Serrated Colorectal Polyps and the Serrated Neoplasia Pathway

An update on the pathology and clinical significance

Mark Bettington

Envoi Specialist Pathologists

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Overview

1. A framework of what is included in the serrated neoplasia pathway and how this fits into colorectal carcinoma as a whole

2. Briefly separating the serrated neoplasia pathway from Lynch syndrome, separating serrated morphology from the serrated neoplasia pathway and discussing serrated polyposis syndrome

3. A more detailed discussion of the polyp precursors of the serrated neoplasia pathway with a focus on:
   a) their pathological features
   b) their molecular biology
   c) the subtypes of colorectal carcinoma that they give rise to

Part I

The Serrated Neoplasia Pathway in the Context of Colorectal Carcinoma

Proposed Major Molecular Subtypes of Colorectal Carcinoma

Molecular subtypes of colorectal carcinoma.

Feature	**MSS**	***KRAS***	***BRAF* MSI**	***BRAF* MSS**	**Lynch**
Phenotype	Traditional pathway	Traditional pathway	Serrated pathway	Serrated Pathway	Familial
Percentage of CRC	50%	30%	10%	5%	2%
Prognosis	Referent	1.5x worse	Favourable	2x worse	0.3x worse

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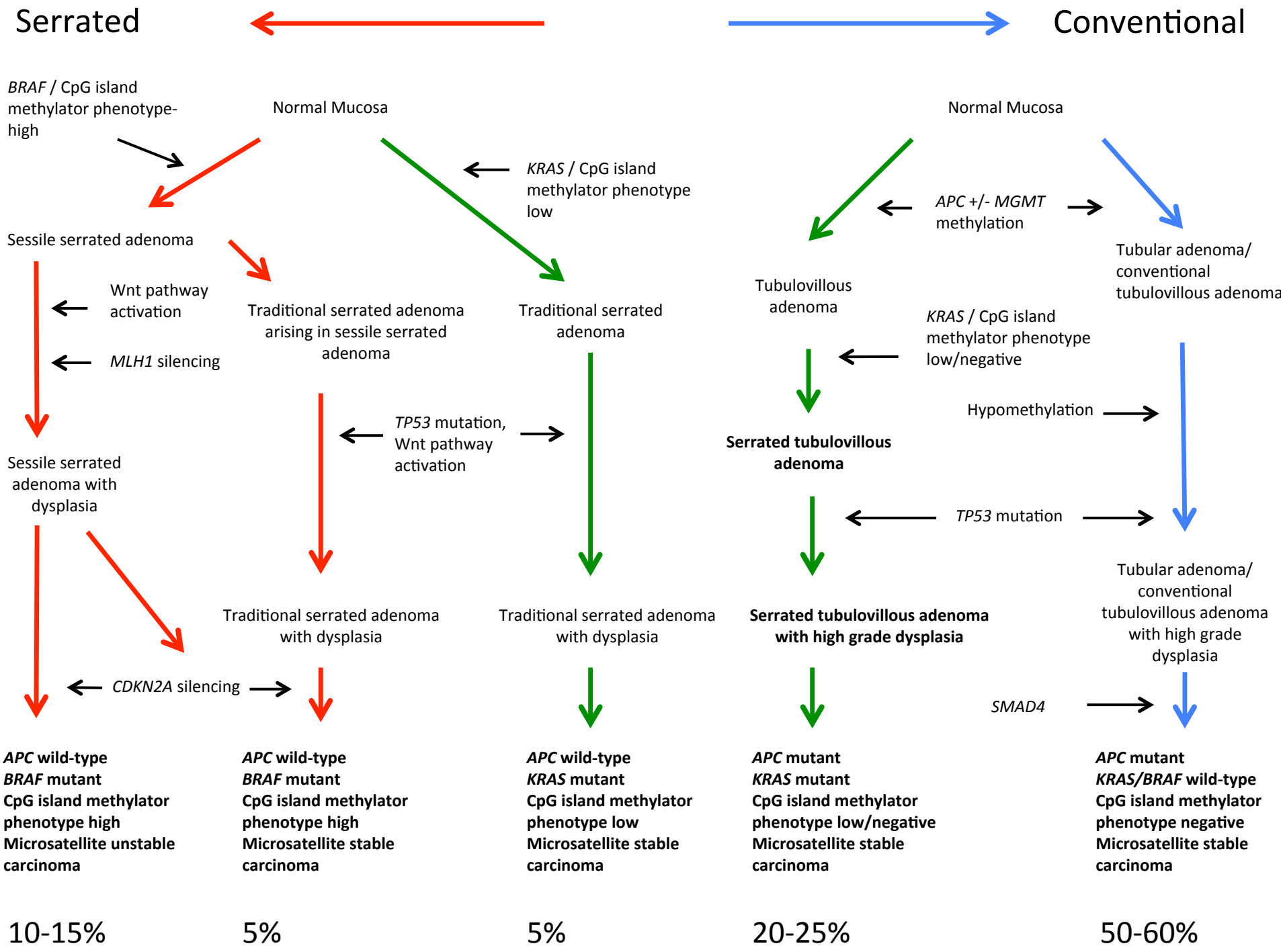
Sinicrope et al, Gastroenterology 2015;148:88-99
Phipps et al, Gastroenterology 2015;148:77-87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Normal Mucosa
KRAS / CpG island methylator phenotype low
Normal Mucosa
APC +/- *MGMT* methylation
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Wnt pathway activation
MLH1 silencing
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TP53 mutation
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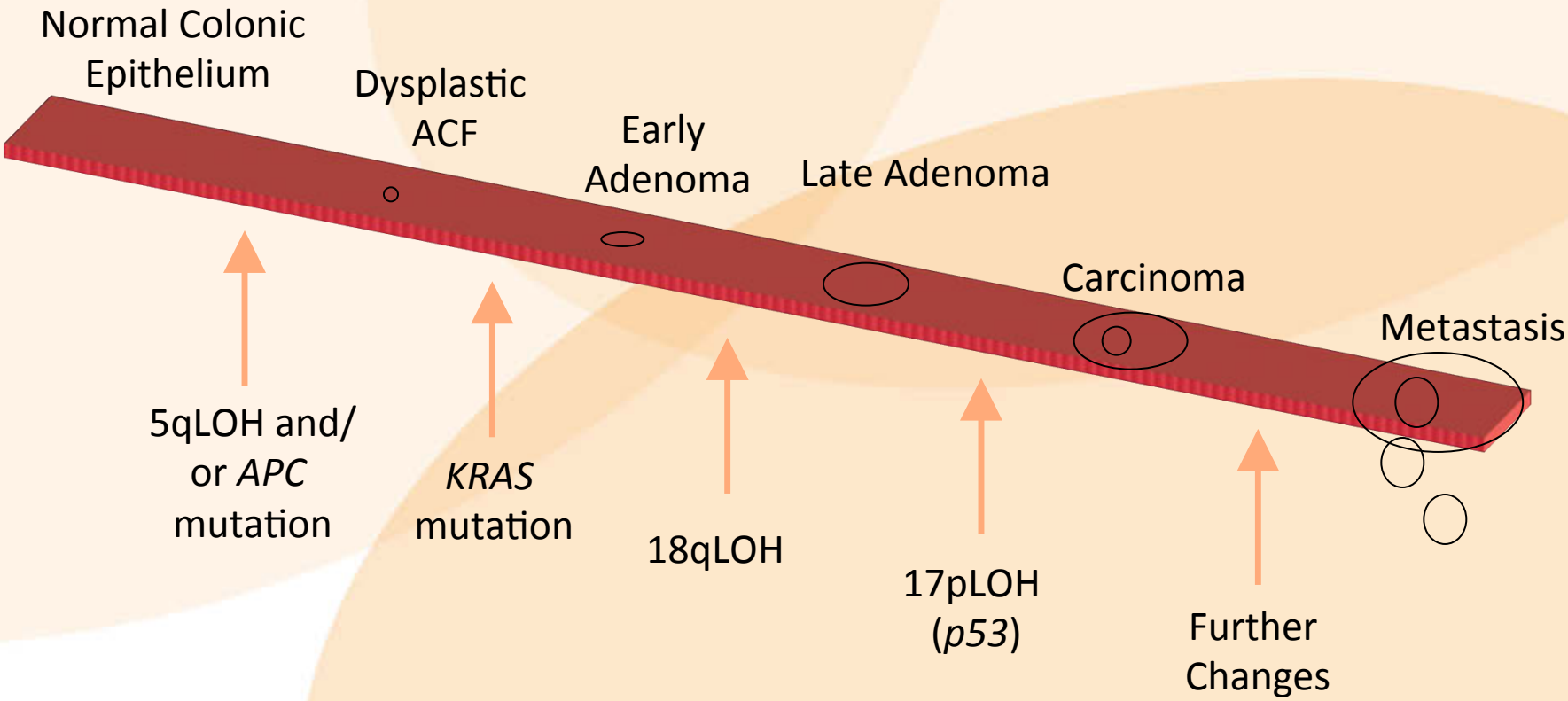
Traditional serrated adenoma with dysplasia
Traditional serrated adenoma with dysplasia
Serrated tubulovillous adenoma with high grade dysplasia
Tubular adenoma/ conventional tubulovillous adenoma with high grade dysplasia
SMAD4
***APC* wild-type**
***BRAF* mutant**
CpG island methylator phenotype high
Microsatellite unstable carcinoma
***APC* wild-type**
***BRAF* mutant**
CpG island methylator phenotype high
Microsatellite stable carcinoma
***APC* wild-type**
***KRAS* mutant**
CpG island methylator phenotype low
Microsatellite stable carcinoma
***APC* mutant**
***KRAS* mutant**
CpG island methylator phenotype low/negative
Microsatellite stable carcinoma
***APC* mutant**
***KRAS/BRAF* wild-type**
CpG island methylator phenotype negative
Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

Traditional Pathway of Tumour Progression



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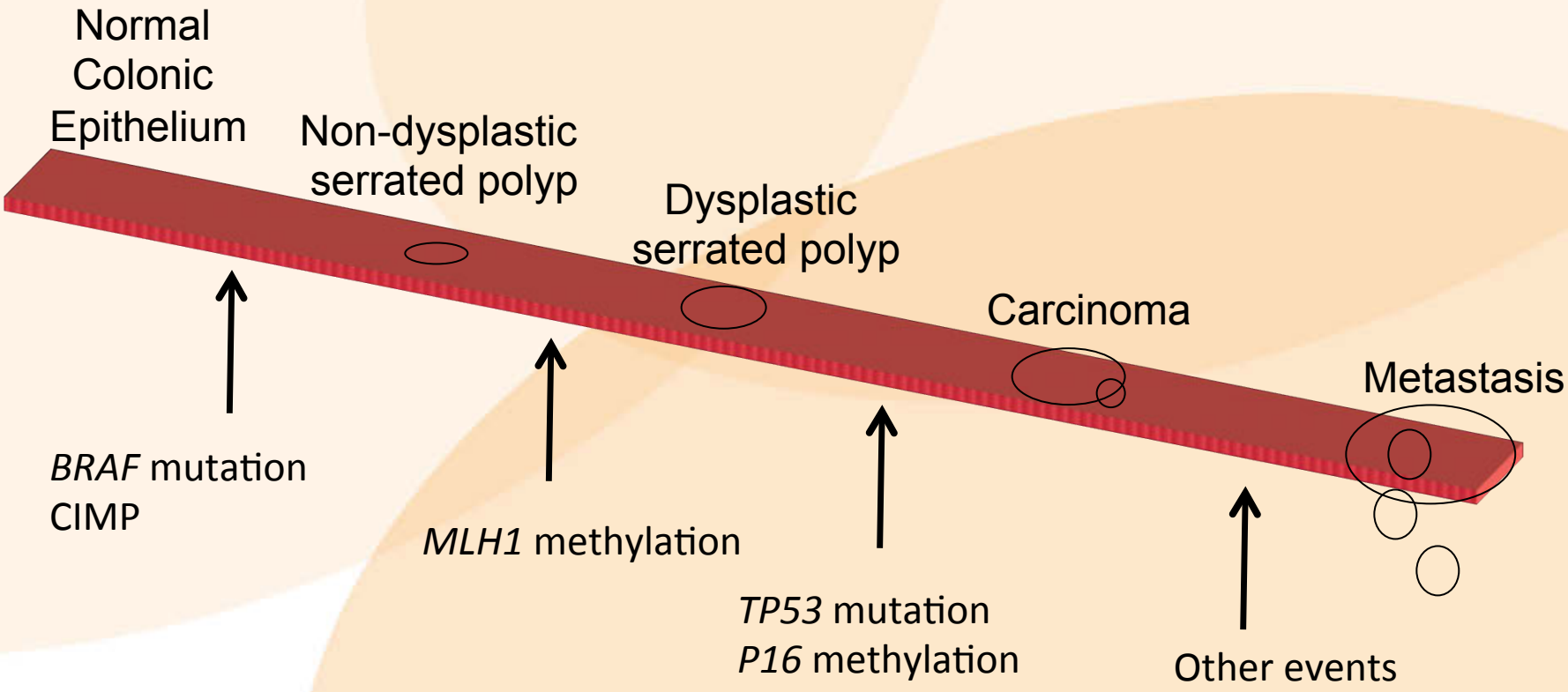
Adapted from Kinzler and Vogelstein, Cell, 1996

Serrated Pathway of Tumour Progression



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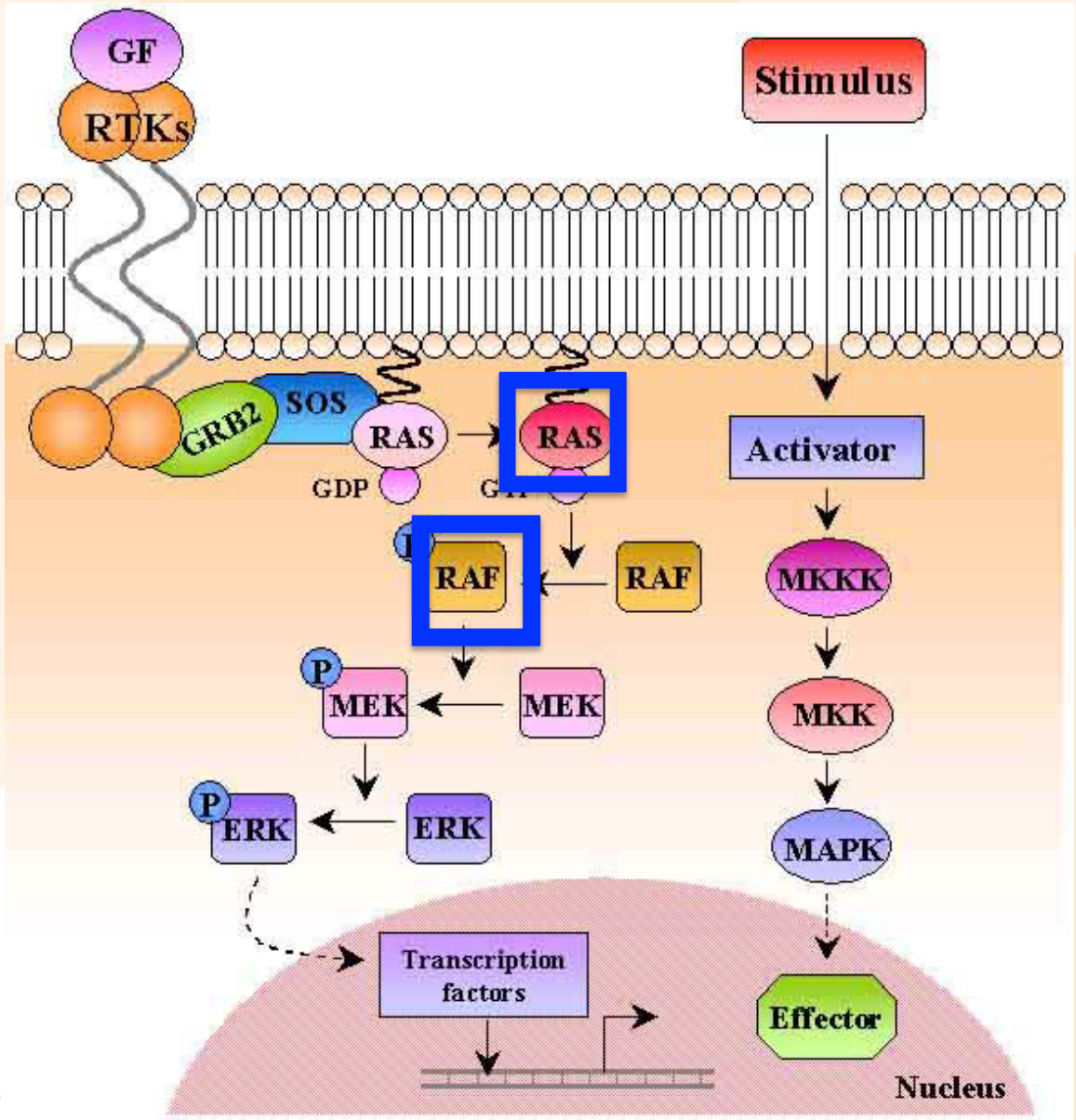
The Serrated Neoplasia Pathway

- The major molecular alterations underpinning the serrated neoplasia pathway are:
  1. MAP kinase pathway activation
  2. The CpG island methylator phenotye

MAPK pathway activation

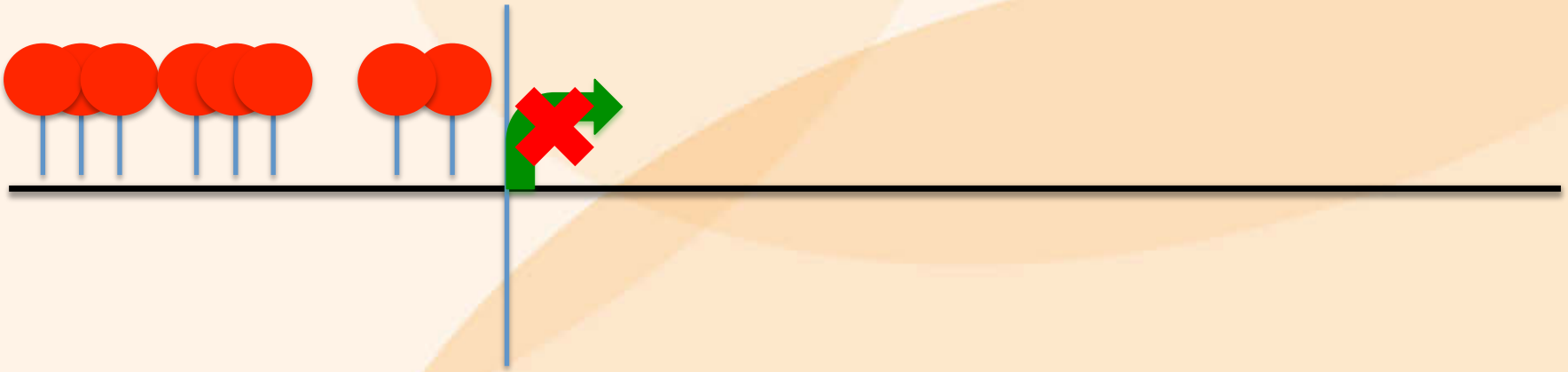


The CpG island methylator phenotype (CIMP)

- This refers to the propensity to methylate CpG islands
- Cytosine followed by Guanine (CpG) is quite uncommon in the genome (far less than expected by chance alone)
- In general CpGs aggregate in the promoter regions of some genes
- These sites are typically hypomethylated
- In cancers with CIMP these sites become increasingly methylated until transcription factors can no longer bind to the promoter
- This change is effectively irreversible and results in gene silencing
- When a tumour suppressor gene (such as MLH1) is methylated, it is oncogenic

CpG Island Methylation

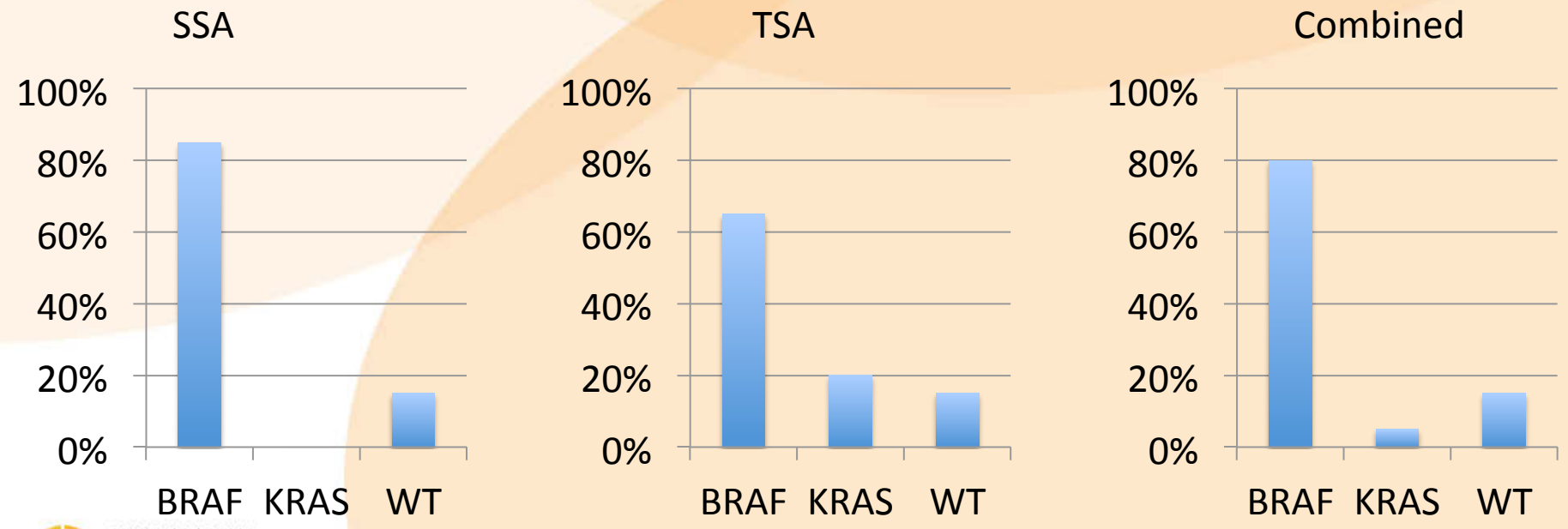
CpG rich promoter region



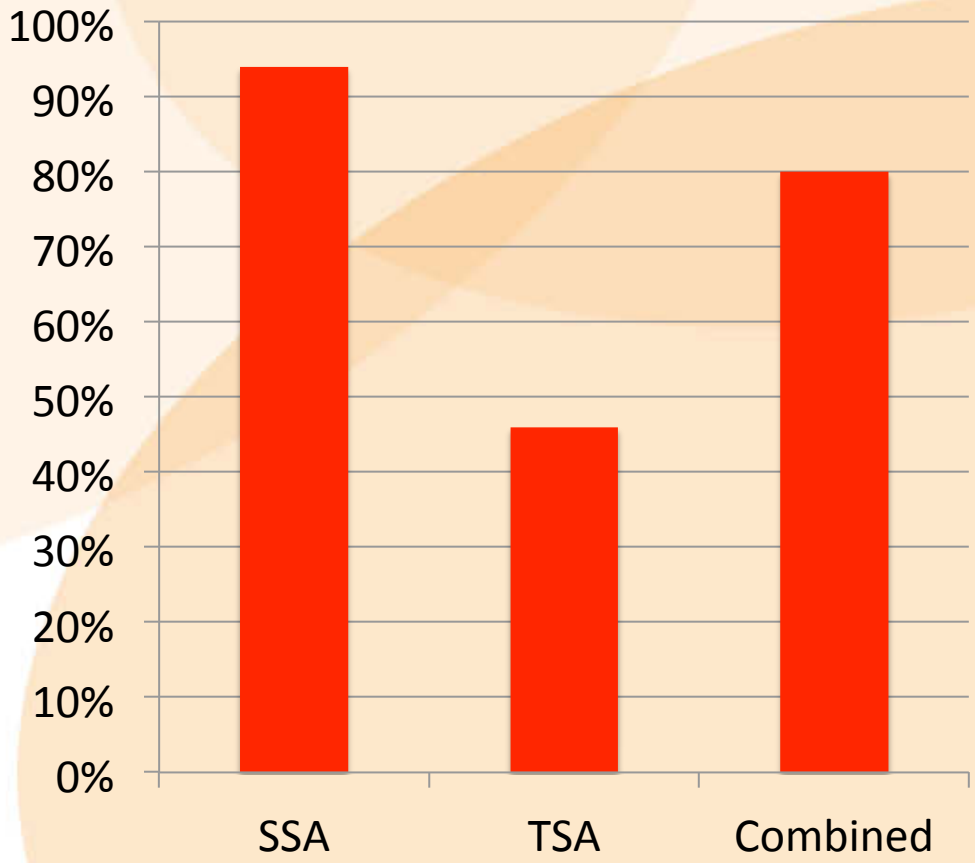
Transcription start site

MAP kinase pathways activation in serrated polyps

- Nearly all serrated polyps have an activating mutation of either *BRAF* or *KRAS*
- Both result in MAP kinase pathway activation



CpG island methylator phenotype high in serrated polyps



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So what makes a “serrated” molecular signature?

- MAP kinase pathway activation?
- MAP kinase and CIMP?
- BRAF mutation?
- BRAF and CIMP?
- CIMP?
- BRAF, CIMP and MSI?

What do I think should be included in the serrated neoplasia pathway?

- Origin in an SSA or TSA is the best definition to me

- Any cancer with a *BRAF* mutation
- A small subset of cancers with a *KRAS* mutation
  - if they have TSA at the edge
  - if they are CIMP-H
- A tiny fraction of *BRAF*/*KRAS* wild-type cancers probably are of the serrated neoplasia pathway (having arisen from null type SSAs or TSAs)

- I do not consider mismatch repair status (and therefore microsatellite instability) to be relevant

Treatment Implications of the Serrated Neoplasia Pathway

- Surgical resection is the primary treatment
- Chemotherapy for stage III / IV (and possibly some stage II)
  - Although the studies are conflicted, standard chemotherapy (e.g. folfox) does not appear to be as effective for MSI cancers as for MSS cancers[1]

- Targeted therapies
  - Monoclonal antibodies to the EGFR (cetuximab, panitumumab) can be used
  - A *BRAF* mutation is not a contraindication
  - Small molecule *BRAF* inhibitors do not appear to be effective as monotherapy[2]
  - Small molecule MEK inhibitors alone or in combination with other targeted therapies are being trialed

- Immunotherapy
  - CTLA-4 antagonists and PD-1 antagonsits have been utilised with great success in melanoma
  - A brisk immune response to the tumour appears to be critical to success of these agents
  - A recent small study has shown efficacy of pembrolizumab in MSI colorectal carcinoma[3]

1. Sargent et al; JCO 2010
2. Prahallad et al; Nature 2012
3. Le et al; NEJM 2015

Summary – Part I

- Cancers of the serrated neoplasia pathway have their origins in SSAs or TSAs
- A *BRAF* mutation is the best molecular evidence of a serrated pathway carcinoma
- If a cancer is *KRAS* mutated or null-type it must have either an unequivocal serrated polyp at the edge, or be CIMP-H to consider it a serrated pathway carcinoma

- Overall about 20-25% of colorectal carcinoma fits this definition
- Amounts to approximately 3000 cancer deaths per year in Australia
- Most (the MSI subset) are associated with a good prognosis
- Approximately 30% have a very poor prognosis

- Targeted therapies may be particularly relevant to the serrated neoplasia pathway, especially the MSI group

Part II

Separating the Serrated Neoplasia Pathway from Lynch Syndrome

Serrated Morphology versus the Serrated Neoplasia Pathway

And

Serrated Polyposis Syndrome

Differentiating serrated neoplasia pathway from Lynch syndrome

Feature	Serrated Neoplasia Pathway	Lynch Syndrome
Genetics	Sporadic	Hereditary
Precursor polyp	SSA or TSA	Conventional adenoma
Age at cancer	Older	Younger
Gender	F>M	F=M
***BRAF* mutation**	Yes	No
Mismatch repair deficiency (MSI)	Approximately 70%	100%
Location	Proximal	Proximal
Histopathology	Poor differentiation Mucinous Tumour infiltrating lymphocytes Crohn's-like reaction Clonal growth *mismatch repair proficient cancers do not have these features	Poor differentiation Mucinous Tumour infiltrating lymphocytes Crohn's-like reaction Clonal growth

The role of the pathologist in separating the serrated neoplasia pathway from Lynch syndrome

1. Polyp type -> SSA or TSA then it is not Lynch

2. Mismatch repair enzyme testing

- we use reflex testing for all new colorectal carcinoma diagnoses
- PMS2, MSH2, MSH6 loss -> suggest referral to a clinical geneticist
- MLH1 (and PMS2) loss we have standard comments

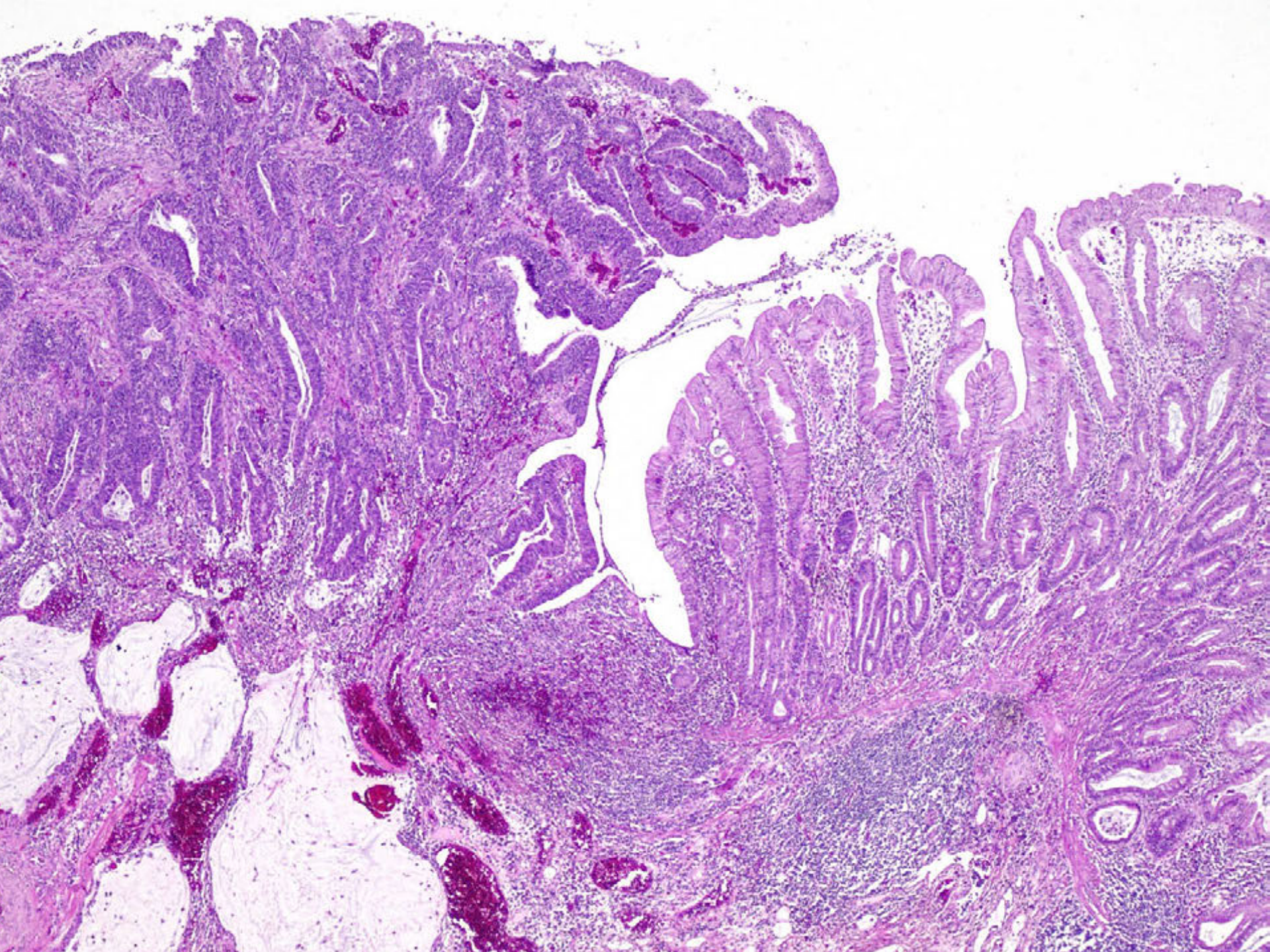
Our standard comments

1. For patients less than 70 years of age

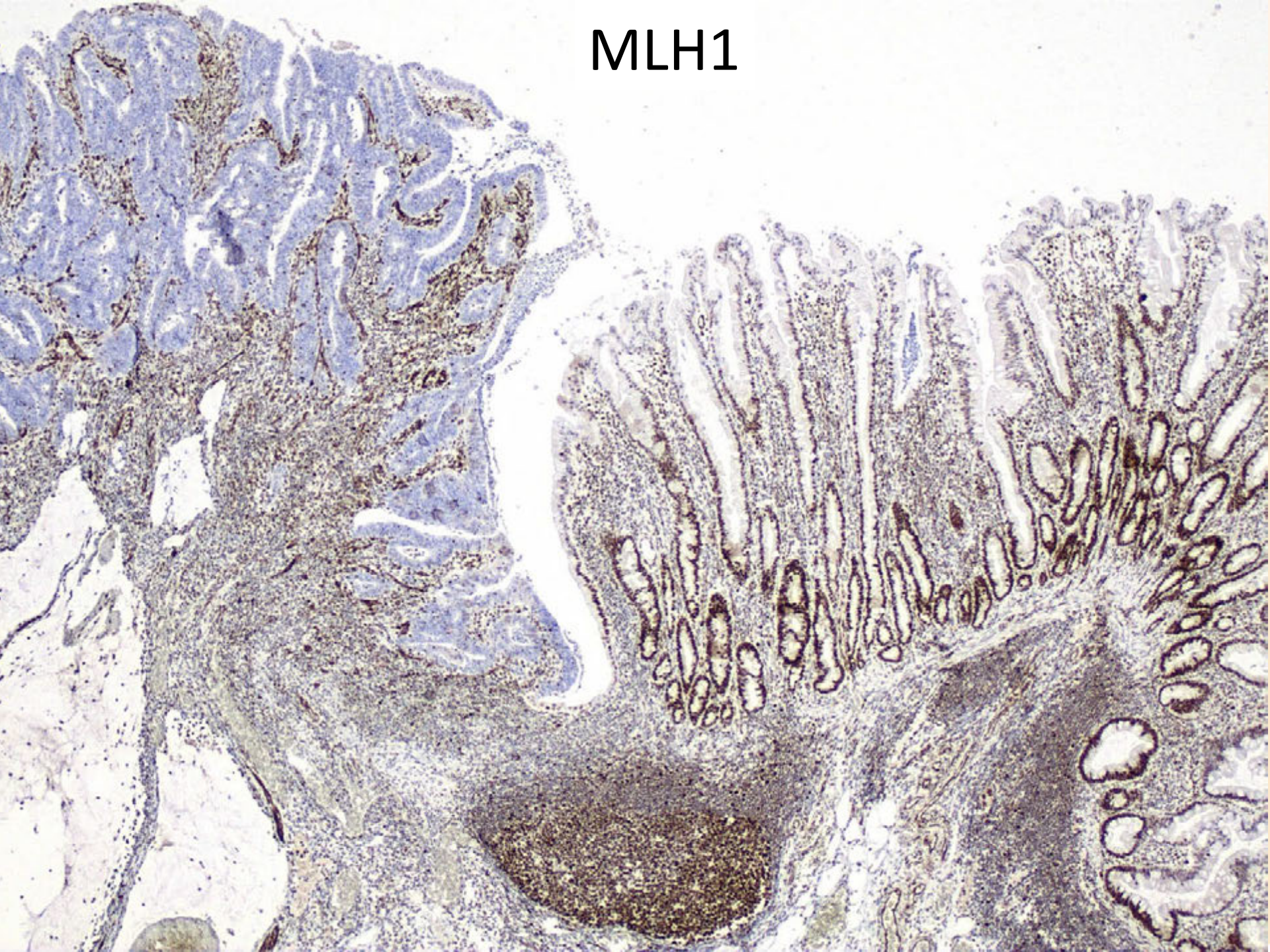
"Immunoperoxidase stains for the mismatch repair enzymes MLH1, PMS2 and MSH6 show loss of staining of carcinoma nuclei for MLH1 and its binding partner PMS2. MLH1-deficient colorectal carcinoma can be caused by Lynch syndrome or by sporadic MLH1 methylation. BRAF gene testing can be performed upon request to distinguish between these two entities."

2. For patients over 70 years of age

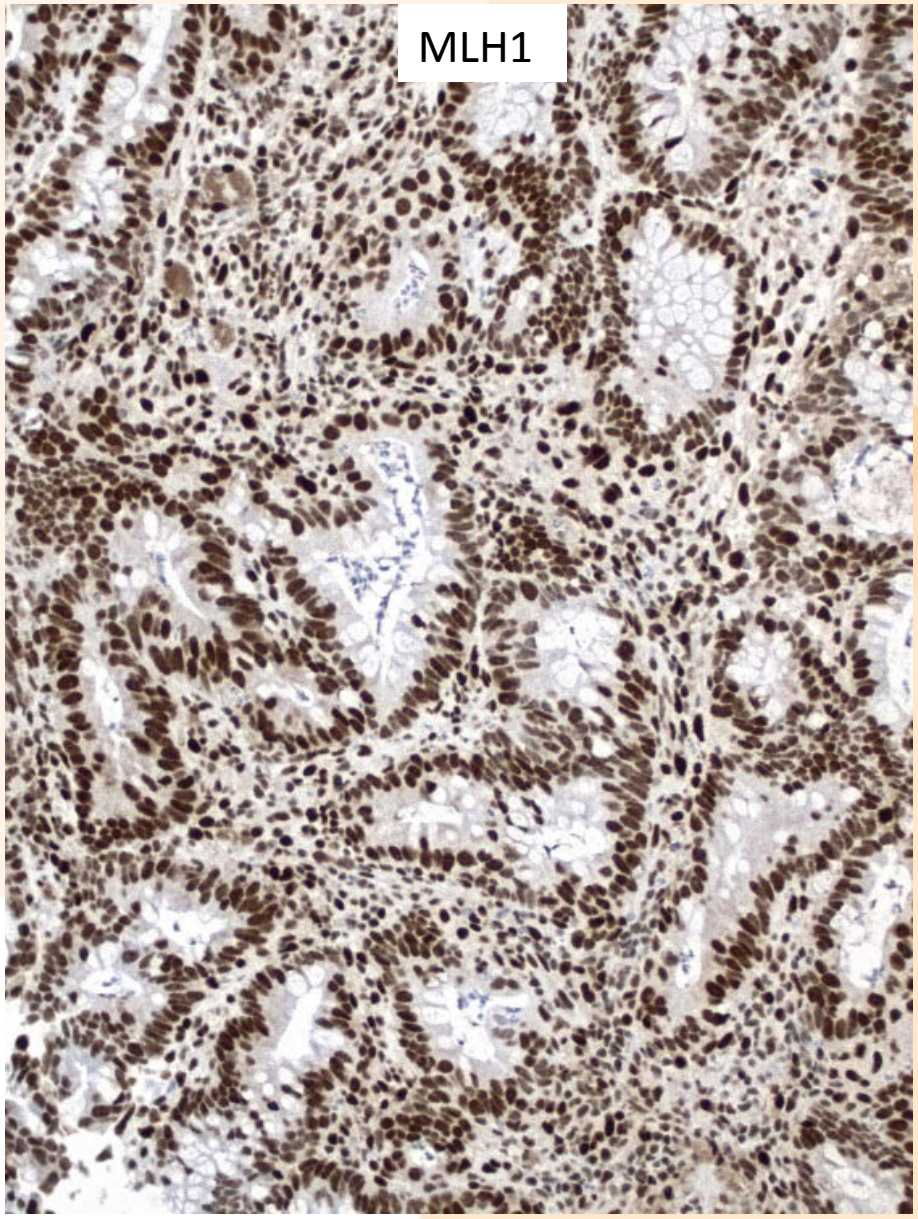
"Immunoperoxidase stains for the mismatch repair enzymes MLH1, PMS2 and MSH6 show loss of staining of carcinoma nuclei for MLH1 and its binding partner PMS2. MLH1-deficient colorectal carcinoma can be caused by Lynch syndrome or by sporadic MLH1 methylation. In patients over 70 years of age, sporadic MSI cancer is usual."

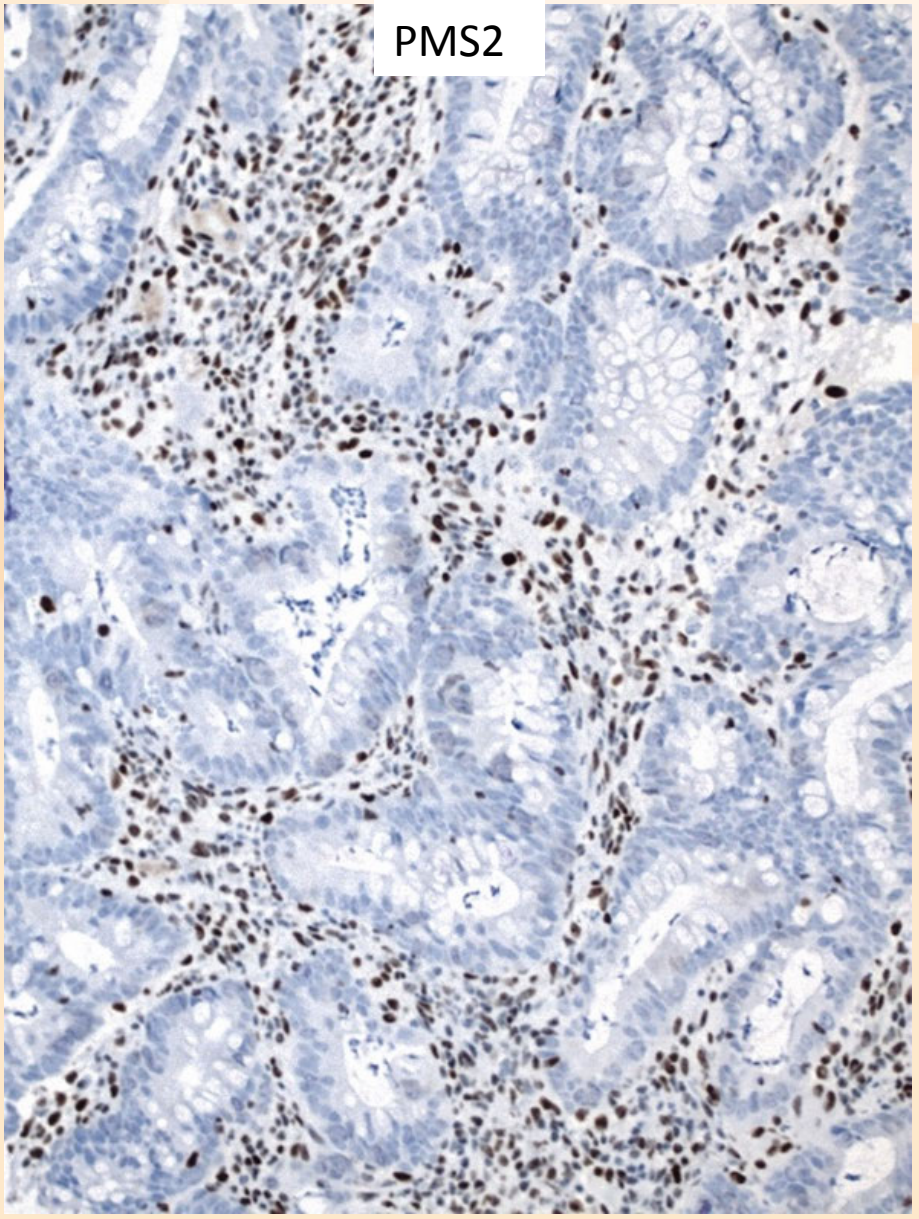


MLH1



CRC in PMS2 mutation carrier



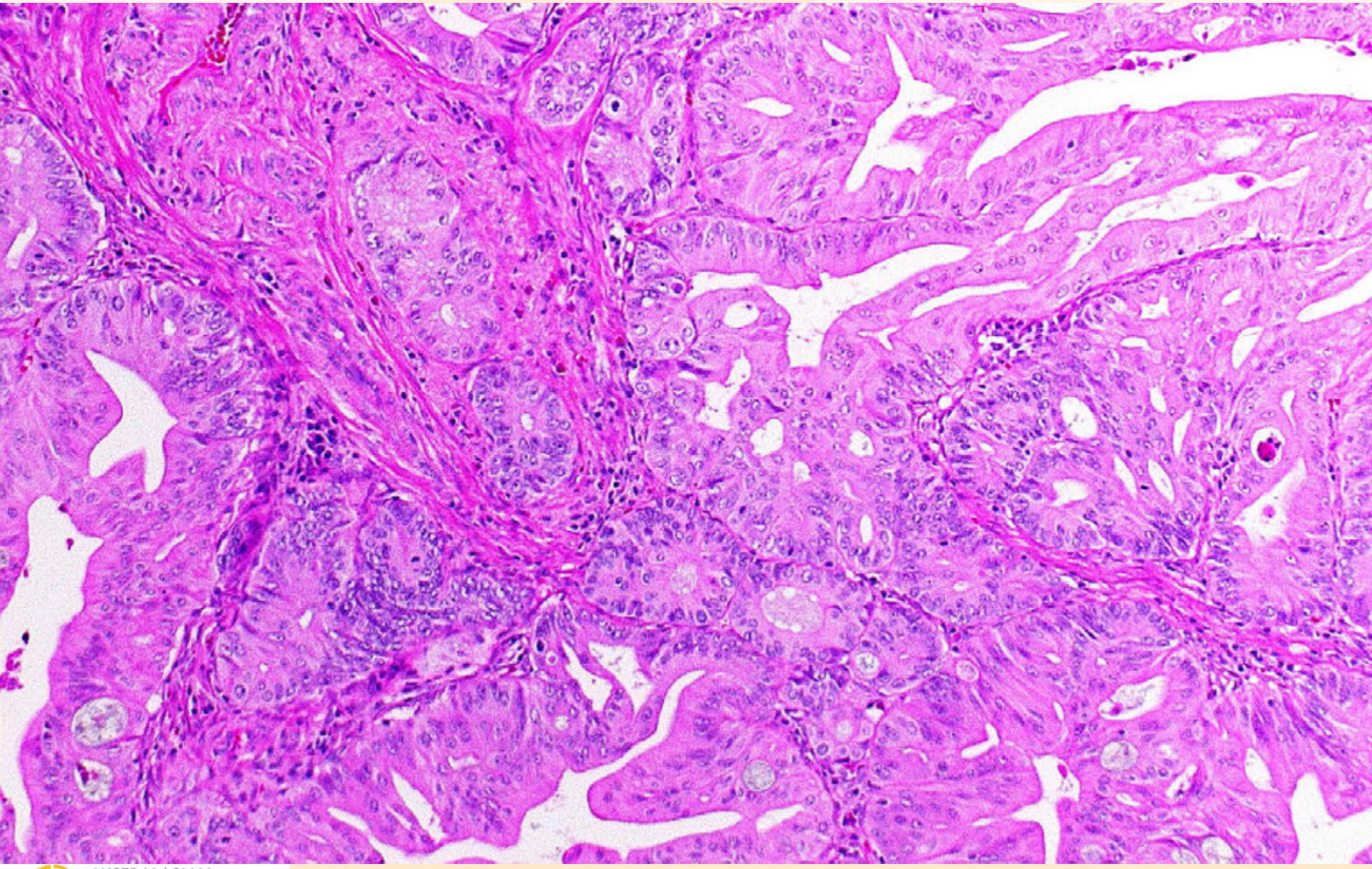


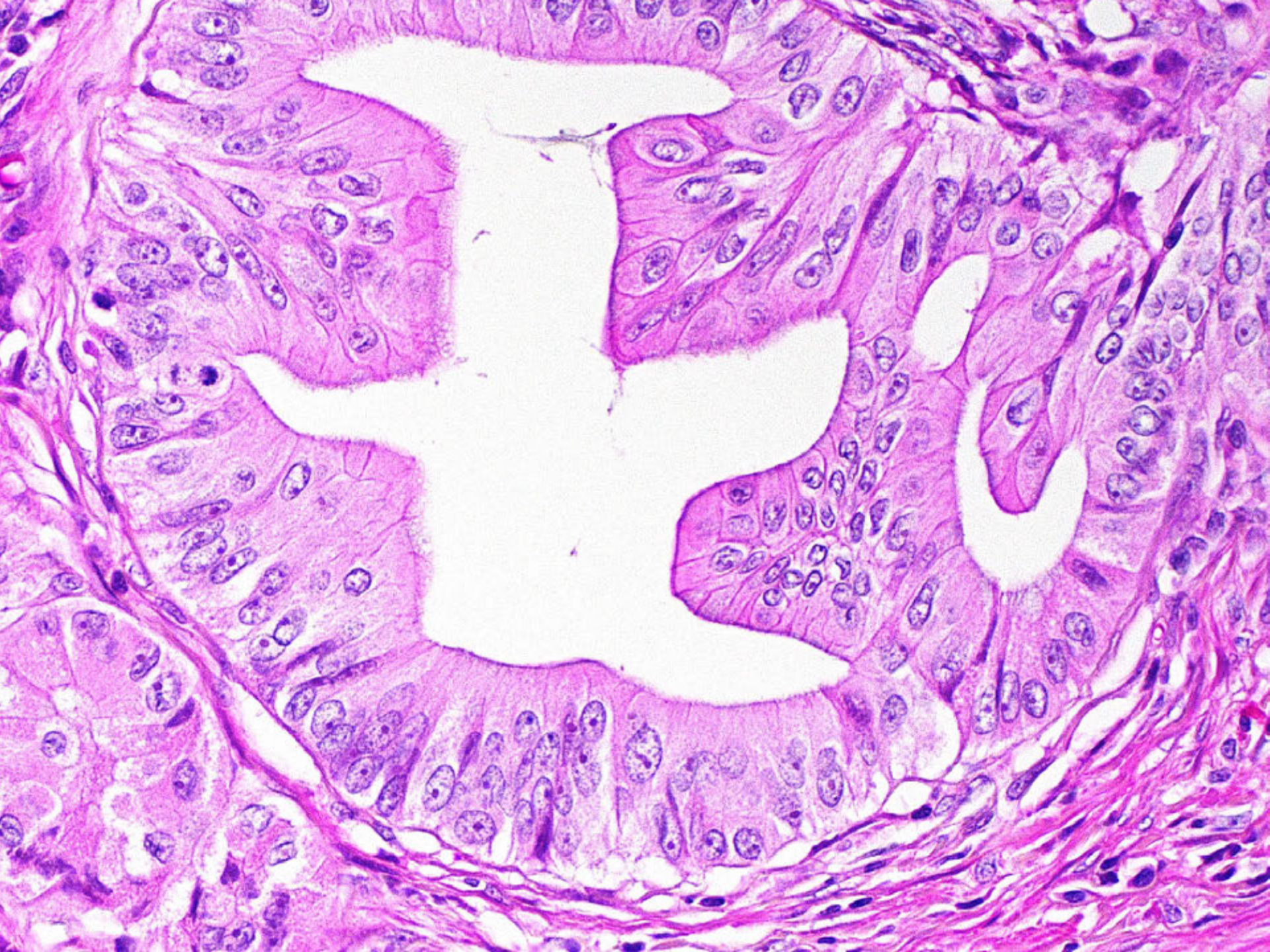
Serrated Morphology versus the Serrated Neoplasia Pathway

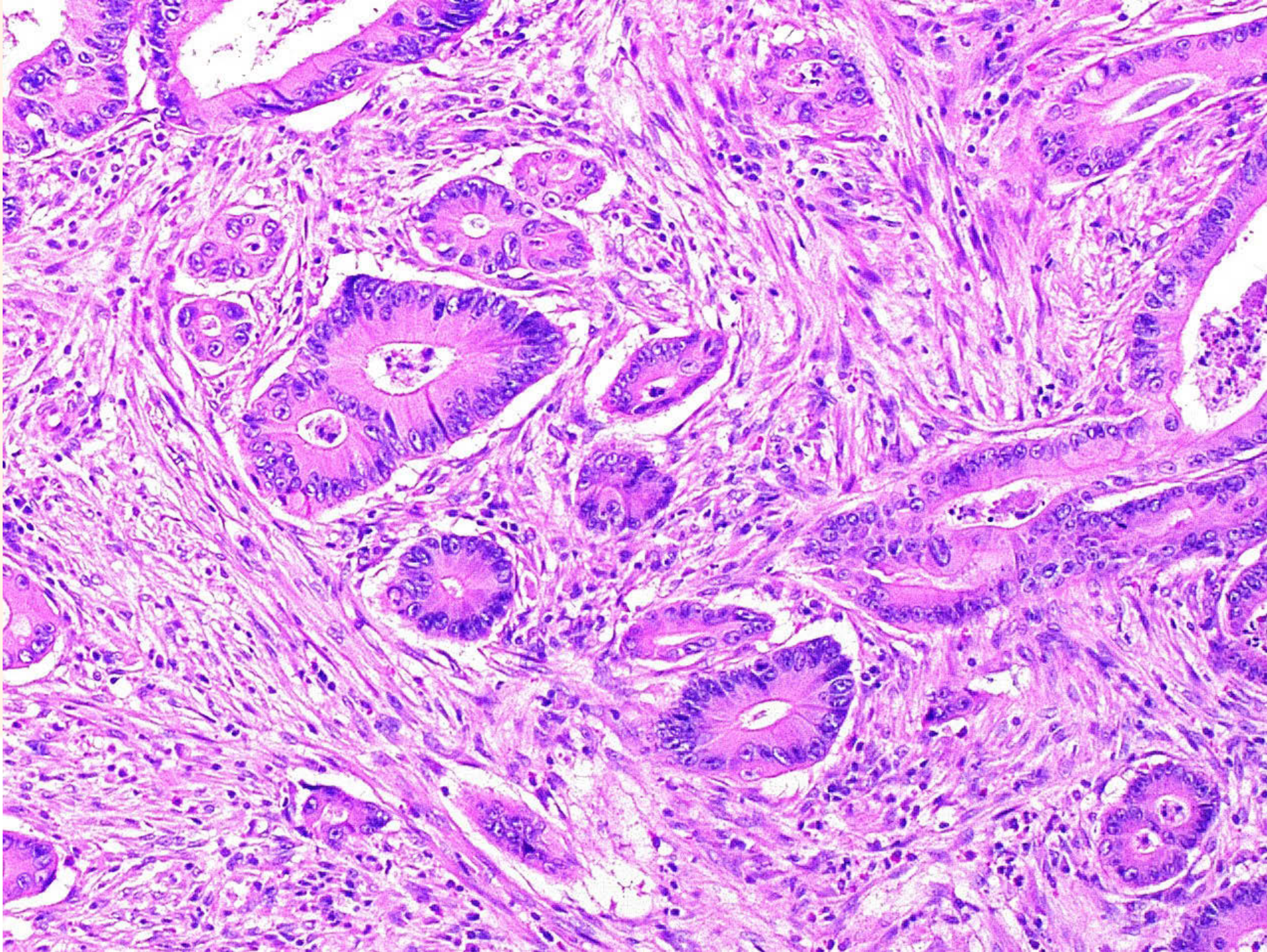
- This is a confusing issue
- "Serrated adenocarcinoma" is now a specific subtype in the WHO, but the significance of the diagnosis is unclear and (in my opinion) the diagnosis lacks reproducibility
- Clinicians usually either don't understand what it means (nor do I), misinterpret what it means or ignore it

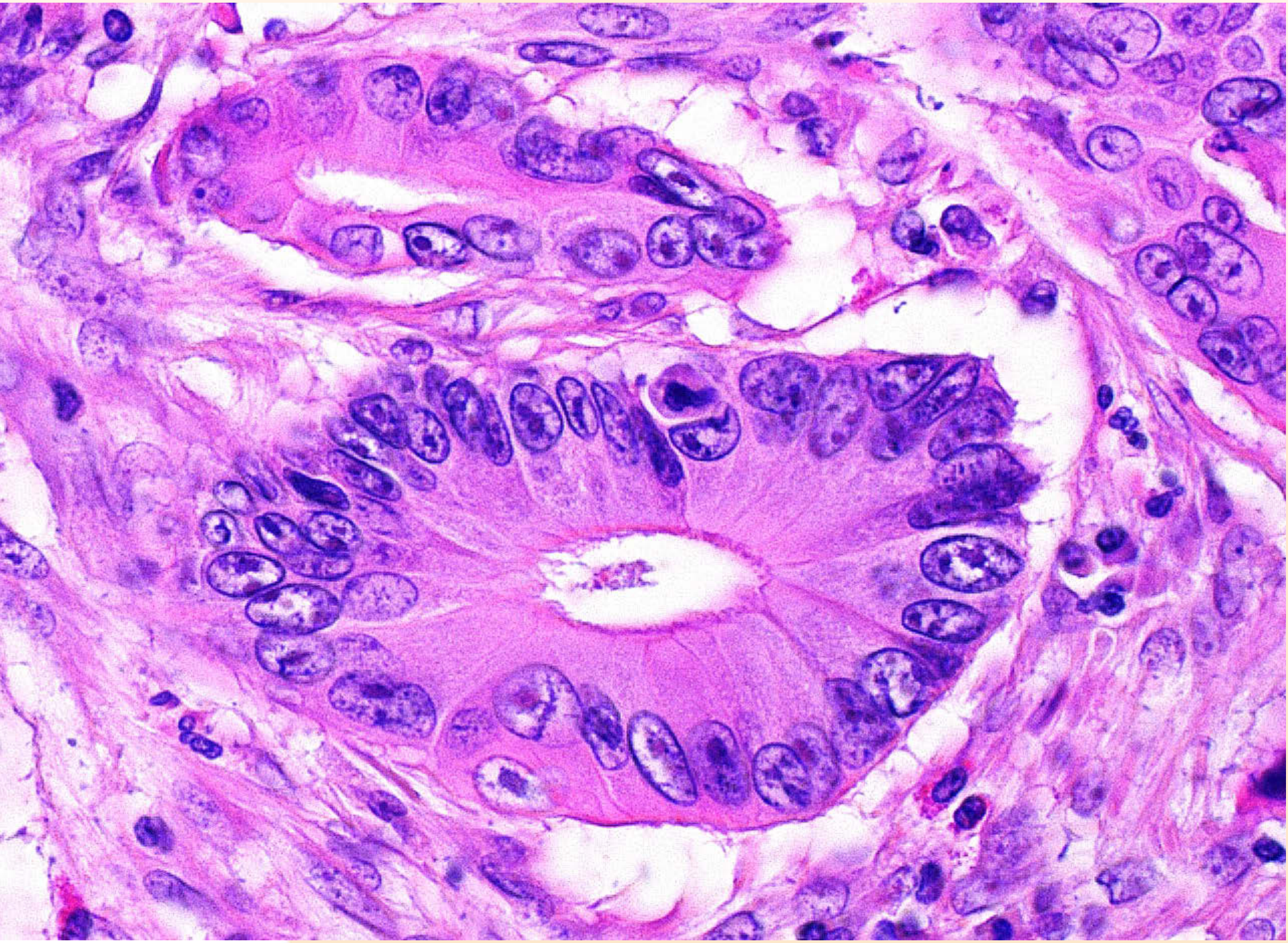
- Serrated morphology does not equal serrated neoplasia pathway

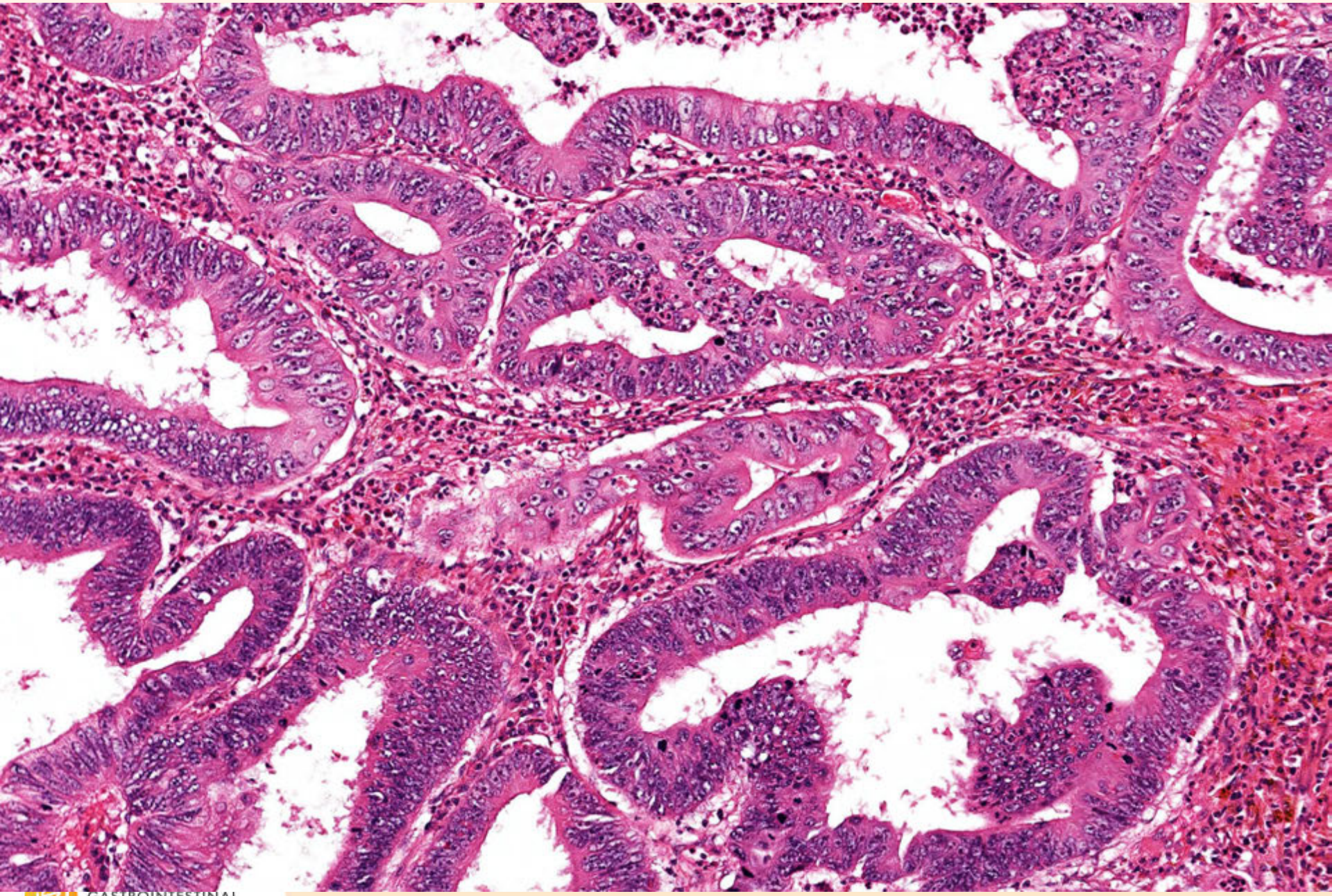
Examples of “Serrated Carcinomas”

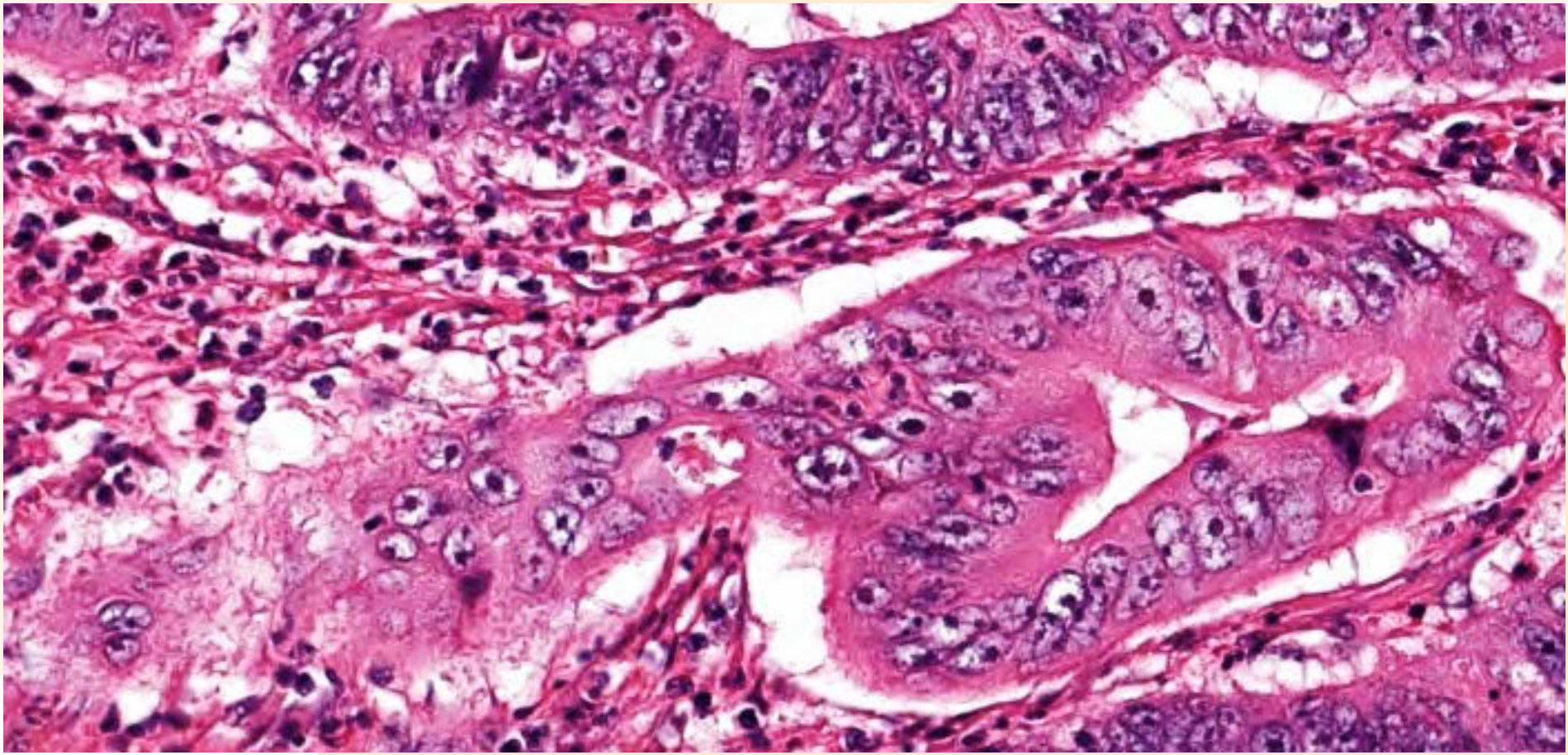












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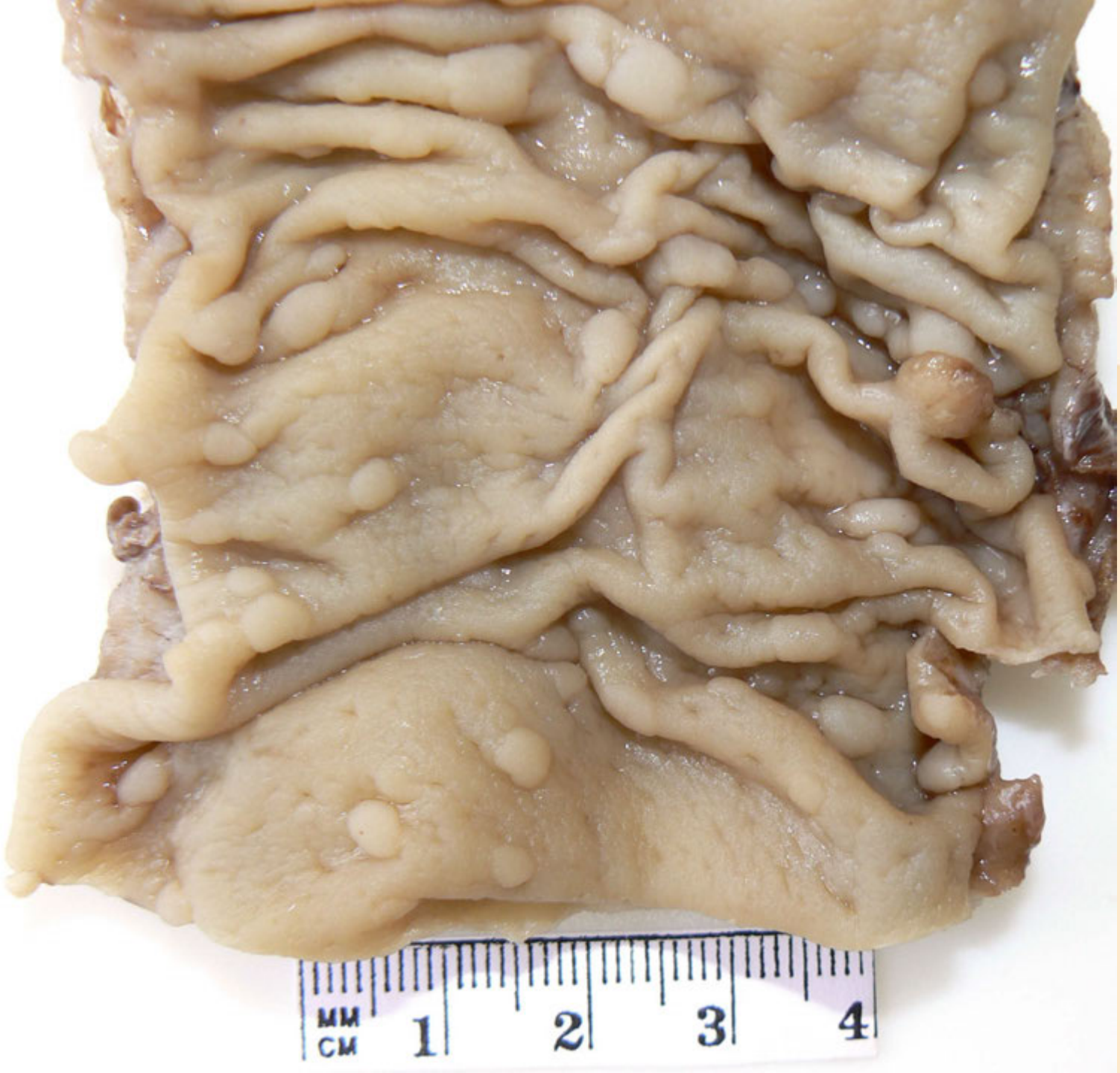
Serrated Polyposis Syndrome

Definition

- Remains clinical and arbitrary

1) At least 5 serrated polyps proximal to the sigmoid colon, at least two of which are >10mm
2) Any serrated polyp in a first degree relative of a patient with serrated polyposis syndrome
3) >20 serrated polyps of any size distributed throughout the colorectum


MM
CM
1
2
3
4

Serrated Polyposis Syndrome

Clinicopathological features

- Most of the cancers are proximal
- The mean age of onset is 50 years
- Most patients will also have conventional adenomas

Surprising facts

- 18% of patients meeting the criteria for SPS had MUTYH syndrome in one paper[1], actual figure is likely to be much less, but still requires consideration
- The cancers in SPS patients are not uniform, less than half have a *BRAF* mutation and only 38% are MMRD[2]

1. Boparai et al; Gastroenterology 2008
2. Rosty et al; AJSP 2013

Serrated Polyposis Syndrome

Cancer risk and surveillance

- The cancer risk is not clear and probably reflects a heterogeneous population
- Probably <50% lifetime risk overall
- No apparent risk of cancer outside the large bowel
- Surveillance colonoscopy every 1-3 years depending on polyp burden
- If adequate colonoscopic control is not possible, prophylactic colectomy is reasonable
- Risk to relatives is unclear, but there does appear to be an increased risk (up to 5 fold)
- Start screening at age of CRC diagnosis or by 40

Summary – Part II

- The serrated neoplasia pathway and Lynch syndrome are very different
- Pathologists should emphasise the distinction in reports, particularly when reporting mismatch repair enzymes
- Serrated morphology does not equal the serrated neoplasia pathway
- Serrated polyposis syndrome remains a clinicopathological entity
- The pathologist has a role in suggesting SPS in reports (and considering MUTYH when appropriate)

Part III

Serrated Colorectal Polyps

WHO classification

WHO Classification of Tumours of the Digestive System (4th edition; 2010):

Hyperplastic polyps

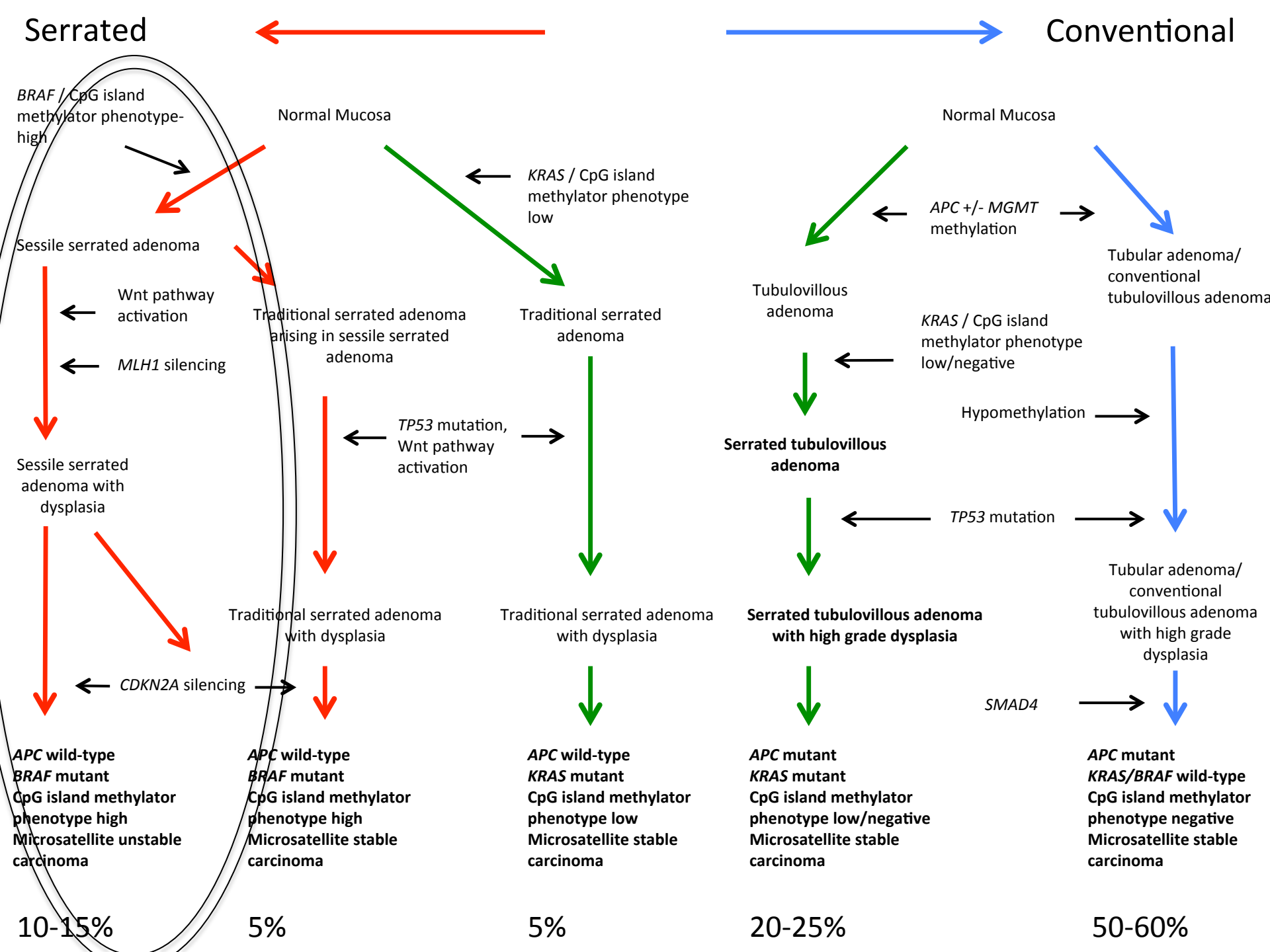
- microvesicular
- goblet cell
- mucin poor

Sessile serrated adenoma

Sessile serrated adenoma with dysplasia

Traditional serrated adenoma

The Sessile Serrated Adenoma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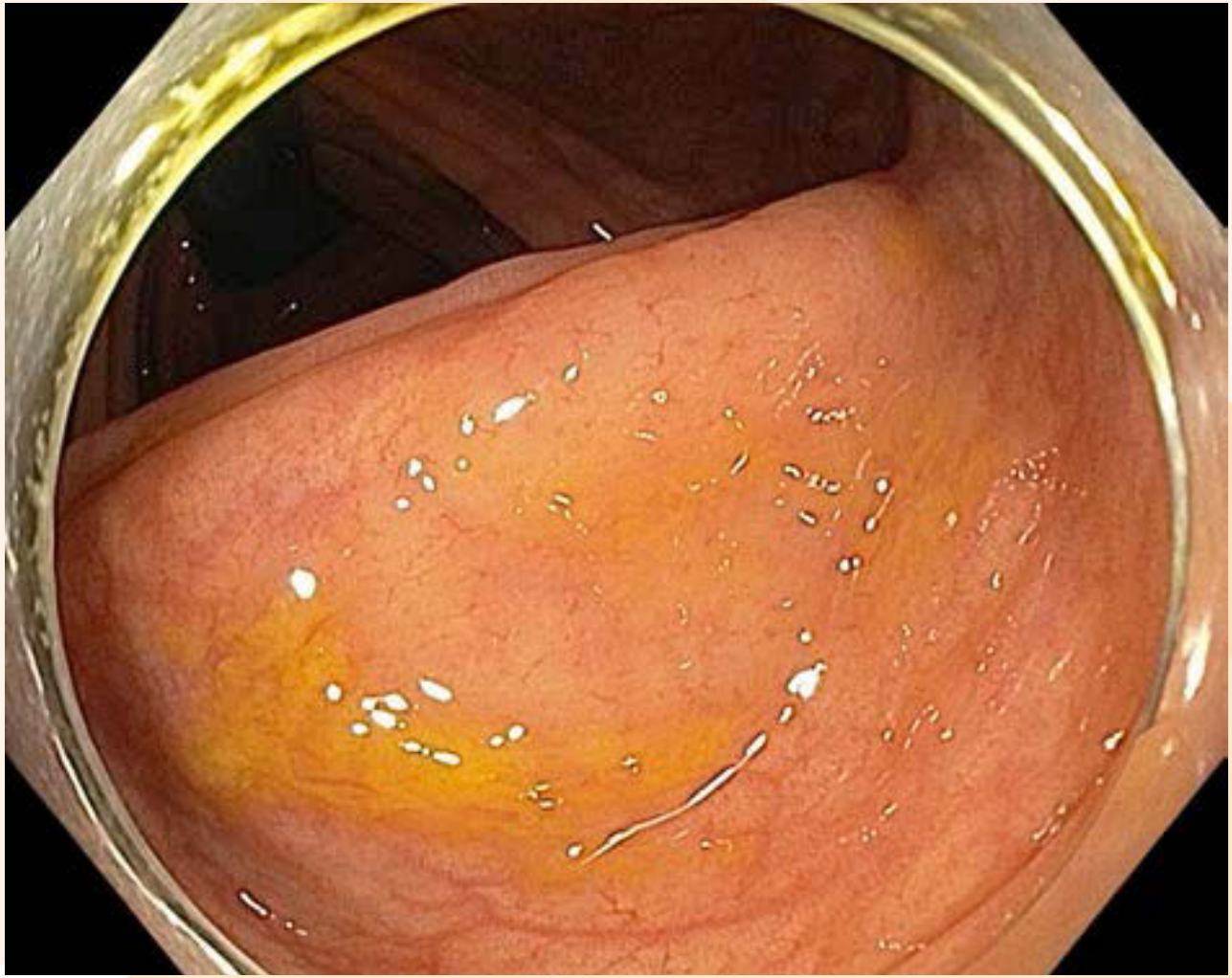
APC mutant KRAS/BRAF wild-type CpG island methylator phenotype negative Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

The sessile serrated adenoma

- First recognised in 2003
- Mostly proximal
- More common in women
- Variable size
- Definite risk of malignancy
- Require surveillance colonoscopy



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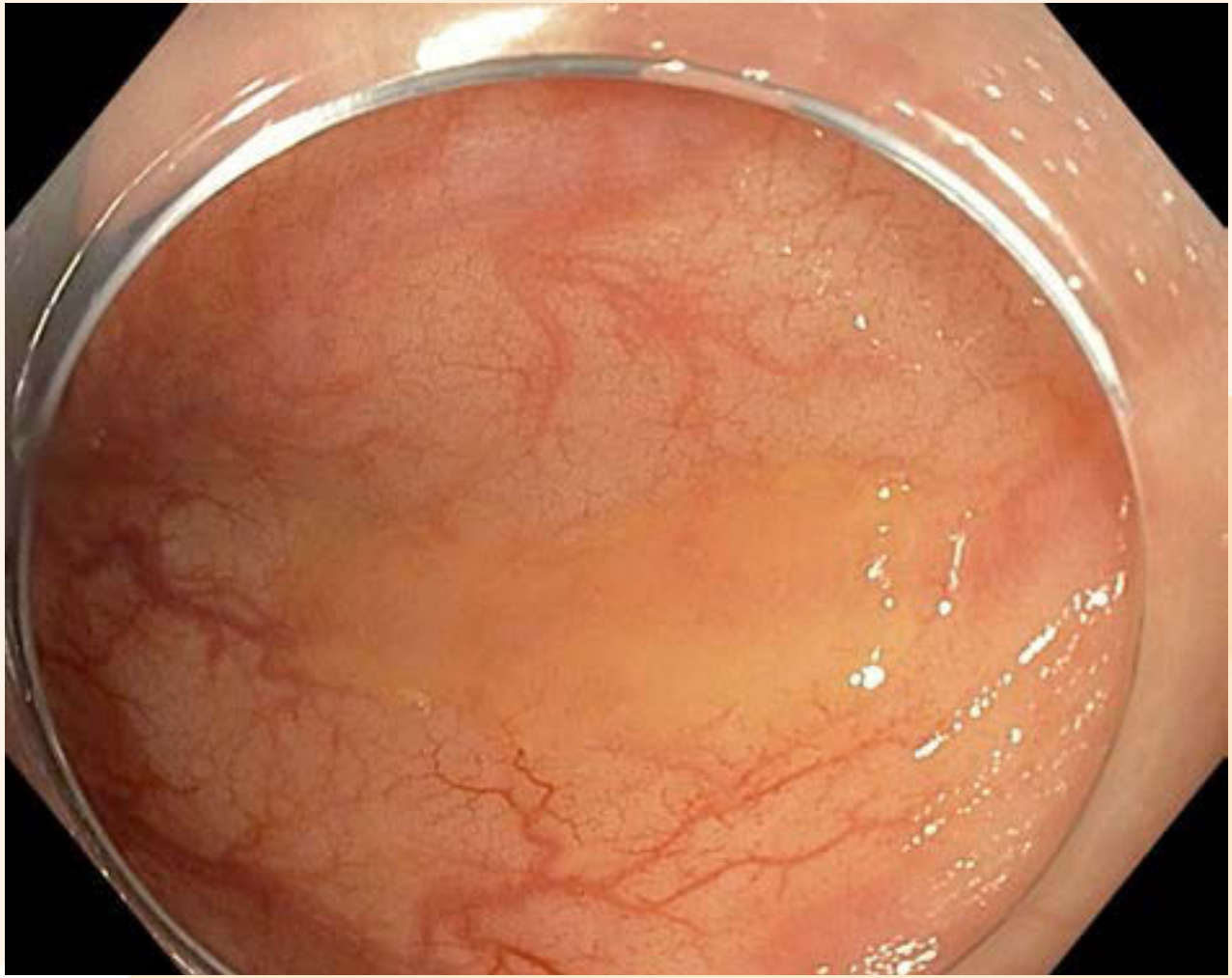

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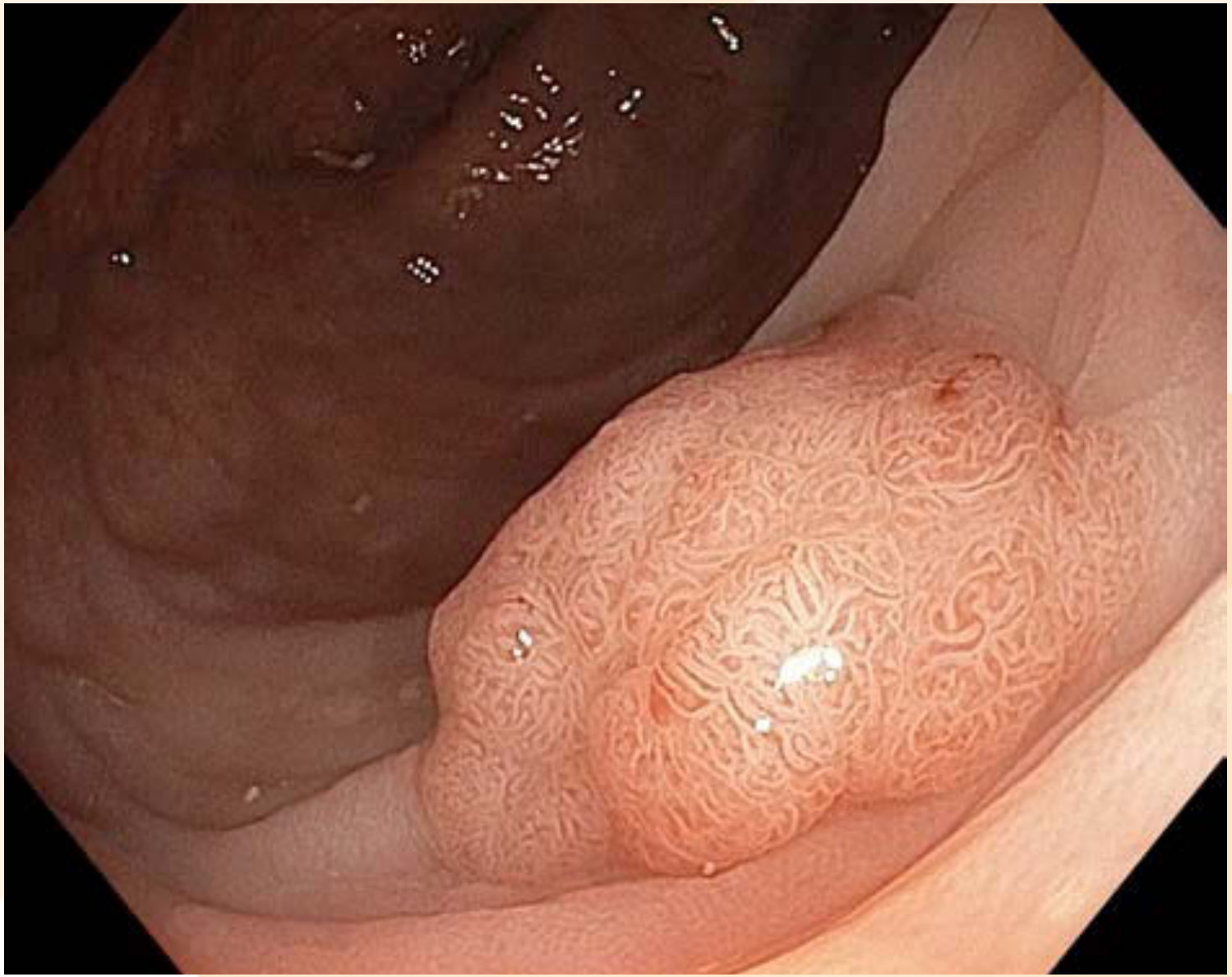

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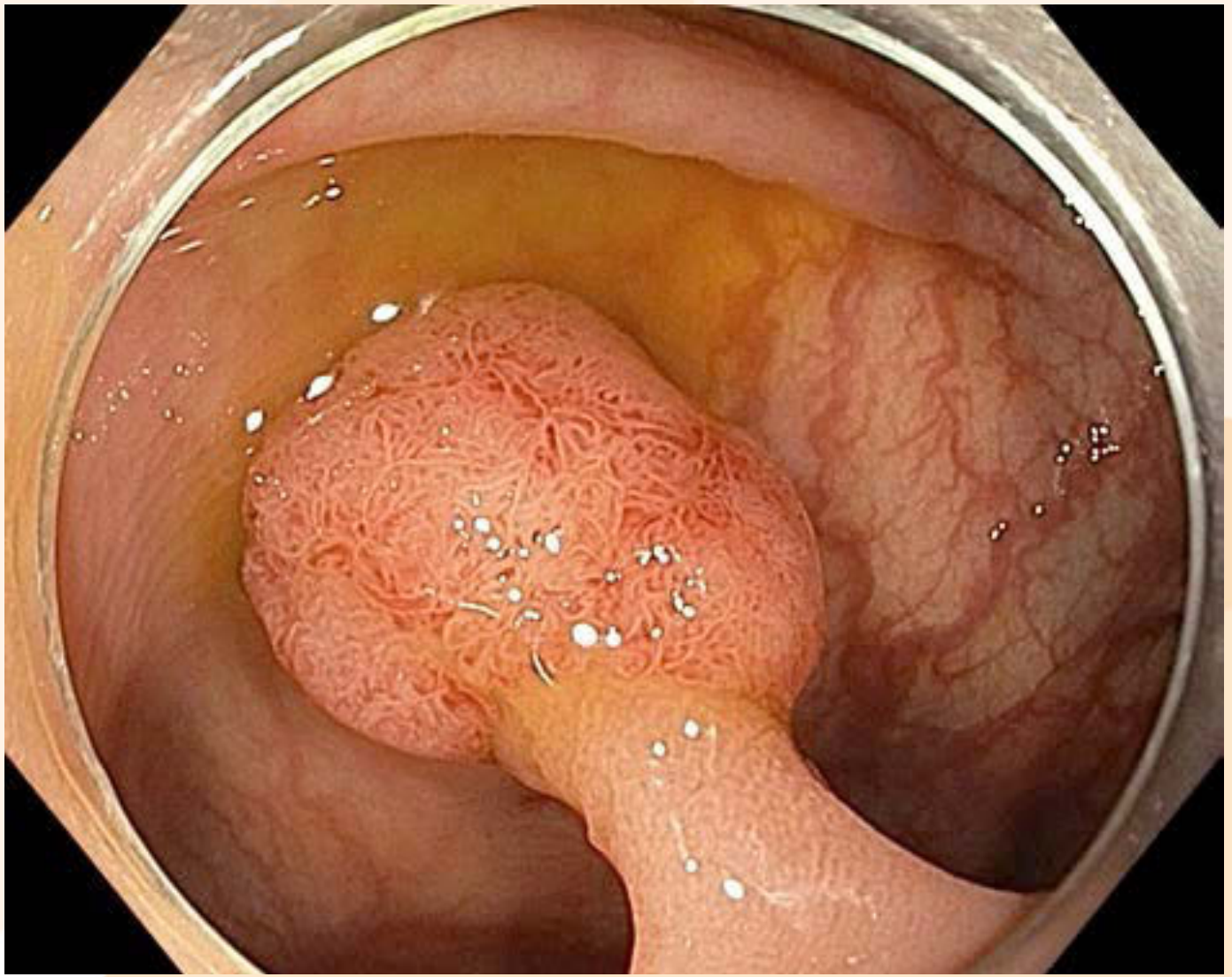

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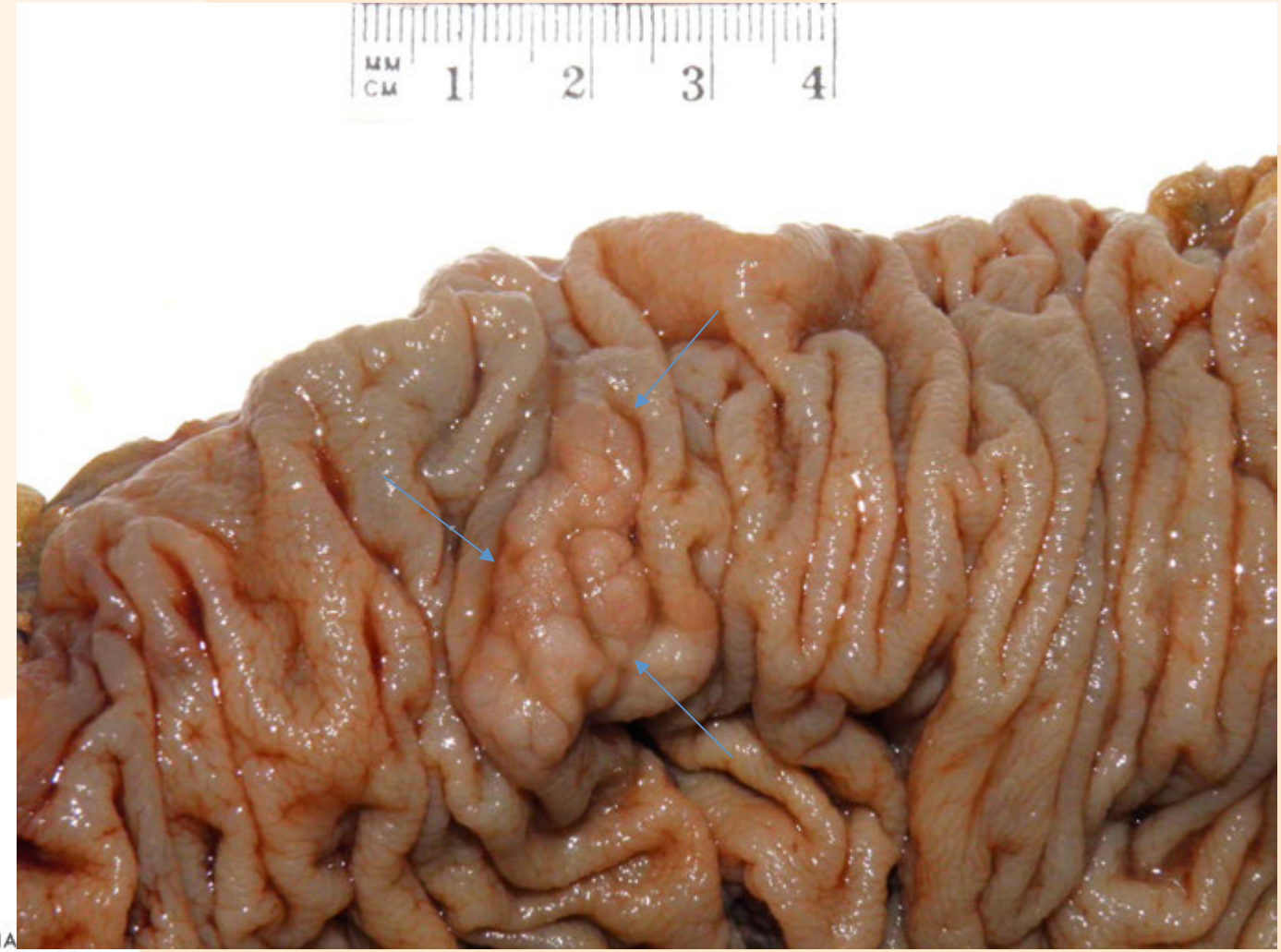
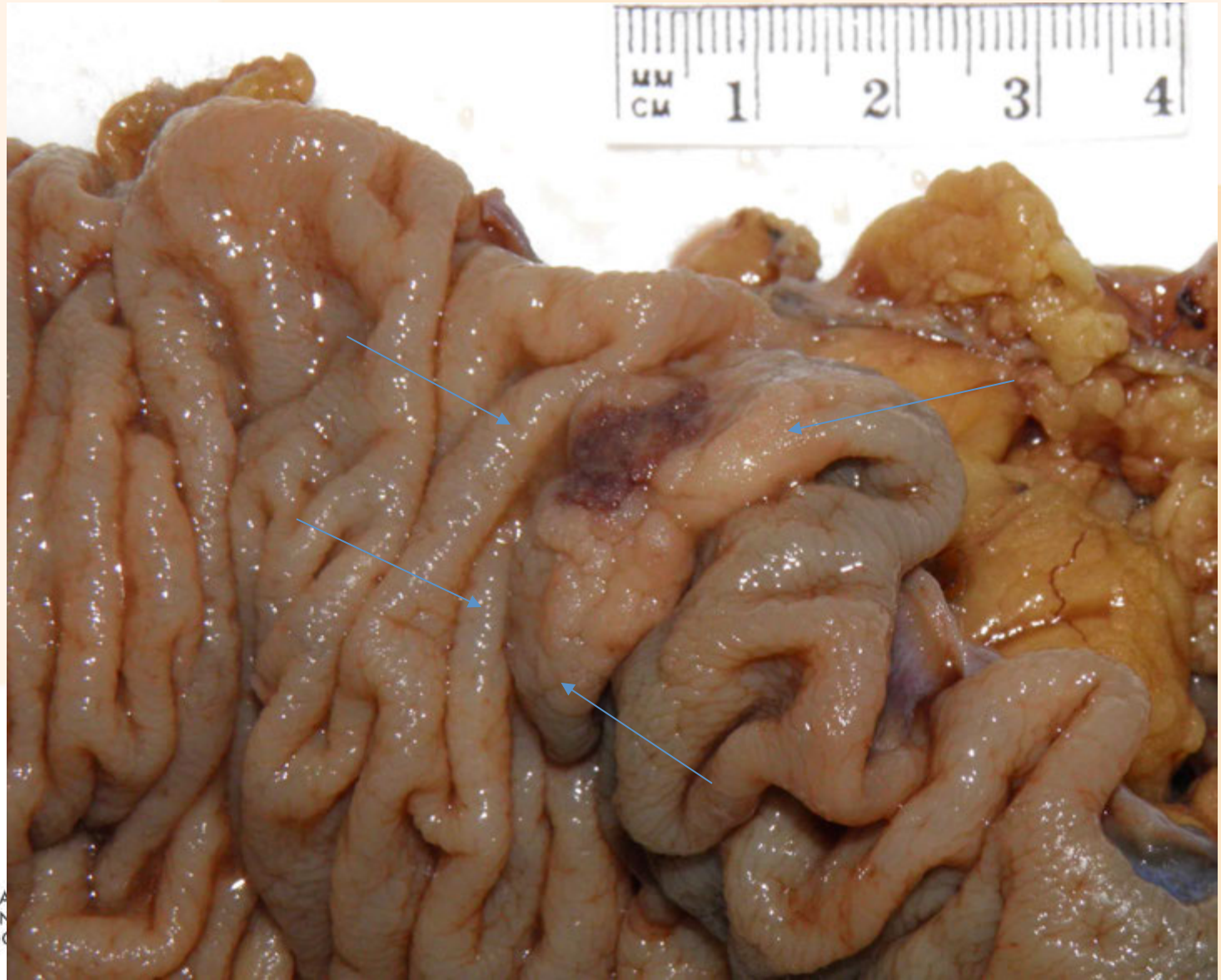
Sessile Serrated Adenoma



Sessile Serrated Adenoma with Dysplasia and Early Carcinoma



Diagnostic criteria

- The diagnostic criteria are variable
- Older criteria were fairly restrictive
- 2010 WHO relaxed the criteria substantially, requiring only **2-3 SSA type crypts**
- More recently Rex et al, have proposed even **one SSA-type crypt** as sufficient for the diagnosis

Diagnostic criteria

- We gathered a consecutive series of colorectal polyps received at our practice over a three month period (n=6340) and undertook a central review of all of the cases.
- For all of the MVHPs and SSAs we further divided them according to the number of SSA type crypts per polyp

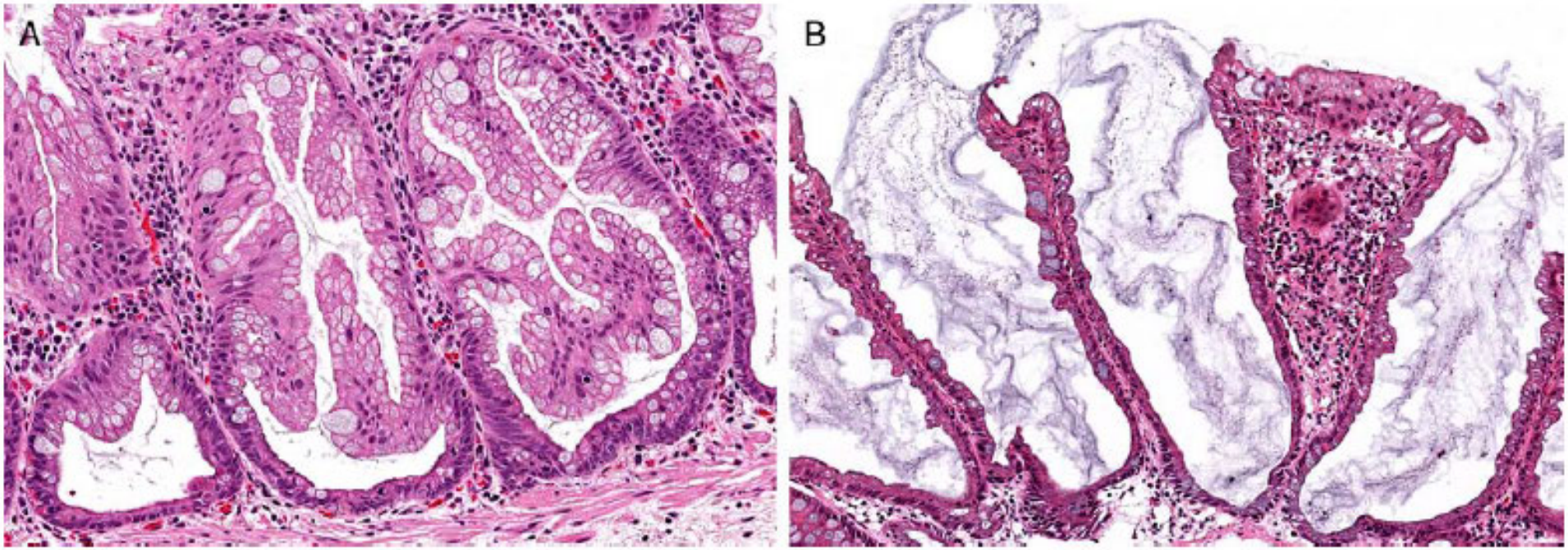


Table 1. Diagnostic subcategories for microvesicular hyperplastic polyps and sessile serrated adenomas.

Subcategory	Definition
MVHP	No SSA-type crypts
pSSA (type 1)	One SSA-type crypt
pSSA (type 2)	Two non-adjacent SSA-type crypts
pSSA (type 3)	Multiple crypts with poorly-developed SSA-type features
SSA (type 1)	Minimal WHO criteria to four SSA-type crypts
SSA (type 2)	Five to nine SSA-type crypts
SSA (type 3)	Ten or more SSA-type crypts

MVHP – microvesicular hyperplastic polyp; pSSA – provisional SSA; SSA – sessile serrated adenoma

Results

- The location and gender distribution of the serrated polyps was most logical using the expert panel criteria to separate MVHP from SSA

Results – location divided by number of SSA type crypts

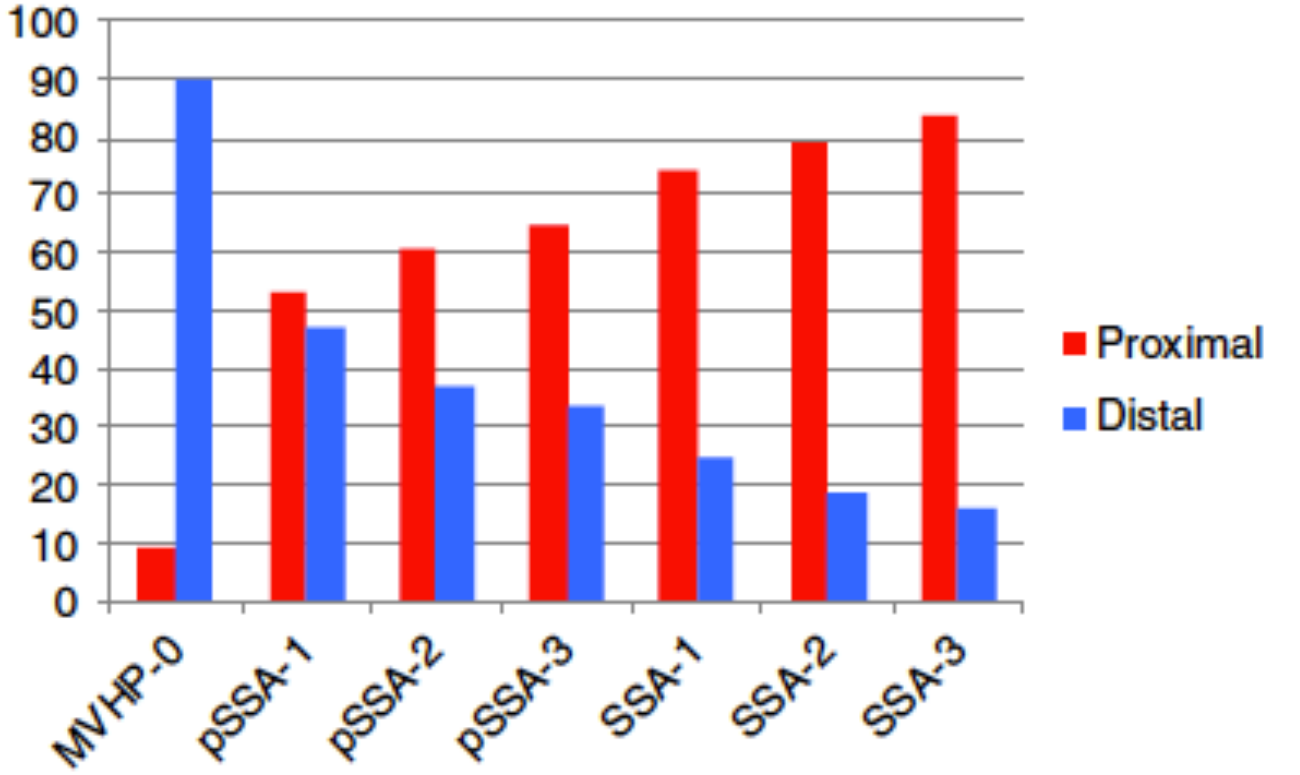


FIGURE 3. Location of subcategories of MVHP, pSSAs, and SSAs by percentage on a per polyp basis.

Results – gender divided by number of SSA type crypts

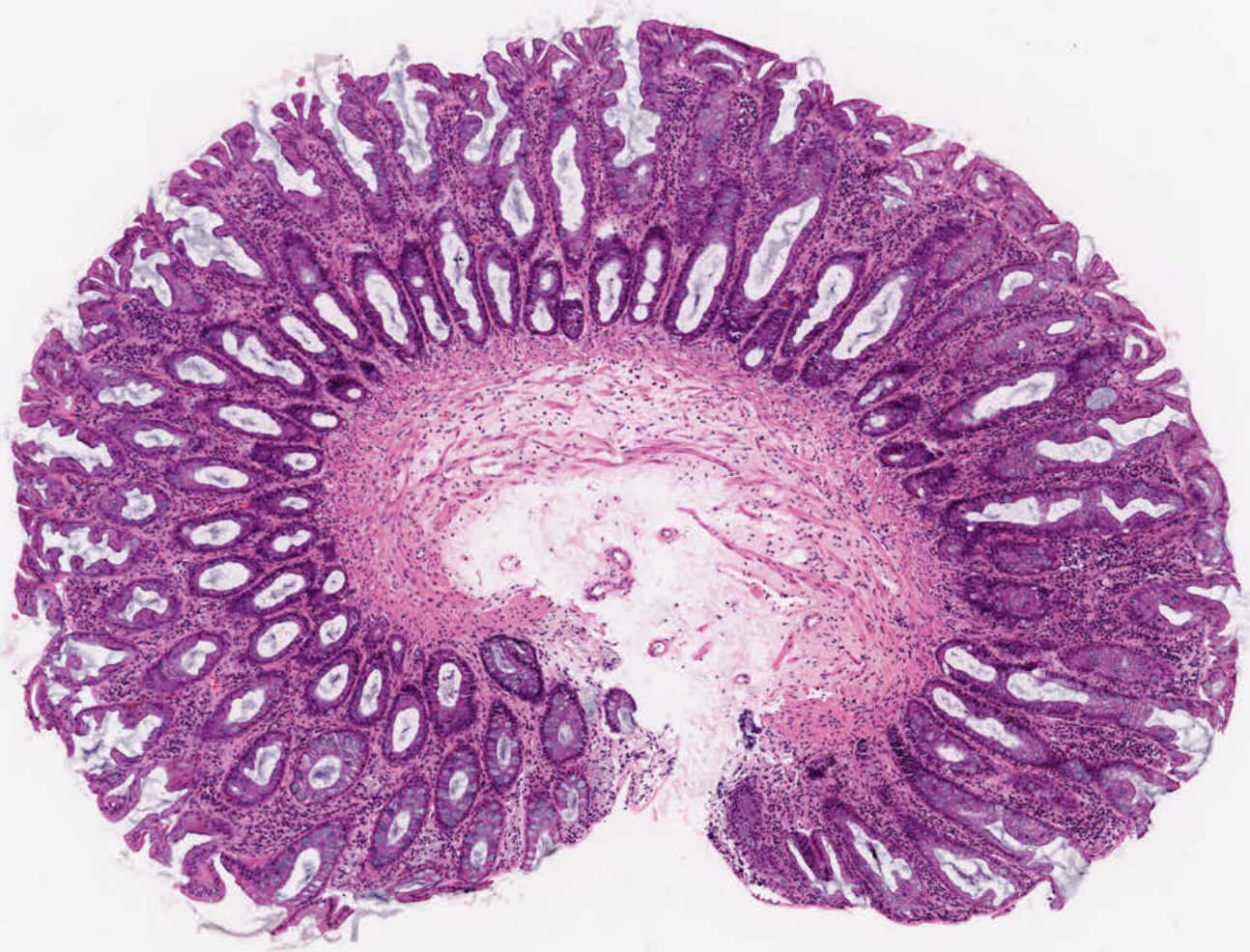
Table 5. Gender of serrated polyps sub-categorised by sessile serrated adenoma-type crypts.

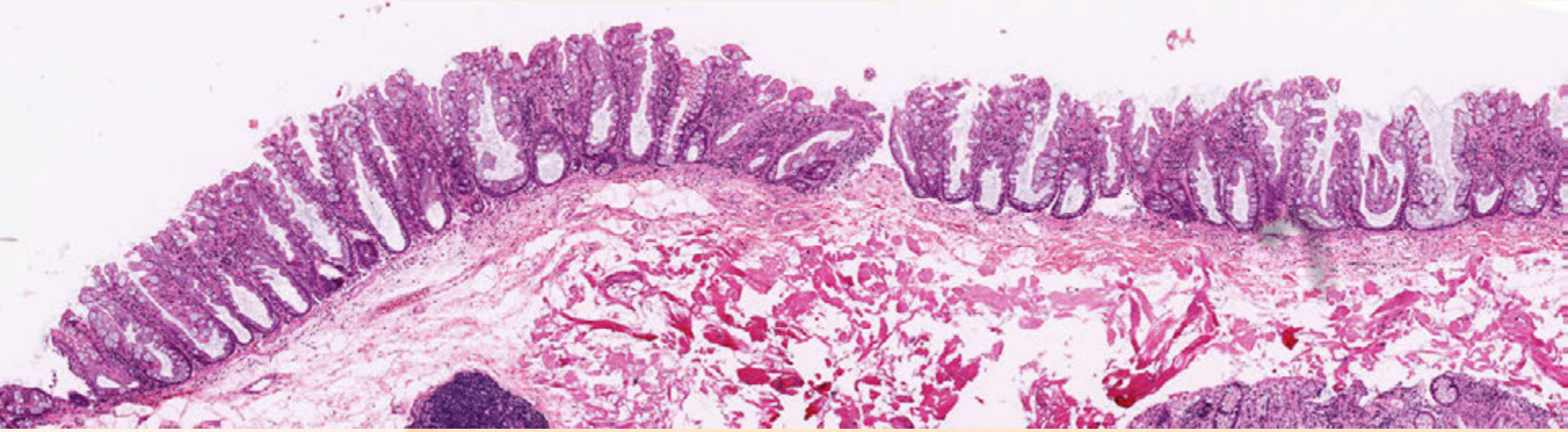
	F	M	Chi-squared test
Has MVHP	443 (46%)	516 (54%)	0.209
Has pSSA (type 1-3)	87 (56%)	68 (44%)	0.037
Has SSA (type 1-3)	321 (55%)	258 (45%)	<0.001
Has SSA (type 1-3)/pSSA (type 1-3)	399 (56%)	315 (44%)	<0.001

MVHP – microvesicular hyperplastic polyp; pSSA – provisional SSA; SSA – sessile serrated adenoma

MVHP versus SSA using the single crypt criteria

Feature	MVHP	SSA
Location	Distal>>Proximal	Proximal>Distal
Gender	M=F	F>M
Size	Mostly <5mm	Variable
Malignant potential	Effectively none	Definite but requires more investigation
Surveillance interval (average risk patients)	Nil	5 years (3 years if >10mm)





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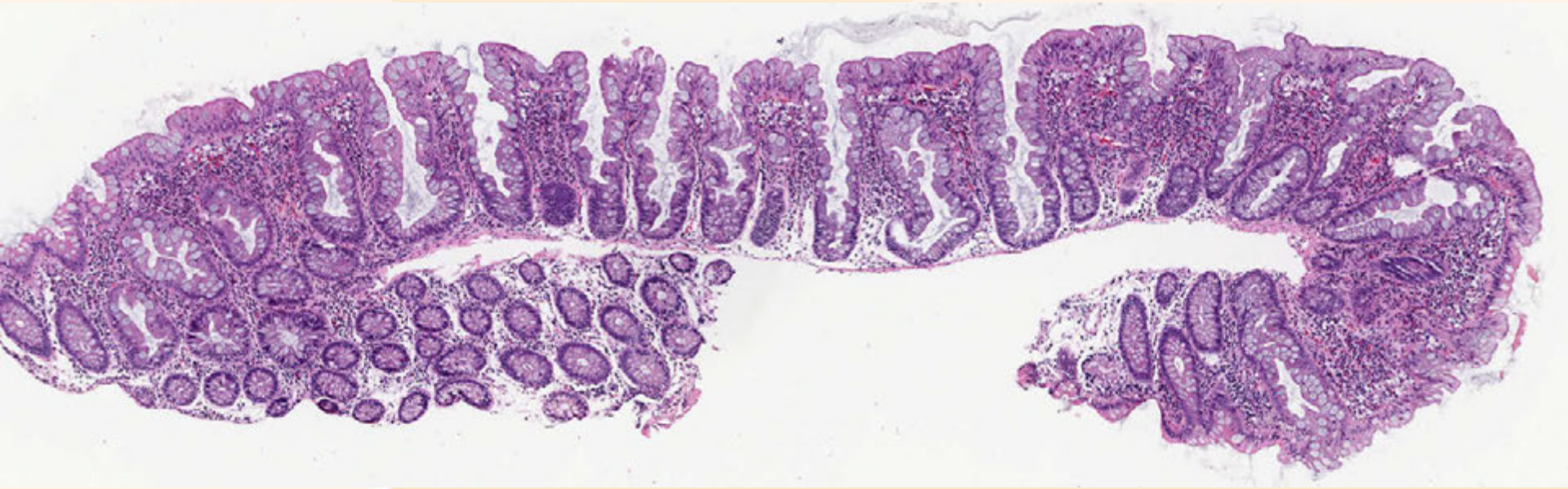

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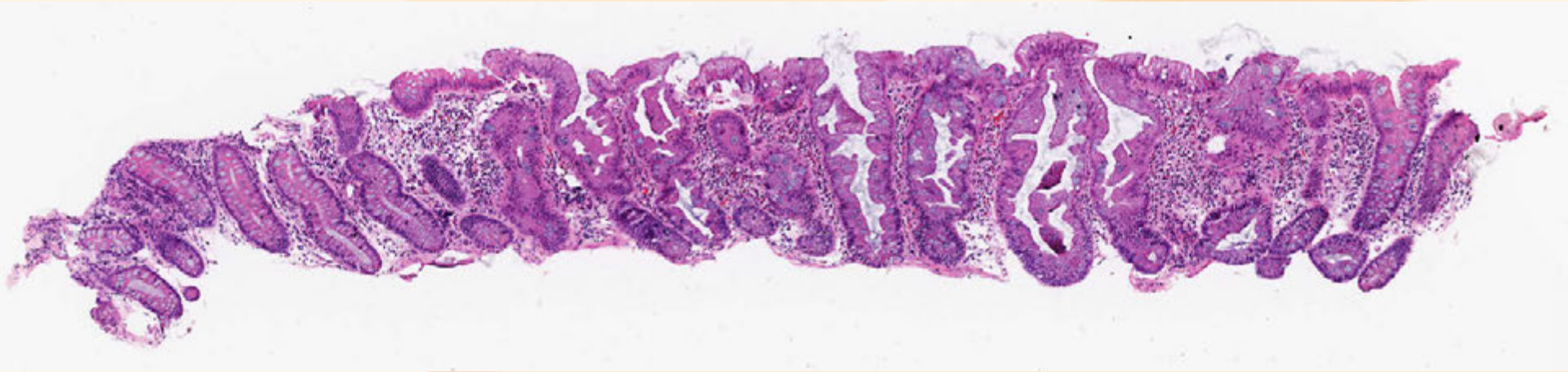
PATHOLOGY SOCIETY



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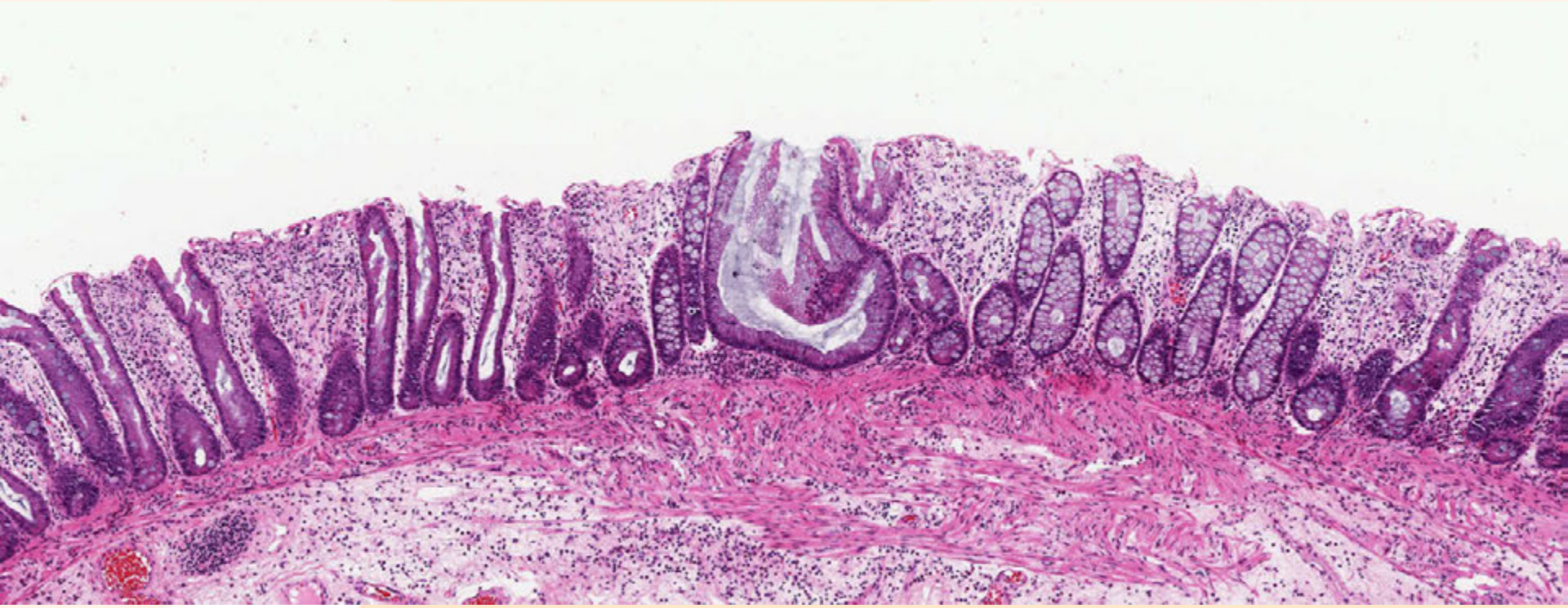

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Results - frequency

- We found that SSAs are common
- Using the WHO criteria 12.1% of all colorectal polyps are SSAs
- Using the criteria of the expert panel 14.7% of colorectal polyps are SSAs

SSA prevalence

- We are conducting a study of SSA prevalence based on data from 707 consecutive colonscopies by an experienced gastroenterologist in a public hospital outpatient setting
- Central pathological review of all polyps
- SSAs diagnosed using the single crypt criteria
- **SSA prevalence is 20.1%**

Factors associated with increased detection of sessile serrated adenomas and conventional adenomas

Factor	Sessile serrated adenoma	Conventional adenoma
Older age	NS	**P=<0.0001**
Male gender	NS	**P=0.0002**
Withdrawal time	**P<0.0001**	**P<0.0001**
Bowel preparation	NS	NS
NBCSP positive result	NS	**P=0.0402**

P-values calculated by comparison with patients without polyps

Risk of malignancy in an SSA

- This is a very difficult question to answer, especially because the goalposts for a diagnosis of an SSA keep changing

- Consider that about 75% of colorectal carcinoma arise from conventional polyps and conventional polyps have a prevalence of approximately 50%
- About 25% of colorectal carcinoma arise from serrated polyps and serrated polyps have a prevalence of approximately 20%

- This would suggest the risk of malignancy in a serrated polyp is slightly less than a conventional adenoma

The sessile serrated adenoma with dysplasia

Much less is known about the SSAD

- Mostly because they are rare (0.4% of colorectal polyps)
- Much comes from small series and indirectly from what we know about ordinary SSAs and *BRAF* mutated colorectal carcinomas
- We have recently undertaken a study of advanced SSAs (SSAs with dysplasia and or carcinoma) to attempt to address some of the knowledge gaps

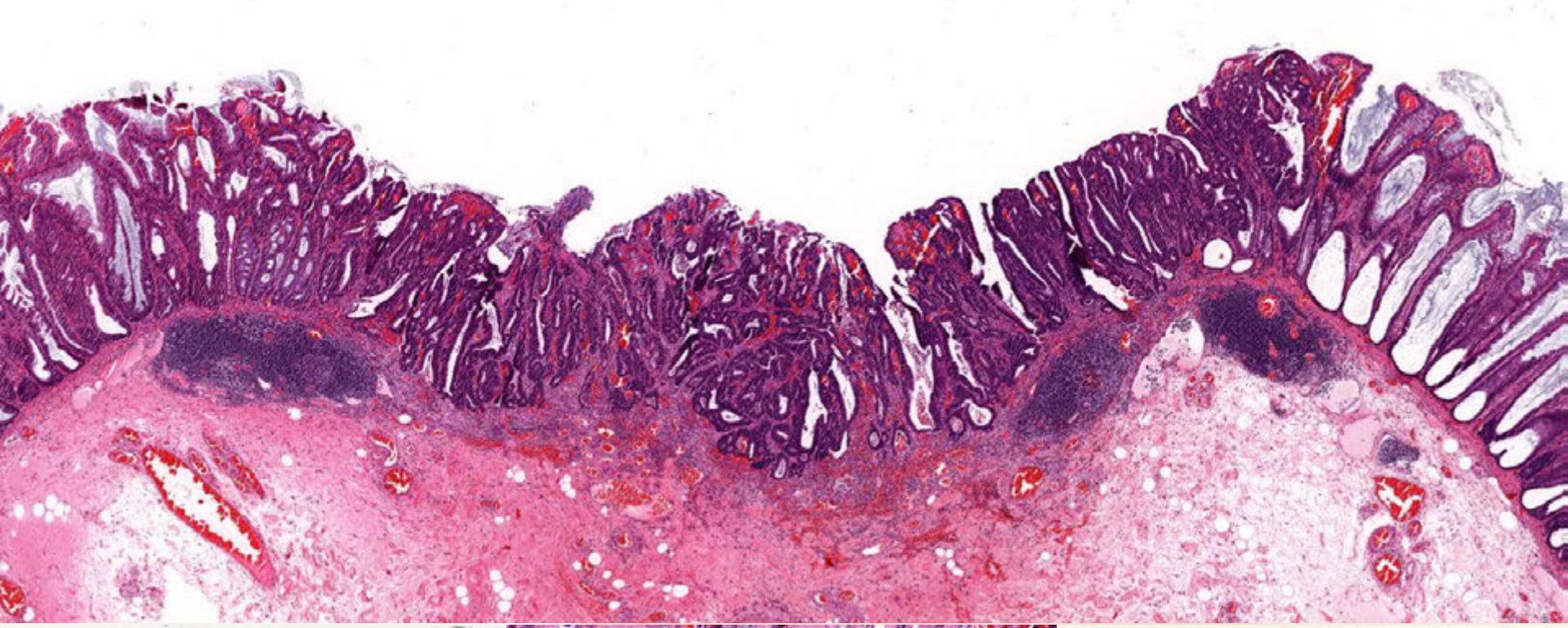
A series of 137 advanced SSAs

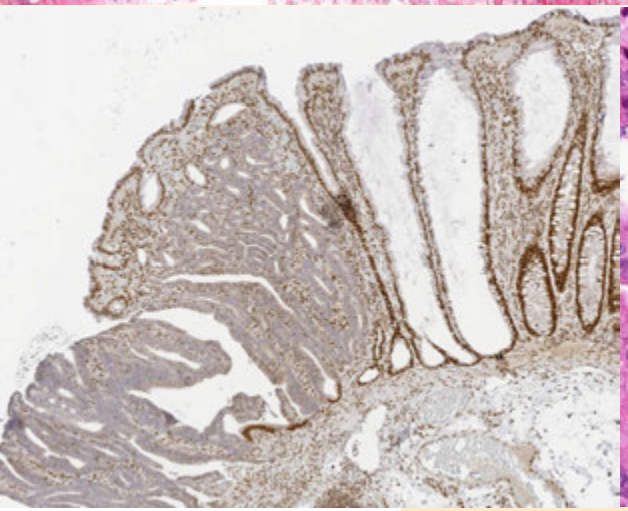
- We performed a detailed clinicopathological and molecular analysis of all cases
- Rigorous inclusion criteria:

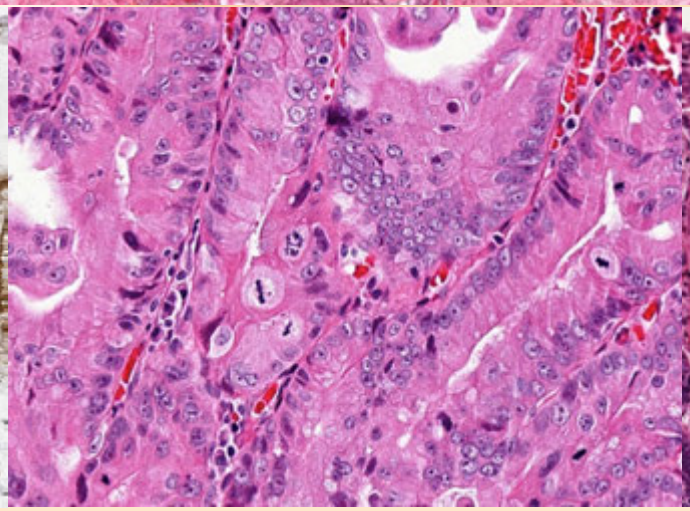
1. Required an abrupt transition from SSA to dysplasia in the one tissue fragment

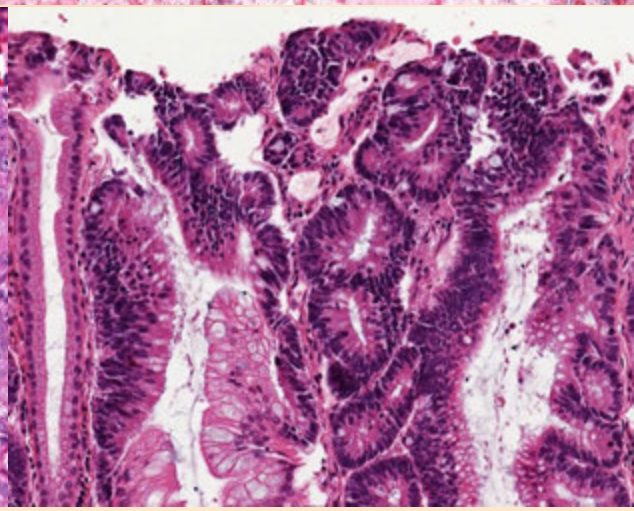
2. Excluded TSA arising in SSA

3. Sufficient tissue for molecular analysis (*BRAF*, *KRAS*, CIMP, IHC)









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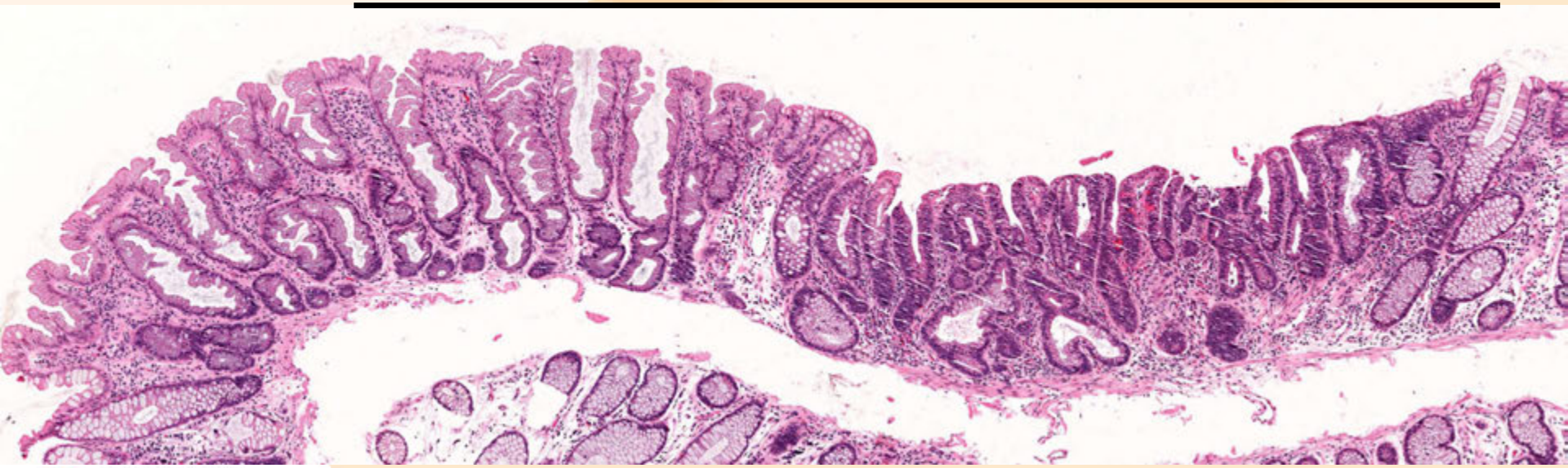
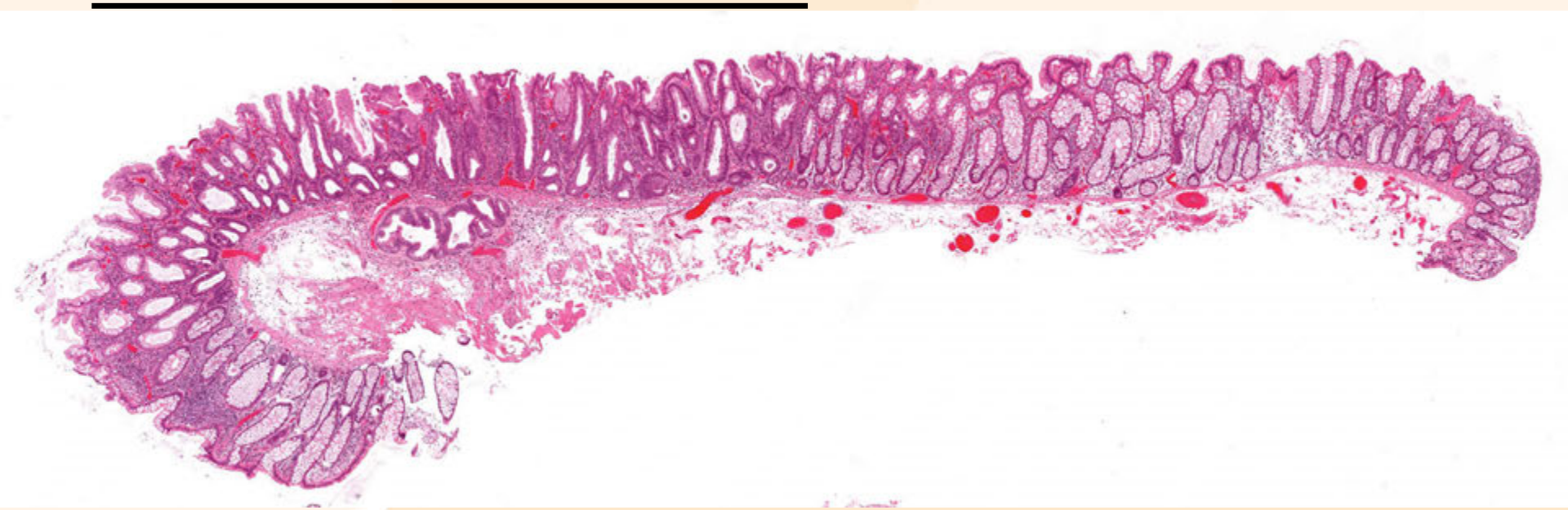
Clinicopathological features

Advanced SSAs are predominantly small and flat polyps

- The median size of the cohort was 9mm
- 86% were flat



2.3mm SSADC



Bar = 2mm

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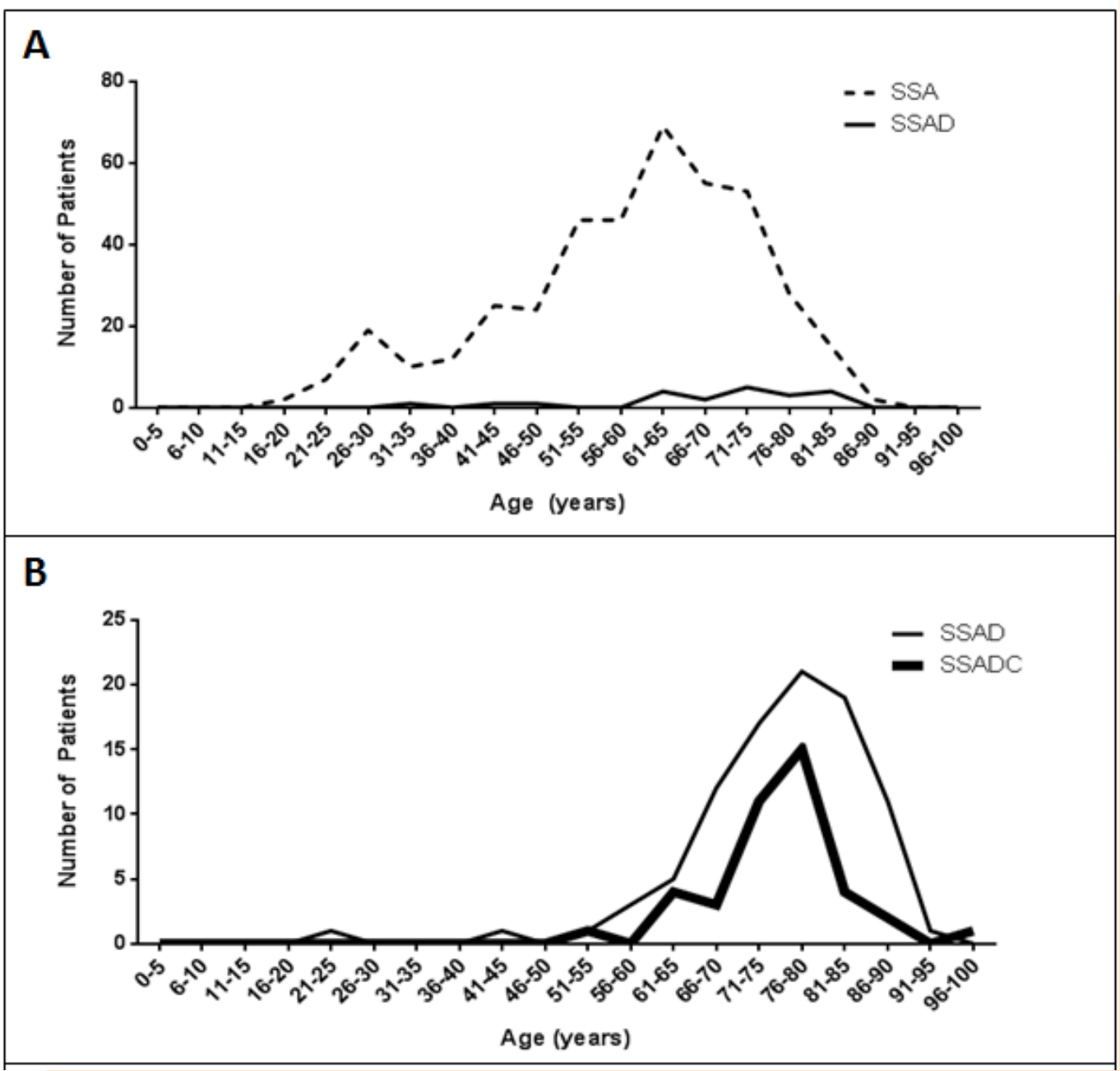
Clinicopathological features

The transition from SSA to SSAD appears to be slow
The transition from SSAD to carcinoma appears to be rapid

- Mean age of patients with ordinary SSAs – 58.6
- Mean age of patients with SSADs – 75.3
- Mean age of patients with SSAs with component of carcinoma – 75.1
- SSA versus SSAD (p-value <0.0001)
- SSAD versus SSA with carcinoma (p-value 0.8820)



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Molecular features

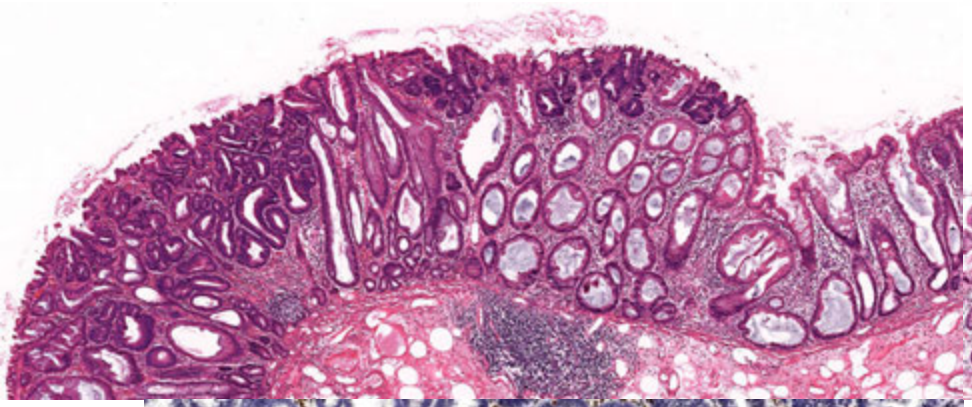
75% of the cohort was mismatch repair deficient

Mismatch repair status divides SSAD/Cs into distinct clinical and molecular entities

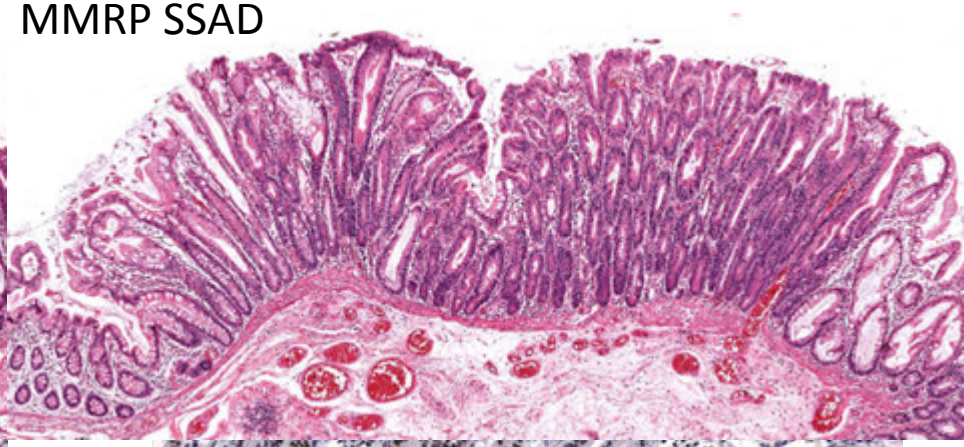
Table 4. Molecular features of the study lesions

Molecular feature	All (n=137)	**MMRD (n=102)**	**MMRP (n=35)**	P-value (MMRD versus MMRP)
BRAF mutation	93%	93%	91%	0.7154
KRAS mutation	1%	1%	0%	1.000
CIMP-H	93%	98%	80%	**0.0010**
P16 loss	43%	43%	43%	1.000
Positive B-catenin	55%	56%	54%	1.000
Positive p53	14%	7%	34%	**0.0002**

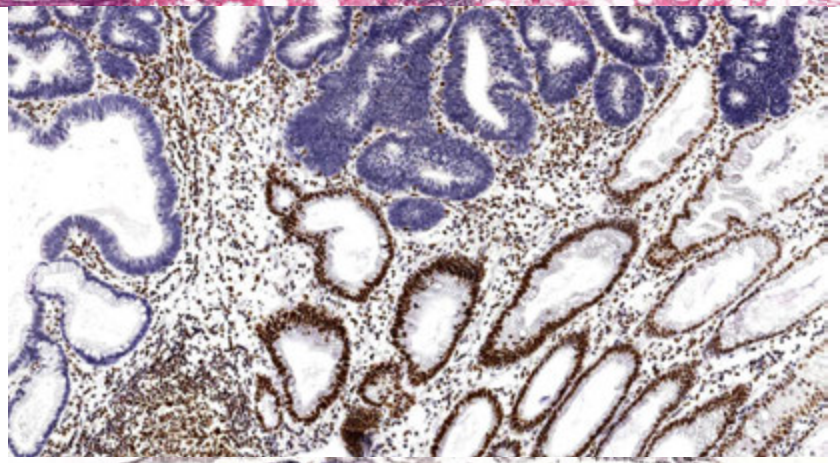
MMRD SSAD



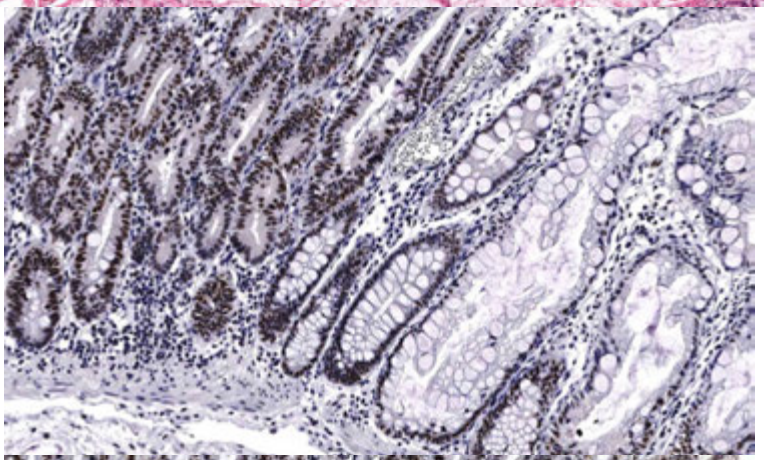
MMRP SSAD



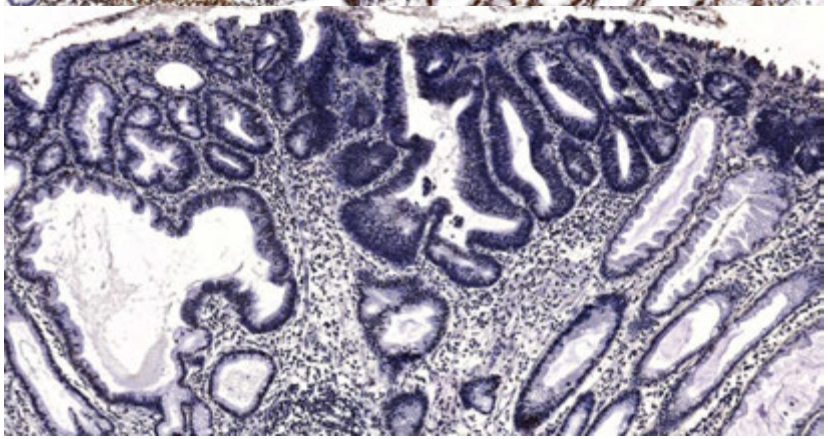
MLH1



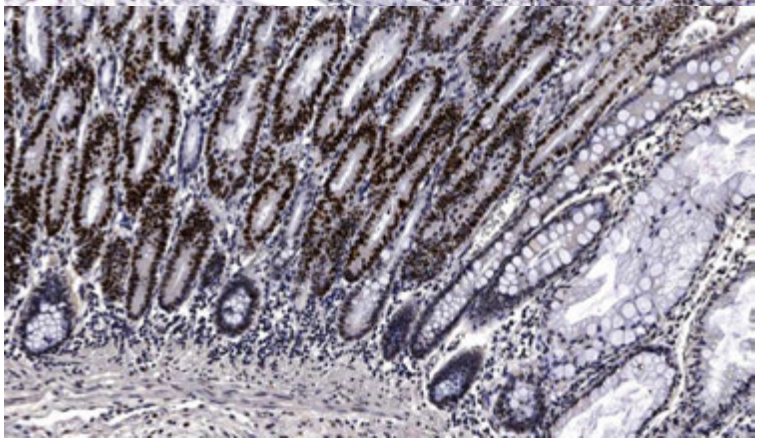
MLH1



p53



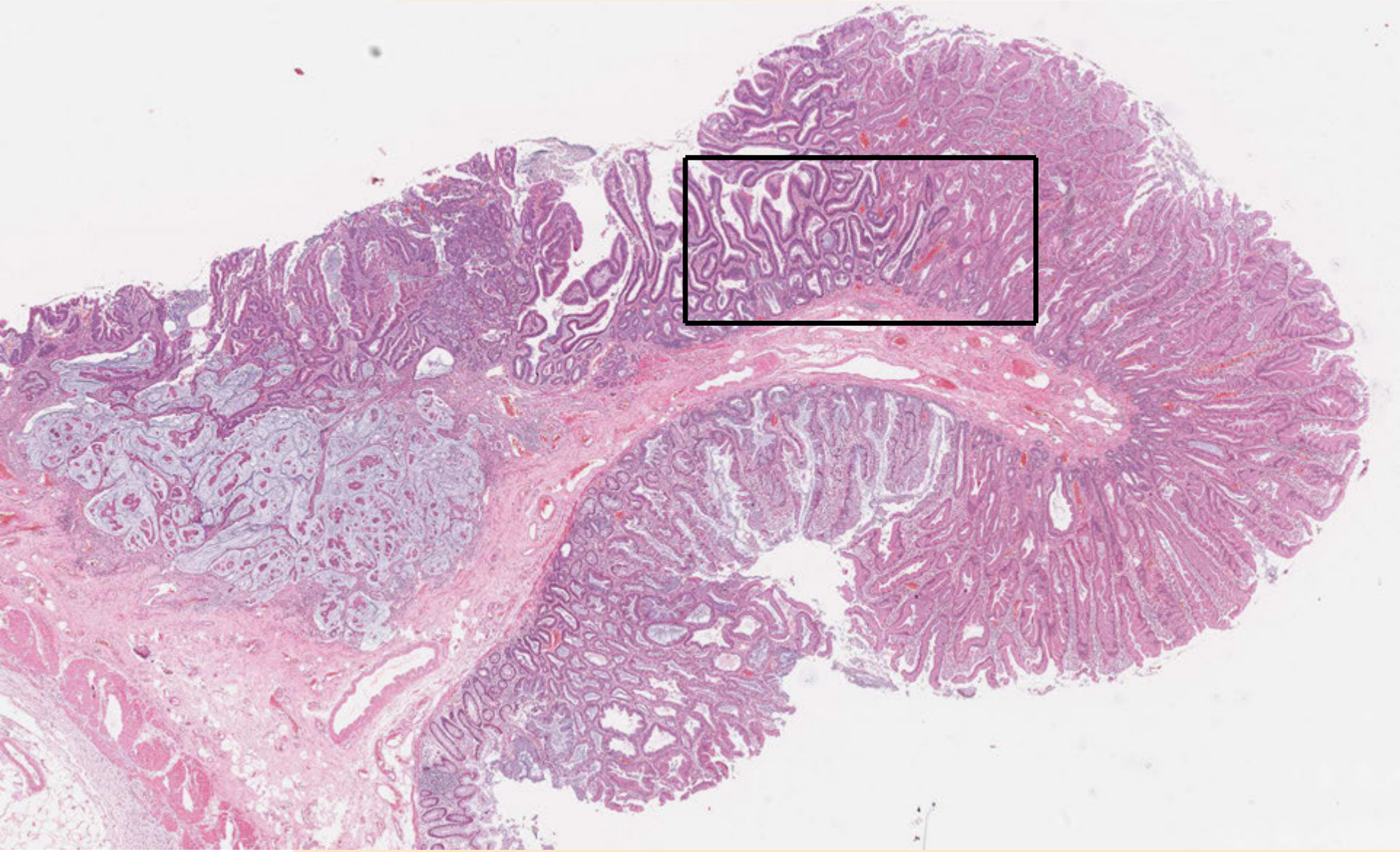
p53

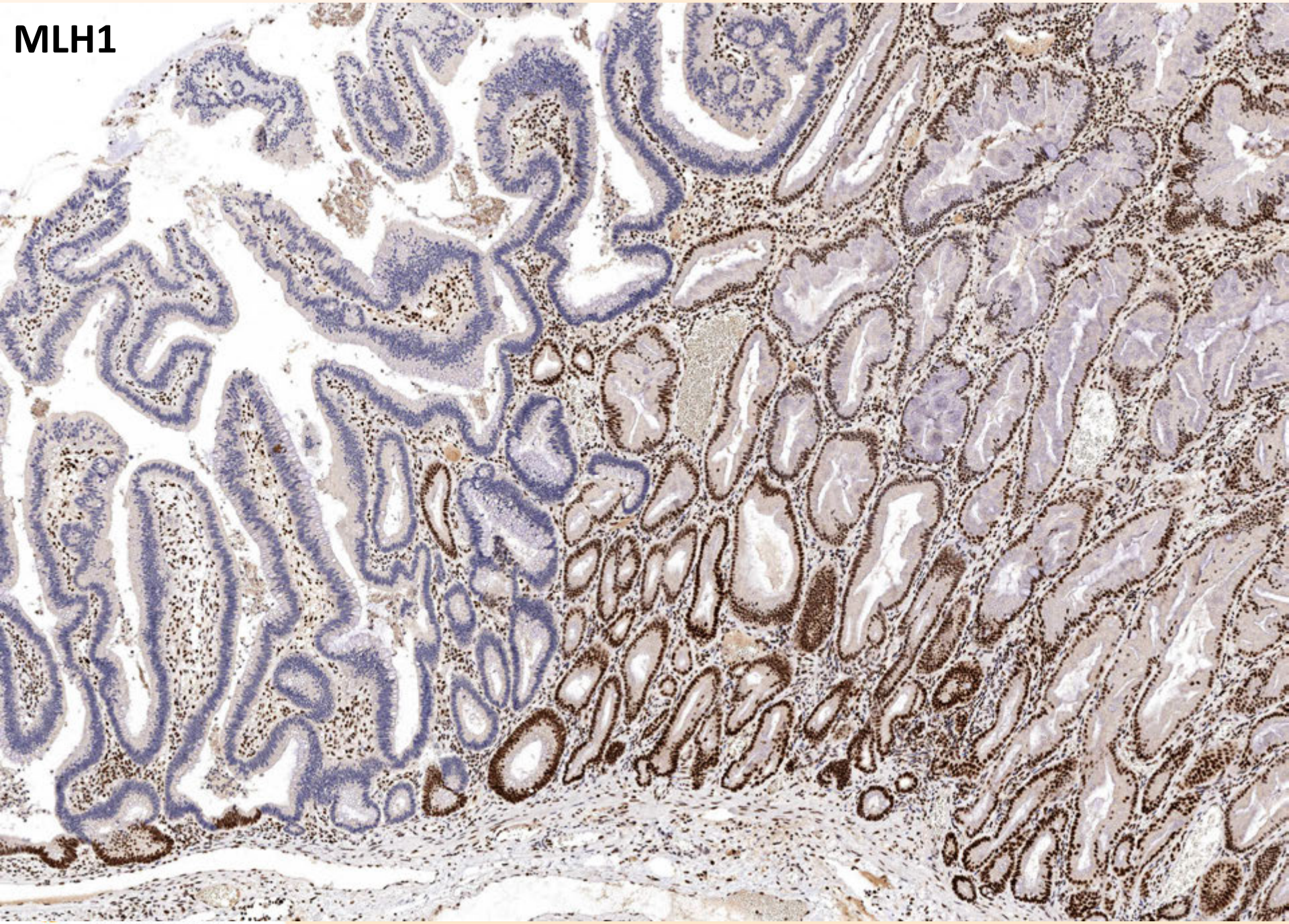


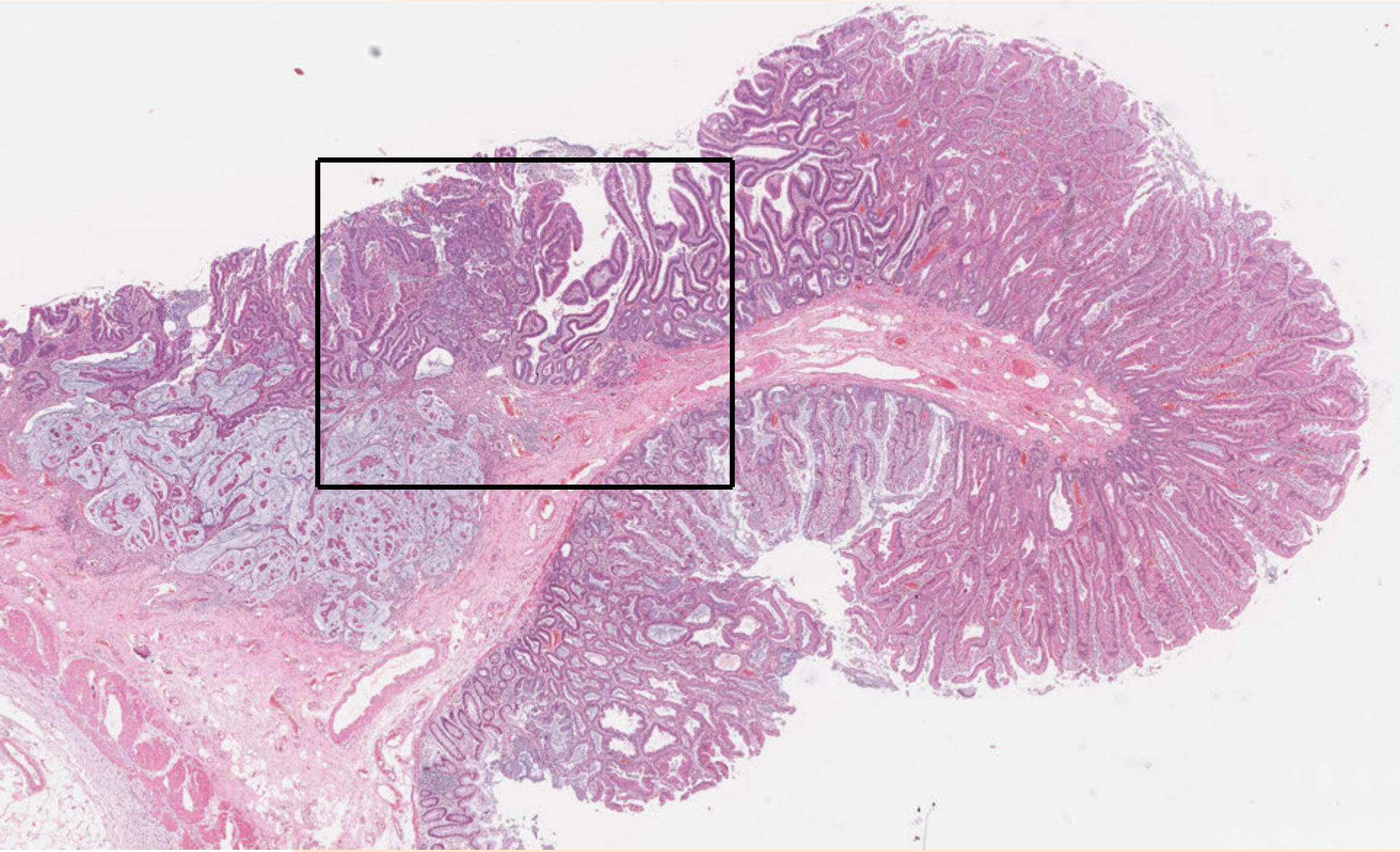
Immunohistochemical features of the lesions divided into ordinary and advanced components.

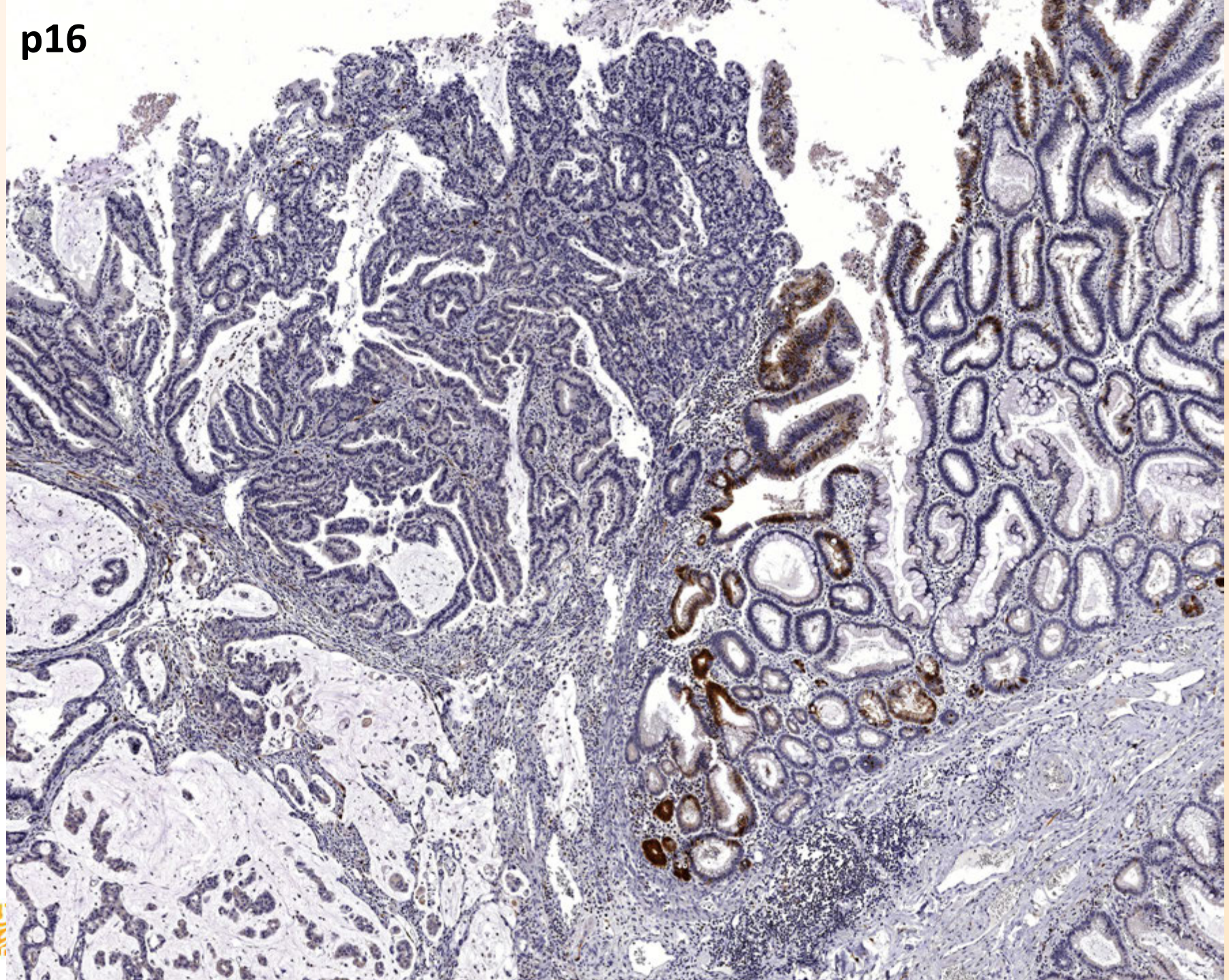
Stain	Ordinary SSA component (n=137)	Advanced components (n=137)	P-value
MLH1 loss	0 (0%)	102 (75%)	**<0.0001**
P16 loss	13 (9%)	59 (43%)	**<0.0001**
β-catenin	15 (11%)	76 (55%)	**<0.0001**
p53	0 (0%)	19 (14%)	**<0.0001**
MGMT	11 (8%)	38 (28%)	**<0.0001**

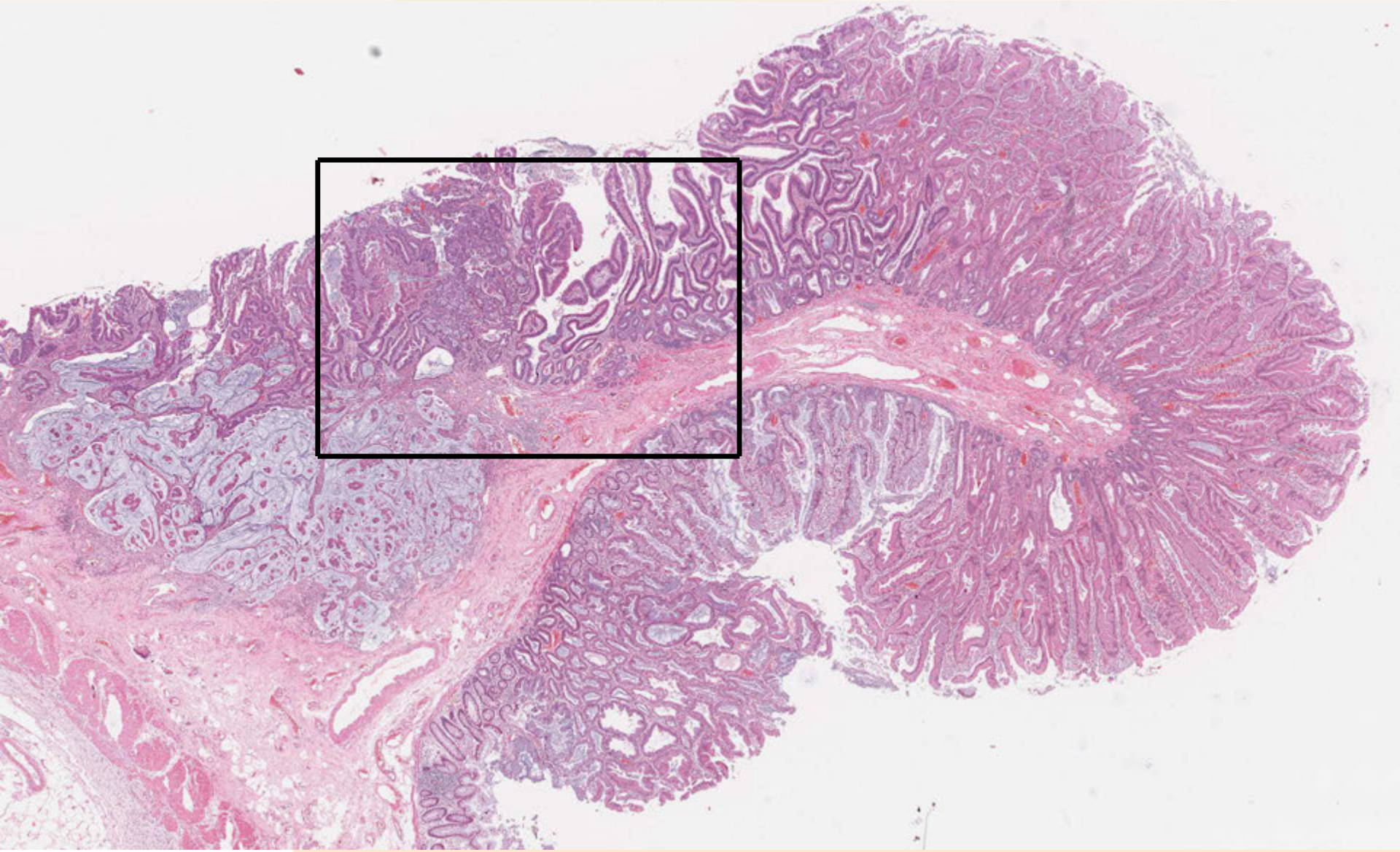
MMRD SSADCa



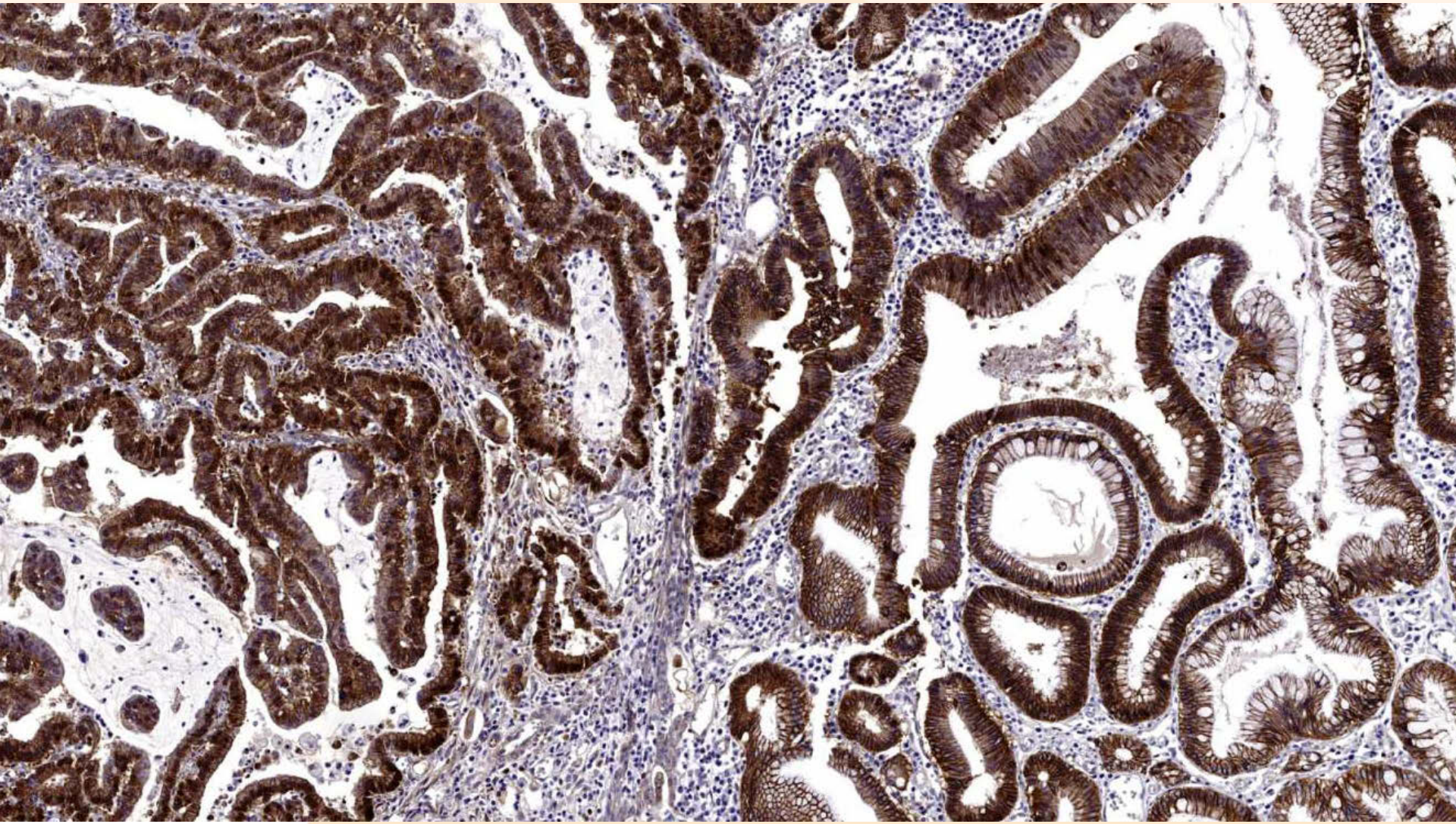

MLH1




p16



β-catenin



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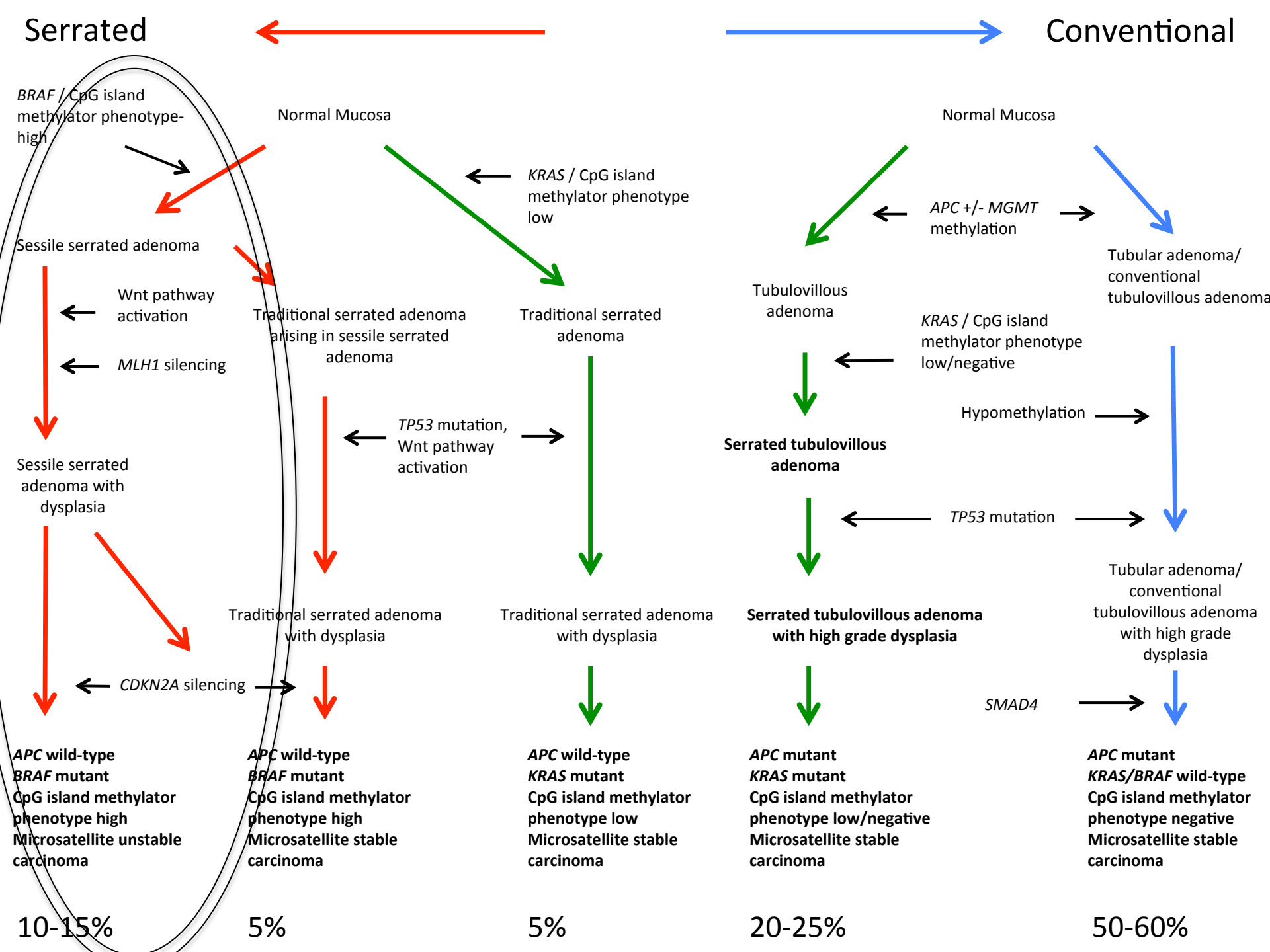
Key findings and clinical implications

1) Most of the study polyps were proximal, small (<10mm) and flat making them difficult to detect at colonoscopy

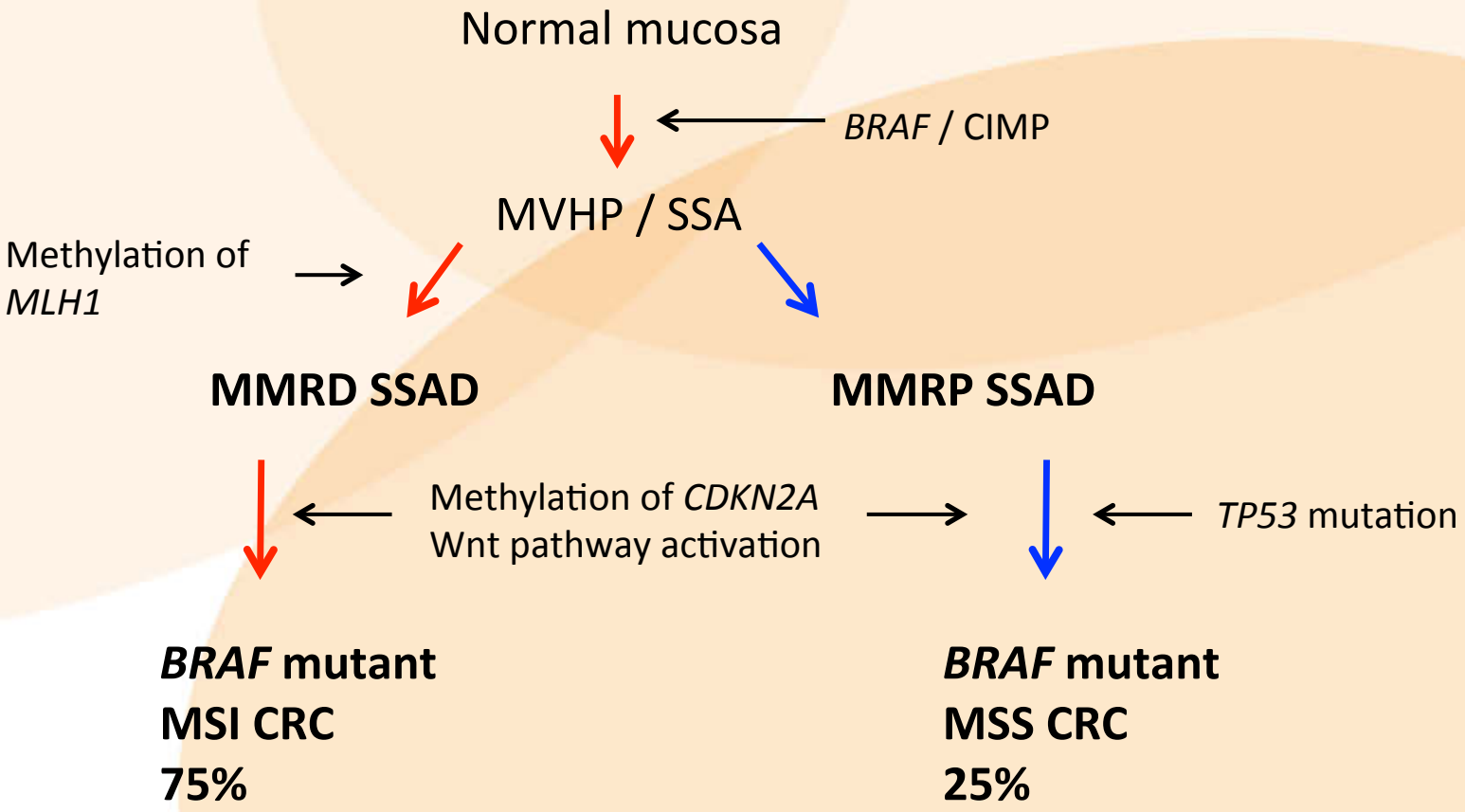
2) Progress to cancer by a combination of *MLH1* and *p16* silencing, WNT pathway activation and *TP53* mutation (and almost certainly other methylation / mutation events)

3) There appears to be a rapid progression from SSAD to cancer meaning if they are missed, cancer can develop in the surveillance interval

4) The mismatch repair status separates these polyps into distinct clinical and molecular subtypes. This is important because the MMRP subtype is the precursor of the most aggressive molecular subtype of colorectal carcinoma


Serrated
Conventional
BRAF / CpG island methylator phenotype-high
Normal Mucosa
KRAS / CpG island methylator phenotype low
Normal Mucosa
APC +/- MGMT methylation
Tubular adenoma/ conventional tubulovillous adenoma
Sessile serrated adenoma
Wnt pathway activation
MLH1 silencing
Traditional serrated adenoma arising in sessile serrated adenoma
Traditional serrated adenoma
Tubulovillous adenoma
KRAS / CpG island methylator phenotype low/negative
TP53 mutation, Wnt pathway activation
Hypomethylation
Sessile serrated adenoma with dysplasia
Serrated tubulovillous adenoma
TP53 mutation
Tubular adenoma/ conventional tubulovillous adenoma with high grade dysplasia
CDKN2A silencing
Traditional serrated adenoma with dysplasia
Traditional serrated adenoma with dysplasia
Serrated tubulovillous adenoma with high grade dysplasia
SMAD4
APC wild-type BRAF mutant CpG island methylator phenotype high Microsatellite unstable carcinoma
APC wild-type BRAF mutant CpG island methylator phenotype high Microsatellite stable carcinoma
APC wild-type KRAS mutant CpG island methylator phenotype low Microsatellite stable carcinoma
APC mutant KRAS mutant CpG island methylator phenotype low/negative Microsatellite stable carcinoma
APC mutant KRAS/BRAF wild-type CpG island methylator phenotype negative Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

Proposed pathways to carcinoma for sessile serrated adenomas



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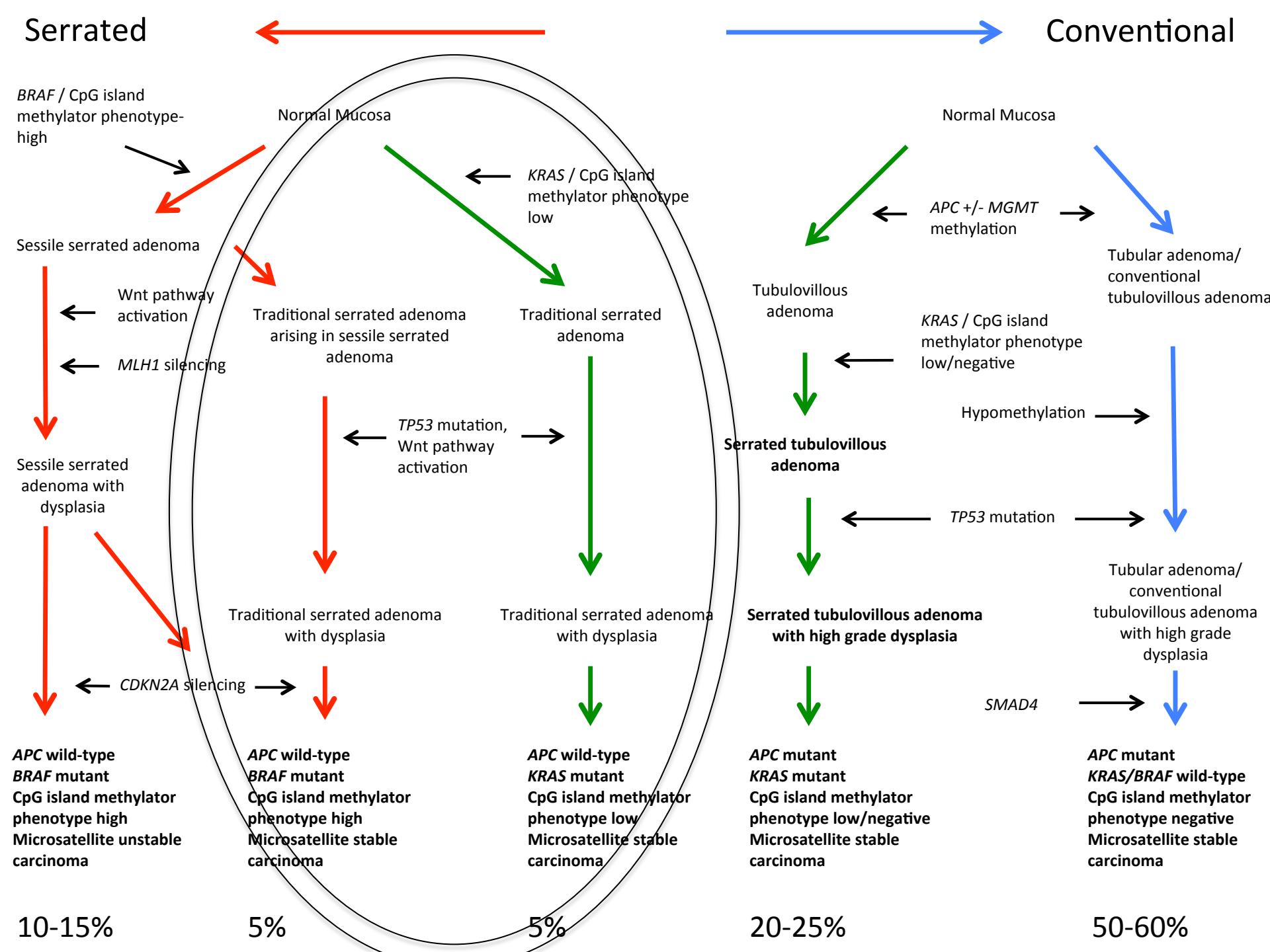
The Traditional Serrated Adenoma


Serrated
Conventional
BRAF / CpG island methylator phenotype-high
Normal Mucosa
KRAS / CpG island methylator phenotype low
Normal Mucosa
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Sessile serrated adenoma
Wnt pathway activation
MLH1 silencing
Traditional serrated adenoma arising in sessile serrated adenoma
Traditional serrated adenoma
Tubulovillous adenoma
Tubular adenoma/ conventional tubulovillous adenoma
KRAS / CpG island methylator phenotype low/negative
TP53 mutation, Wnt pathway activation
Hypomethylation
Sessile serrated adenoma with dysplasia
Serrated tubulovillous adenoma
TP53 mutation
Traditional serrated adenoma with dysplasia
Traditional serrated adenoma with dysplasia
Serrated tubulovillous adenoma with high grade dysplasia
Tubular adenoma/ conventional tubulovillous adenoma with high grade dysplasia
CDKN2A silencing
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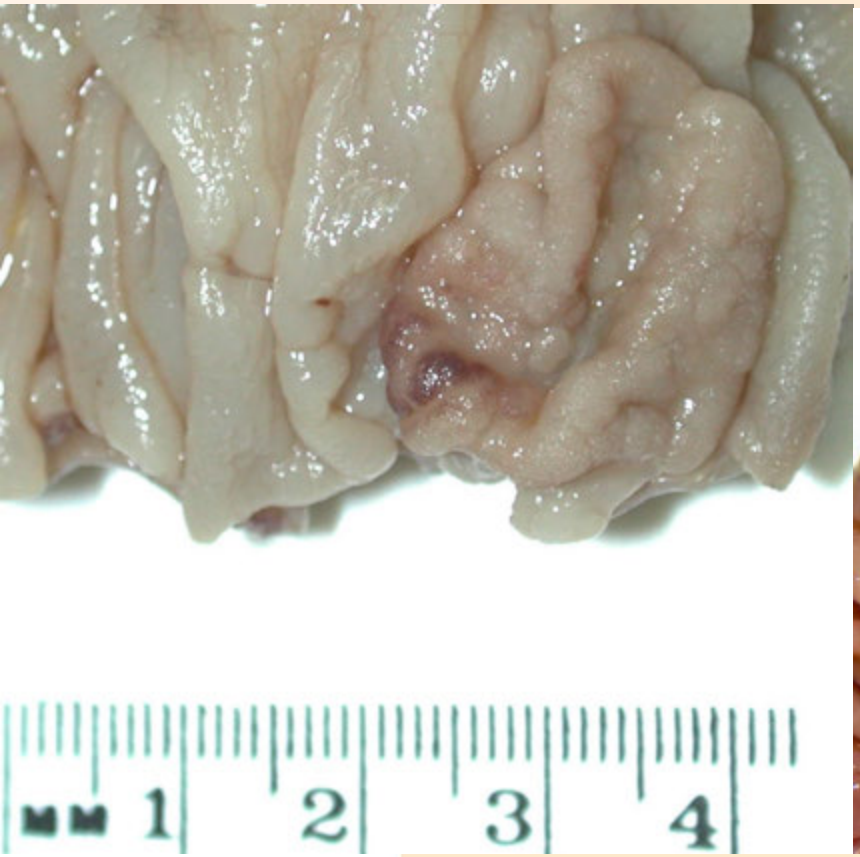
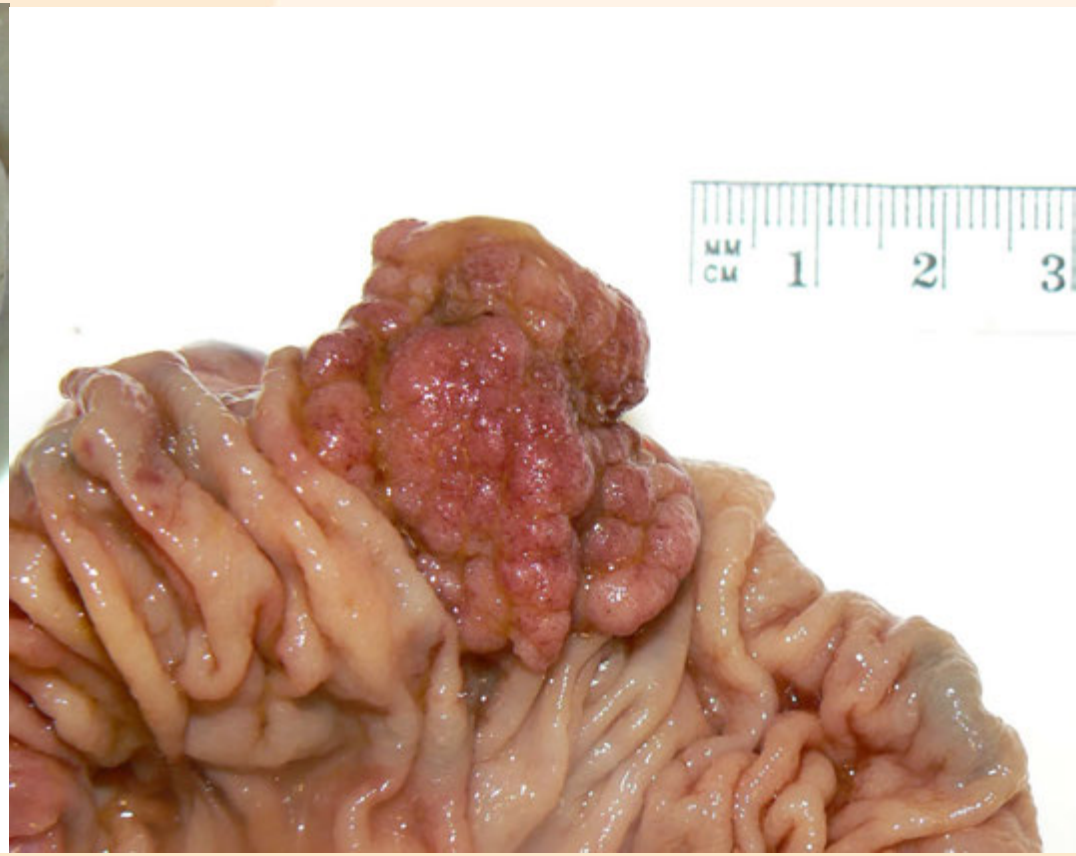
APC wild-type BRAF mutant CpG island methylator phenotype high Microsatellite unstable carcinoma
APC wild-type BRAF mutant CpG island methylator phenotype high Microsatellite stable carcinoma
APC wild-type KRAS mutant CpG island methylator phenotype low Microsatellite stable carcinoma
APC mutant KRAS mutant CpG island methylator phenotype low/negative Microsatellite stable carcinoma
APC mutant KRAS/BRAF wild-type CpG island methylator phenotype negative Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

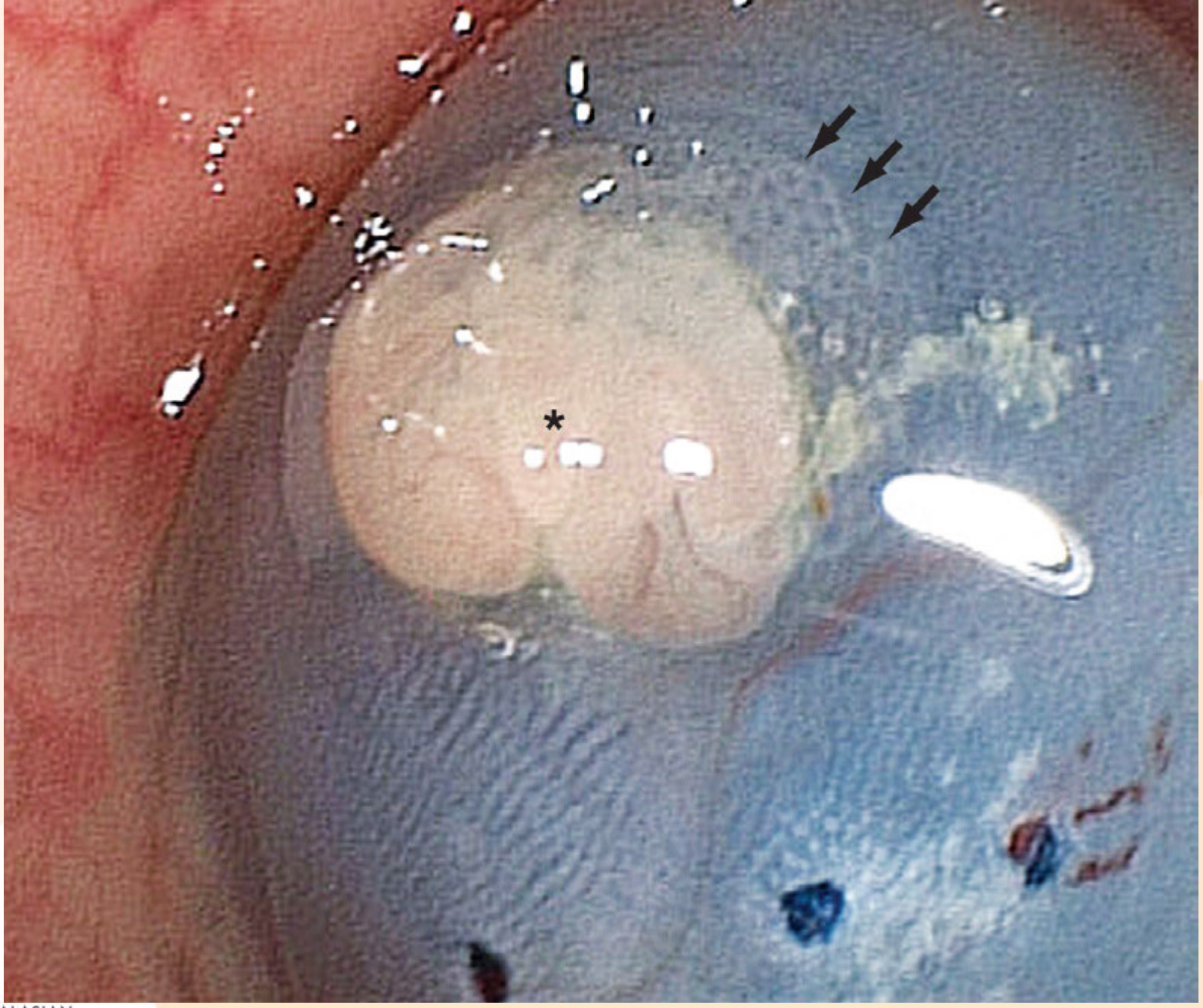
A controversial entity

- For a lesion that is seemingly straightforward to diagnose, the TSA is quite controversial
- The diagnostic criteria are not all that clear
- The molecular features are quite variable
- The malignant risk is not known
- The pathways and types of cancer that arise from them are also not entirely clear

Traditional Serrated Adenoma







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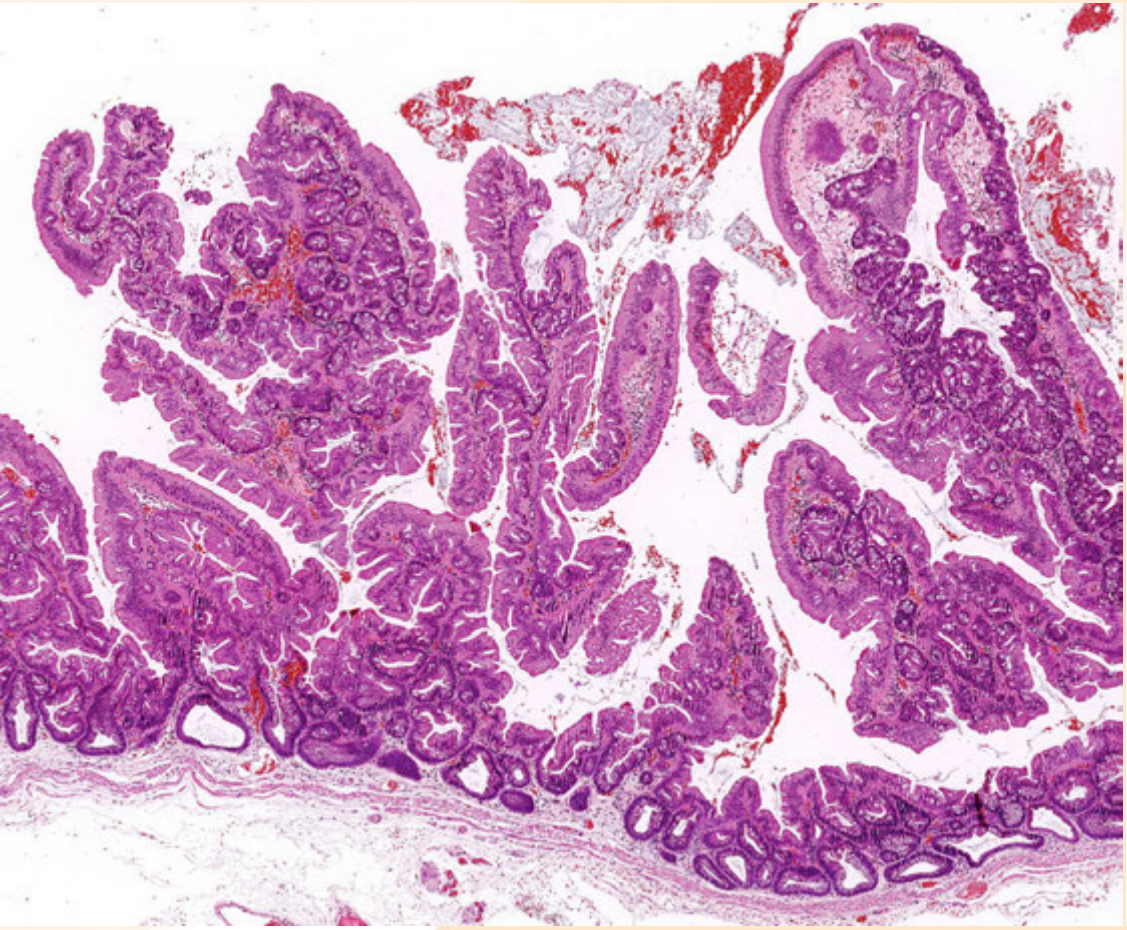
Kim MJ, Lee EJ, et al. Am J Clin Pathol 2013; 140: 898-911

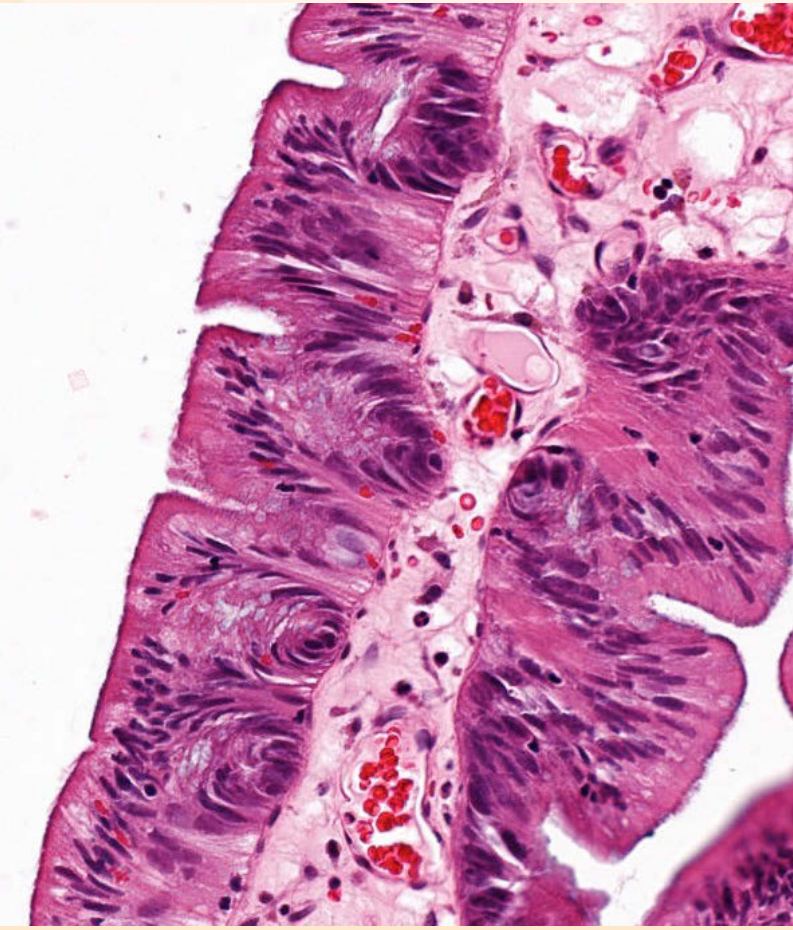
Morphological features

- We like to see at least two of;

1. Eosinophilic cells
2. Ectopic crypt formations
3. Slit-like serrations

- To call overt dysplasia we have similar criteria to an SSA i.e. an abrupt transition from ordinary TSA to overt cytological dysplasia





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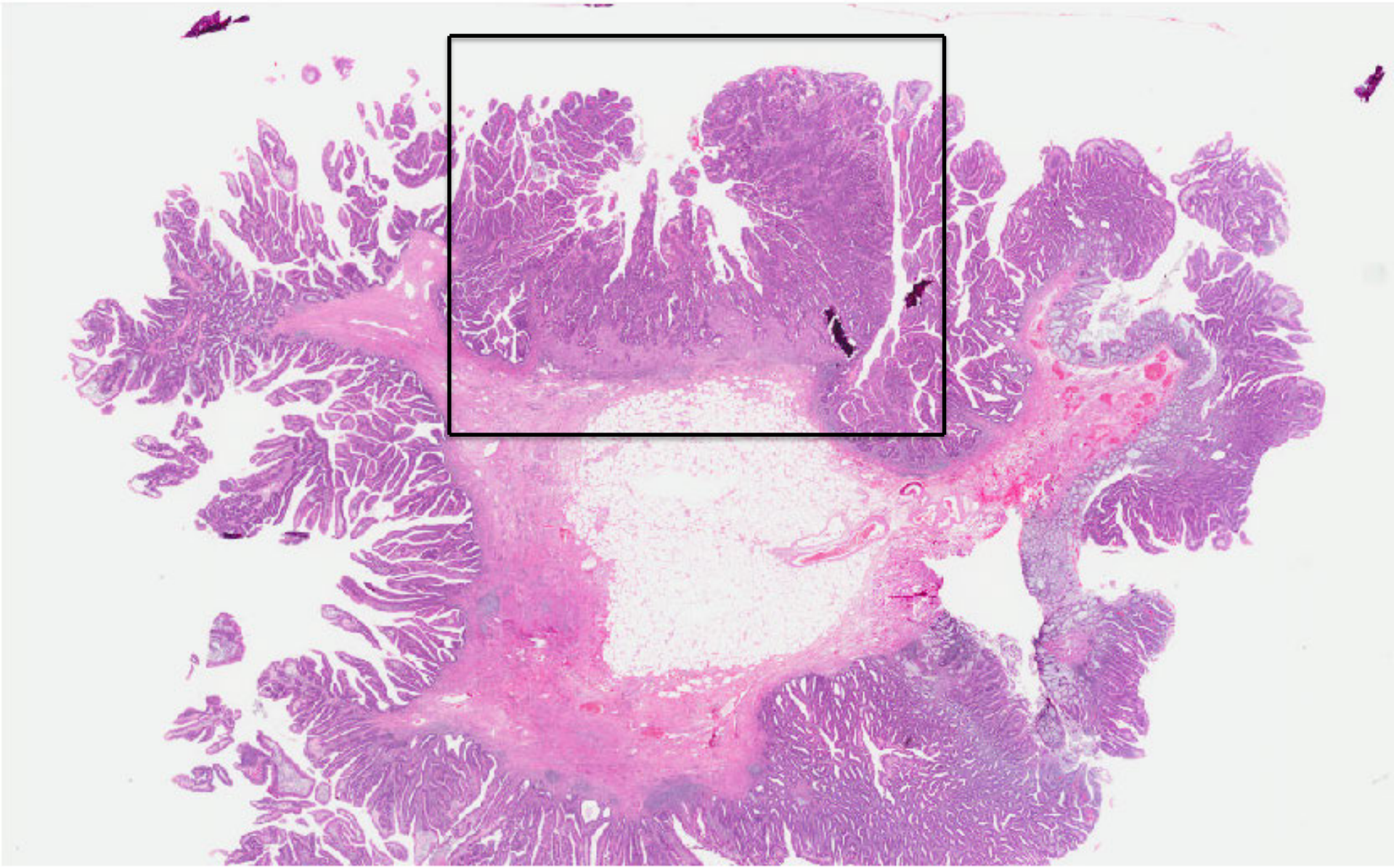
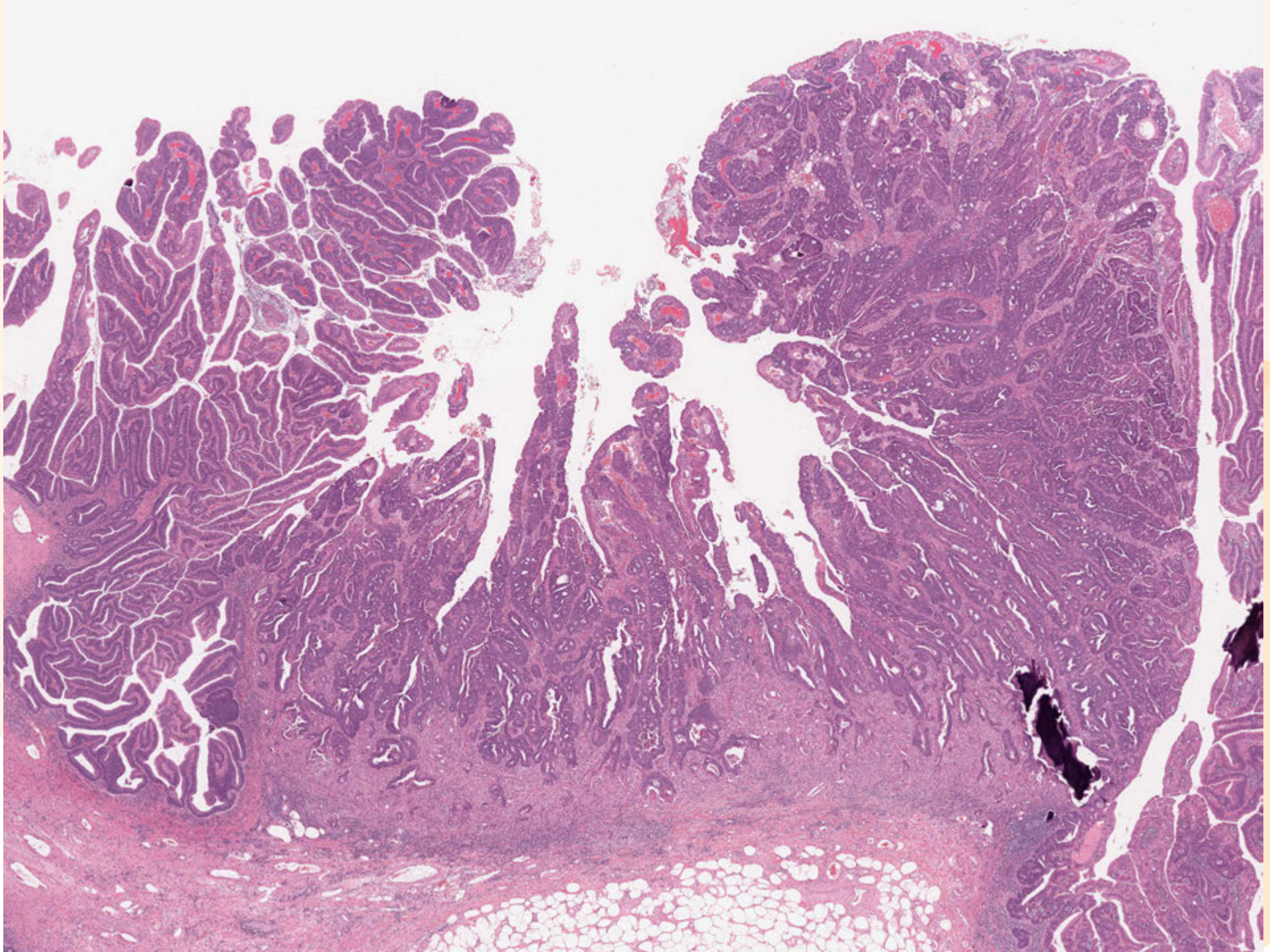
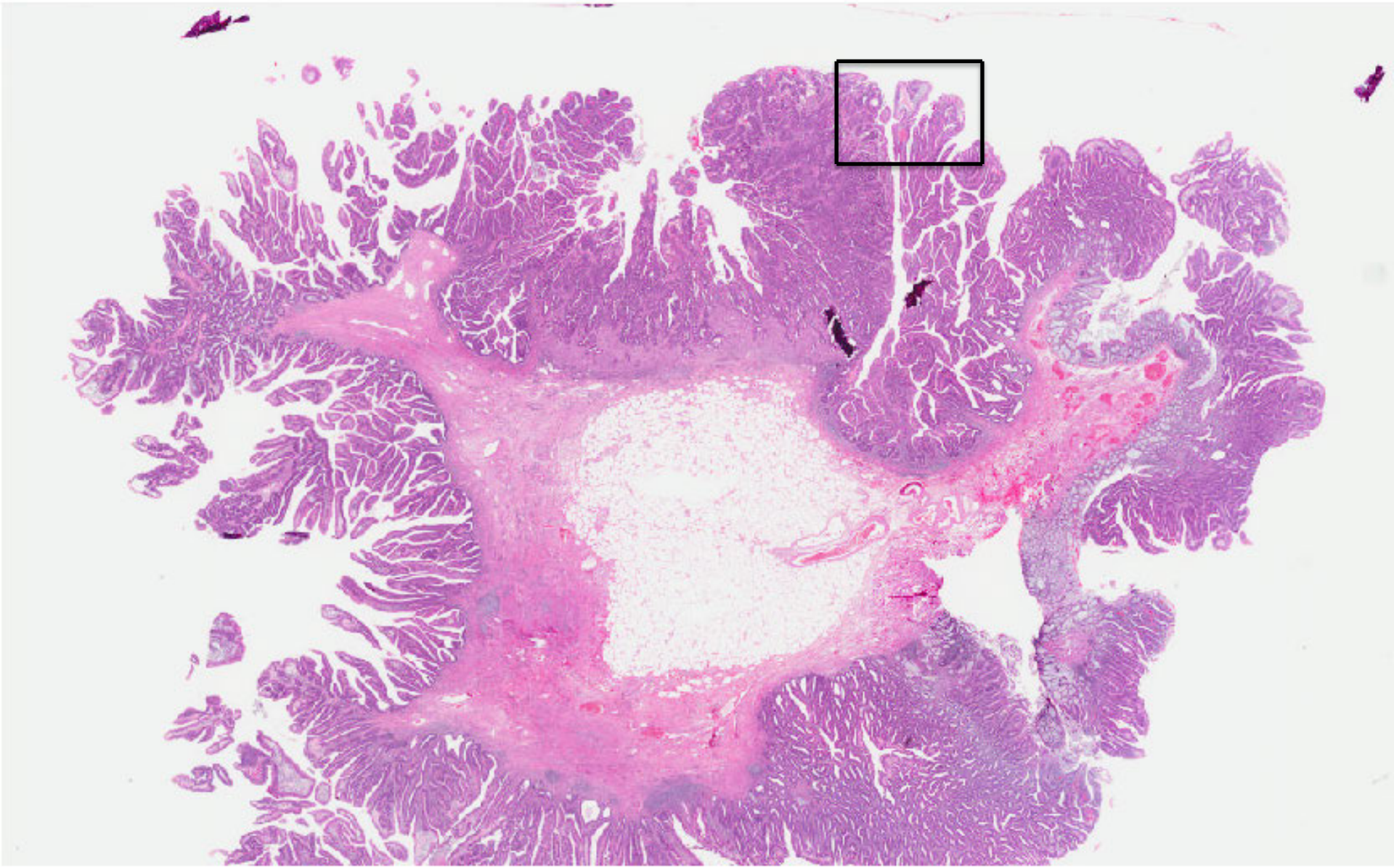
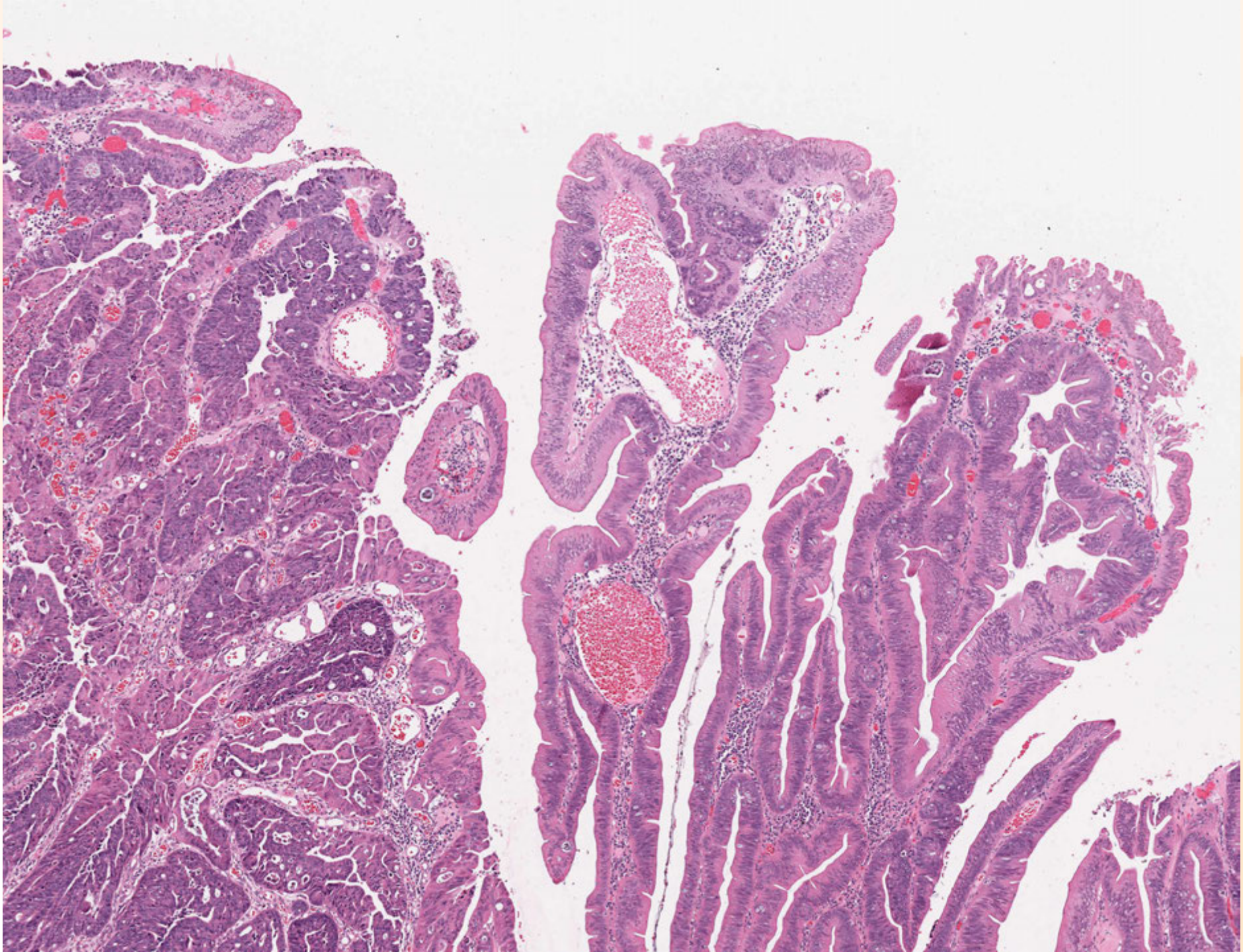








TSAs can be broadly divided by either their *BRAF* and *KRAS* mutation status or by the presence of advanced histology

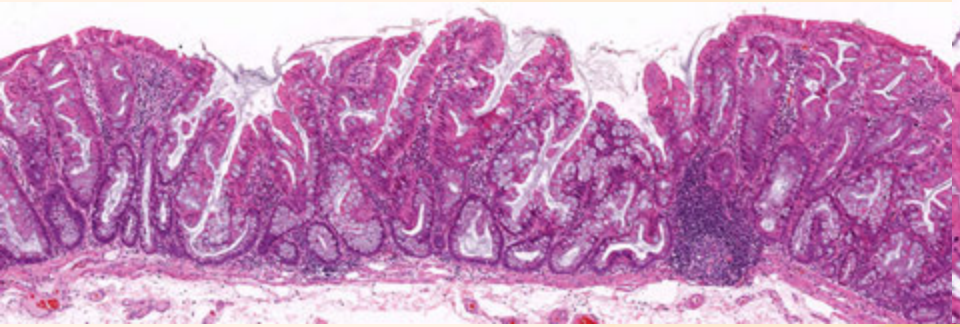
Clinicopathological features by advanced histology				
	All TSAs (n=200)	**Ordinary TSAs (n=162)**	**Advanced TSAs (n=38)**	**P-value (ordinary versus advanced)**
Age	64 (27-89)	64 (27-89)	65 (27-85)	0.8069
Female	50%	51%	45%	0.5891
Mean size (mm)	16 (3-95) (median 12)	14 (3-95) (median 11)	25 (5-70) (median 21)	**<0.0001**
Distal location	71%	68%	82%	0.1153
Precursor polyp	38%	44%	13%	**0.0003**
- SSA	31%	36%	11%	**0.0018**
- MVHP	7%	8%	3%	0.4769

TSAs can be flat

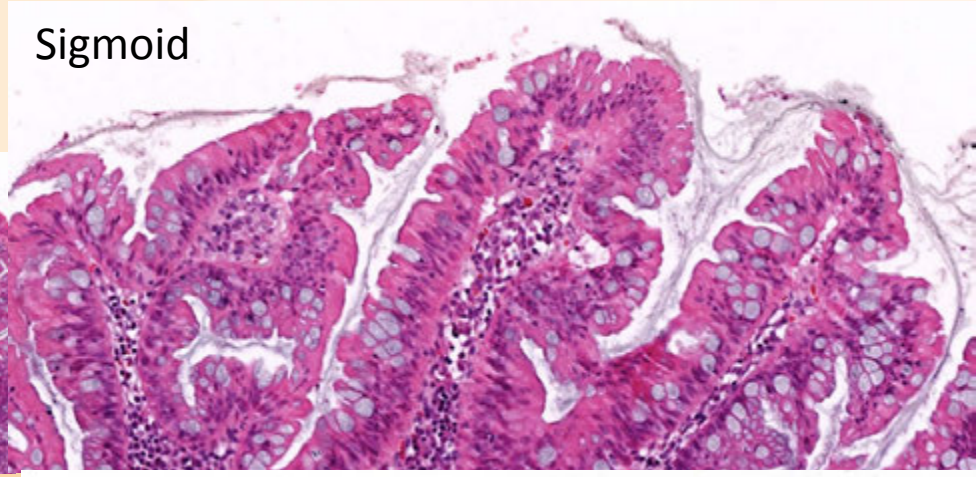
- In our study 38% of the TSAs were flat
- This has also been demonstrated by others

- More likely in the proximal colon and may be a reflection of intraluminal factors

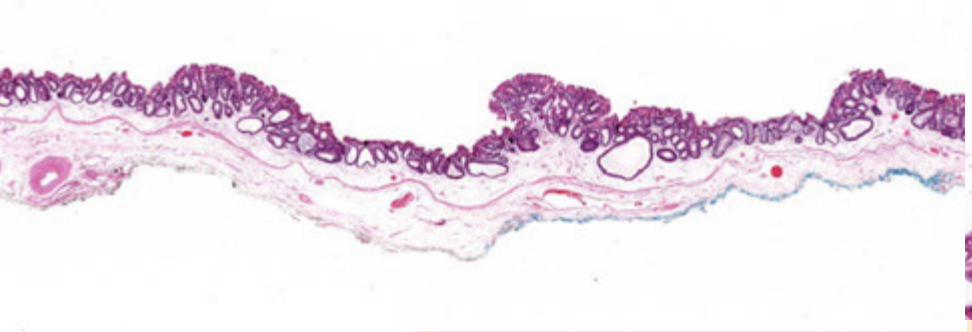
Sigmoid



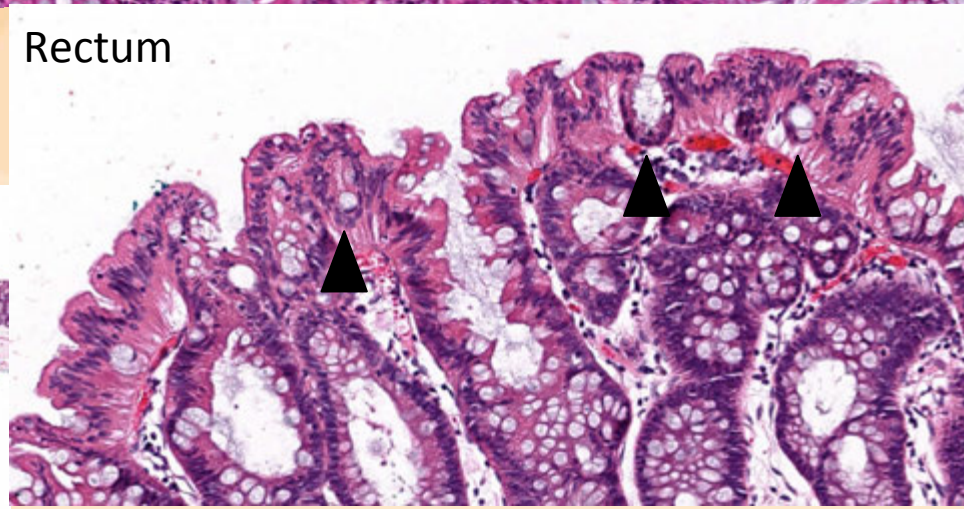
Sigmoid



Rectum



Rectum



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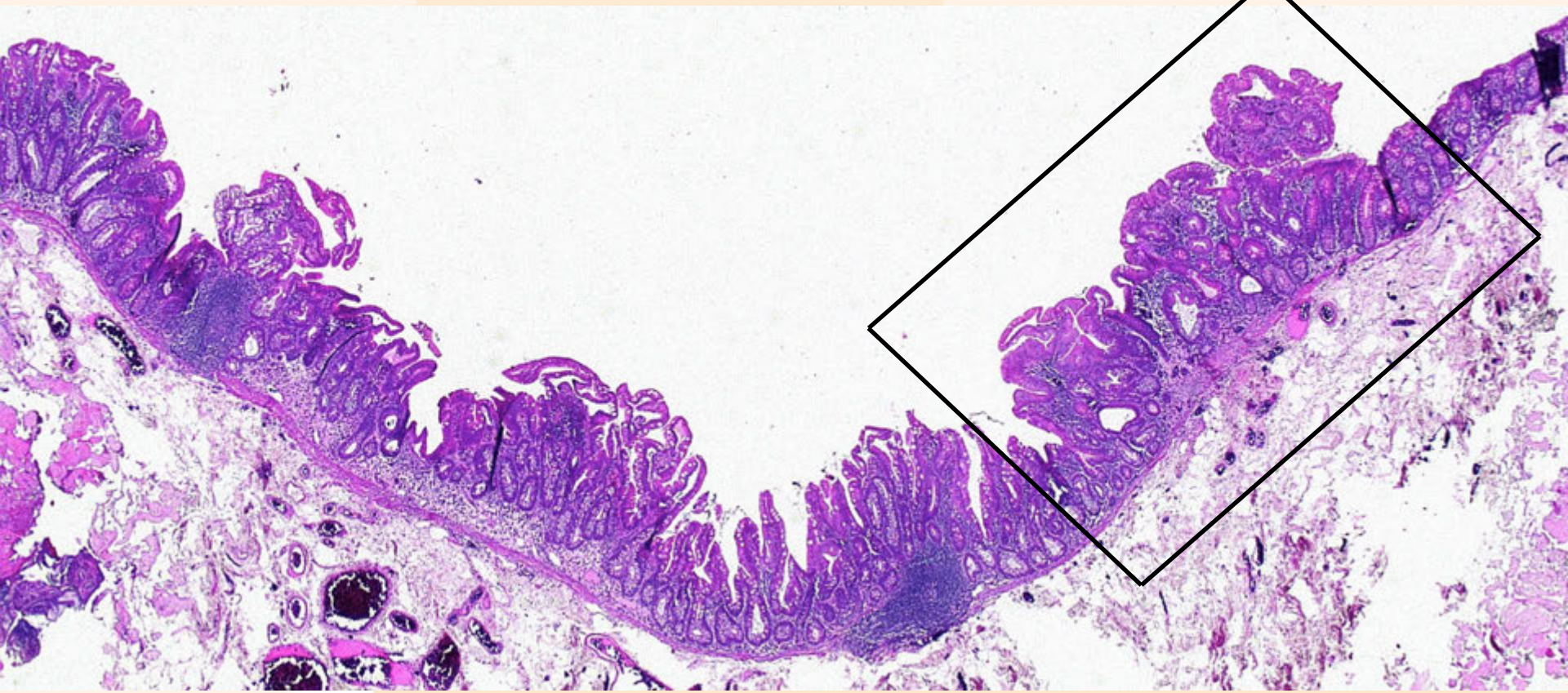

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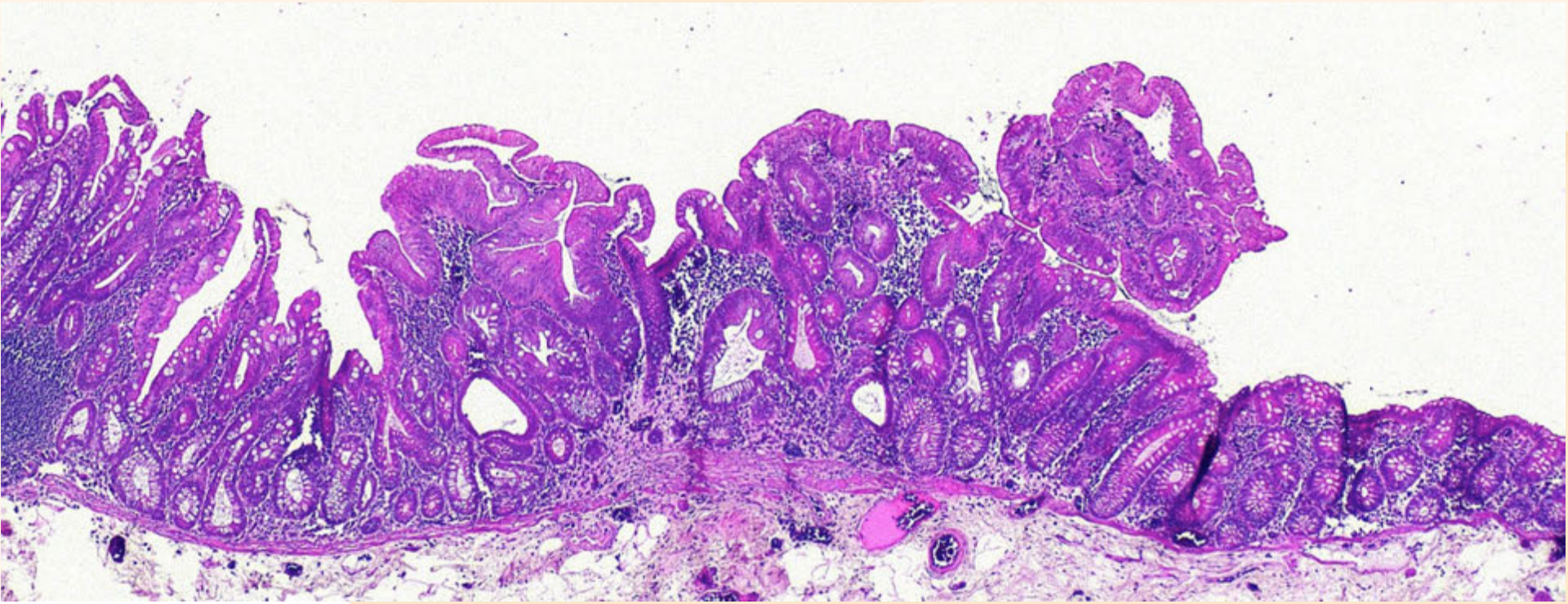

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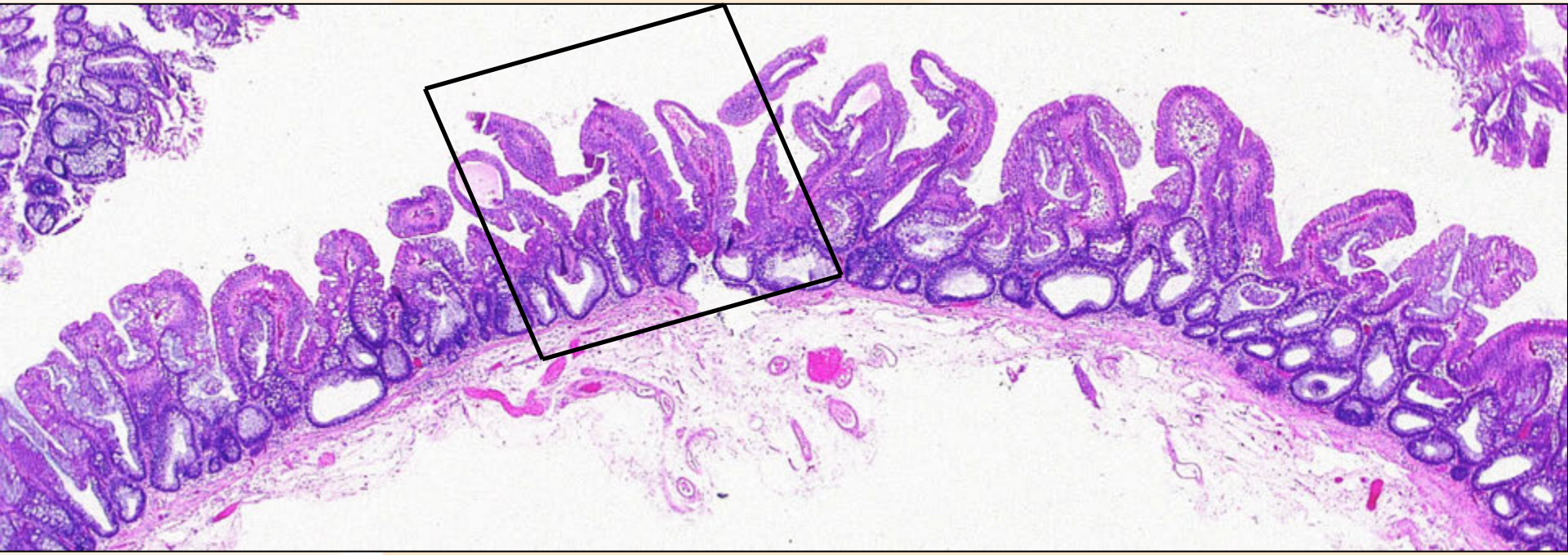

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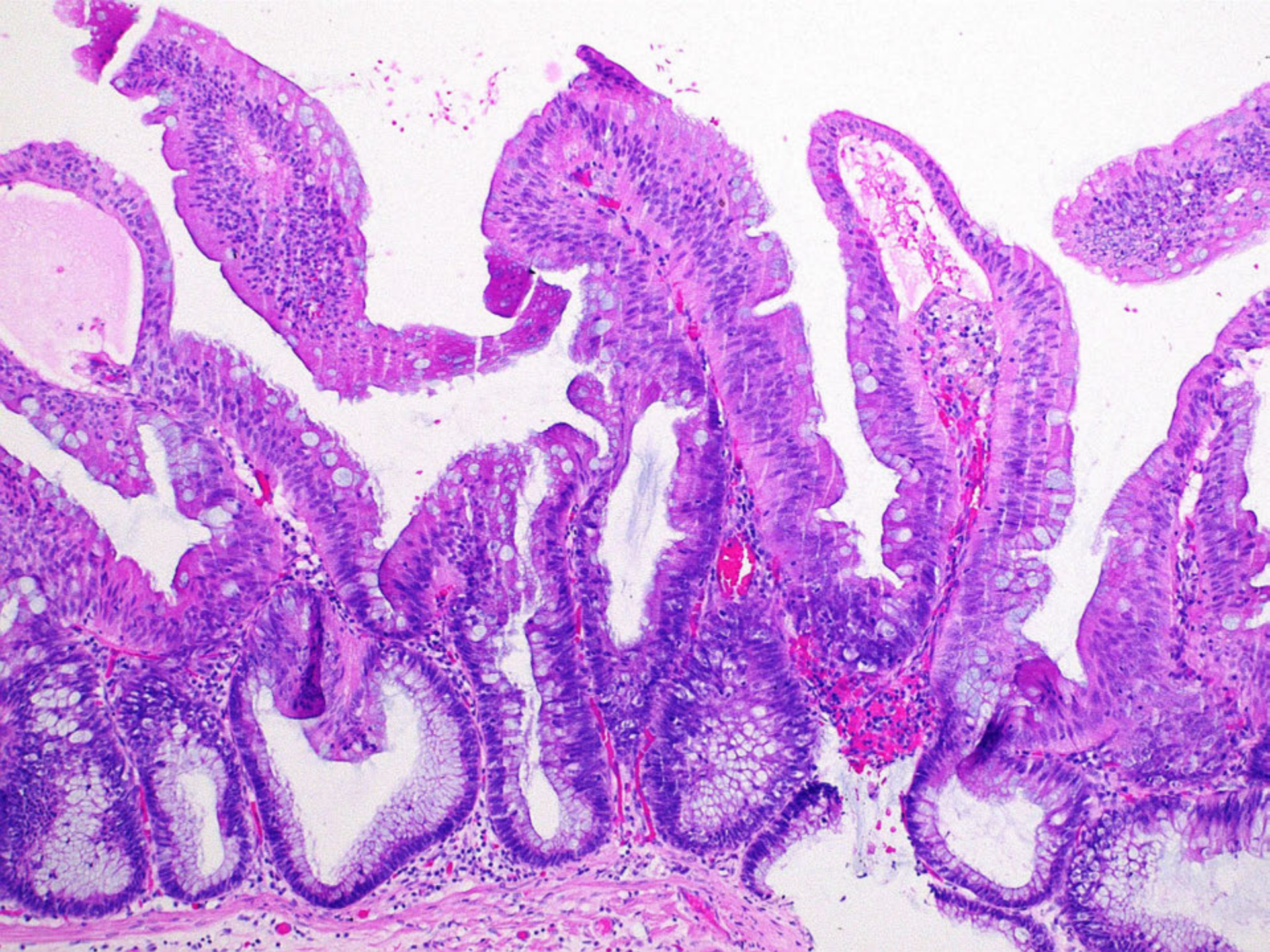
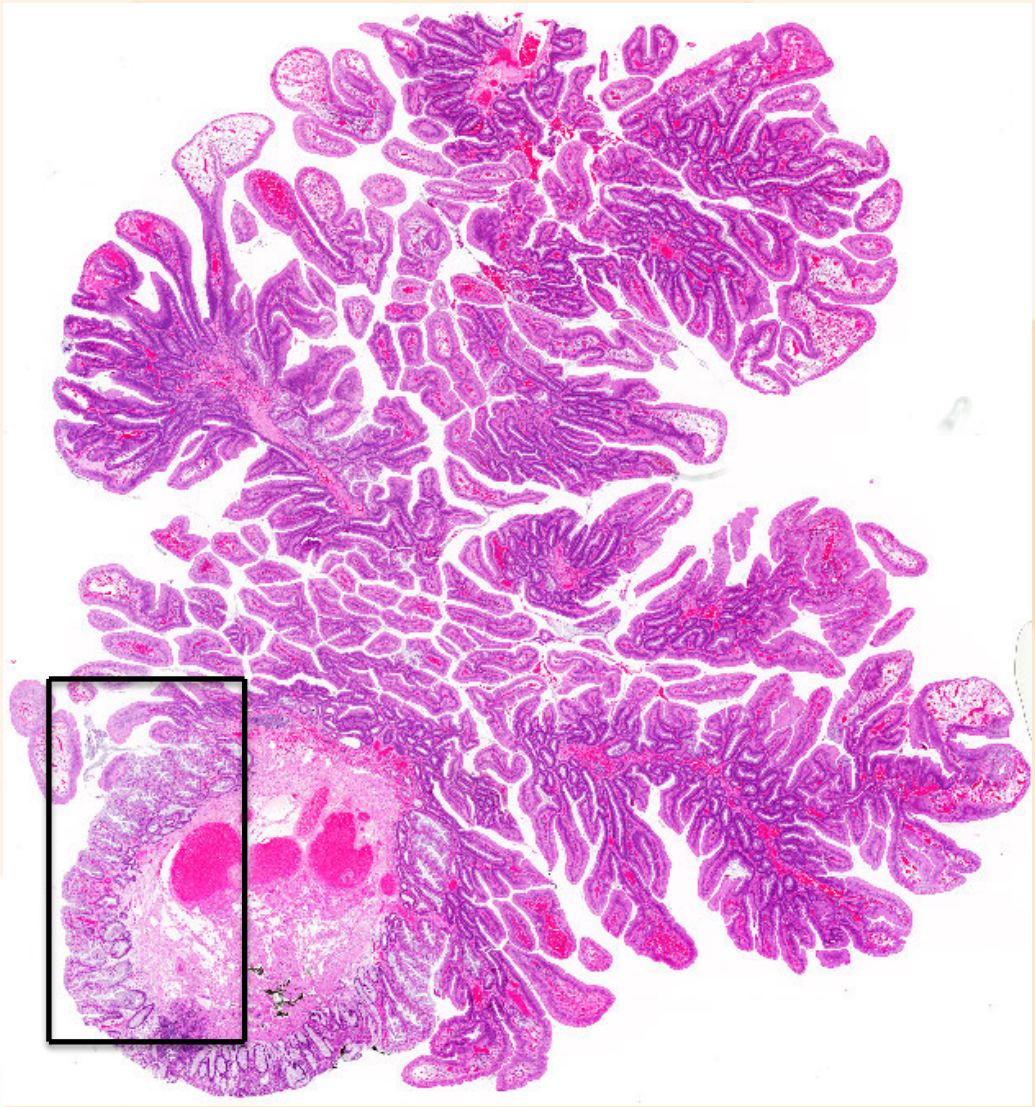


TSAs have frequent origin in an SSA or MVHP

- In our study 38% of the TSAs were had origin in an SSA or MVHP
- But it was restricted to *BRAF* mutated cases (57%)
- This has also been demonstrated by others



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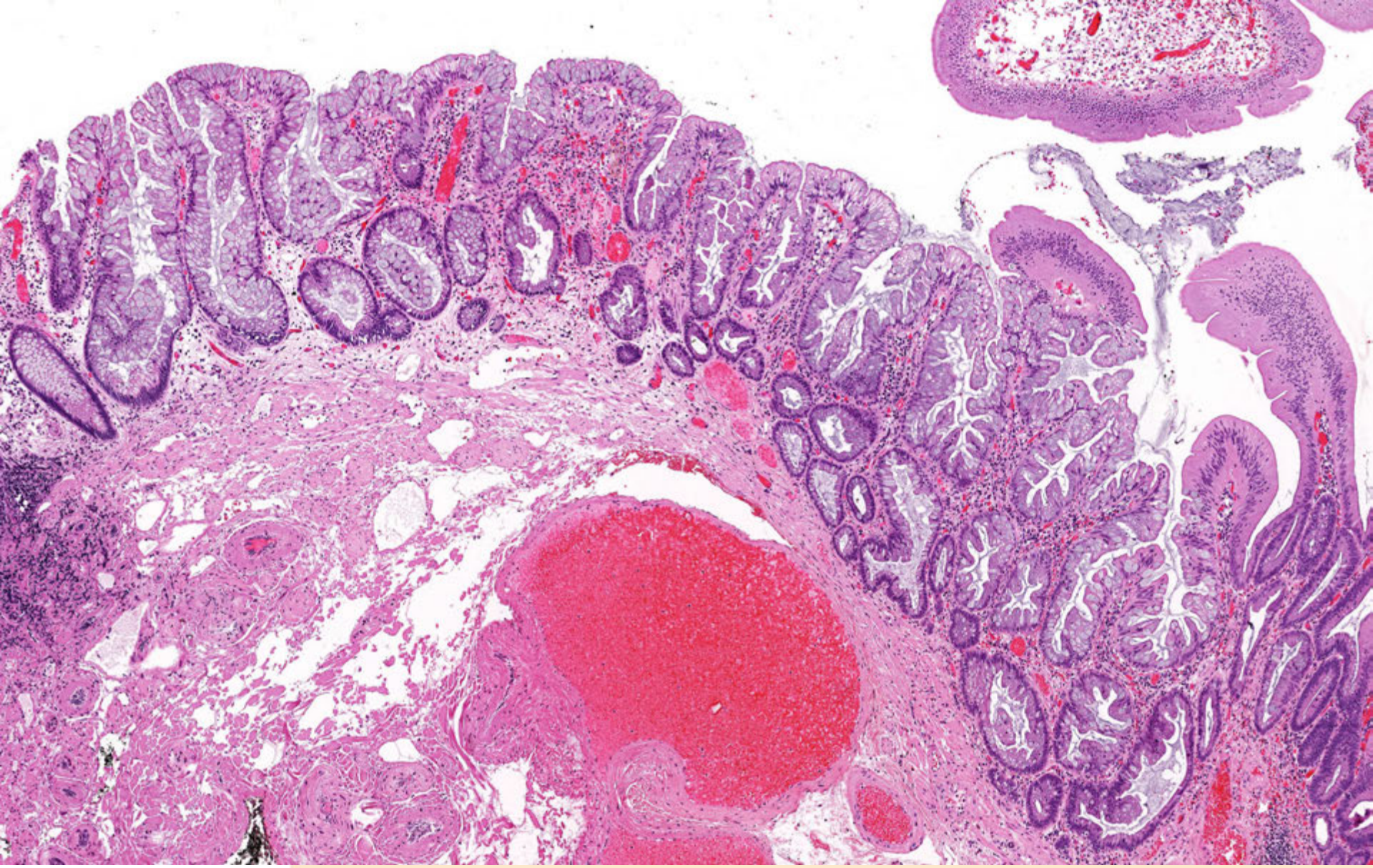

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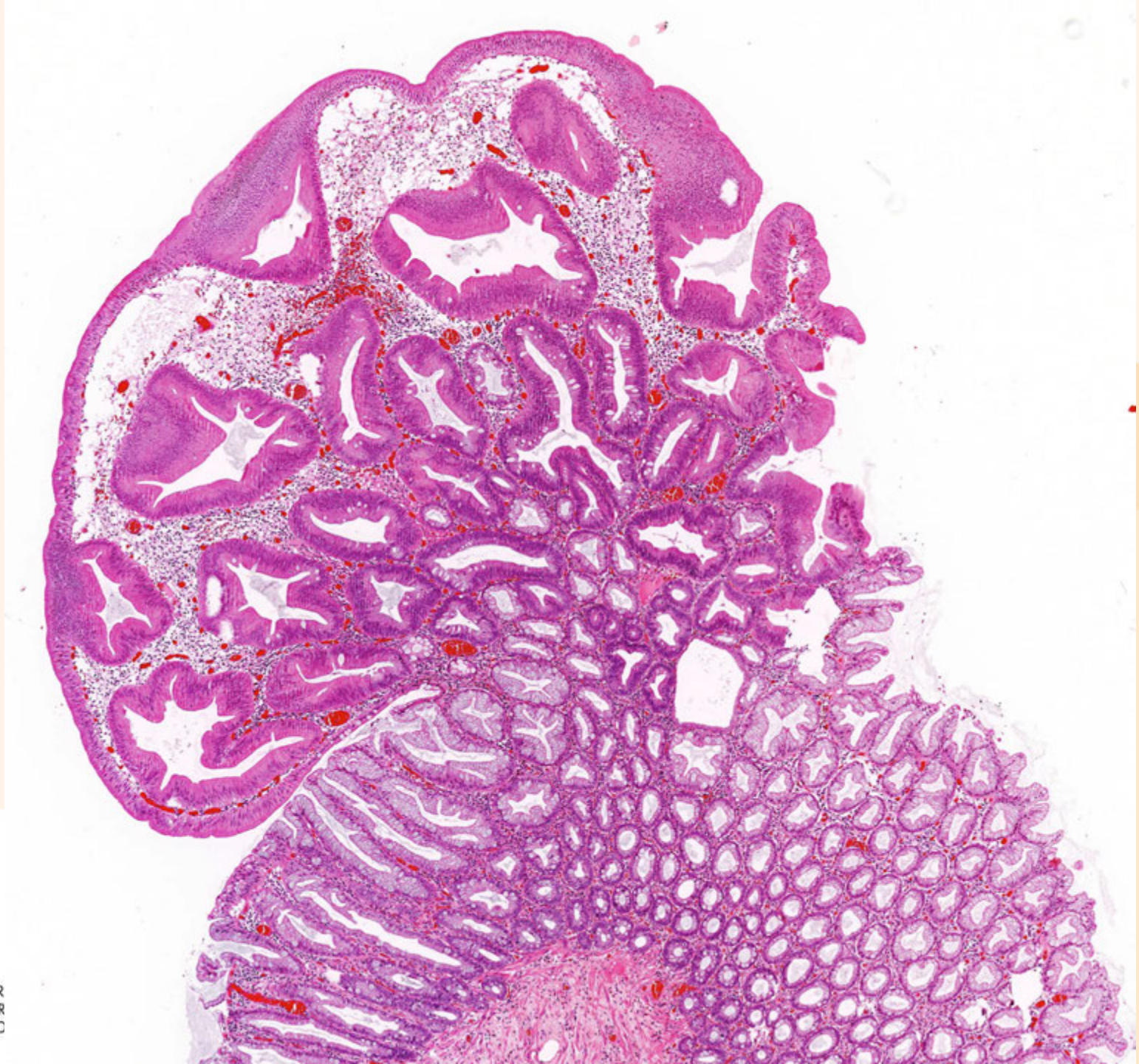

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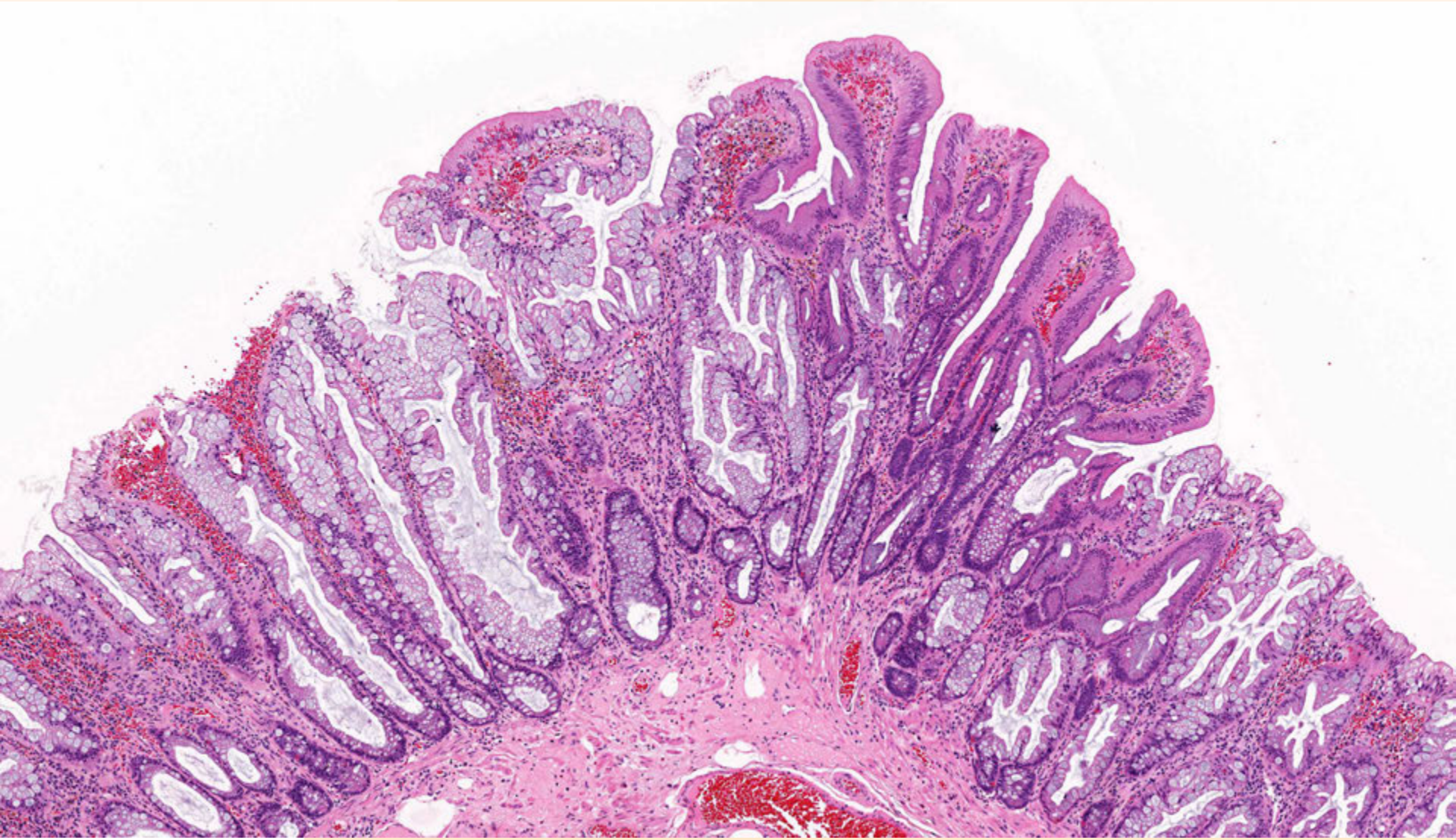

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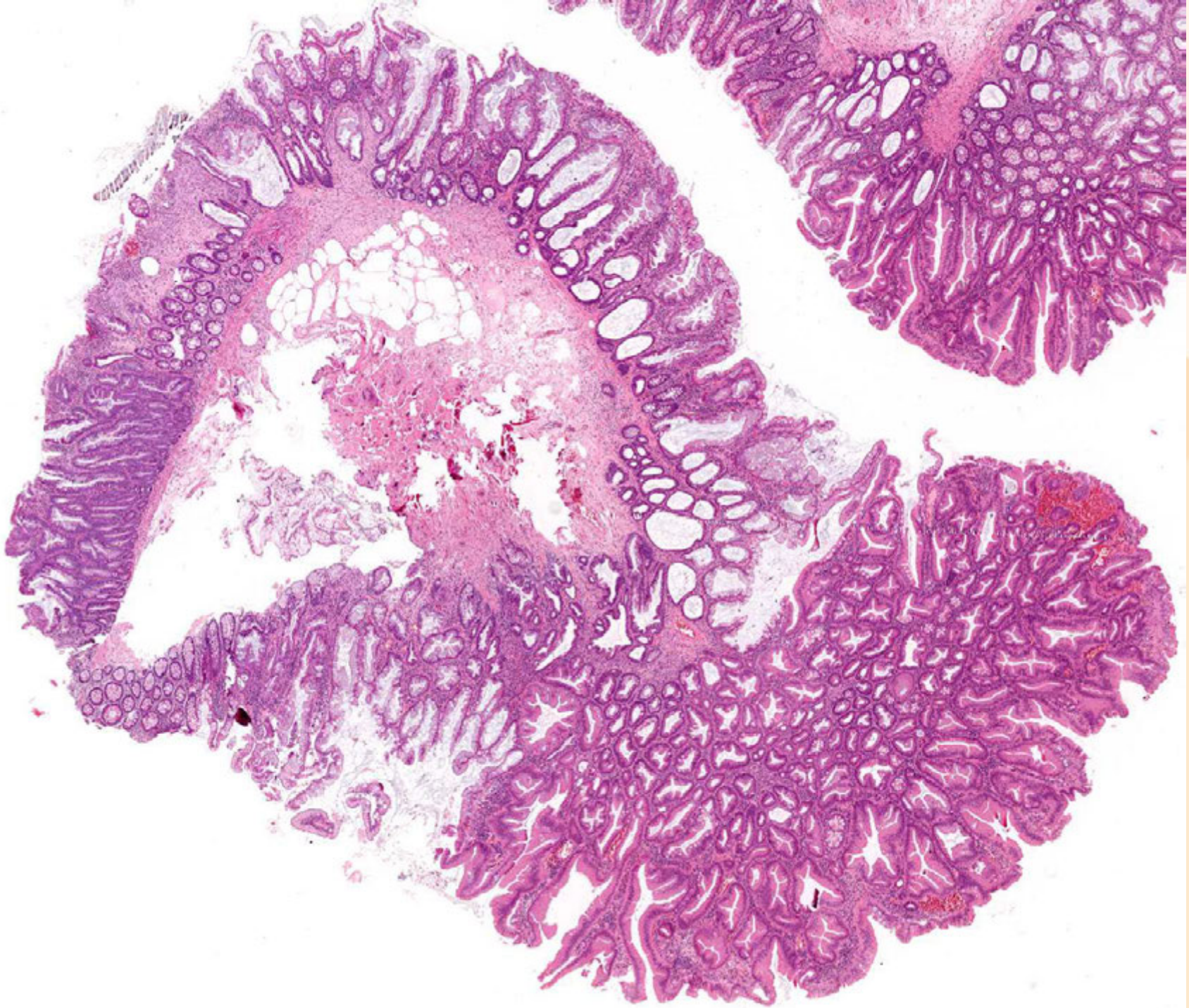


Molecular features of TSAs – *BRAF*/*KRAS* mutation status and CIMP

Ordinary TSAs

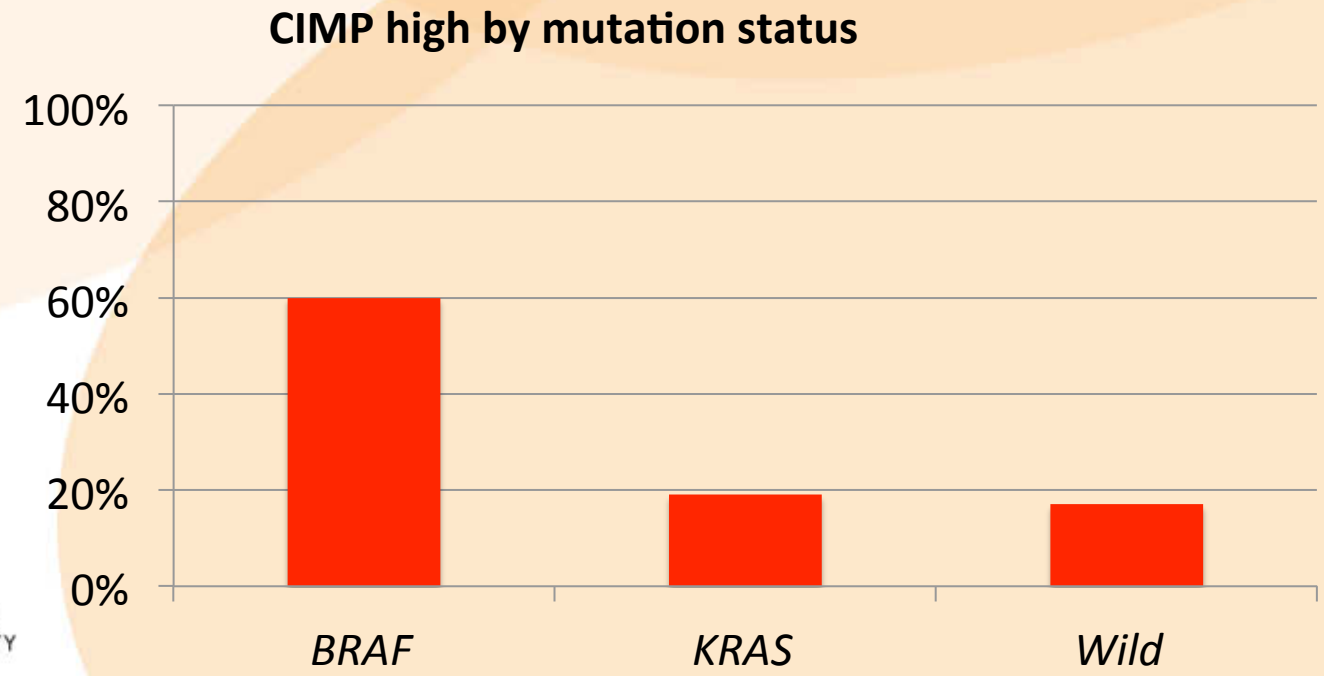
Mutation and CIMP status

- *BRAF* mutation in 112 (69.1%)
- *KRAS* mutation in 33 (20.4%)
- Wild type in 17 (10.5%)
- CIMP+ 46.3%

Advanced TSAs

Mutation and CIMP status

- *BRAF* mutation in 22 (57.9%)
- *KRAS* mutation in 10 (26.3%)
- Wild type in 6 (15.8%)
- CIMP+ 44.7%



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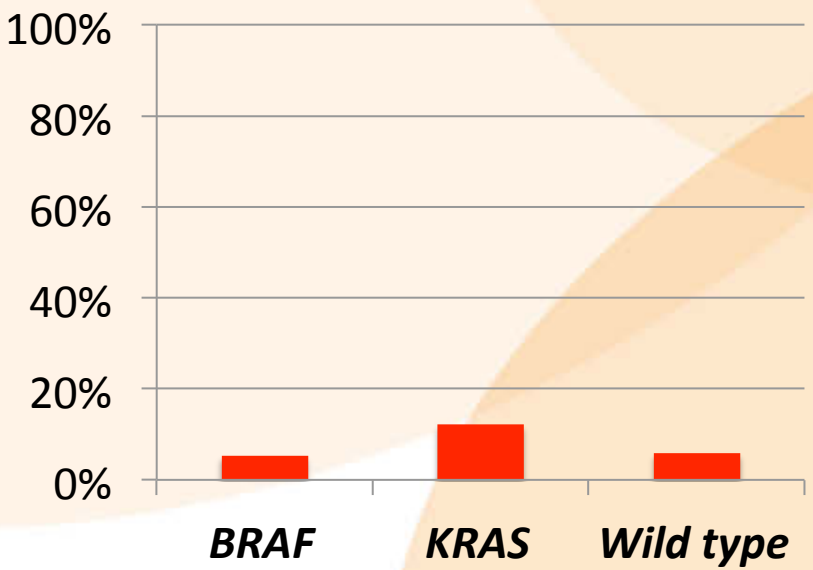
Molecular features of TSAs – MMR function

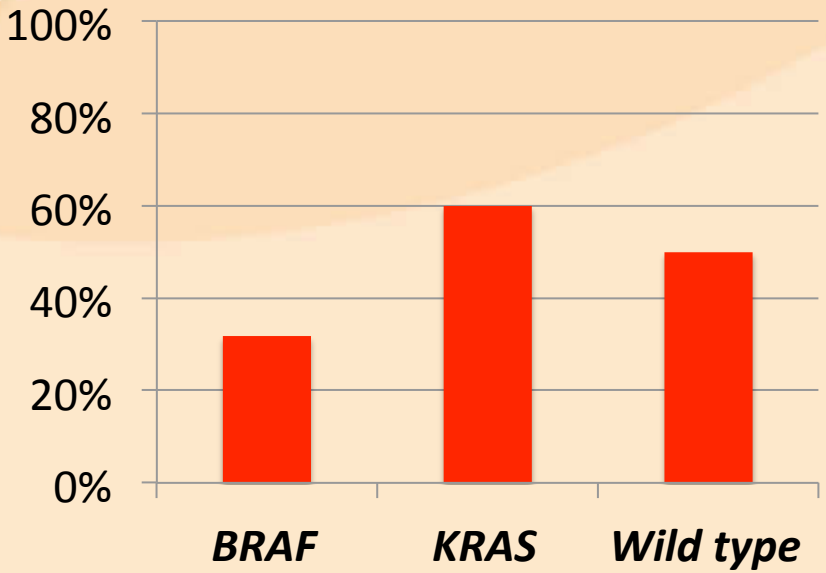
MLH1 expression is retained in 99.5% of TSAs indicating a MICROSATELLITE STABLE PHENOTYPE

Molecular features of TSAs – WNT signaling

Nuclear β-catenin (ordinary TSAs)



Nuclear β-catenin (advanced TSAs)



p=<0.0001
(ordinary vs advanced)

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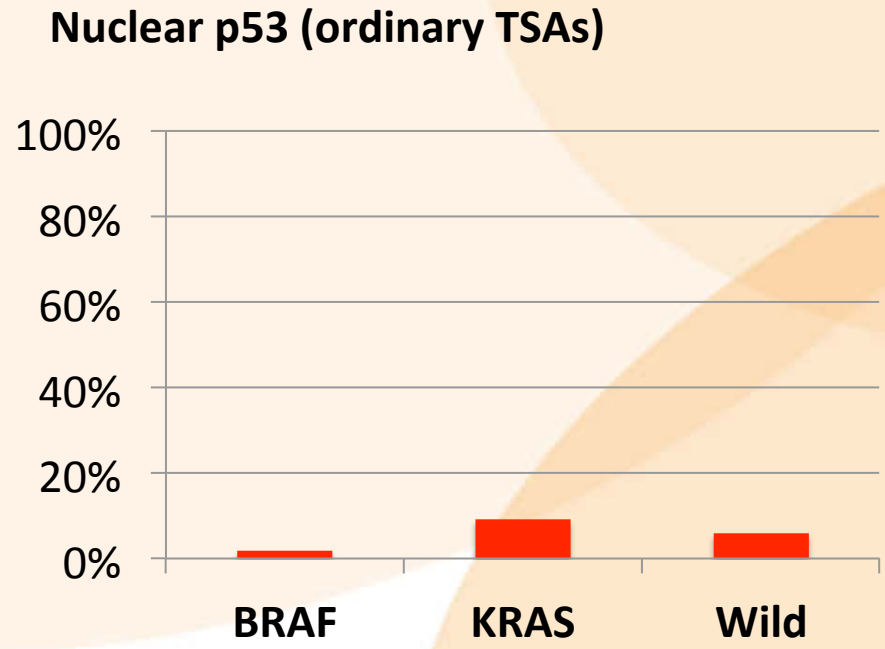
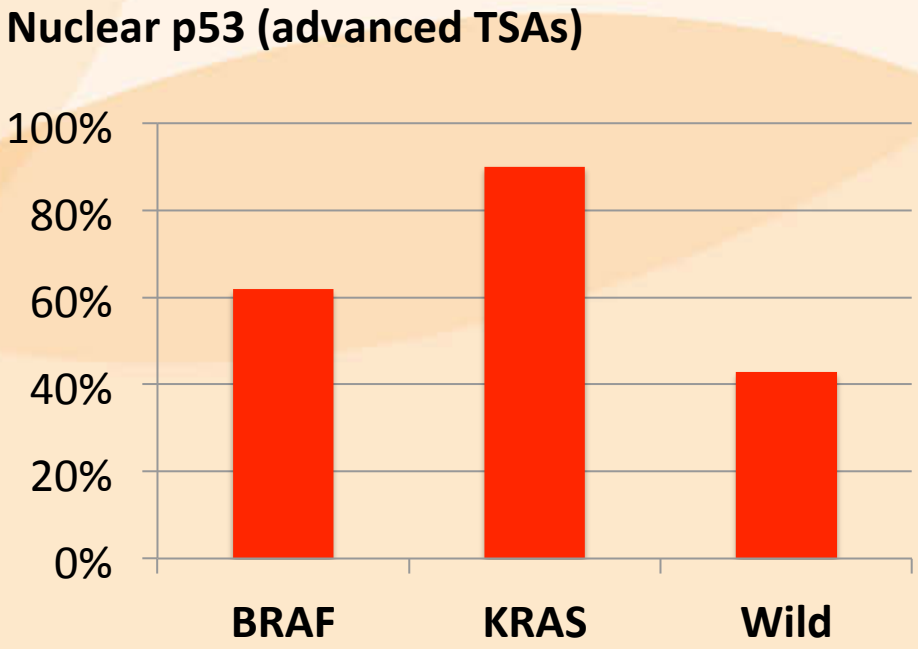
Molecular features of TSAs – p53





p=<0.0001
(ordinary vs advanced)

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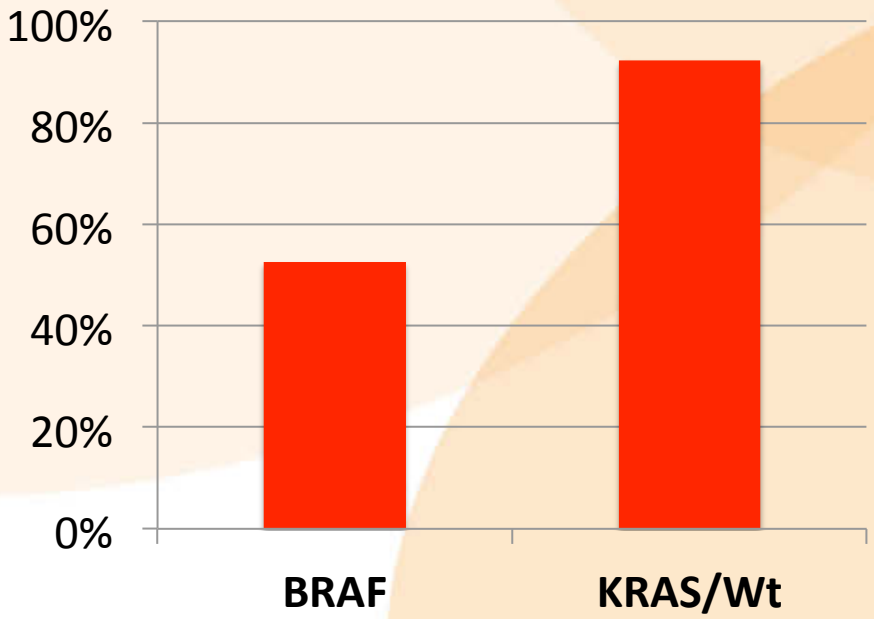
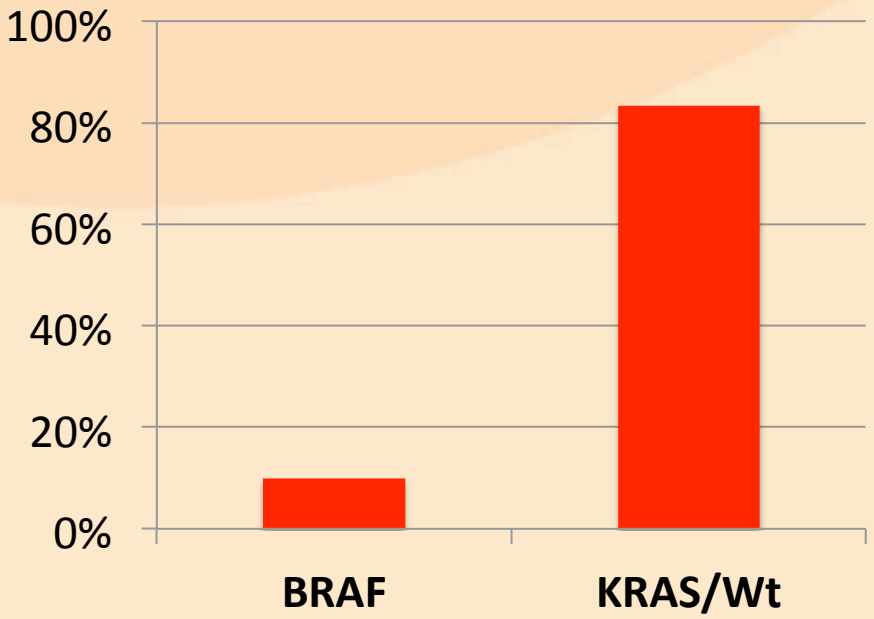
Molecular features of TSAs – p16

P16 staining in TSAs with dysplasia



P16 staining in TSAs with CRC



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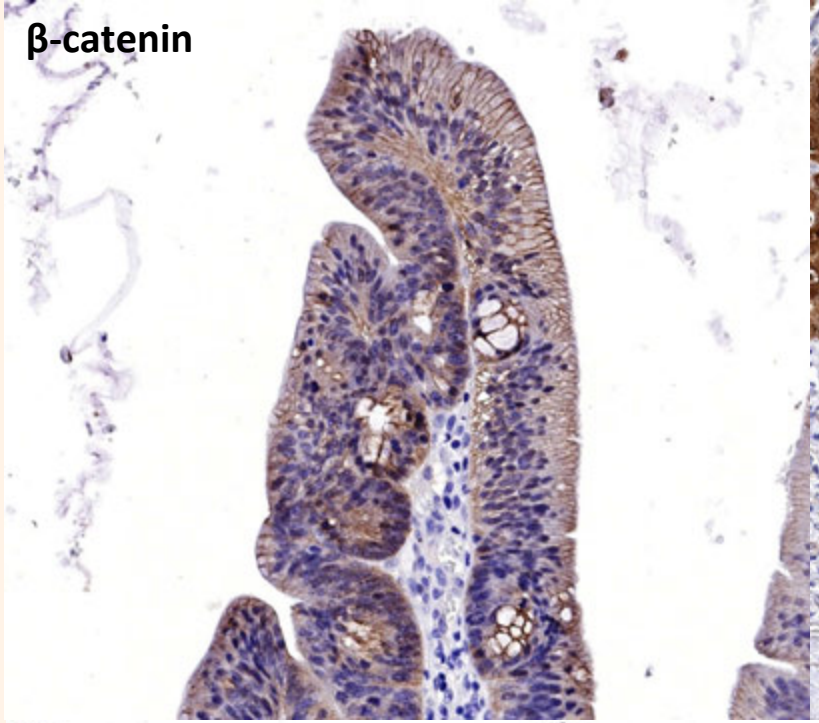
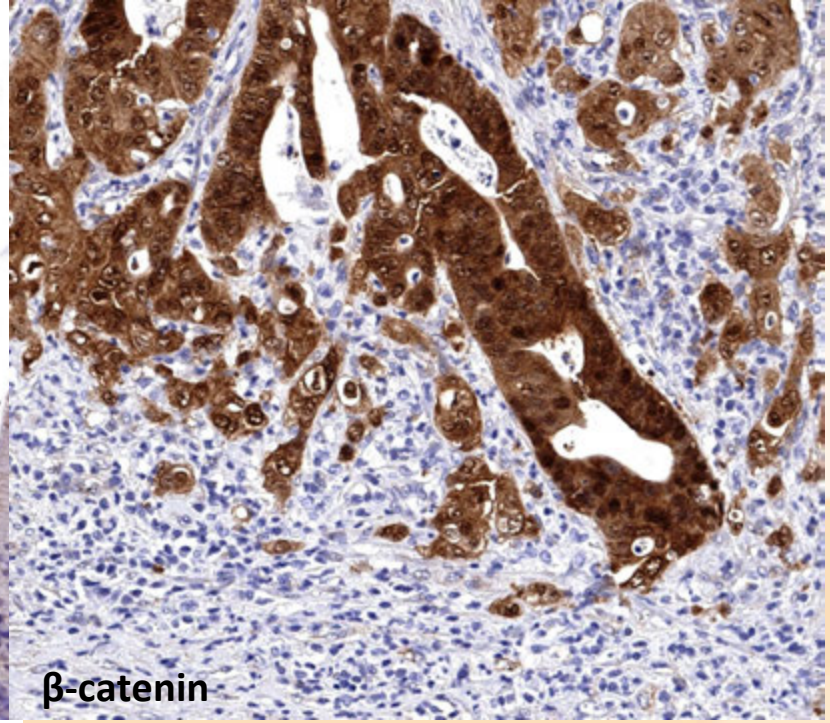
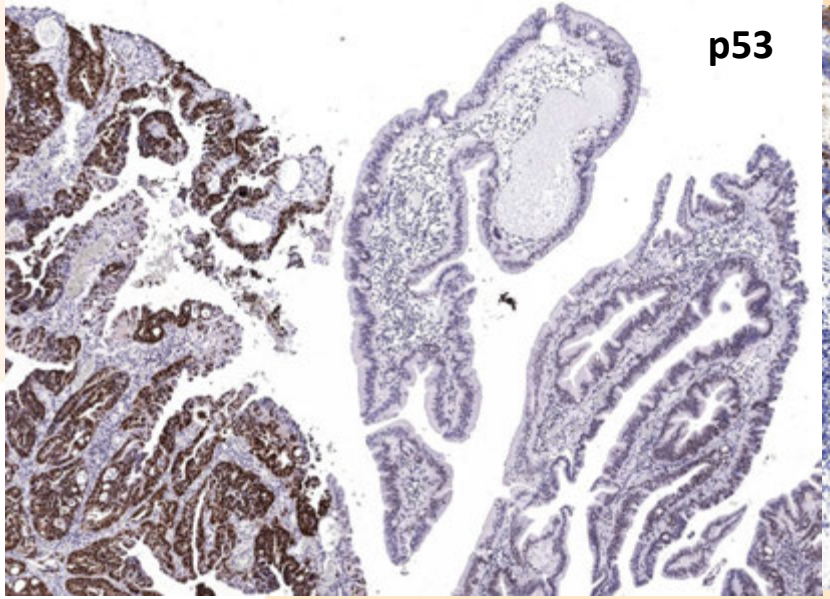
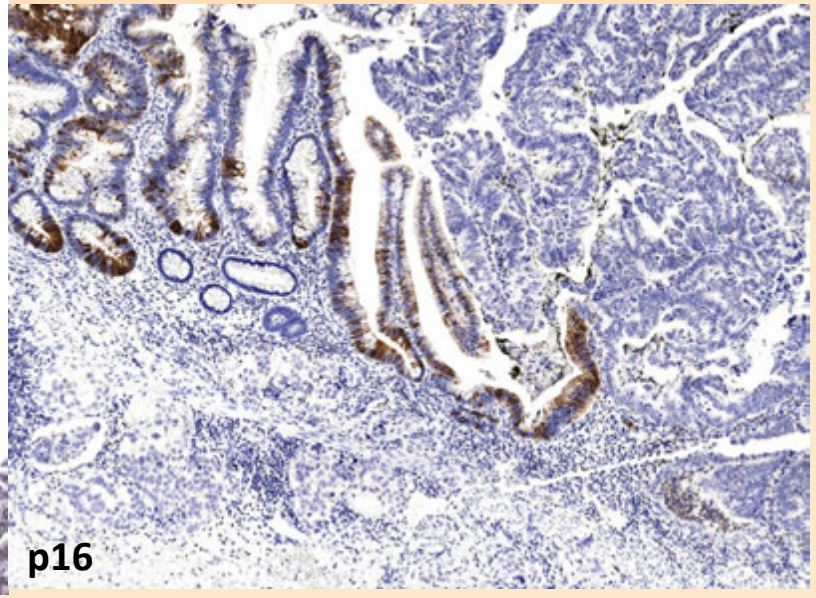
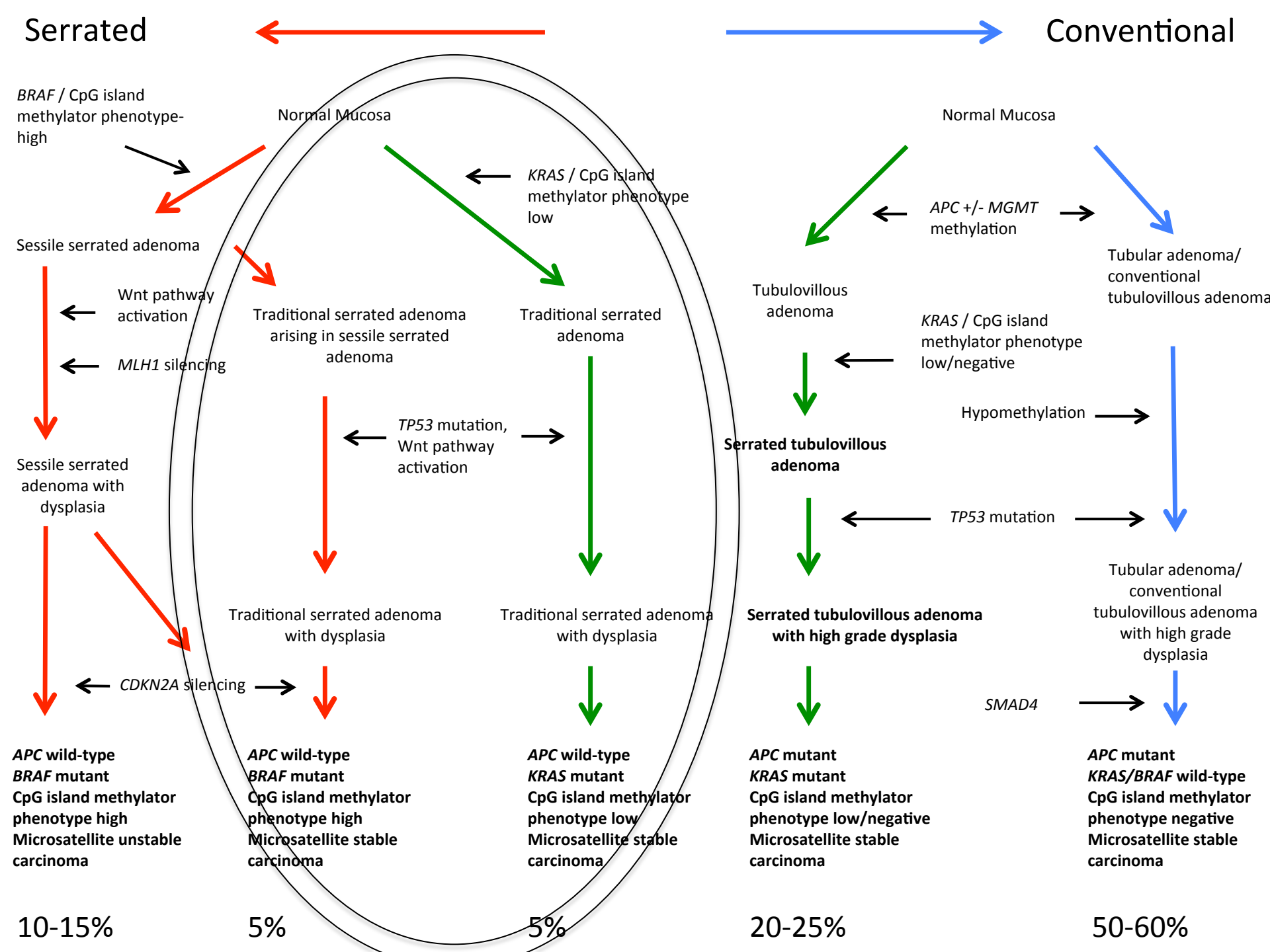

β-catenin


β-catenin


p53


p16


Serrated
Conventional
BRAF / CpG island methylator phenotype-high
Normal Mucosa
KRAS / CpG island methylator phenotype low
Normal Mucosa
APC +/- MGMT methylation
Sessile serrated adenoma
Wnt pathway activation
MLH1 silencing
Traditional serrated adenoma arising in sessile serrated adenoma
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Tubular adenoma/ conventional tubulovillous adenoma
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SMAD4
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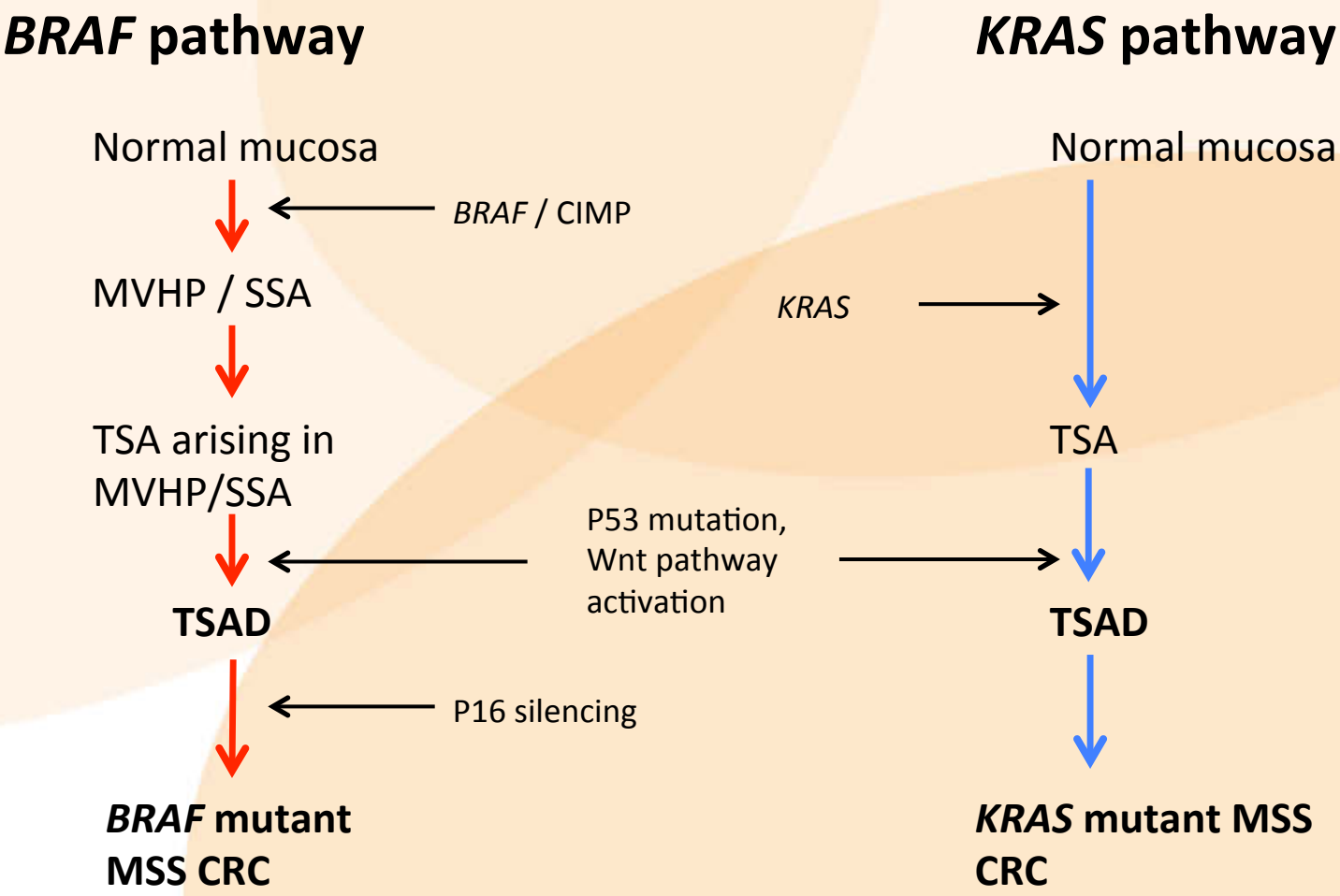
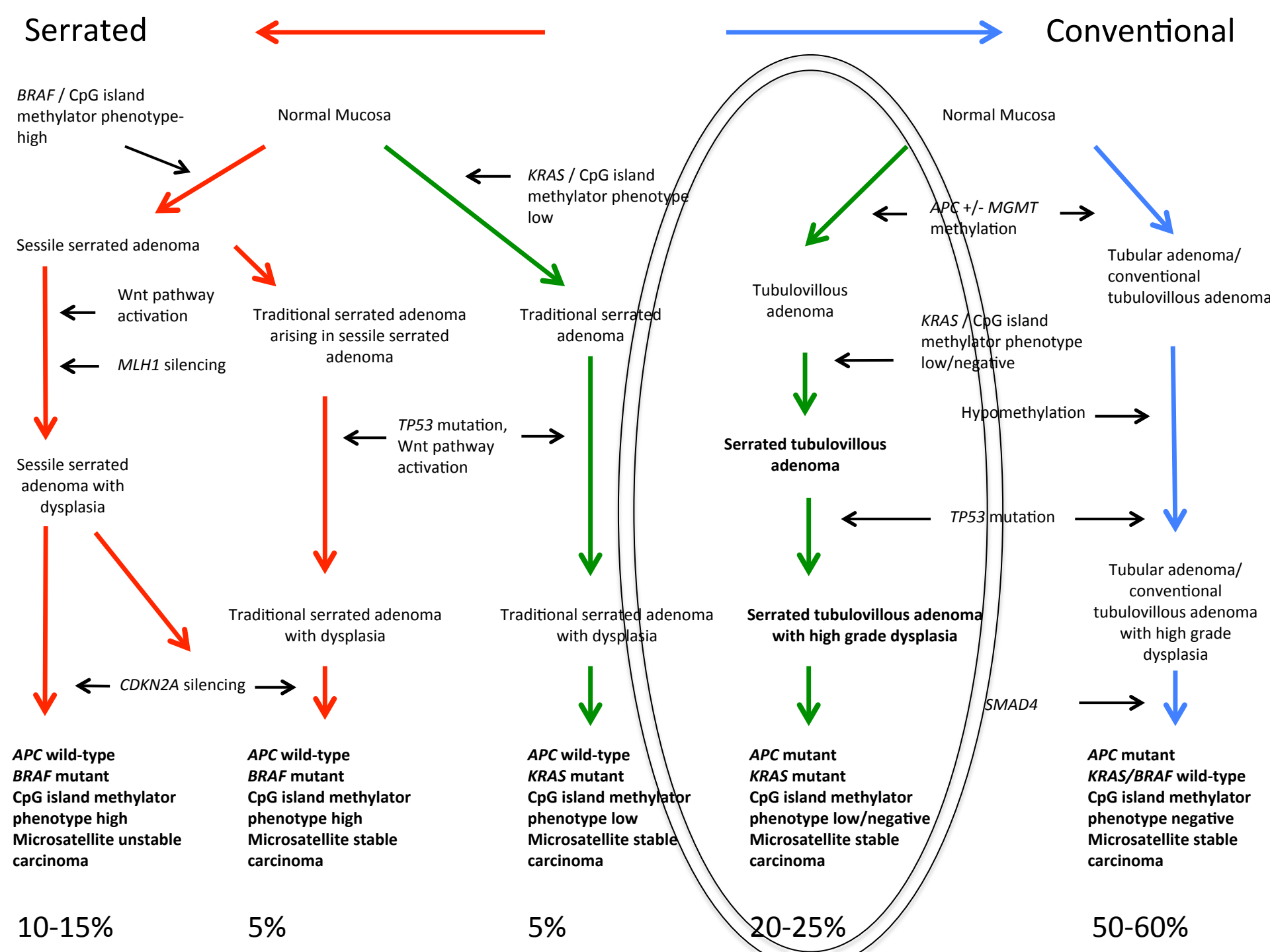
APC mutant KRAS mutant CpG island methylator phenotype low/negative Microsatellite stable carcinoma
APC mutant KRAS/BRAF wild-type CpG island methylator phenotype negative Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

Proposed pathways to carcinoma for traditional serrated adenomas



Serrated tubulovillous adenoma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APC mutant KRAS/BRAF wild-type CpG island methylator phenotype negative Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

Serrated Tubulovillous Adenoma (sTVA)

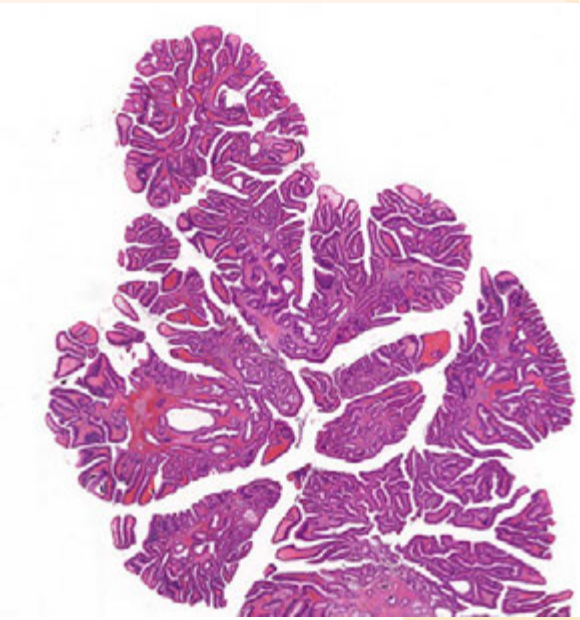
- a variant of conventional tubulovillous adenoma (cTVA)
- confused morphologically with traditional serrated adenoma
- postulated to be a precursor of *KRAS* mutated colon cancer
- no detailed studies available

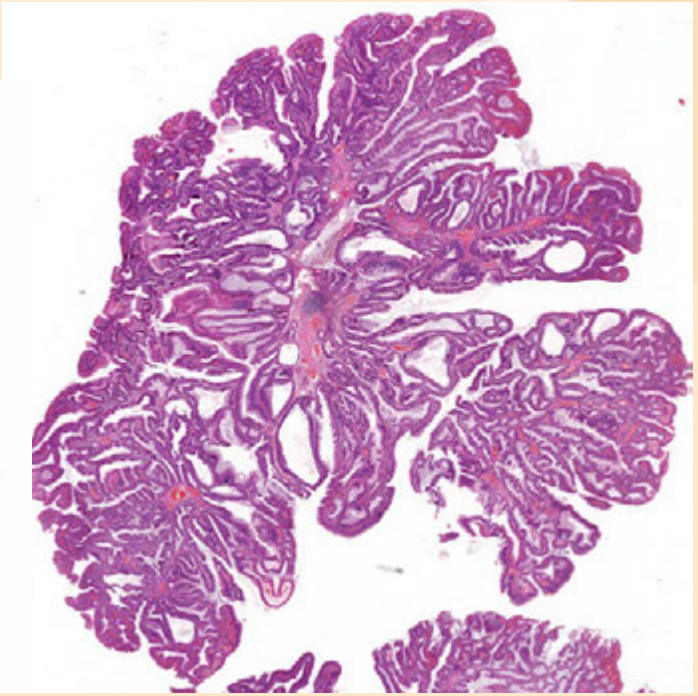
A subset of colorectal polyps are difficult to classify

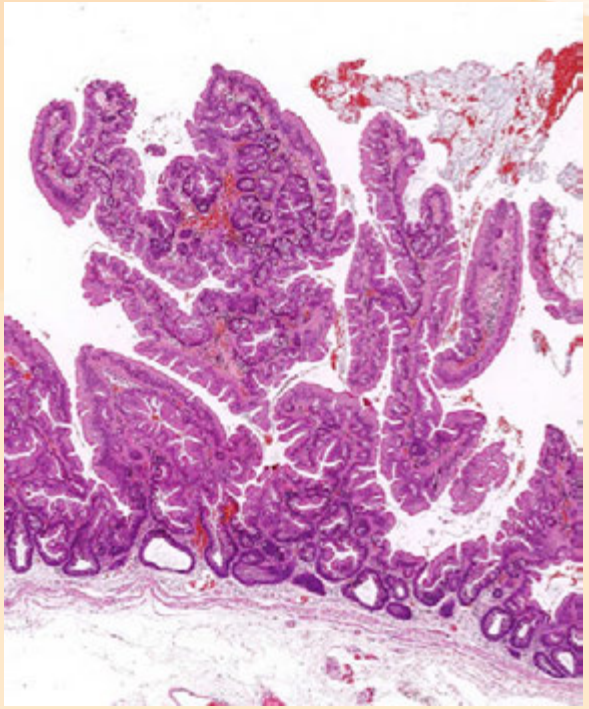
Tubulovillous adenoma

Intermediate polyp
(serrated tubulovillous adenoma)

Traditional serrated adenoma







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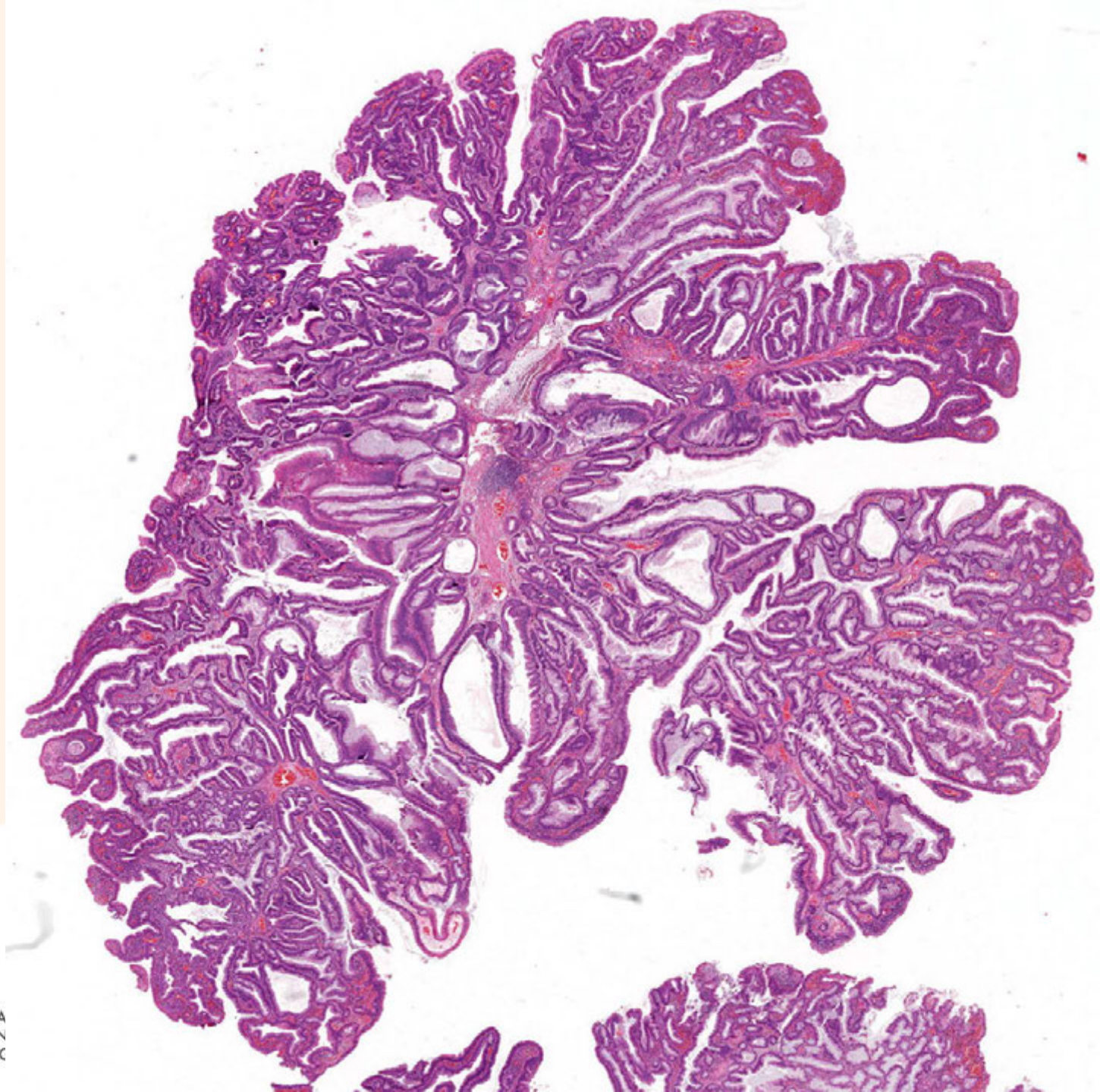
Serrated tubulovillous adenoma

- We collected a series of these difficult to classify polyps for clinicopathological and molecular analysis

- Diagnostic criteria
  1. Villous architecture in >25% of the polyp
  2. Serration in >50% of the polyp
  3. Lack of cytology and slit-like serrations seen in traditional serrated adenomas

Diagnostic criteria

1. Villous architecture in >25% of the polyp
2. Serration in >50% of the polyp
3. Lack of cytology and slit-like serrations seen in traditional serrated adenomas



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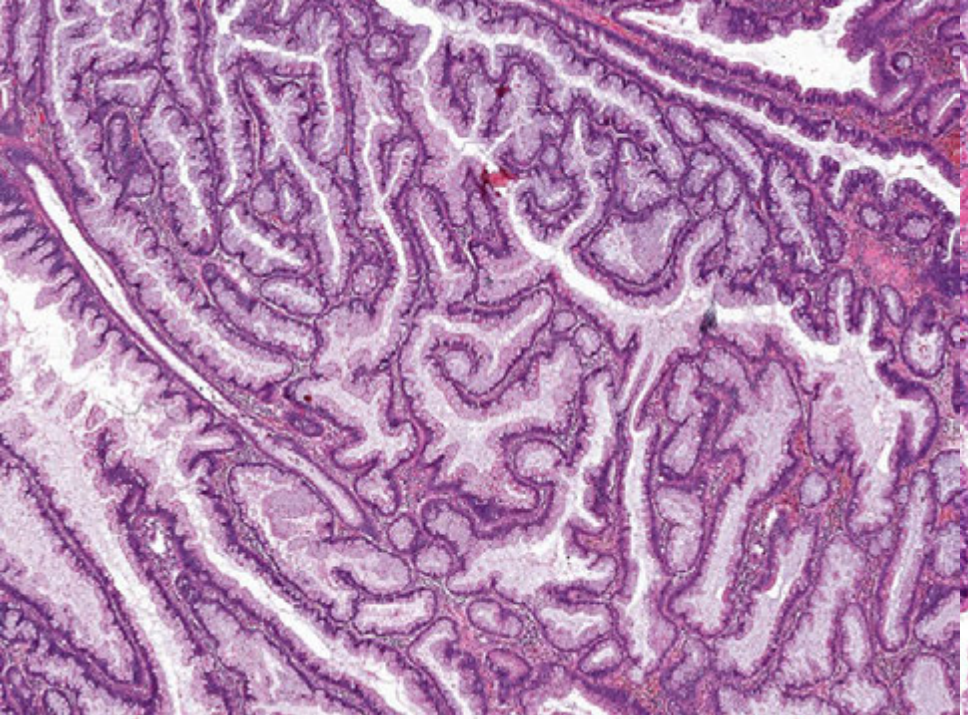
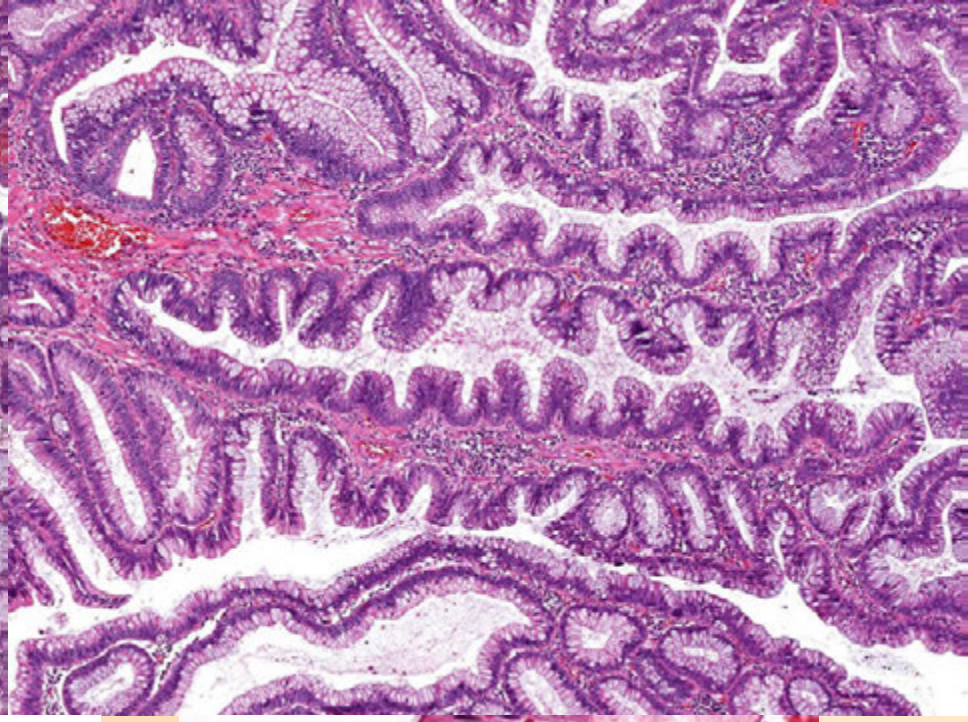
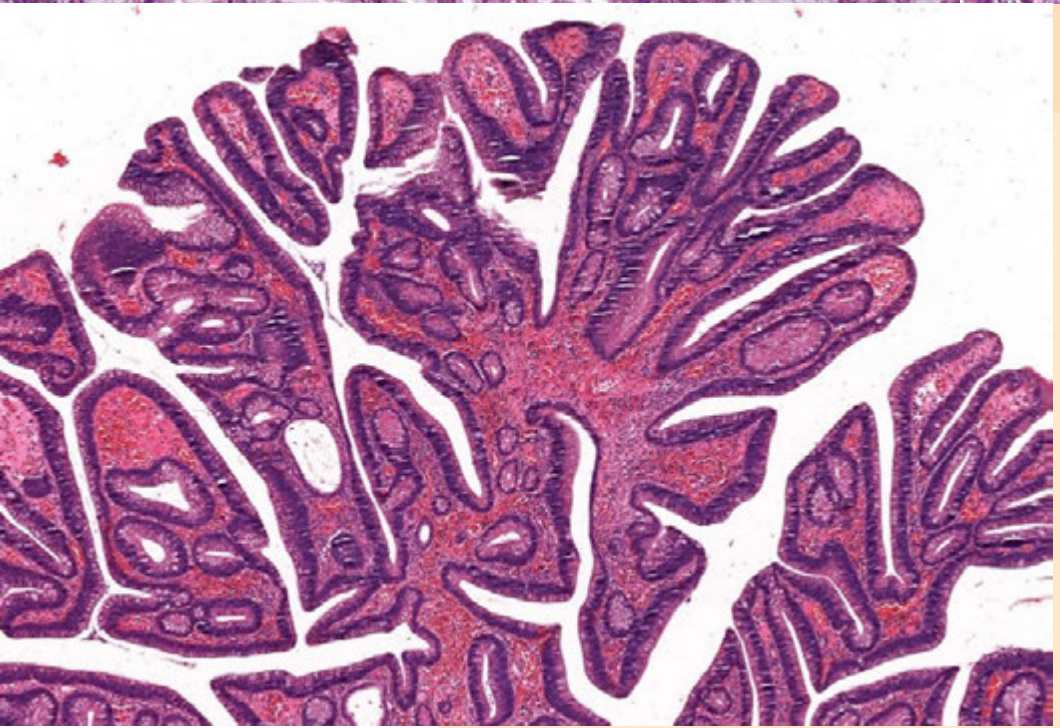
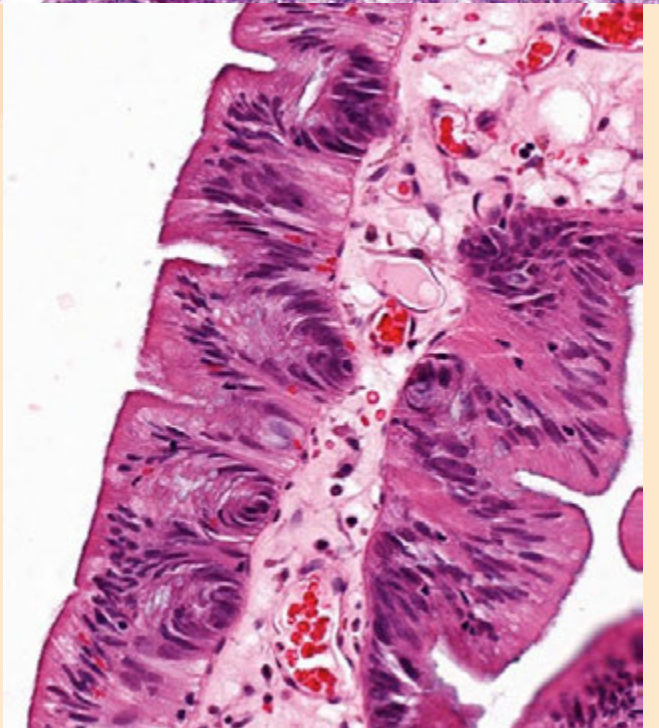





Serrated TVAs are rare

- When using our criteria:
  - 27 of 412 tubulovillous adenomas met the inclusion criteria
  - This represents 0.3% of all colorectal polyps
- We achieved a high level of diagnostic reproducibility
  - Light's kappa value of 0.85 (0.81-0.89)
  - This indicates excellent concordance
  - But this was optimal conditions

Clinicopathological comparison

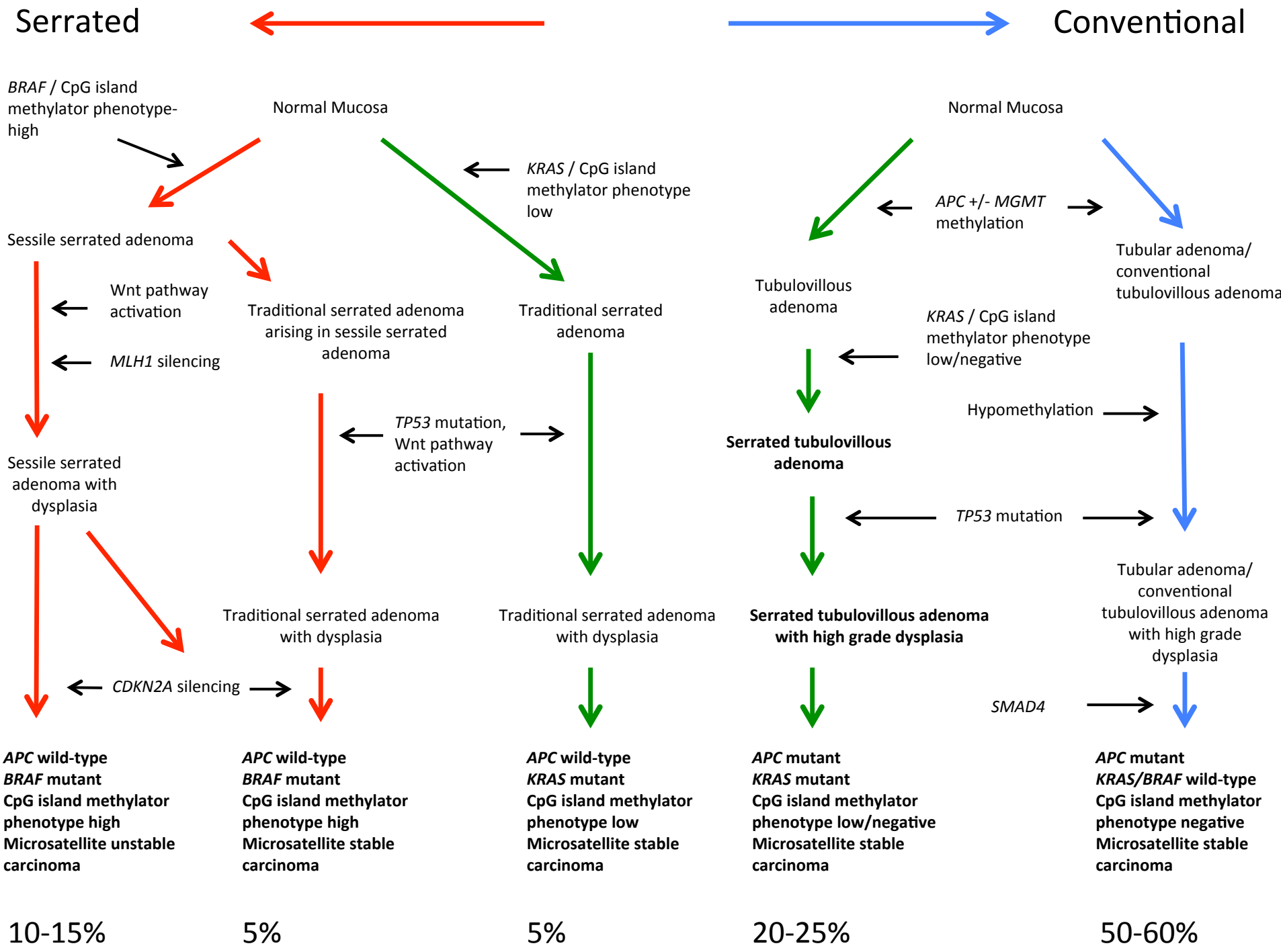
Feature	cTVA (n=50)	P-value	sTVA (n=27)	TSA (n=66)	P-value
Age	59.9	0.2460	63.4	63.8	0.9163
Female	44%	0.4667	33%	52%	0.1687
Size (mm)	13.4	**<0.0001**	21.6	18.9	0.4684
Distal	90%	**0.0027**	59%	89%	**0.0104**
Advanced	8%	**0.0088**	33%	24%	0.4417

Molecular comparison

Feature	cTVA (n=50)	P-value	sTVA (n=27)	TSA (n=66)	P-value
BRAF	0	NA	0	0	NA
KRAS	18%	**<0.0001**	67%	65%	1.0000
CIMP-H/L	6%	**0.0279**	26%	62%	**0.0026**
Nuclear β-catenin	84%	0.2384	70%	24%	**<0.0001**
P53	6%	0.2322	15%	18%	0.7723
P16	80%	0.5248	89%	79%	0.3772
MLH1 loss	0%	1.000	0%	0%	1.0000

Summary of the serrated TVA

- The serrated tubulovillous adenomas is a rare polyp that can be reliably diagnosed
- It has distinct clinicopathological and molecular features
- Compared to the conventional tubulovillous adenoma the serrated tubulovillous adenoma is
    - **larger**
    - **more often proximal**
    - **more often displays advanced histology**
    - **shows more frequent CIMP and**
    - **much more likely to harbour a *KRAS* mutation**
- Compared to the traditional serrated adenoma, the serrated tubulovillous adenoma is
    - **more often proximal**
    - **has less CIMP and**
    - **has far more frequent Wnt pathway activation**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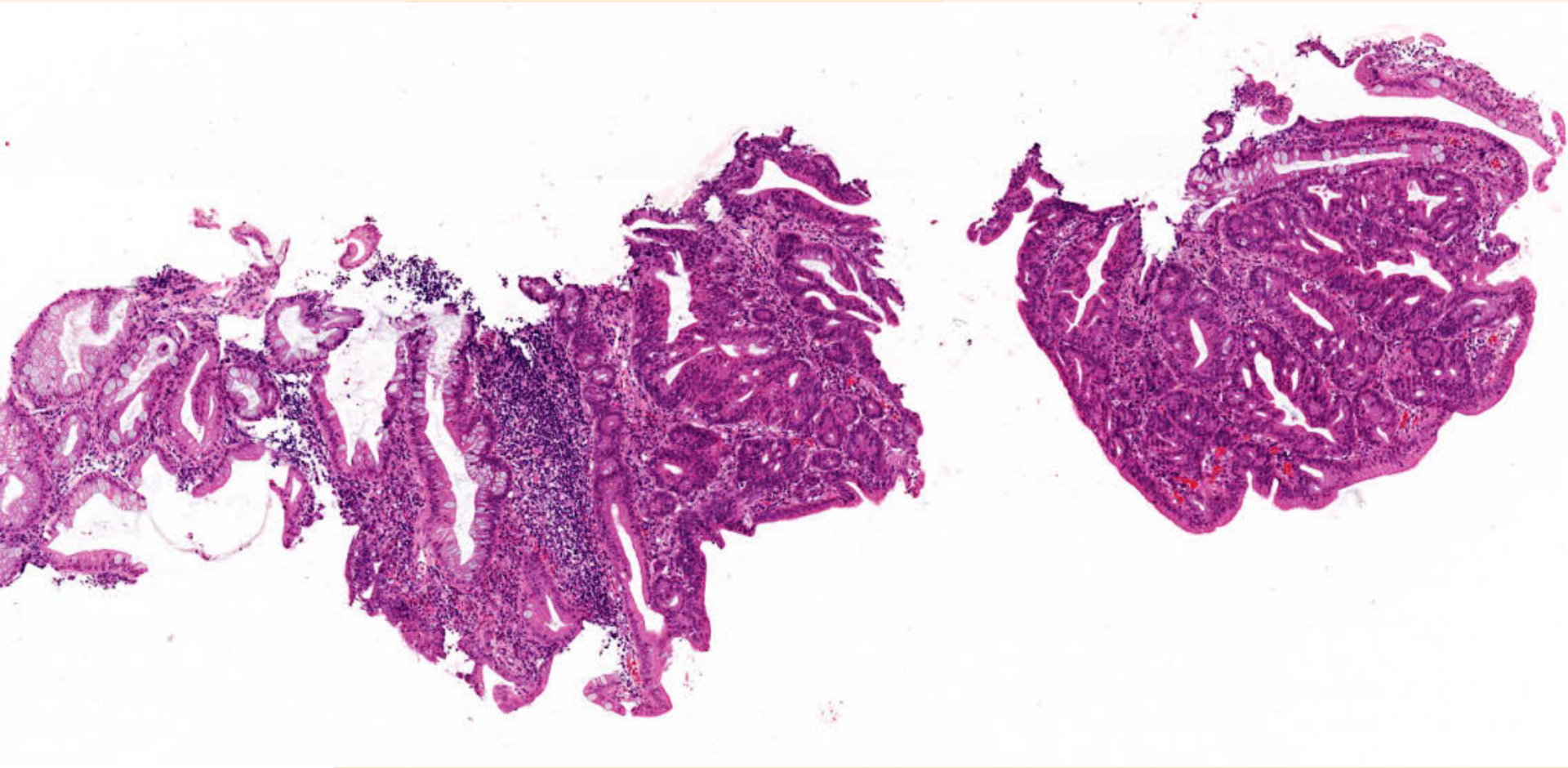
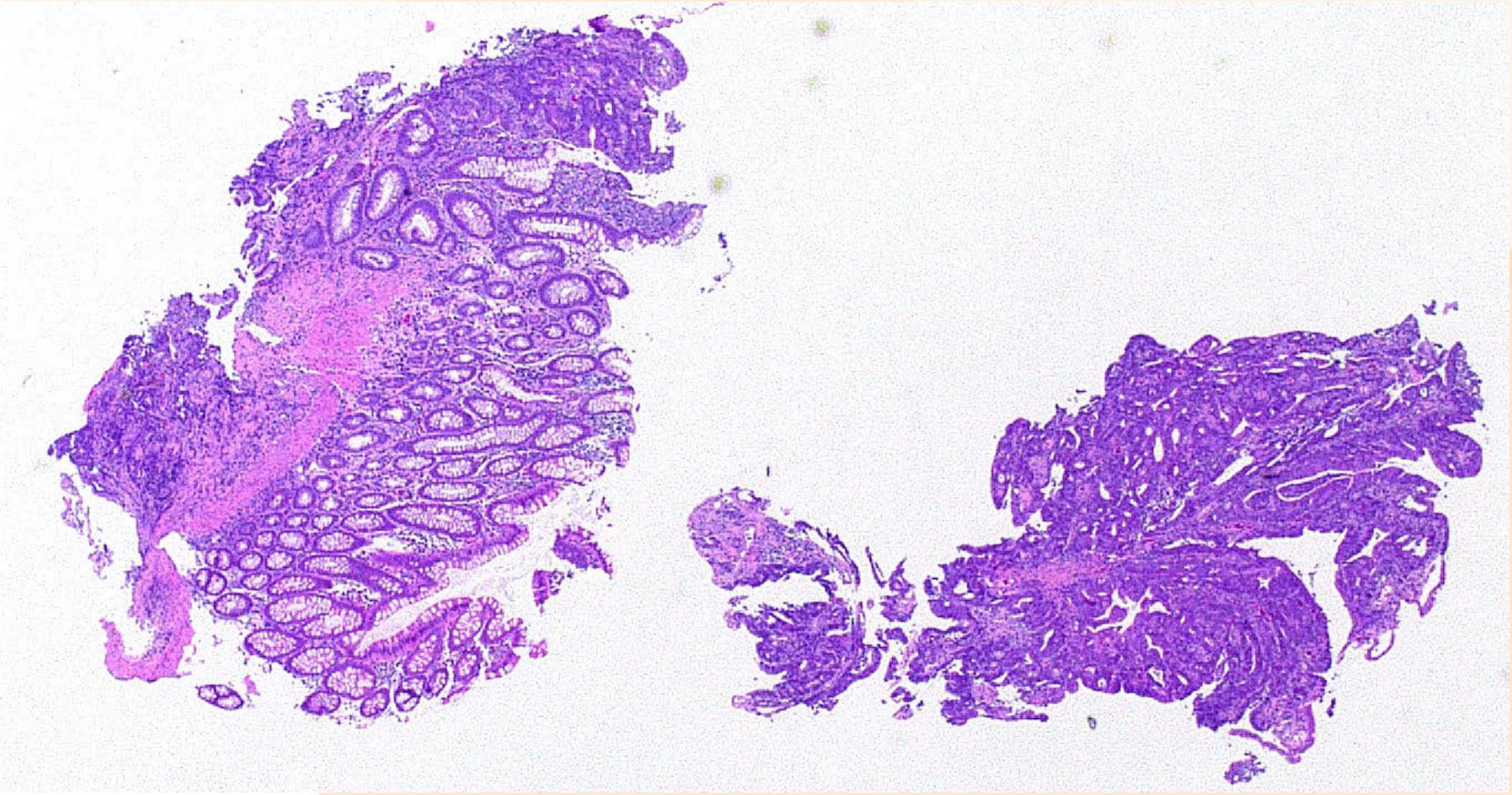
CpG island methylator phenotype low
Microsatellite stable carcinoma
***APC* mutant**
***KRAS* mutant**
CpG island methylator phenotype low/negative
Microsatellite stable carcinoma
***APC* mutant**
***KRAS/BRAF* wild-type**
CpG island methylator phenotype negative
Microsatellite stable carcinoma
10-15%
5%
5%
20-25%
50-60%

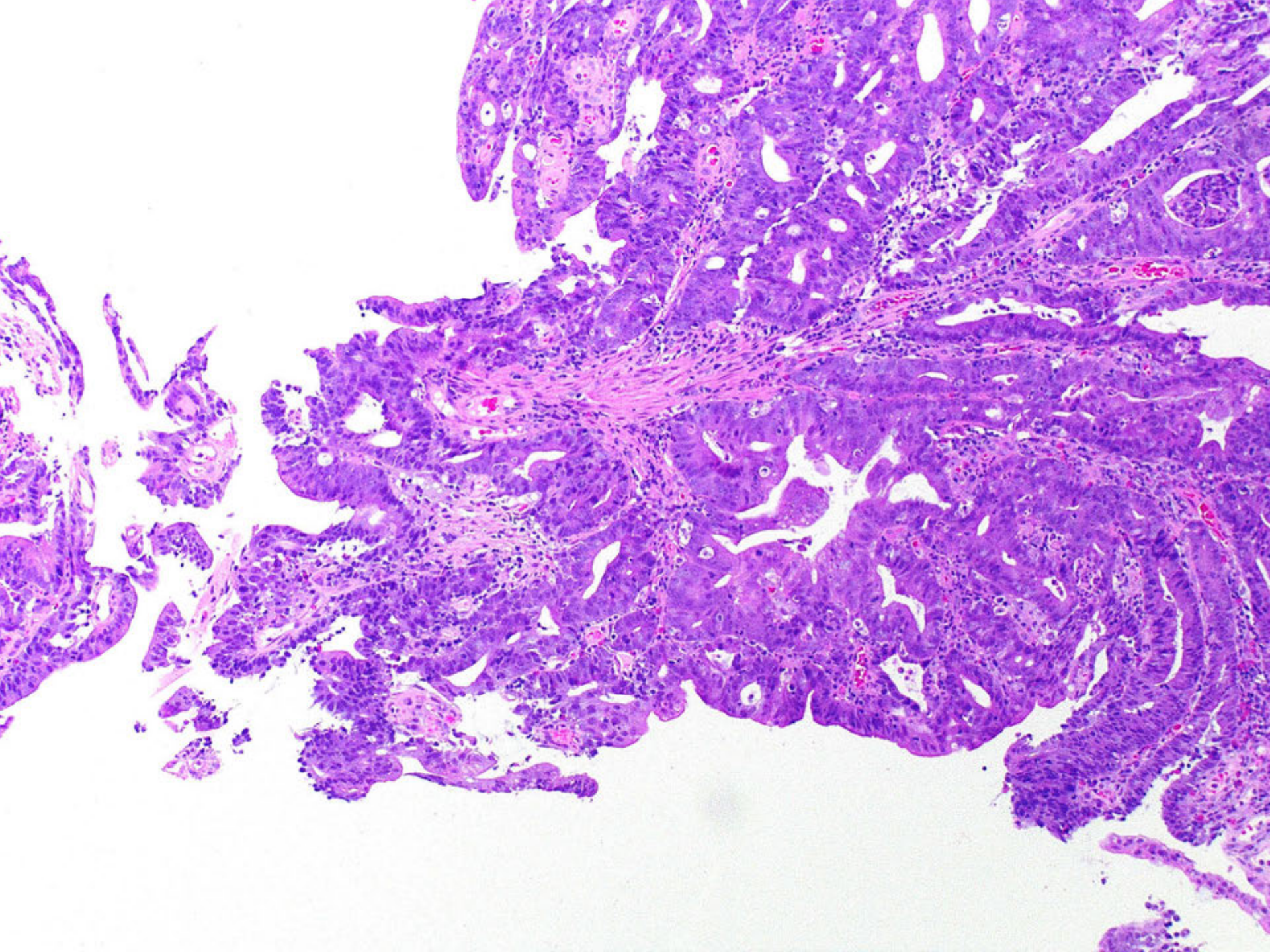
Some illustrative cases

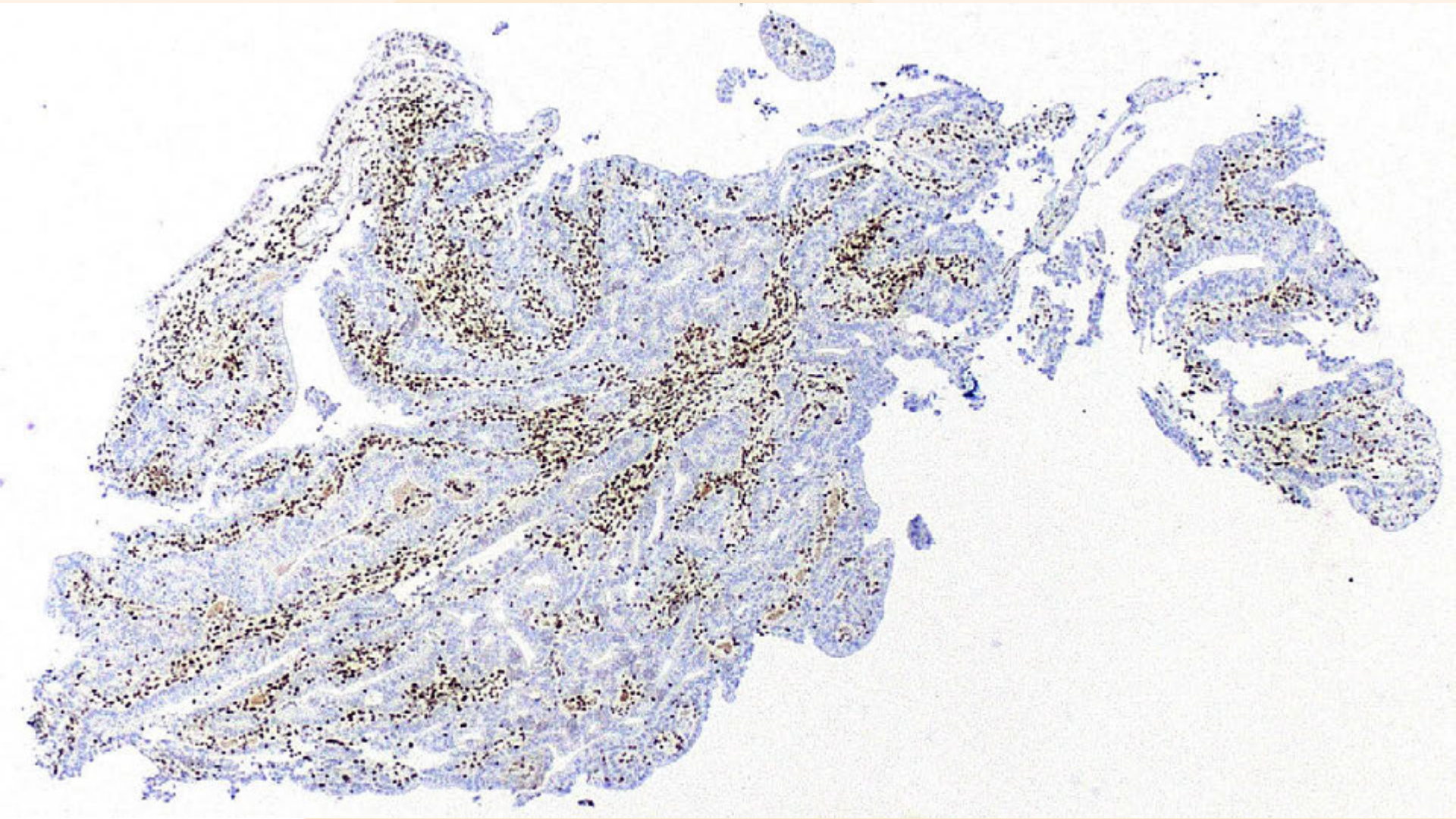
Female 76 – Biopsy of caecal polyp (10.01.2013)



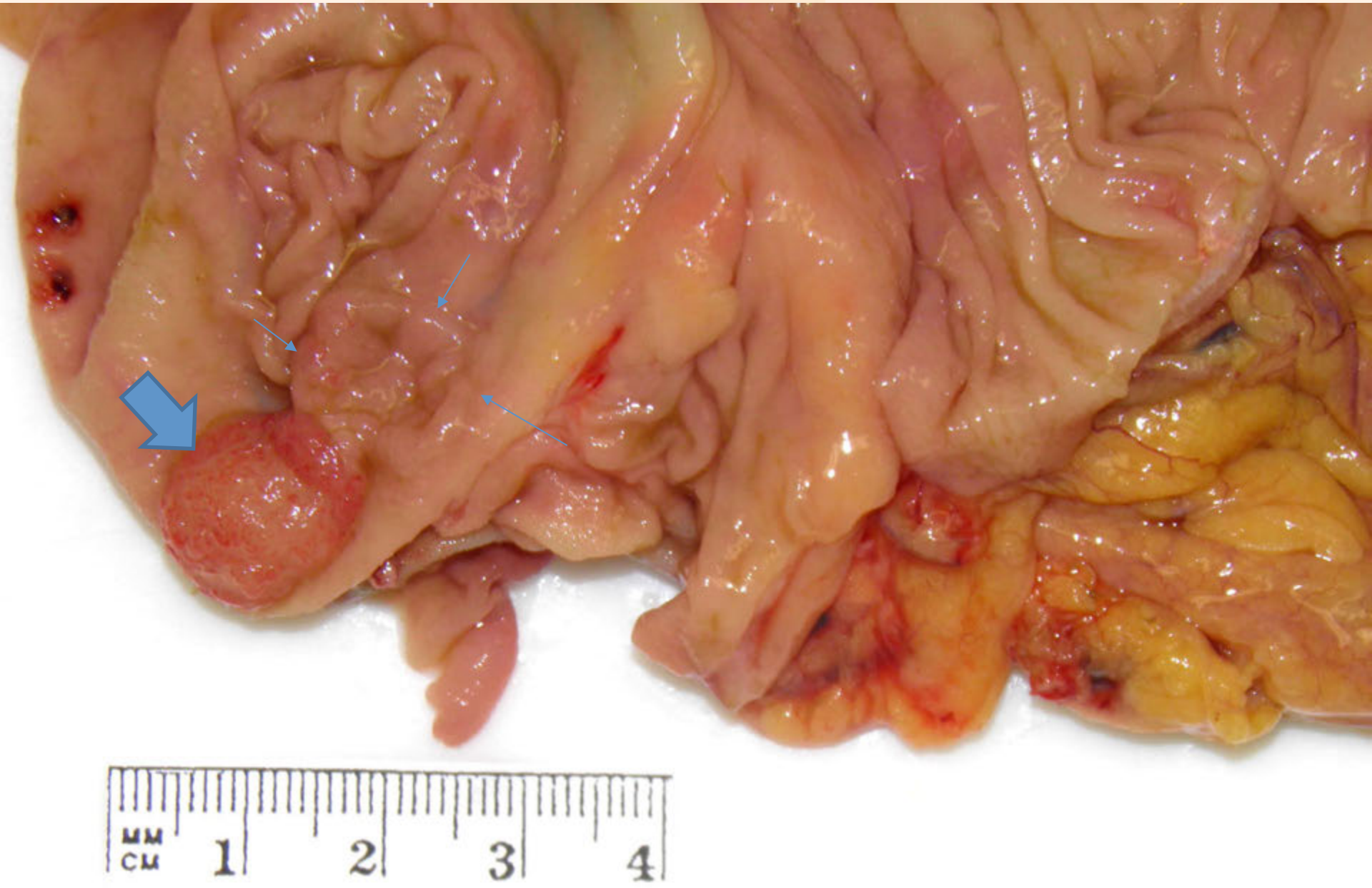
Female now 77 – Biopsy of caecal lesion (10.06.2014)

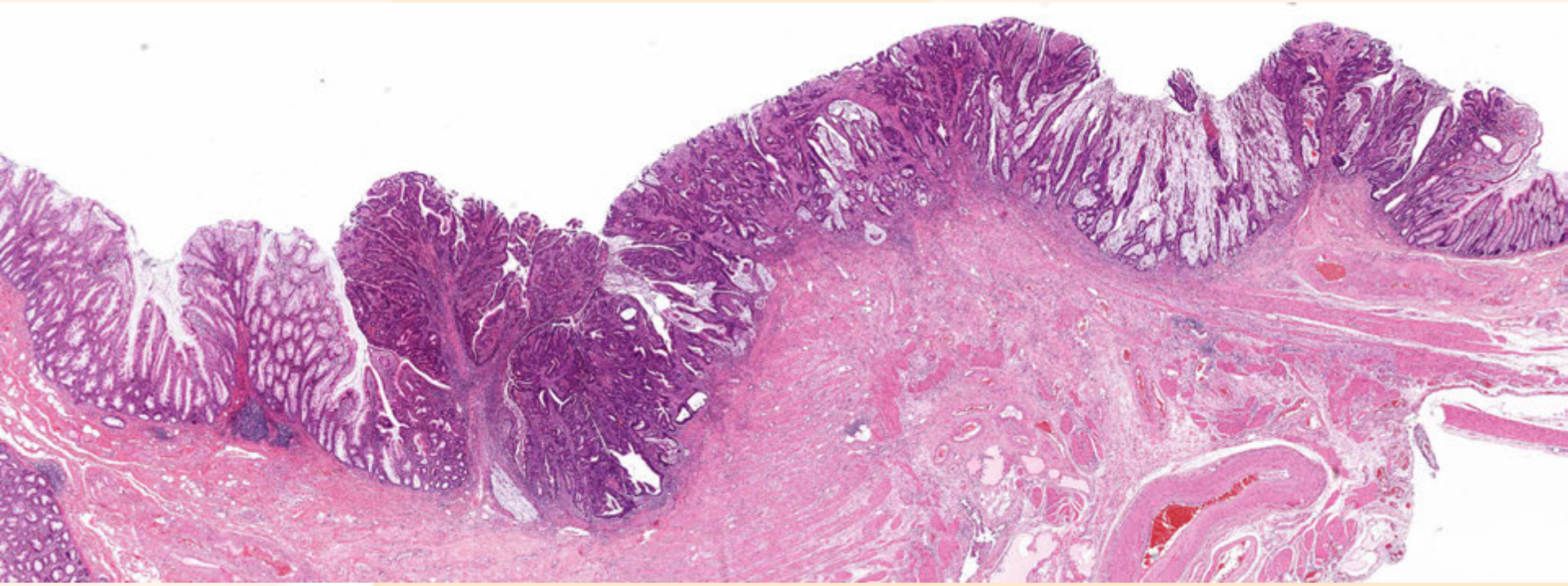






3 days later





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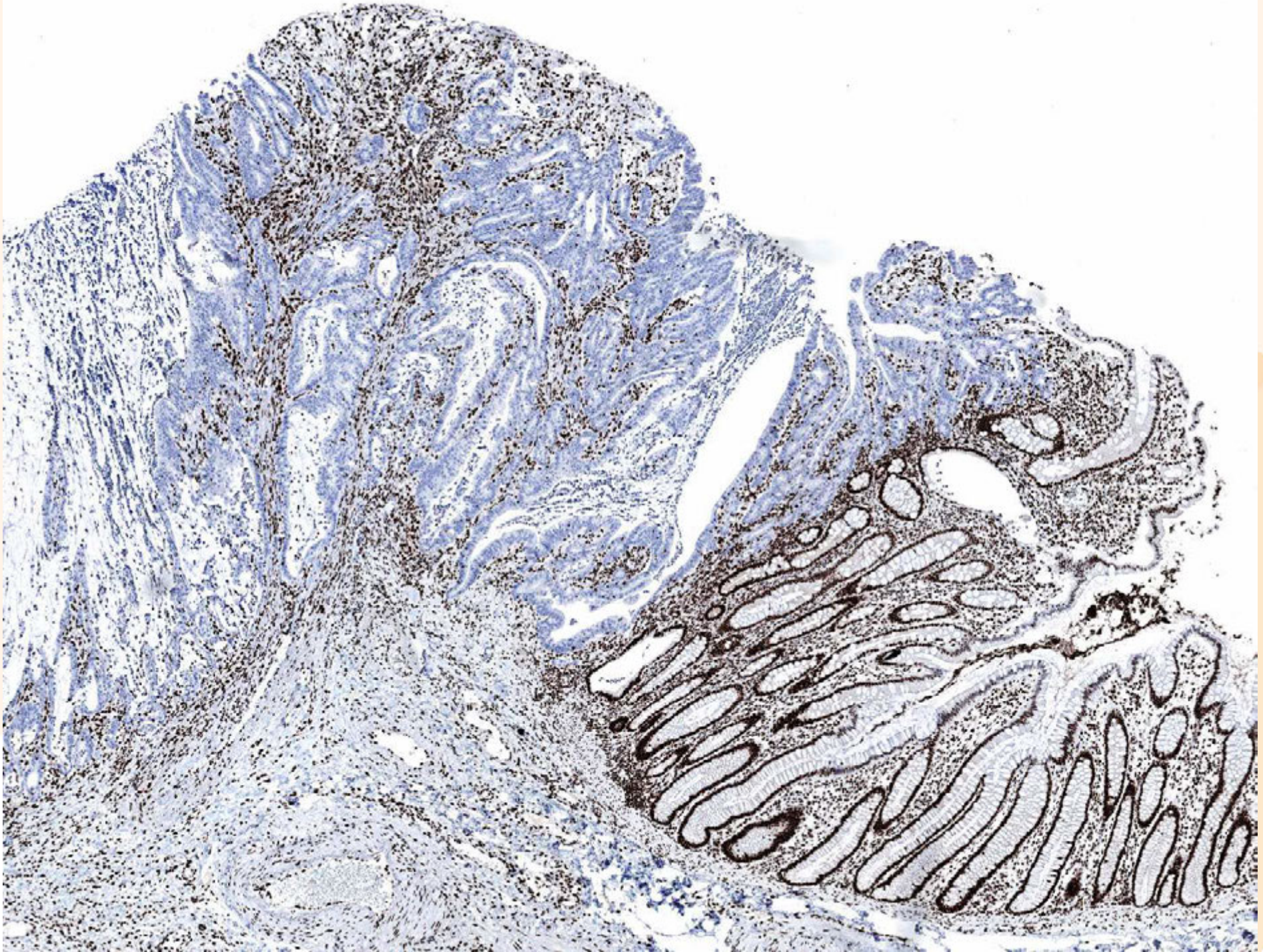
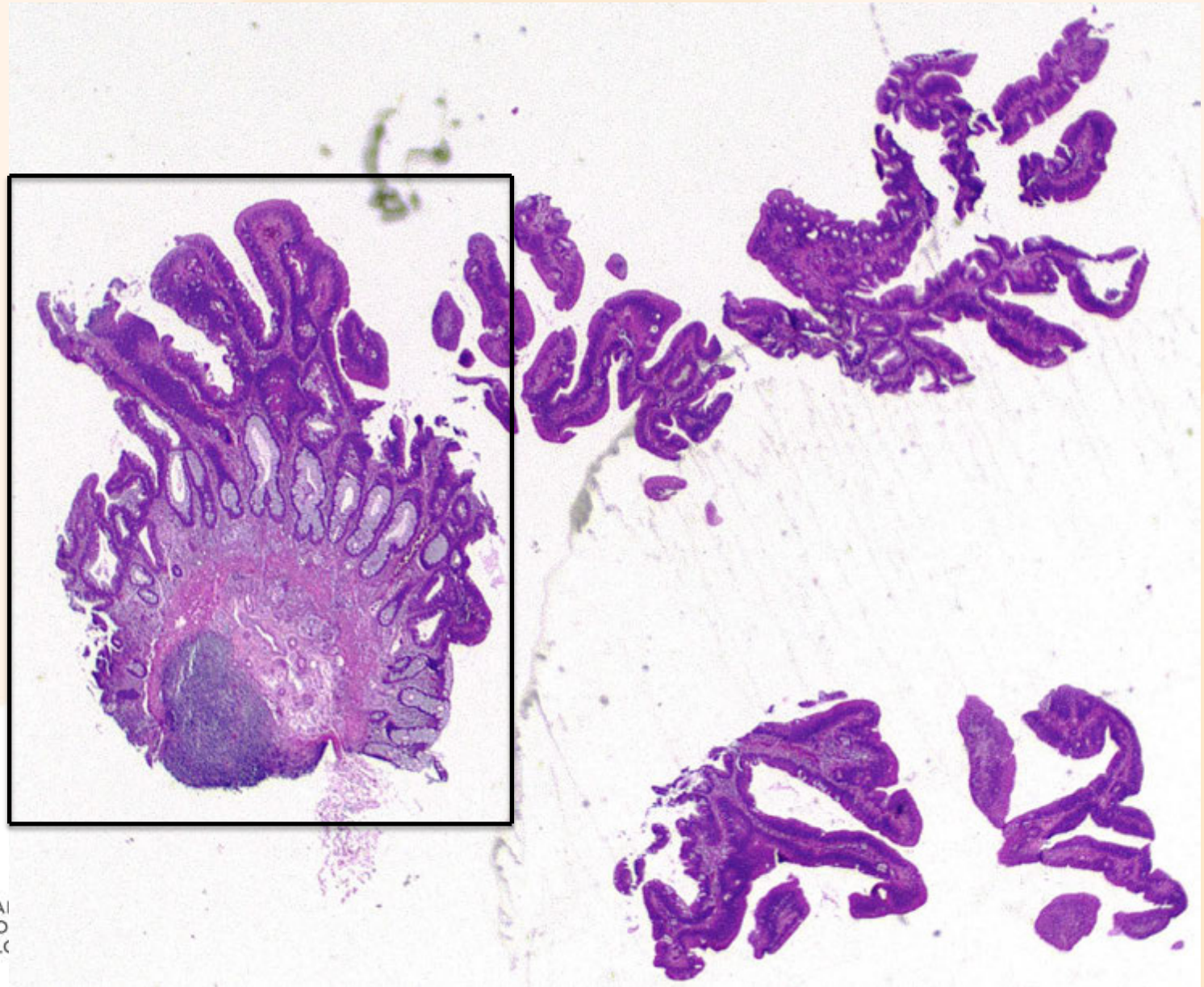
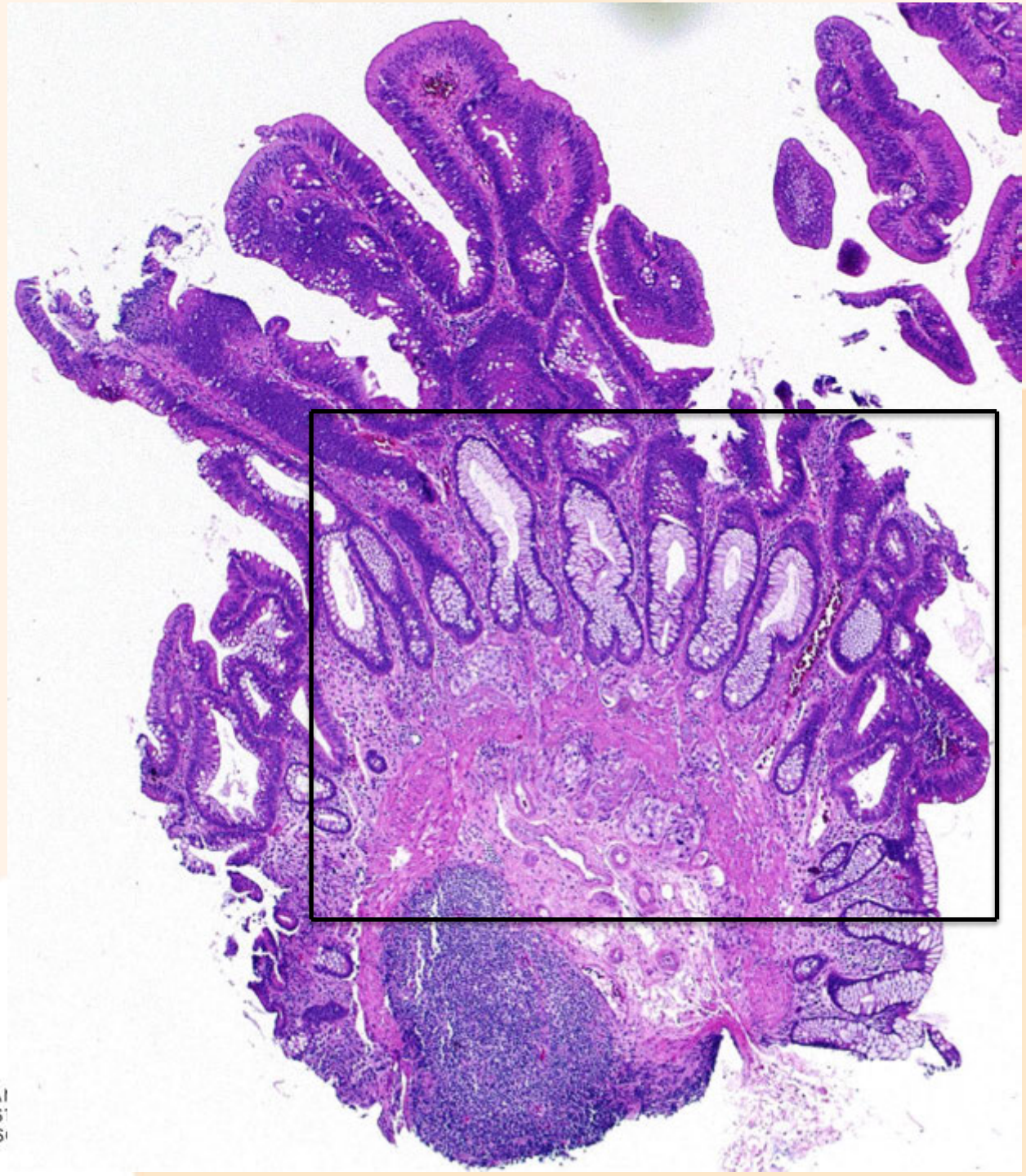


Female 27 – Biopsy of transverse colon polyp





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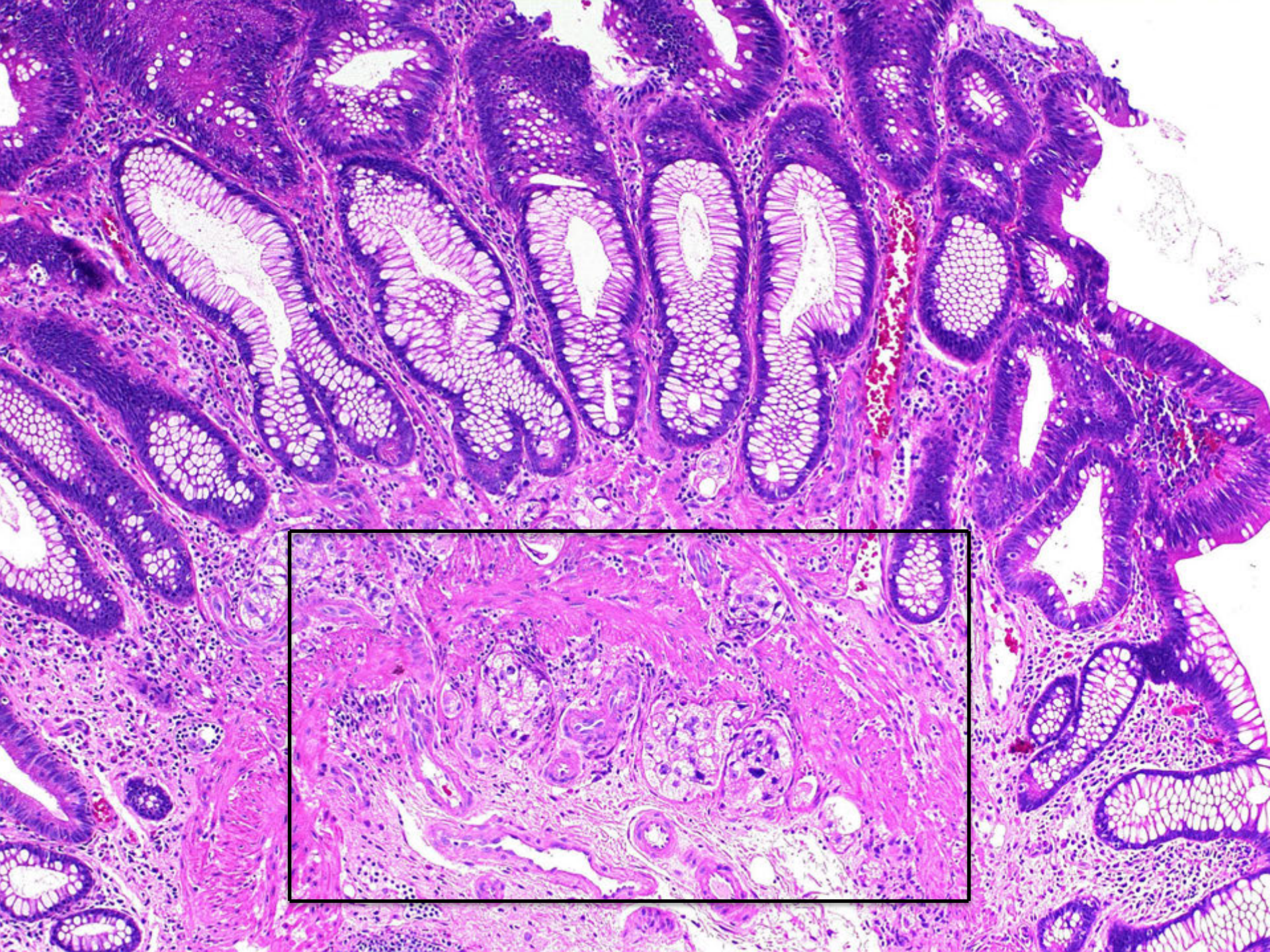

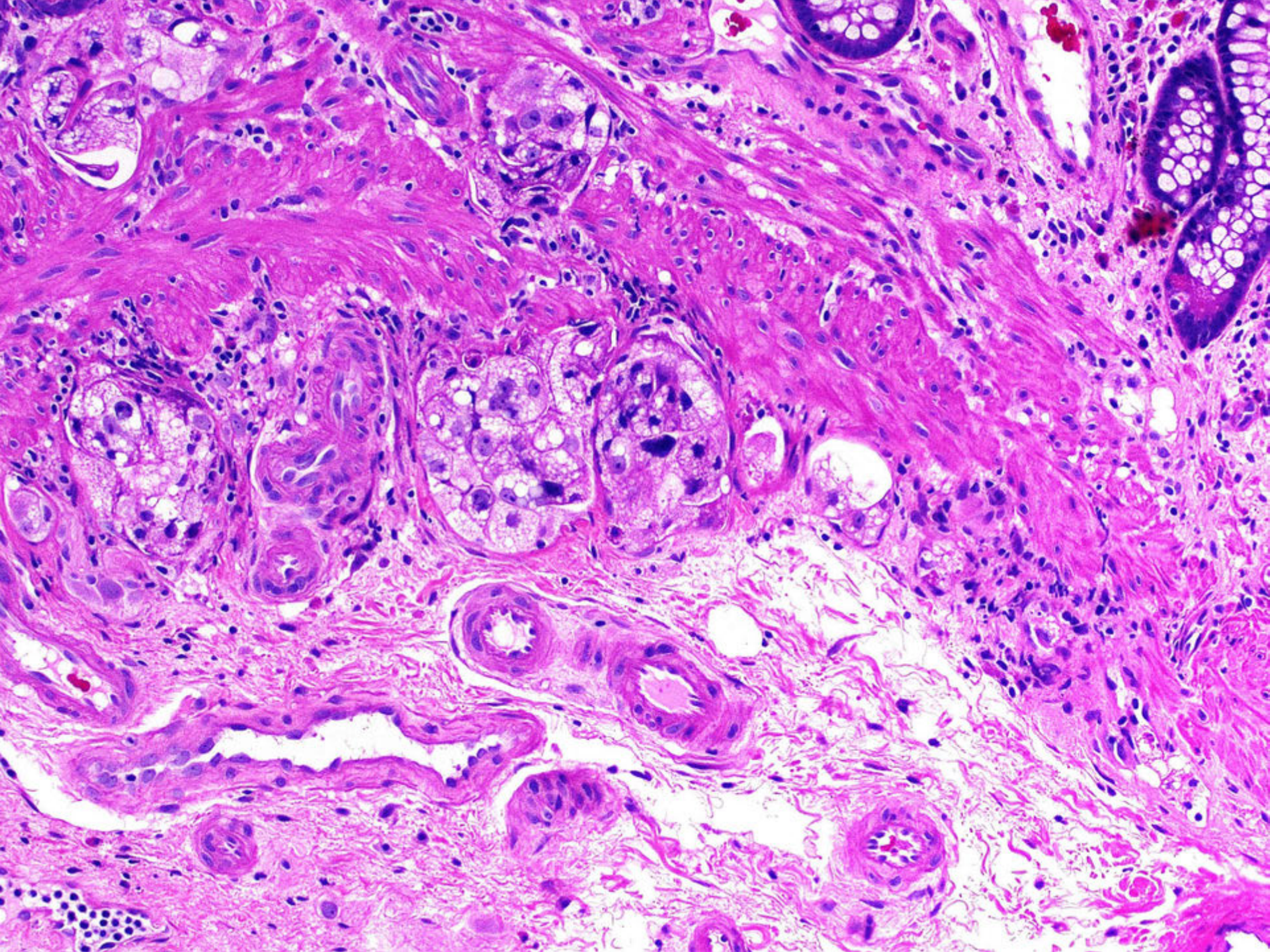

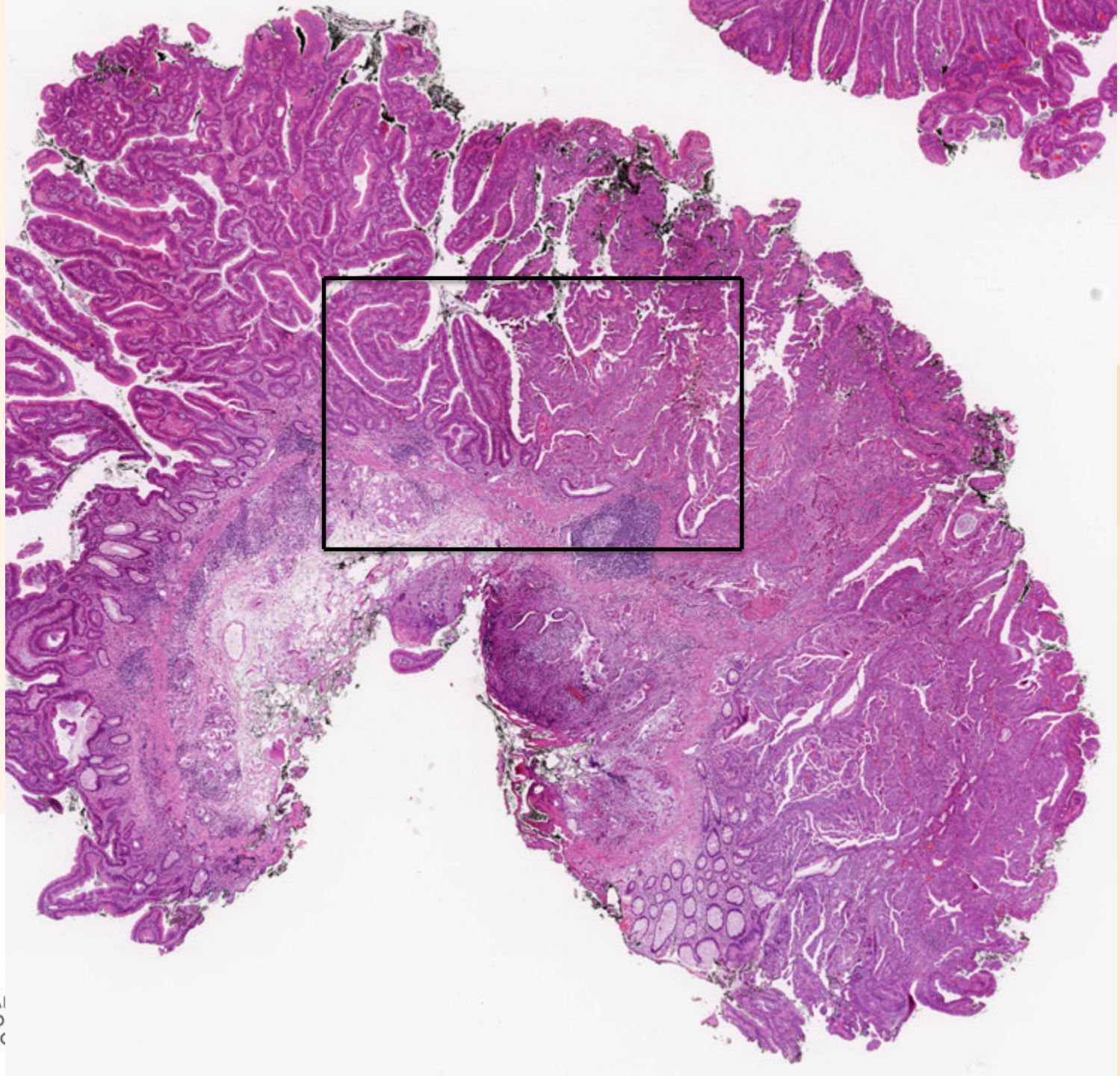

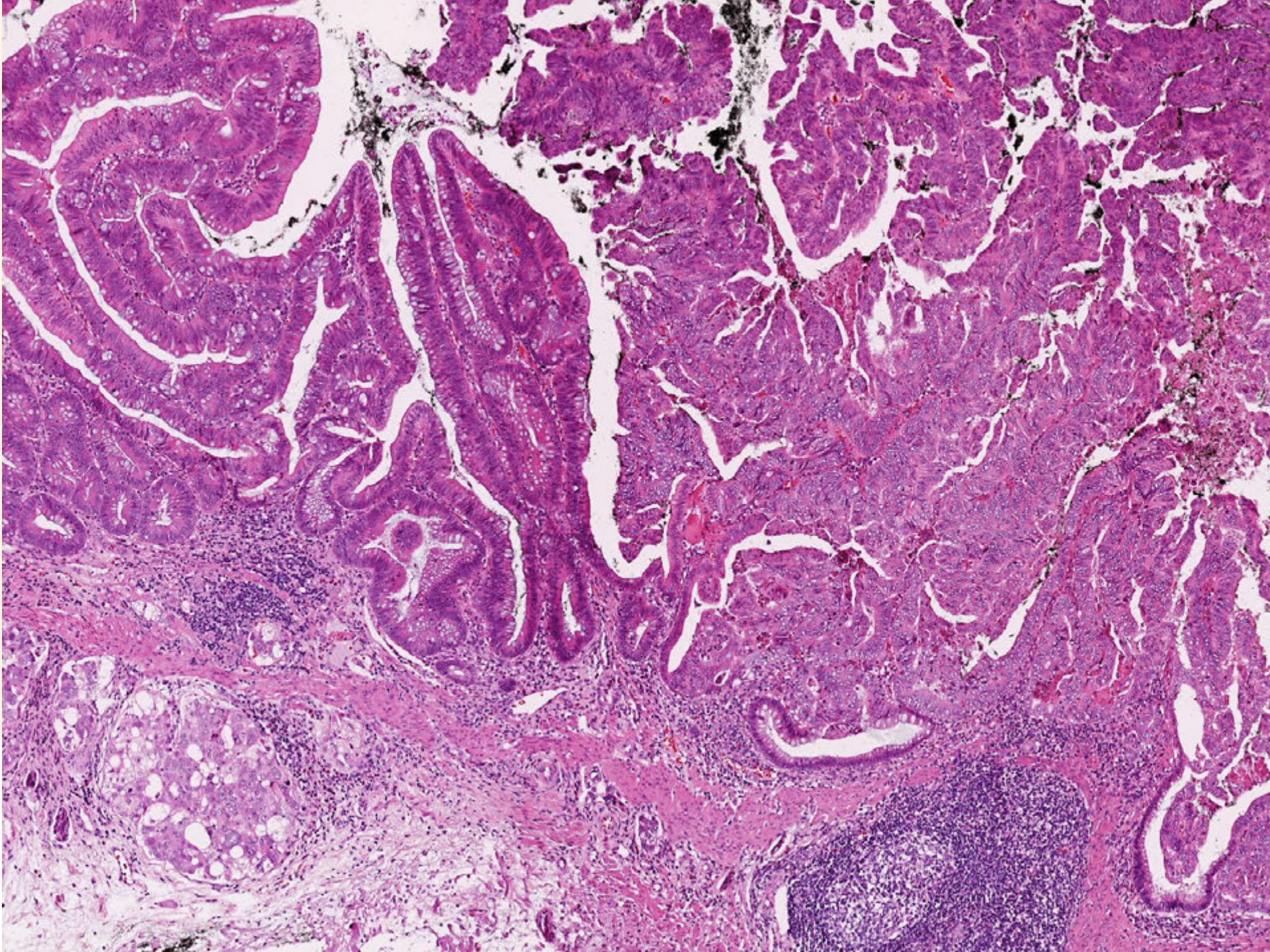

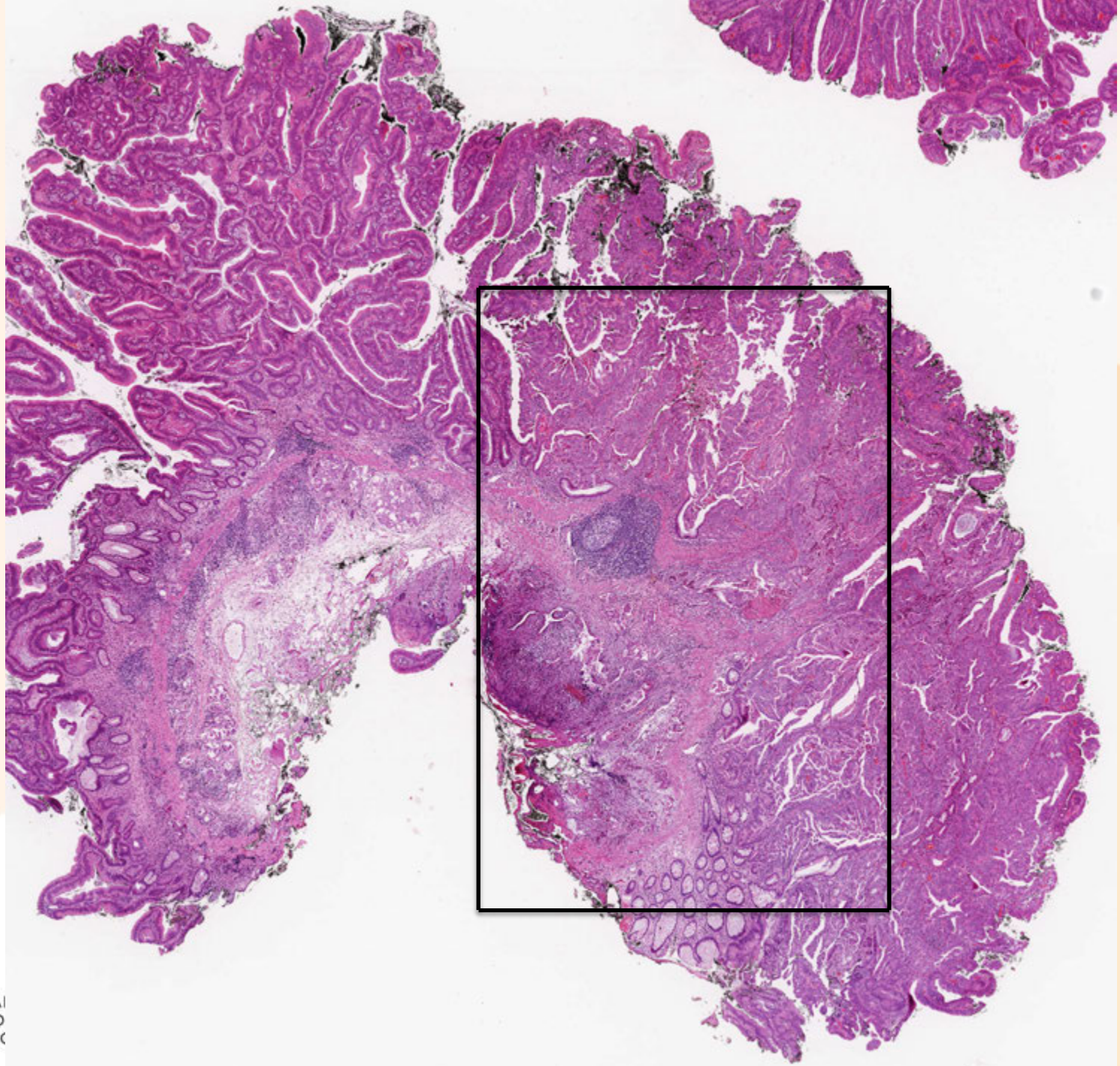

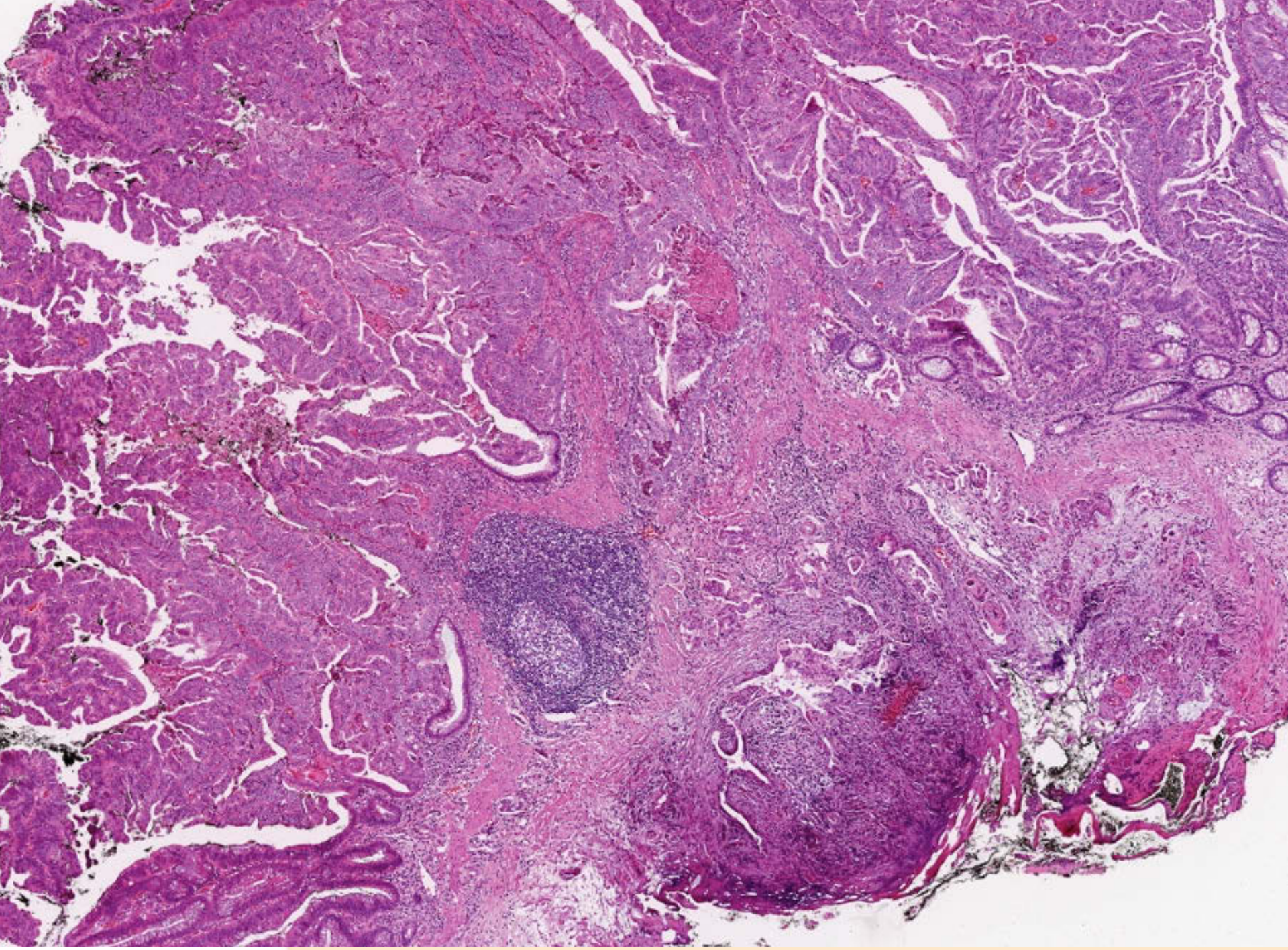

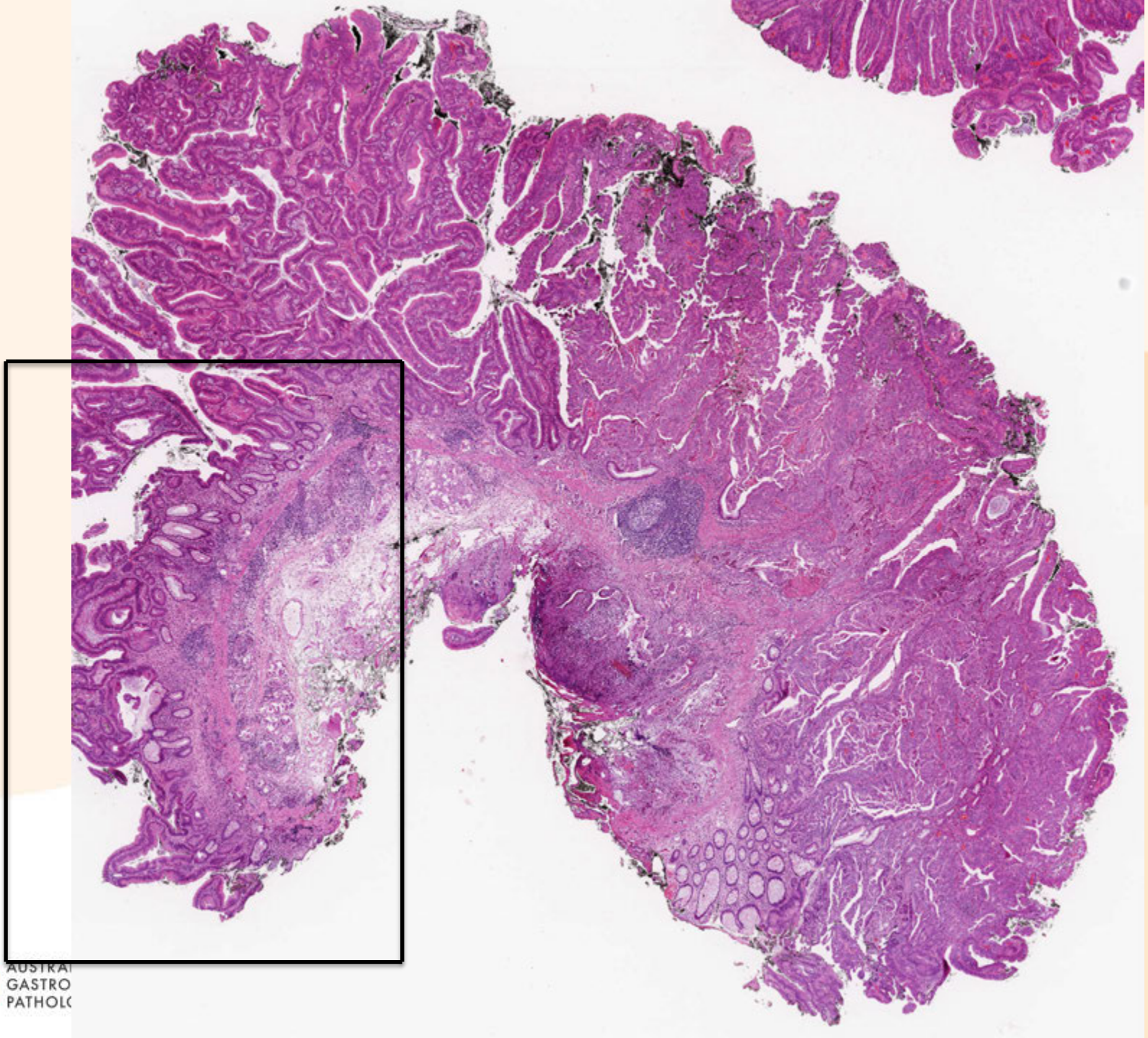

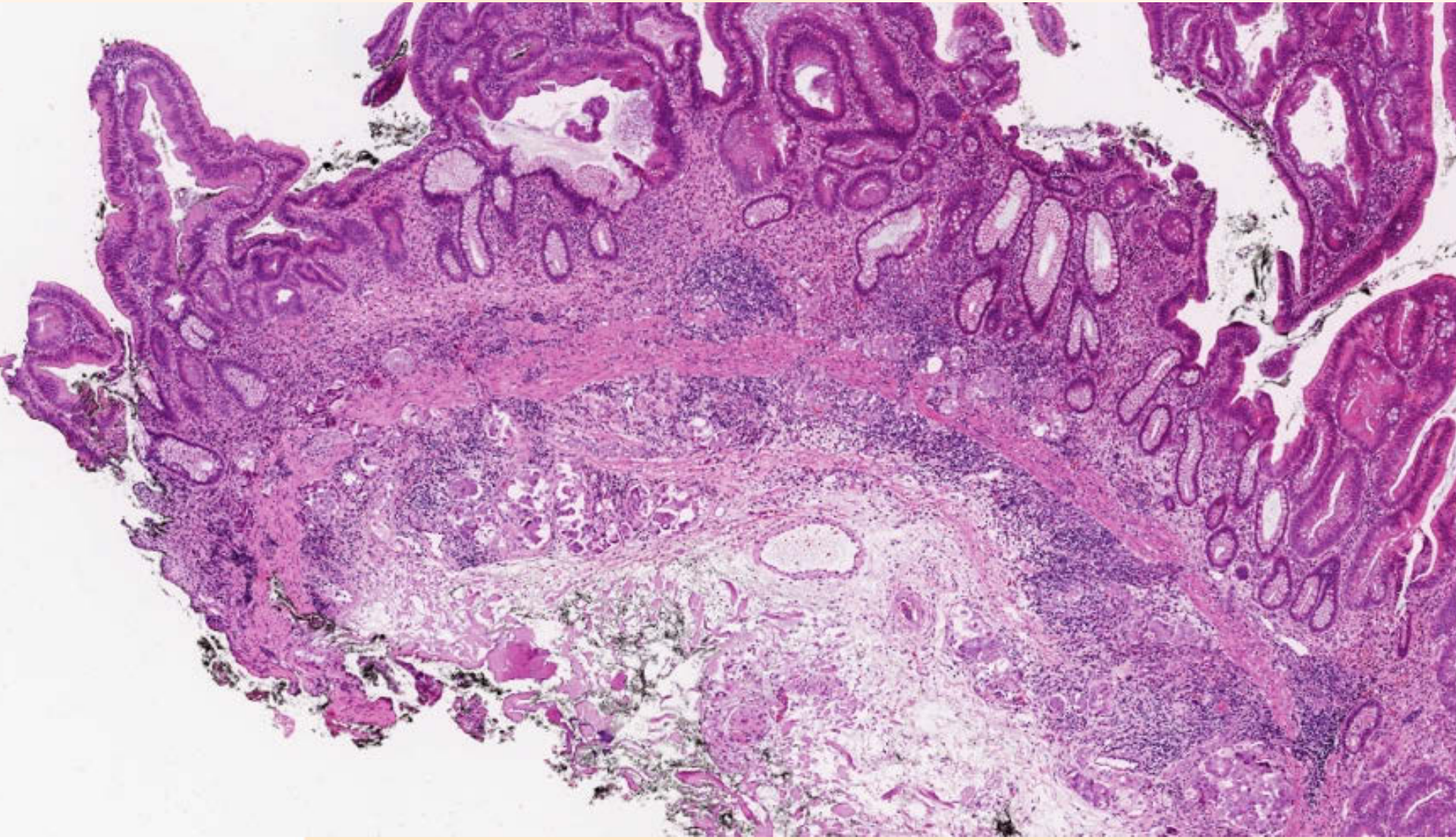

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