

# When Morphology Meets Molecular: The Evaluation of Small Biopsy Specimens of the Pancreas, Bile Duct, and Ampulla (Part 2)

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# Objectives

- Provide an algorithmic approach to the preoperative evaluation of pancreatobiliary lesions/neoplasms.
- Discuss next-generation needles for the evaluation of solid lesions of the pancreas.
- Review pancreatic cysts and the clinical utility of molecular testing.
- Present data on molecular testing of bile duct specimens for the assessment of associated strictures.



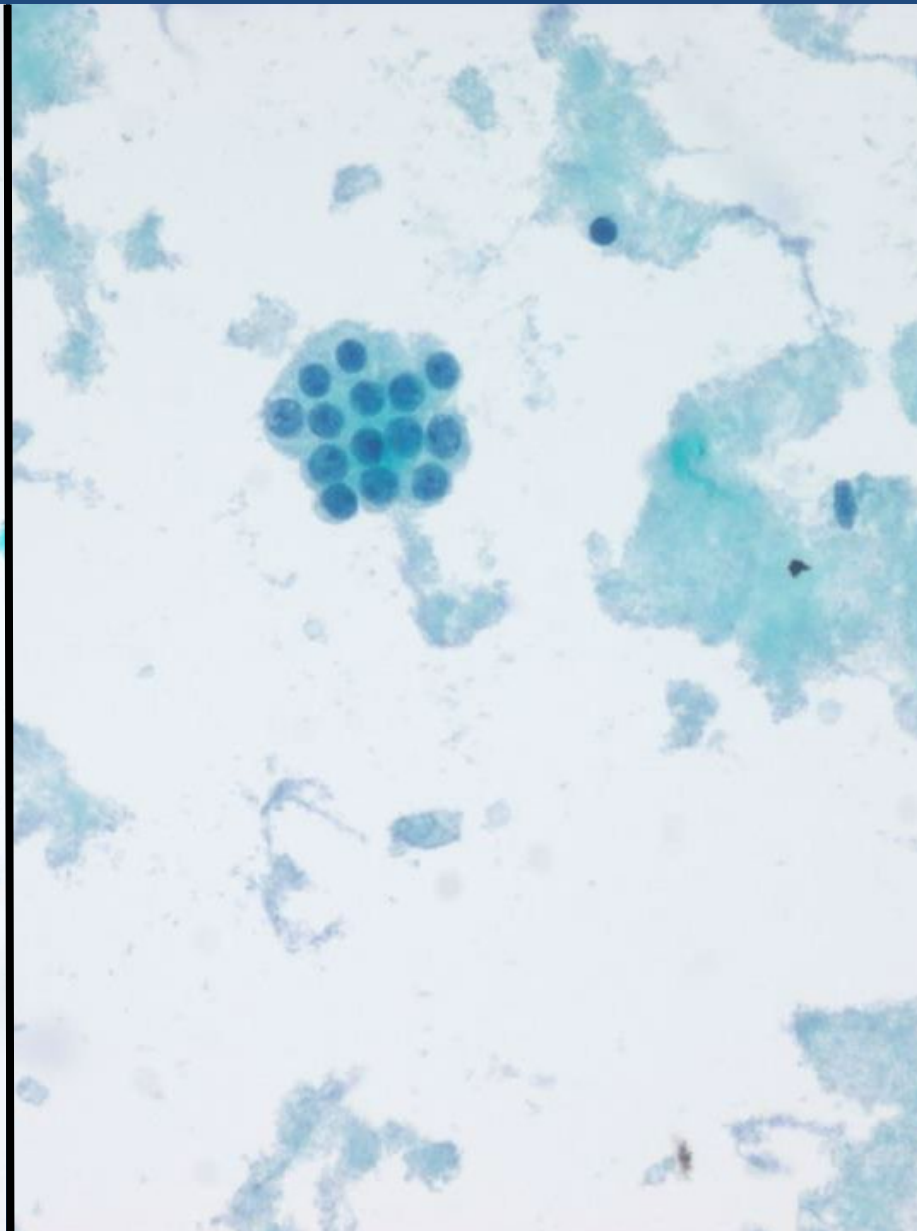
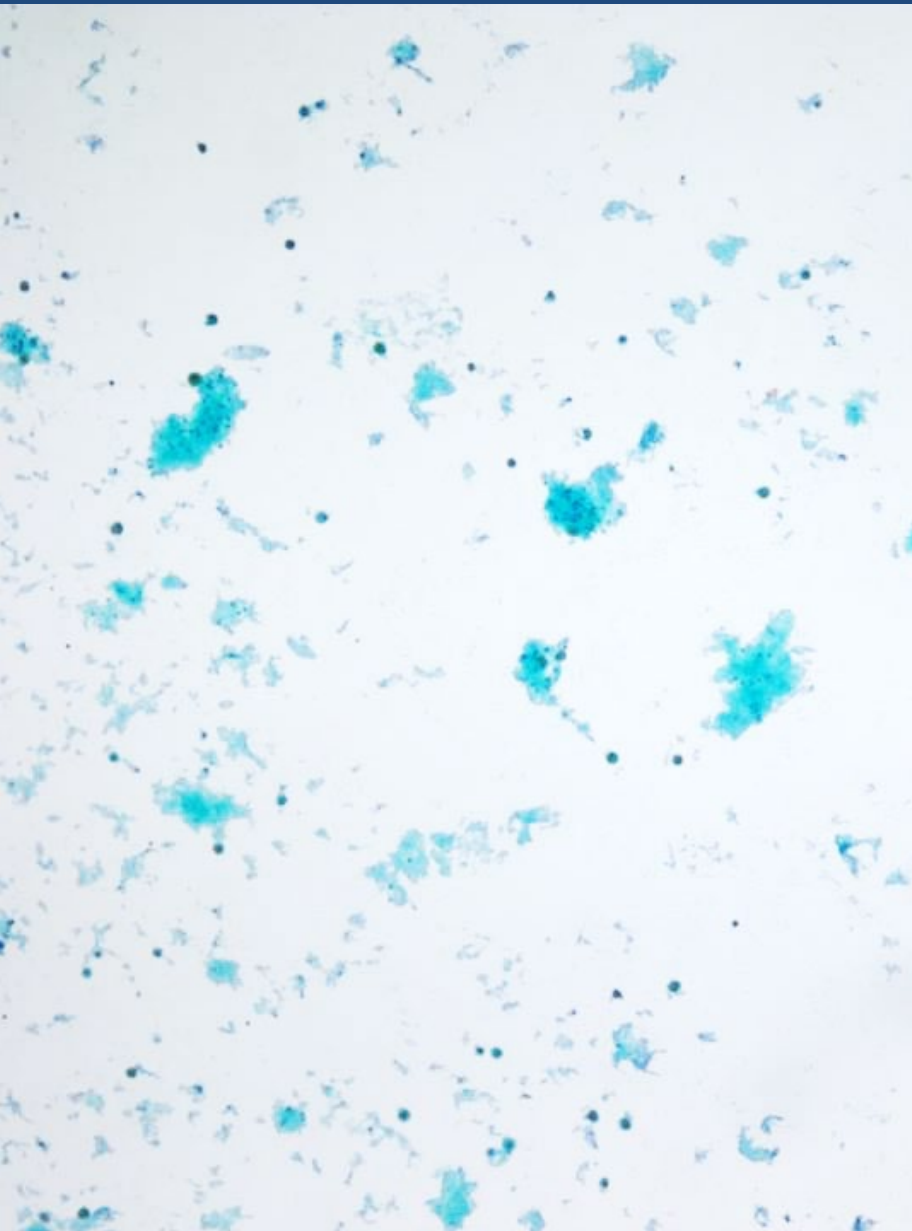
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# Case 5

- A 44-year-old female with a history of breast cancer had a staging CT scan that identified a 3.1 cm cyst within the pancreatic head.
- An endoscopic ultrasound (EUS) confirmed the presence of a 3.1 cm pancreatic cyst that was multiseptated by imaging.
- A fine-needle aspiration (FNA) yielded thin fluid, which was submitted for cytopathologic examination and molecular testing.

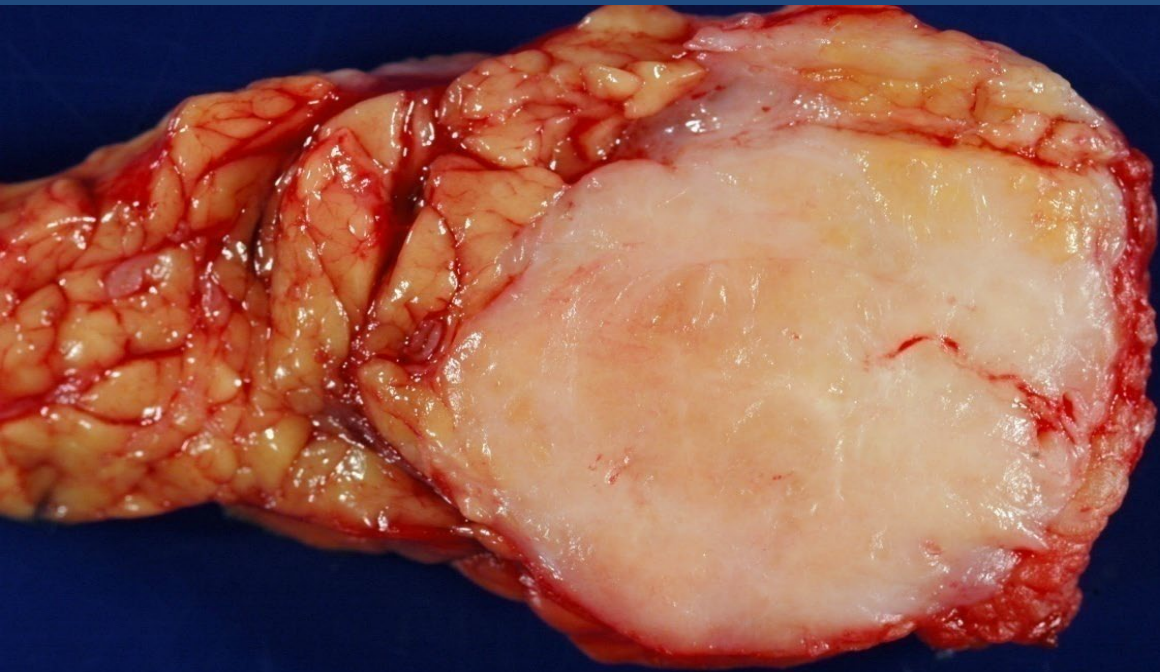
# Case 5



- 44-year-old female with a 3.1 cm pancreatic head cyst
- **Cytopathologic findings:**
  - Paucicellular smears with significant debris
  - Rare clusters of cuboidal cells with round nuclei, and scant cytoplasm
  - Nuclear contours are smooth
  - No definitive evidence of high-grade atypia
- **Molecular testing:**
  - A mutations in **VHL** was identified
  - No other genomic alterations were seen



# Solid



# Cystic





# Cystic



# Differential Dx



60,430

Estimated new cases of pancreatic cancer (equivalent number of deaths).

9,065

Pancreatic cancers arising from a mucinous pancreatic cyst (IPMN/MCN).

6,462,000

Number of Americans with a pancreatic cyst.

Pseudocyst  
Foregut Cyst  
Retention Cyst  
Lymphoepithelial Cyst  
Acinar Cell Cystadenoma

Squamoid Cyst  
Serous Cystadenoma (SCA)  
Cystic PanNET  
Solid-Pseudopapillary Neoplasm

**IPMN = Intraductal Papillary Mucinous Neoplasm**

**MCN = Mucinous Cystic Neoplasm**



60,430

Estimated new cases of pancreatic cancer (equivalent number of deaths).

9,065

Pancreatic cancers arising from a mucinous pancreatic cyst (IPMN/MCN).

6,462,000

Number of Americans with a pancreatic cyst.

3,231,000

Number of Americans with a mucinous pancreatic cyst.

**Not all mucinous pancreatic cysts (IPMNs and MCNs) will transform into Pancreatic Cancer.**

60,430

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6,462,000

Number of Americans with a pancreatic cyst.

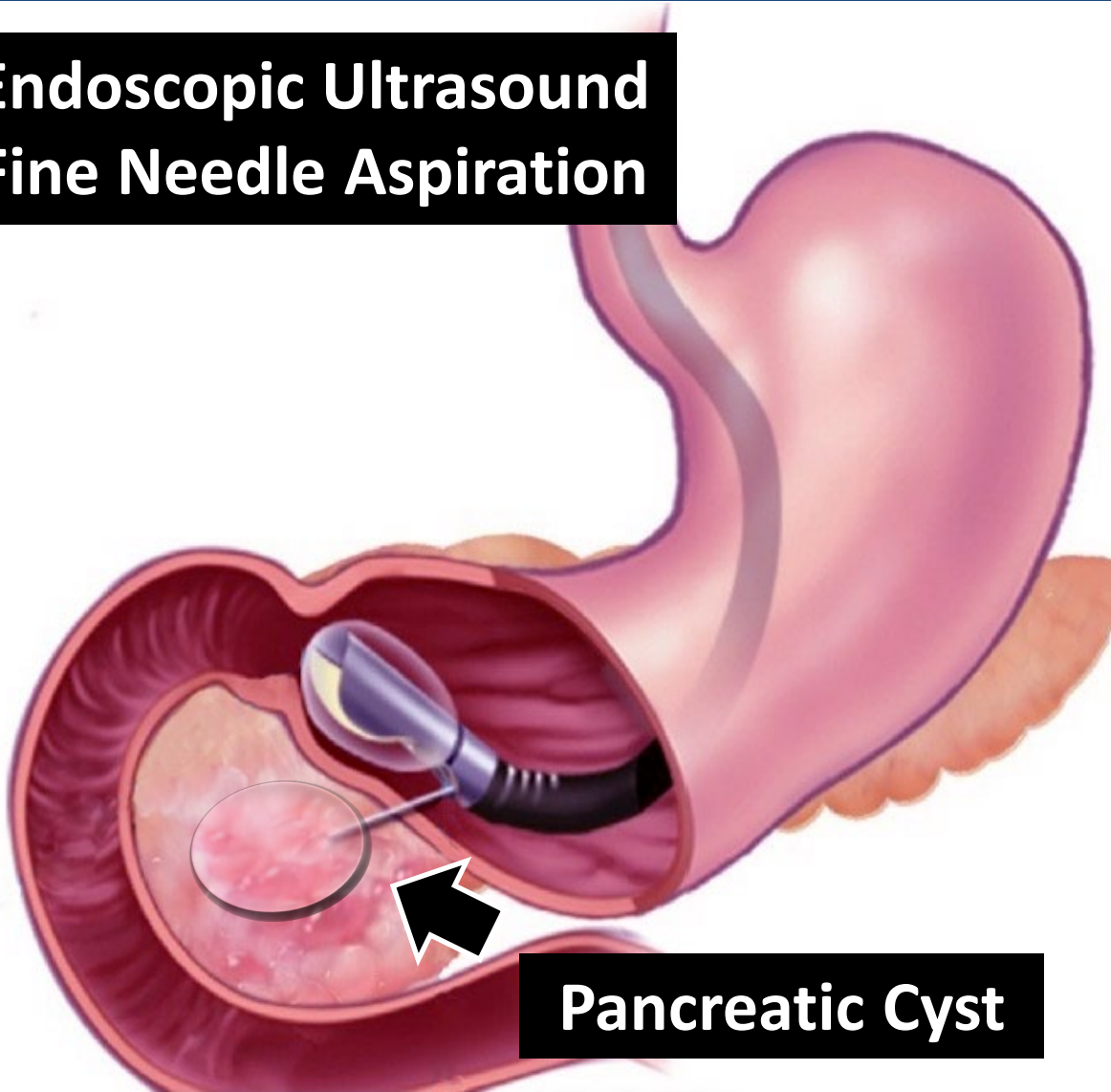
3,231,000

Number of Americans with a mucinous pancreatic cyst.



# Pancreatic Cysts

**Endoscopic Ultrasound  
Fine Needle Aspiration**



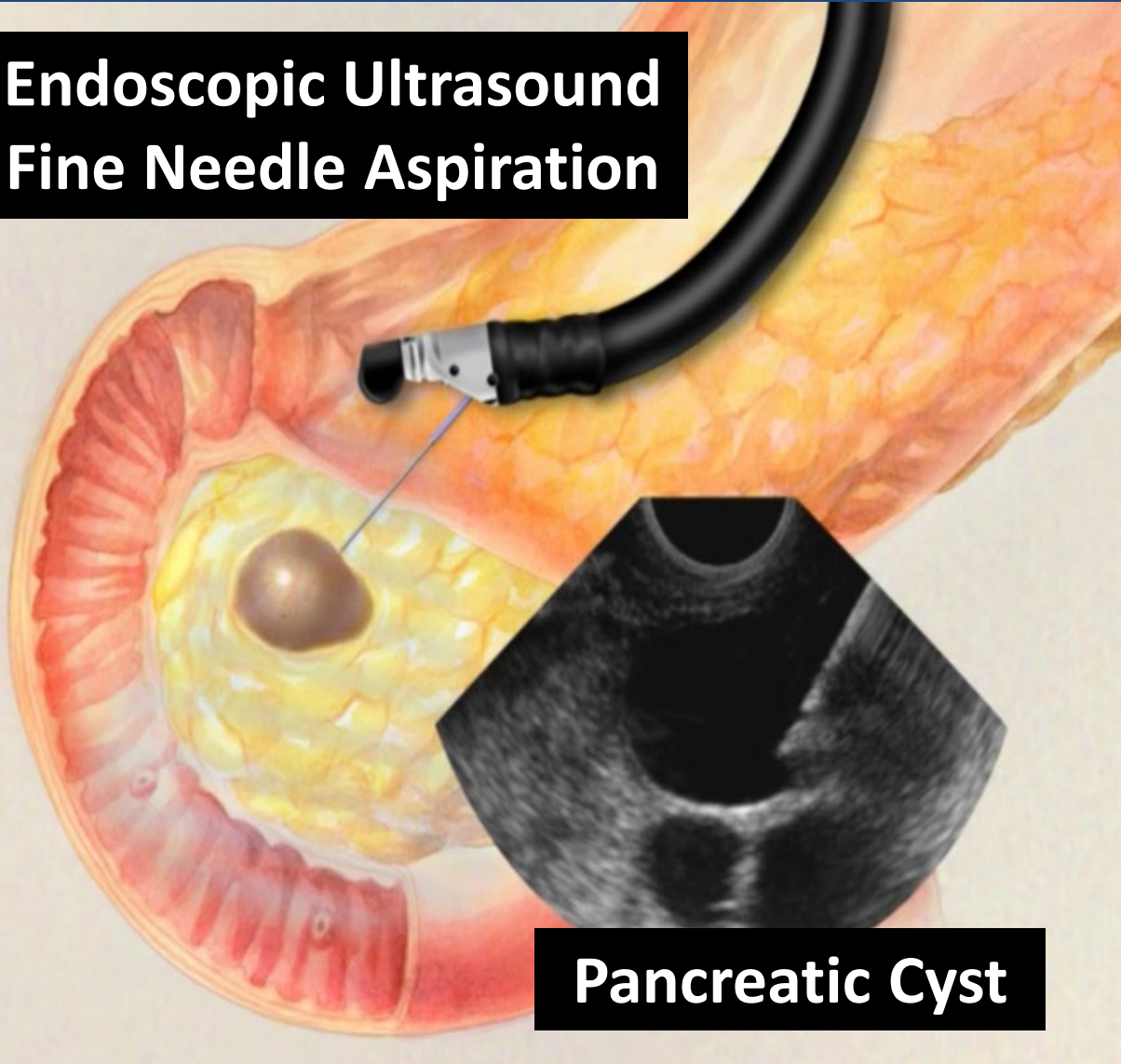
**Pancreatic Cyst**

## **Multidisciplinary Approach:**

- Clinical presentation
- Cross-sectional imaging
- Endoscopic ultrasound (EUS)
- Fine needle aspiration (FNA)
- Chemistry: CEA, Amylase
- Cytopathology

# Pancreatic Cysts

Endoscopic Ultrasound  
Fine Needle Aspiration



Pancreatic Cyst

- Endoscopic ultrasound (EUS) allows for high resolution imaging of the pancreatic cyst and relationship to the main pancreatic duct.
- Ancillary studies using **aspirated cyst fluid** include: **CEA** (marker of mucinous cysts), **glucose**, **amylase** and **cytologic evaluation**.

# Pancreatic Cysts

	<b>IPMN</b>	<b>MCN</b>	<b>SCA*</b>	<b>SPN*</b>	<b>Pseudocyst</b>
<b>Gender</b>	<b>M&gt;F</b>	<b>F&gt;&gt;M</b>	<b>F&gt;M</b>	<b>F&gt;&gt;M</b>	<b>M&gt;F</b>
<b>Location</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;&gt;Head</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;Head</b>	<b>Head=Tail</b>
<b>Viscosity</b>	<b>Increased</b>	<b>Increased</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>
<b>CEA</b>	<b>&gt;192 ng/mL</b>	<b>&gt;192 ng/mL</b>	<b>&lt;0.5 ng/mL</b>	<b>&lt;192 ng/mL</b>	<b>&lt;192 ng/mL</b>
<b>Amylase</b>	<b>High</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>	<b>High</b>
<b>Cytology</b>	<b>Mucinous</b>	<b>Mucinous</b>	<b>Scant, Bland PAS+</b>	<b>Papillary &amp; Vascular</b>	<b>Pigmented Histiocytes</b>

**SCA** = Serous Cystadenoma

**SPN** = Solid Pseudopapillary Neoplasm

# Pancreatic Cysts

	IPMN	MCN	SCA*	SPN*	Pseudocyst
Gender	M>F	F>>M	F>M	F>>M	M>F
Location	Head>Tail	Tail>>Head	Head>Tail	Tail>Head	Head=Tail
Viscosity	Increased	Increased	Low	Low	Low
CEA	>192 ng/mL	>192 ng/mL	<0.5 ng/mL	<192 ng/mL	<192 ng/mL
Amylase	High	Low	Low	Low	High
Cytology	Mucinous	Mucinous	Scant, Bland PAS+	Papillary & Vascular	Pigmented Histiocytes

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# Pancreatic Cysts

**IPMN**

**MCN**

**SCA\***

**SPN\***

**Pseudocyst**

## Scant-to-No Cellularity

<b>Viscosity</b>	<b>Increased</b>	<b>Increased</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>
<b>CEA</b>	<b>&gt;192 ng/mL</b>	<b>&gt;192 ng/mL</b>	<b>&lt;0.5 ng/mL</b>	<b>&lt;192 ng/mL</b>	<b>&lt;192 ng/mL</b>
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**SCA** = Serous Cystadenoma

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# Pancreatic Cysts

**IPMN**

**MCN**

**SCA\***

**SPN\***

**Pseudocyst**

**Scant-to-No Cellularity**

**Gastrointestinal Tract Contamination**

**Amylase**

**High**

**Low**

**Low**

**Low**

**High**

**Cytology**

**Mucinous**

**Mucinous**

**Scant,  
Bland PAS+**

**Papillary &  
Vascular**

**Pigmented  
Histiocytes**

**SCA** = Serous Cystadenoma

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# Pancreatic Cysts

IPMN

MCN

SCA\*

SPN\*

Pseudocyst

Scant-to-No Cellularity

Gastrointestinal Tract Contamination

Inaccuracies with CEA and Amylase

**SCA** = Serous Cystadenoma

**SPN** = Solid Pseudopapillary Neoplasm



# Pancreatic Cysts

IPMN

MCN

SCA\*

SPN\*

Pseudocyst

## American Gastroenterological Association Institute Guideline on the Diagnosis and Management of Asymptomatic Neoplastic Pancreatic Cysts

Santhi Swaroop Vege,<sup>1</sup> Barry Ziring,<sup>2</sup> Rajeev Jain,<sup>3</sup> Paul Moayyedi,<sup>4</sup> and the Clinical Guidelines Committee

## Management of Incidental Pancreatic Cysts: A White Paper of the ACR Incidental Findings Committee

EC: Editor's Choice

SA-CME

Alec J. Megibow, MD, MPH<sup>a</sup>, Mark E. Baker, MD<sup>b</sup>, Desiree E. Morgan, MD<sup>c</sup>, Ihab R. Kamel, MD, PhD<sup>d</sup>, Dushyant V. Sahani, MD<sup>e</sup>, Elliot Newman, MD<sup>f</sup>, William R. Brugge, MD<sup>g</sup>, Lincoln L. Berland, MD<sup>h</sup>, Sandharipande, MD, MPH<sup>e, h</sup>

Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas

Masao Tanaka<sup>a, \*</sup>, Carlos Fernández-del Castillo<sup>b</sup>, Terumi Kamada<sup>c</sup>, Philippe Levy<sup>e</sup>, Takao Ohtsuka<sup>f</sup>, Roberto Salvia<sup>g</sup>, Yasuhiro Shiota<sup>h</sup>, Christopher L. Wolfgang<sup>j</sup>

## ACG Clinical Guideline: Diagnosis and Management of Pancreatic Cysts

Grace H. Elta, MD, FACP<sup>1</sup>, Brintha K. Enestvedt, MD, MBA<sup>2</sup>, Bryan G. Sauer, MD, MSc, FACP (GRADE Methodologist)<sup>3</sup> and Anne Marie Lennon, MD, PhD, FACP<sup>4</sup>

Inaccuracies w

## European evidence-based guidelines on pancreatic cystic neoplasms

SC The European Study Group on Cystic Tumours of the Pancreas

Contamination

udopapillary Neoplasm

# Pancreatic Cyst Biomarkers

## Recurrent GNAS Mutations Define an Unexpected Pathway for Pancreatic Cyst Development

Jian Wu,<sup>1\*</sup> Hanno Matthaei,<sup>2\*</sup> Anirban Maitra,<sup>2</sup> Marco Dal Molin,<sup>2</sup> Laura D. Wood,<sup>2</sup> James R. Eshleman,<sup>2</sup> Michael Goggins,<sup>2</sup> Marcia I. Canto,<sup>3</sup> Richard D. Schulick,<sup>3</sup> Barish H. Edil,<sup>3</sup> Christopher L. Wolfgang,<sup>3</sup> Alison P. Klein,<sup>2</sup> Luis A. Diaz Jr.,<sup>1</sup> Peter J. Allen,<sup>4</sup> C. Max Schmidt,<sup>5</sup> Kenneth W. Kinzler,<sup>1</sup> Nickolas Papadopoulos,<sup>1</sup> Ralph H. Hruban,<sup>2</sup> Bert Vogelstein<sup>1†</sup>

## Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways

Jian Wu<sup>a,1</sup>, Yuchen Jiao<sup>a,1</sup>, Marco Dal Molin<sup>b,1</sup>, Anirban Maitra<sup>b</sup>, Roeland F. de Wilde<sup>b</sup>, Laura D. Wood<sup>b</sup>, James R. Eshleman<sup>b</sup>, Michael G. Goggins<sup>b,c</sup>, Christopher L. Wolfgang<sup>d</sup>, Marcia I. Canto<sup>c</sup>, Richard D. Schulick<sup>d</sup>, Barish H. Edil<sup>d</sup>, Michael A. Choti<sup>d</sup>, Volkan Adsay<sup>e</sup>, David S. Klimstra<sup>f</sup>, G. Johan A. Offerhaus<sup>g</sup>, Alison P. Klein<sup>b</sup>, Levy Kopelovich<sup>h</sup>, Hannah Carter<sup>i</sup>, Rachel Karchin<sup>i</sup>, Peter J. Allen<sup>j</sup>, C. Max Schmidt<sup>k</sup>, Yoshiki Naito<sup>l</sup>, Luis A. Diaz, Jr.<sup>a</sup>, Kenneth W. Kinzler<sup>a</sup>, Nickolas Papadopoulos<sup>a</sup>, Ralph H. Hruban<sup>b,2</sup>, and Bert Vogelstein<sup>a,2</sup>

# DNA Sequencing is a Promising Biomarker Assay

## A Combination of Molecular Markers and Clinical Features Improve the Classification of Pancreatic Cysts



Simeon Springer,<sup>1,2,\*</sup> Yuxuan Wang,<sup>1,2,\*</sup> Marco Dal Molin,<sup>2,3,\*</sup> David L. Masica,<sup>2,4,5,\*</sup> Yuchen Jiao,<sup>1,2</sup> Isaac Kinde,<sup>1,2</sup> Amanda Blackford,<sup>6</sup> Siva P. Raman,<sup>7</sup> Christopher L. Wolfgang,<sup>2,8,9</sup> Tyler Tomita,<sup>4,5</sup> Noushin Niknafs,<sup>4,5</sup> Christopher Douville,<sup>4,5</sup> Janine Ptak,<sup>1,2</sup> Lisa Dobbyn,<sup>1,2</sup> Peter J. Allen,<sup>10</sup> David S. Klimstra,<sup>11</sup> Mark A. Schattner,<sup>12</sup> C. Max Schmidt,<sup>13</sup> Michele Yip-Schneider,<sup>14</sup> Oscar W. Cummings,<sup>14</sup> Randall E. Brand,<sup>15</sup> Herbert J. Zeh,<sup>16</sup> Aatur D. Singhi,<sup>17</sup> Aldo Scarpa,<sup>18,19</sup> Roberto Salvia,<sup>20</sup> Giuseppe Malleo,<sup>20</sup> Giuseppe Zamboni,<sup>19,21</sup> Massimo Falconi,<sup>22</sup> Jin-Young Jang,<sup>23</sup> Sun-Whe Kim,<sup>23</sup> Wooil Kwon,<sup>23</sup> Seung-Mo Hong,<sup>24</sup> Ki-Byung Song,<sup>25</sup> Song Cheol Kim,<sup>25</sup> Niall Swan,<sup>26</sup> Jean Murphy,<sup>26</sup> Justin Geoghegan,<sup>27</sup> William Brugge,<sup>28</sup> Carlos Fernandez-Del Castillo,<sup>29</sup> Mari Mino-Kenudson,<sup>30</sup> Richard Schulick,<sup>31</sup> Barish H. Edil,<sup>31</sup> Volkan Adsay,<sup>32</sup> Jorge Paulino,<sup>33</sup> Jeanin van Hooft,<sup>34</sup> Shinichi Yachida,<sup>35</sup> Satoshi Nara,<sup>35</sup> Nobuyoshi Hiraoka,<sup>35</sup> Kenji Yamao,<sup>36</sup> Susuma Hijioka,<sup>36</sup> Schalk van der Merwe,<sup>37</sup> Michael Goggins,<sup>2,9,38</sup> Marcia Irene Canto,<sup>38</sup> Nita Ahuja,<sup>8</sup> Kenzo Hirose,<sup>8</sup> Martin Makary,<sup>8</sup> Matthew J. Weiss,<sup>8</sup> John Cameron,<sup>8</sup> Meredith Pittman,<sup>2,3</sup> James R. Eshleman,<sup>1,2</sup> Luis A. Diaz Jr.,<sup>1,2,8</sup> Nickolas Papadopoulos,<sup>1,2</sup> Kenneth W. Kinzler,<sup>1,2</sup> Rachel Karchin,<sup>2,4,5,9</sup> Ralph H. Hruban,<sup>1,2,3,9</sup> Bert Vogelstein,<sup>1,2</sup> and Anne Marie Lennon<sup>2,8,38</sup>

## A multimodality test to guide the management of patients with a pancreatic cyst

Simeon Springer<sup>1,2,\*</sup>, David L. Masica<sup>2,3,4,\*</sup>, Marco Dal Molin<sup>2,5,\*</sup>, Christopher Douville<sup>1,2,3,4</sup>, Christopher J. Thoburn<sup>1,2</sup>, Bahman Afsari<sup>2,6</sup>, Lu Li<sup>1,2</sup>, Joshua D. Cohen<sup>1,2,3</sup>, Elizabeth Thompson<sup>2,5</sup>, Peter J. Allen<sup>7</sup>, David S. Klimstra<sup>8</sup>, Mark A. Schattner<sup>9</sup>, C. Max Schmidt<sup>10</sup>, Michele Yip-Schneider<sup>10</sup>, Rachel E. Simpson<sup>10</sup>, Carlos Fernandez-Del Castillo<sup>11</sup>, Mari Mino-Kenudson<sup>12</sup>, William Brugge<sup>13</sup>, Randall E. Brand<sup>14</sup>, Aatur D. Singhi<sup>15</sup>, Aldo Scarpa<sup>16,17</sup>, Rita Lawlor<sup>16,17</sup>, Roberto Salvia<sup>18</sup>, Giuseppe Zamboni<sup>19</sup>, Seung-Mo Hong<sup>20</sup>, Dae Wook Hwang<sup>21</sup>, Jin-Young Jang<sup>22</sup>, Wooil Kwon<sup>22</sup>, Niall Swan<sup>23</sup>, Justin Geoghegan<sup>24</sup>, Massimo Falconi<sup>25</sup>, Stefano Crippa<sup>25</sup>, Claudio Doglioni<sup>26</sup>, Jorge Paulino<sup>27</sup>, Richard D. Schulick<sup>28</sup>, Barish H. Edil<sup>28</sup>, Walter Park<sup>29</sup>, Shinichi Yachida<sup>30</sup>, Susumu Hijioka<sup>31</sup>, Jeanin van Hooft<sup>32</sup>, Jin He<sup>33</sup>, Matthew J. Weiss<sup>33</sup>, Richard Burkhardt<sup>33</sup>, Martin Makary<sup>33</sup>, Marcia I. Canto<sup>34</sup>, Michael G. Goggins<sup>2,5,6,34</sup>, Janine Ptak<sup>1,2</sup>, Lisa Dobbyn<sup>1,2</sup>, Joy Schaefer<sup>1,2</sup>, Natalie Sillman<sup>1,2</sup>, Maria Popoli<sup>1,2</sup>, Alison P. Klein<sup>1,2,6</sup>, Cristian Tomasetti<sup>2,35†</sup>, Rachel Karchin<sup>2,3,4,6†</sup>, Nickolas Papadopoulos<sup>1,2</sup>, Kenneth W. Kinzler<sup>1,2</sup>, Bert Vogelstein<sup>1,2†</sup>, Christopher L. Wolfgang<sup>2,6,33†</sup>, Ralph H. Hruban<sup>1,2,5,6†</sup>, Anne Marie Lennon<sup>1,2,6,33,34,36†</sup>

# Pancreatic Cysts

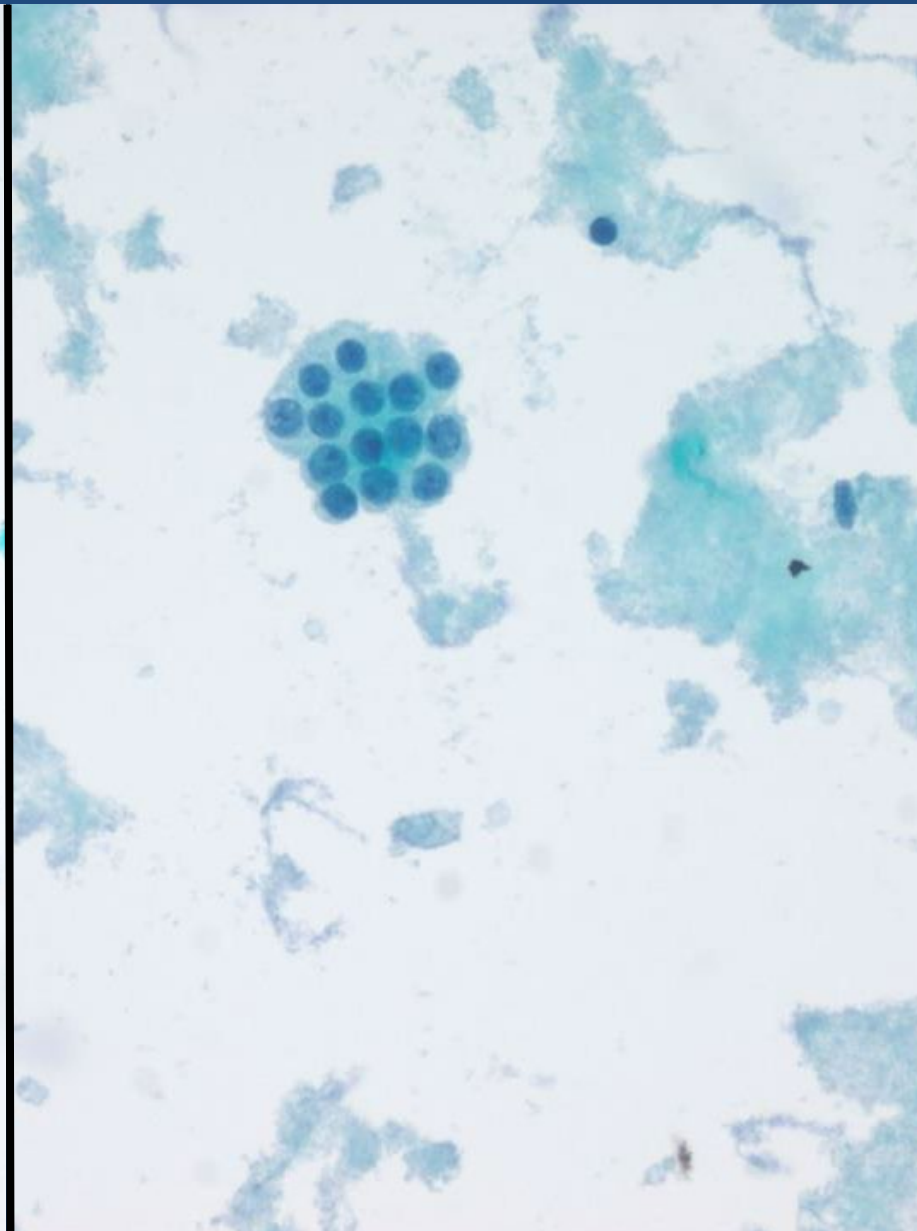
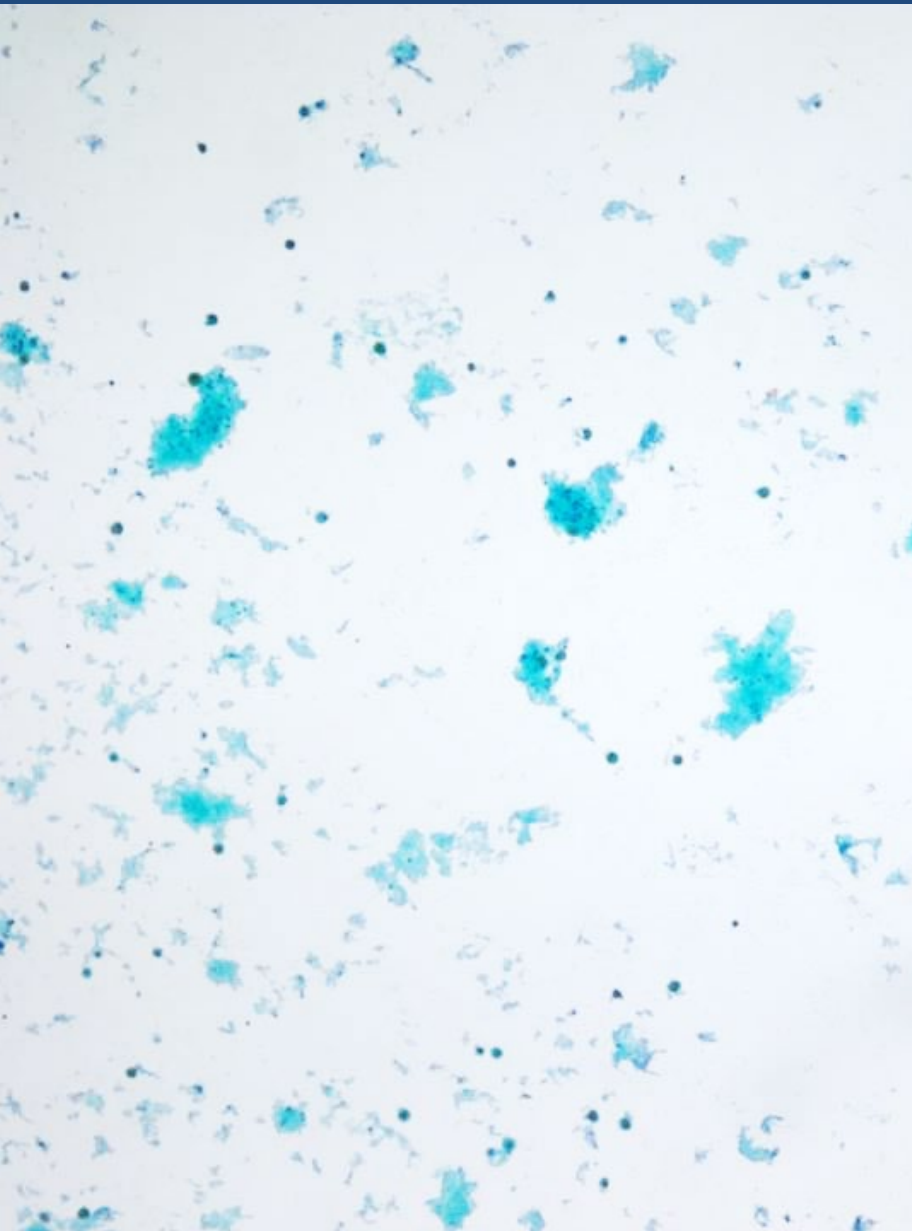
	<b>IPMN</b>	<b>MCN</b>	<b>SCA</b>	<b>SPN</b>	<b>Pseudocyst</b>
<b>Gender</b>	<b>M&gt;F</b>	<b>F&gt;&gt;M</b>	<b>F&gt;M</b>	<b>F&gt;&gt;M</b>	<b>M&gt;F</b>
<b>Location</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;&gt;Head</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;Head</b>	<b>Head=Tail</b>
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<b>CEA</b>	<b>&gt;192 ng/mL</b>	<b>&gt;192 ng/mL</b>	<b>&lt;0.5 ng/mL</b>	<b>&lt;192 ng/mL</b>	<b>&lt;192 ng/mL</b>
<b>Amylase</b>	<b>High</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>	<b>High</b>
<b>Cytology</b>	<b>Mucinous</b>	<b>Mucinous</b>	<b>Scant, Bland PAS+</b>	<b>Papillary &amp; Vascular</b>	<b>Pigmented Histiocytes</b>

# Molecular Analysis (of Pancreatic Cyst Fluid)

	<b>IPMN</b>	<b>MCN</b>	<b>SCA</b>	<b>SPN</b>	<b>Non-SPN</b>	<b>Pseudocyst</b>
<b>Genetics</b>	<b>IPMN</b> • Intraductal papillary mucinous neoplasms (IPMN): <b>KRAS</b> + and <b>GNAS</b> +	<b>MCN</b> • Mucinous cystic neoplasms (MCN): <b>KRAS</b> +	<b>SCA</b> • Serous cystadenomas (SCA): <b>VHL</b> -	<b>SPN</b> <b>CTNNB1</b> +	<b>Non-SPN</b> <b>VHL</b> -	<b>Pseudocyst</b>
<b>Gender</b>	M	F	F	F >> M	M	M > F
<b>Location</b>	Head	Body/Tail	Head	Low	Low	Head=Tail
<b>Viscosity</b>	High	High	Low	Low	Low	Low
<b>CEA</b>	>192 ng/mL	>192 ng/mL	<192 ng/mL	<192 ng/mL	<192 ng/mL	<192 ng/mL
<b>Amylase</b>	High	Low	Low	Low	Low	High
<b>Cytology</b>	<b>CTNNB1</b> Mucinous	Mucinous	Scant, Bland PAS+	Papillary & Vascular	Pigmented Histiocytes	
<b>Genetics</b>	<b>IPMN</b> <b>KRAS</b> , <b>GNAS</b>	<b>MCN</b> <b>KRAS</b>	<b>SCA</b> <b>VHL</b>	<b>SPN</b> <b>CTNNB1</b>	<b>Non-SPN</b> <b>VHL</b>	<b>Absent</b>

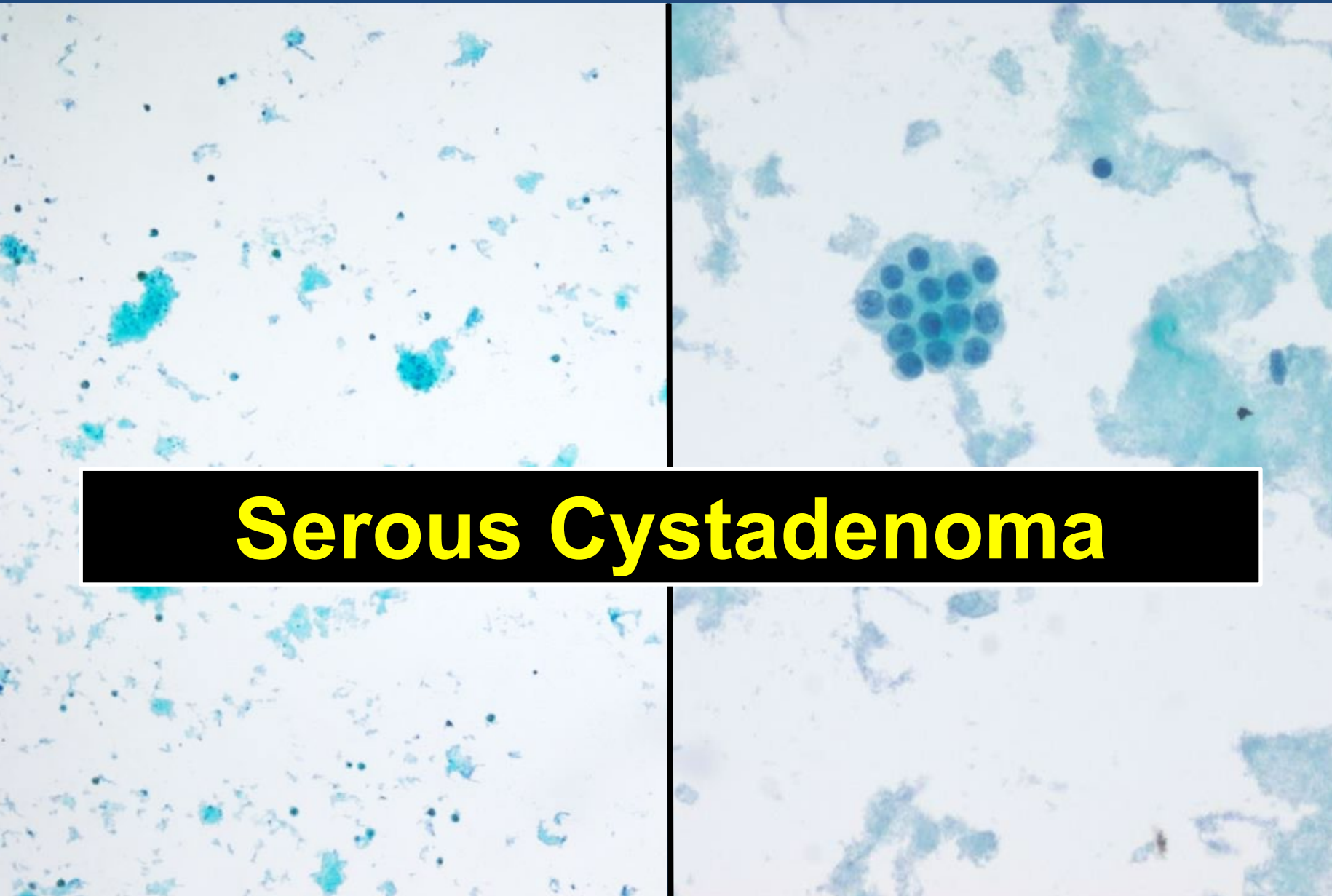


# Case 5



- 44-year-old female with a 3.1 cm pancreatic head cyst
- **Cytopathologic findings:**
  - Paucicellular smears with significant debris
  - Rare clusters of cuboidal cells with round nuclei, and scant cytoplasm
  - Nuclear contours are smooth
  - No definitive evidence of high-grade atypia
- **Molecular testing:**
  - A mutations in **VHL** was identified
  - No other genomic alterations were seen

# Case 5



## Serous Cystadenoma

- 54-year-old female with a 3.1 cm pancreatic head cyst
- **Cytopathologic findings:**
  - Paucicellular smears with significant debris
  - Rare clusters of cuboidal cells with round nuclei, and scant cytoplasm
  - Nuclear contours are smooth
  - No definitive evidence of high-grade atypia
- **Molecular testing:**
  - A mutations in **VHL** was identified
  - No other genomic alterations were seen



# Take Home Points: Case 5

	<b>IPMN</b>	<b>MCN</b>	<b>SCA</b>	<b>SPN</b>	<b>Pseudocyst</b>
<b>Gender</b>	<b>M&gt;F</b>	<b>F&gt;&gt;M</b>	<b>F&gt;M</b>	<b>F&gt;&gt;M</b>	<b>M&gt;F</b>
<b>Location</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;&gt;Head</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;Head</b>	<b>Head=Tail</b>
<b>Viscosity</b>	<b>Increased</b>	<b>Increased</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>
<b>CEA</b>	<b>&gt;192 ng/mL</b>	<b>&gt;192 ng/mL</b>	<b>&lt;0.5 ng/mL</b>	<b>&lt;192 ng/mL</b>	<b>&lt;192 ng/mL</b>
<b>Amylase</b>	<b>High</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>	<b>High</b>
<b>Cytology</b>	<b>Mucinous</b>	<b>Mucinous</b>	<b>Scant, Bland PAS+</b>	<b>Papillary &amp; Vascular</b>	<b>Pigmented Histiocytes</b>

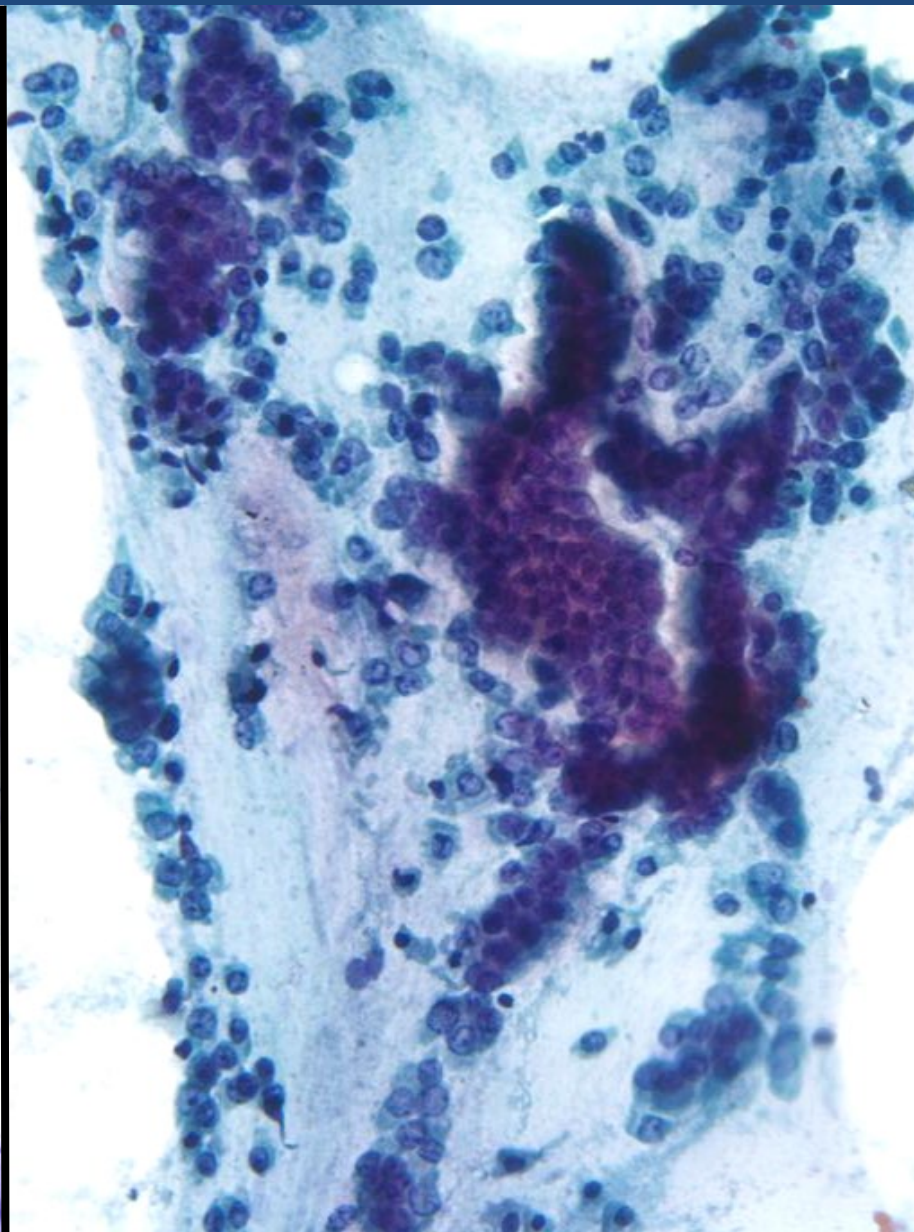
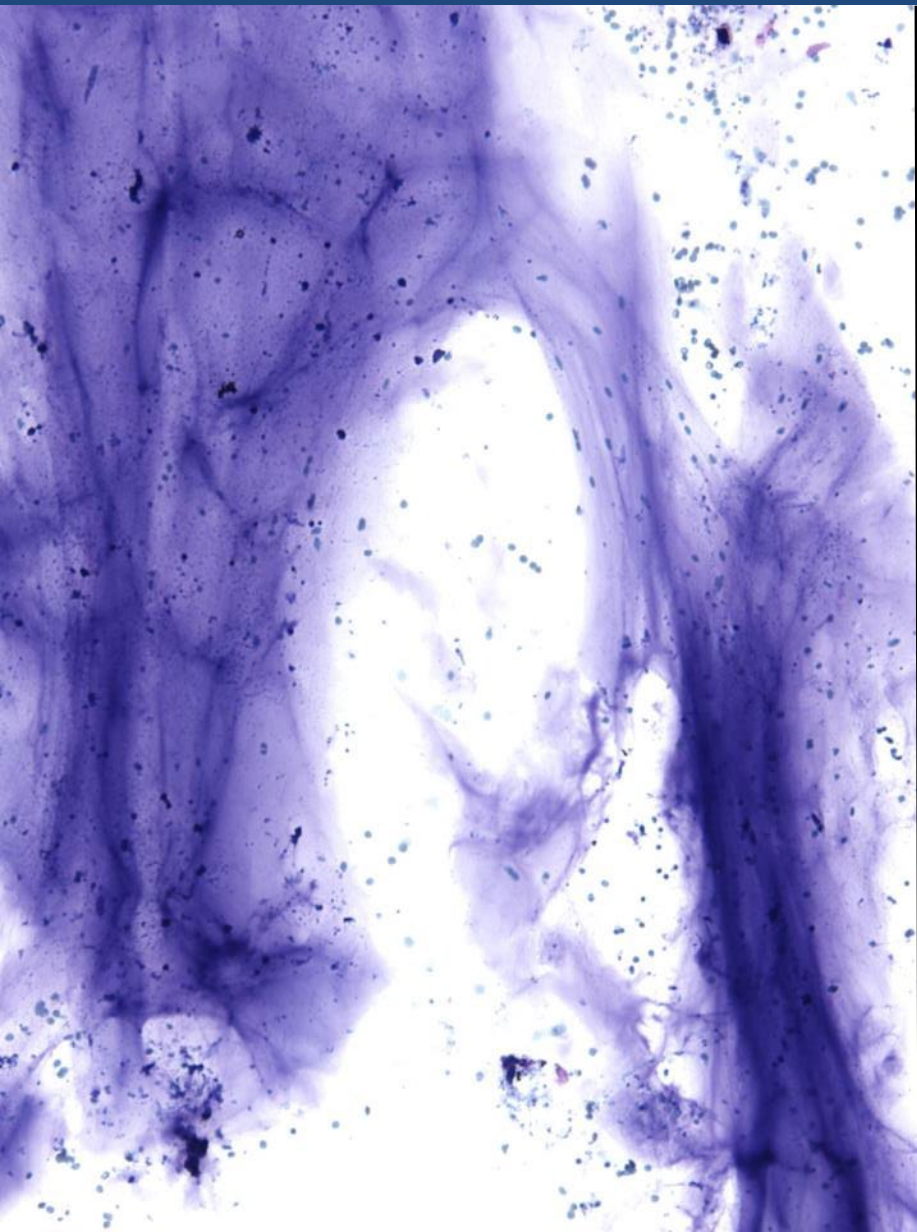
# Take Home Points: Case 5

	<b>IPMN</b>	<b>MCN</b>	<b>SCA</b>	<b>SPN</b>	<b>Pseudocyst</b>
<b>Gender</b>	<b>M&gt;F</b>	<b>F&gt;&gt;M</b>	<b>F&gt;M</b>	<b>F&gt;&gt;M</b>	<b>M&gt;F</b>
<b>Location</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;&gt;Head</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;Head</b>	<b>Head=Tail</b>
<b>Viscosity</b>	<b>Increased</b>	<b>Increased</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>
<b>CEA</b>	<b>&gt;192 ng/mL</b>	<b>&gt;192 ng/mL</b>	<b>&lt;0.5 ng/mL</b>	<b>&lt;192 ng/mL</b>	<b>&lt;192 ng/mL</b>
<b>Amylase</b>	<b>High</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>	<b>High</b>
<b>Cytology</b>	<b>Mucinous</b>	<b>Mucinous</b>	<b>Scant, Bland PAS+</b>	<b>Papillary &amp; Vascular</b>	<b>Pigmented Histiocytes</b>
<b>Genetics</b>	<b><i>KRAS,GNAS</i></b>	<b><i>KRAS</i></b>	<b><i>VHL</i></b>	<b><i>CTNNB1</i></b>	<b>Absent</b>

# Case 6

- A 52-year-old female complaining of abdominal pain and had a CT scan that identified a 4.1 cm cyst within the pancreatic body.
- An endoscopic ultrasound (EUS) confirmed the presence of a 4.1 cm pancreatic cyst that did not communicate with the main pancreatic duct.
- A fine-needle aspiration (FNA) yielded thin fluid, which was submitted for cytopathologic examination and molecular testing.

# Case 6



- 52-year-old female with a 4.1 cm pancreatic body cyst
- **Cytopathologic findings:**
  - Thick colloid like mucin with scant cellularity
  - Rare groups of epithelial clusters of mucinous epithelium
  - No definitive evidence of high-grade atypia
- **Molecular testing:**
  - Mutations in **KRAS** and **GNAS** were identified
  - Copy number alterations were also detected and involved **TP53** and **SMAD4**

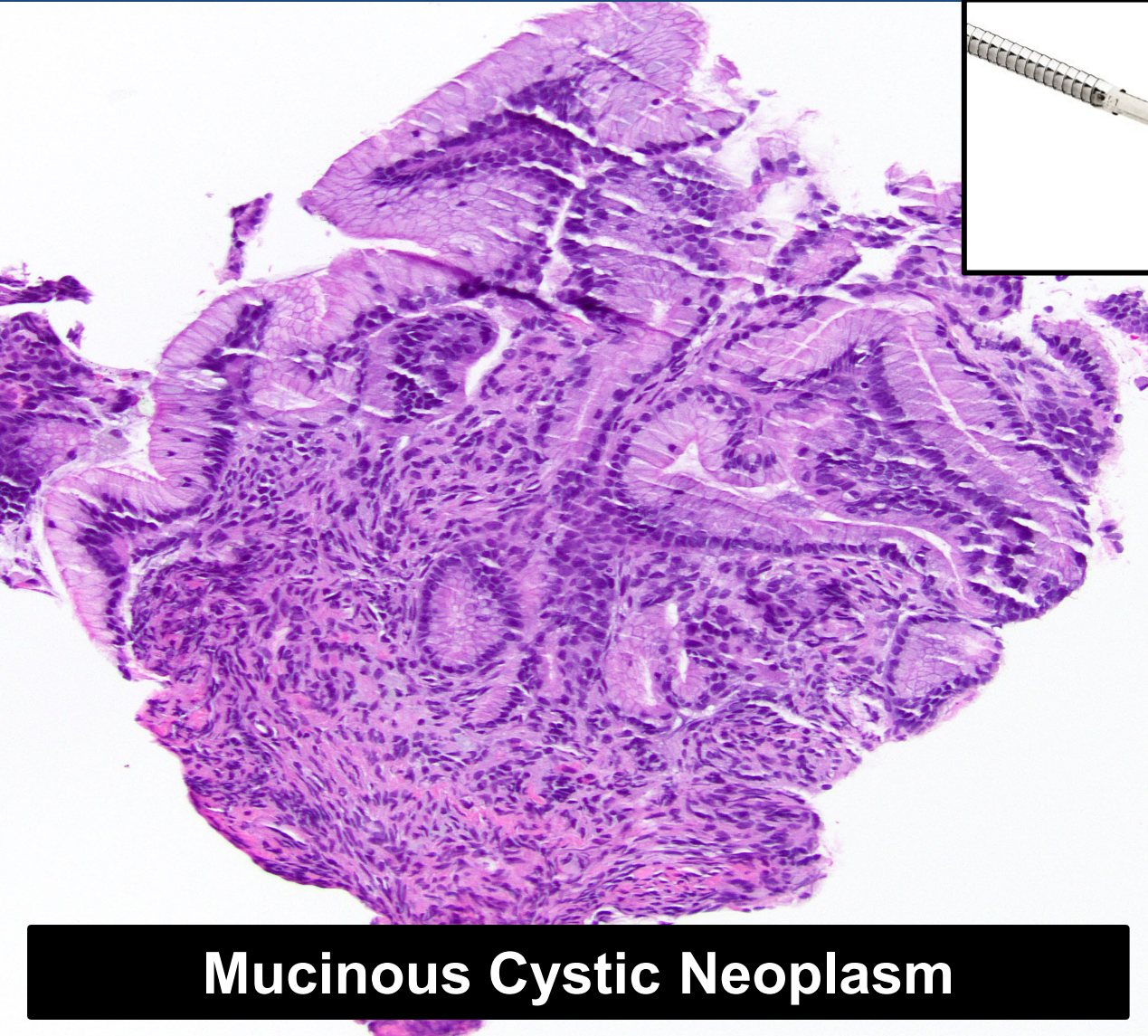


# Case 6

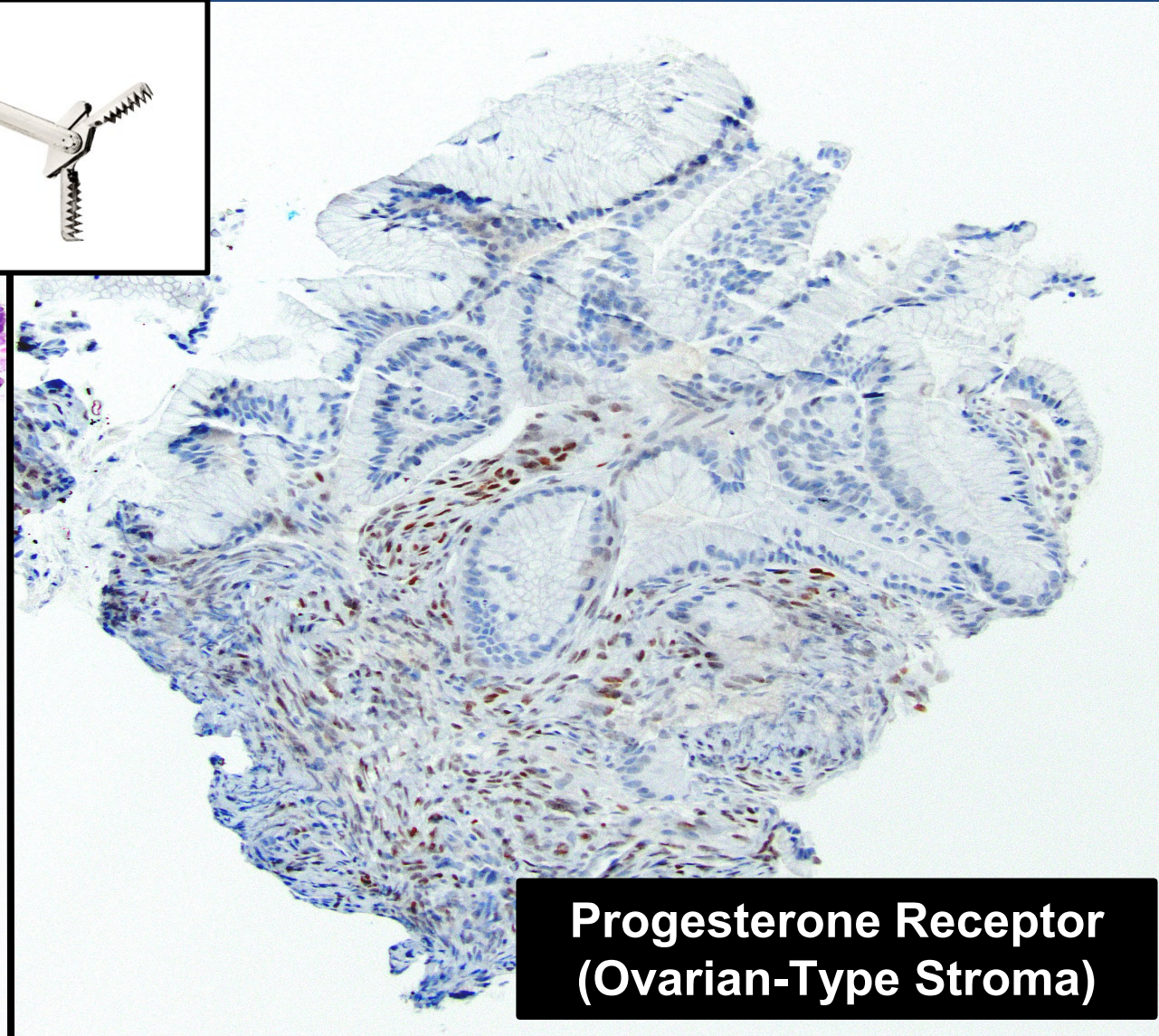
- A **52-year-old female** complaining of abdominal pain and had a CT scan that identified a 4.1 cm cyst within the **pancreatic body**.
- An endoscopic ultrasound (EUS) confirmed the presence of a 4.1 cm pancreatic cyst that **did not communicate with the main pancreatic duct**.
- A fine-needle aspiration (FNA) yielded thin fluid, which was submitted for cytopathologic examination and molecular testing.



# Moray Micro Forceps



**Mucinous Cystic Neoplasm**



**Progesterone Receptor  
(Ovarian-Type Stroma)**



# Pancreatic Cysts

	<b>IPMN</b>	<b>MCN</b>	<b>SCA</b>	<b>SPN</b>	<b>Pseudocyst</b>
<b>Gender</b>	<b>M&gt;F</b>	<b>F&gt;&gt;M</b>	<b>F&gt;M</b>	<b>F&gt;&gt;M</b>	<b>M&gt;F</b>
<b>Location</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;&gt;Head</b>	<b>Head&gt;Tail</b>	<b>Tail&gt;Head</b>	<b>Head=Tail</b>
<b>Viscosity</b>	<b>Increased</b>	<b>Increased</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>
<b>CEA</b>	<b>&gt;192 ng/mL</b>	<b>&gt;192 ng/mL</b>	<b>&lt;0.5 ng/mL</b>	<b>&lt;192 ng/mL</b>	<b>&lt;192 ng/mL</b>
<b>Amylase</b>	<b>High</b>	<b>Low</b>	<b>Low</b>	<b>Low</b>	<b>High</b>
<b>Cytology</b>	<b>Mucinous</b>	<b>Mucinous</b>	<b>Scant, Bland PAS+</b>	<b>Papillary &amp; Vascular</b>	<b>Pigmented Histiocytes</b>

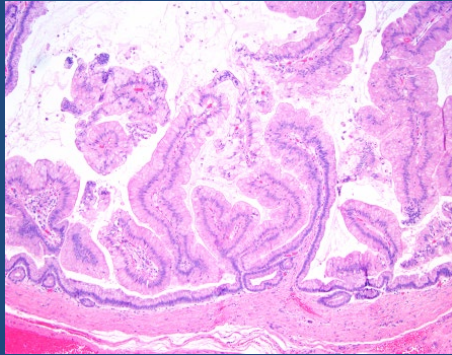
# Molecular Analysis (of Pancreatic Cyst Fluid)

	<b>IPMN</b>	<b>MCN</b>	<b>SCA</b>	<b>SPN</b>	<b>Non-SPN</b>
<b>Genetics</b>	<b>IPMN</b> • Intraductal papillary mucinous neoplasms (IPMN): <b>KRAS</b> + and <b>GNAS</b> +	<b>MCN</b> • Mucinous cystic neoplasms (MCN): <b>KRAS</b> +	<b>SCA</b> • Serous cystadenomas (SCA): <b>VHL</b> -	<b>SPN</b> <b>CTNNB1</b> +	<b>Non-SPN</b> <b>VHL</b> -
<b>Gender</b>	M>F	M>F	M>F	F>>M	M>F
<b>Location</b>	Head	Body/Tail	Head	Low	Head=Tail
<b>Viscosity</b>	High	High	High	Low	Low
<b>CEA</b>	>192 ng/mL	>192 ng/mL	>192 ng/mL	<192 ng/mL	<192 ng/mL
<b>Amylase</b>	High	Low	Low	Low SPN	High
<b>Cytology</b>	<b>CTNNB1</b> Mucinous	Mucinous	Scant, Bland PAS+	Papillary & Vascular	Pigmented Histiocytes
<b>Genetics</b>	<b>Absent</b>	<b>Absent</b>	<b>KRAS</b>	<b>VHL</b>	<b>CTNNB1</b>
					<b>Absent</b>

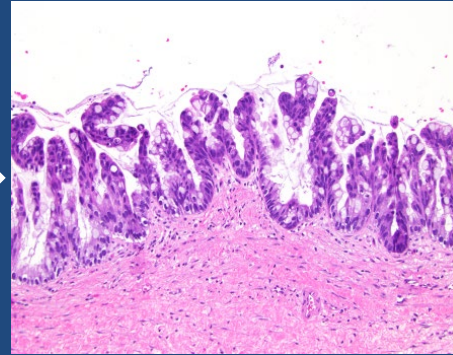


# Molecular Analysis (of Panc. Cyst Fluid)

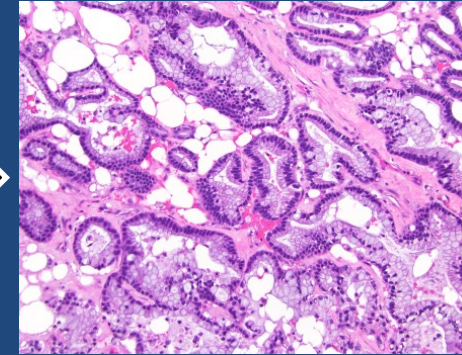
	<i>KRAS</i>	<i>GNAS</i>	<i>VHL</i>	<i>CTNNB1</i>
IPMN	+	+	-	-
MCN	+	-	-	-



Low-grade  
Dysplasia



High-grade  
Dysplasia



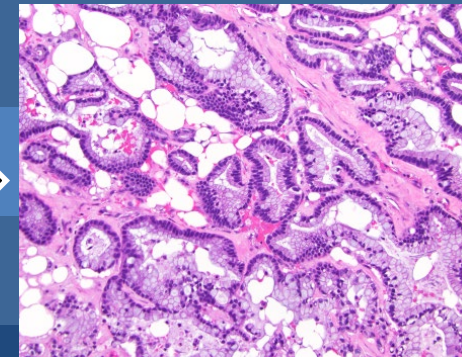
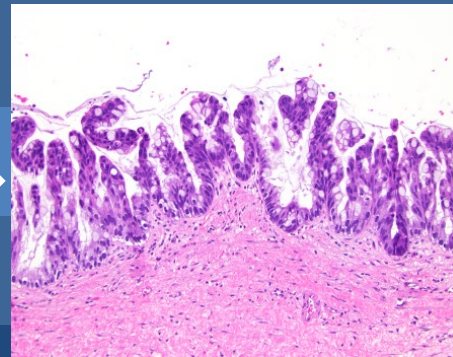
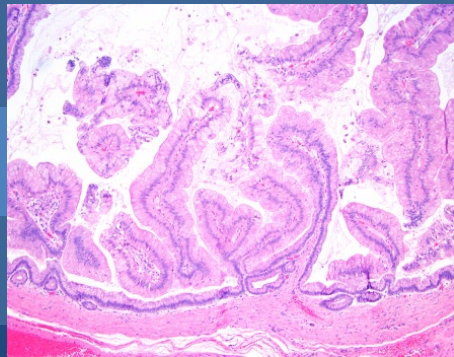
AdenoCA

*KRAS* & *GNAS*



# Molecular Analysis (of Panc. Cyst Fluid)

	<i>KRAS</i>	<i>GNAS</i>	<i>VHL</i>	<i>CTNNB1</i>	<i>TP53</i>	<i>PIK3CA</i>	<i>PTEN</i>
IPMN	+	+	-	-	HR	HR	HR
MCN	+	-	-	-	HR	HR	HR



Low-grade  
Dysplasia

HR = High-risk of malignancy

High-grade  
Dysplasia

AdenoCA

*KRAS* & *GNAS*



*TP53*, *PIK3CA*  
& *PTEN*



# Single Institutional Studies

## Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study

Asif Khalid, MD, Maliha Zahid, MD, Sydney D. Finkelstein, MD, Julia K. LeBlanc, MD, Neeraj Kaushik, MD, Nuzhat Ahmad, MD, William R. Brugge, MD, Steven A. Edmundowicz, MD, Robert H. Hawes, MD, Kevin M. McGrath, MD

Pittsburgh, Philadelphia, Pennsylvania, Indianapolis, Indiana, Boston, Massachusetts, St Louis, Missouri, Charleston, South Carolina, USA

## Integration of *KRAS* testing in the diagnosis of pancreatic cystic lesions: a clinical experience of 618 pancreatic cysts

Marina N Nikiforova<sup>1</sup>, Asif Khalid<sup>2</sup>, Kenneth E Fasanella<sup>2</sup>, Kevin M McGrath<sup>2</sup>, Randall E Brand<sup>2</sup>, Jennifer S Chennat<sup>2</sup>, Adam Slivka<sup>2</sup>, Herbert J Zeh<sup>3</sup>, Amer H Zureikat<sup>3</sup>, Alyssa M Krasinskas<sup>1</sup>, N Paul Ohori<sup>1</sup>, Karen E Schoedel<sup>1</sup>, Sarah Navina<sup>1</sup>, Geeta S Mantha<sup>1</sup>, Reetesh K Pai<sup>1</sup> and Aatur D Singhi<sup>1</sup>

## Next-Generation Sequencing Adds Value to the Preoperative Diagnosis of Pancreatic Cysts

Matthew W. Rosenbaum, MD<sup>1</sup>; Martin Jones, MBBS<sup>2</sup>; Jonathan C. Dudley, MD, PhD<sup>3</sup>; Long P. Le, MD<sup>1</sup>; A. John Iafrate, MD, PhD<sup>1</sup>; and Martha B. Pitman, MD<sup>1</sup>

## Preoperative next-generation sequencing of pancreatic cyst fluid is highly accurate in cyst classification and detection of advanced neoplasia

Aatur D Singhi,<sup>1</sup> Kevin McGrath,<sup>2</sup> Randall E Brand,<sup>2</sup> Asif Khalid,<sup>2</sup> Herbert J Zeh,<sup>3</sup> Jennifer S Chennat,<sup>2</sup> Kenneth E Fasanella,<sup>2</sup> Georgios I Papachristou,<sup>2</sup> Adam Slivka,<sup>2</sup> David L Bartlett,<sup>3</sup> Anil K Dasyam,<sup>4</sup> Melissa Hogg,<sup>3</sup> Kenneth K Lee,<sup>3</sup> James Wallis Marsh,<sup>3</sup> Sara E Monaco,<sup>1</sup> N Paul Ohori,<sup>1</sup> James F Pingpank,<sup>3</sup> Allan Tsung,<sup>3</sup> Amer H Zureikat,<sup>3</sup> Abigail I Wald,<sup>1</sup> Marina N Nikiforova<sup>1</sup>

## Somatic molecular analysis augments cytologic evaluation of pancreatic cyst fluids as a diagnostic tool

Ali Sakhdari<sup>1,5</sup>, Parnian Ahmadi Moghaddam<sup>1,4,6</sup>, Chi Young Ok<sup>1,5</sup>, Otto Walter<sup>1</sup>, Keith Tomaszewicz<sup>1</sup>, Mandi-Lee Caporelli<sup>1</sup>, Xiuling Meng<sup>1</sup>, Jennifer LaFemina<sup>2</sup>, Giles Whalen<sup>2</sup>, Edward Belkin<sup>3</sup>, Jaroslav Zivny<sup>3</sup>, Wahid Wassef<sup>3</sup>, Bruce A. Woda<sup>1</sup>, Lloyd M. Hutchinson<sup>1</sup> and Ediz F. Cosar<sup>1</sup>

## Impact of next-generation sequencing on the clinical diagnosis of pancreatic cysts

Martin Jones, MBBS,<sup>1</sup> Zongli Zheng, MD, PhD,<sup>1</sup> Jessica Wang, MD,<sup>1</sup> Jonathan Dudley, MD, PhD,<sup>1</sup> Emily Albanese,<sup>1</sup> Abdurrahman Kadayifci, MD,<sup>2</sup> Dora Dias-Santagata, PhD,<sup>1</sup> Long Le, MD,<sup>1</sup> William R. Brugge, MD,<sup>2</sup> Carlos Fernandez-del Castillo, MD,<sup>3</sup> Mari Mino-Kenudson, MD,<sup>1</sup> A. John Iafrate, MD, PhD,<sup>1,\*</sup> Martha B. Pitman, MD<sup>1,\*</sup>

## Preoperative *GNAS* and *KRAS* Testing in the Diagnosis of Pancreatic Mucinous Cysts

Aatur D. Singhi<sup>1</sup>, Marina N. Nikiforova<sup>1</sup>, Kenneth E. Fasanella<sup>2</sup>, Kevin M. McGrath<sup>2</sup>, Reetesh K. Pai<sup>1</sup>, N. Paul Ohori<sup>1</sup>, Tanner L. Bartholow<sup>1</sup>, Randall E. Brand<sup>2</sup>, Jennifer S. Chennat<sup>2</sup>, Xuong Lu<sup>2</sup>, Georgios I. Papachristou<sup>2,4</sup>, Adam Slivka<sup>2</sup>, Herbert J. Zeh<sup>3</sup>, Amer H. Zureikat<sup>3</sup>, Kenneth K. Lee<sup>3</sup>, Allan Tsung<sup>3</sup>, Geeta S. Mantha<sup>1</sup>, and Asif Khalid<sup>2,4</sup>



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# Prospective Testing (of Panc. Cyst Fluid)

- PancreaSeq testing consisted of **22 genes** at a depth of **>1000**

- Between  
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- Among

from 1832 patients were satisfactory for molecular analysis (**PancreaSeq**).



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# Molecular Analysis (of Panc. Cyst Fluid)

<b>Surgical Resection Dx</b>	<b>Total, n = 251 (15%)</b>
<b>IPMN with advance neoplasia</b>	<b>90</b>
<b>MCN with advanced neoplasia</b>	<b>6</b>
<b>IPMN with LGD</b>	<b>77</b>
<b>MCN with LGD</b>	<b>13</b>
<b>IOPN</b>	<b>3</b>
<b>ITPN</b>	<b>1</b>
<b>Acinar cell carcinoma</b>	<b>2</b>
<b>Solid-pseudopapillary neoplasm</b>	<b>2</b>
<b>Cystic PanNET</b>	<b>34</b>
<b>Serous cystadenoma</b>	<b>14</b>
<b>Paraganglioma</b>	<b>1</b>
<b>Cystic schwannoma</b>	<b>1</b>
<b>Non-neoplastic cysts</b>	<b>2</b>
<b>Metastatic neoplasms</b>	<b>5</b>

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Non-neoplastic cysts	2
Metastatic neoplasms	5

**186 Mucinous Cysts:  
167 IPMNs  
20 MCNs**

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Cystic schwannoma	1
Non-neoplastic cysts	2
Metastatic neoplasms	5

**186 Mucinous Cysts:**  
**167 IPMNs**  
**20 MCNs**

**60 Non-Mucinous  
Cysts**

# Molecular Analysis (of Panc. Cyst Fluid)

Surgical Resection Dx	Total, n = 251 (15%)
IPMN with advance neoplasia	90
MCN with advanced neoplasia	6
IPMN with LGD	77
MCN with LGD	13
IOPN	3
ITPN	1
Acinar cell carcinoma	2
Solid-pseudopapillary neoplasm	2
Cystic PanNET	34
Serous cystadenoma	14
Paraganglioma	1
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Non-neoplastic cysts	2
Metastatic neoplasms	5

**186 Mucinous Cysts:  
167 IPMNs  
20 MCNs**

**60 Non-Mucinous  
Cysts**

**5 Metastases**

# ***KRAS, GNAS, & BRAF*** alterations

Surgical Resection Dx	Total, n = 246 (14%)	Wild-type	Mutant
IPMN with advance neoplasia	90	3 (3%)	87 (97%)
MCN with advanced neoplasia	6	0 (0%)	6 (100%)
IPMN with LGD	77	5 (6%)	72 (94%)
MCN with LGD	13	10 (77%)	3 (23%)
IOPN	3	3 (100%)	0 (0%)
ITPN	1	1 (100%)	0 (0%)
Acinar cell carcinoma	2	2 (100%)	0 (0%)
Solid-pseudopapillary neoplasm	2	2 (100%)	0 (0%)
Cystic PanNET	34	34 (100%)	0 (0%)
Serous cystadenoma	14	14 (100%)	0 (0%)
Paraganglioma	1	1 (100%)	0 (0%)
Cystic schwannoma	1	1 (100%)	0 (0%)
Non-neoplastic cysts	2	2 (100%)	0 (0%)



# KRAS, GNAS, & BRAF alterations

Surgical Resection Dx	Total, n = 246 (14%)	Wild-type	Mutant
IPMN with advance neoplasia	90	3 (3%)	87 (97%)
MCN with advanced neoplasia	6	0 (0%)	6 (100%)
IPMN with LGD	77	5 (6%)	72 (94%)
MCN with LGD	13	10 (77%)	3 (23%)
IOPN	3	3 (100%)	0 (0%)
ITPN	1	1 (100%)	0 (0%)
		2 (100%)	0 (0%)
		2 (100%)	0 (0%)
		34 (100%)	0 (0%)
		14 (100%)	0 (0%)
		1 (100%)	0 (0%)
Cystic schwannoma	1	1 (100%)	0 (0%)
Non-neoplastic cysts	2	2 (100%)	0 (0%)

## IPMNs & MCNs

### KRAS, GNAS, & BRAF mutations

- Sensitivity: 90%
- Specificity: 100%

# KRAS, GNAS, & BRAF alterations

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MCN with LGD	13	10 (77%)	3 (23%)
IOPN	3	3 (100%)	0 (0%)
ITPN	1	1 (100%)	0 (0%)
Cystic schwannoma	1	1 (100%)	0 (0%)
Non-neoplastic cysts	2	2 (100%)	0 (0%)

## IPMNs & MCNs

### KRAS, GNAS, & BRAF mutations

- Sensitivity: 90%
- Specificity: 100%

### Fluid Viscosity\*

- Sensitivity: 77%
- Specificity: 92%

### Elevated CEA\*

- Sensitivity: 73%
- Specificity: 94%

# TP53, SMAD4, CTNNB1, & mTOR

Surgical Resection Dx	Total, n = 246 (18%)	Wild-type	Mutant
IPMN with advance neoplasia	90	11 (12%)	79 (88%)
MCN with advanced neoplasia	6	0 (0%)	6 (100%)
IPMN with LGD	77	74 (96%)	3 (4%)
MCN with LGD	13	13 (100%)	0 (0%)
		3 (100%)	0 (0%)
		1 (100%)	0 (0%)
		2 (100%)	0 (0%)
		2 (100%)	0 (0%)
		34 (100%)	0 (0%)
		14 (100%)	0 (0%)
		1 (100%)	0 (0%)
		1 (100%)	0 (0%)
Non-neoplastic cysts	2	2 (100%)	0 (0%)

**Advanced Neoplasia**  
**TP53, SMAD4, CTNNB1,**  
**and/or mTOR genes**

- Sensitivity: 89%
- Specificity: 98%

# TP53, SMAD4, CTNNB1, & mTOR

Surgical Resection Dx	Total, n = 246 (18%)	Wild-type	Mutant
IPMN with advance neoplasia	90	41 (46%)	49 (54%)
MCN with advanced neoplasia	6	0 (0%)	6 (100%)
IPMN with LGD	77	11 (14%)	66 (86%)
MCN with LGD	13	0 (0%)	13 (100%)
Non-neoplastic cysts	2	2 (100%)	0 (0%)

**Advanced Neoplasia**  
**TP53, SMAD4, CTNNB1,**  
**and/or mTOR genes**

- Sensitivity: 89%
- Specificity: 98%

**Cytology**

- Sensitivity: 46%
- Specificity: 97%

**Main duct dilation**

- Sensitivity: 68%
- Specificity: 65%

**Mural nodule**

- Sensitivity: 45%
- Specificity: 81%

# TP53, SMAD4, CTNNB1, & mTOR

Surgical Resection Dx	Total, n = 246 (18%)	Wild-type	Mutant
IPMN with advance neoplasia	90	11 (12%)	79 (88%)
MCN with advanced neoplasia	6	0 (0%)	6 (100%)
IPMN with LGD	77	74 (96%)	3 (4%)
MCN with LGD	13	13 (100%)	0 (0%)
Non-neoplastic cysts	2	2 (100%)	0 (0%)

## Advanced Neoplasia

**TP53, SMAD4, CTNNB1, and/or mTOR genes**

- Sensitivity: 89%
- Specificity: 98%

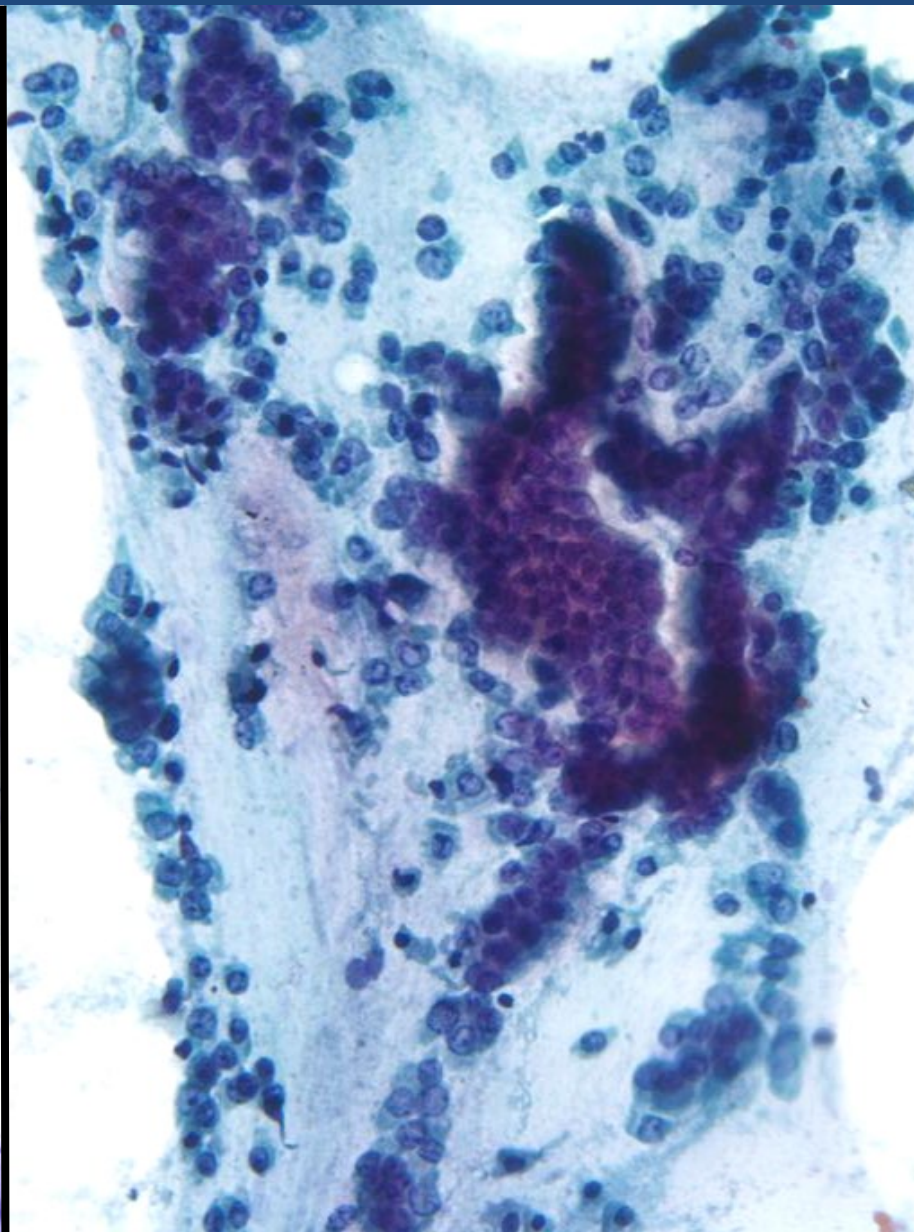
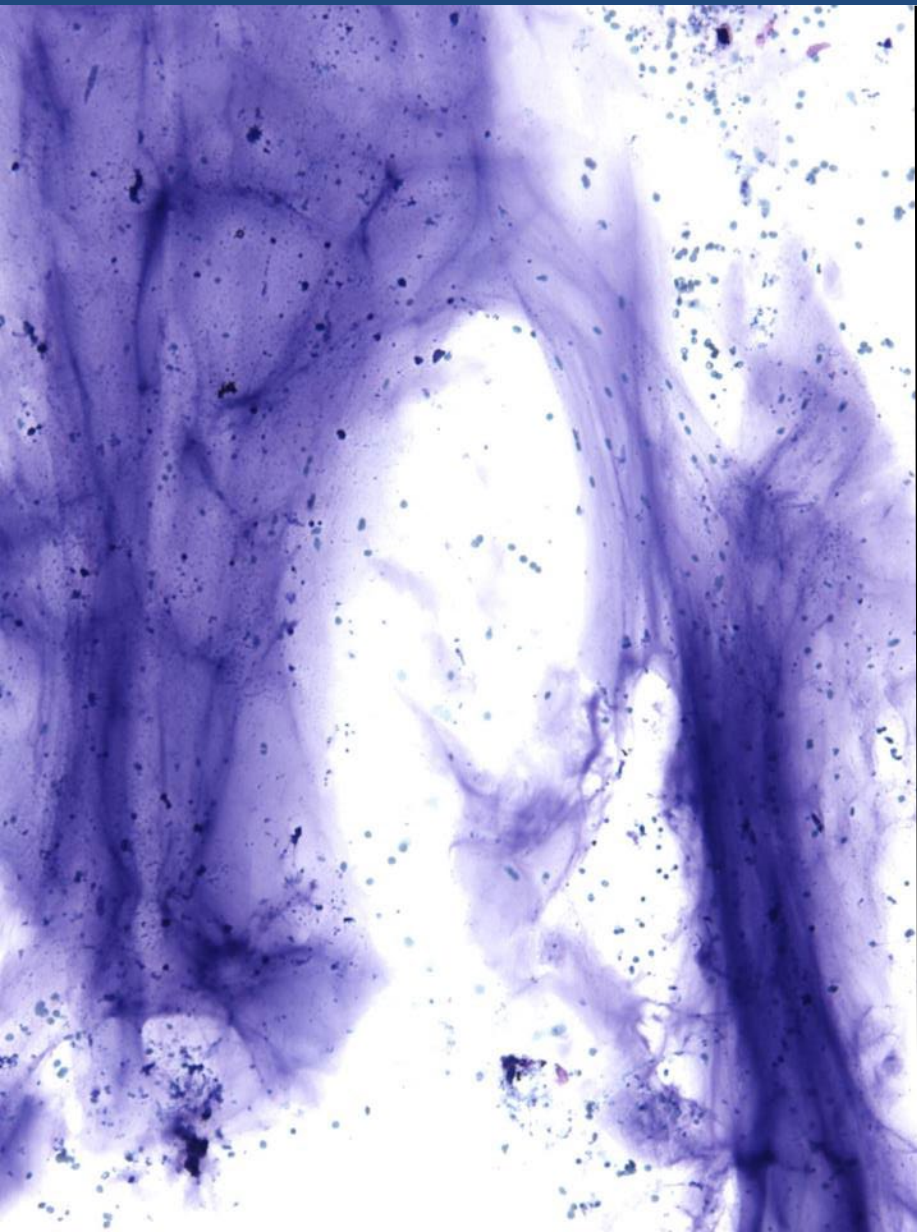
## Advanced Neoplasia

**TP53, SMAD4, CTNNB1, and/or mTOR genes & Cytology**

- Sensitivity: 93%
- Specificity: 95%



# Case 6

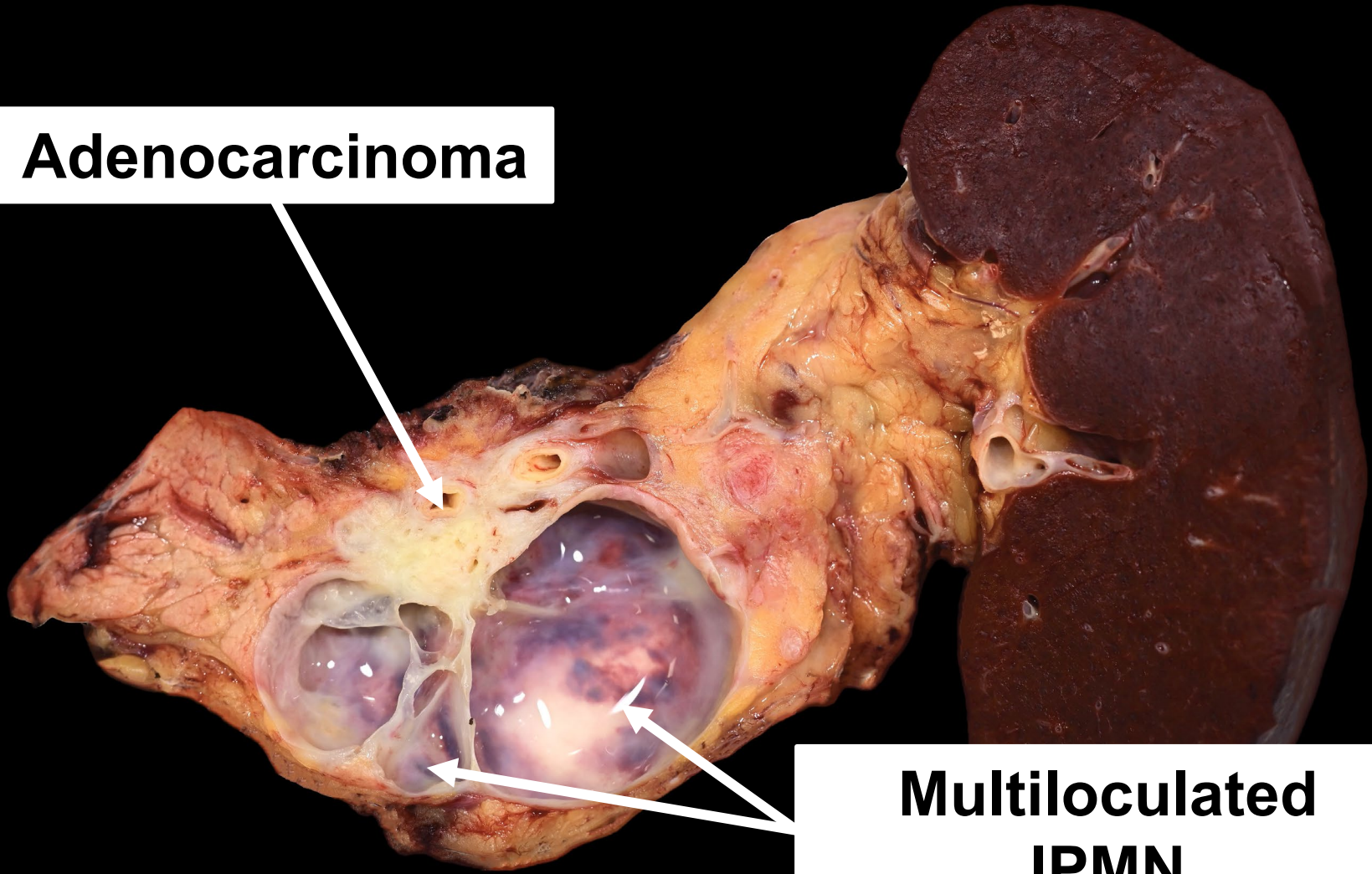


- 52-year-old female with a 4.1 cm pancreatic body cyst
- **Cytopathologic findings:**
  - Thick colloid like mucin with scant cellularity
  - Rare groups of epithelial clusters of mucinous epithelium
  - No definitive evidence of high-grade atypia
- **Molecular testing:**
  - Mutations in **KRAS** and **GNAS** were identified
  - Copy number alterations were also detected and involved **TP53** and **SMAD4**

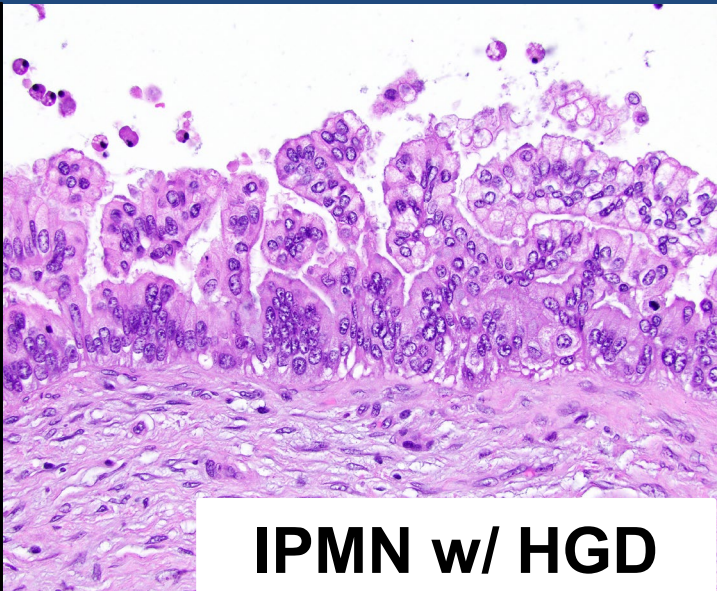


# Case 6: IPMN-associated PDAC

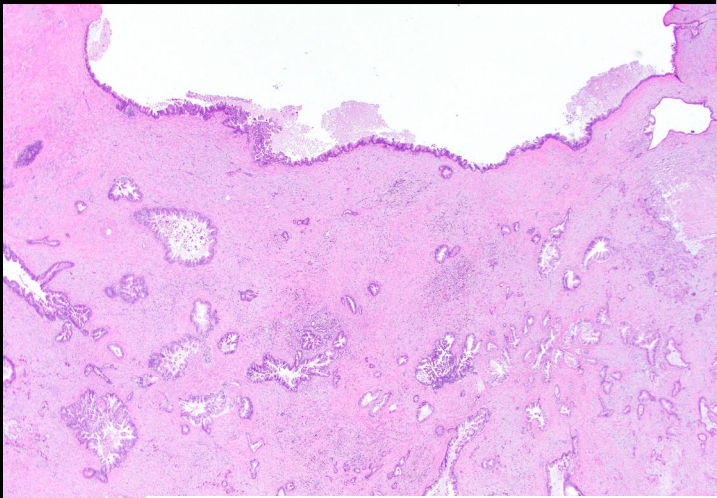
**Adenocarcinoma**



**Multiloculated IPMN**



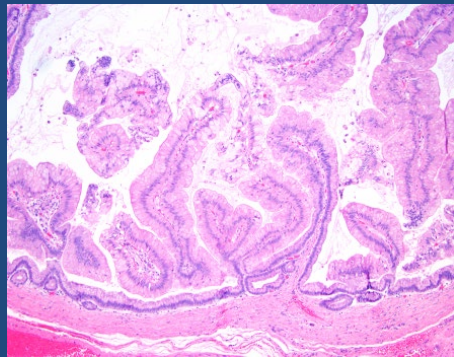
**IPMN w/ HGD**



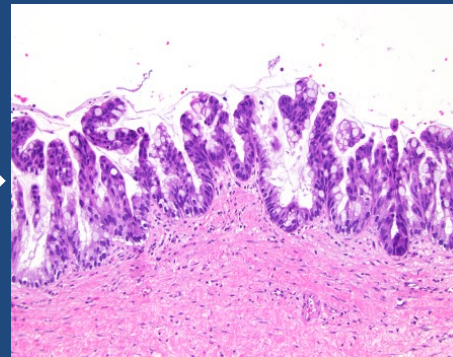
**Adenocarcinoma**

# Take Home Points: Case 6

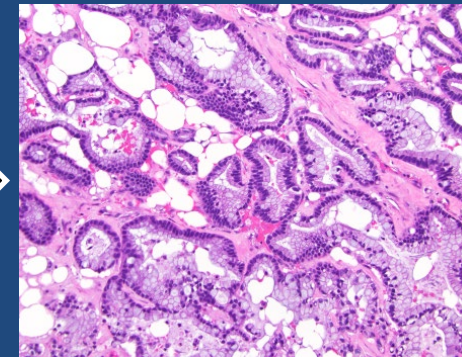
	<i>KRAS</i>	<i>GNAS</i>	<i>VHL</i>	<i>CTNNB1</i>	<i>TP53</i>	<i>PIK3CA</i>	<i>PTEN</i>
IPMN	+	+	-	-	HR	HR	HR
MCN	+	-	-	-	HR	HR	HR



Low-grade  
Dysplasia



High-grade  
Dysplasia



AdenoCA

*KRAS* & *GNAS*



*TP53*, *PIK3CA*  
& *PTEN*



HR = High-risk of malignancy

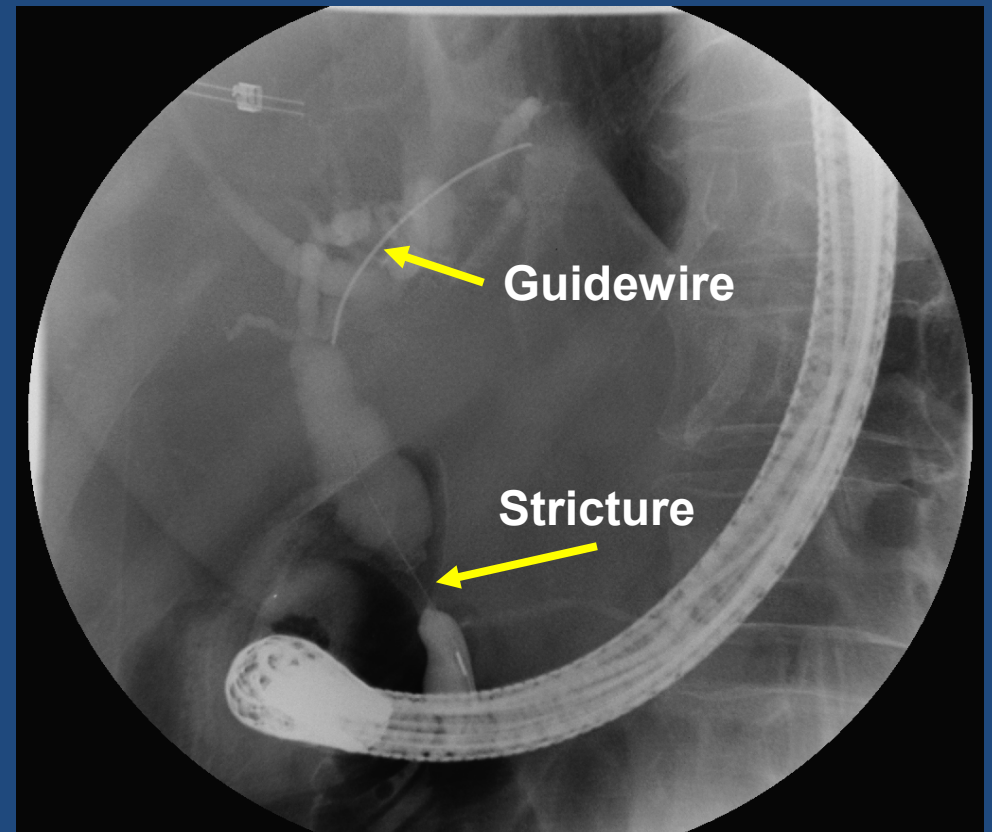
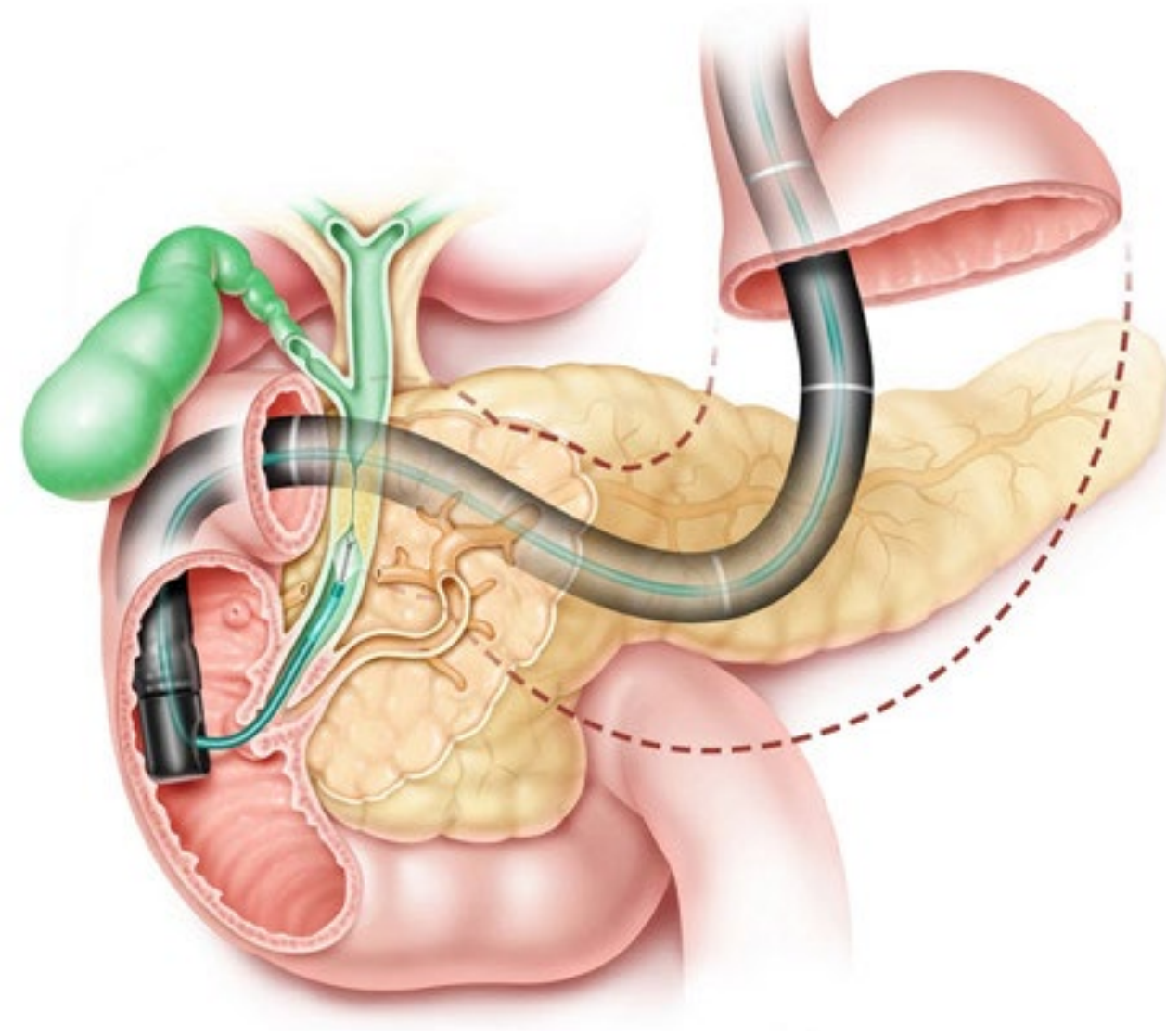


# Case 7

- A 59-year-old male with a history of primary sclerosing cholangitis, multiple stent changes, and continued hilar bile duct stricture.
- Previous brushing specimens were signed out as “atypical cells present” and current brushing showed similar findings as previously.
- Next-generation sequencing of a separate brushing specimen demonstrated missense mutations in *KRAS* and *TP53*.

# Bile Duct Stricture

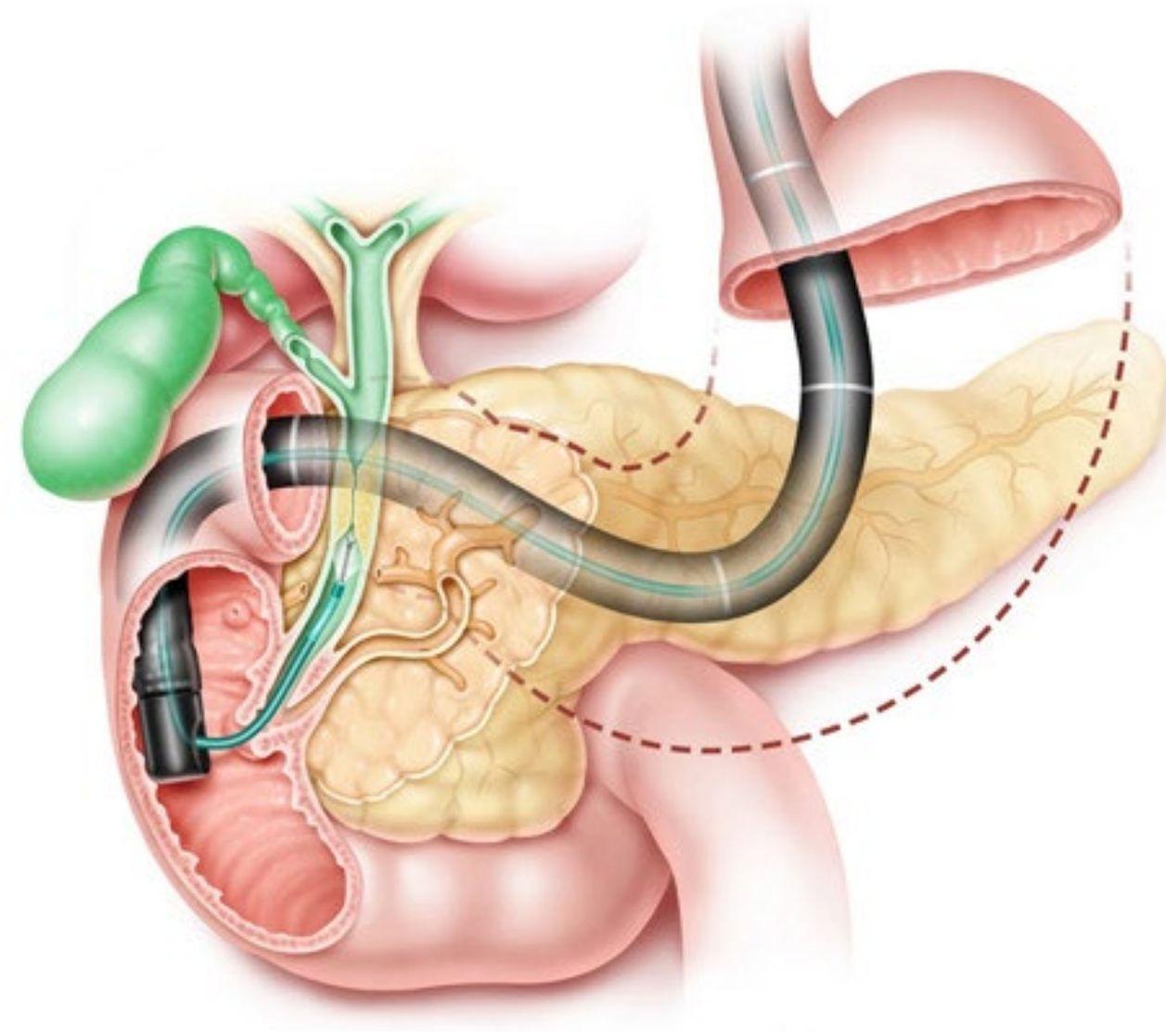
- How is a bile duct stricture evaluated?
  - **Imaging:**
    - ERCP/MRCP





# Bile Duct Stricture

- How is a bile duct stricture evaluated?
  - **Imaging:**
    - ERCP/MRCP
    - Cholangioscopy



# Bile Duct Stricture

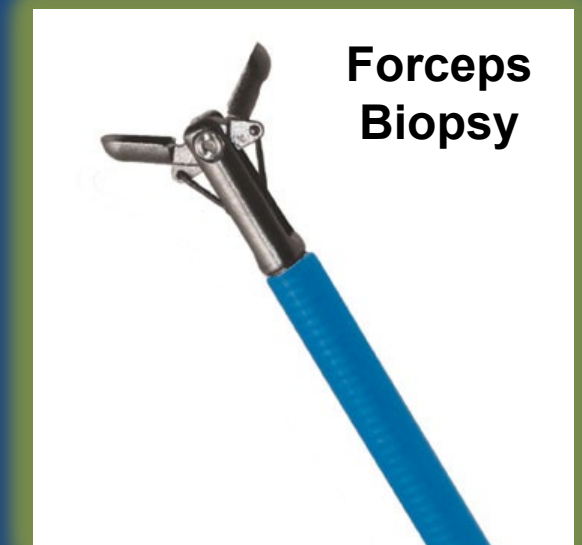
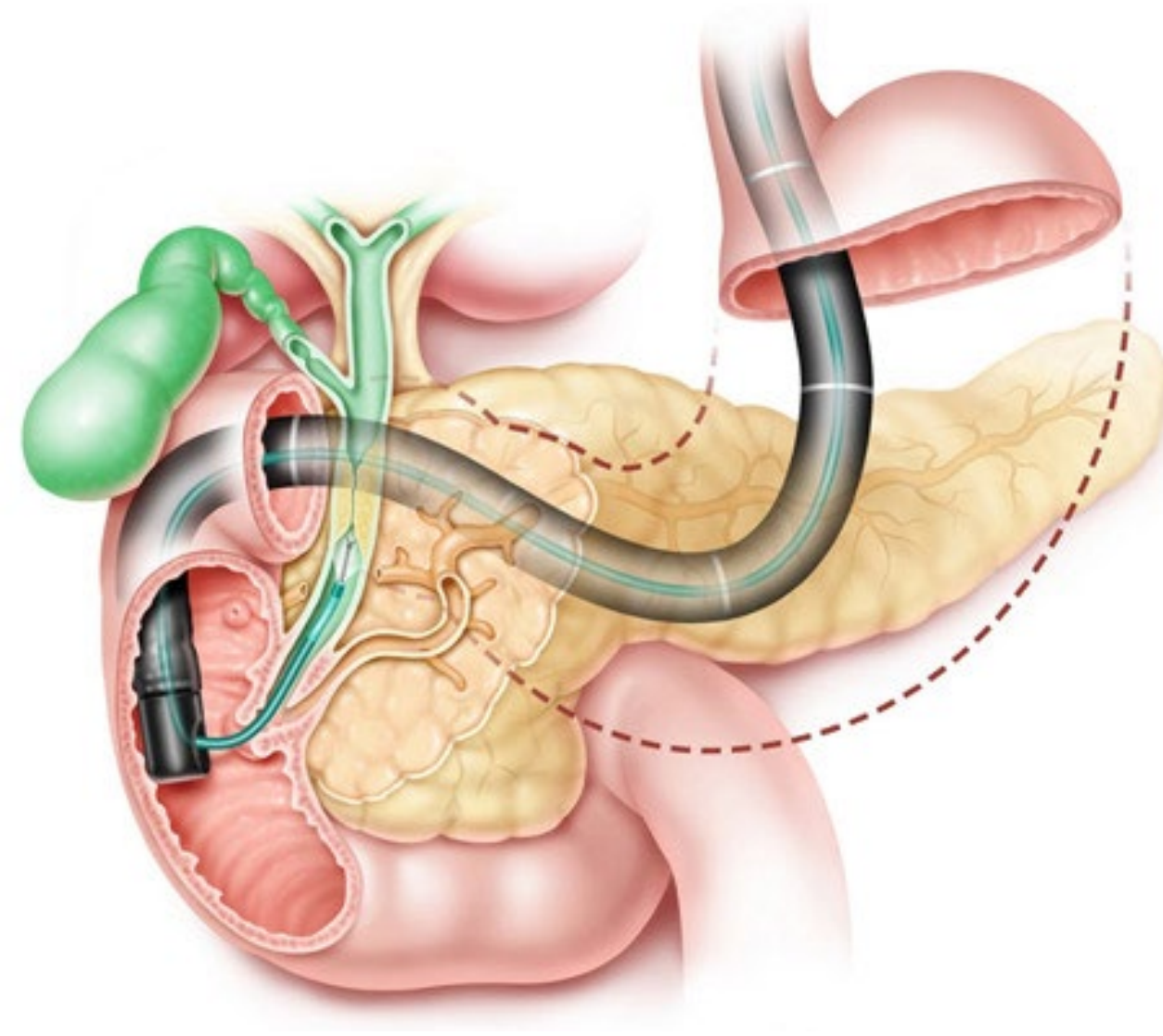
- How is a bile duct stricture evaluated?

- **Imaging:**

- ERCP/MRCP
- Cholangioscopy

- **Pathology:**

- Brushing/Biopsy



# Bile Duct Stricture

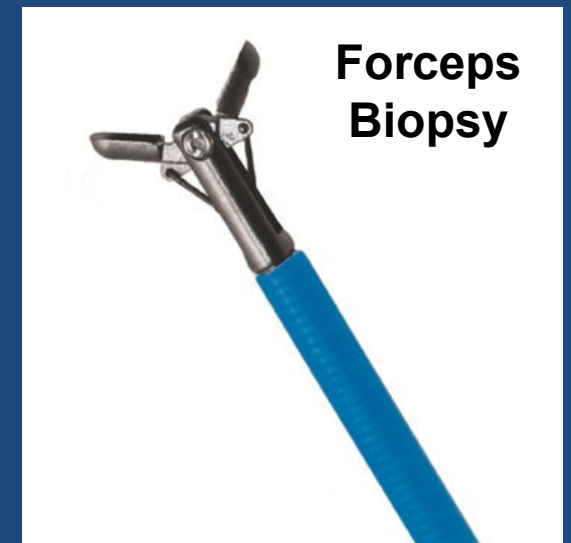
- How is a bile duct stricture evaluated?

- **Imaging**

- ERCP
- Cholangiography

- **Pathology**

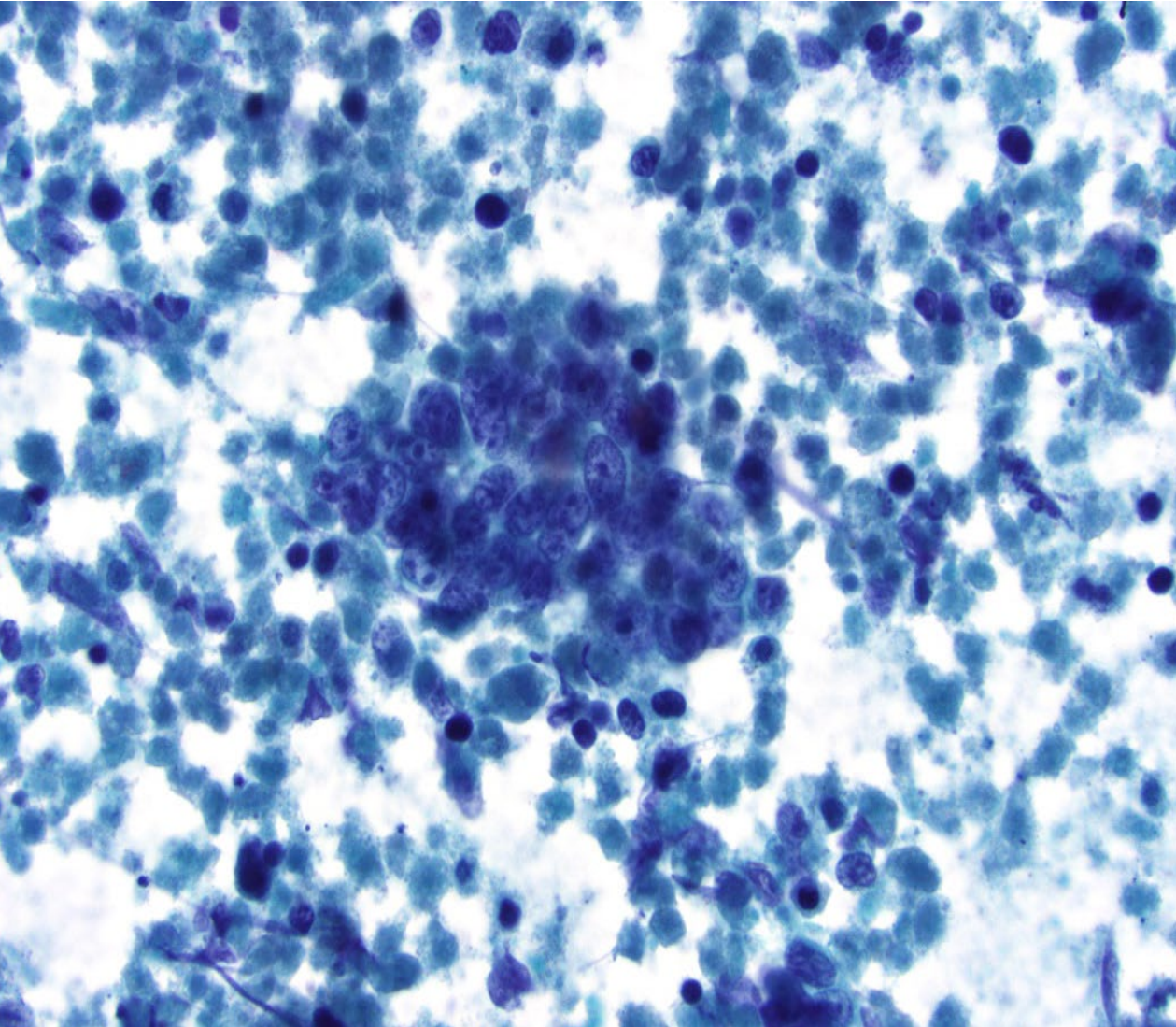
- Brushing biopsy



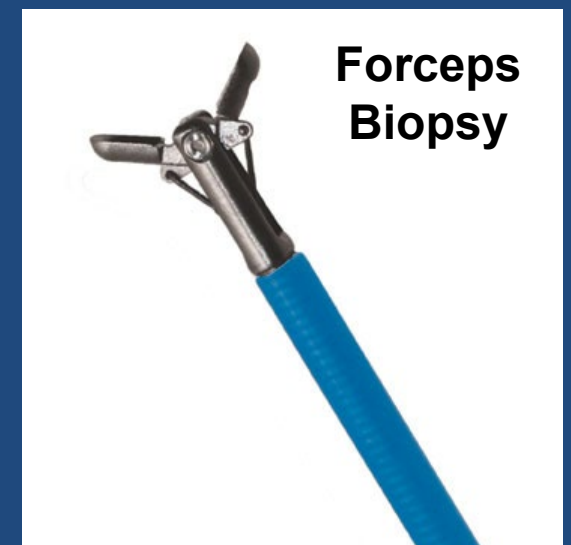


# Bile Duct Stricture

- How is a bile duct stricture evaluated?
  - **Imaging:**
    - ERCP/MRCP
    - Cholangioscopy
  - **Pathology:**
    - Brushing/Biopsy



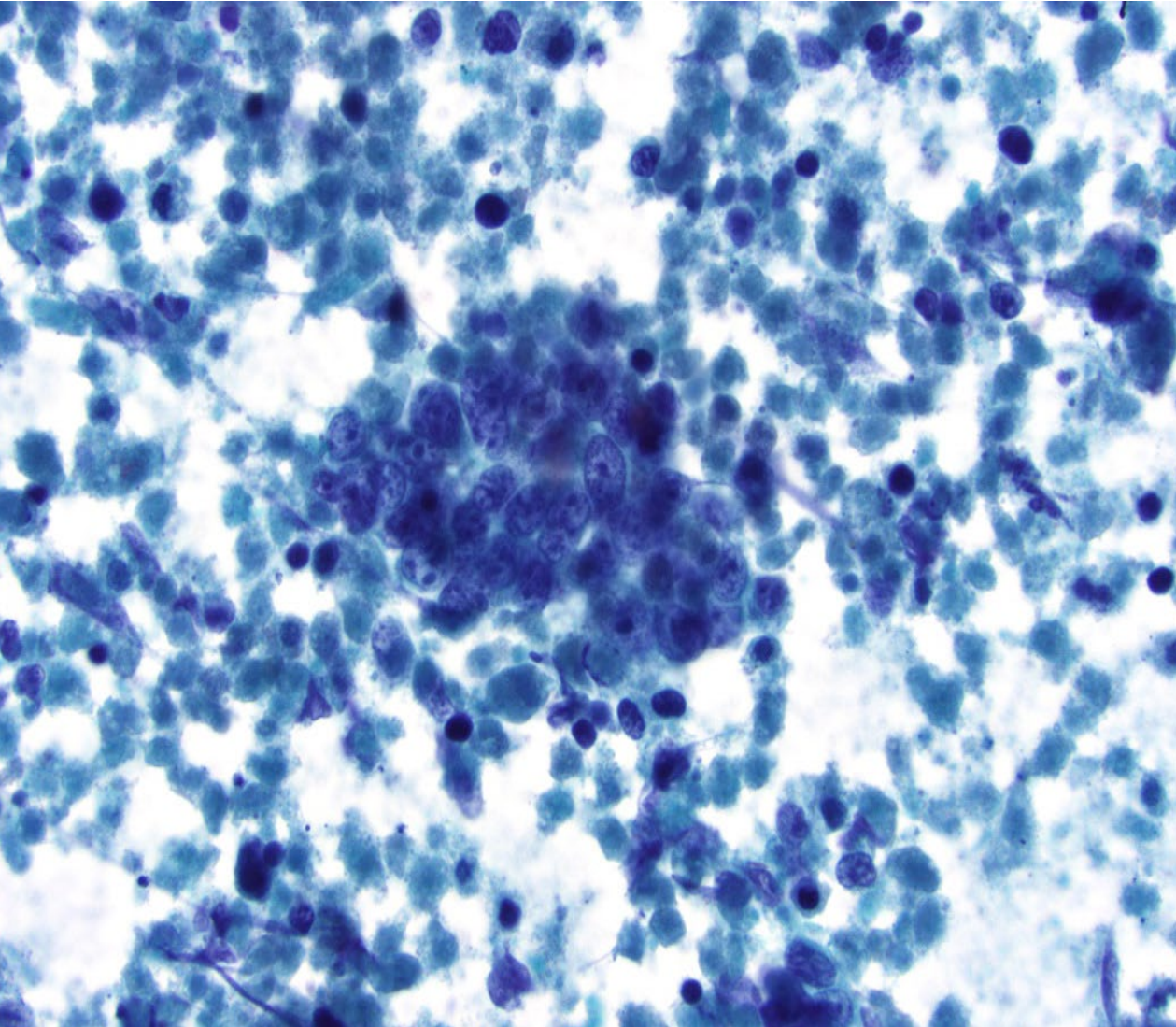
**Biliary Brushing**





# Bile Duct Stricture

- How is a bile duct stricture evaluated?
  - **Imaging:**
    - ERCP/MRCP
    - Cholangioscopy
  - **Pathology:**
    - Brushing/Biopsy



**Biliary Brushing**



**Sensitivity:**  
8% to 60%

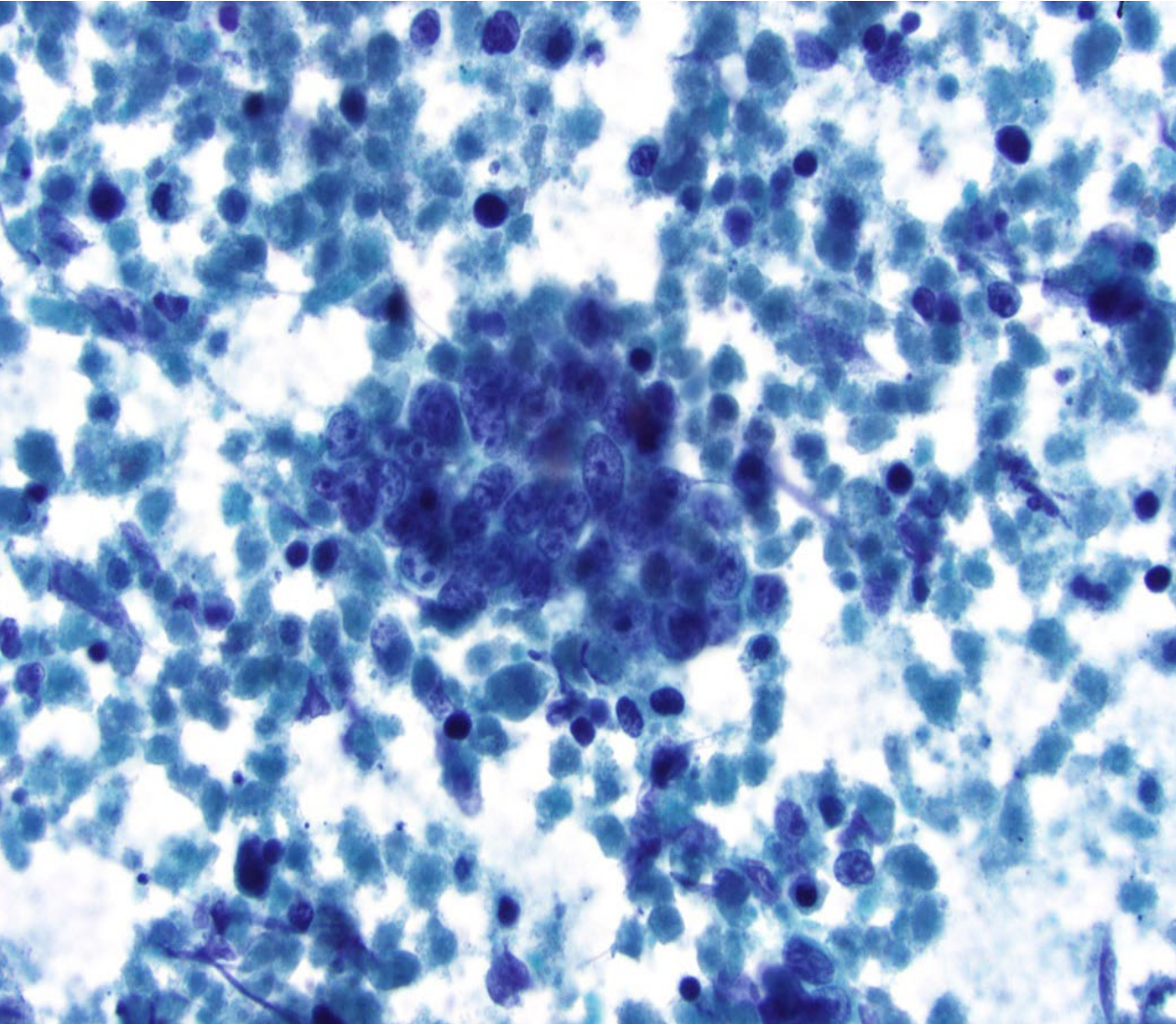
**Specificity:**  
96% to 100%



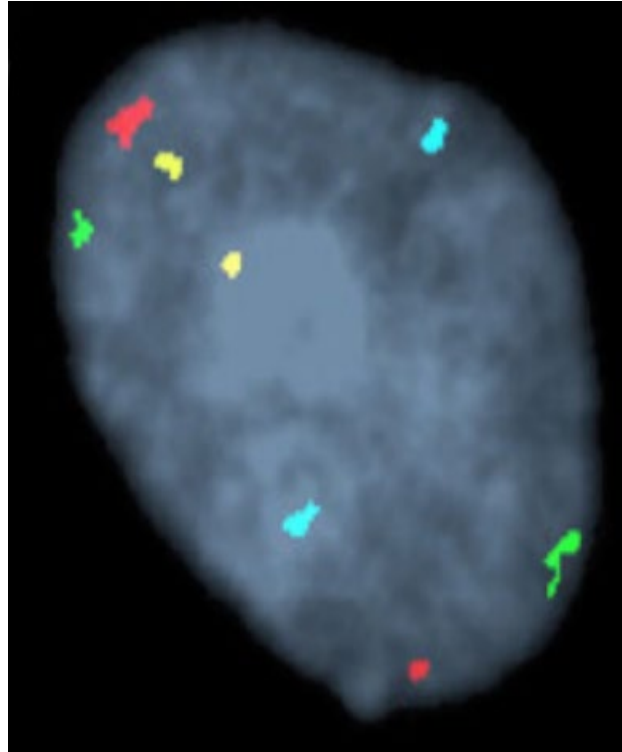
# Bile Duct Stricture

**Benign**

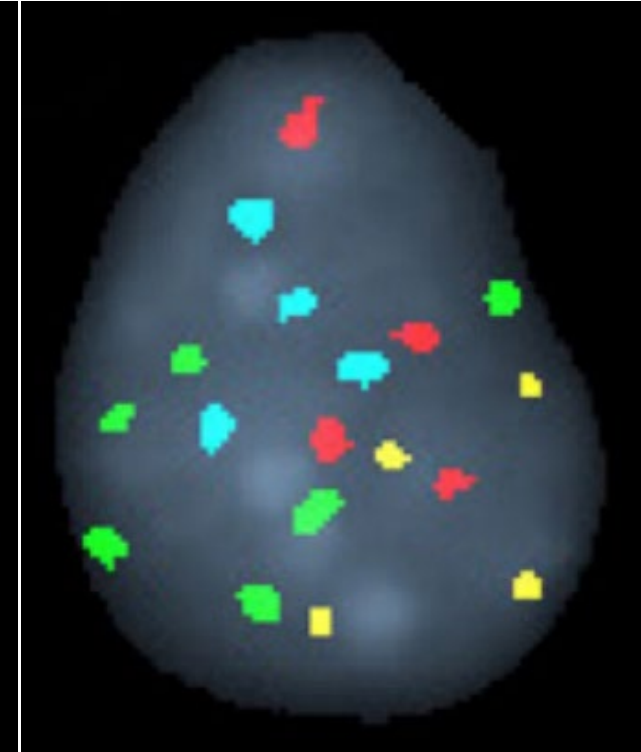
**Malignant**



**Biliary Brushing**



**Disomic**



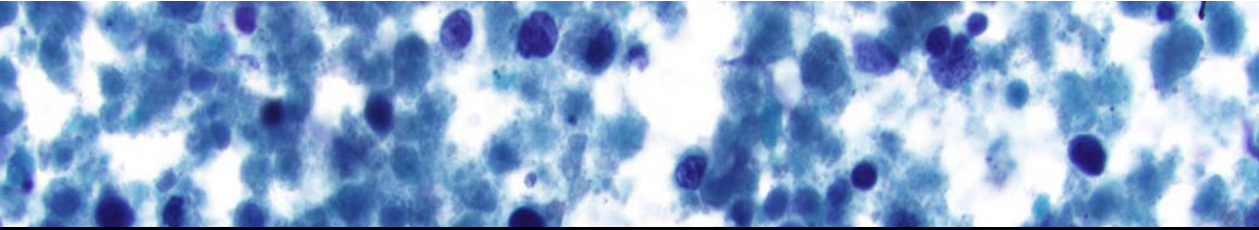
**Polysomy**

**FISH**

# Bile Duct Stricture

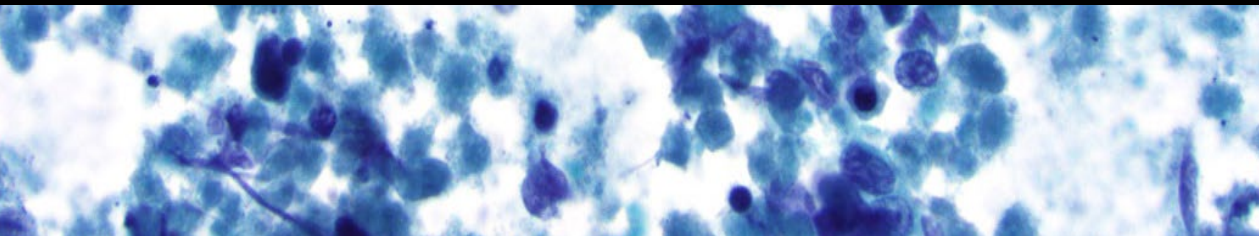
**Benign**

**Malignant**

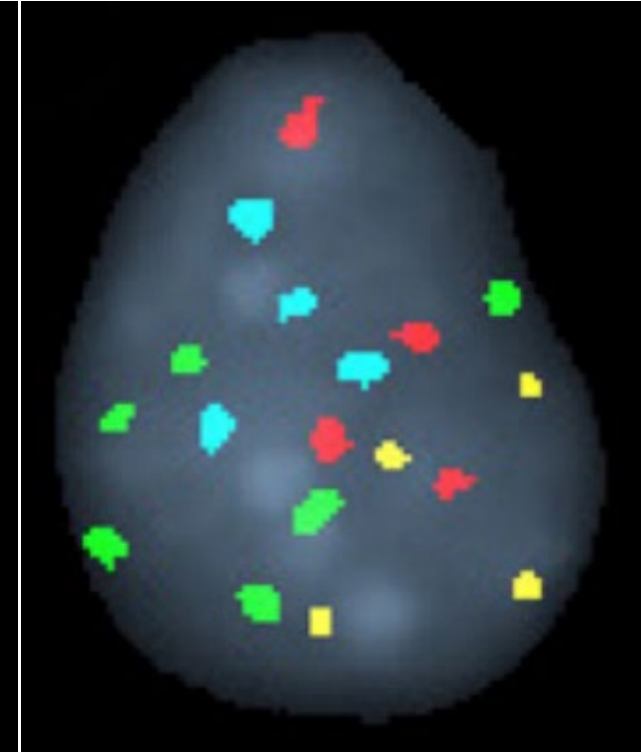
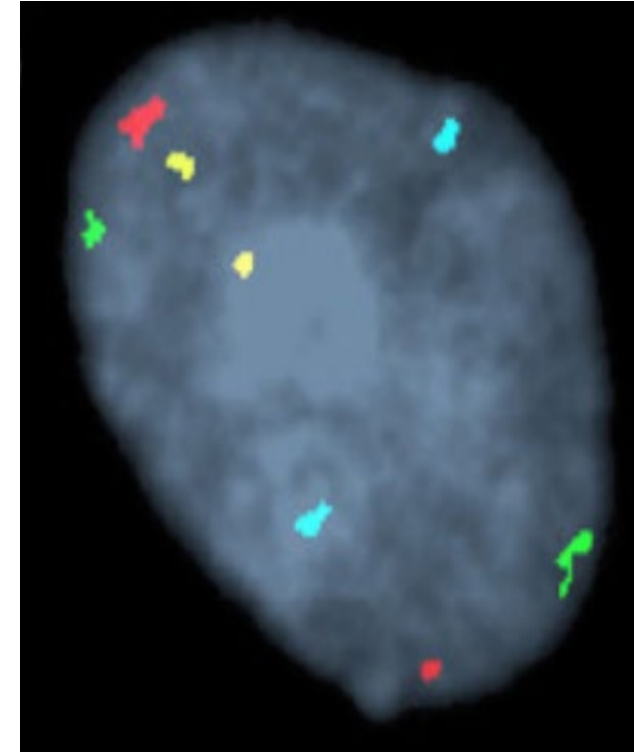


**Increase  
Sensitivity:  
~10 to 20%**

**Specificity: ~100%**



**Biliary Brushing**



**Disomic**

**Polysomy**

**FISH**




# Next-Generation Sequencing and Fluorescence *in Situ* Hybridization Have Comparable Performance Characteristics in the Analysis of Pancreaticobiliary Brushings for Malignancy



Jonathan C. Dudley,\* Zongli Zheng,\* Thomas McDonald,\* Long P. Le,\* Dora Dias-Santagata,\* Darrell Borger,\* Julie Batten,\* Kathy Vernovsky,\* Brenda Sweeney,\* Ronald N. Arpin,\* William R. Brugge,<sup>††</sup> David G. Forcione,<sup>††</sup> Martha B. Pitman,\* and A. John Iafrate\*

ORIGINAL ARTICLE

## Integrating next-generation sequencing to endoscopic retrograde cholangiopancreatography (ERCP)-obtained biliary specimens improves the detection and management of patients with malignant bile duct strictures

Aatur D Singhi ,<sup>1</sup> Marina N Nikiforova,<sup>1</sup> Jennifer Chennat,<sup>2</sup> Georgios I Papachristou,<sup>2</sup> Asif Khalid,<sup>2</sup> Mordechai Rabinovitz,<sup>2</sup> Rohit Das,<sup>3</sup> Savreet Sarkaria,<sup>3</sup> M Samir Ayasso,<sup>3</sup> Abigail I Wald,<sup>1</sup> Sara E Monaco,<sup>1</sup> Michael Nalesnik,<sup>4</sup> N Paul Ochori,<sup>1</sup> David Geller,<sup>5</sup> Allan Tsung,<sup>5</sup> Amer H Zureikat,<sup>5</sup> Herbert Zeh,<sup>6</sup> J Wallis Marsh,<sup>7</sup> Melissa Hogg,<sup>8</sup> Kenneth Lee,<sup>9</sup> David L Bartlett,<sup>5</sup> James F Pingpank,<sup>5</sup> Abhinav Humar,<sup>10</sup> Nathan Bahary,<sup>11</sup> Anil K Dasyam,<sup>12</sup> Randall Brand,<sup>2</sup> Kenneth E Fasanella,<sup>2</sup> Kevin McGrath,<sup>2</sup> Adam Slivka<sup>2</sup>

## Molecular diagnosis of pancreaticobiliary tract cancer by detecting mutations and methylation changes in bile samples





Shun He,<sup>ah</sup> Fanxin Zeng,<sup>bh</sup> Huihui Yin,<sup>ch</sup> Pei Wang,<sup>ch</sup> Yinlei Bai,<sup>dh</sup> Qianqian Song,<sup>ch</sup> Jiangtao Chu,<sup>a</sup> Zhen Huang,<sup>ef</sup> Yumeng Liu,<sup>g</sup> Hong Liu,<sup>g</sup> Qichen Chen,<sup>ef</sup> Li Liu,<sup>d</sup> Jun Zhou,<sup>b</sup> Hanjie Hu,<sup>ef</sup> Xingchen Li,<sup>ef</sup> Tengyan Li,<sup>ef</sup> Guiqi Wang,<sup>a,\*\*\*\*</sup> Jianqiang Cai,<sup>ef,\*\*\*\*</sup> Yuchen Jiao,<sup>c,\*\*\*</sup> and Hong Zhao<sup>ef,†\*</sup>







Article

## Bile-Based Cell-Free DNA Analysis Is a Reliable Diagnostic Tool in Pancreatobiliary Cancer

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



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# Clinical Evaluation of **Next-Generation Sequencing**

- Between 2019 to 2022, **1208 ERCP-obtained bile duct specimens (751 brushings and 457 biopsies)** from 754 patients were submitted for **NGS** testing from **7 academic institutions**.
- Parallel **ERCP-obtained biliary brushings and biopsies** were submitted for pathologic evaluation.
- **Only two biliary specimens were insufficient** for NGS-based testing.
- Findings were correlated with clinicopathologic features and designated as benign or neoplastic based on diagnostic pathology or clinical course of >12 months.



# Follow-up ( $n = 548$ , 73%)

Diagnostic Pathology/Clinical Follow-up of >12 Months	Total, $n = 548$
Intrahepatic cholangiocarcinoma	107
Extrahepatic cholangiocarcinoma	73
Hepatocellular carcinoma	3
Mixed HCC-cholangiocarcinoma	2
Pancreatic adenocarcinoma	88
Gallbladder adenocarcinoma	7
Ampullary adenocarcinoma	28
Metastatic neoplasms	15
High/low-grade dysplasia	10
Benign cholangiopathy	215

**333 Neoplastic Strictures**

**215 Benign Strictures**

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Benign cholangiopathy	215

## Neoplastic Strictures

### NGS Testing

- Sensitivity: 82%
- Specificity: 96%\*

### Pathologic Evaluation

- Sensitivity: 49%
- Specificity: 100%

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## Neoplastic Strictures

### NGS Testing

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**\*Are genomic alterations present prior to the histologic findings?**

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## Neoplastic Strictures

### NGS Testing

- Sensitivity: 82%
- Specificity: 96%\*

### Pathologic Evaluation

- Sensitivity: 49%
- Specificity: 100%

### NGS + Pathology

- Sensitivity: 88%
- Specificity: 96%

# Case 7

- A 59-year-old male with a history of primary sclerosing cholangitis, multiple stent changes, and continued hilar bile duct stricture.
- Previous brushing specimens were signed out as “atypical cells present” and current brushing showed similar findings as previously.
- Next-generation sequencing of a separate brushing specimen demonstrated missense mutations in *KRAS* and *TP53*.



# NGS/PSC: Follow-up ( $n = 548, 73\%$ )

## Primary Sclerosing Cholangitis ( $n = 96$ )

### Neoplastic Strictures

#### NGS Testing

- Sensitivity: 84%
- Specificity: 95%\*

#### Pathologic Evaluation

- Sensitivity: 30%
- Specificity: 100%

#### NGS + Pathology

- Sensitivity: 89%
- Specificity: 95%

Next-generation sequencing in the evaluation of biliary strictures in patients with primary sclerosing cholangitis

Joha  
Krishn

umar  
sdraji,

## Case 7: Adenocarcinoma

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performs when it is added to BB cytology and FISH in patients with PSC. **METHODS:** This study reports the authors' experience with NGS performed as a prospective cotest with cytology and FISH on BBs obtained from 60 patients with PSC followed at Massachusetts General Hospital. A duct with malignancy was defined as a high-risk (HR) stricture with either high-grade dysplasia or CCA. **RESULTS:** NGS was better than FISH and cytology in detecting HR strictures, which showed multiple genetic mutations in all cases. NGS provided specific mutational information, and NGS results were reproducible in longitudinal samples. **CONCLUSIONS:** Adding NGS to BB cytology and FISH in the evaluation of biliary strictures for patients with PSC may provide additional information that could help to inform clinical management. *Cancer Cytopathol* 2022;130:215-230.

© 2021 American Cancer Society.

# Case 8

- A 79-year-old male, status post cholecystectomy, with a distal bile duct stent presents with continued abdominal pain and distal biliary obstruction.
- A previous distal common bile duct biopsy was signed out as “reactive biliary epithelium with erosion and marked acute inflammation.”
- A repeat bile duct biopsy was performed along with parallel molecular (next-generation sequencing) testing: **negative for mutations.**

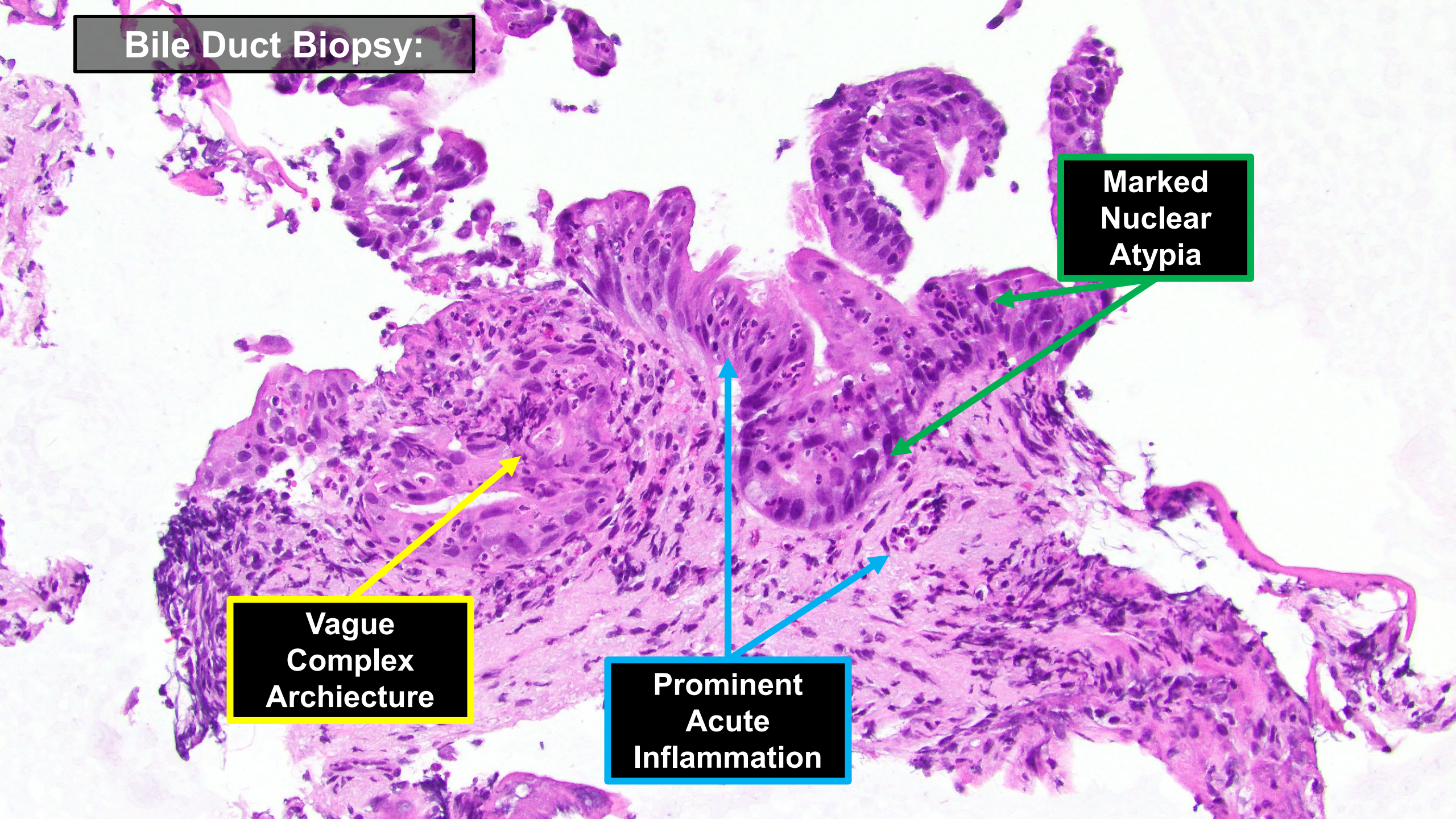


**Bile Duct Biopsy:**

**Marked  
Nuclear  
Atypia**

**Vague  
Complex  
Architecture**

**Prominent  
Acute  
Inflammation**





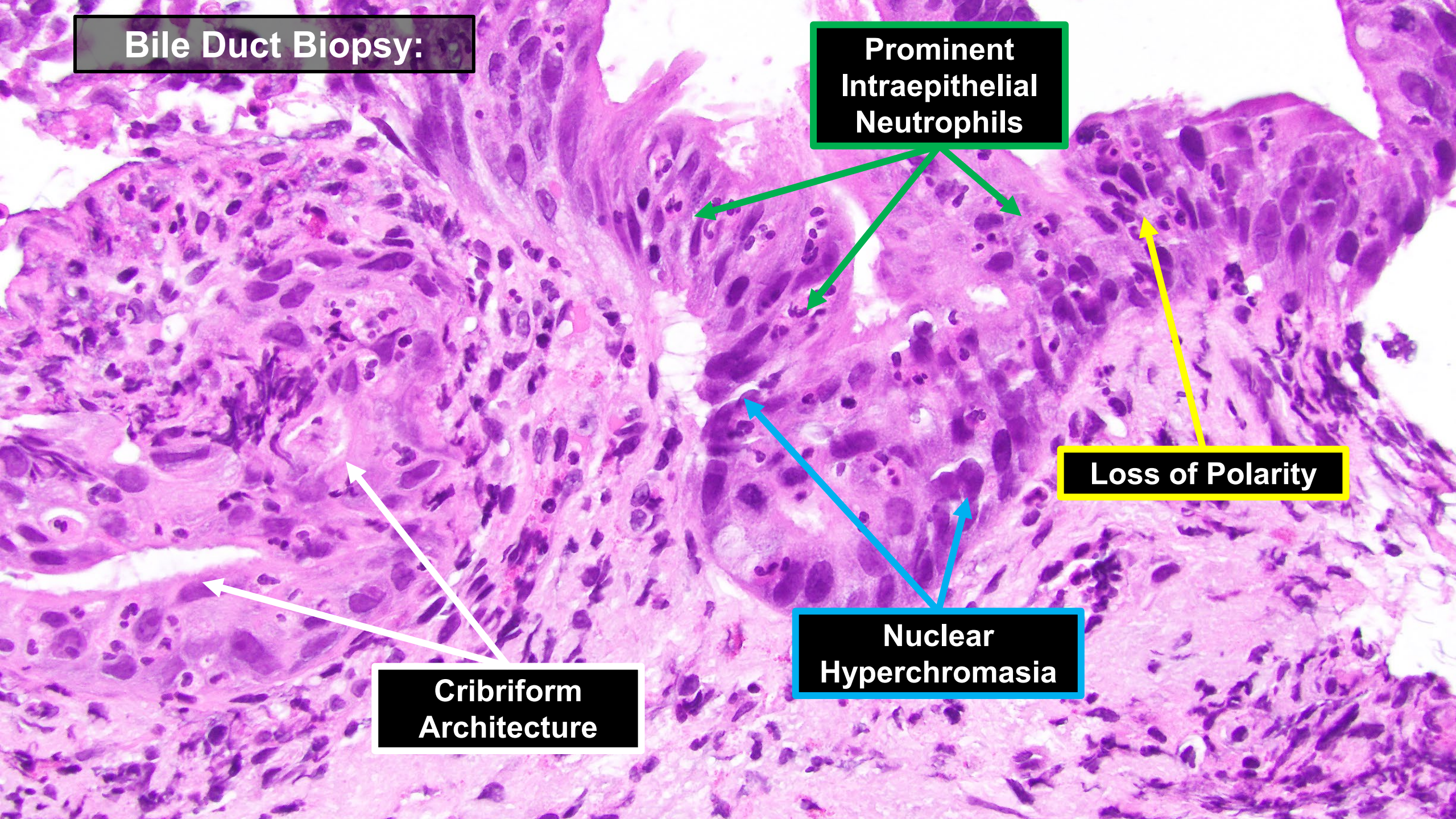
**Bile Duct Biopsy:**

**Prominent  
Intraepithelial  
Neutrophils**

**Loss of Polarity**

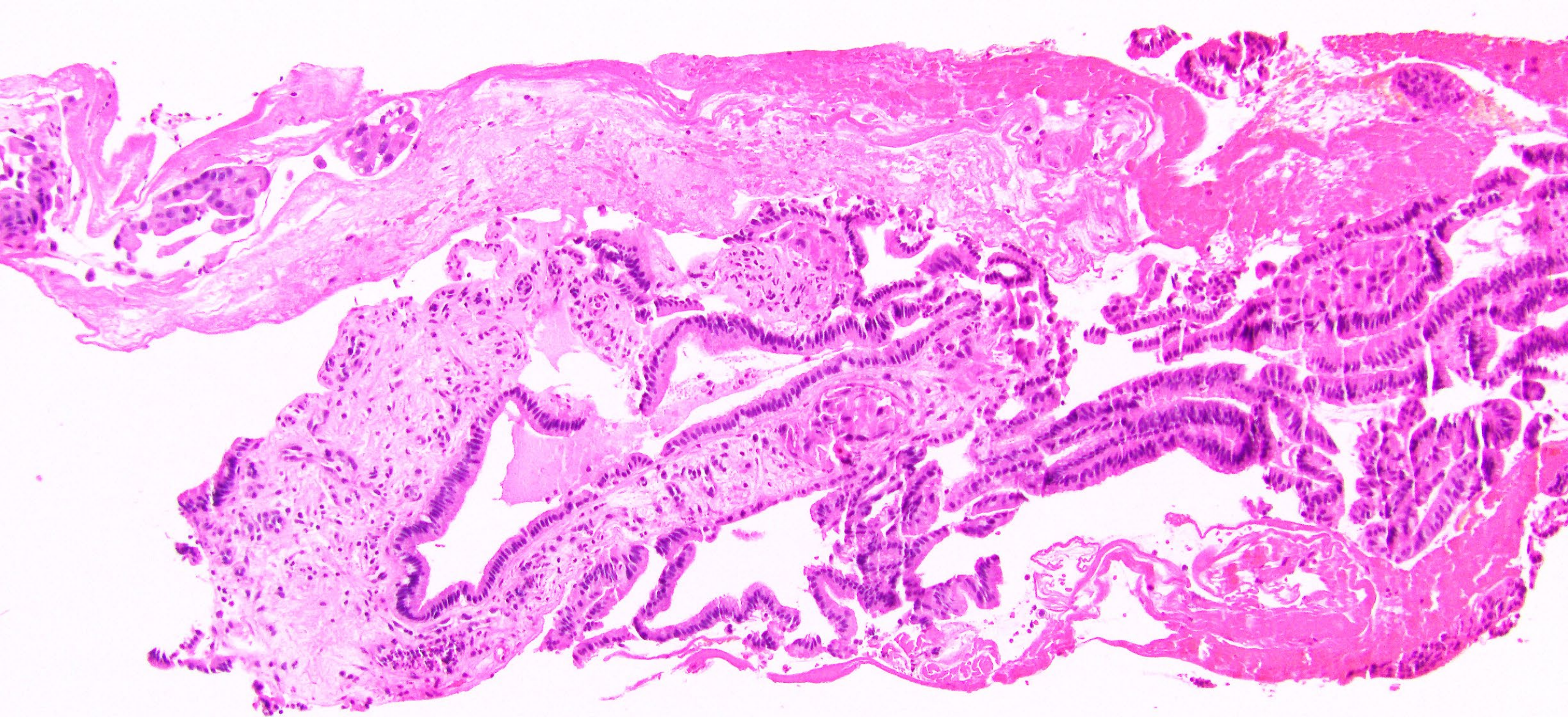
**Nuclear  
Hyperchromasia**

**Cribriform  
Architecture**

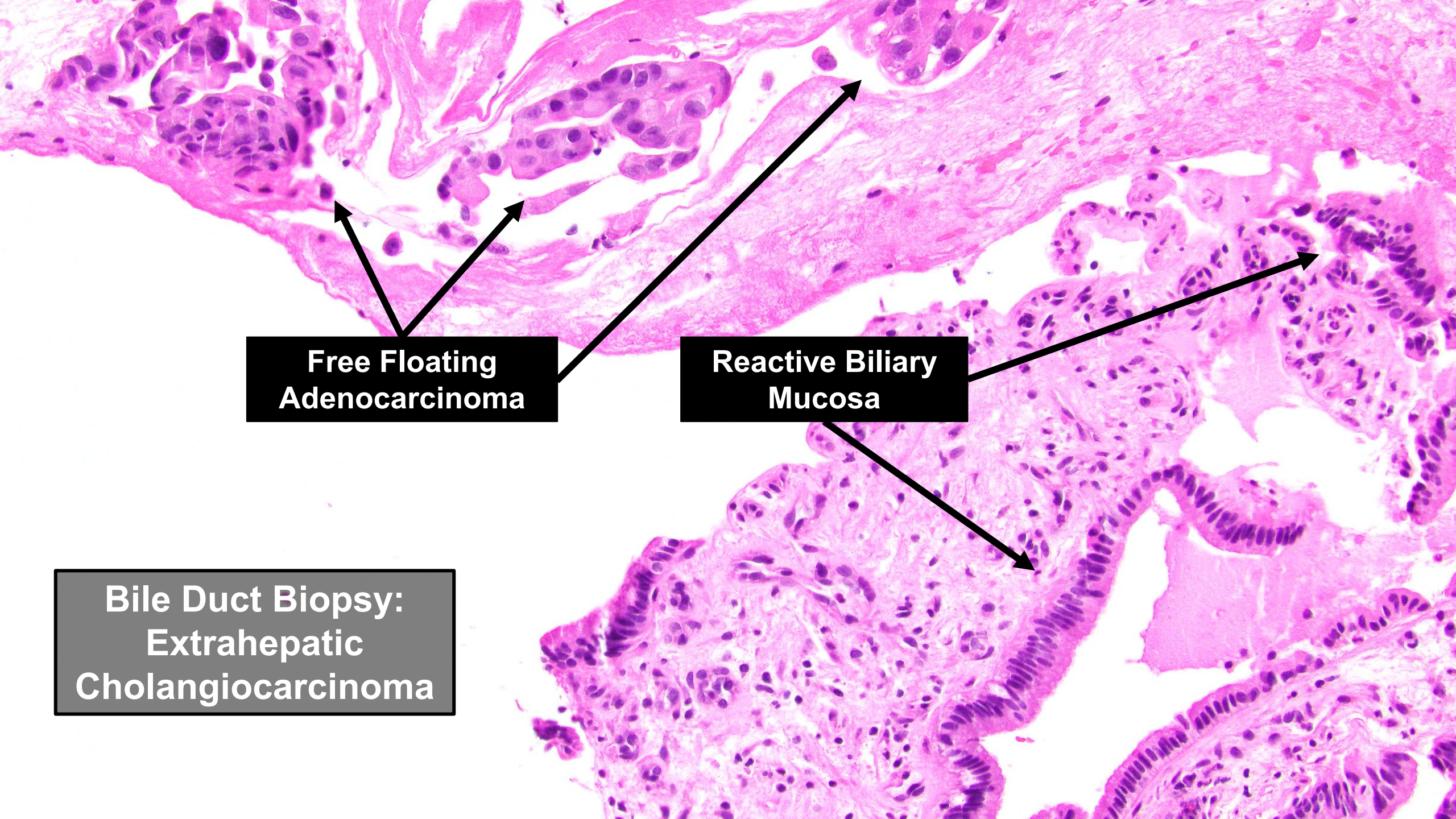




# Bile Duct Biopsies







**Free Floating  
Adenocarcinoma**

**Reactive Biliary  
Mucosa**

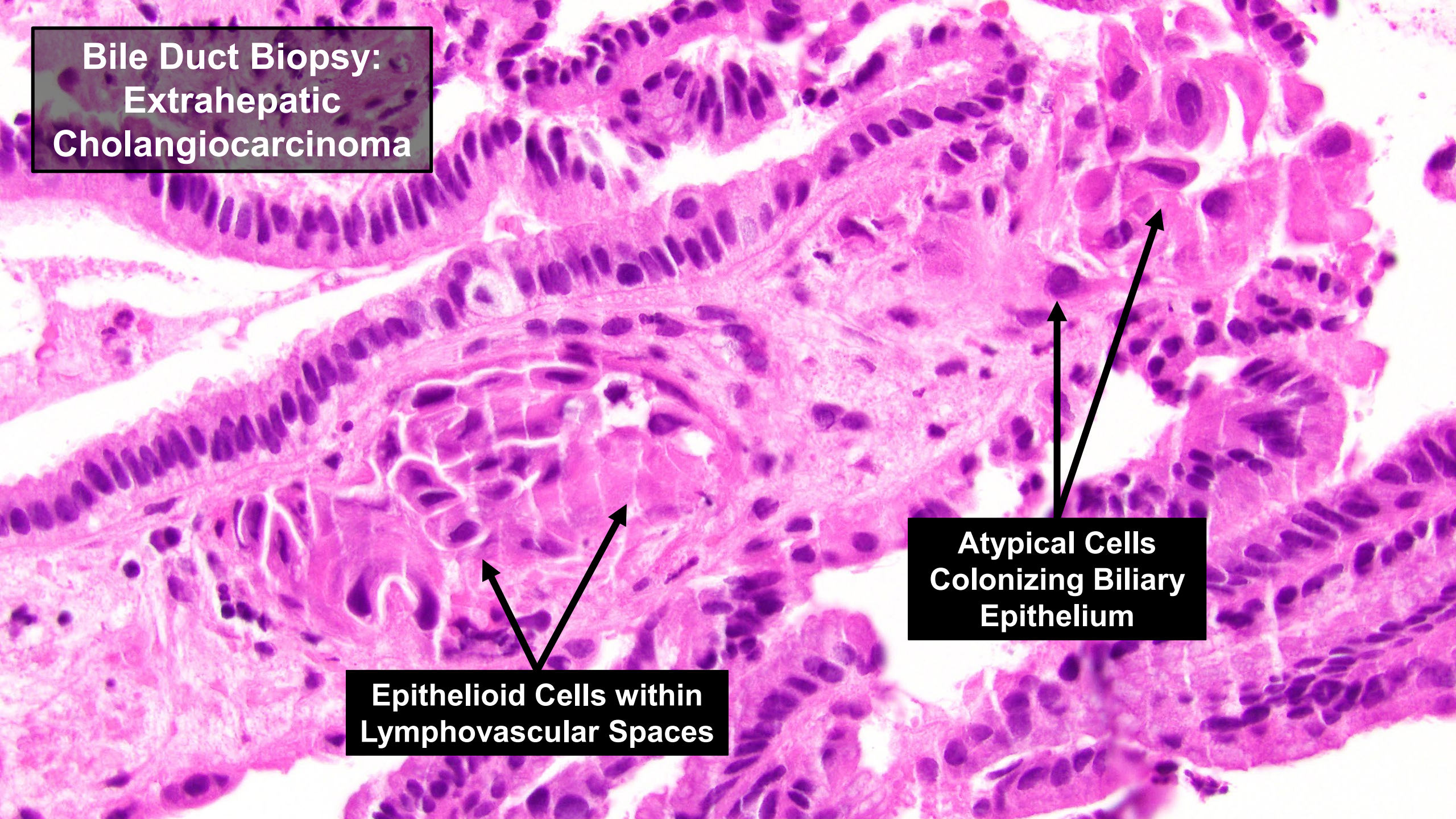
**Bile Duct Biopsy:  
Extrahepatic  
Cholangiocarcinoma**



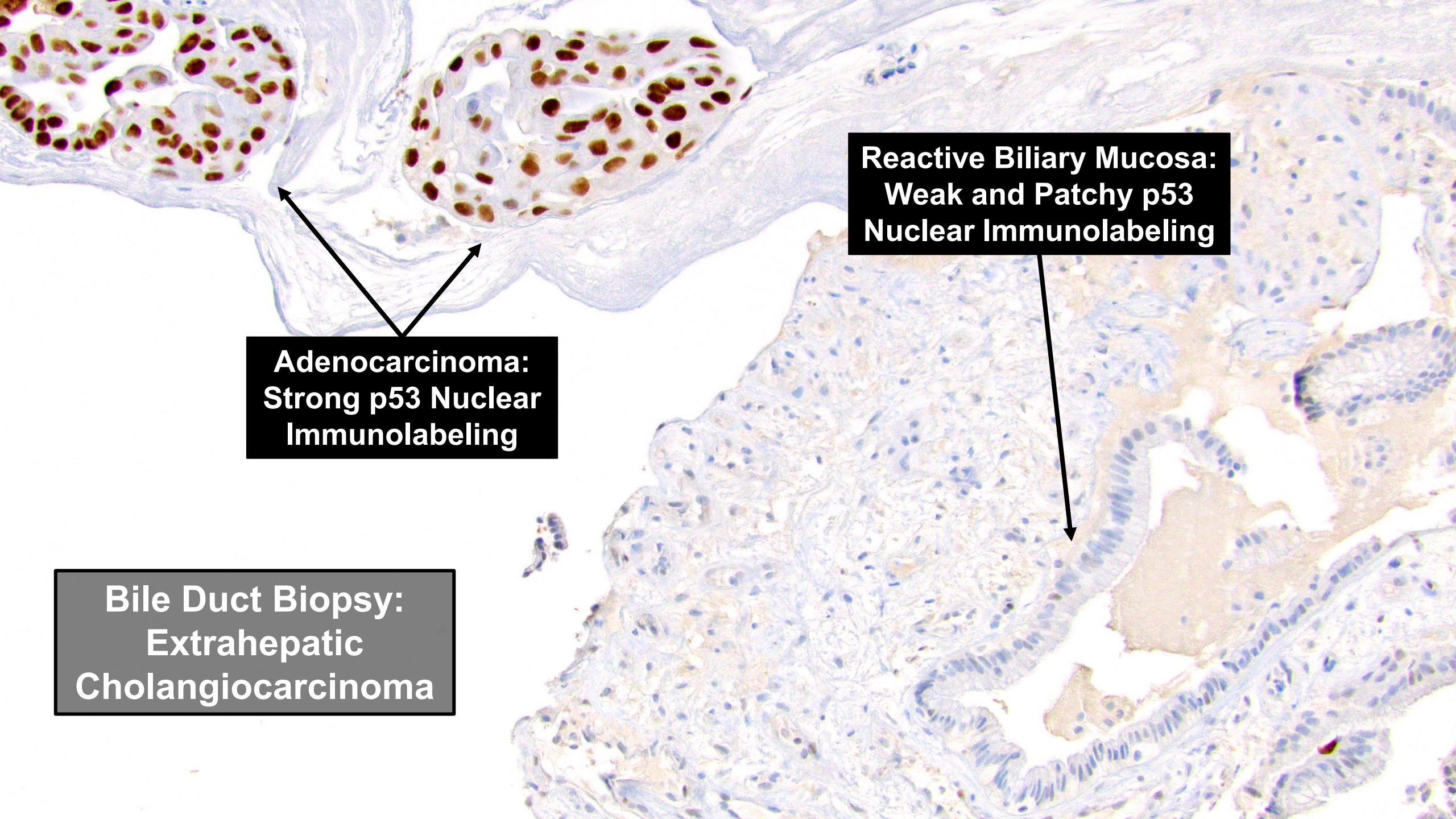
**Bile Duct Biopsy:  
Extrahepatic  
Cholangiocarcinoma**

**Epithelioid Cells within  
Lymphovascular Spaces**

**Atypical Cells  
Colonizing Biliary  
Epithelium**







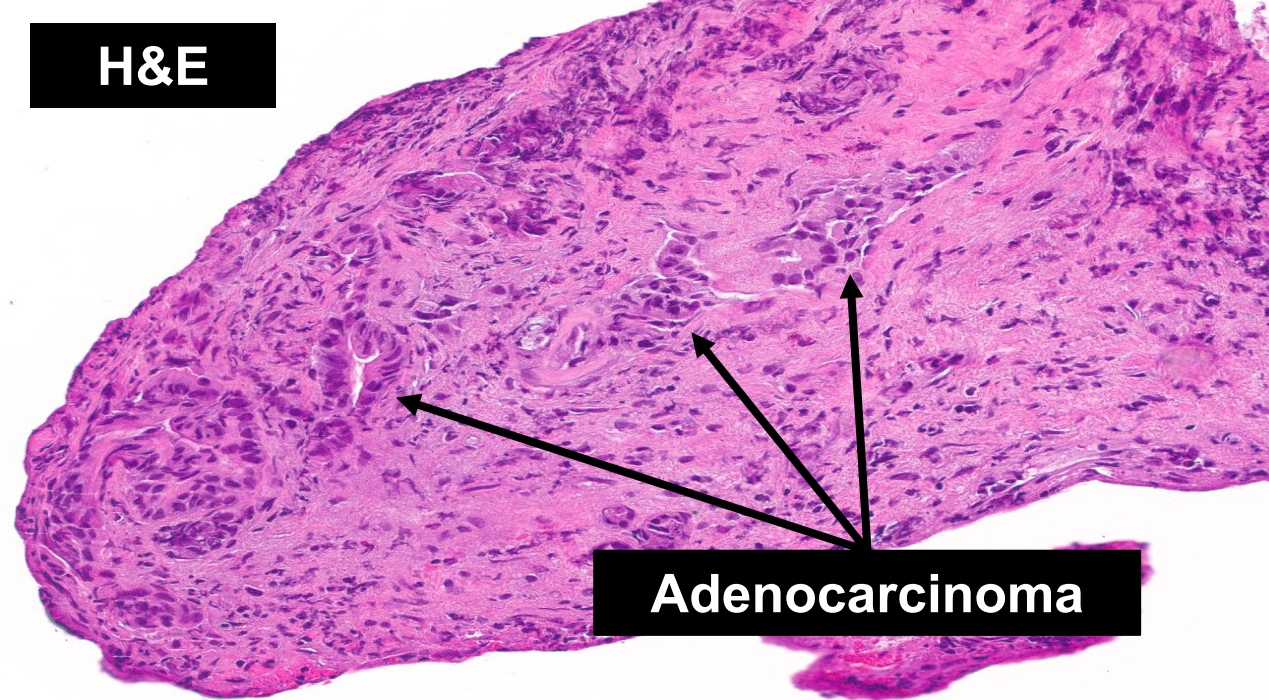
**Adenocarcinoma:  
Strong p53 Nuclear  
Immunolabeling**

**Reactive Biliary Mucosa:  
Weak and Patchy p53  
Nuclear Immunolabeling**

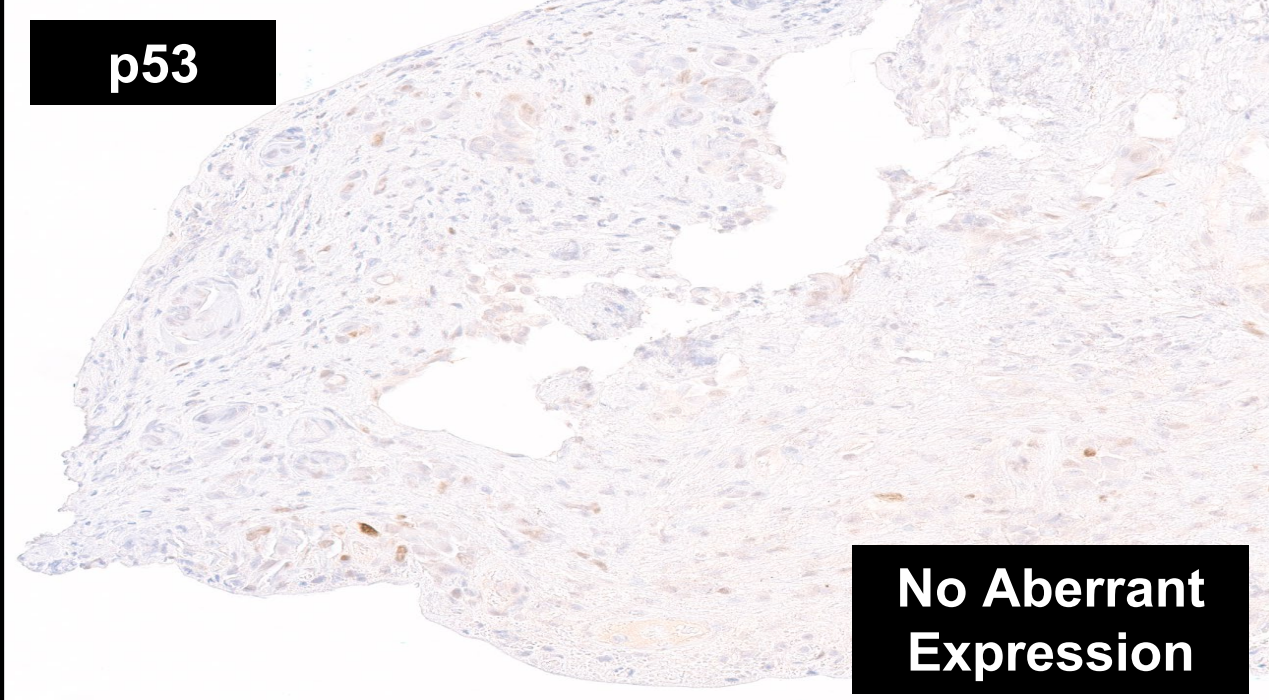
**Bile Duct Biopsy:  
Extrahepatic  
Cholangiocarcinoma**



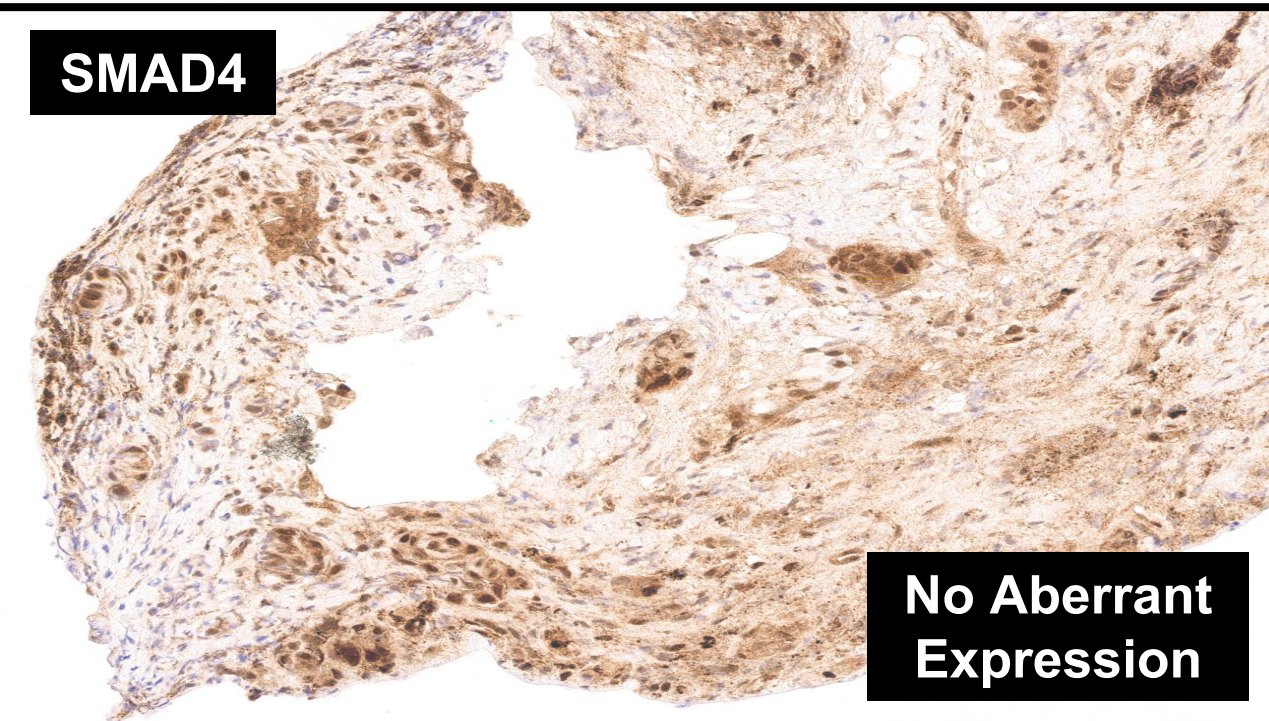
**H&E**



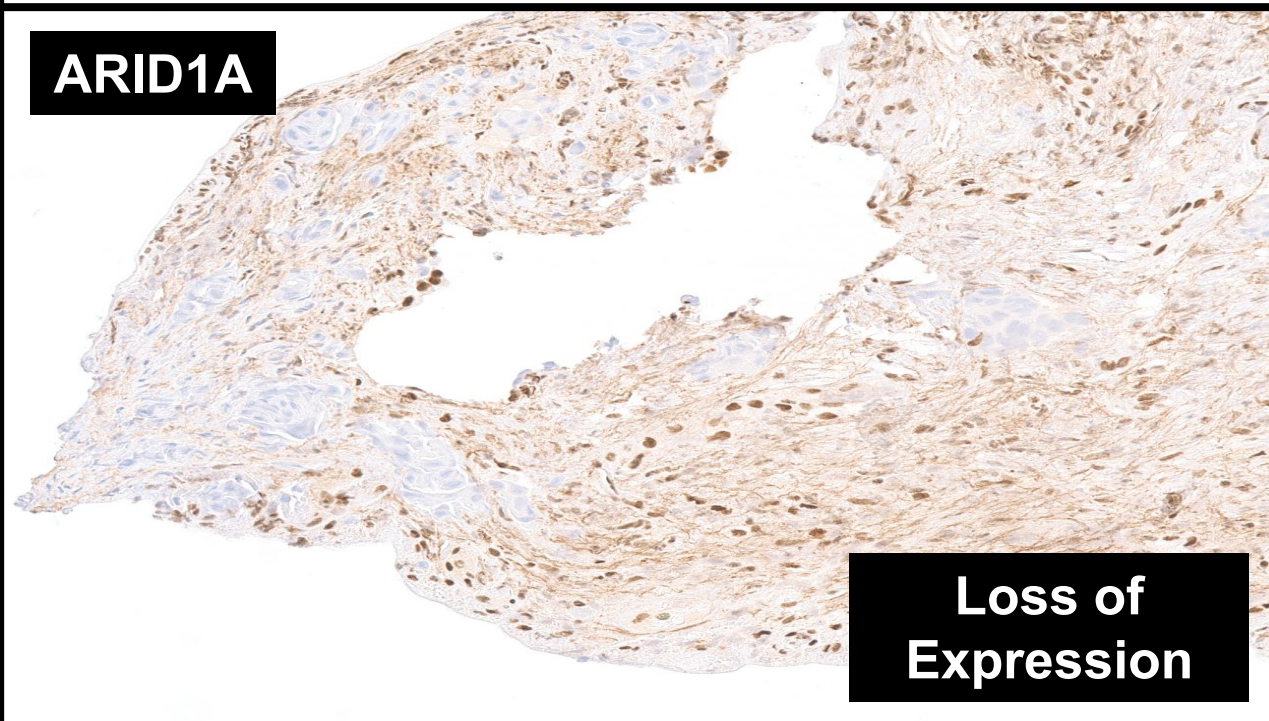
**p53**



**SMAD4**

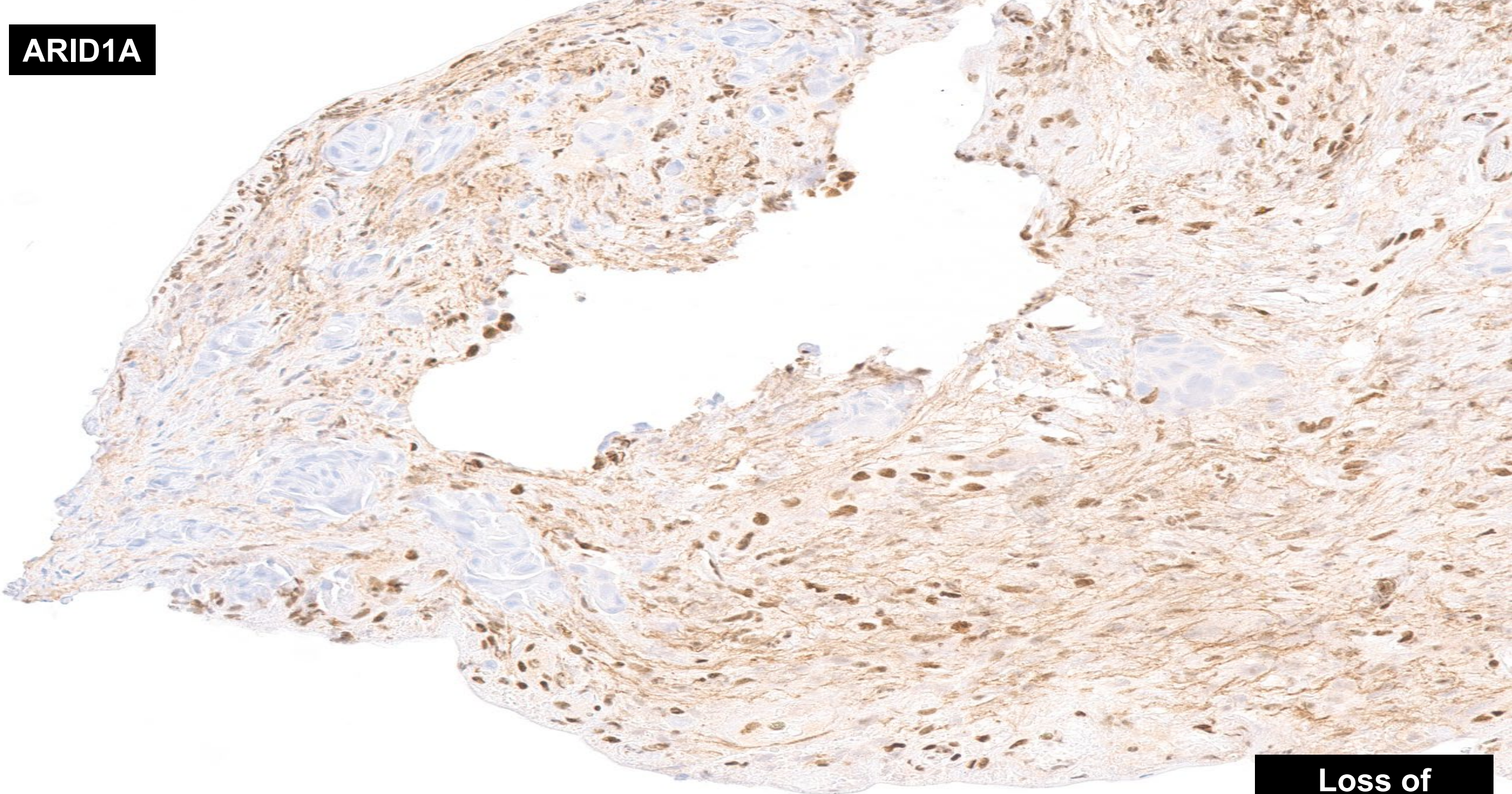


**ARID1A**





**ARID1A**



**Loss of  
Expression**



# Case 8

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- A previous distal common bile duct biopsy was signed out as “reactive biliary epithelium with erosion and marked acute inflammation.”
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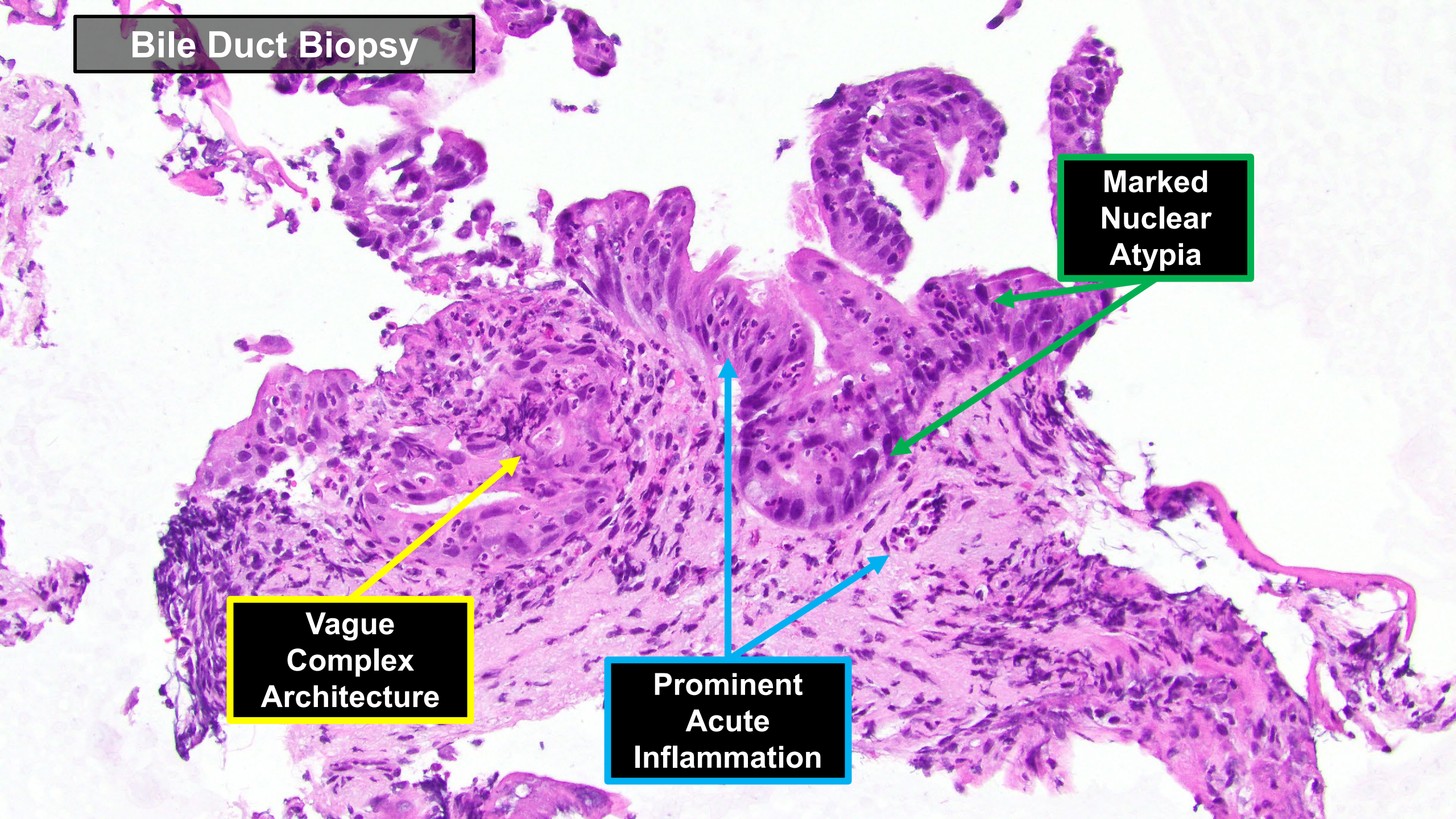


**Bile Duct Biopsy**

**Marked  
Nuclear  
Atypia**

**Vague  
Complex  
Architecture**

**Prominent  
Acute  
Inflammation**





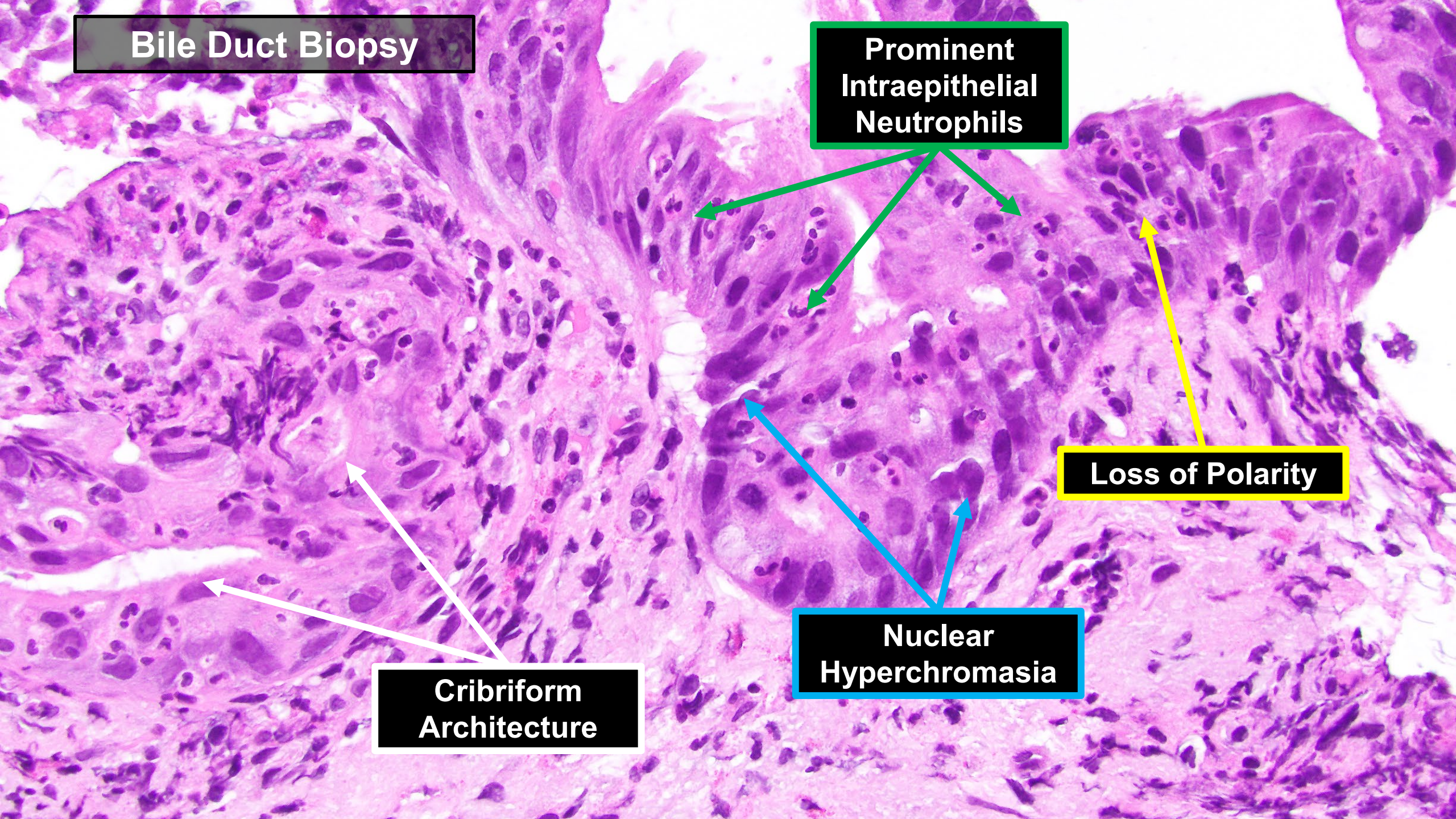
**Bile Duct Biopsy**

**Prominent  
Intraepithelial  
Neutrophils**

**Loss of Polarity**

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Architecture**

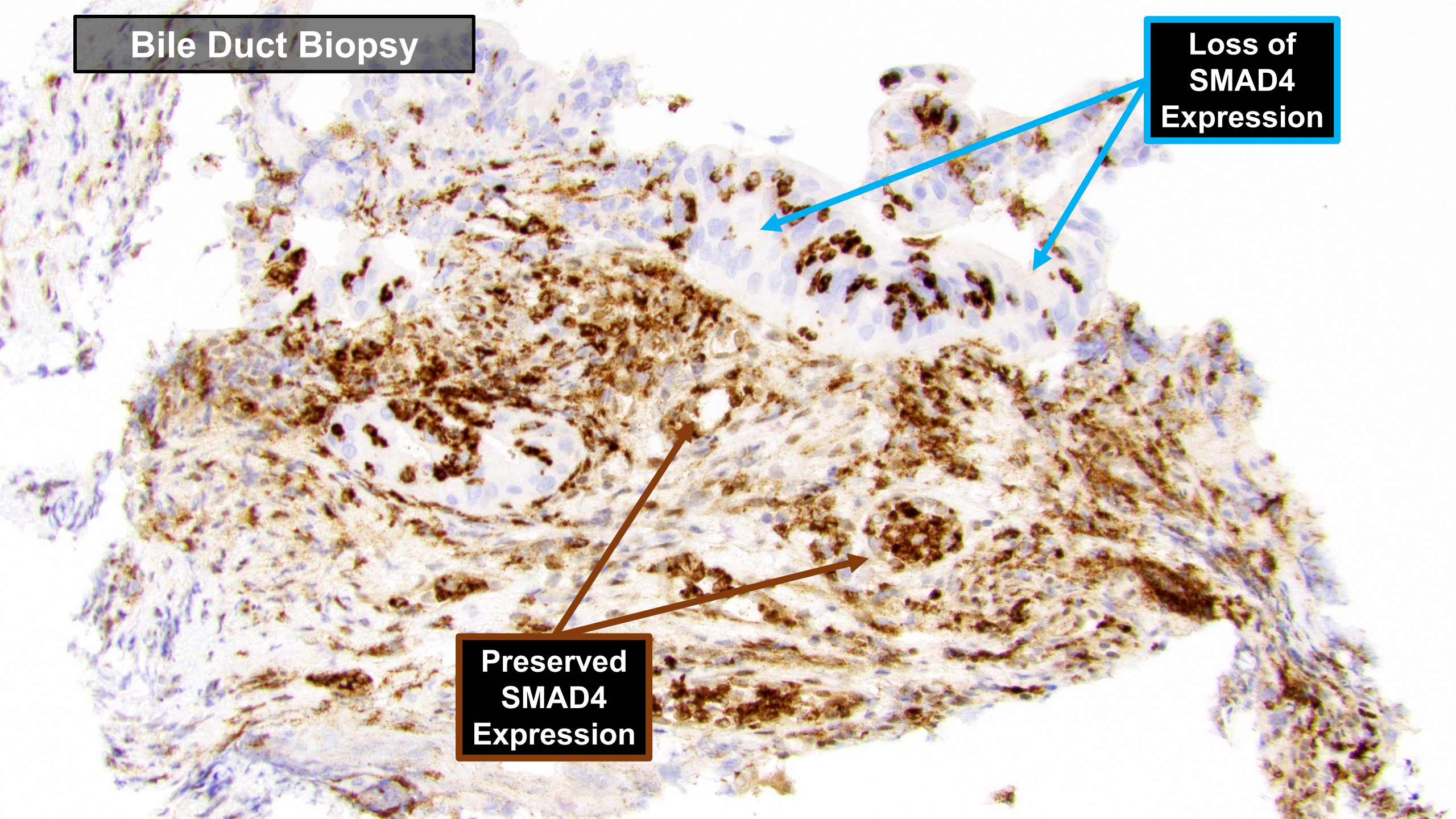




**Bile Duct Biopsy**

**Loss of  
SMAD4  
Expression**

**Preserved  
SMAD4  
Expression**



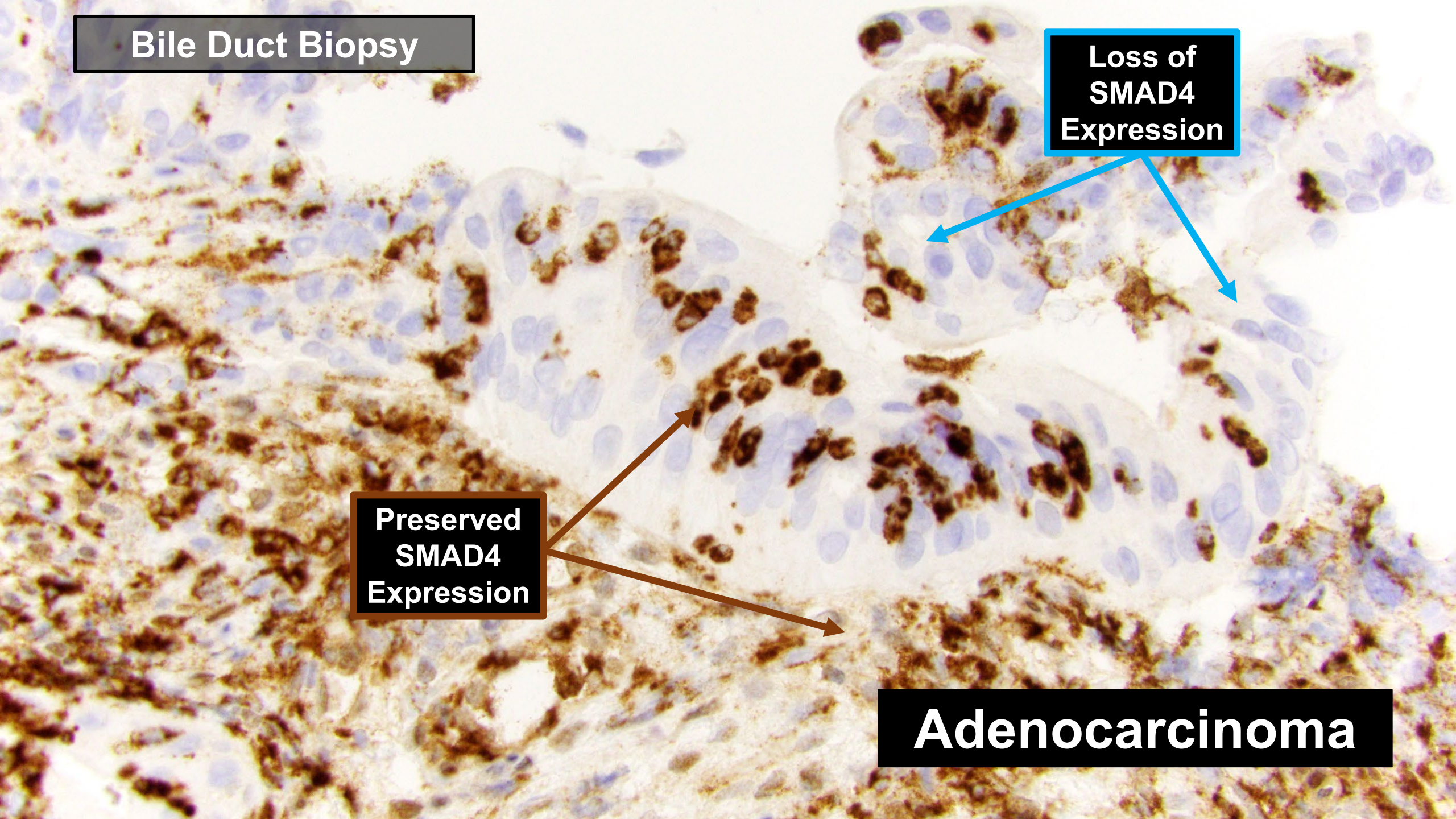


**Bile Duct Biopsy**

**Loss of  
SMAD4  
Expression**

**Preserved  
SMAD4  
Expression**

**Adenocarcinoma**





# Take Home Points: Case 7 & 8

Diagnostic Pathology/Clinical Follow-up of >12 Months	Total, n = 548
Intrahepatic cholangiocarcinoma	107
Extrahepatic cholangiocarcinoma	73
Hepatocellular carcinoma	3
Mixed HCC-cholangiocarcinoma	2
Pancreatic adenocarcinoma	88
Gallbladder adenocarcinoma	7
Ampullary adenocarcinoma	28
Metastatic neoplasms	15
High/low-grade dysplasia	10
Benign cholangiopathy	215

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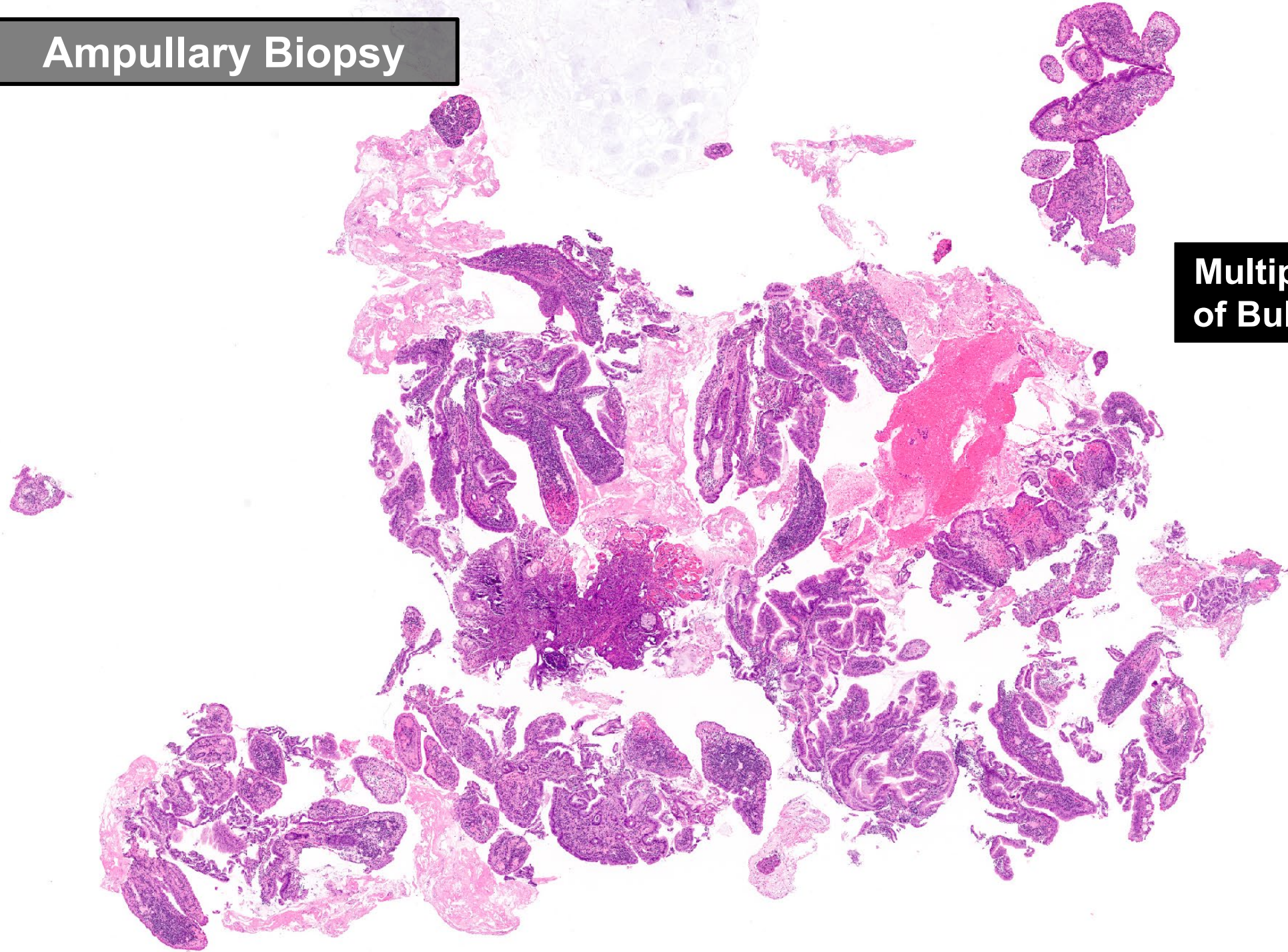
# Case 9

- A 56-year-old male with epigastric pain that underwent an esophagogastroduodenoscopy (EGD).
- No abnormal findings were present in the esophagus and stomach.
- However, the gastroenterologist noted a **2.0 cm polypoid lesion** involving the **ampulla**.
- Biopsies of the ampulla were taken and submitted for pathologic evaluation.



# Ampullary Biopsy

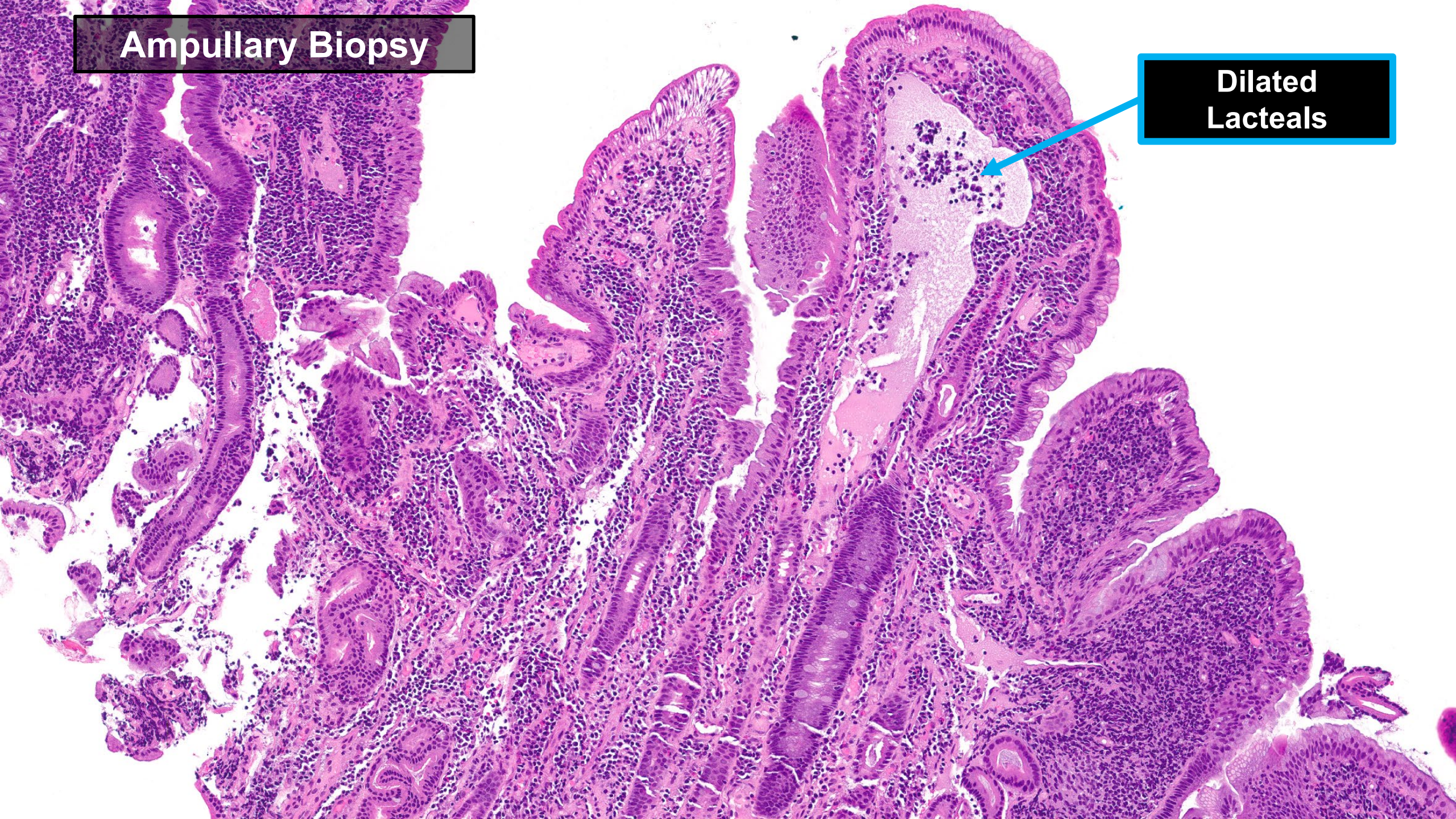
**Multiple Fragments  
of Bulbous Mucosa**





# Ampullary Biopsy

Dilated  
Lacteals



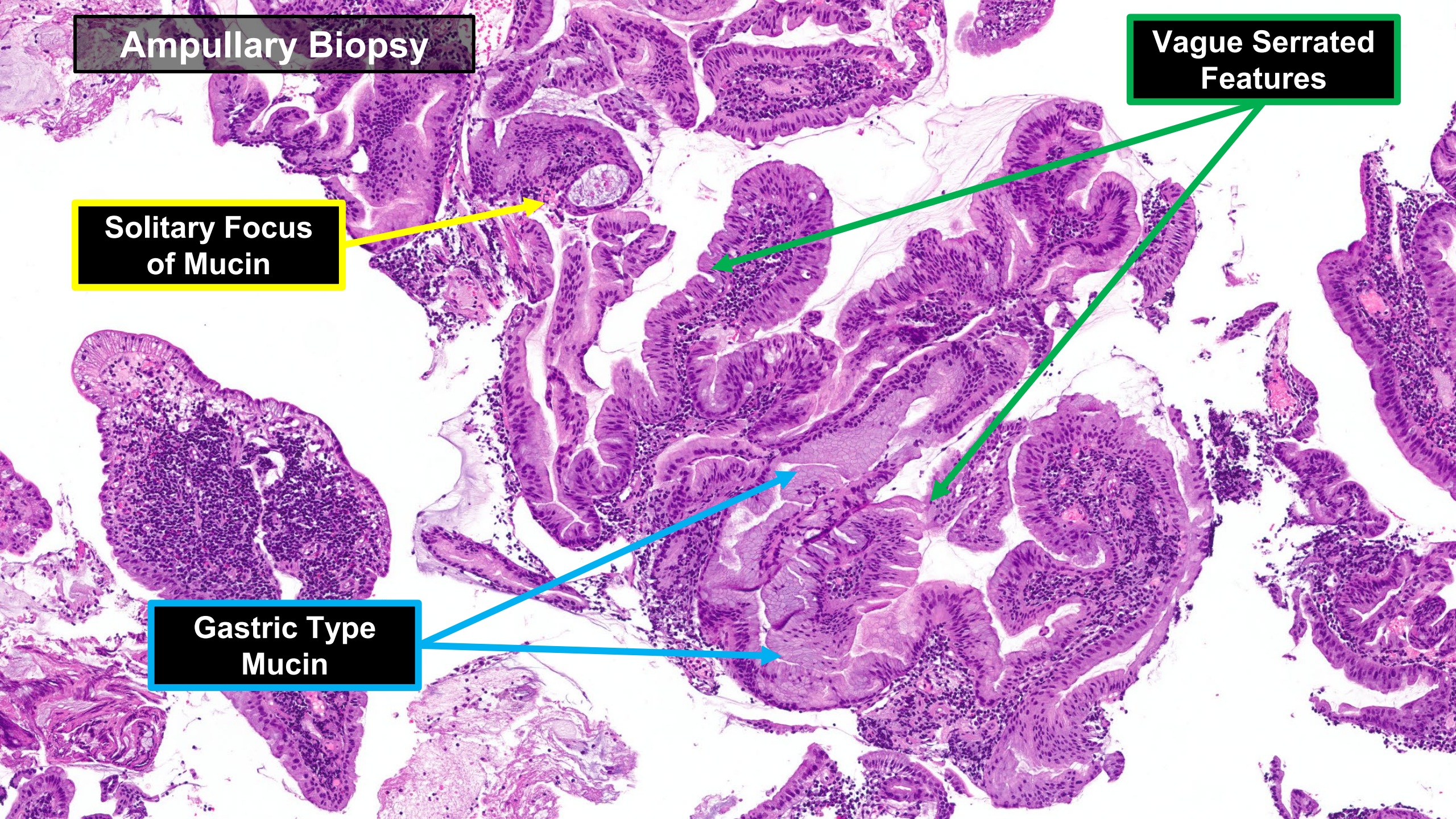


**Ampullary Biopsy**

**Vague Serrated Features**

**Solitary Focus of Mucin**

**Gastric Type Mucin**



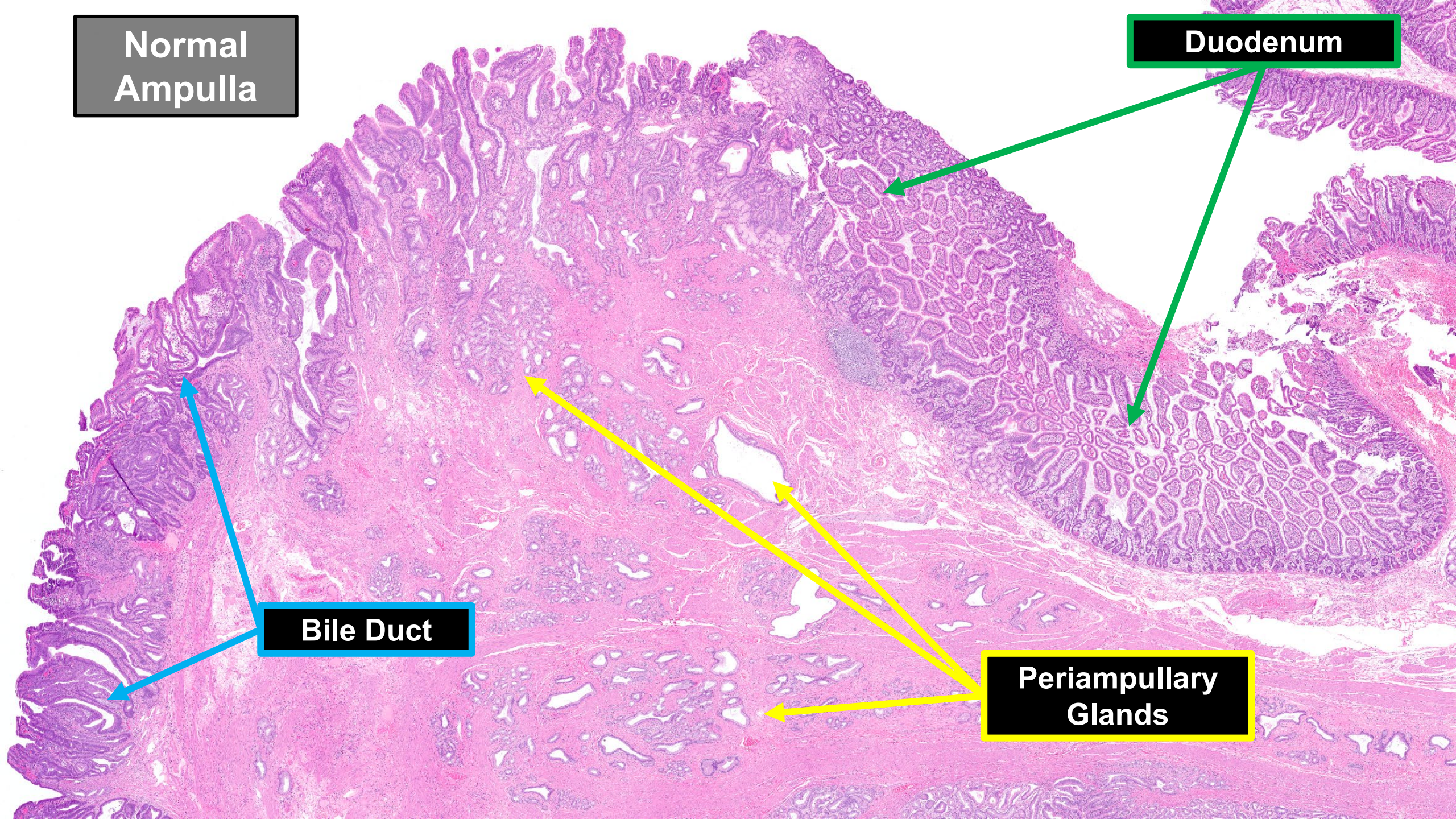


**Normal  
Ampulla**

**Duodenum**

**Bile Duct**

**Periampullary  
Glands**



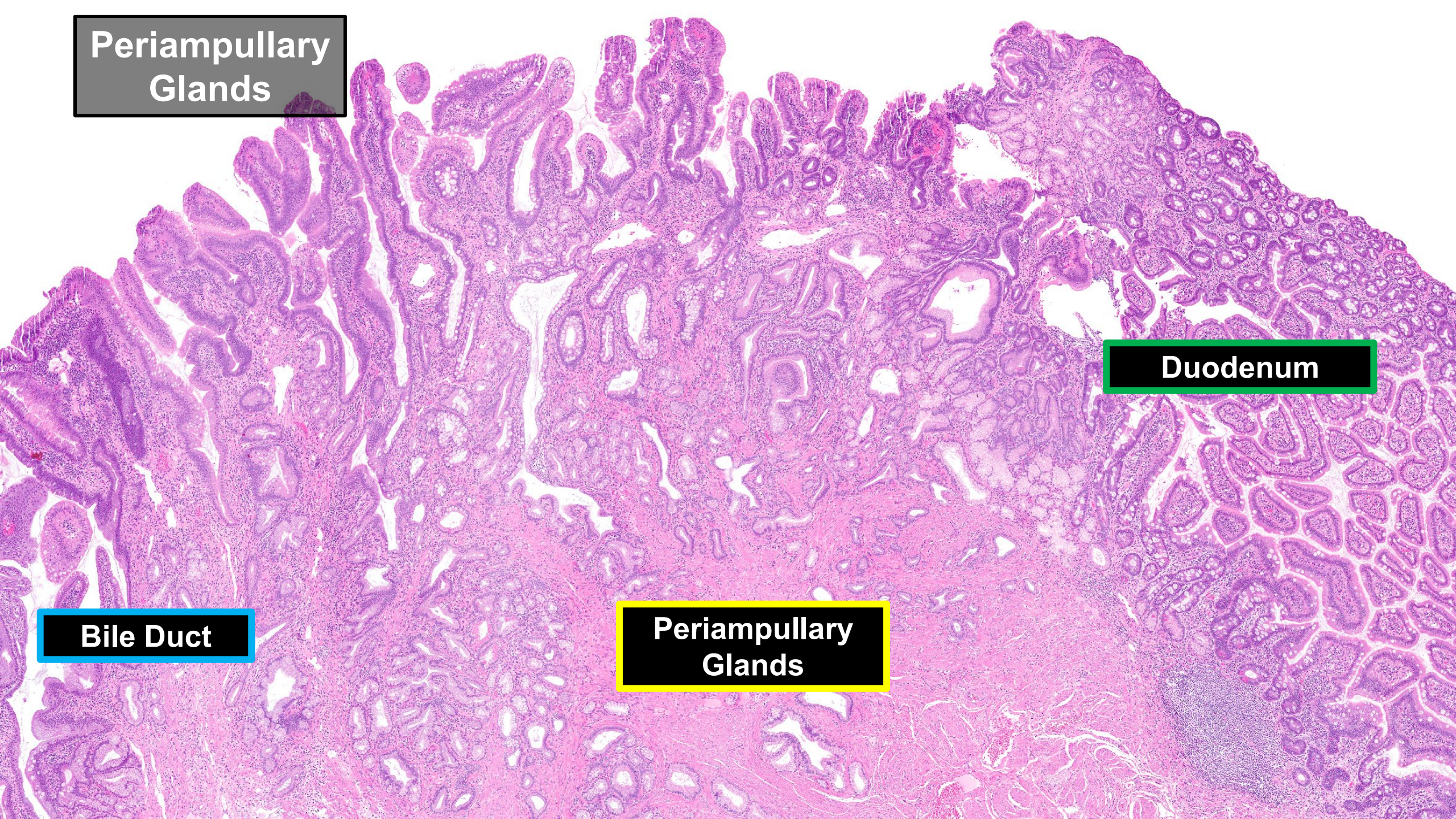


**Periampullary  
Glands**

**Duodenum**

**Bile Duct**

**Periampullary  
Glands**



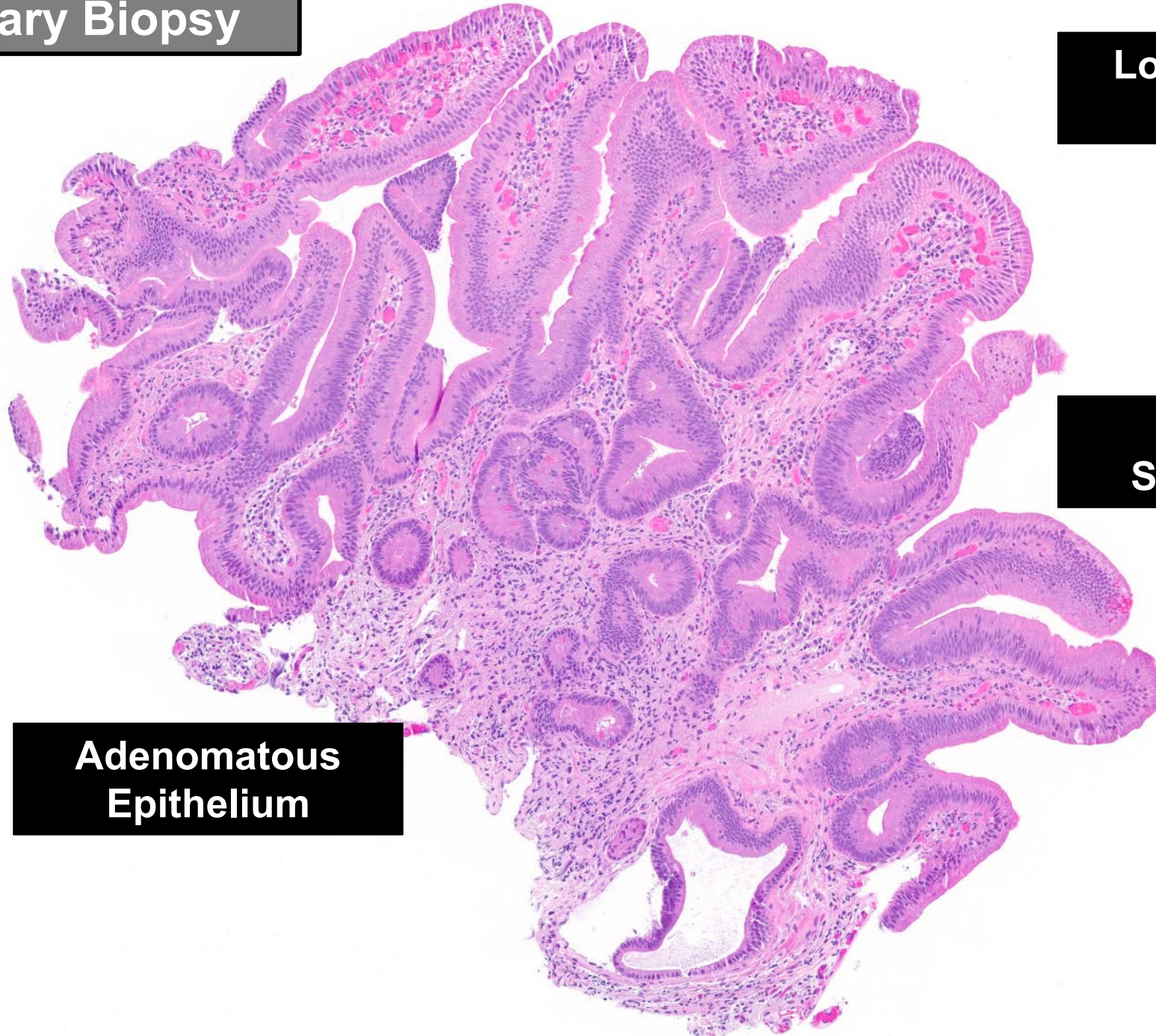


# Ampullary Biopsy

Loss of Goblet  
Cells

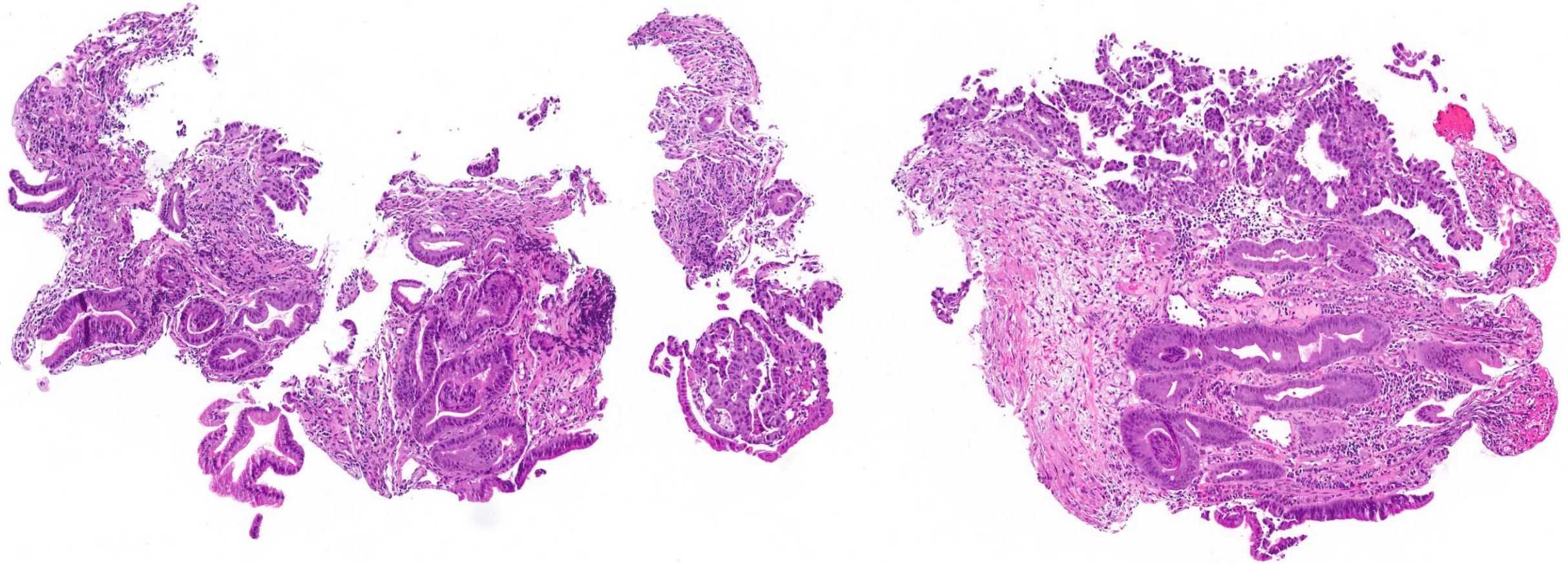
Nuclear  
Stratification

Adenomatous  
Epithelium





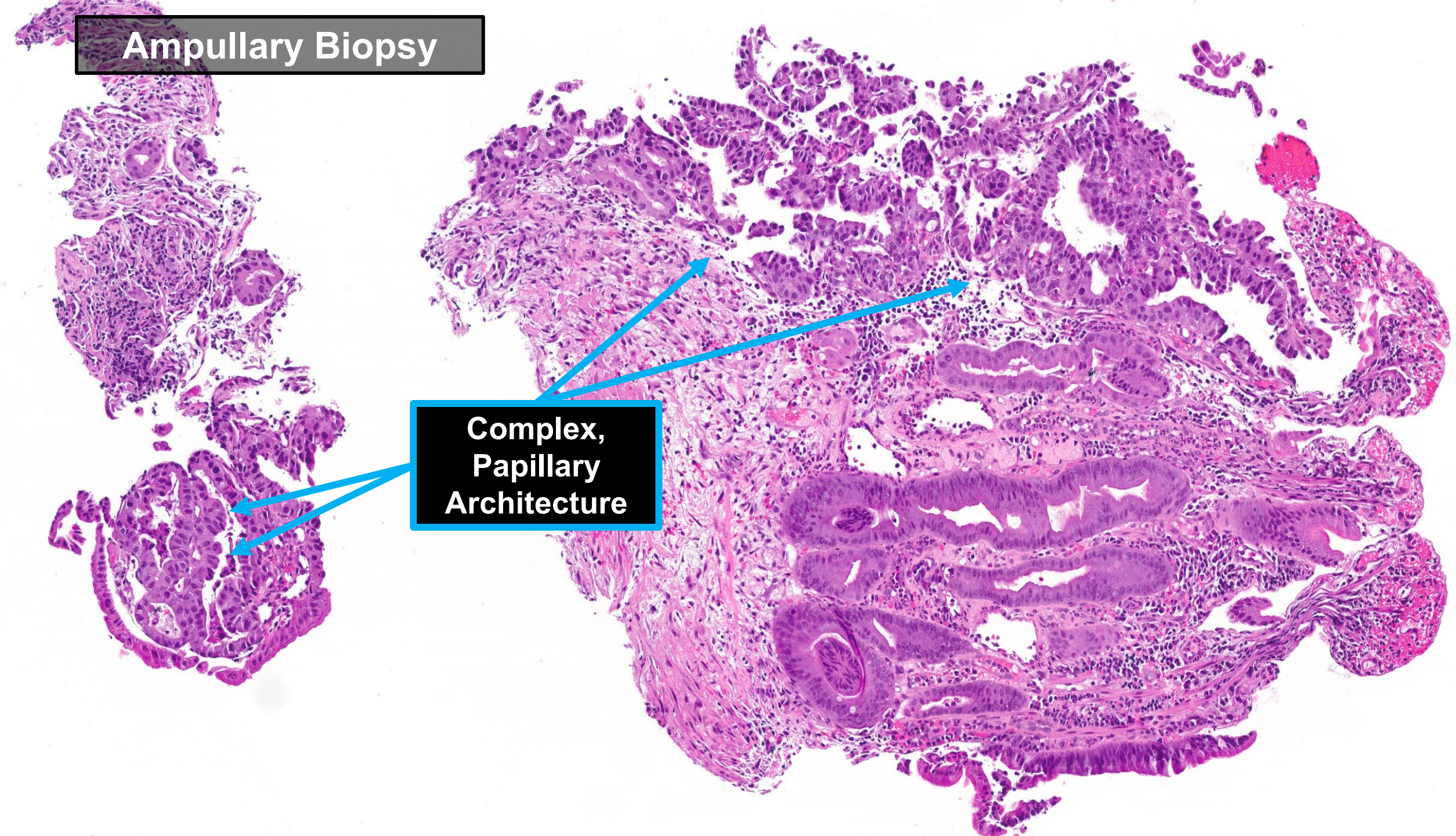
# Ampullary Biopsy





# Ampullary Biopsy

**Complex,  
Papillary  
Architecture**





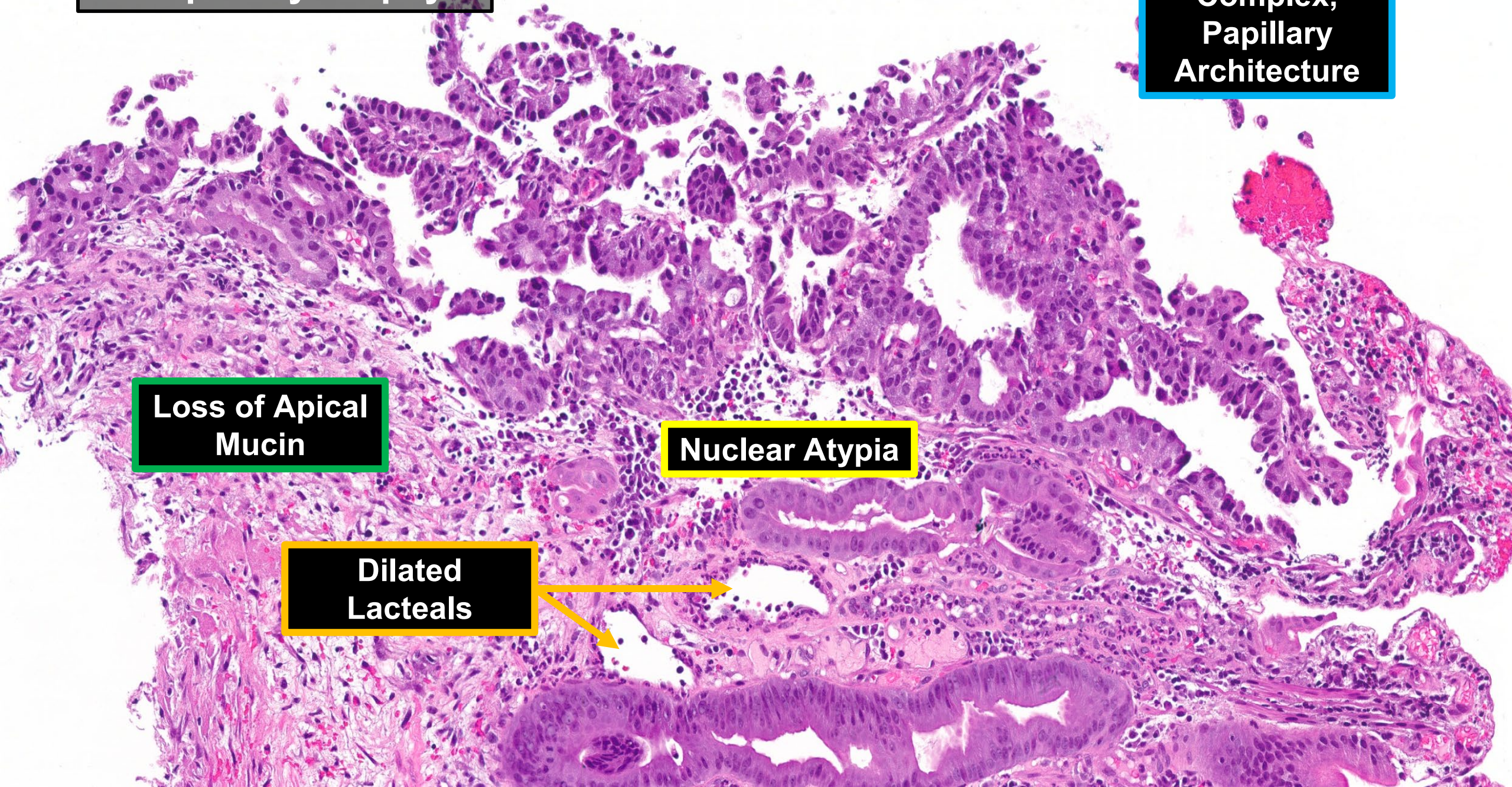
# Ampullary Biopsy

Complex,  
Papillary  
Architecture

Loss of Apical  
Mucin

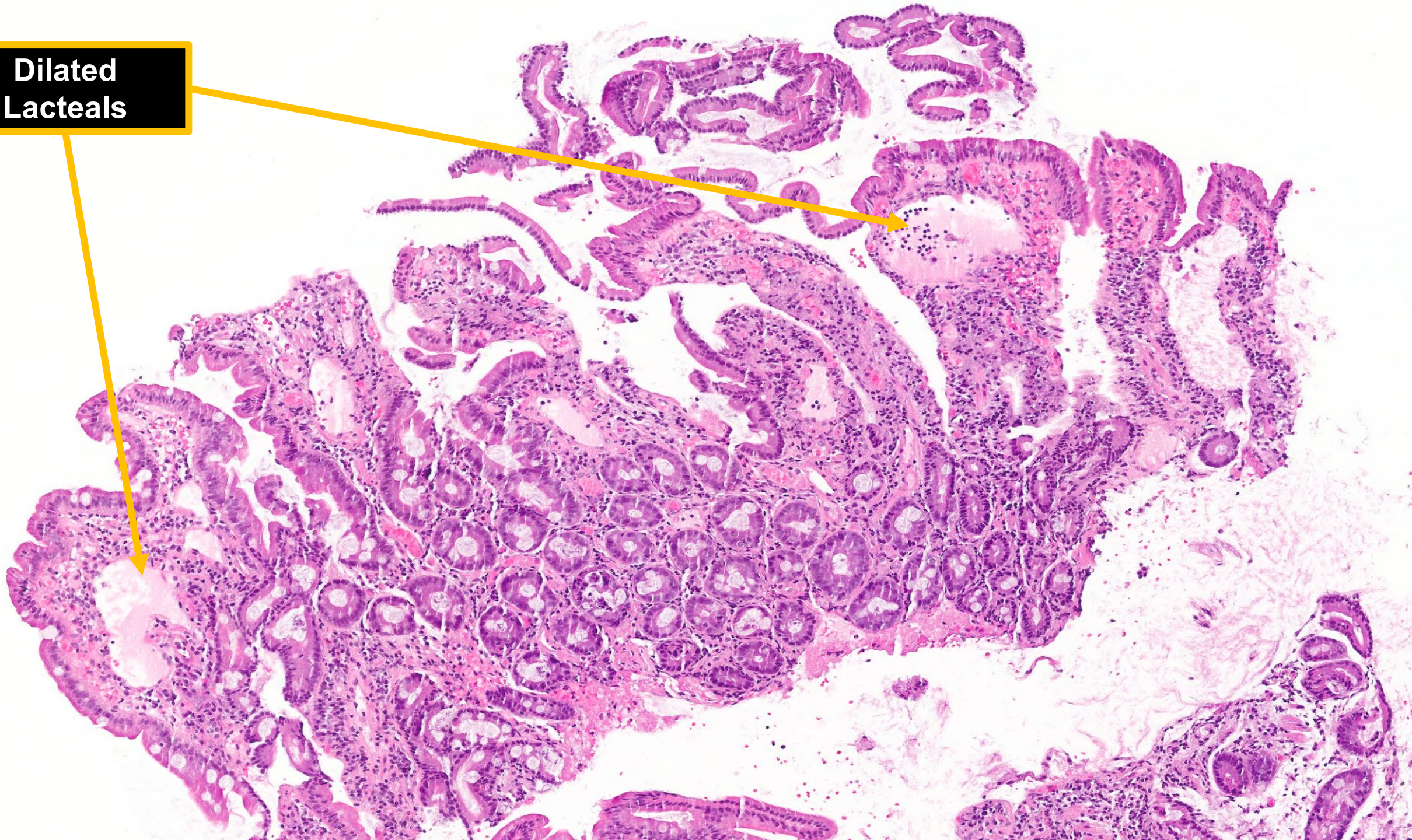
Nuclear Atypia

Dilated  
Lacteals





**Dilated  
Lacteals**





# Case 9

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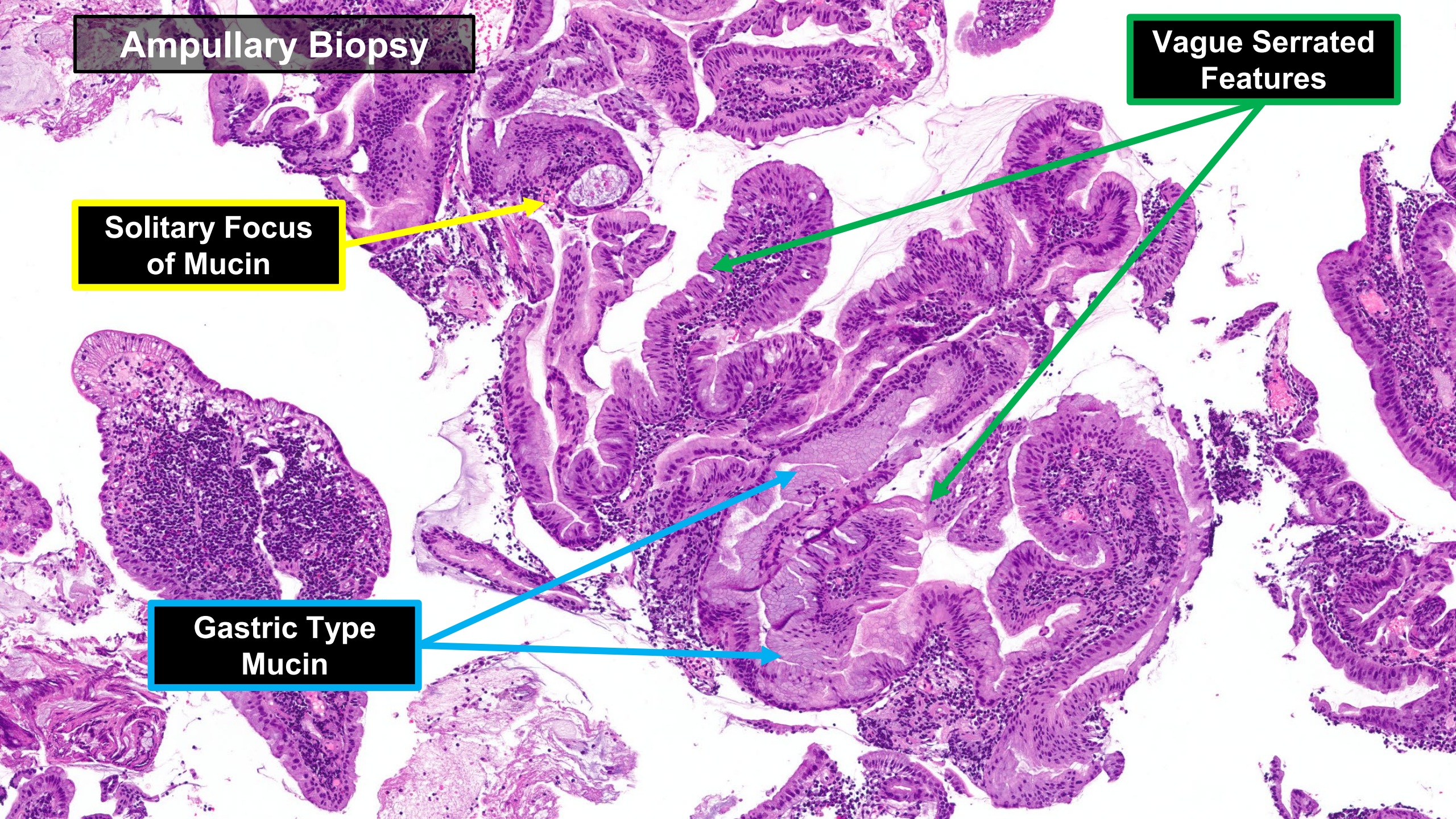


**Ampullary Biopsy**

**Vague Serrated Features**

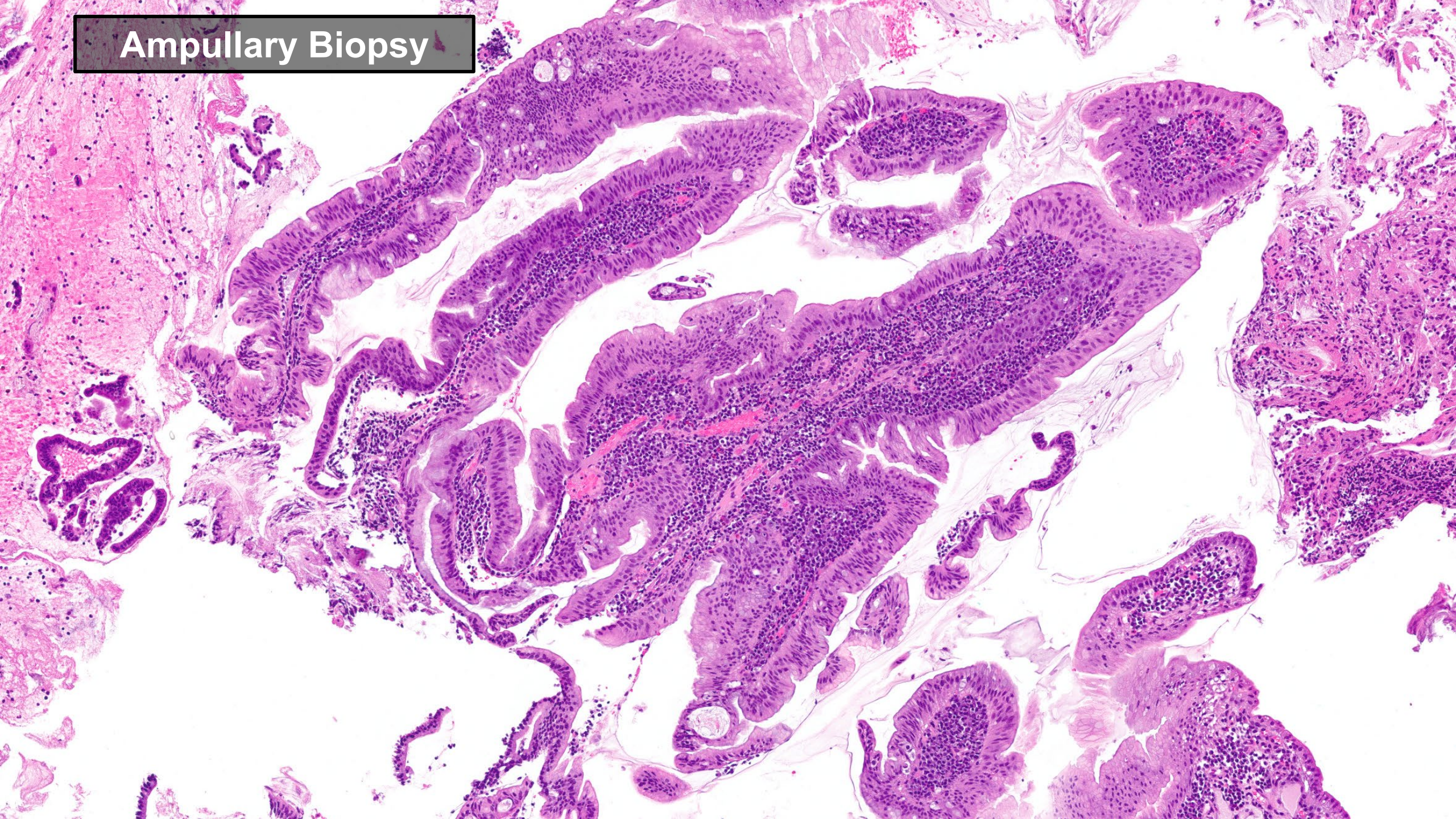
**Solitary Focus of Mucin**

**Gastric Type Mucin**





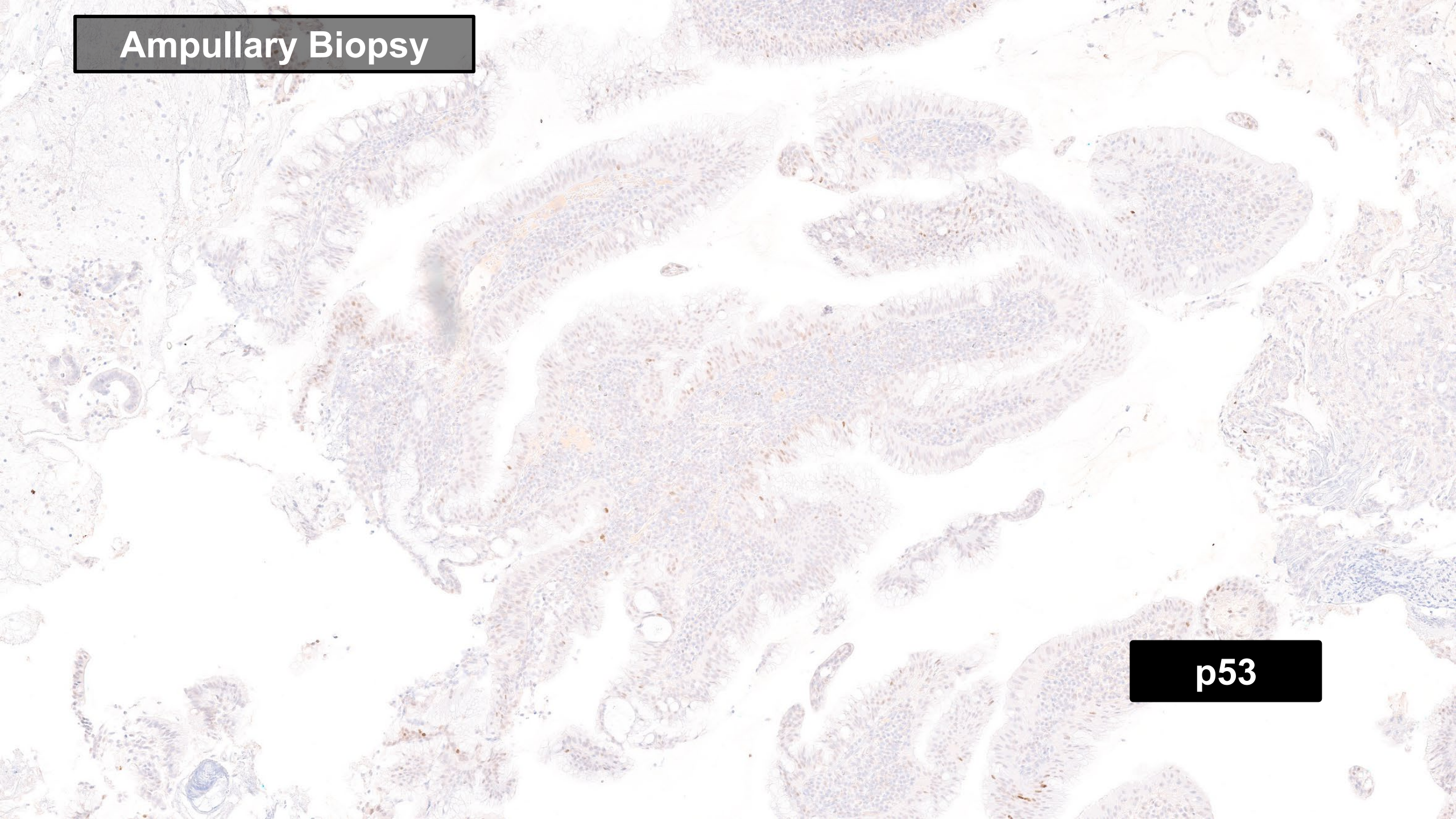
# Ampullary Biopsy





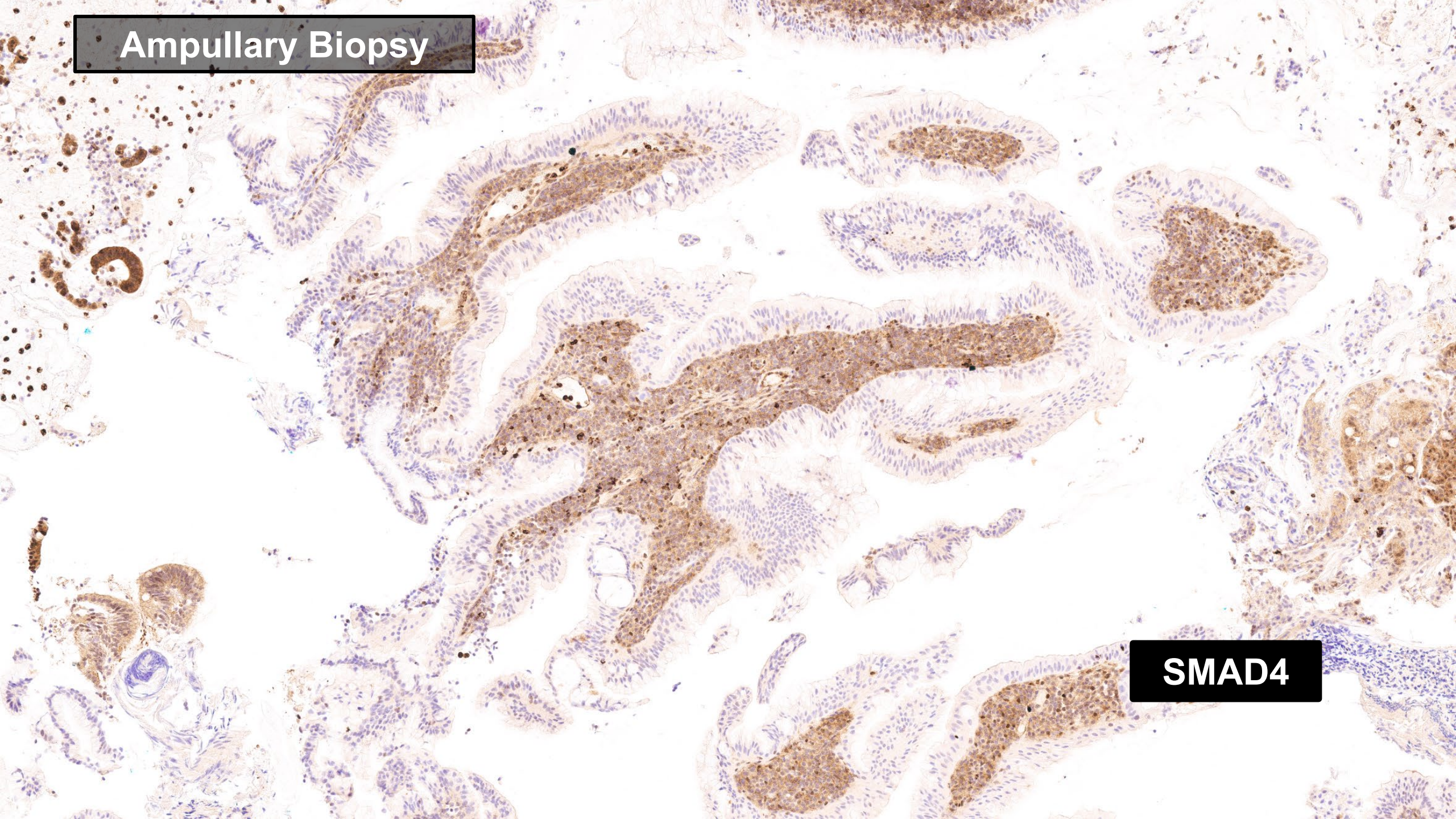
# Ampullary Biopsy

p53





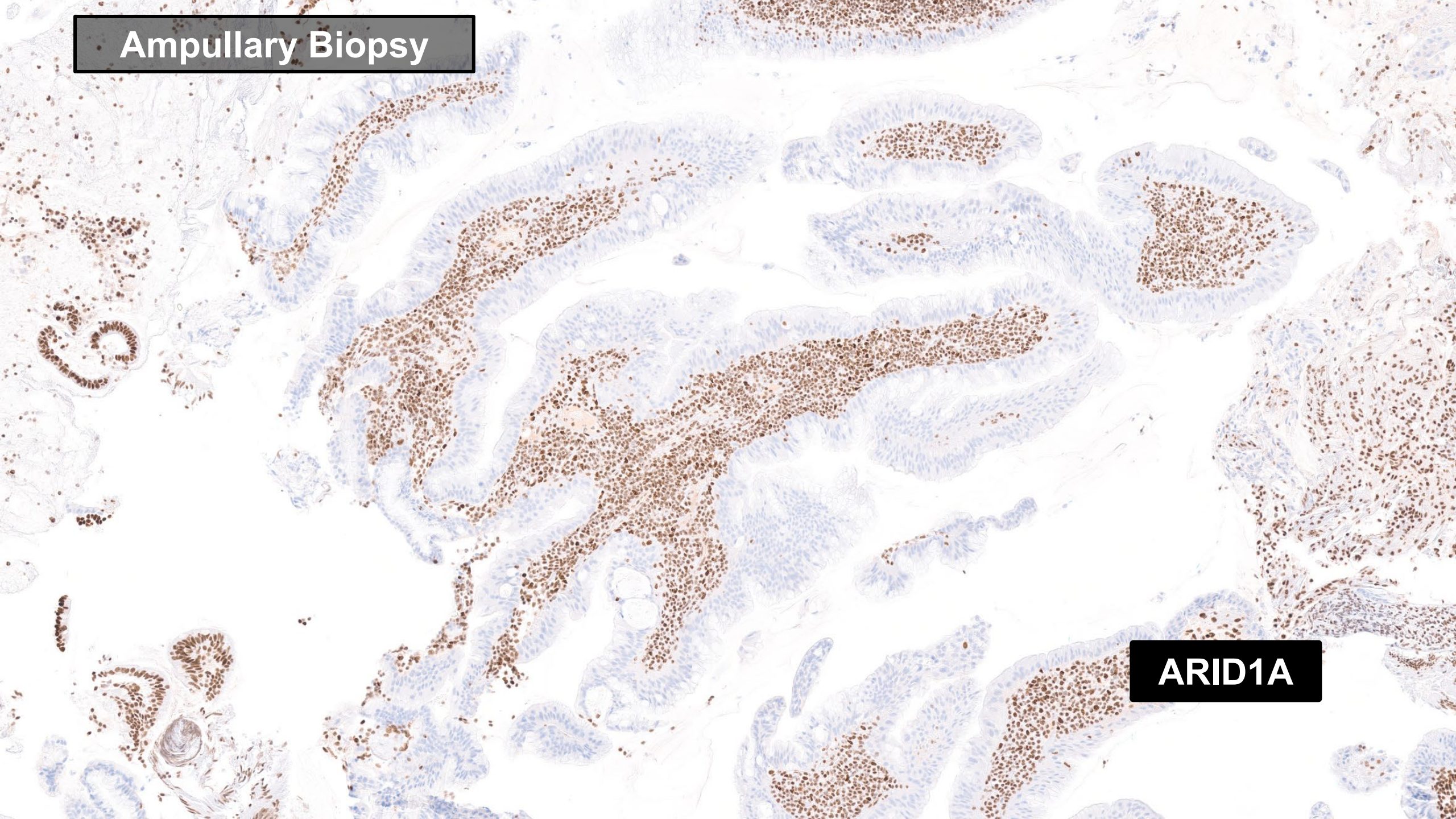
# Ampullary Biopsy



**SMAD4**



# Ampullary Biopsy

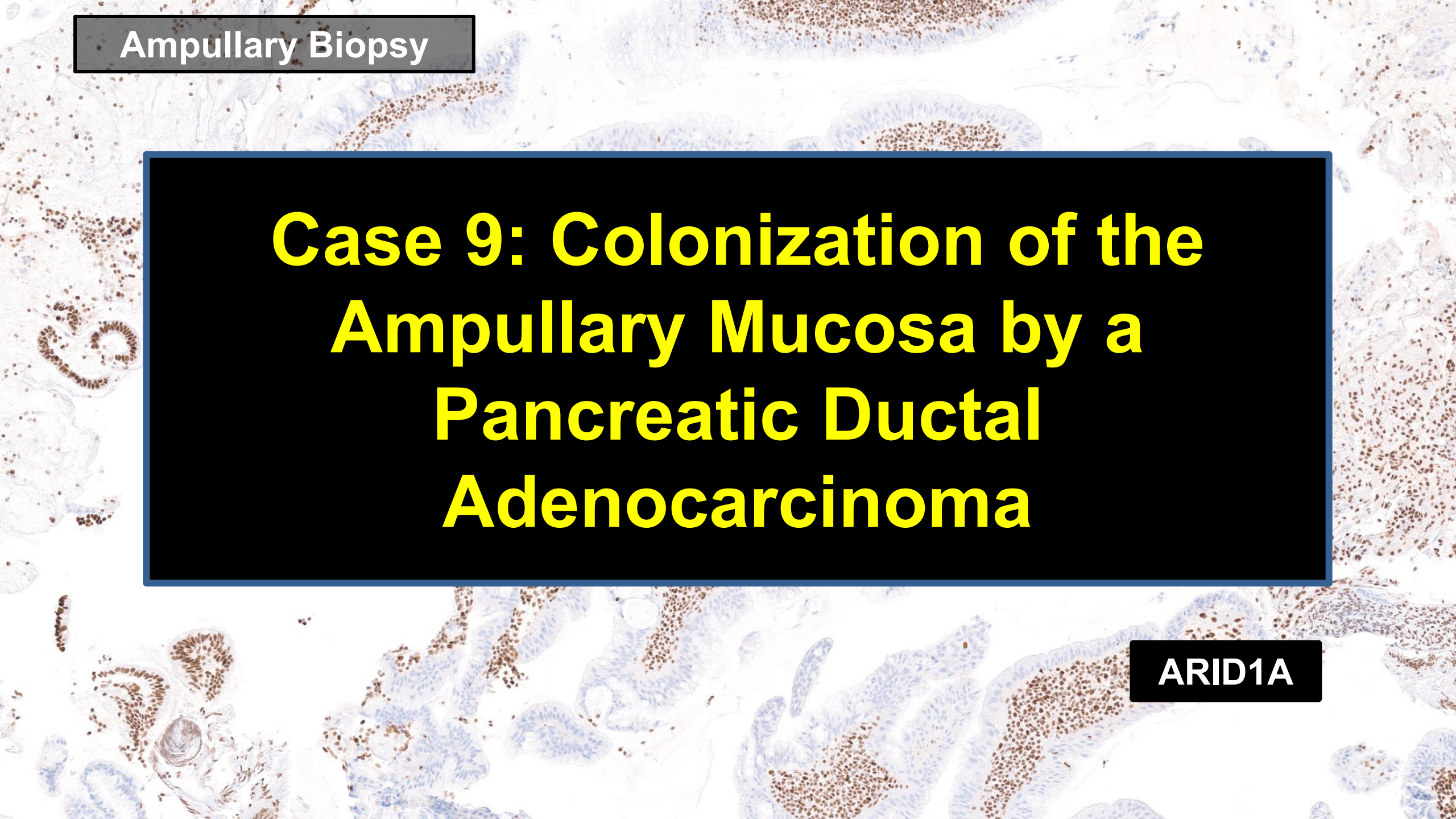


**ARID1A**



**Case 9: Colonization of the  
Ampullary Mucosa by a  
Pancreatic Ductal  
Adenocarcinoma**

ARID1A

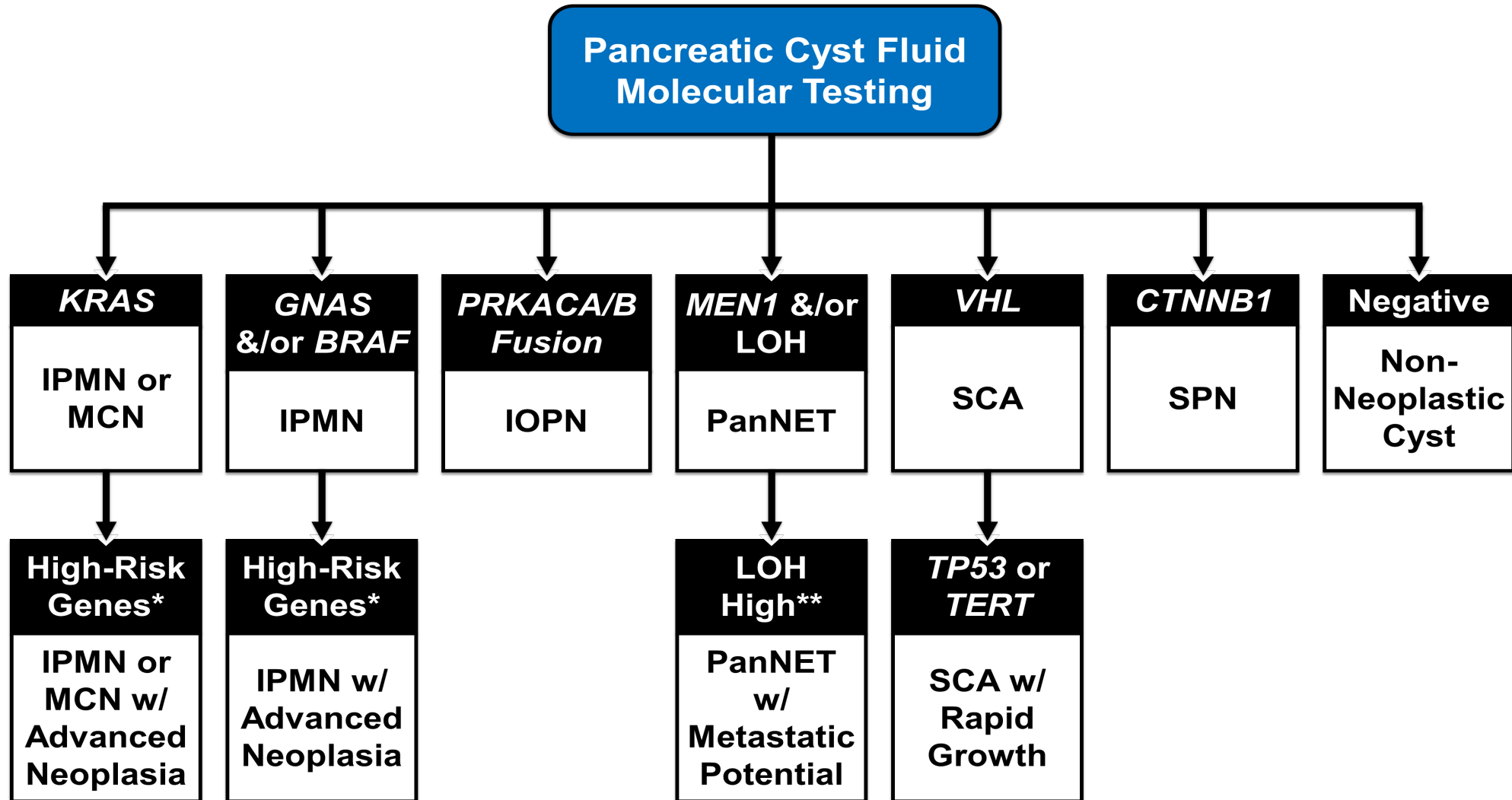


# Summary

- Next-generation fine-needle biopsies and aspiration of cyst fluid improves the evaluation of solid and cystic pancreatic lesions, respectively.
- Regarding pancreatic cysts, ***KRAS***, ***GNAS***, and/or ***BRAF*** mutations/ are highly sensitive and highly specific for mucinous cysts, specifically IPMNs (and also MCNs).
- Alterations in ***TP53***, ***SMAD4***, ***CTNNB1***, and/or **the mTOR genes** are highly sensitive and highly specific for mucinous cysts with advanced neoplasia.



# Summary

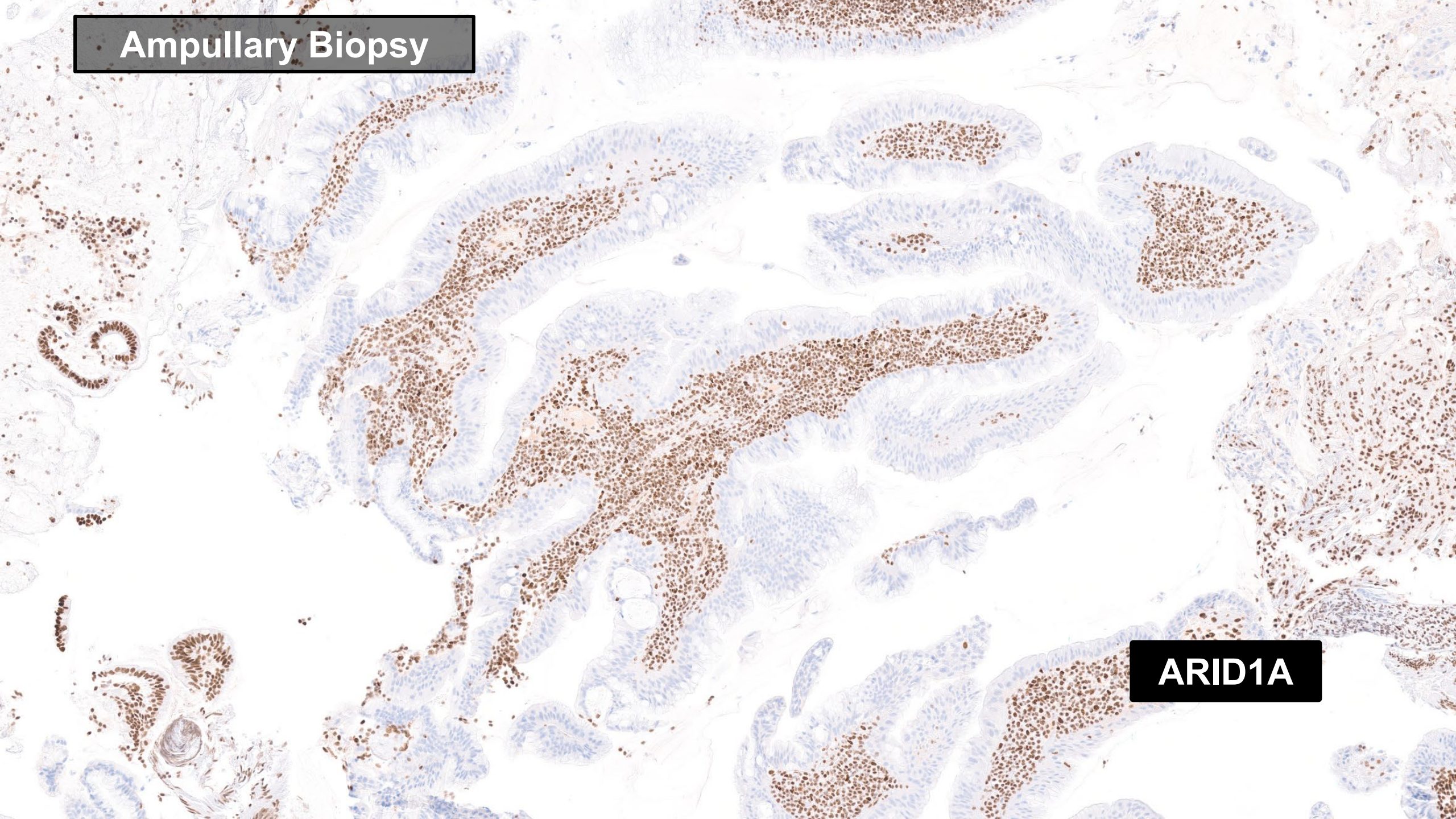


# Summary

- In addition to pancreatic cysts, **next-generation sequencing can improve the evaluation of bile duct strictures.**
- **Genomic alterations** identified in bile, bile duct brushings, and bile duct biopsies **are highly specific for a neoplastic process.**
- For both pancreatic cysts and bile duct specimens, the incorporation of **cytopathologic/pathologic findings is essential to establishing the diagnosis.**



# Ampullary Biopsy



**ARID1A**



UPMC

PANCREATIC  
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ACTION  
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Goal: \$450,000