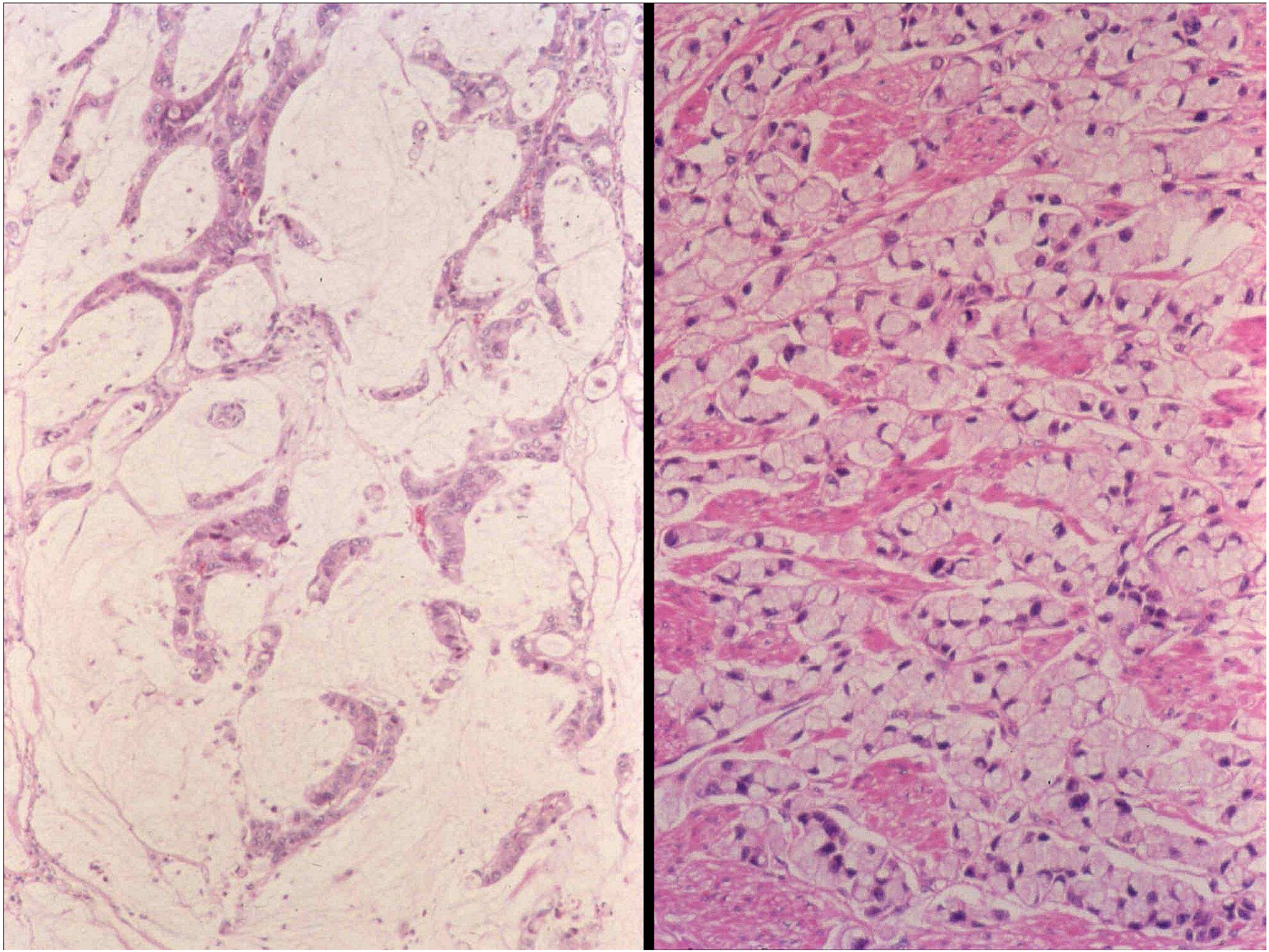
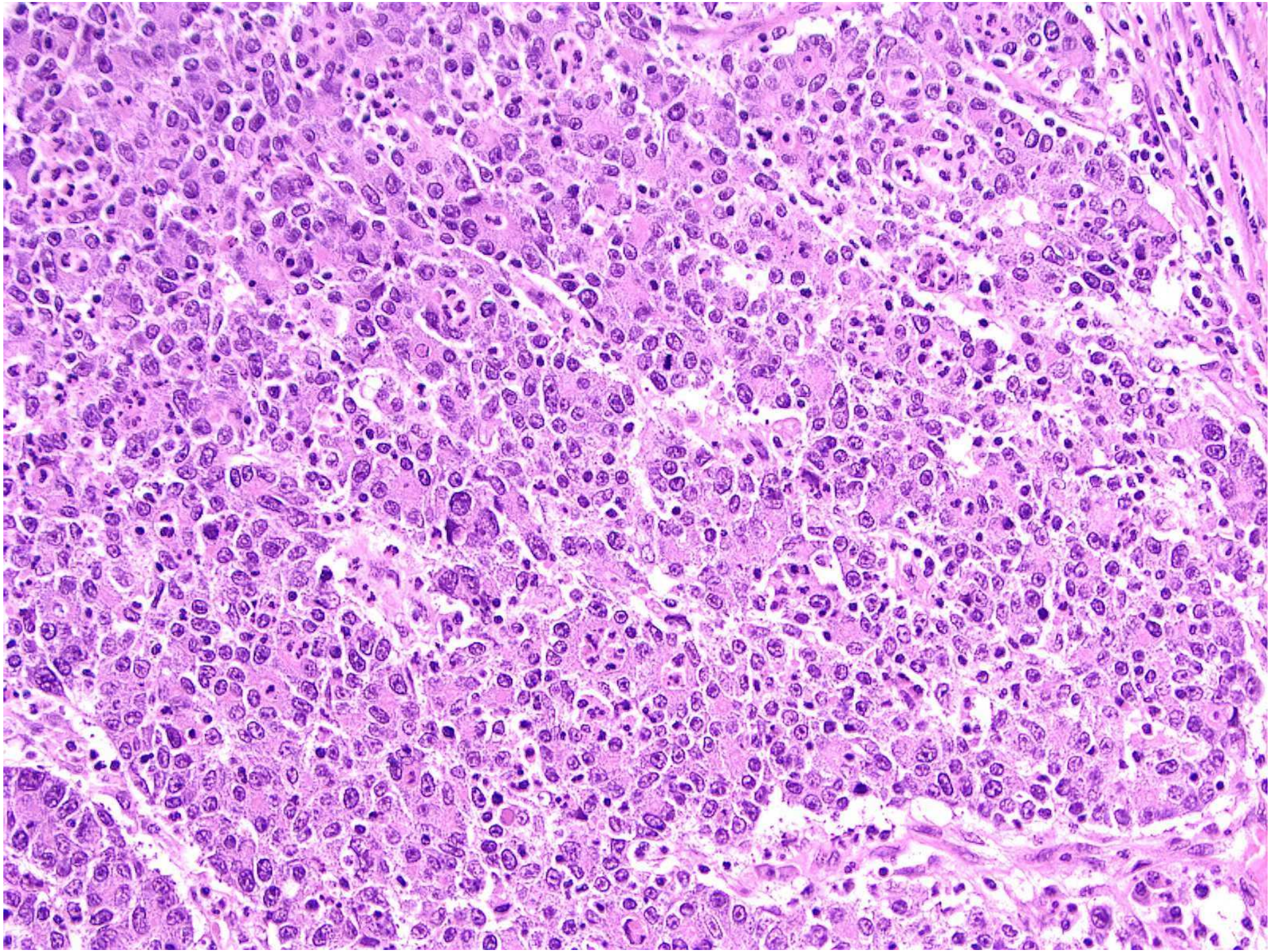
Lynch/MSI-H Colorectal Cancer

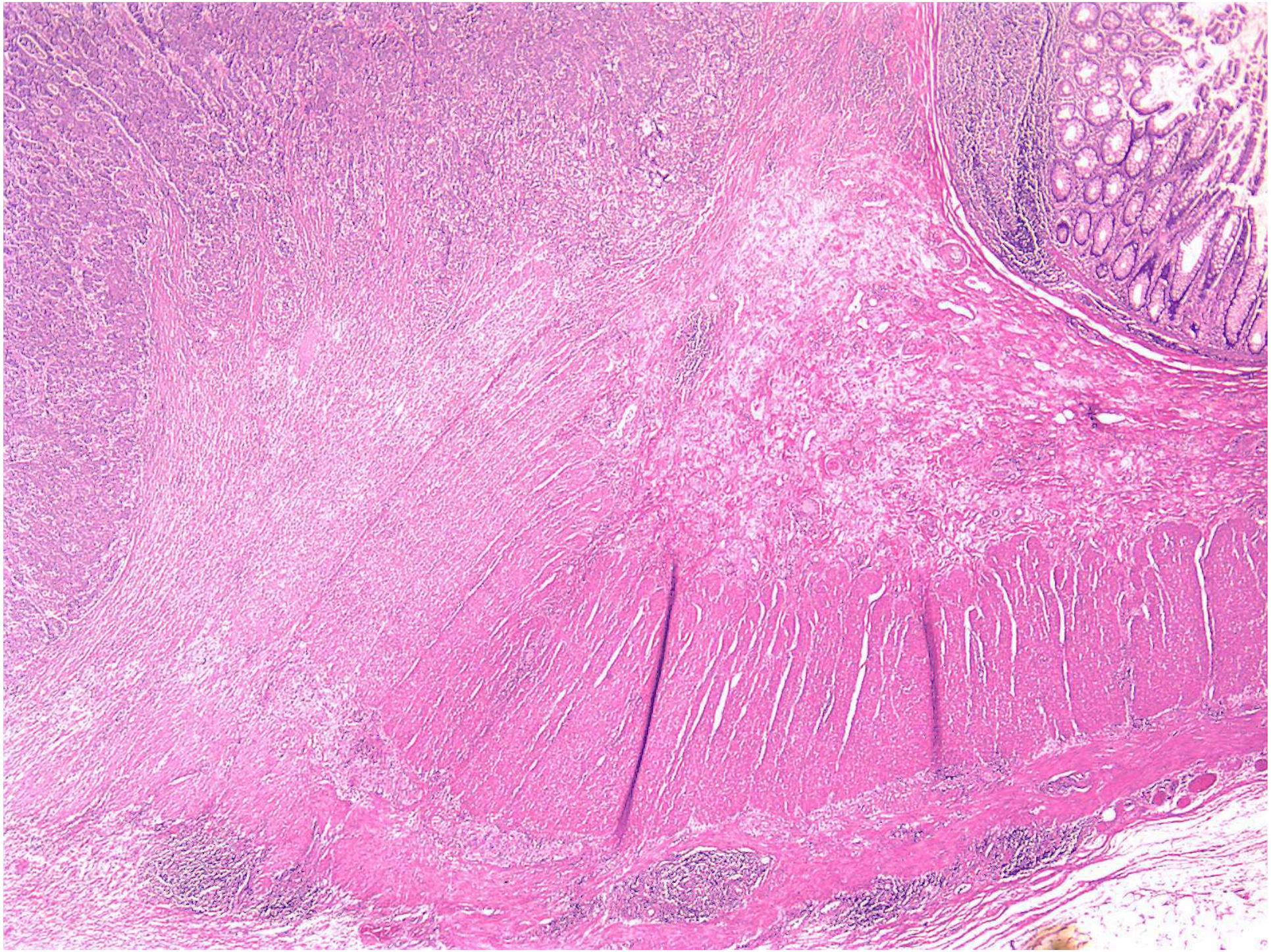
Pathological Features

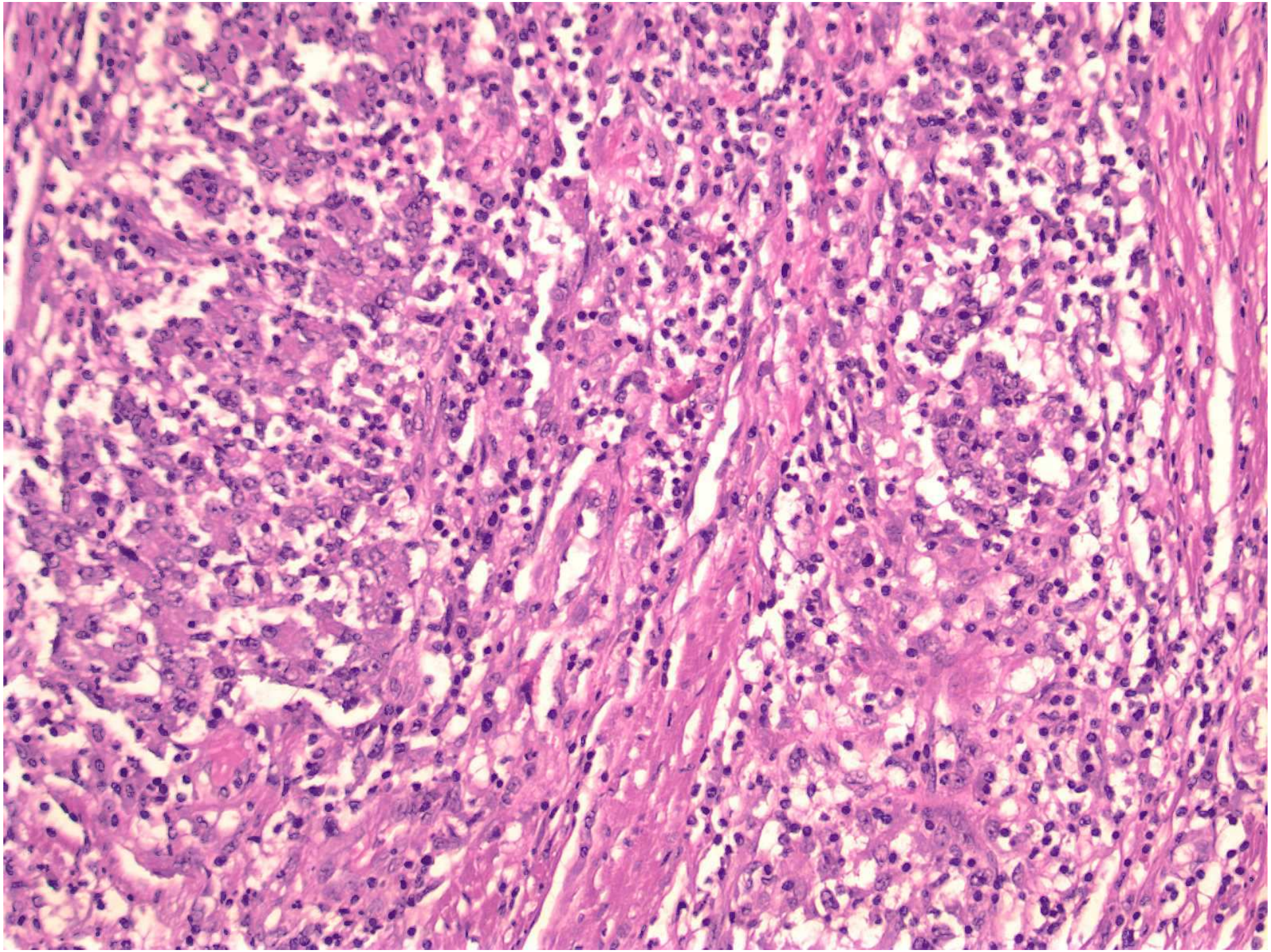
- Right sided location
- Synchronous or metachronous cancers
- Large bulky tumors
- Prominent lymphoid infiltration
- Poor differentiation
- Mucinous and signet ring histology











HNPCC

Identifying Patients and Families

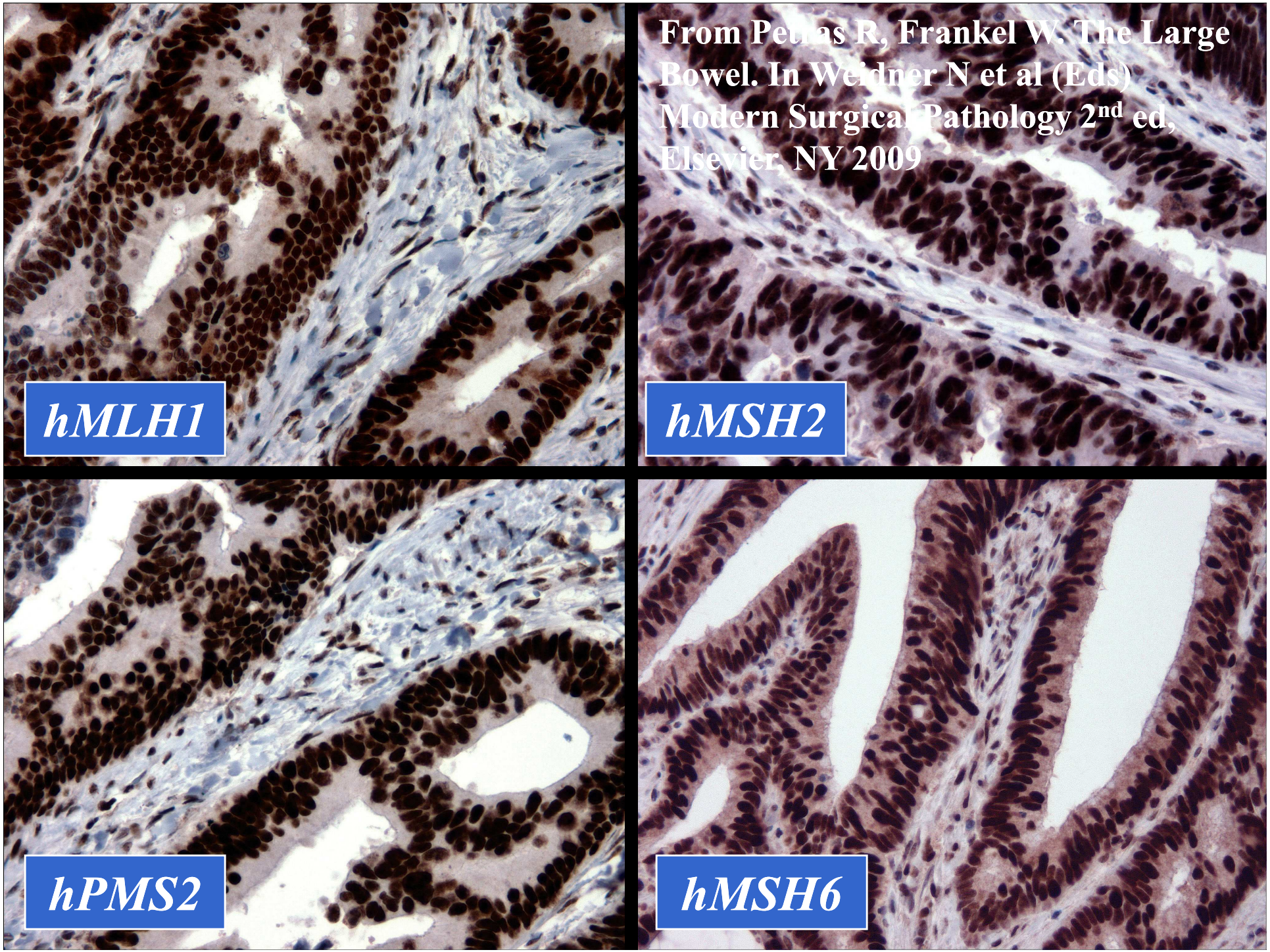
- Patient and family medical history
- Pathologic findings in tumors
- Special testing

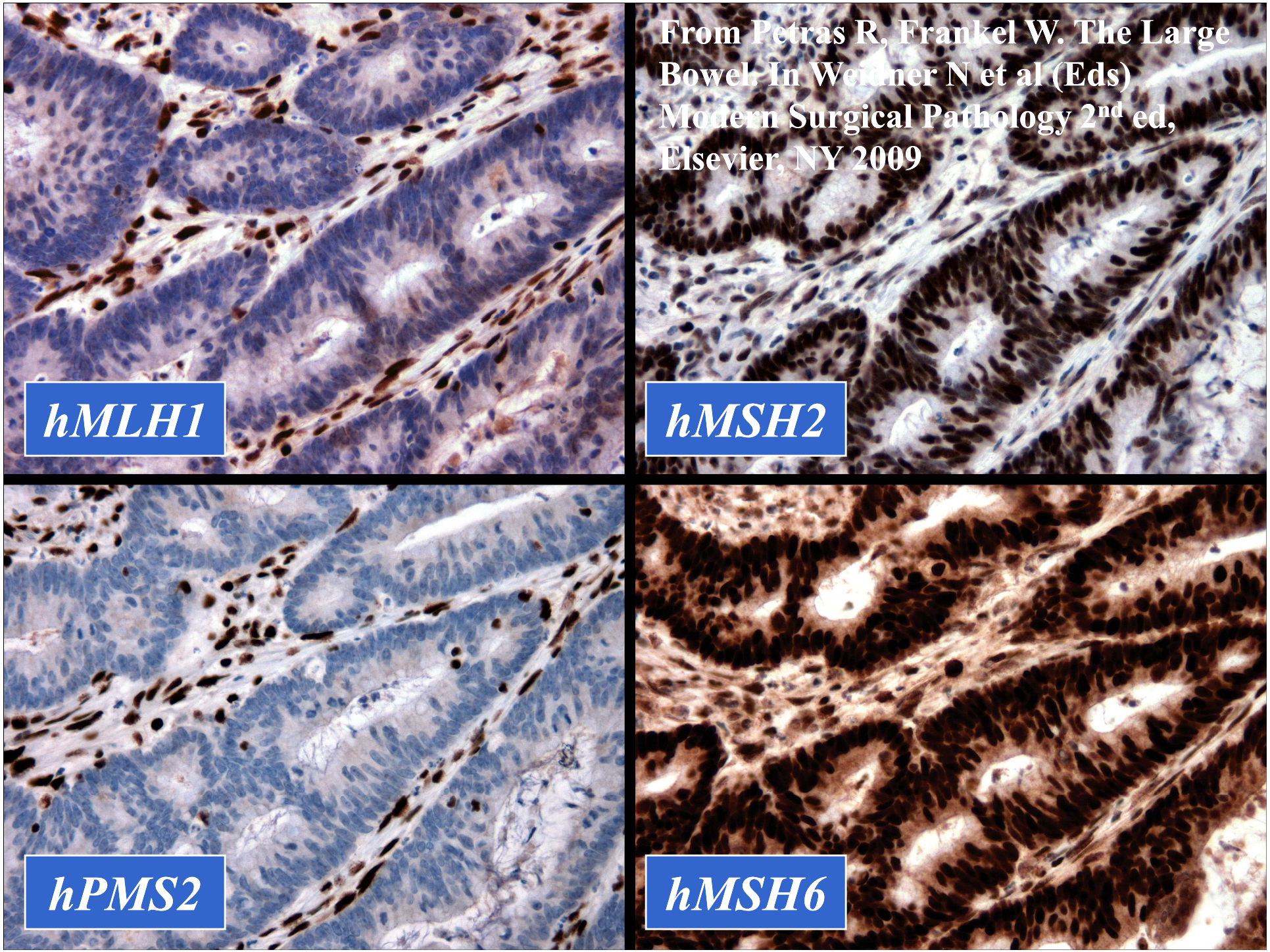
Testing Colorectal Cancers for MSI

Revised Bethesda Criteria

- Just one criterion need be met:
  - Colorectal cancer before age 50
  - Synchronous or metachronous HNPCC related tumor, regardless of age
  - Colorectal cancer with MSI-H histology before age 60
  - Colorectal cancer and a 1st degree relative with a HNPCC cancer detected before age 50
  - Colorectal cancer with ≥2 relatives with HNPCC cancer regardless of age

Modified Umar A et al *J Natl Cancer Inst* 96:261,2004.


From Petras R, Frankel W. The Large Bowel. In Weidner N et al (Eds) Modern Surgical Pathology 2nd ed, Elsevier, NY 2009
hMLH1
hMSH2
hPMS2
hMSH6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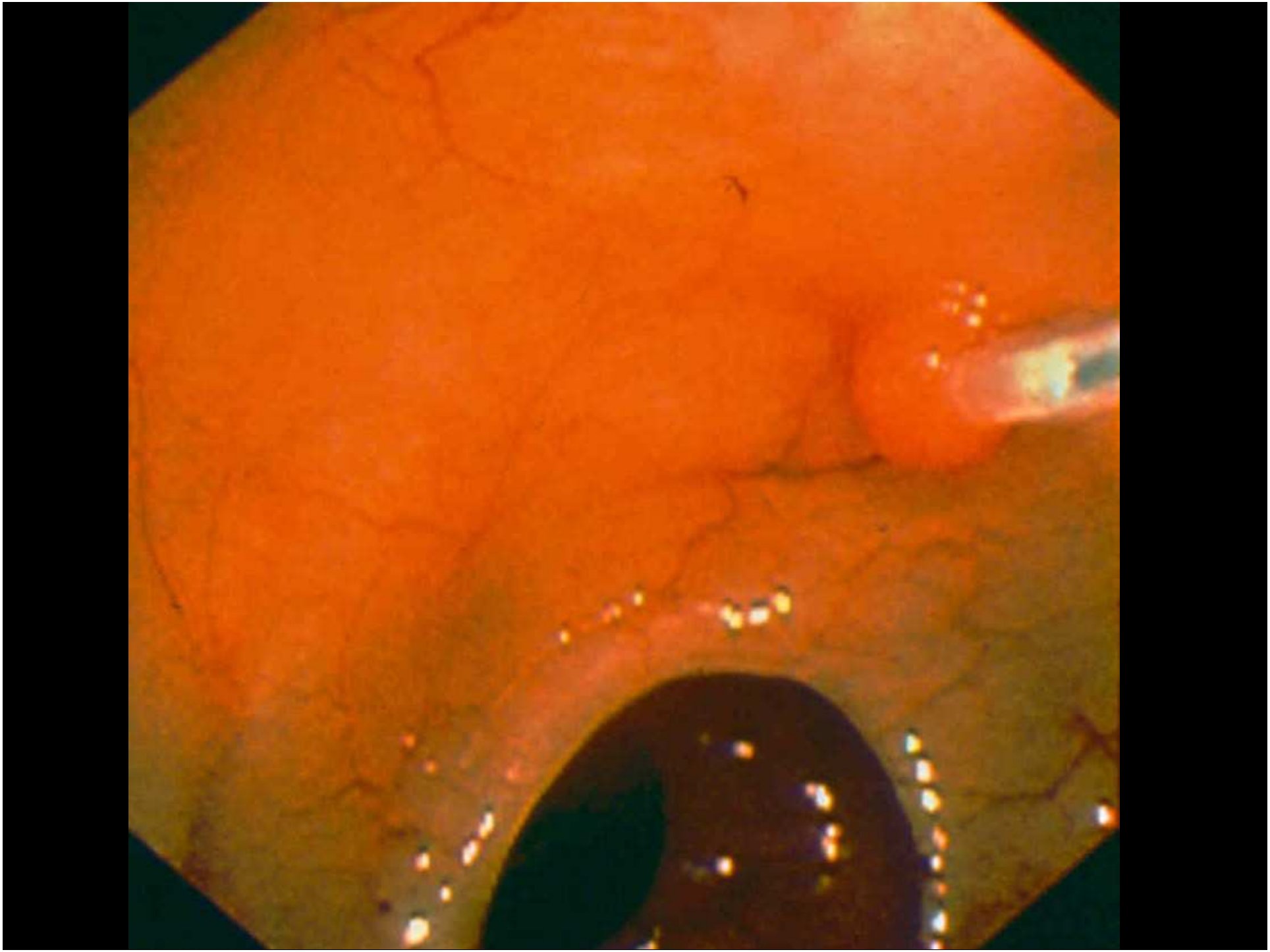
Immunohistochemistry for Mismatch Repair Gene Proteins

- Relatively inexpensive
- Loss of expression correlates highly with MSI
- Majority of MSI high sporadic colorectal cancer related to somatic hypermethylation of hMLH1 promoter
- Absent expression of hMSH2, hMSH6, hPMS2 correlates with gene mutation
- Some absent hMLH1 due to gene mutation
- MSI-H may not be seen in hMSH6 mutation

Testing for MSI and IH for Mismatch Repair Gene Proteins

Should We Do it Routinely?

- Survival advantage; Increased metachronous tumors
- May predict response to chemotherapy
  - Improved survival in MSS, MSI-L stage II and III cancer with fluorouracil (*NEJM* 349:247, 2003; *Gastroenterol* 126:394, 2004)
  - Improved survival with irinotecan (*J Clin Oncol* 27:1814, 2009)
- Could aid in detecting Lynch Syndrome
  - 44% probands > 50; 22% did not meet



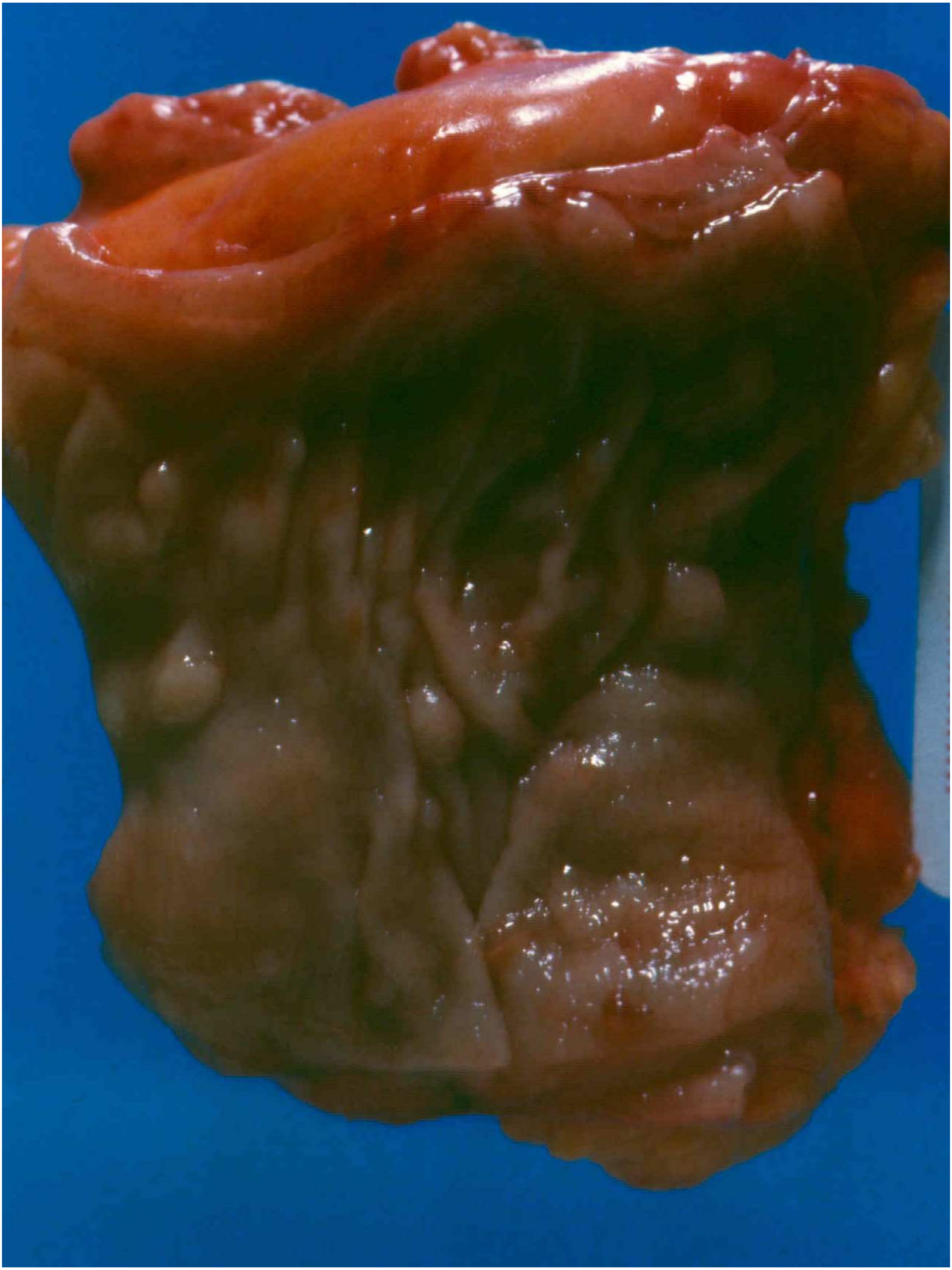
Hyperplastic Polyps

- Most common colorectal polyp
- Usually multiple
- Increased prevalence with increasing age
  - < 40 – 40%
  - > 40 – 75%

Riddell RH, Petras RE, Williams GT, Sobin LH.
Tumors of the intestines. AFIP, Washington, D.C. 2003

Colonic Hyperplastic Polyps

- Gross Appearance:
  - Small (<5 mm)
  - Sessile, usually same color as colon
- Histologic Features:
  - Absorptive and goblet cells
  - Elongate and dilated crypts
  - Serration/micropapillation
  - Thickened basement membrane
  - Regenerative epithelial changes



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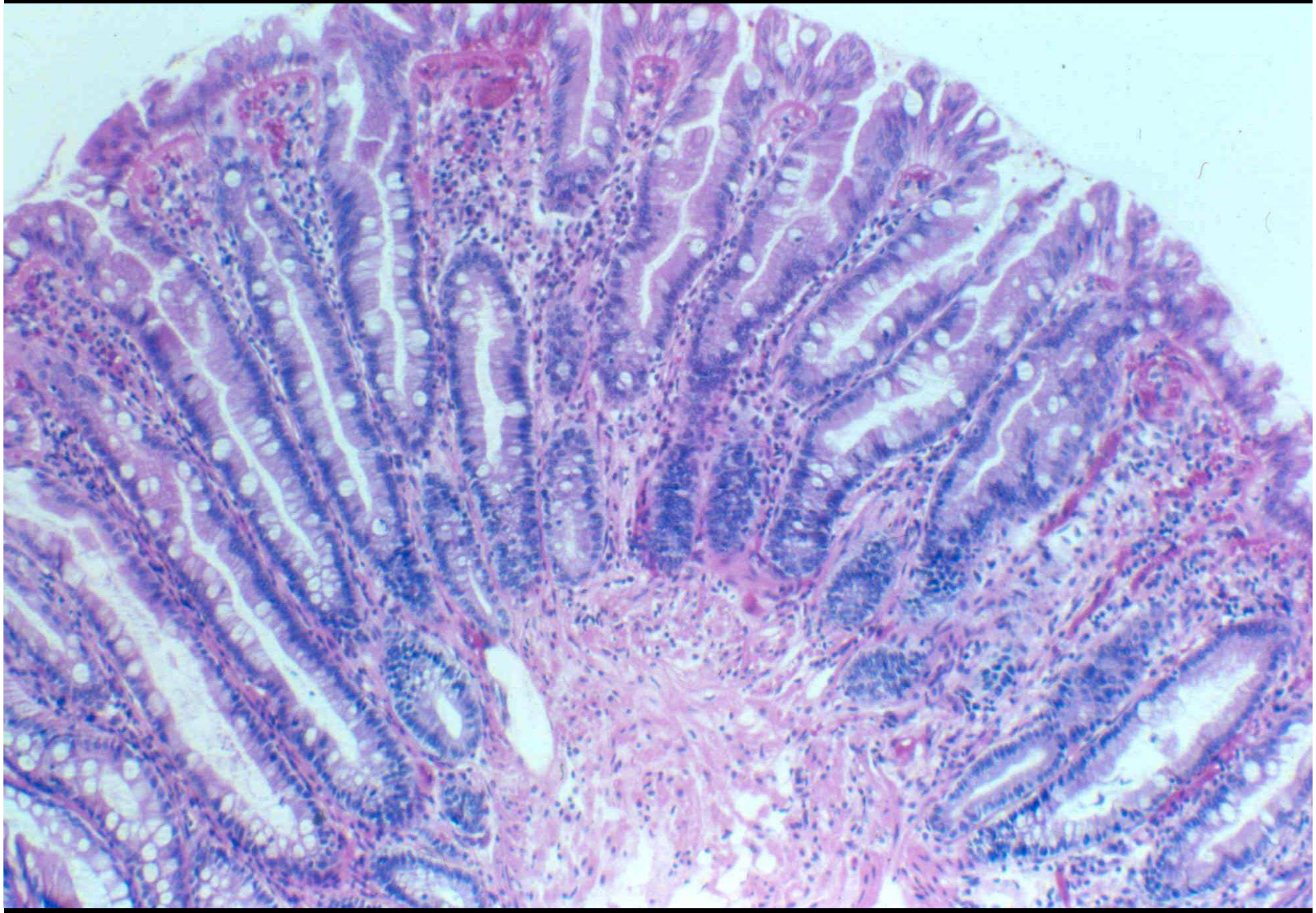

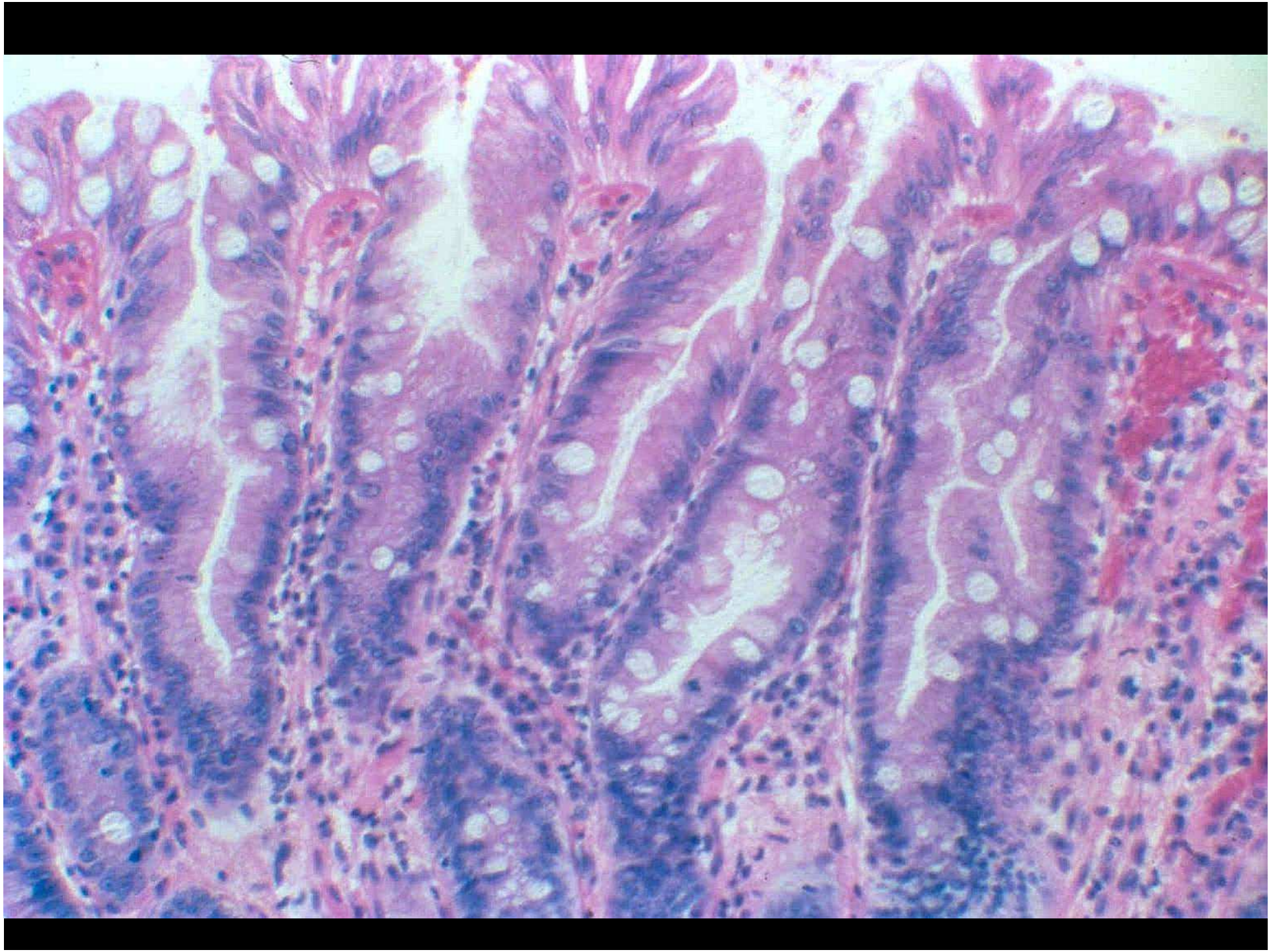

Serrated Polyps and Carcinoma

- Patients with MSI-H cancers have a high rate of serrated polyps but not typical adenomas
- Clear association of carcinoma with "hyperplastic (serrated) polyposis"
- Case reports and small series of cancers reported in association with SSP
- Report of colon cancer detected at site of previously sampled SSP
- Methylation induced inactivation of hMLH1 and MGMT, BRAF mutations in HPs, SSPs and cancers

Serrated Polyps and MSI Colorectal Adenocarcinoma

- 29 MSI Carcinomas
  - 114 “hyperplastic” polyps; 12 serrated adenomas; 14 conventional adenomas
- 29 MSS Carcinomas
  - 4 “hyperplastic” polyps; 3 serrated adenomas; 15 conventional adenomas
- Conclusions:
  - MSI more likely to harbor at least one serrated polyp (OR = 4.0; 95% CI = 1.1-14.2 P = 0.03)
  - Frequency of adenomas same in both groups (P = 0.52 Mann-Whitney test)

Hawkins NJ, Ward RL. *J Natl Cancer Inst* 93:1307, 2001

Serrated Polyps and Carcinoma

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“Hyperplastic (Serrated) Polyposis”

Diagnostic Criteria

- **Five or more serrated polyps proximal to sigmoid colon of which 2 or more are $\geq$10 mm.**
- **Any number of serrated polyps proximal to the sigmoid colon in an individual who has a 1st degree relative with serrated polyposis.**
- **More than 20 serrated polyps of any size but distributed throughout the colon.**

WHO Classification of Tumours of the Digestive System, IARC P

Hyperplastic (Serrated) Polyposis

- Type I
  - Larger (≥1 cm) polyps with sessile serrated polyp morphology
  - Associated with carcinoma
- Type II
  - 20 or more serrated polyps without sessile serrated polyp morphology
  - Probably not associated with carcinoma

Jass JR. *Gastro Clin N Am* 36:927, 2007

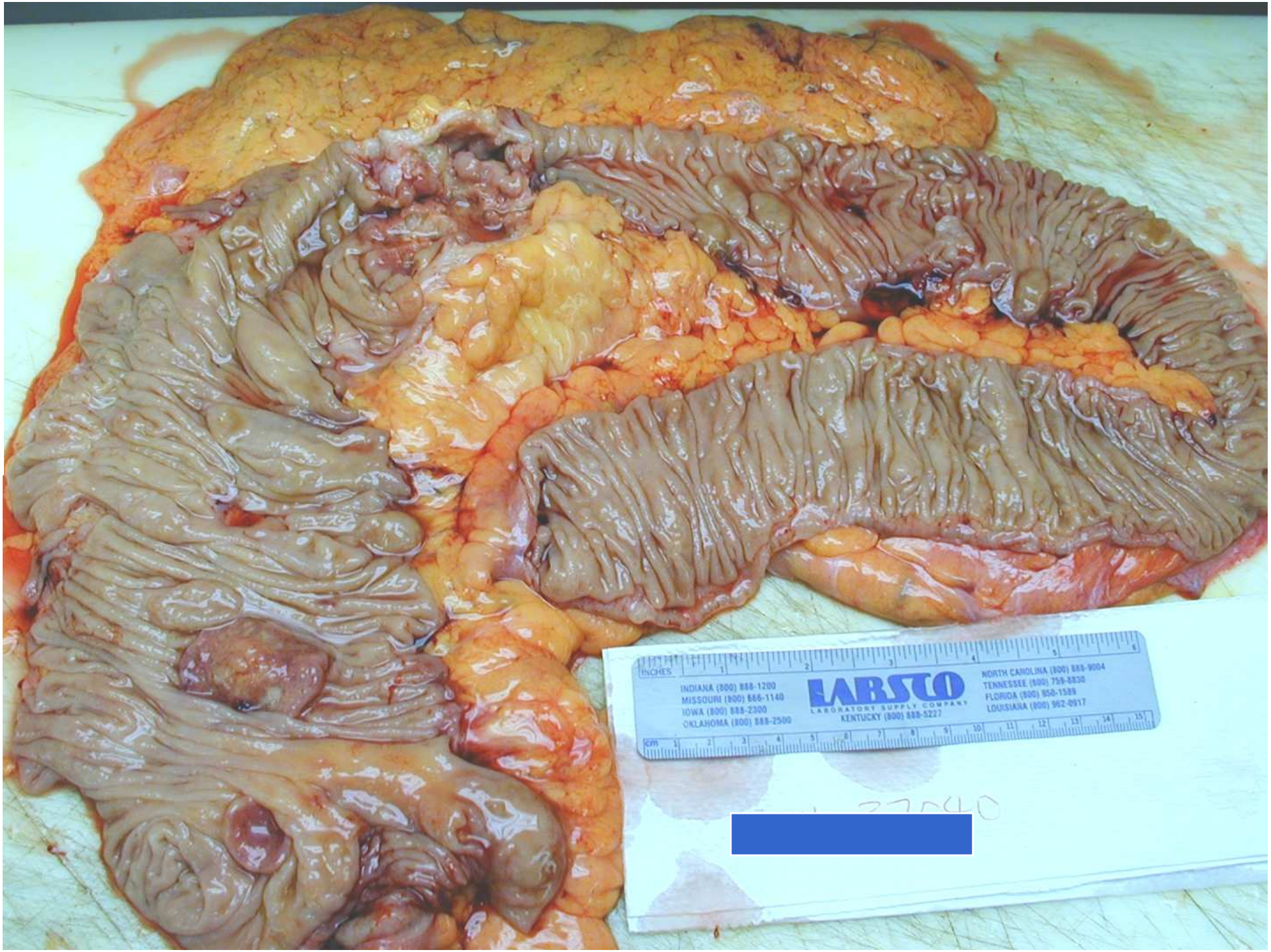
- Type III (MUTYH-associated polyposis)
  - Hyperplastic (serrated) polyposis with multiple (>25) adenomas

Boparai KS, et al. *Gastroenterol* 135:2014, 2008

"Hyperplastic (Serrated) Polyposis"
Risk of Colorectal Cancer

- Thirteen patients with "hyperplastic polyposis"
  - Four with cancer at presentation
  - Three developed cancer at 2, 3 and 8 years following diagnosis
  - Five cancers right sided
  - Coexisting serrated adenomas

Hyman NH et al *Dis Colon Rectum* 47:2101, 2004


INCHES
INDIANA (800) 888-1200
MISSOURI (800) 866-1140
IOWA (800) 888-2300
OKLAHOMA (800) 888-2500
LABSCO
LABORATORY SUPPLY COMPANY
KENTUCKY (800) 888-5227
NORTH CAROLINA (800) 888-9004
TENNESSEE (800) 758-8830
FLORIDA (800) 850-1589
LOUISIANA (800) 962-0917
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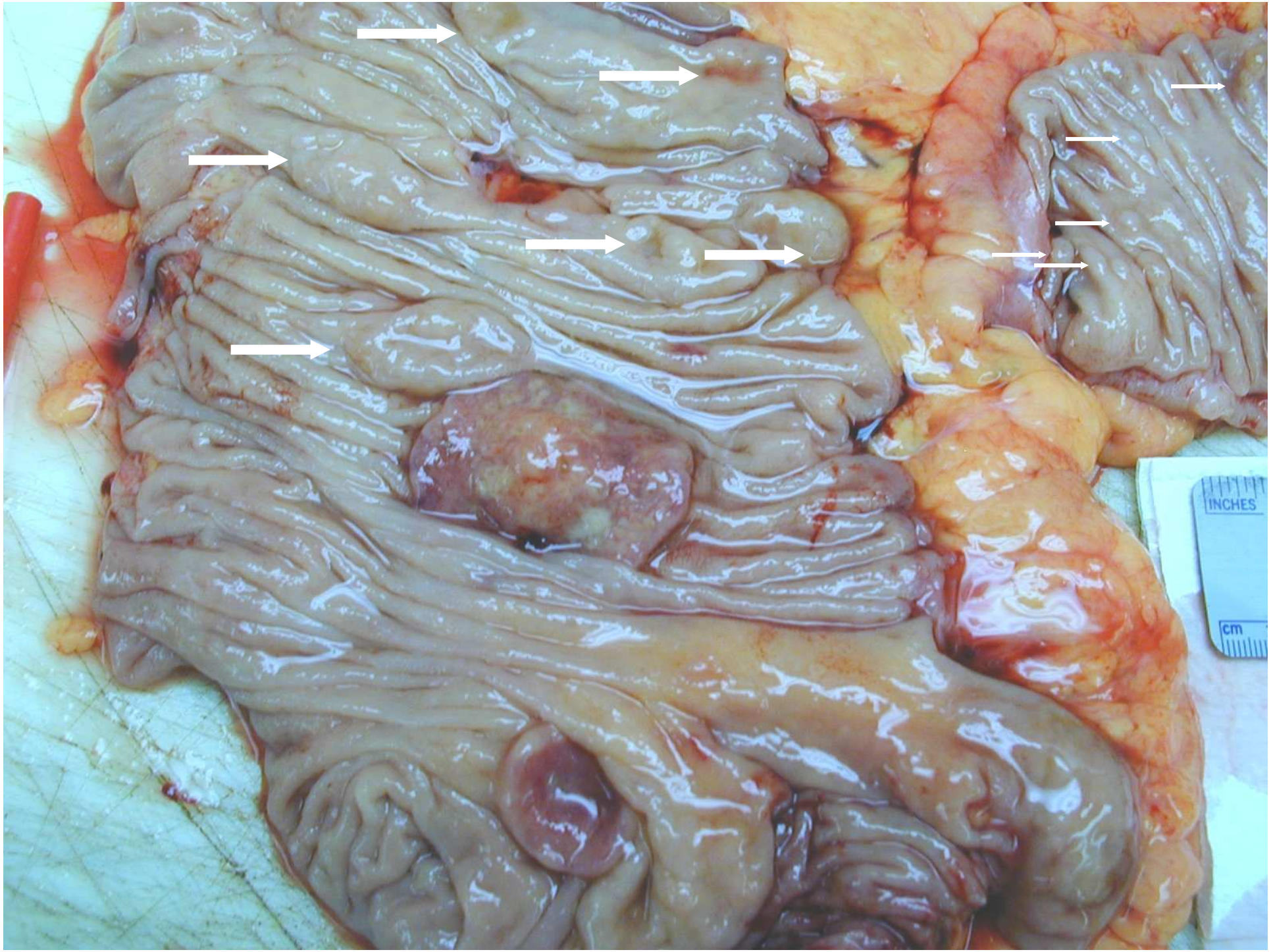
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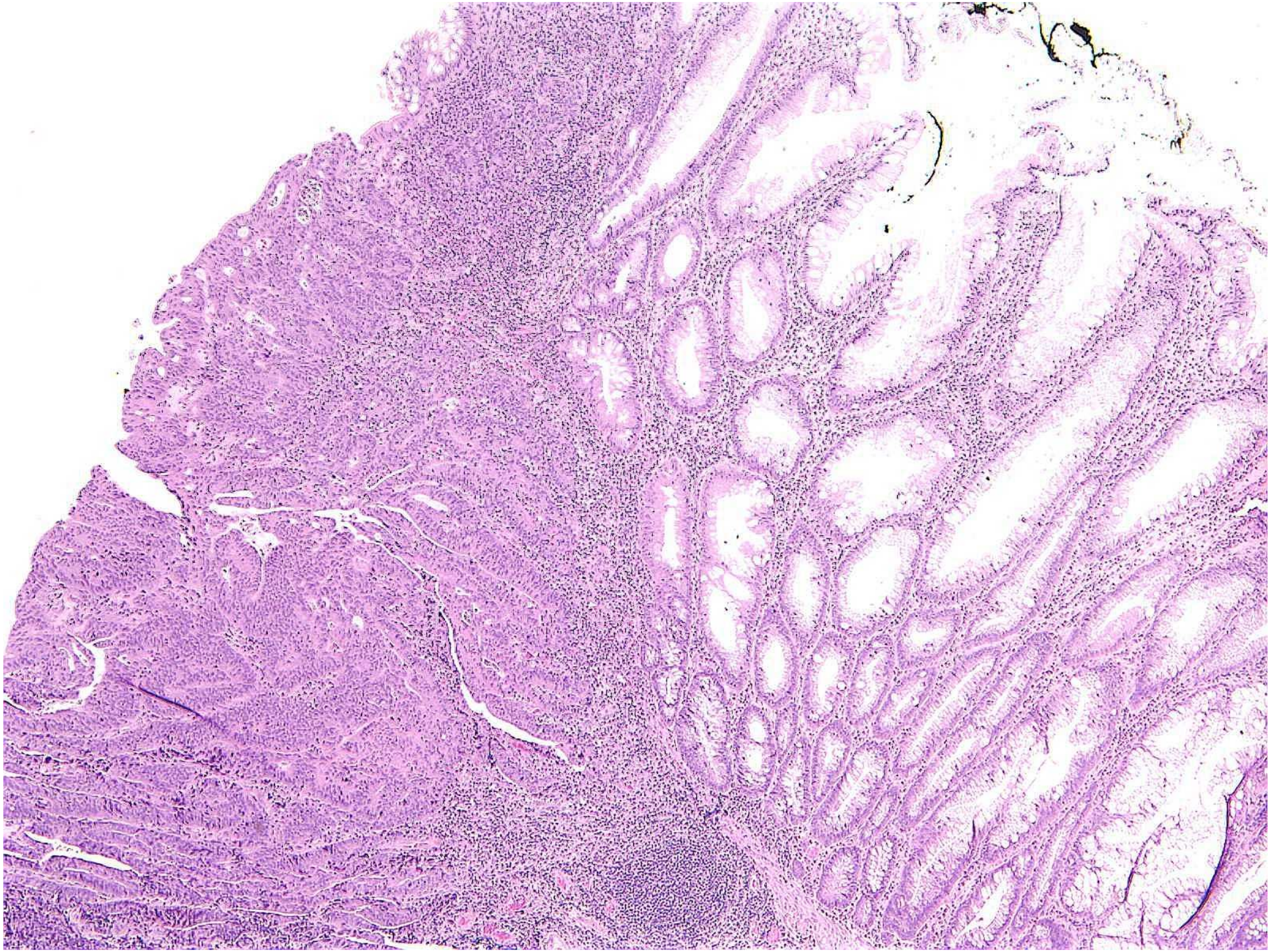
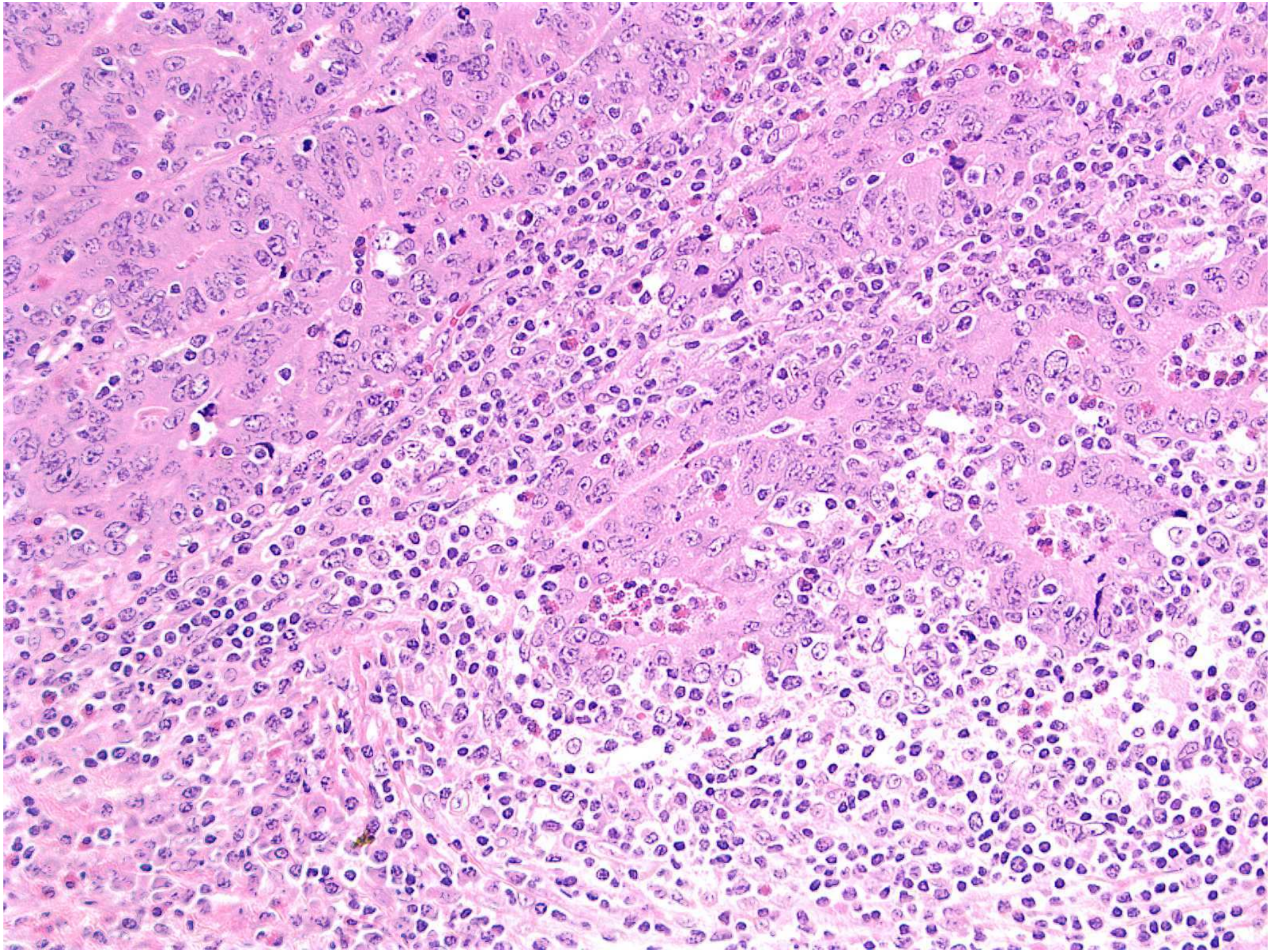
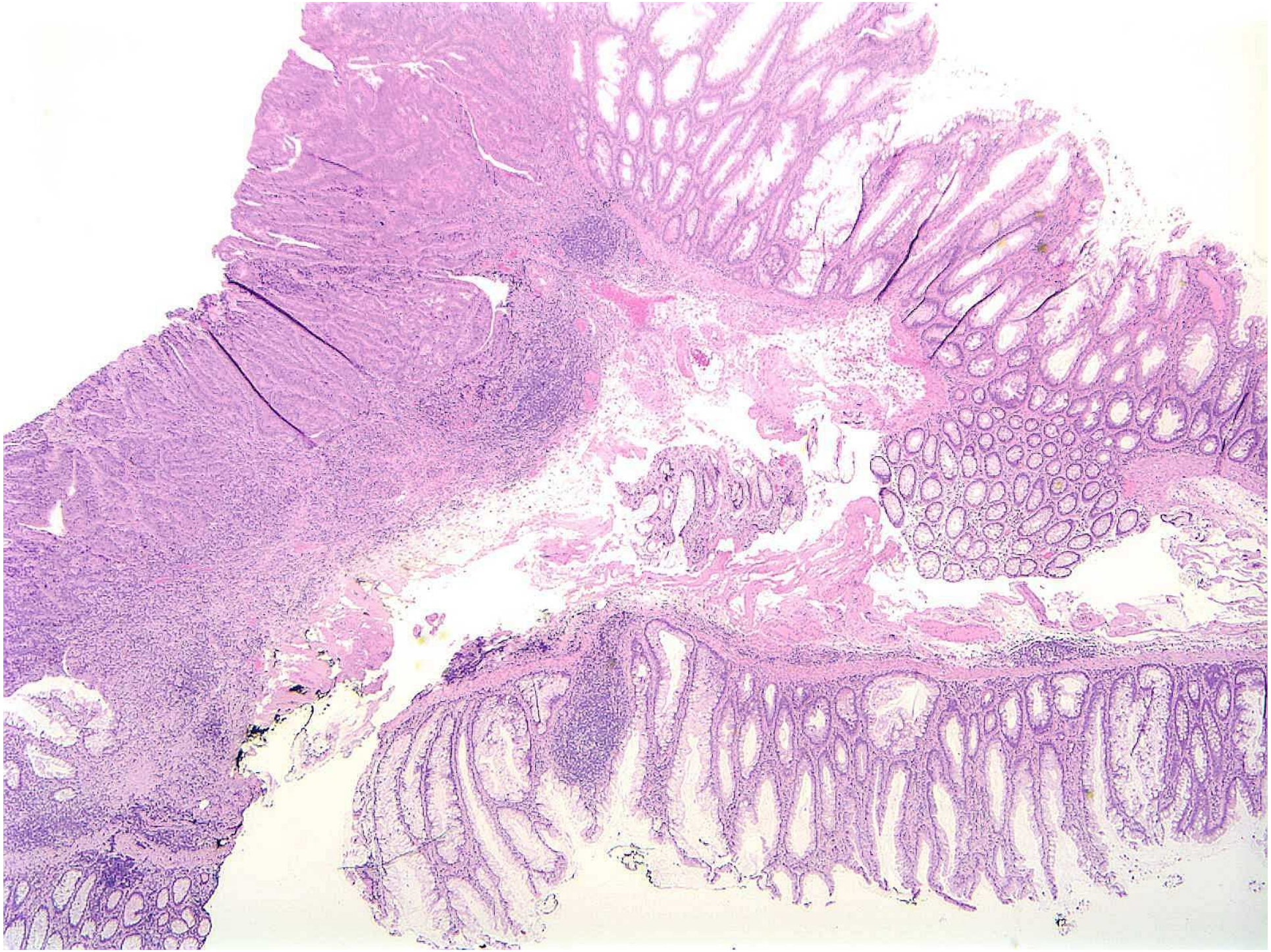
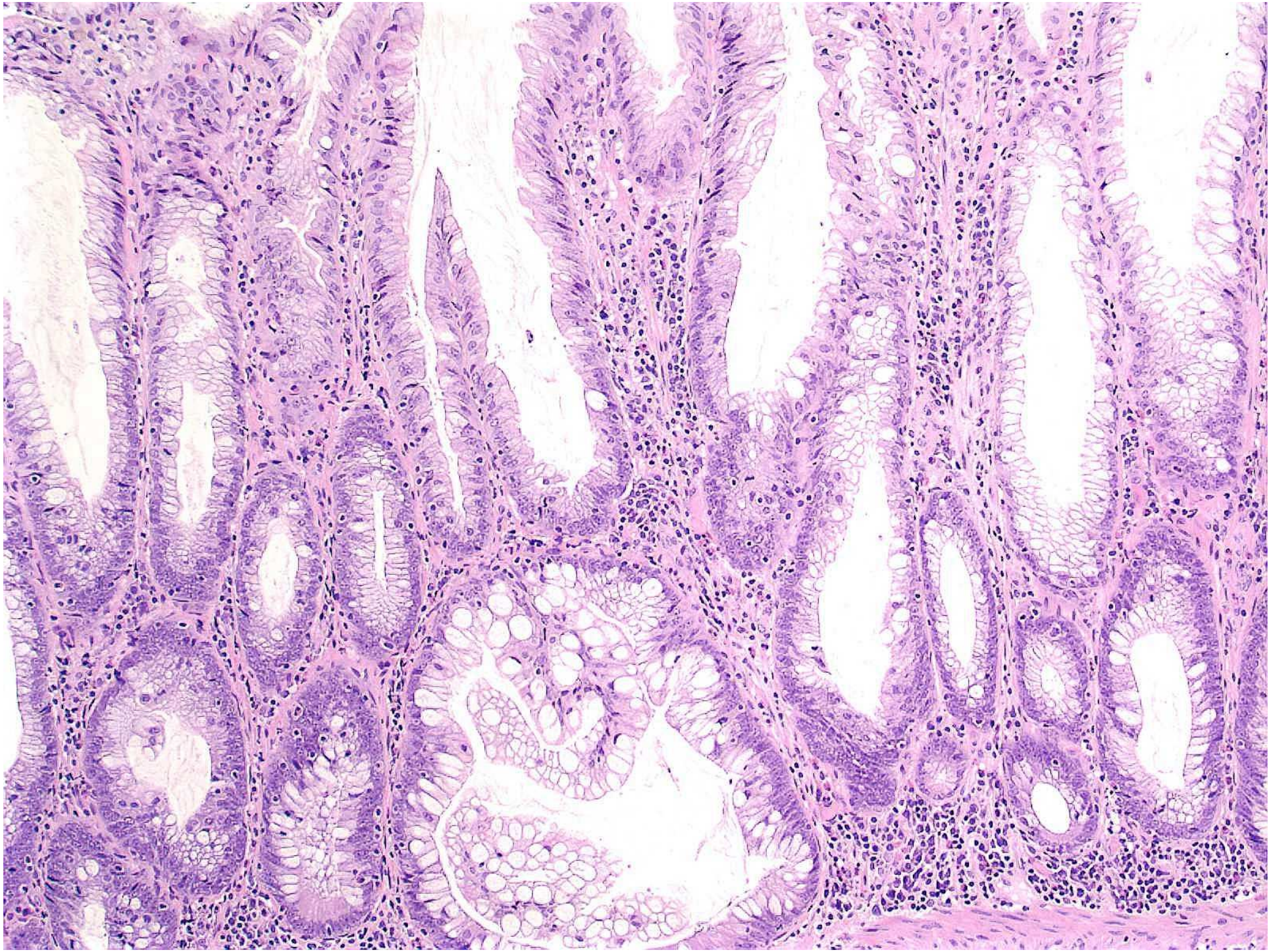
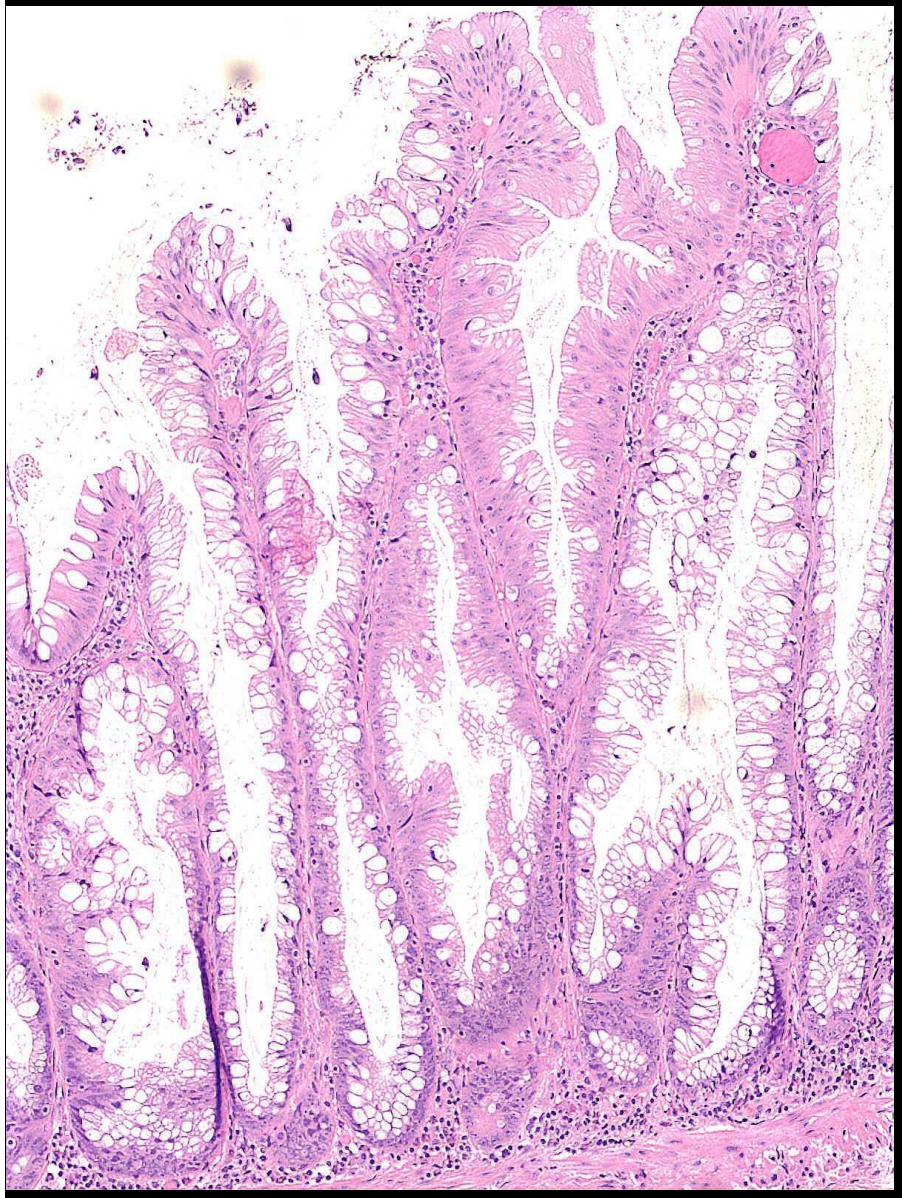
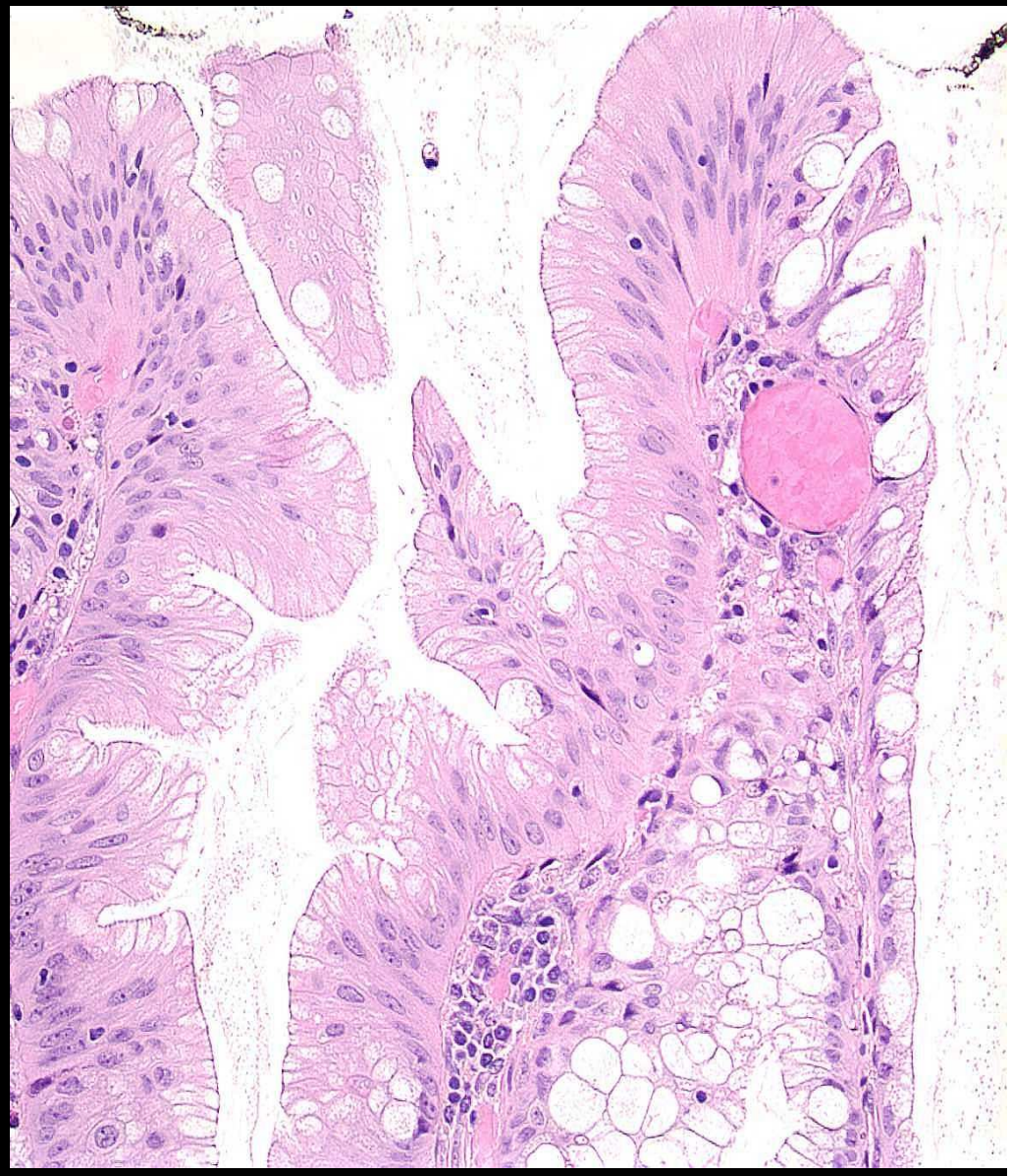










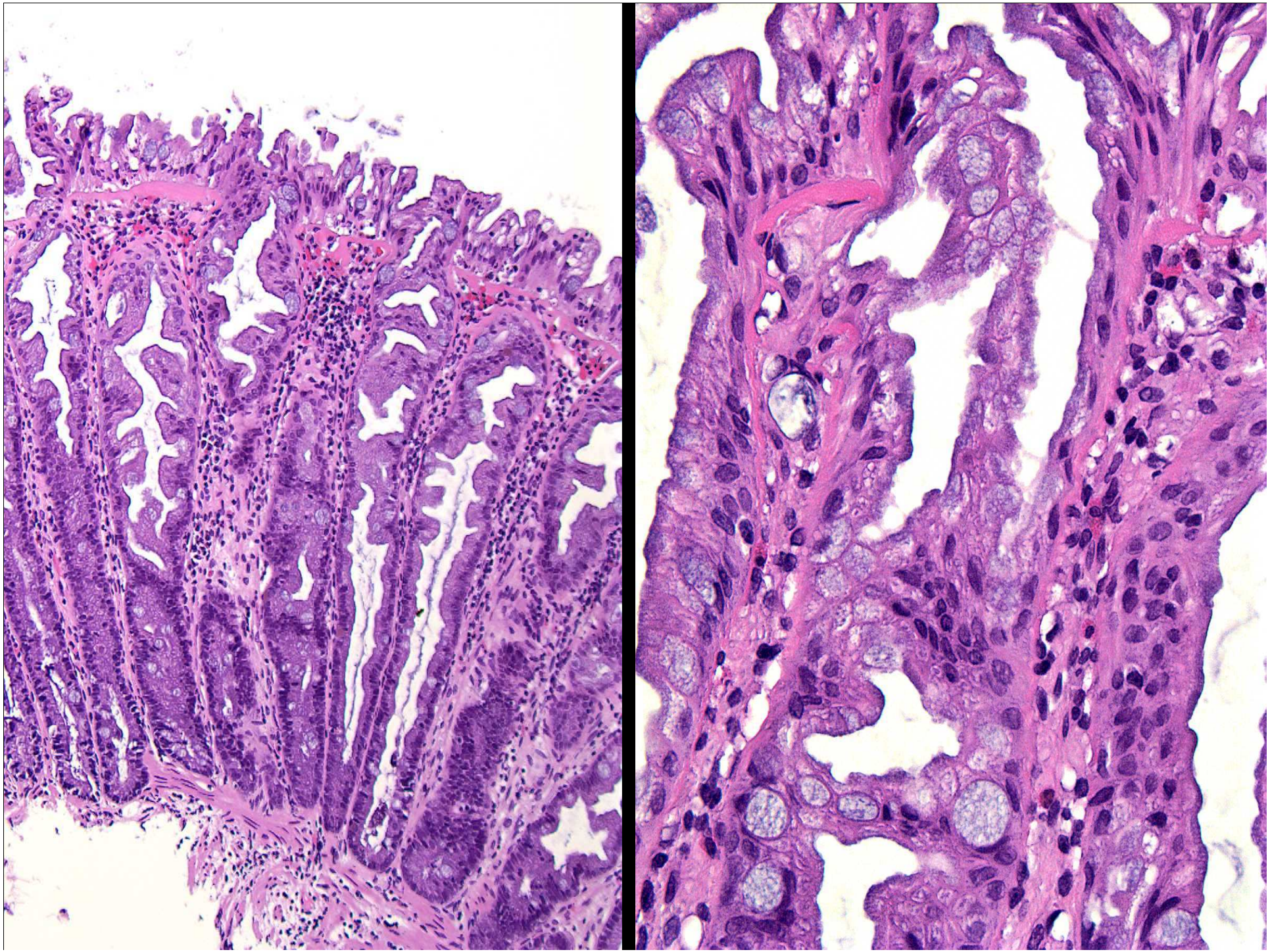


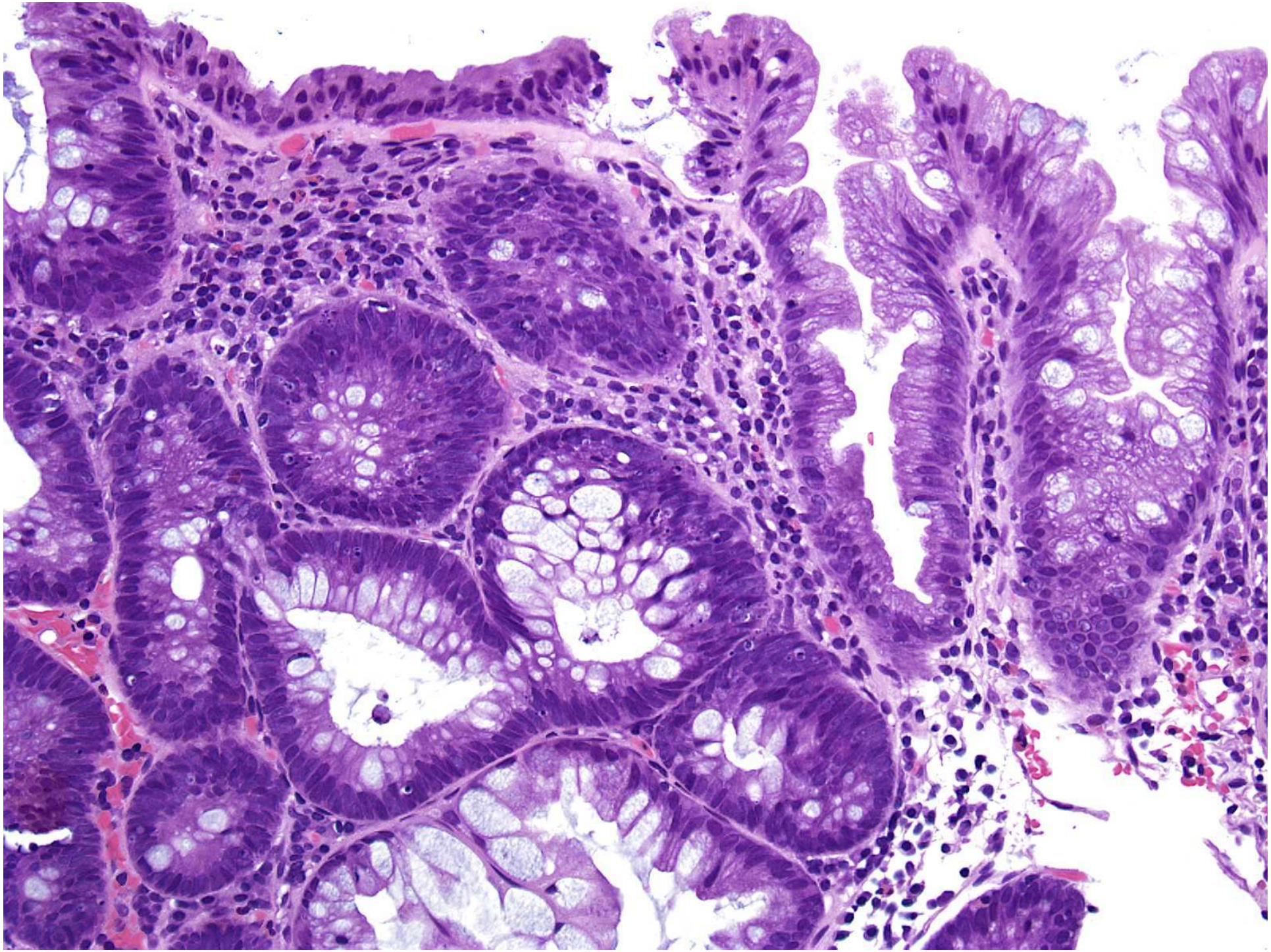
Unusual Hyperplastic Polyps

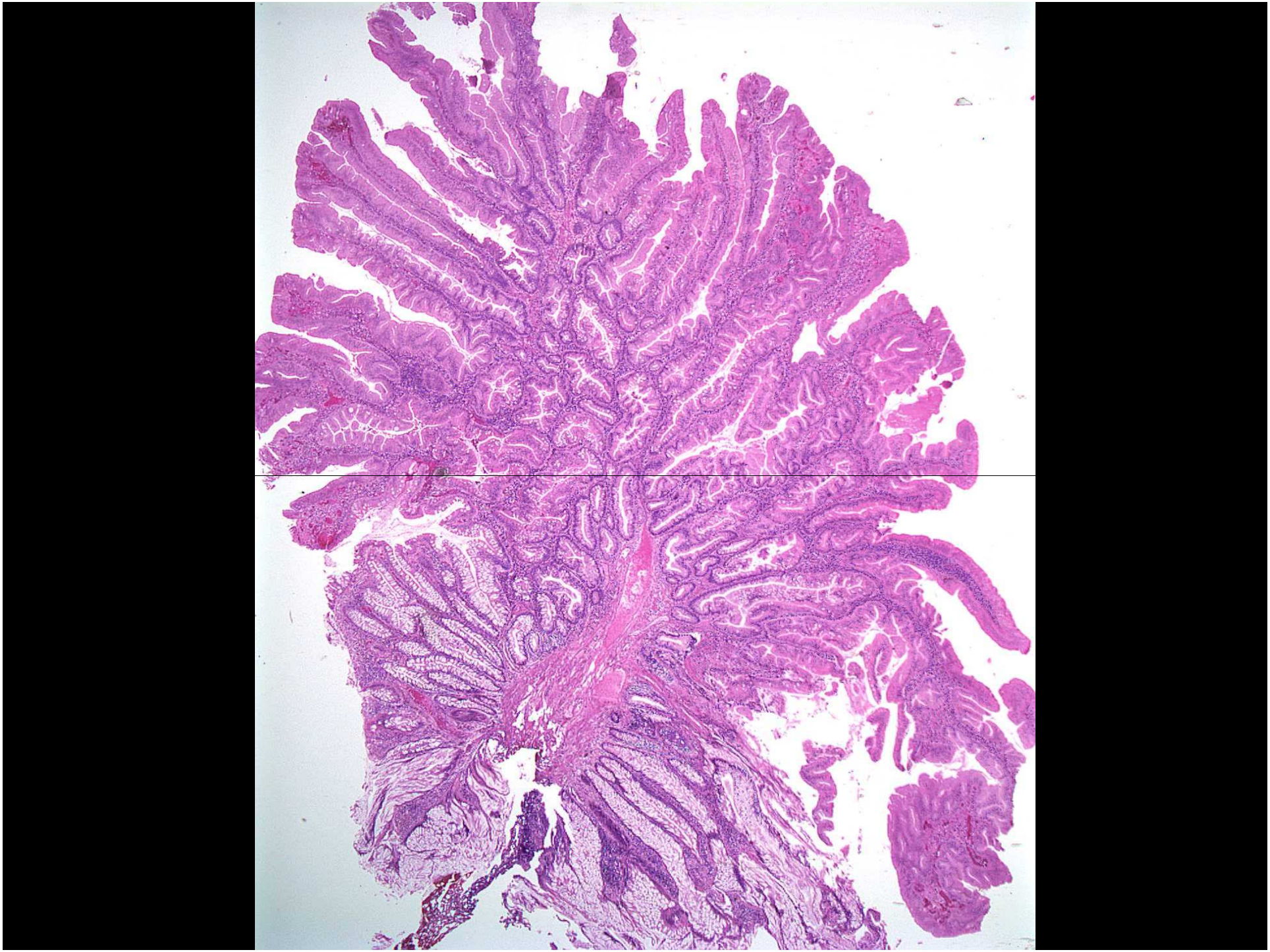
- Giant hyperplastic polyps
- Large hyperplastic polyps
- Sessile serrated adenomas
- Sessile serrated polyps
- Sessile serrated lesions
- Serrated adenomas
- Inverted hyperplastic polyps
- Polyps with epithelial serrated proliferation

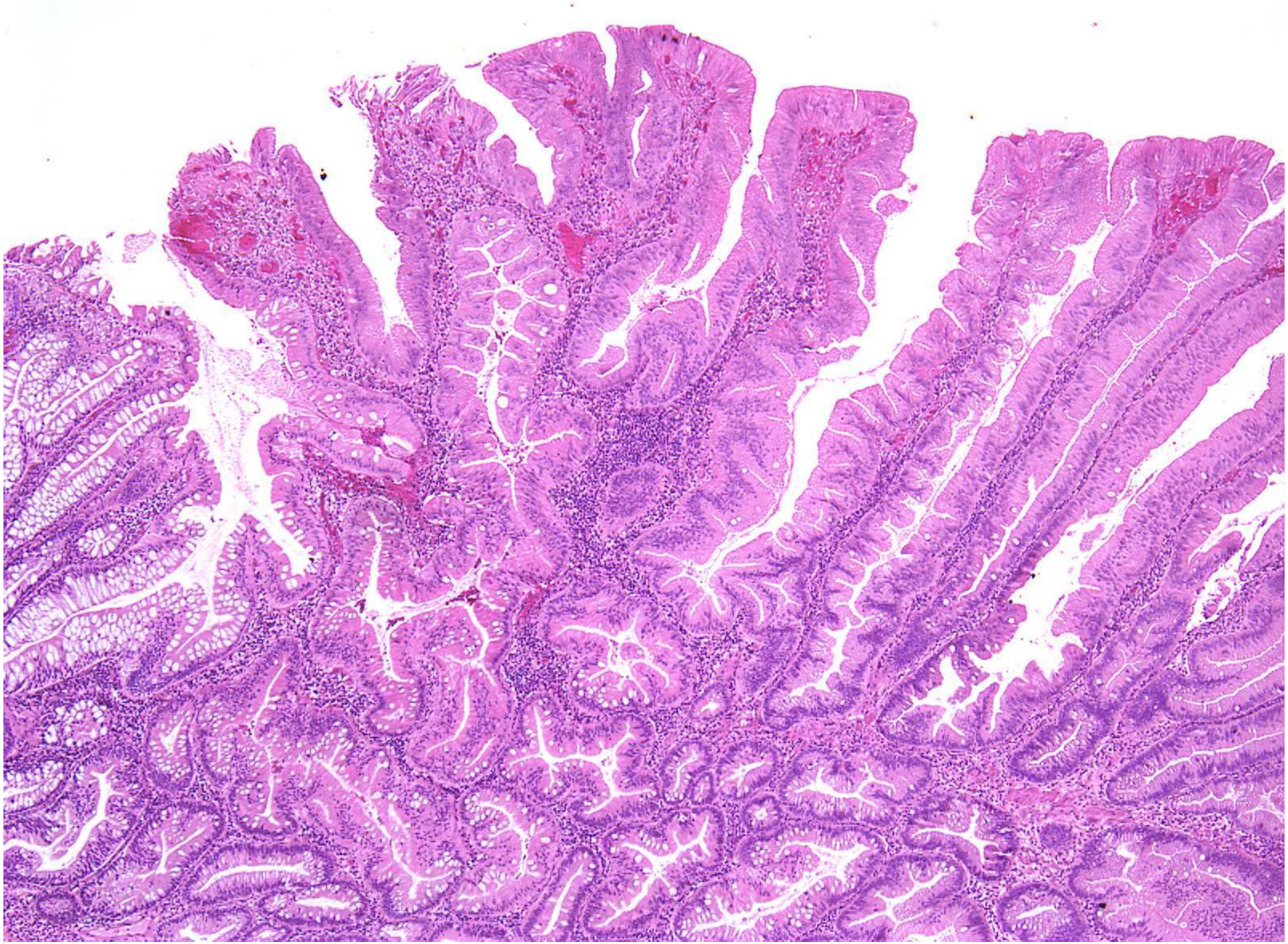
Serrated Polyp Family

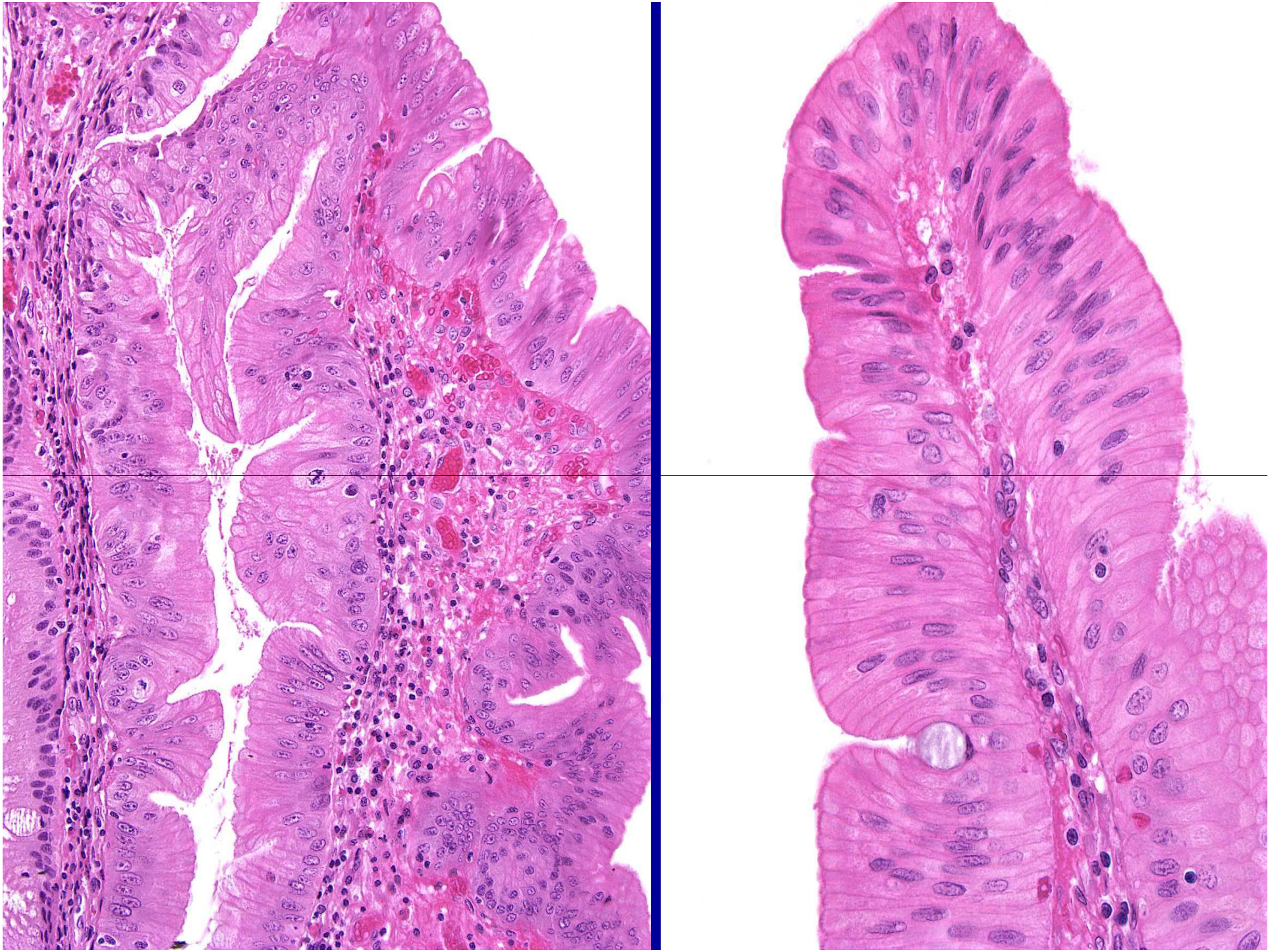
- Conventional hyperplastic polyp
- Mixed hyperplastic polyp and adenoma
- Serrated adenoma
- Sessile serrated polyp









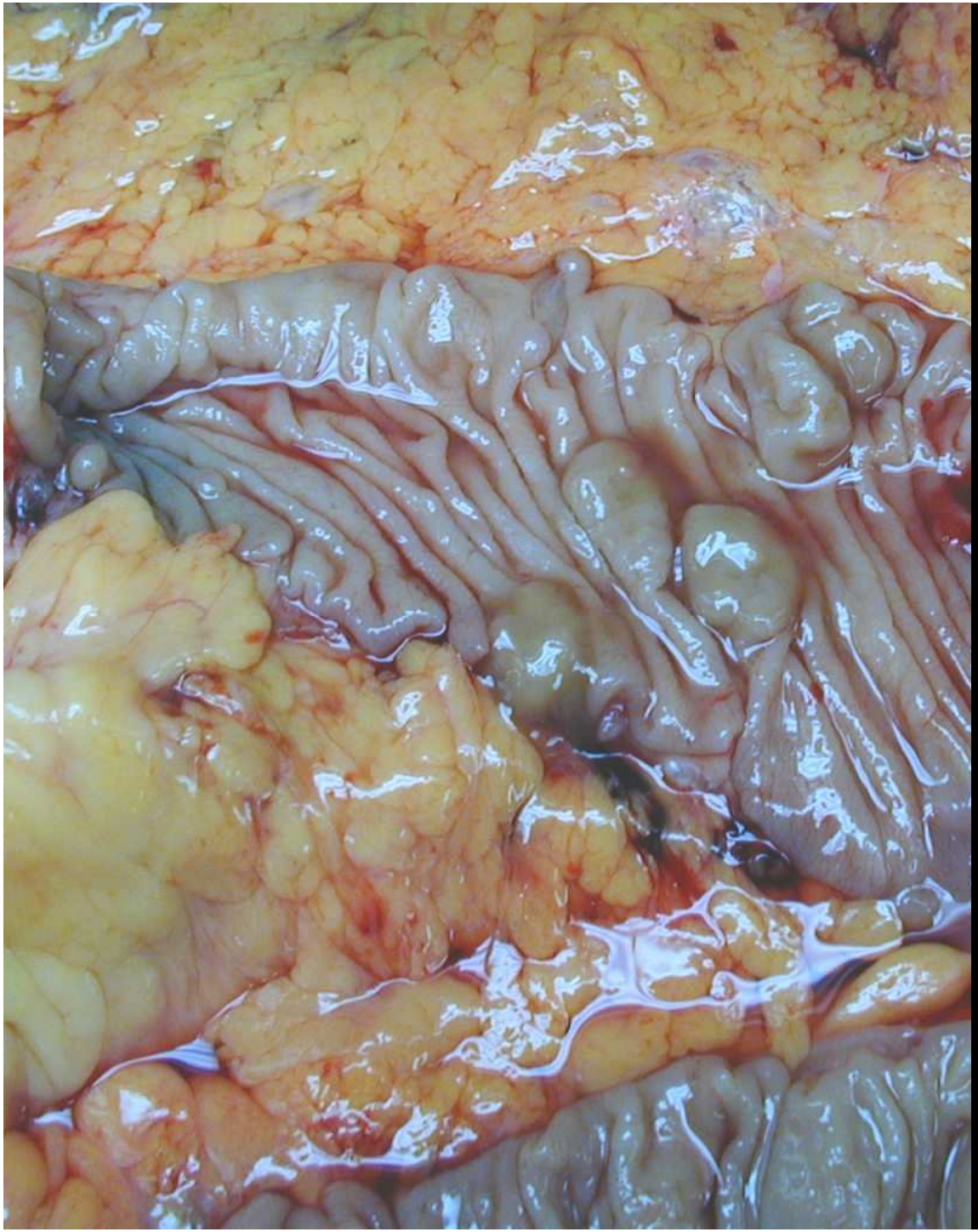


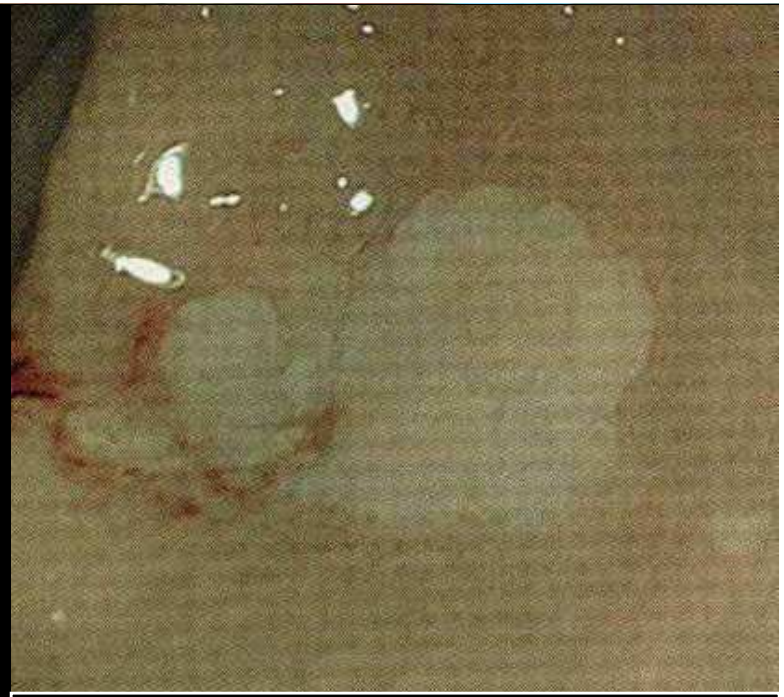
Serrated Polyp Family

- Conventional hyperplastic polyp
- Mixed hyperplastic polyp and adenoma
- Serrated adenoma
- Sessile serrated polyp

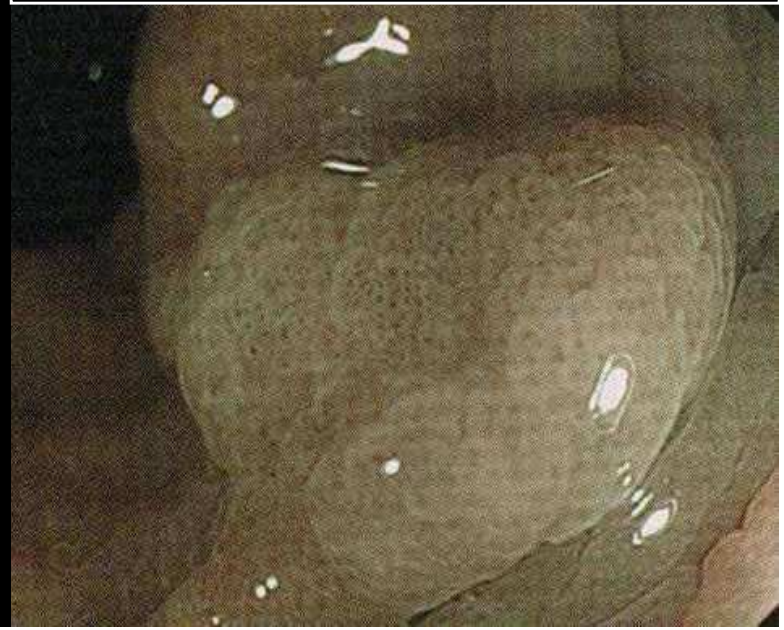
Sessile Serrated Polyp

- Right sided
- Large
- Sessile
- Poor endoscopic circumscription, often mimicking mucosal fold





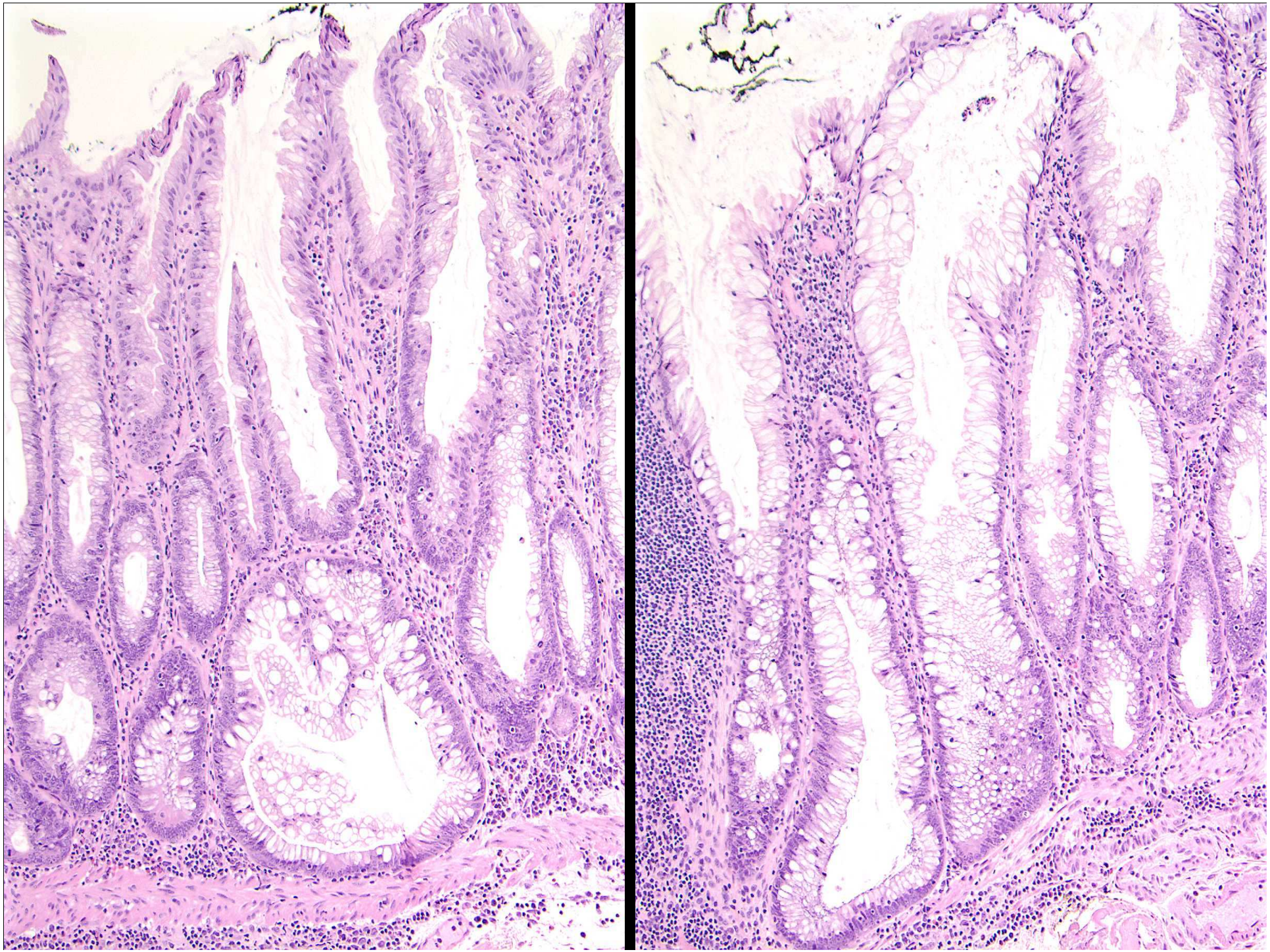
Boparai KS, et al.
Gastrointest Endosc 70:947, 2009

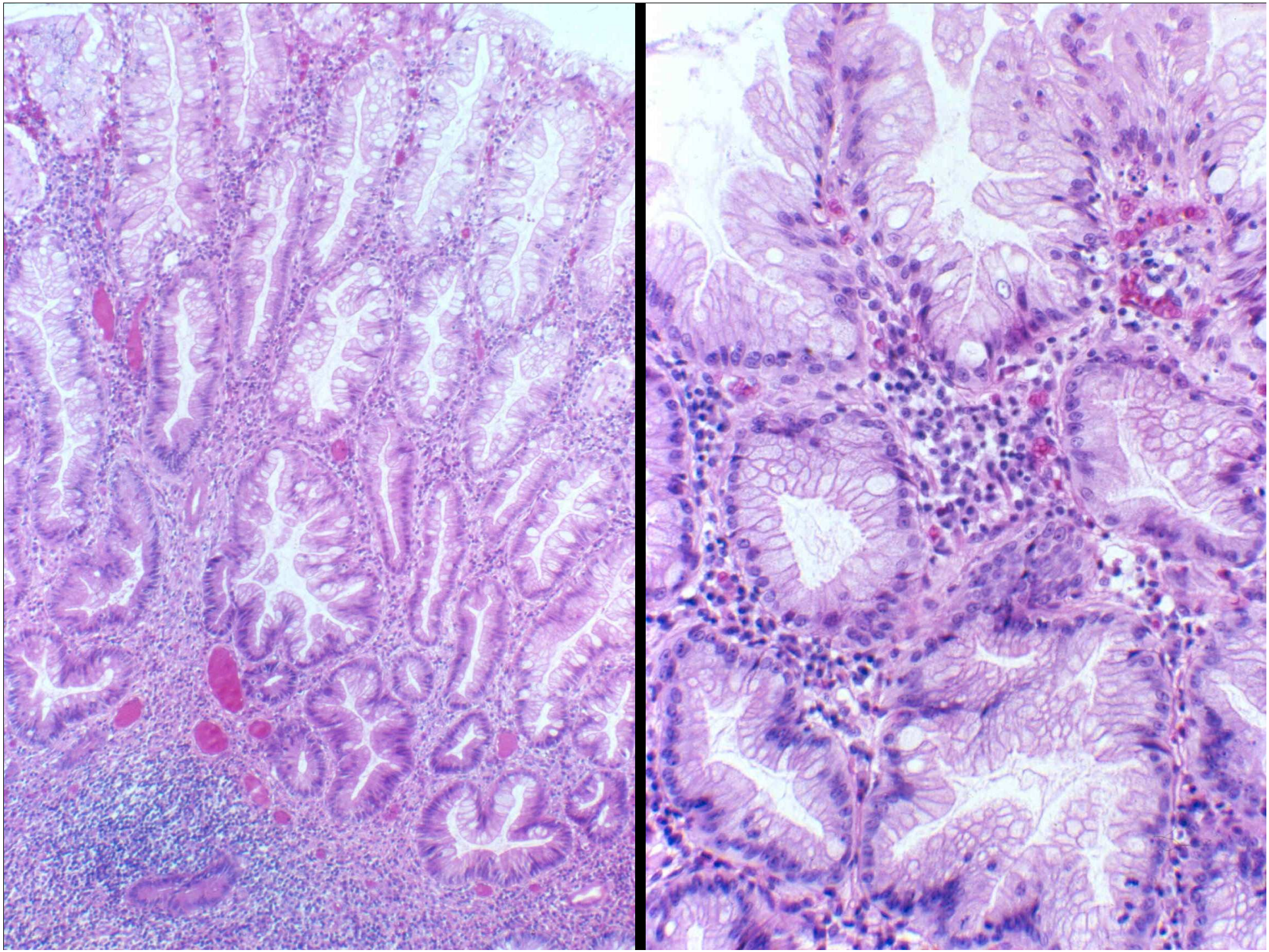


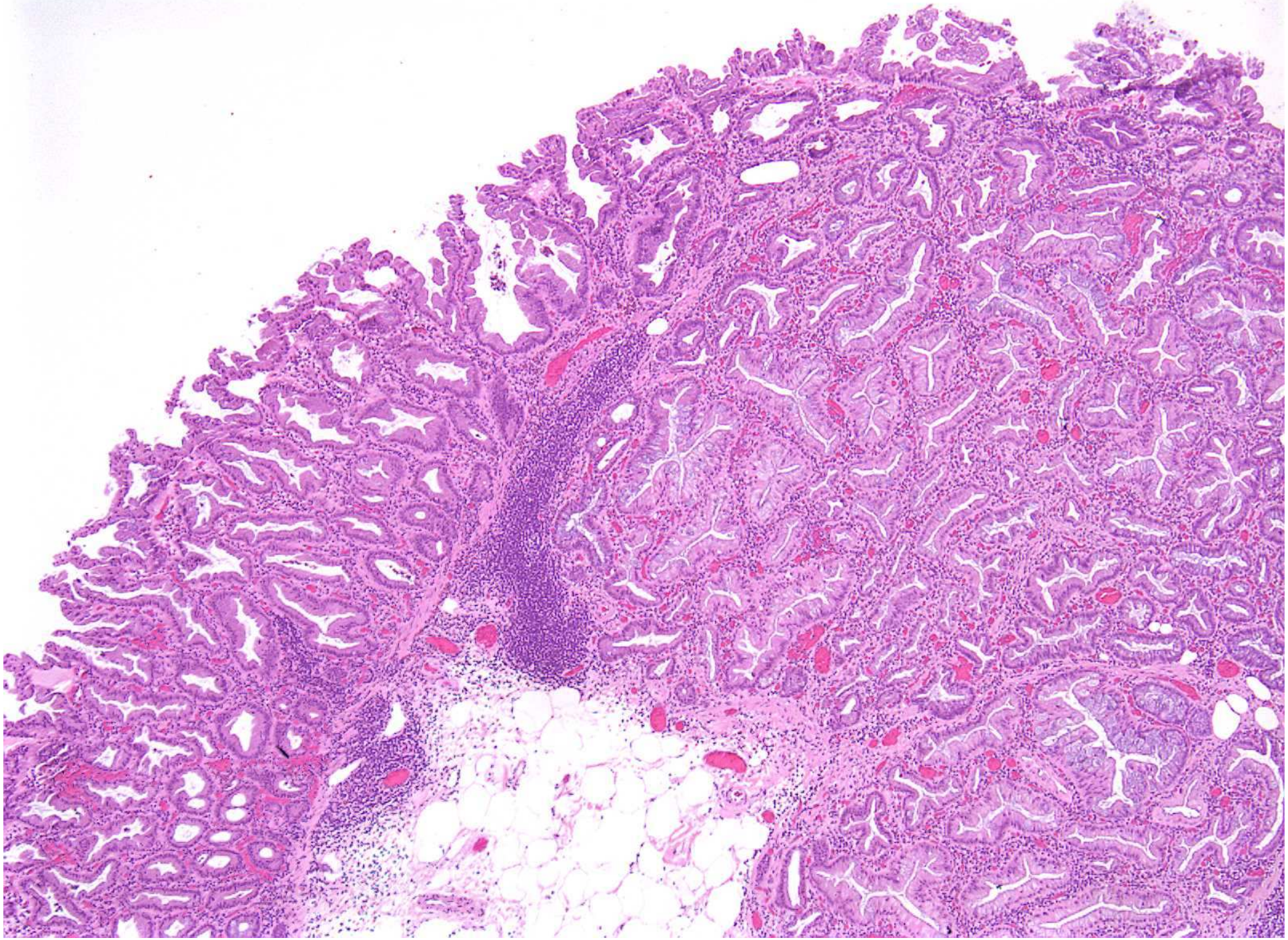
Sessile Serrated Polyps

Morphological Features

- Architectural abnormalities
  - Basal crypt dilatation
  - Crypt branching, horizontal crypts, inverted crypts
  - Prominent serration
  - Increased epithelial:stromal ratio (>50%)
  - Lack of surface basement membrane thickening
- Abnormal proliferation/dysmaturation
  - Persisting nuclear atypia with nucleoli high in crypt
  - High mitosis
  - Abnormal distribution of dystrophic goblet







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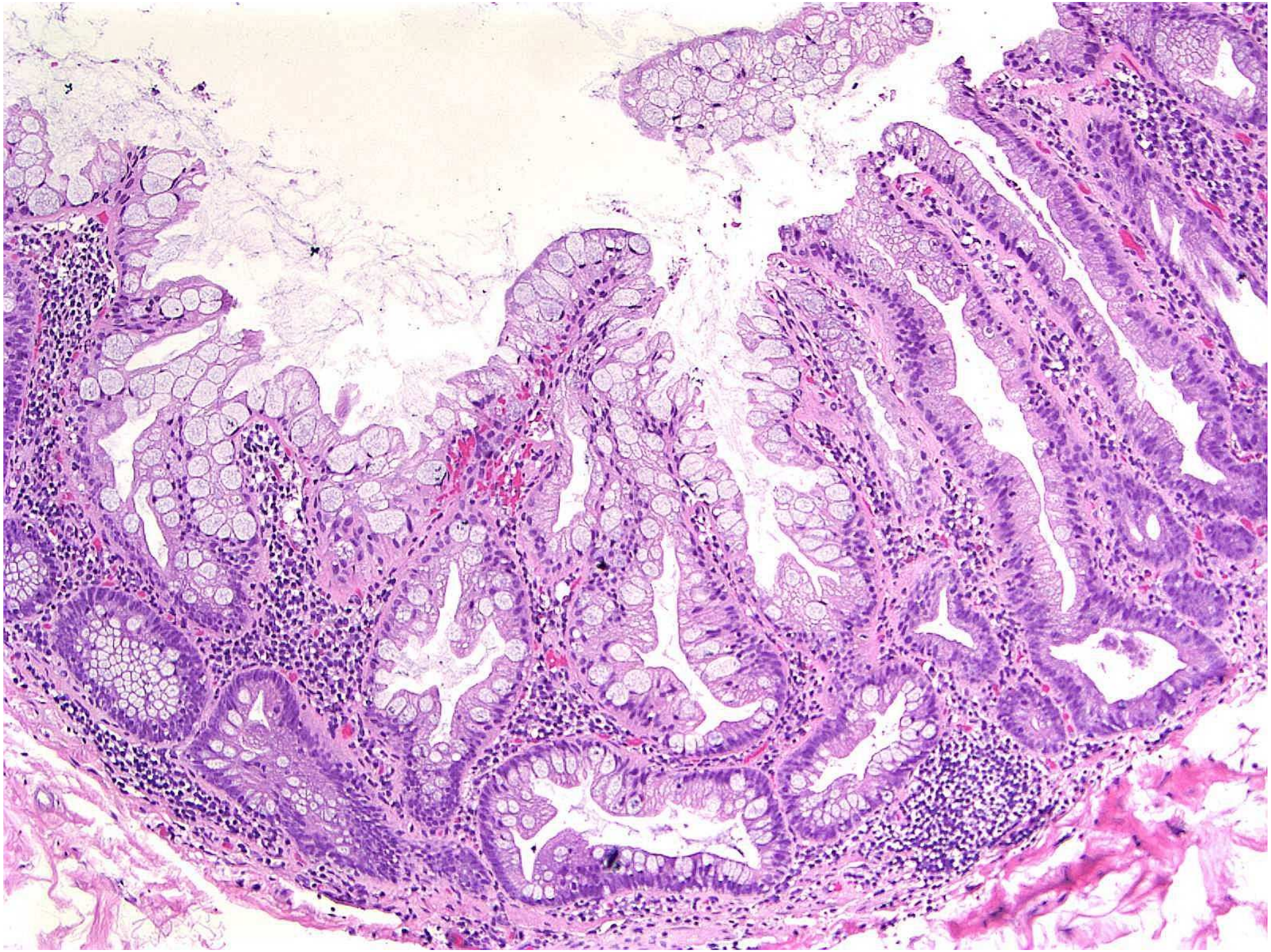

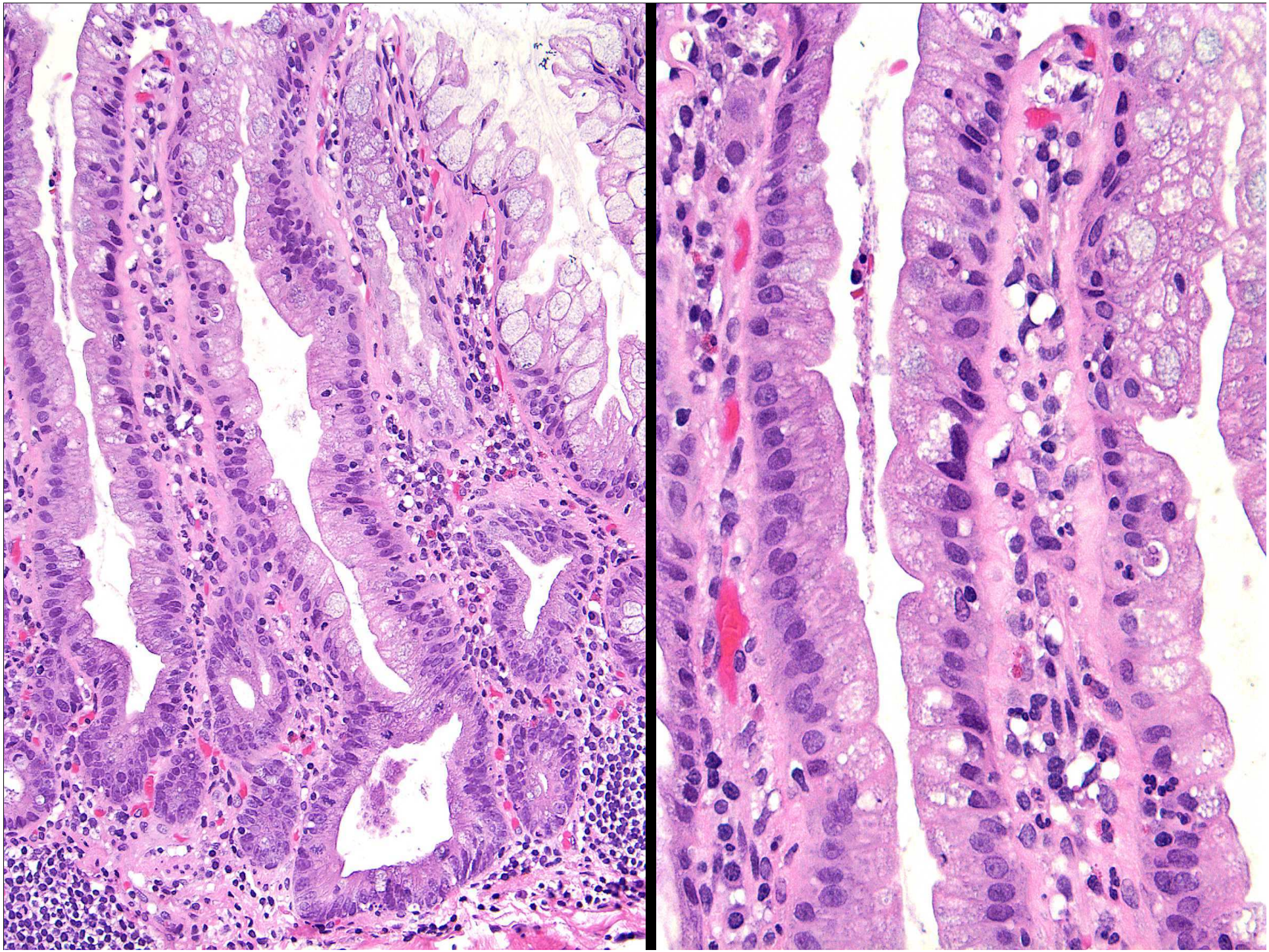

Serrated Polyps and Carcinoma

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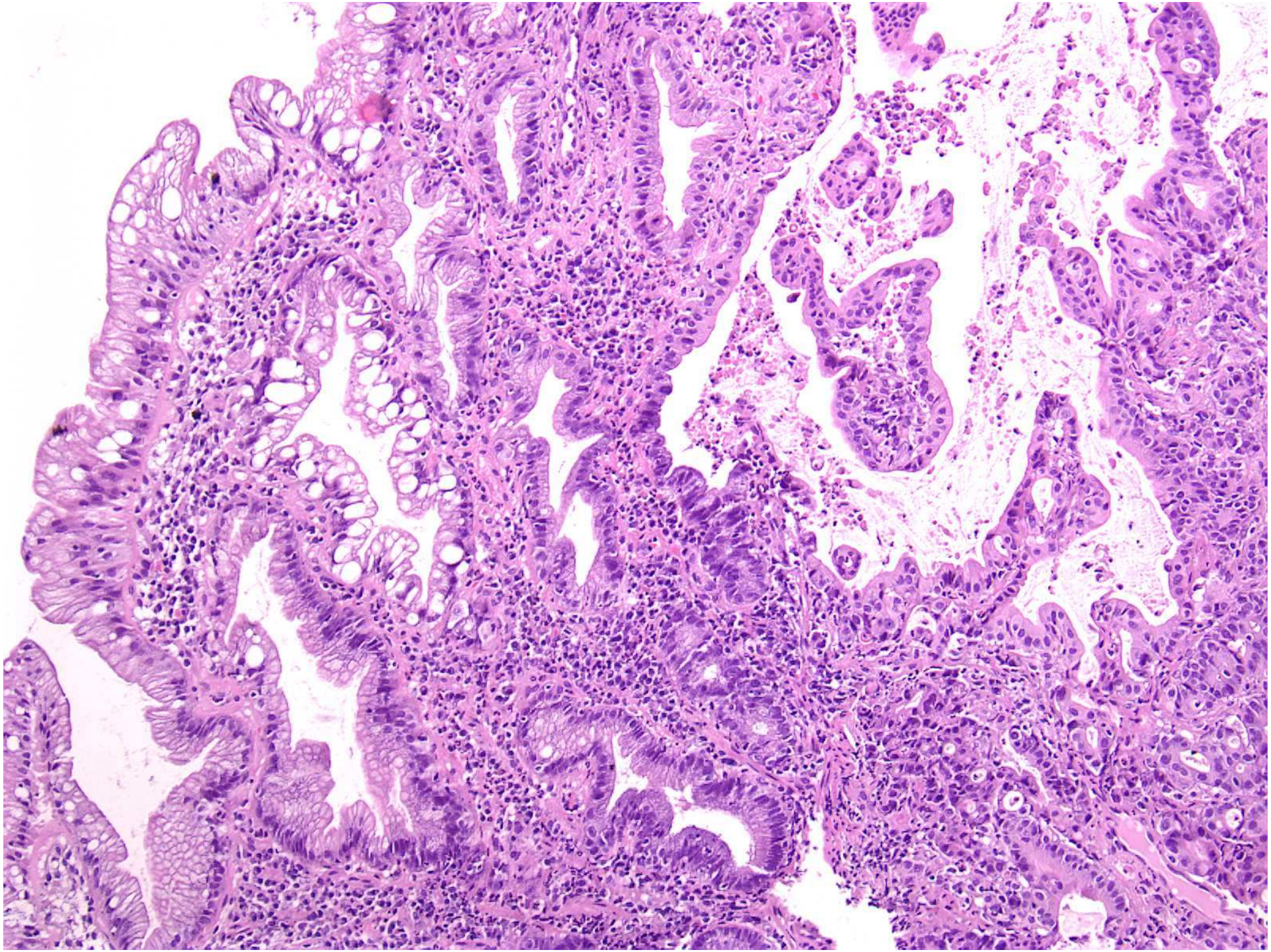
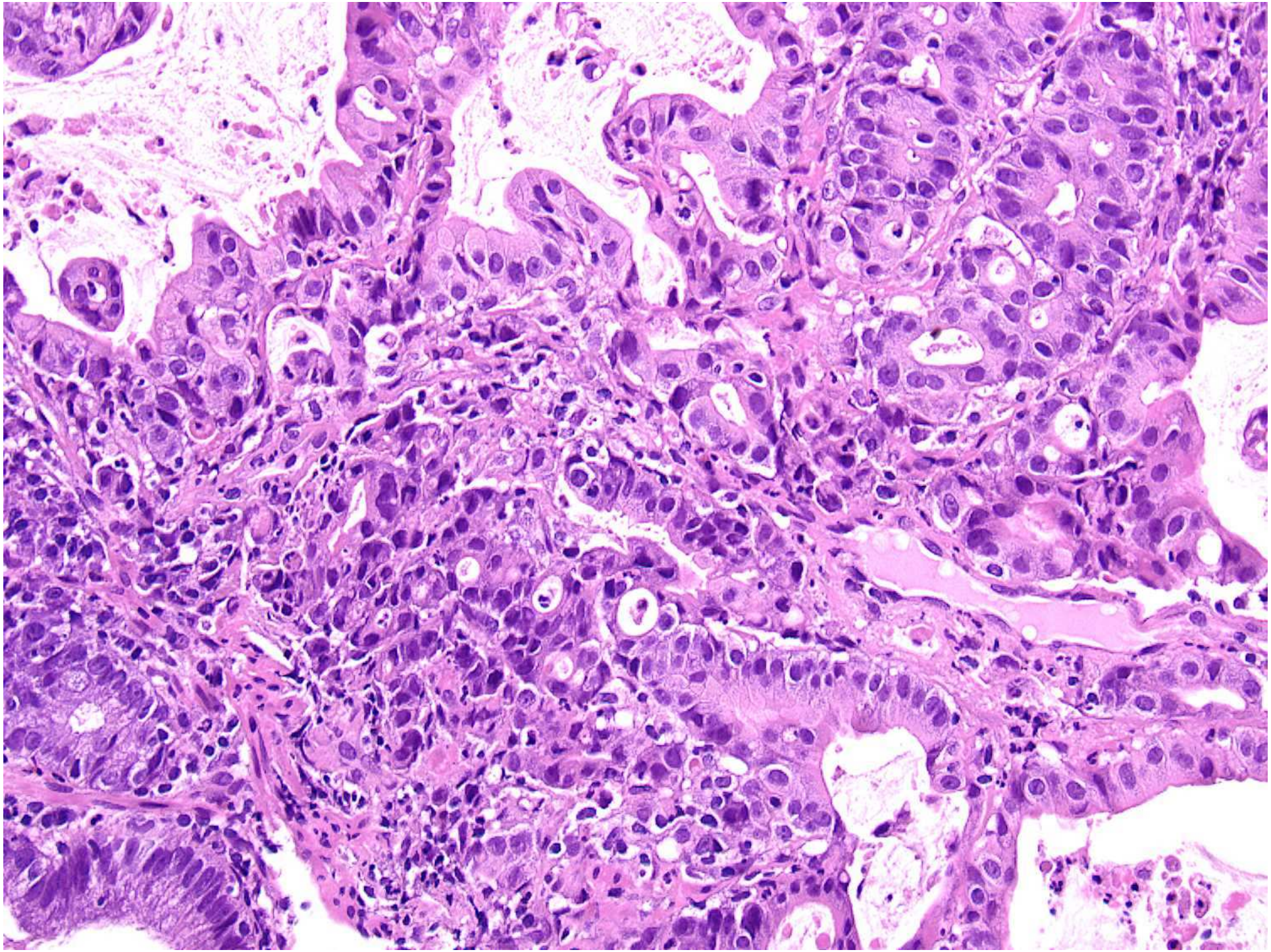
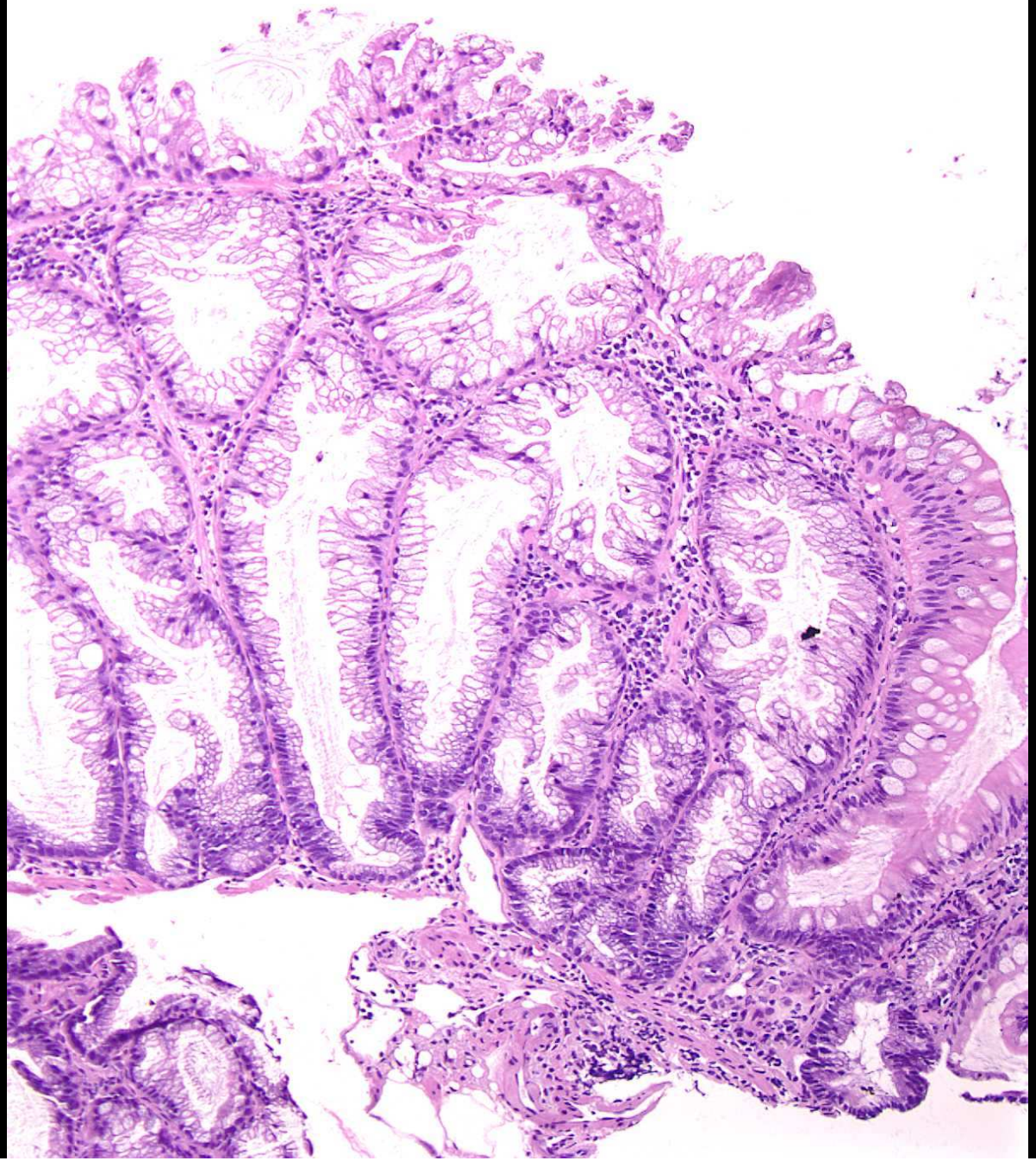
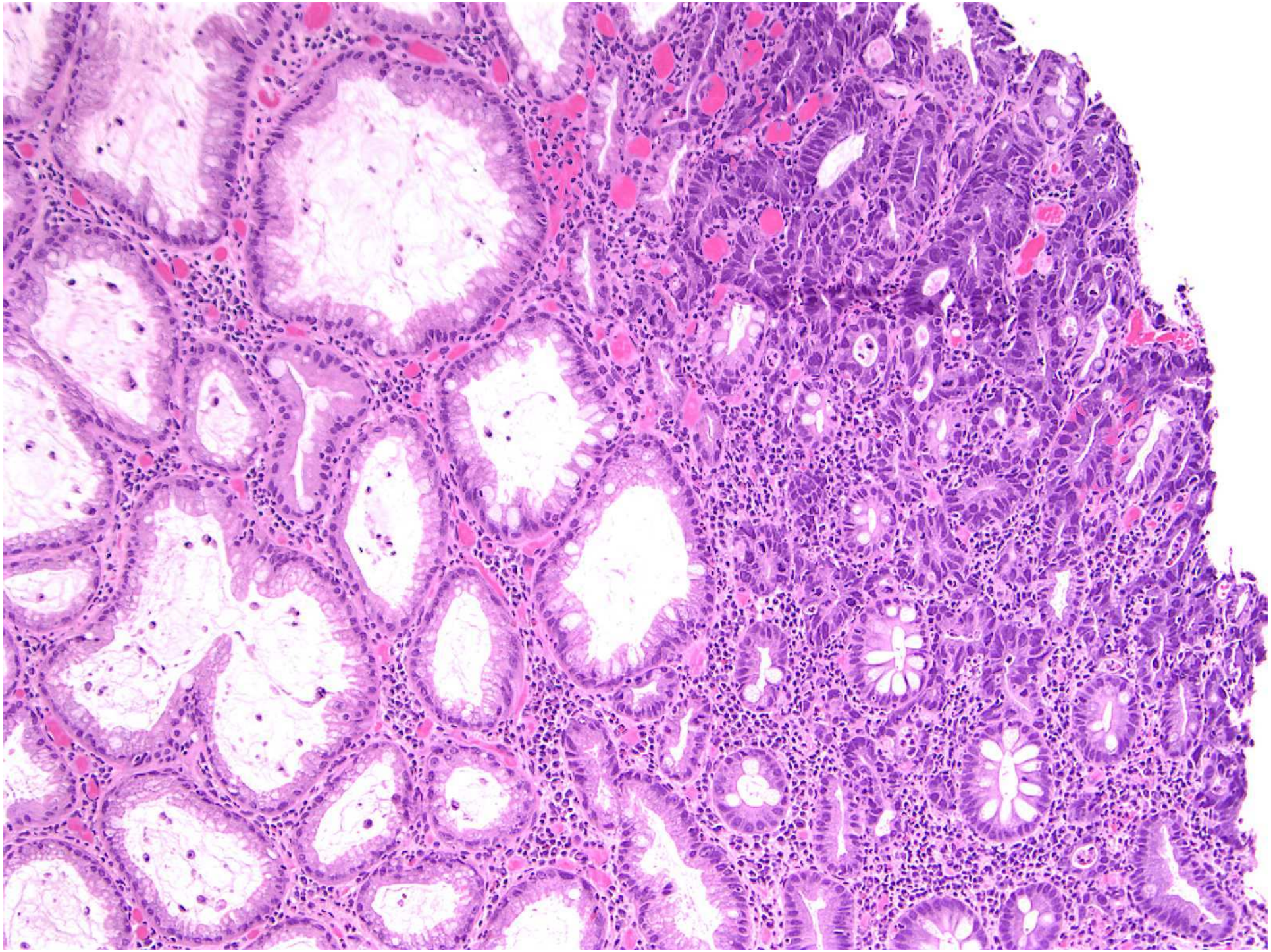
Serrated Polyps and Carcinoma

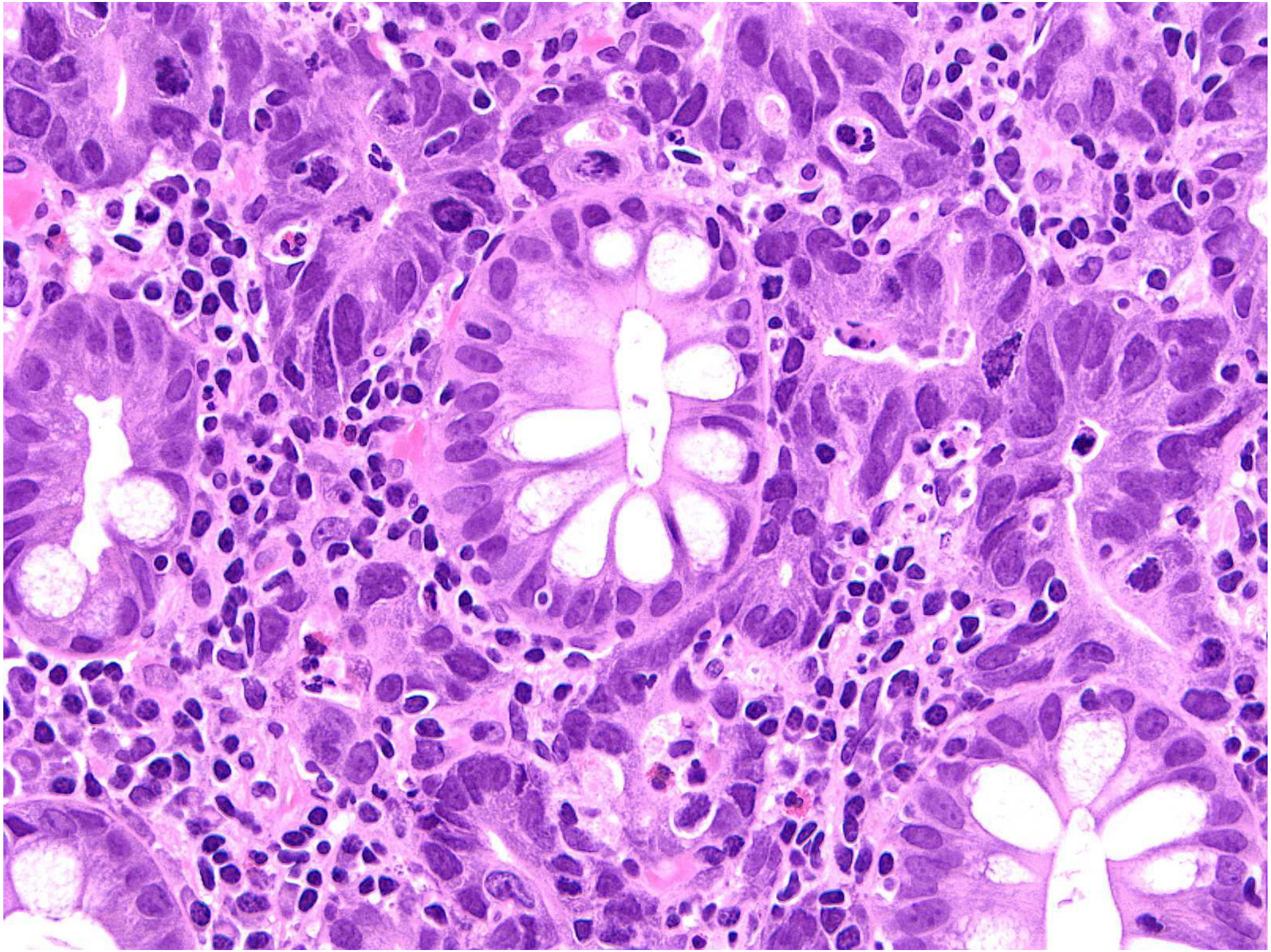
- Goldstein NS. *Am J Clin Pathol* 125:132, 2006
  - Small colonic microsatellite unstable adenocarcinomas and high-grade epithelial dysplasia in sessile serrated adenoma polypectomy specimens: a study of eight cases.
- Sheridan TB, et al. *Am J Clin Pathol* 126:564, 2006
  - Sessile serrated adenomas with low- and high-grade dysplasia and early carcinoma: an immunohistochemical study of serrated lesions “caught in the act”.

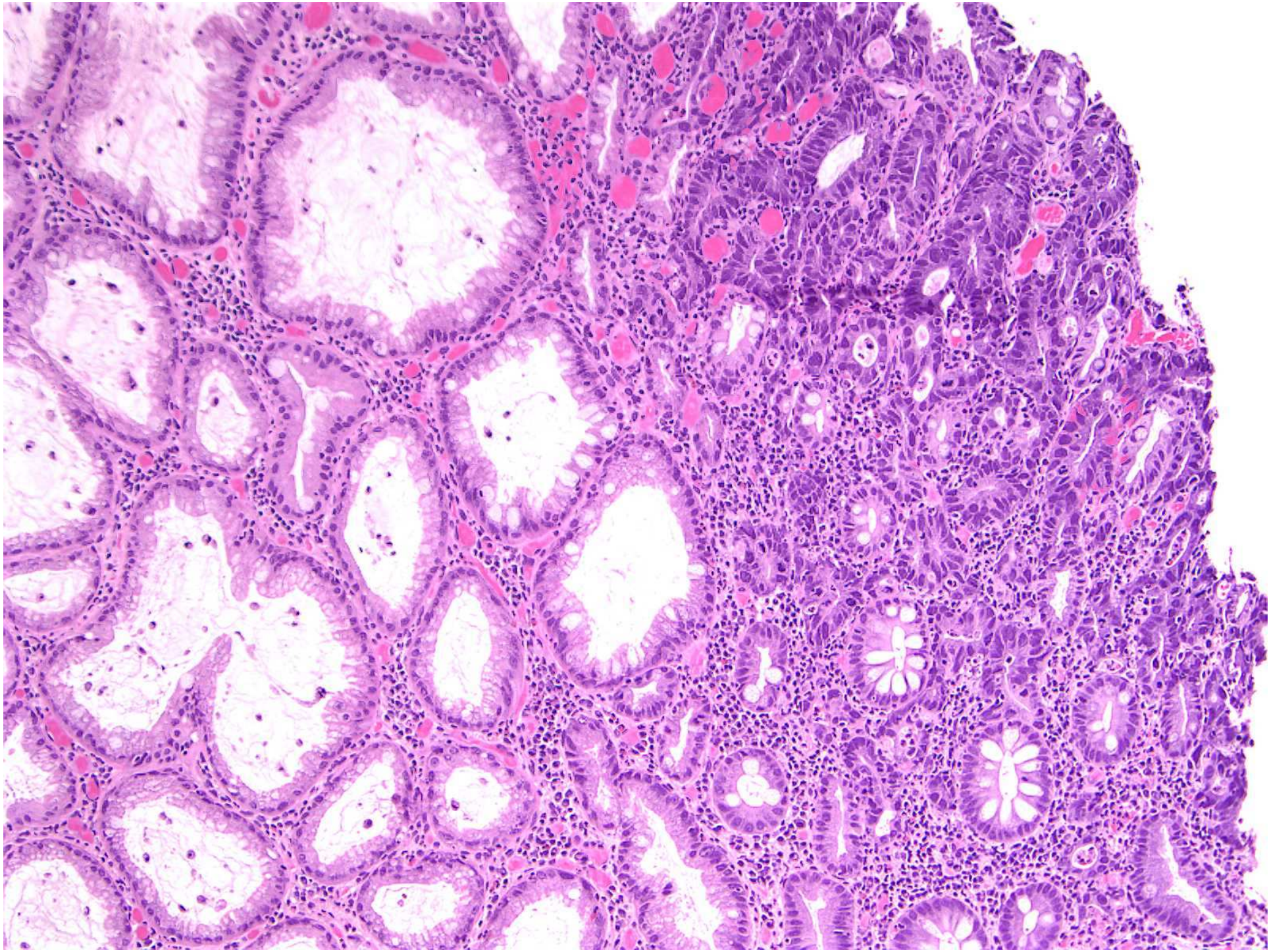


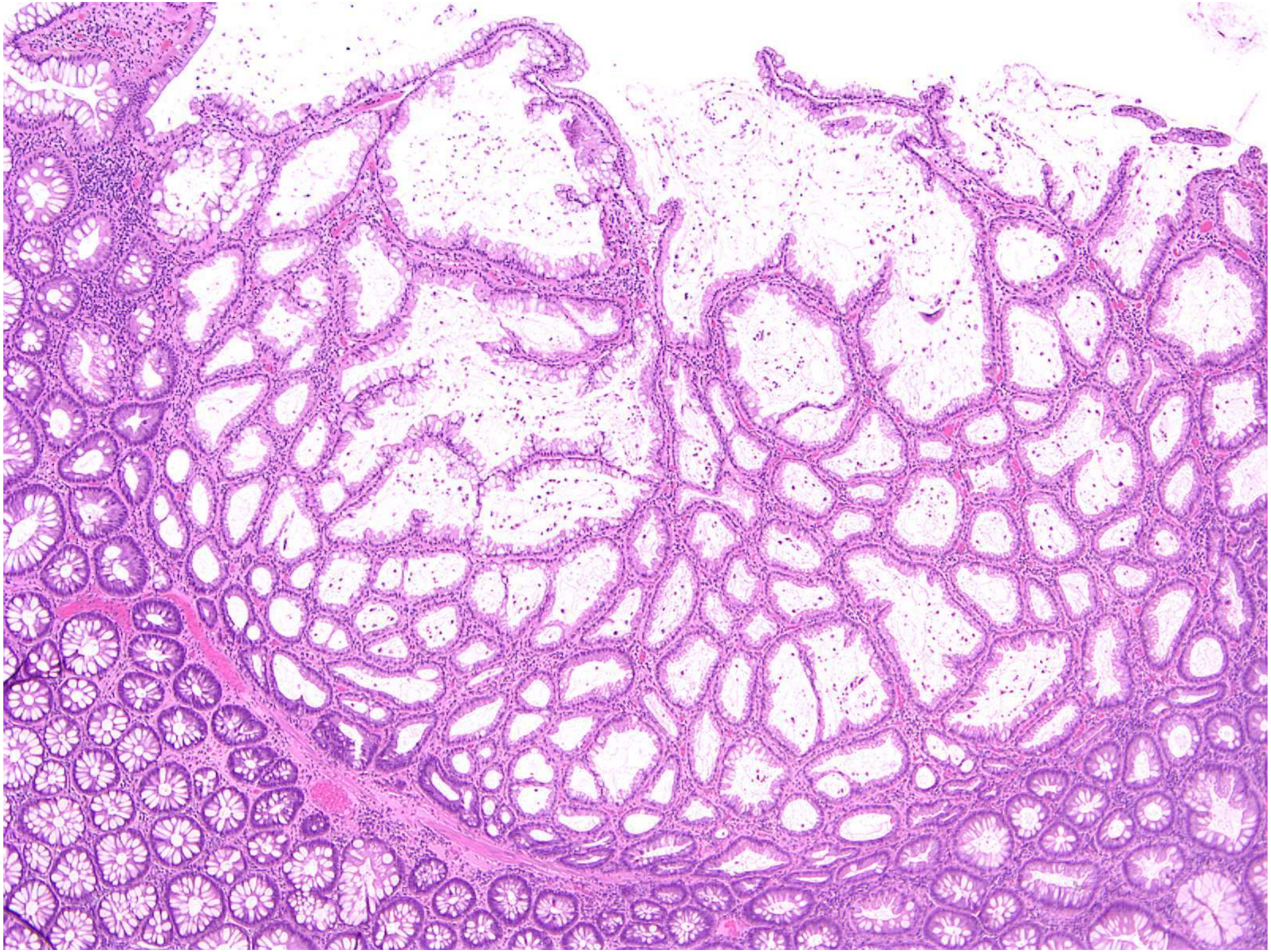


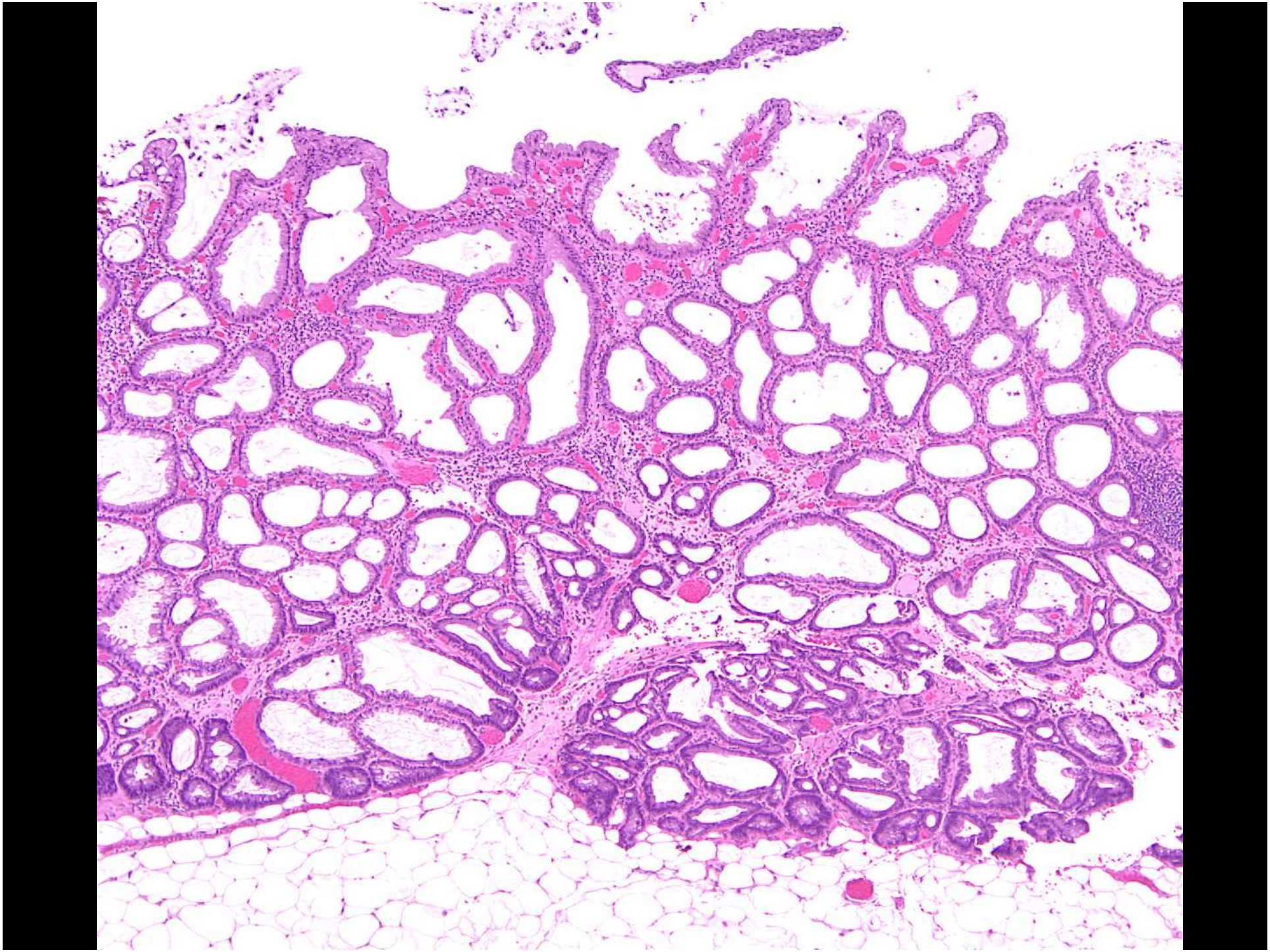


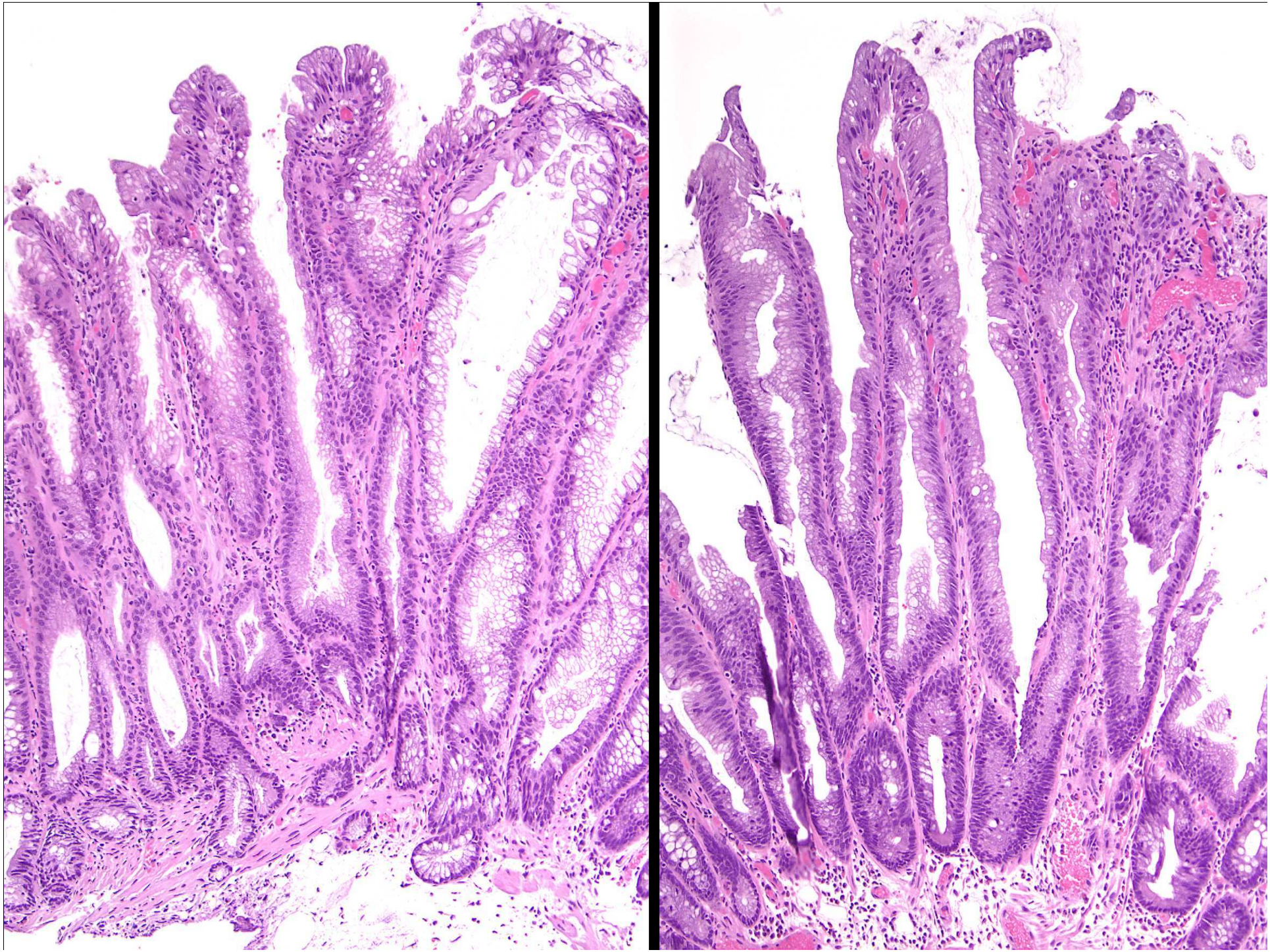












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Hyperplastic-like Polyps That Preceded Microsatellite-Unstable Adenocarcinomas

- 106 polyps in 91 patients with MSI-H cancer
  - Location: Cecum (23%), Ascending colon (70%), Transverse colon (6%)
  - Median interval to cancer – 6.5 years
- Polyps had features of sessile serrated polyp

Goldstein NS et al. *Am J Clin Pathol* 119;778, 2003.

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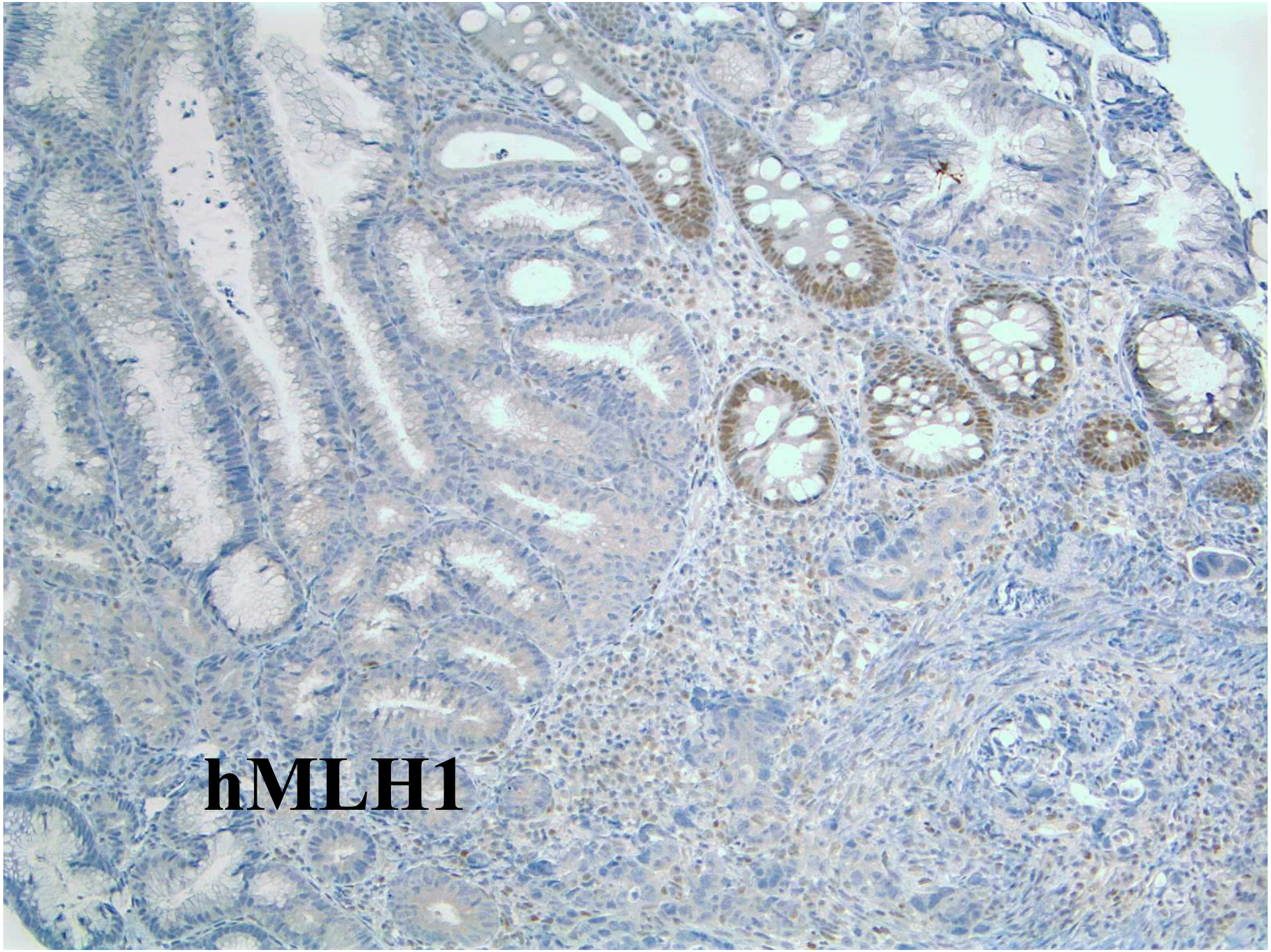
Methylation/Mutations in Serrated Polyp Family

	HP(%)	SSP(%)	Mixed(%)
MINT1	23	30	100
MINT2	32	70	100
MINT31	23	70	100
hMLH1	0	13	70
MGMT	36	57	60
K*ras*(mut)	18	13	0
BRAF(mut)	19	75	89

Wynter CVA et al. *Gut* 53: 573, 2004.
Kambara T et al. *Gut* 53: 1137, 2004.


hMLH1

Sessile Serrated Polyp

Why Not Call It An Adenoma?

- According to WHO and the AFIP adenoma is defined by cytologic dysplasia
- Sessile serrated polyps are often only sampled
  - If considered adenoma, incomplete excision is an indication for operative resection
- Sessile serrated polyps occur in IBD patients
  - If called adenoma the lesion may be interpreted as a DALM and lead to resection
- Term sessile serrated adenoma is frequently mistaken for traditionally defined serrated adenoma and considered a variant of villous adenoma
  - Could lead to inadequate surveillance

Sessile Serrated Polyps
Management Dilemma

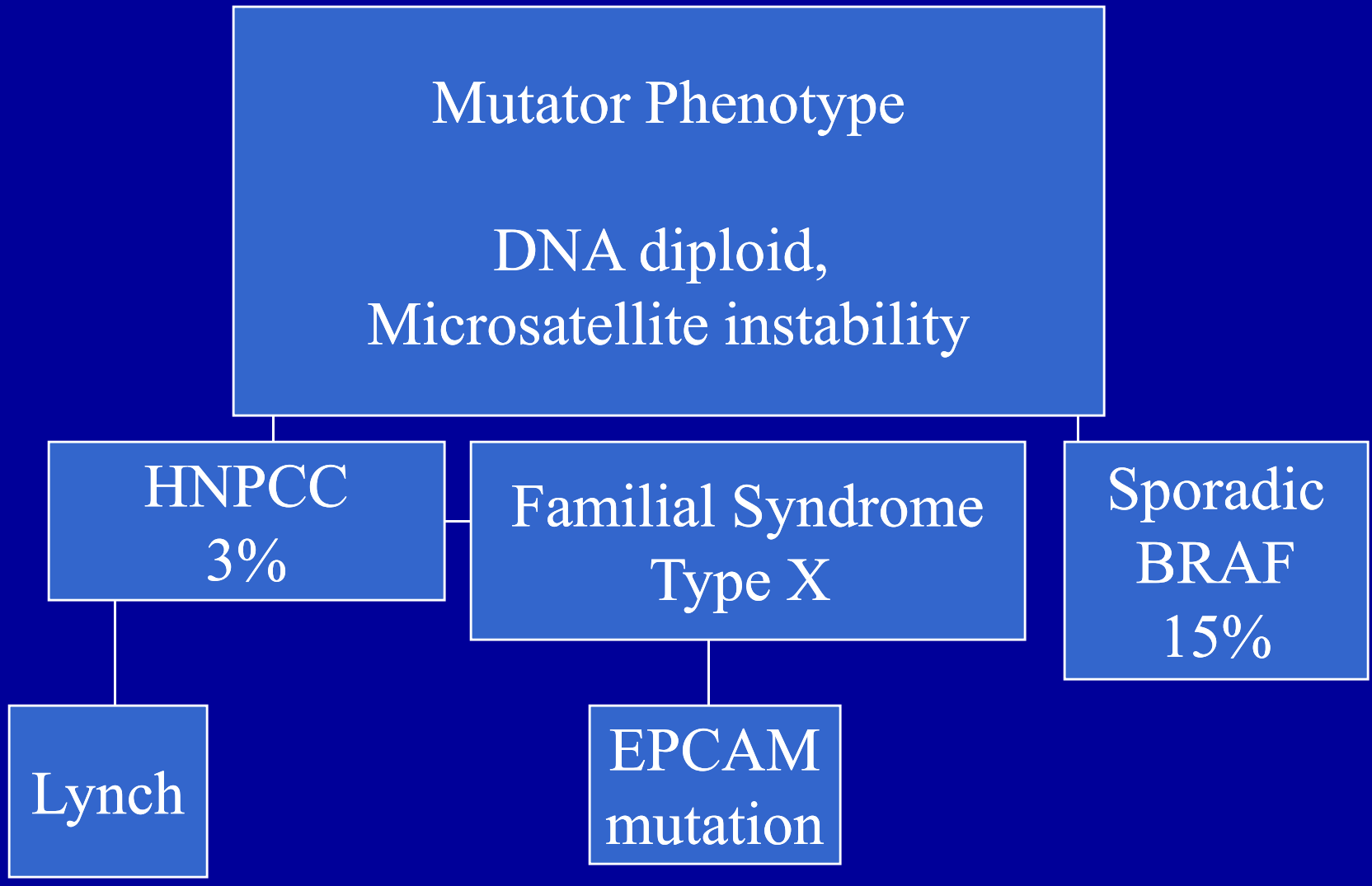
- Serrated adenoma (villous adenoma) may not be an appropriate default diagnosis
  - Resections for incomplete excision
  - Change surveillance interval to 3 years
- In a cohort of 91 patients with sessile serrated polyps (SSP) preceding MSI-H cancers, 19 (21%) SSPs predated MSI-H cancers at same site by less than 3

Ascending Colon, Biopsy: Sessile Serrated Polyp

Comment: This polyp type can be seen in hyperplastic (serrated) polyposis syndrome. Recent data suggest that patients with these polyps, if incompletely excised or associated with additional similar unsampled polyps, may benefit from a shorter surveillance interval (e.g., 1-2 years).

References: Goldstein NS, et al. *Am J Clin Pathol* 119:78, 2003. Jass JR. *Am J Clin Pathol* 119:773, 2003. Jass JR *Clin Gastro Hepatol* 2:1, 2004.

Colorectal Cancer in the Molecular Era



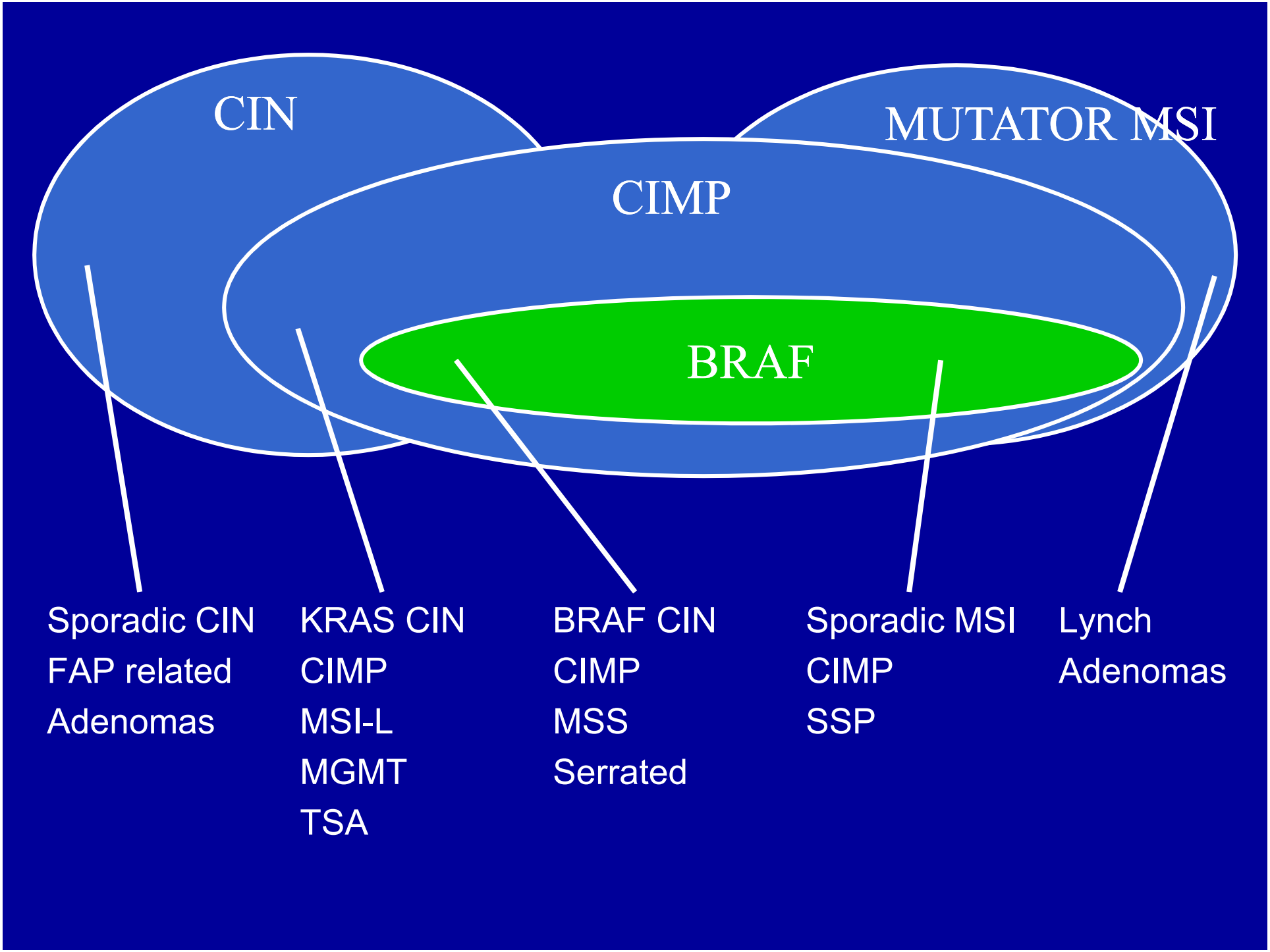
DNA Methylation

- CpG islands – regions of DNA > 0.5 kb with high G:C ratio; promoter regions of 70% of genes contain CpG islands
  - Prone to methylation of cytosine in aging and in certain cancers (CIMP)
  - CpG island methylation leads to conformational changes that render DNA inaccessible to transcription factors
    - Net effect – downstream silencing
- More than 100 genes with CpG island hypermethylation described in colorectal cancer

Colorectal Cancer Stratification Based on CIMP and MSI

Kim JH et al. *Virchows Arch* 455:485, 2009

Combination	Proportion	5 yr
CIMP+/MSI-H (Sporadic MSI-H)	10%	92%
CIMP-/MSI-H (Lynch)	5%	90%
CIMP-/MSS or low (CIN)	75%	65%
CIMP+/MSS (Subset of CIN)	10%	44%


CIN
MUTATOR MSI
CIMP
BRAF
Sporadic CIN
FAP related
Adenomas
KRAS CIN
CIMP
MSI-L
MGMT
TSA
BRAF CIN
CIMP
MSS
Serrated
Sporadic MSI
CIMP
SSP
Lynch
Adenomas

Colorectal Carcinoma Recommendations

- **MSI testing on all resected colorectal carcinomas**
- **KRAS with reflex to NRAS and BRAF on all stage IV colorectal carcinomas**