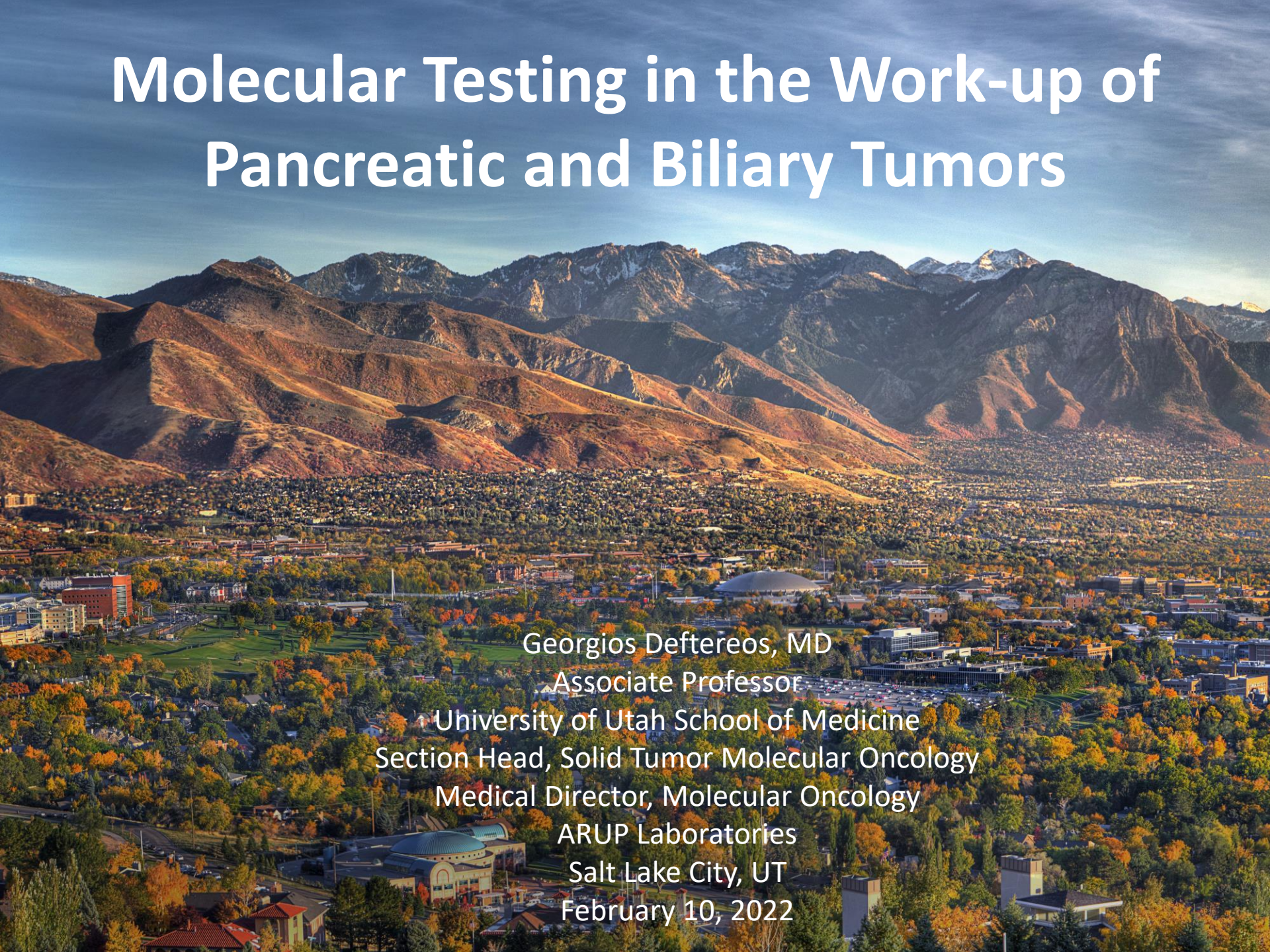
Molecular Testing in the Work-up of Pancreatic and Biliary Tumors



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Outline

- Pancreatic cysts
  - Subtypes
  - Ancillary testing available
    - For differential diagnosis
    - For assessment of malignancy potential
- Solid pancreatic tumors
- Pancreaticobiliary exfoliative cytology and biliary tumors
- Molecular testing for targeted therapies

Pancreatic Cysts - Background

- Pancreatic cysts are diverse and common lesions that vary in clinical, radiological and pathological characteristics
- Extremely common, affecting approximately 2.6% of asymptomatic adults
- More than 8% in people older than 80 years of age
- Most cysts are benign, including mucinous cysts

Pancreatic Cysts - Background

- Cytological evaluation is the first step in the assessment of the pancreatic cyst fluid
  - Often hampered by high rates of false negative and indeterminate diagnoses, due mainly to hypocellular or acellular specimens
  - Small core needle biopsy available mostly for solid tumors
- Biochemical and molecular analysis of pancreatic cyst fluid can be extremely helpful in the assessment of pancreatic cystic lesions

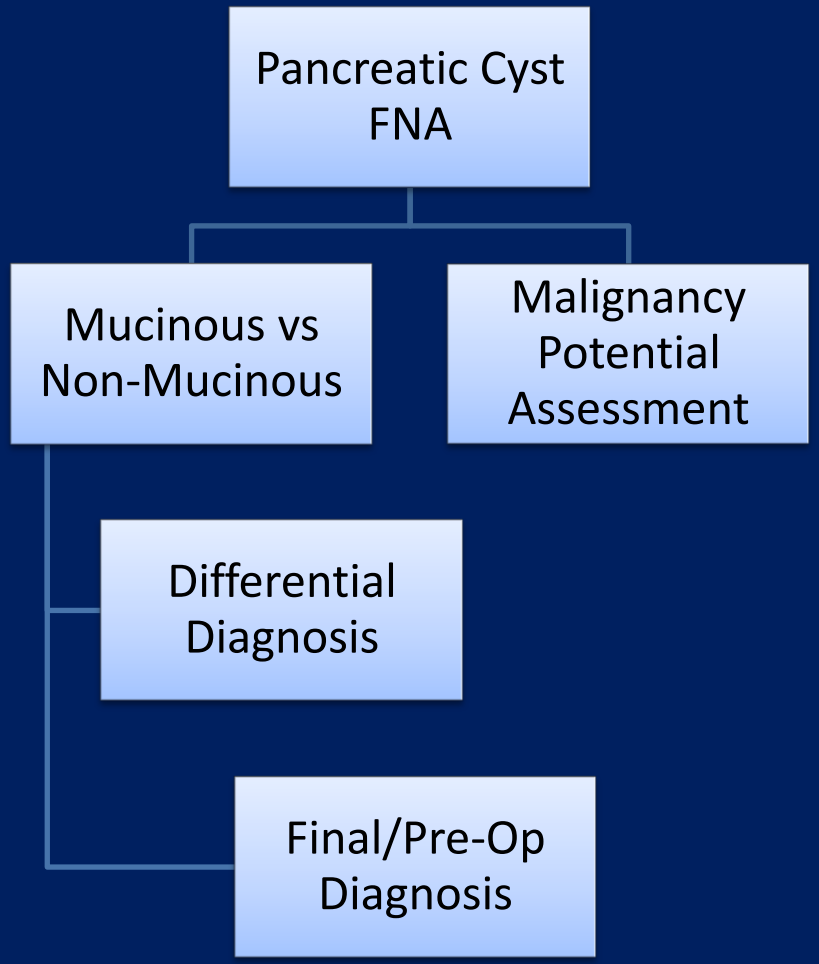
Pancreatic Cysts - Background

- The three types of cysts most commonly encountered are:
  - Serous cystadenoma (SCA), considered to have low malignant potential
  - Mucinous cystic neoplasm (MCN)
  - Intraductal papillary mucinous neoplasm (IPMN)
    - Last two considered to have malignant potential
- These three types account for circa 80% of resected specimens

Pancreatic Cyst FNA

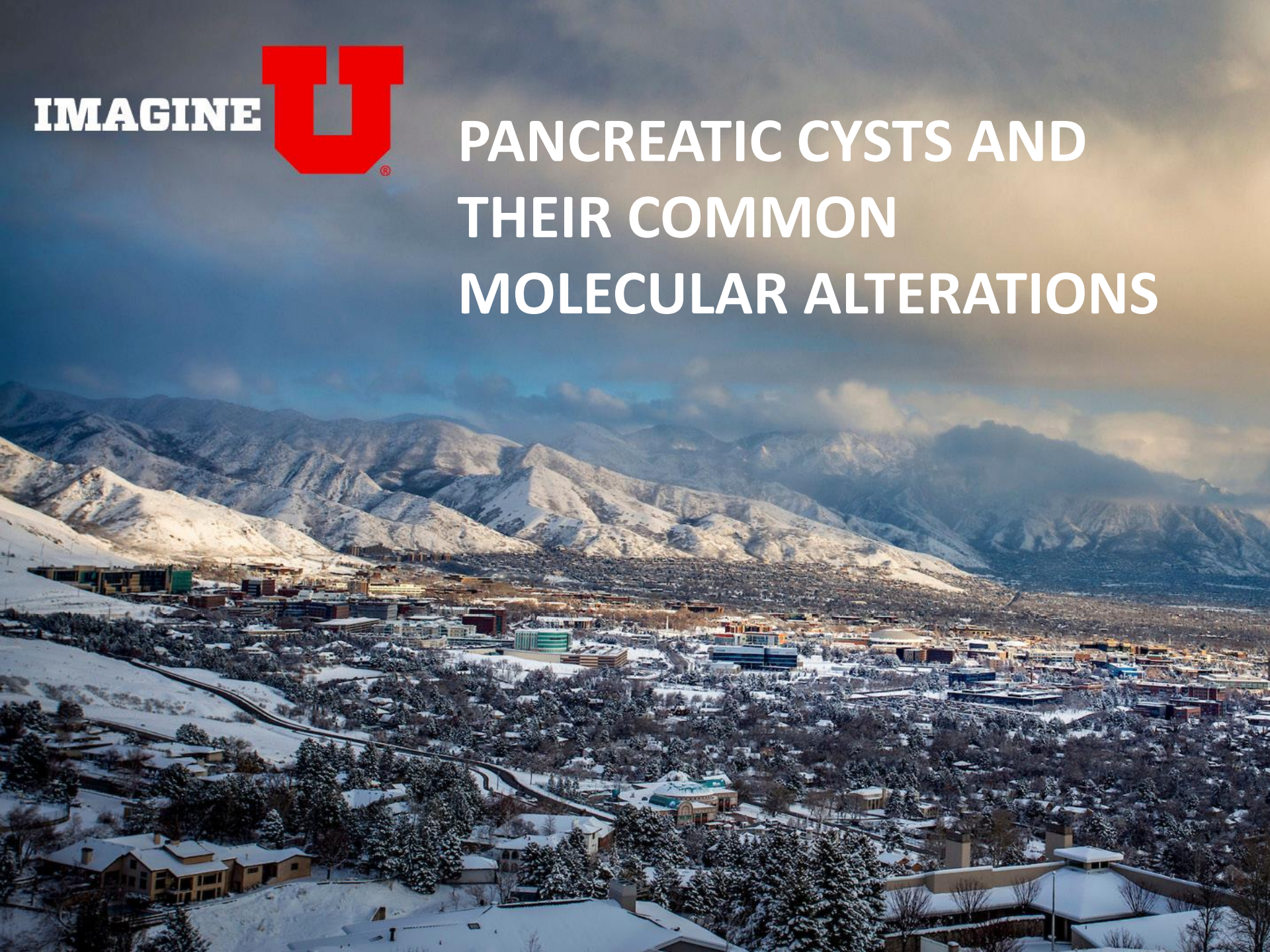
- Approximately 50% of cases of FNA of pancreatic cysts have an indefinite diagnosis frequently related to paucicellular or acellular specimens
- Cystic fluid carcinoembryogenic antigen (CEA) levels over 192 ng/ml correlates with a mucinous cyst
- However, only a positive cytology can definitely separate a malignant cyst from those with benign behavior
  - Does so with relatively low sensitivity

Pancreatic Cyst FNA



CEA Testing

- A cystic fluid CEA concentration higher than 192 ng/ml strongly correlates with a mucinous cyst (Brugge et al. 2004)
  - Using this CEA threshold value for diagnosing a mucinous cyst, yields a sensitivity and specificity of 75% and 84%, respectively
  - No difference in CEA values is seen between mucinous premalignant and malignant cysts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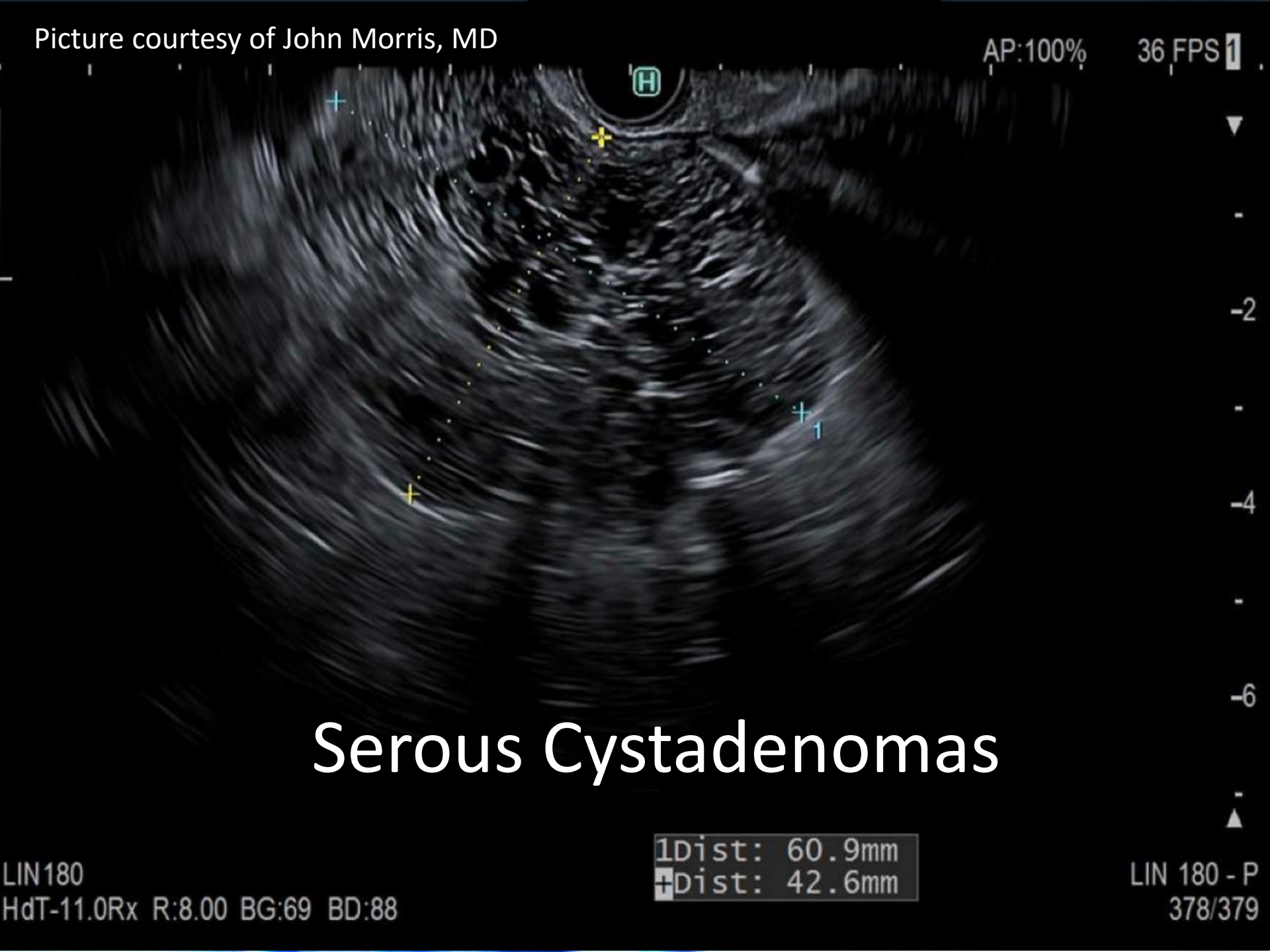
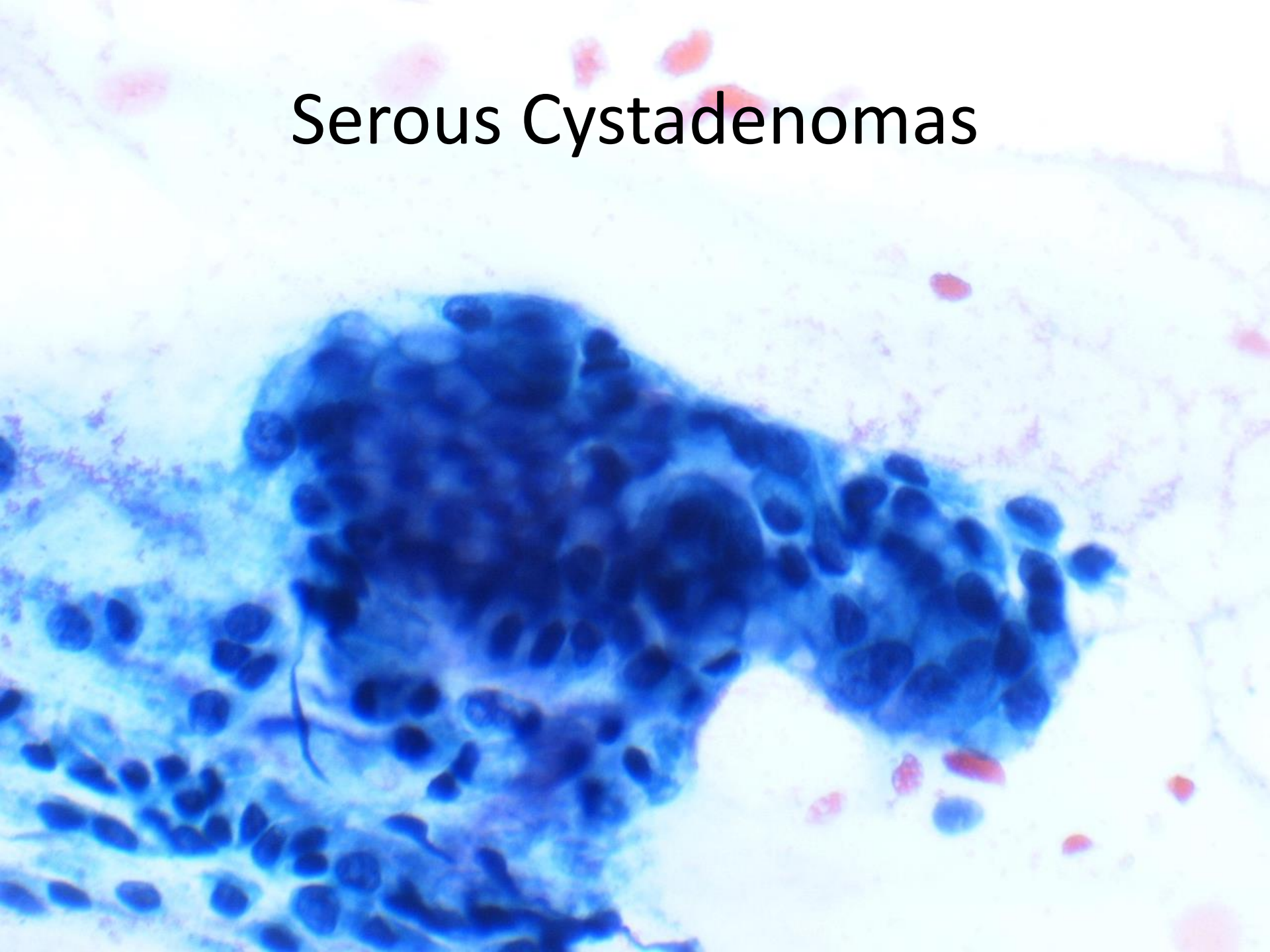
PANCREATIC CYSTS AND THEIR COMMON MOLECULAR ALTERATIONS



Serous Cystadenomas

Serous Cystadenomas



Serous Cystadenomas

- 1-2% of pancreatic neoplasms
- SCAs most commonly occur in patients in their 7th decade of life
- Significant predilection for women (70%)
- 80% found in the head and body of the pancreas
- Histologically, SCAs are composed of multiple small compartments, lined by glycogen containing cuboidal cells

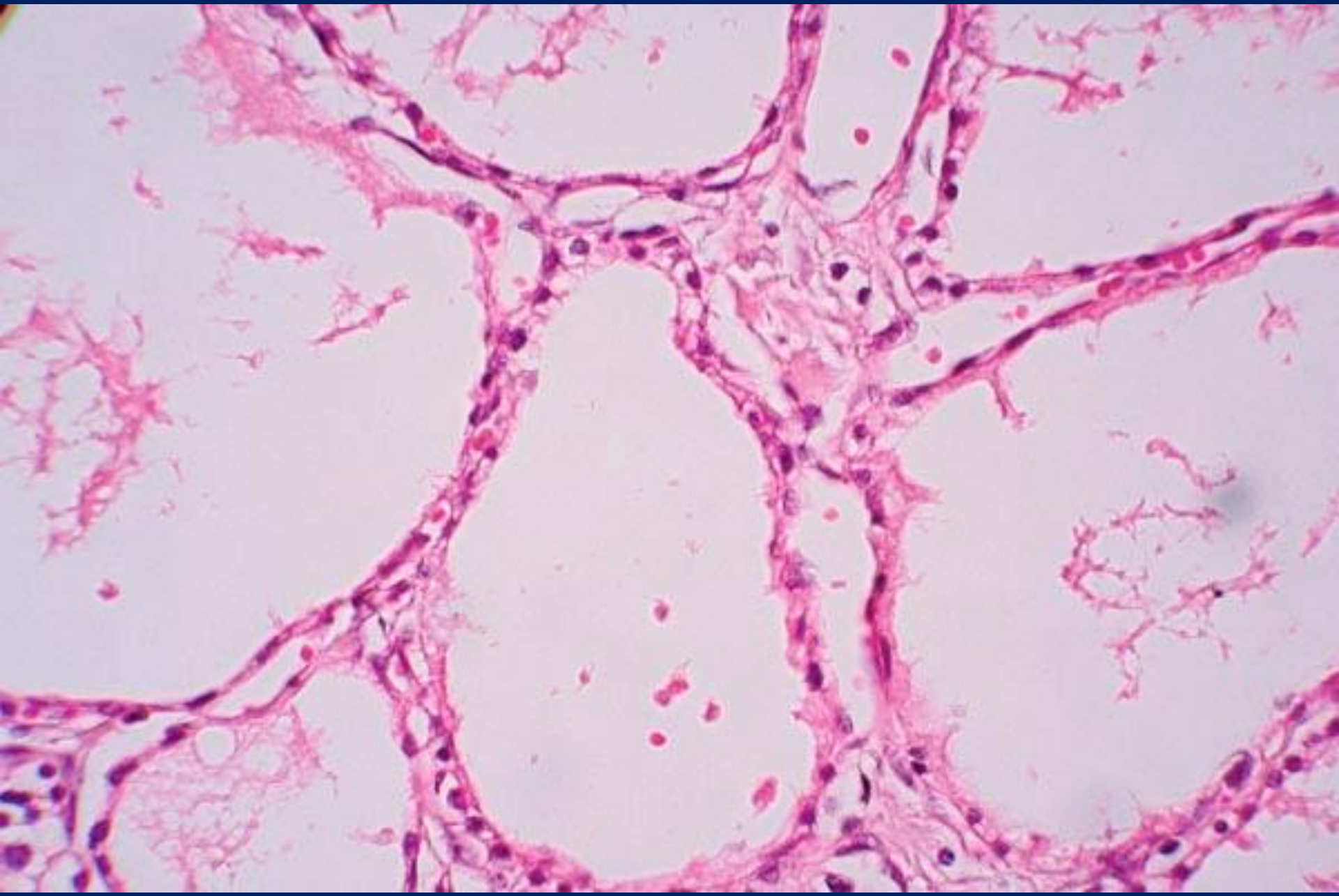


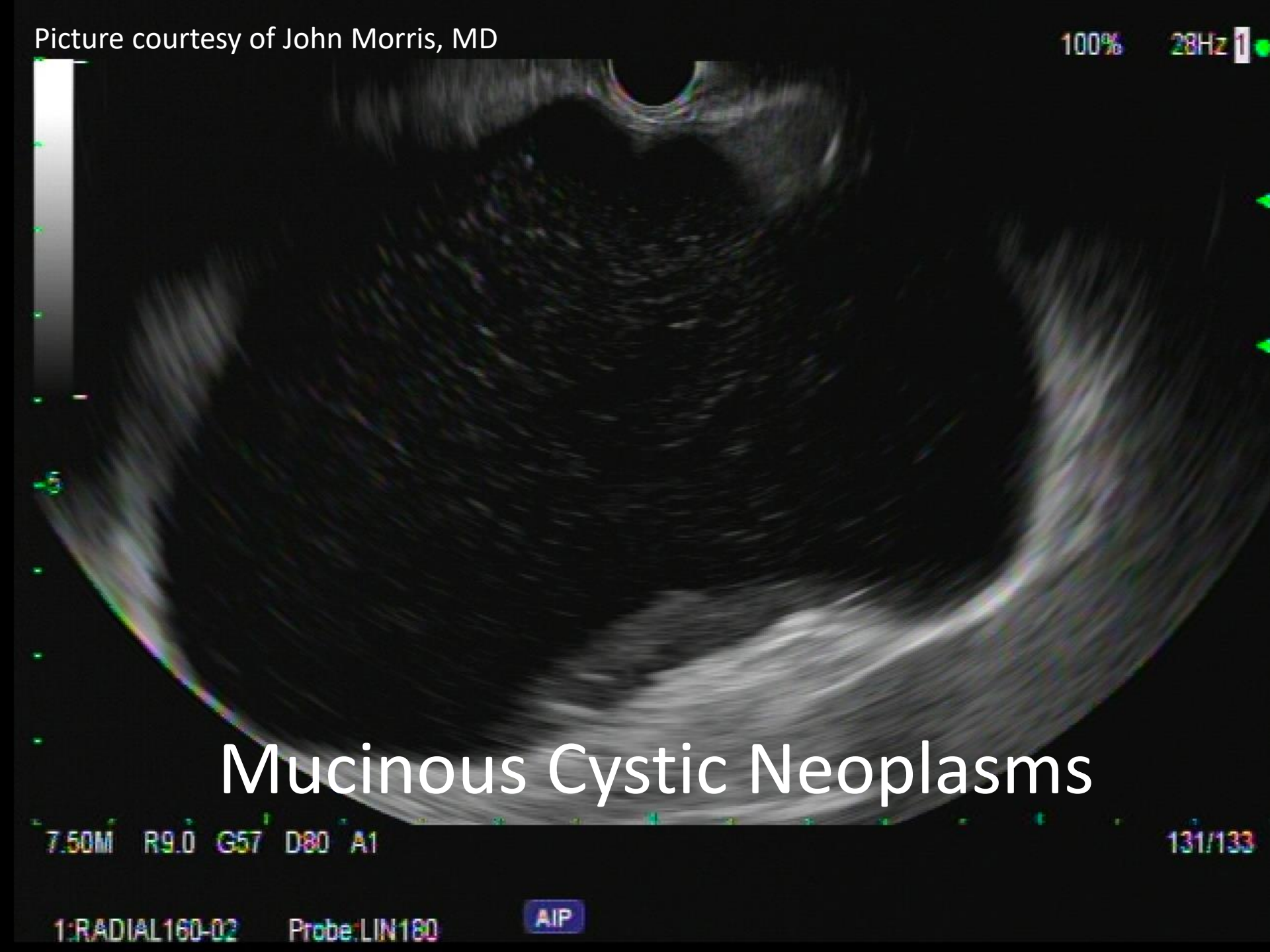
Photo courtesy of Mary Bronner, MD

Serous Cystadenomas

- The risk of malignant transformation of SCA is very small, with only 0.7% of patients developing liver metastases
  - Metastatic disease is correlated with a cyst diameter over 10 cm
- Surgical resection of SCA should be considered if cysts are symptomatic or when differentiation from a mucinous cyst is not possible
- The vast majority of patients with SCA will undergo surveillance.

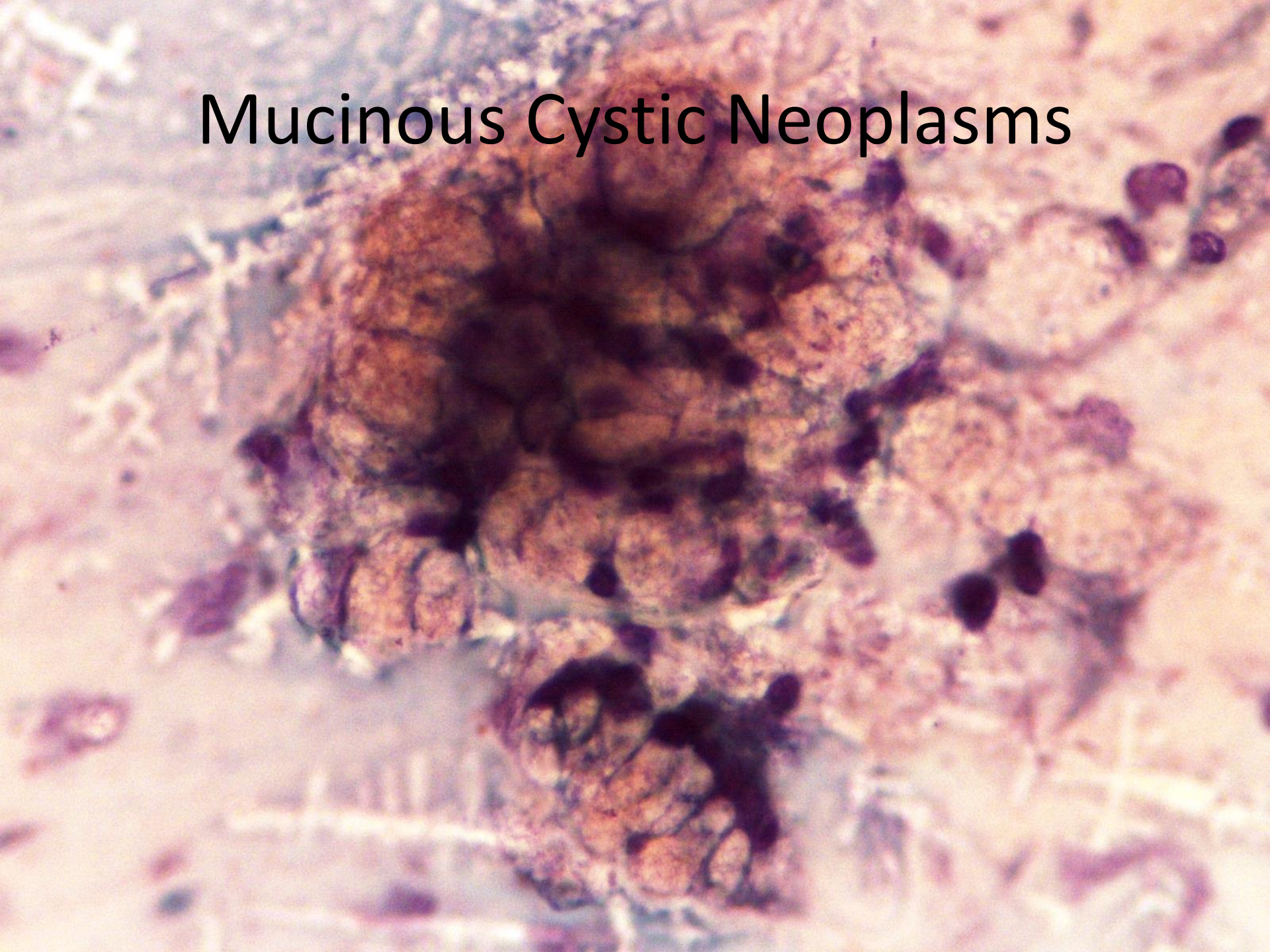
Serous Cystadenomas

- 10% sensitivity on cytology alone
- 67% have “VHL-related alterations”
  - VHL mutations
  - LOH at the VHL locus (3p25)
  - Aneuploidy of chromosome 3
  - Not seen in mucinous neoplasms
  - Can be seen in up to 25% of pancreatic neuroendocrine tumors (PanNETs)



Mucinous Cystic Neoplasms

Mucinous Cystic Neoplasms

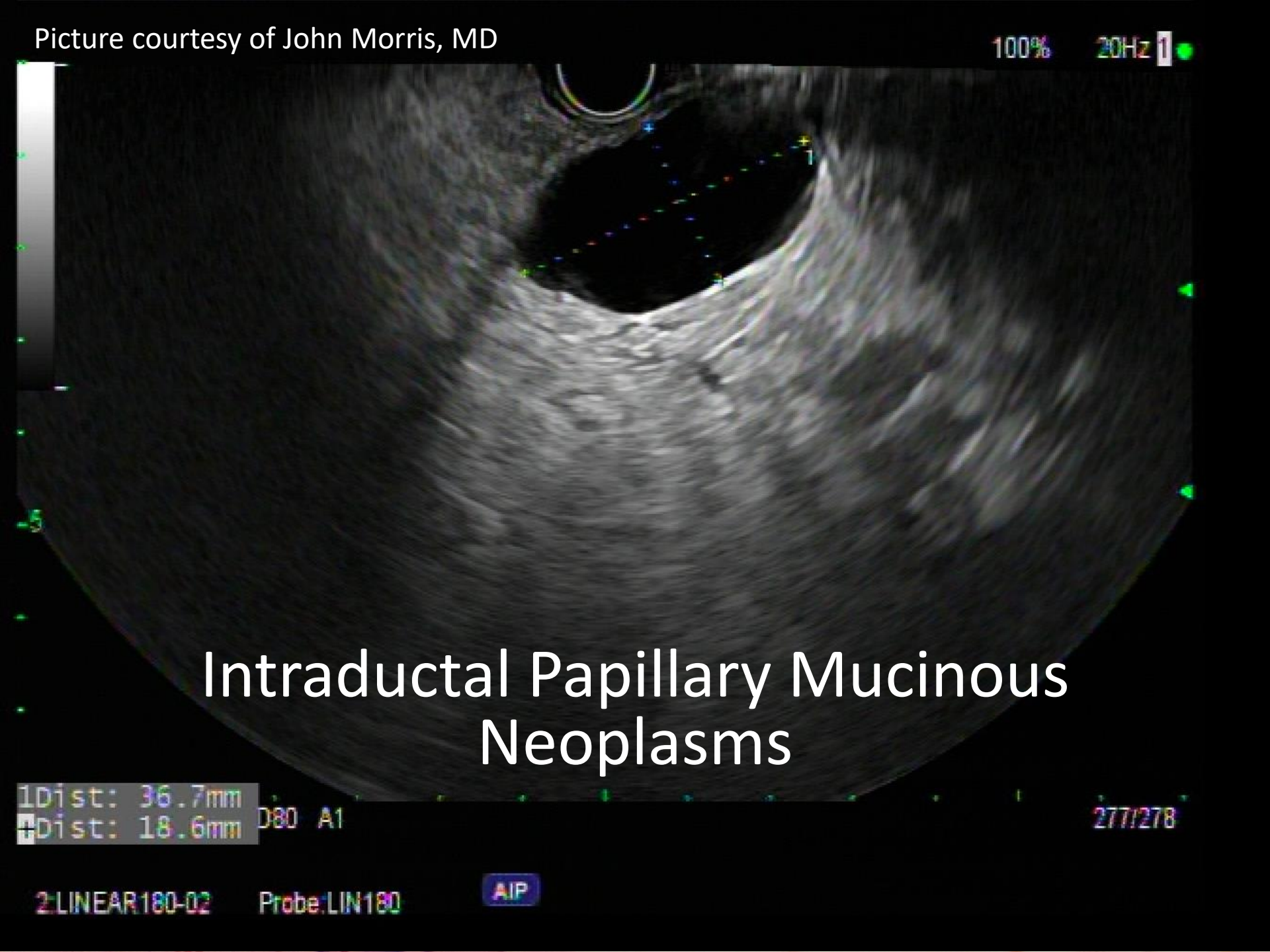


Mucinous Cystic Neoplasms

- 20% of resected pancreatic cysts
- Histologically characterized by the presence of ovarian-type stroma, with overlying cyst lining consistent of mucin-secreting columnar cells
- ER/PR positive
- MCNs occur almost exclusively in female patients (95-98%)
- Peak age distribution in the late 40's (range: 19-80)
- 99% found in the body or tail of the pancreas
- Reported 13.4% with high-grade dysplasia (HGD) and 3.9% with associated invasive cancer component
- International consensus guidelines recommend surgical resection for MCN cases

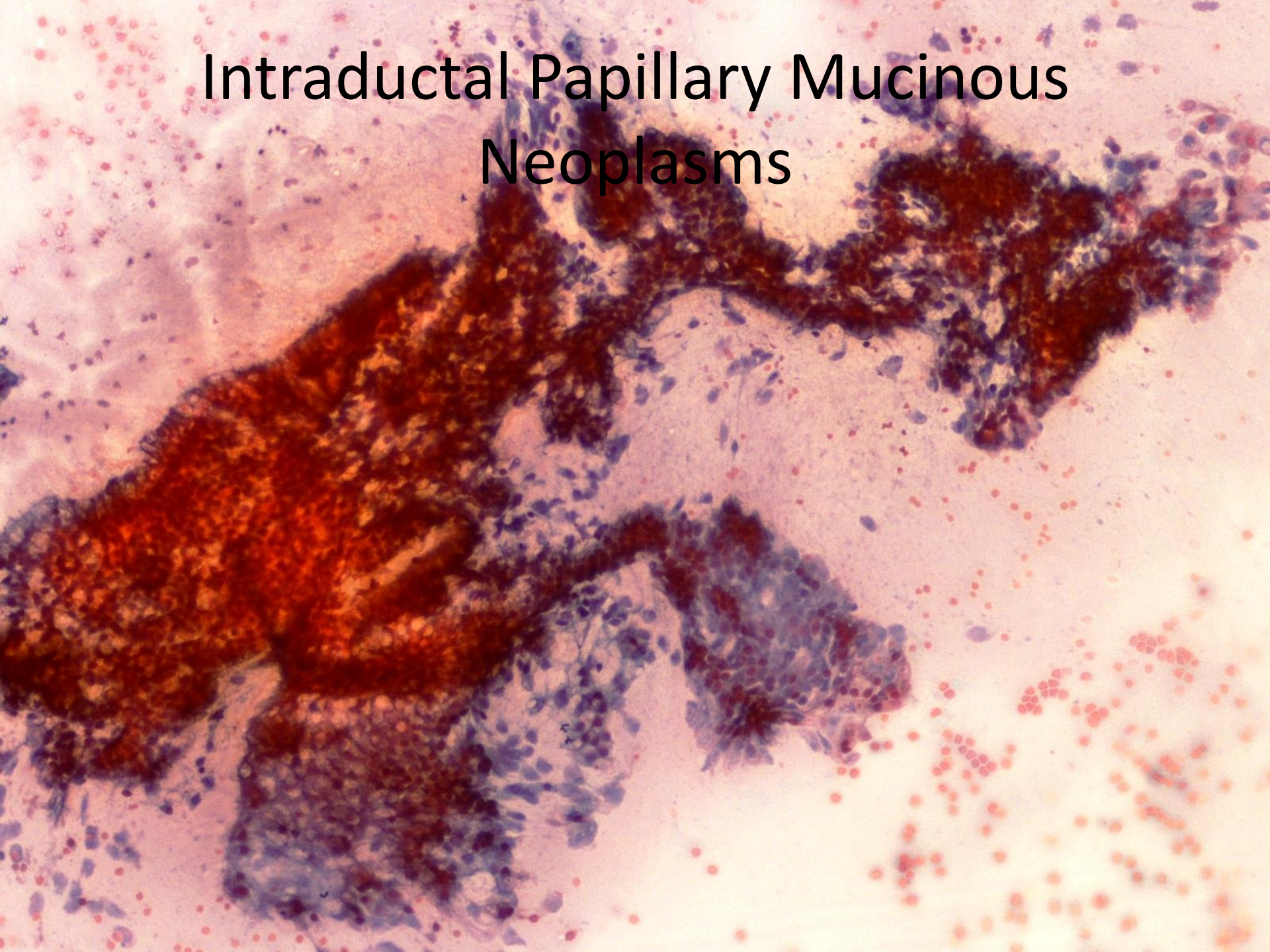
Mucinous Cystic Neoplasms

- Thick, colloid-like extracellular mucin
- Mucinous cyst lining
- Elevated CEA (≥192 ng/ml)
- Presence of dysplasia (high or low)
- Presence or absence of invasion
- Resection regardless of grade of dysplasia
- No known specific mutation for MCN



Intraductal Papillary Mucinous Neoplasms

Intraductal Papillary Mucinous Neoplasms



Intraductal Papillary Mucinous Neoplasms

- 50% of the pancreatic cysts resected
- Equal frequencies in men and women
- Peak age in the 7th decade of life
- Sub-classified into three types, based on which duct structures are involved:
  - Main-duct IPMNs (MD-IPMN)
  - Branch-duct IPMNs (BD-IPMN)
  - Mixed IPMNs (mixed IPMN)
- MD-IPMNs are most often lined by intestinal-type secretory epithelium, and tend to demonstrate more cytologic atypia when compared to BD-IPMNs, which most often have a gastric-type secretory epithelium

Intraductal Papillary Mucinous Neoplasms

- International consensus guidelines define MD-IPMN as a segmental or diffuse dilation of the MPD greater than 5 mm
- Incidence of malignant change was significantly higher in MD-IPMN (69.7%) compared with BD-IPMN cases (17.9%)
  - Same study showed that patients with an invasive carcinoma had a significantly worse outcome if it was derived from BD-IPMN compared with those derived from MD-IPMN (Okabayashi et al. 2013)

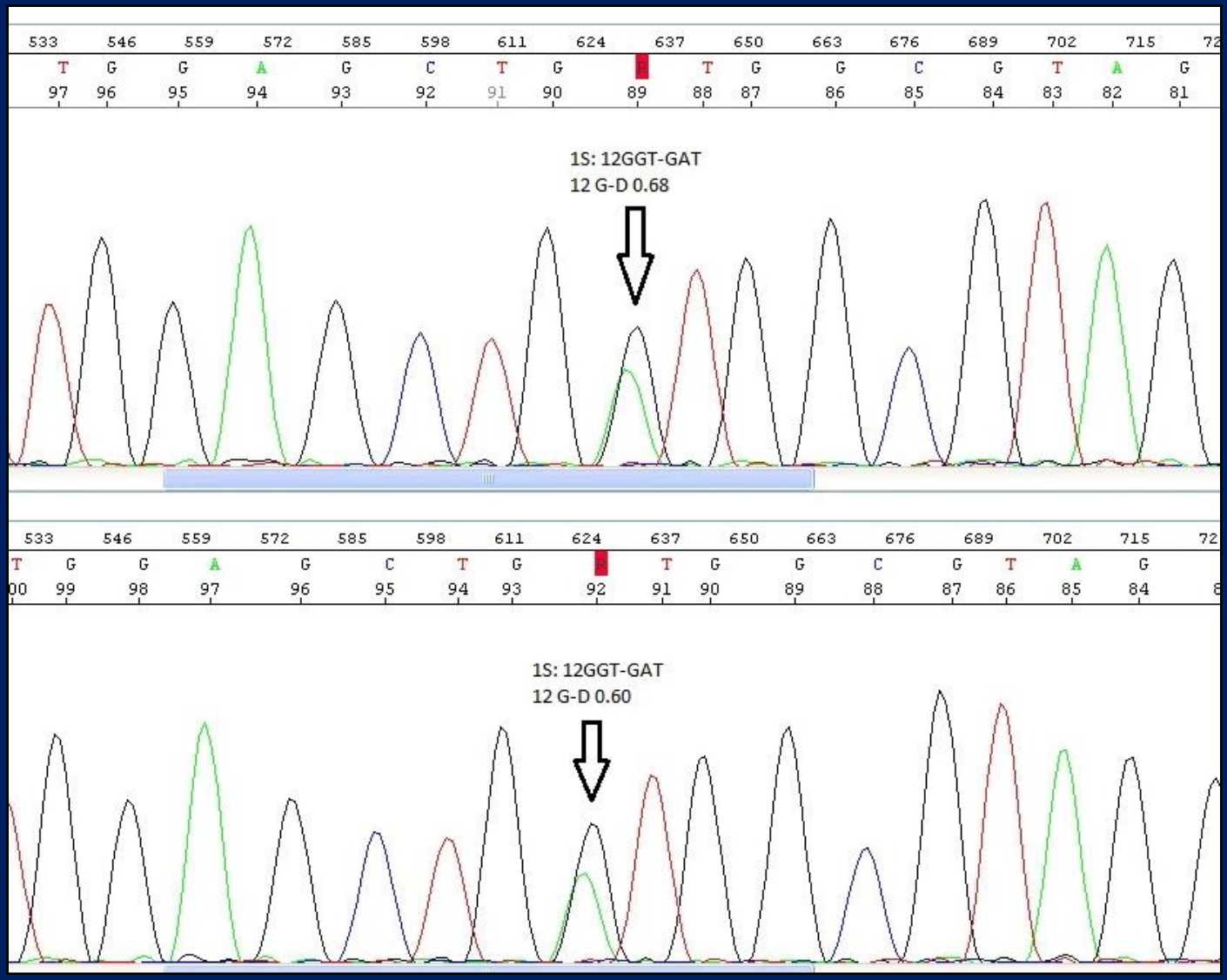
Intraductal Papillary Mucinous Neoplasms

- 3-5% of pancreatic tumors
- 20% of neoplastic pancreatic cysts
- 70% arise in the head of the pancreas
- Prognosis excellent for non-invasion
- High risk features include:
  - Main duct involvement
  - Mural nodule
  - High-grade dysplasia or carcinoma

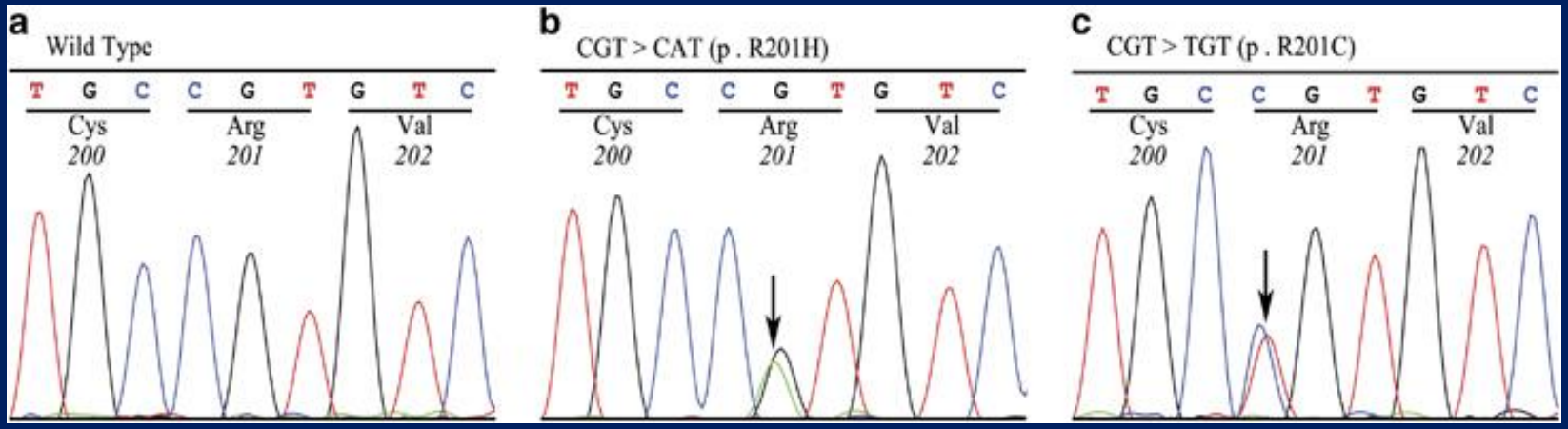
KRAS Mutation and CEA Analysis

- *KRAS* mutations (codon 12 or 13) are present in the fluid of about 30% of pancreatic cystic lesions
- *KRAS* testing of the cyst fluid is valuable, especially in those cases where the CEA level is low, as the presence of *KRAS* mutations supports the diagnosis of a mucinous cyst
- The added value of molecular testing can be small compared with the combination of cytology and CEA testing, at least in cases where the CEA levels are elevated
  - A negative *KRAS* test may be due to insufficient and possibly non-representative DNA and therefore does not exclude a neoplastic mucinous cyst.

KRAS Sequencing



GNAS Mutation Analysis



- Guanine nucleotide protein, alpha stimulating (*GNAS*) mutations differentiate MCN from IPMN (Wu et al. 2011)
- Although, like *KRAS*, *GNAS* does not distinguish benign from malignant cysts, this finding may be helpful in patient management
  - MCNs are generally resected regardless of grade
- Mutations at codon 201 of the *GNAS* gene where found in 66% of IPMNs
- Codon 227 the second most commonly mutated
- Moreover, *GNAS* mutations were not found in other types of cystic neoplasms of the pancreas or in PDACs not associated with IPMNs

Image: Shi et al. Mod Pathol 2013;26:1023-31.

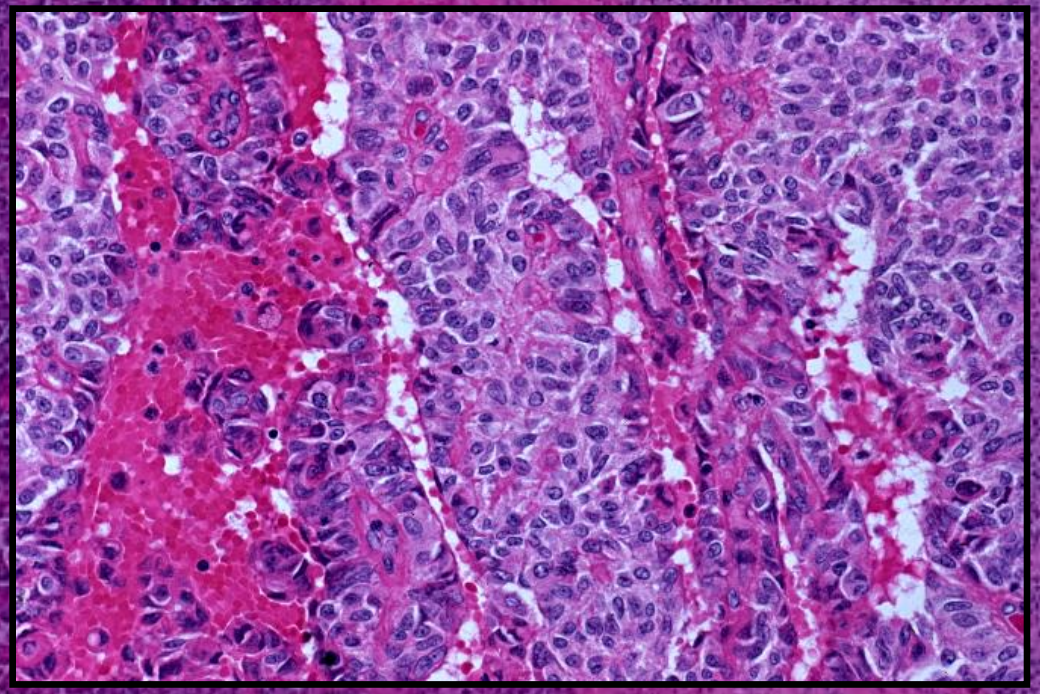
GNAS Mutations

- Prospective study running NGS on IPMN cyst fluid
- KRAS and/or GNAS mutations found in 100% of IPMNs (Singhi et al. 2016)
- GNAS mutations 100% specific for IPMNs
- KRAS/GNAS mutations show 89% sensitivity and 100% specificity for mucinous cysts

Intraductal Papillary Mucinous Neoplasms

- KRAS mutations in 66% of cases
- GNAS mutations in 96% of cases
- SMAD/DPC4 and CDKN2A mutations/loss of function or expression seen in high-grade dysplasia or carcinoma related IPMNs
- Preoperative detection of mutations in TP53, PIK3CA, PTEN and AKT1 have high sensitivity and specificity for IPMN with high-grade dysplasia
- Same for GNAS mutations with high VAF (55% or higher)

Solid Pseudopapillary Neoplasm



B-Catenin

Photos courtesy of Mary Bronner, MD

Solid Pseudopapillary Neoplasms

- <2% of pancreatic neoplasms
- Account for <5% of the resected pancreatic cysts
- Primarily young women
- β-catenin (CTNNB1) activating mutations (95-100% of cases)
- Nuclear and cytoplasmic accumulation of β-catenin
- Allelic loss of chromosome 11q the most common abnormality
- APC mutations have also been described

KRAS/GNAS/VHL/RNF43/CTNNB1 Panel Analysis

- Developed a panel of markers that appears to distinguish the major types of pancreatic cysts (Vogelstein et al.)
- Sequenced the exomes of the four most common types of pancreatic cysts and identified five genes:
  - *VHL*, *RNF43*, *CTNNB1*, *GNAS* and *KRAS*

KRAS/GNAS/VHL/RNF43/CTNNB1 Panel Analysis

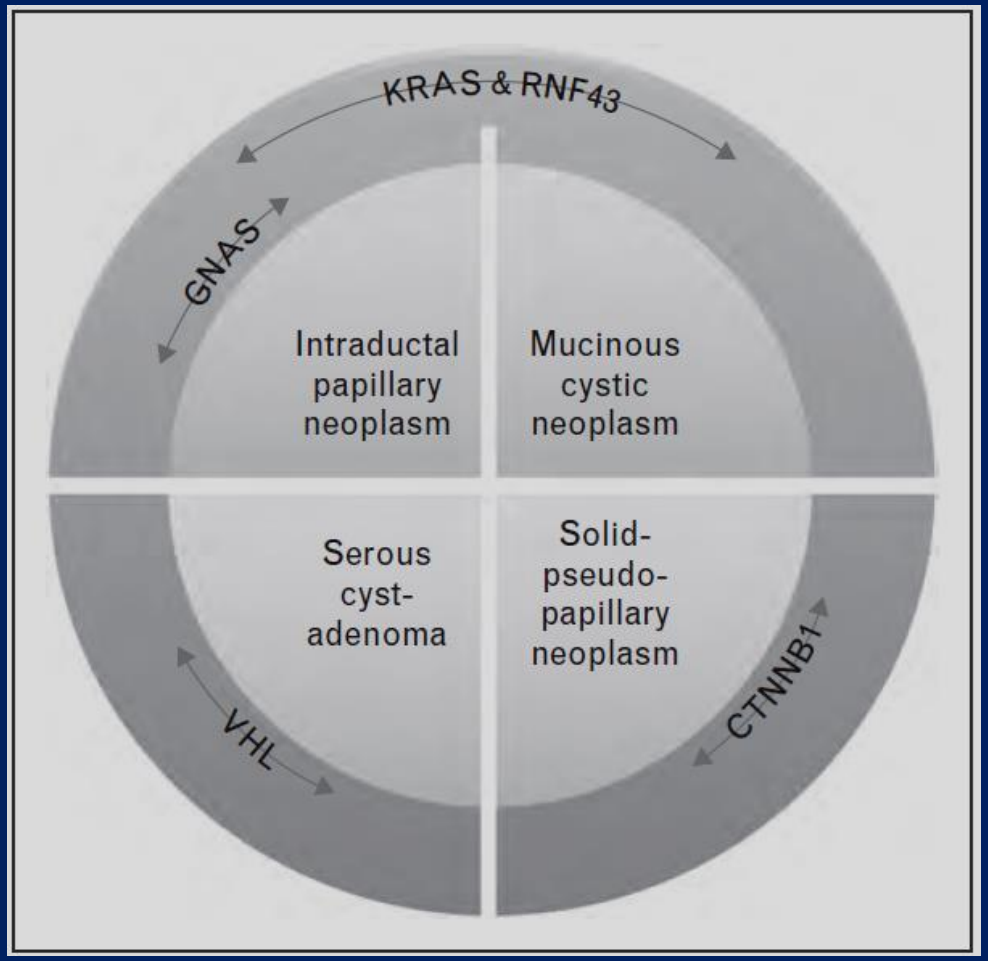
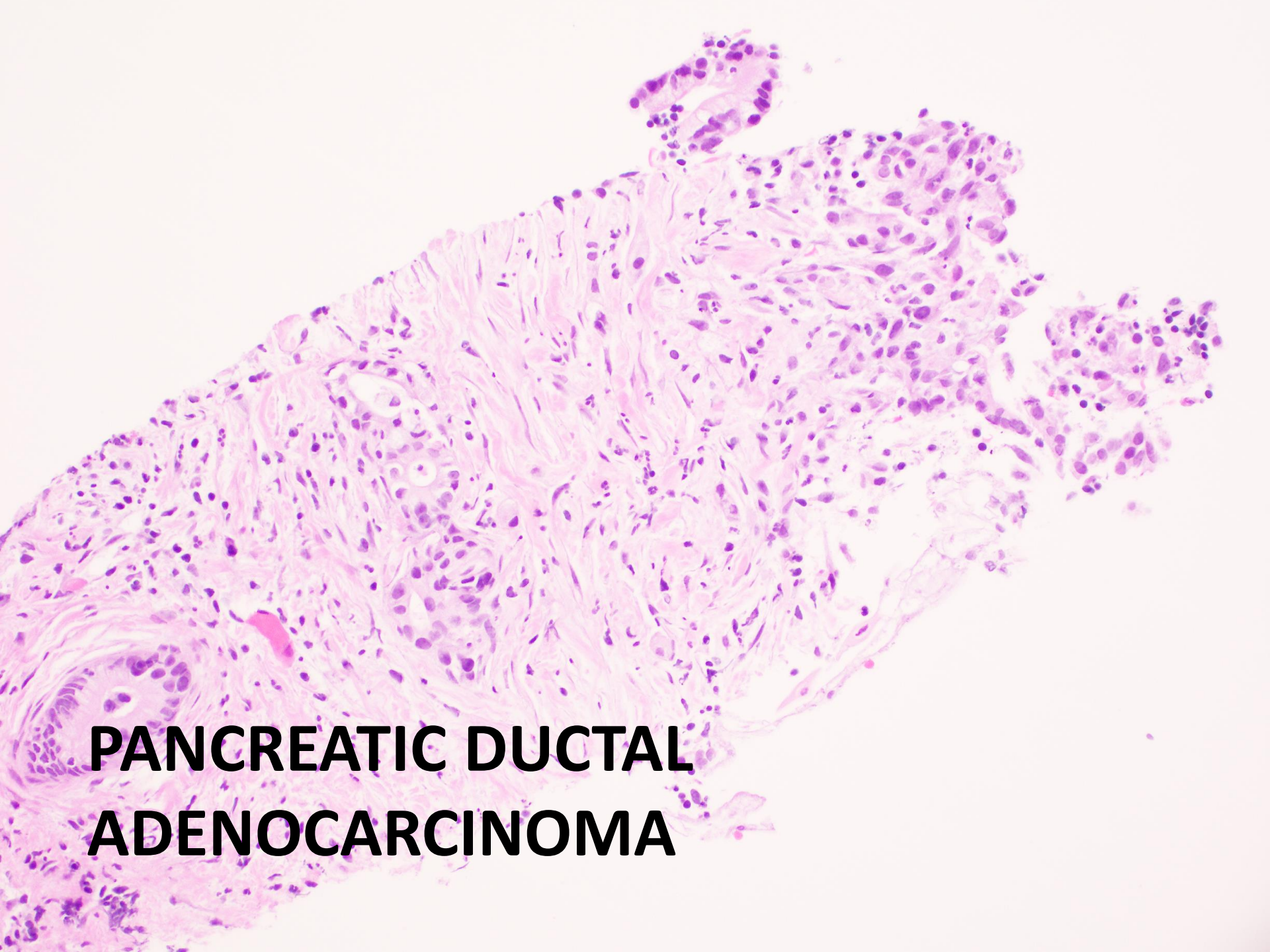


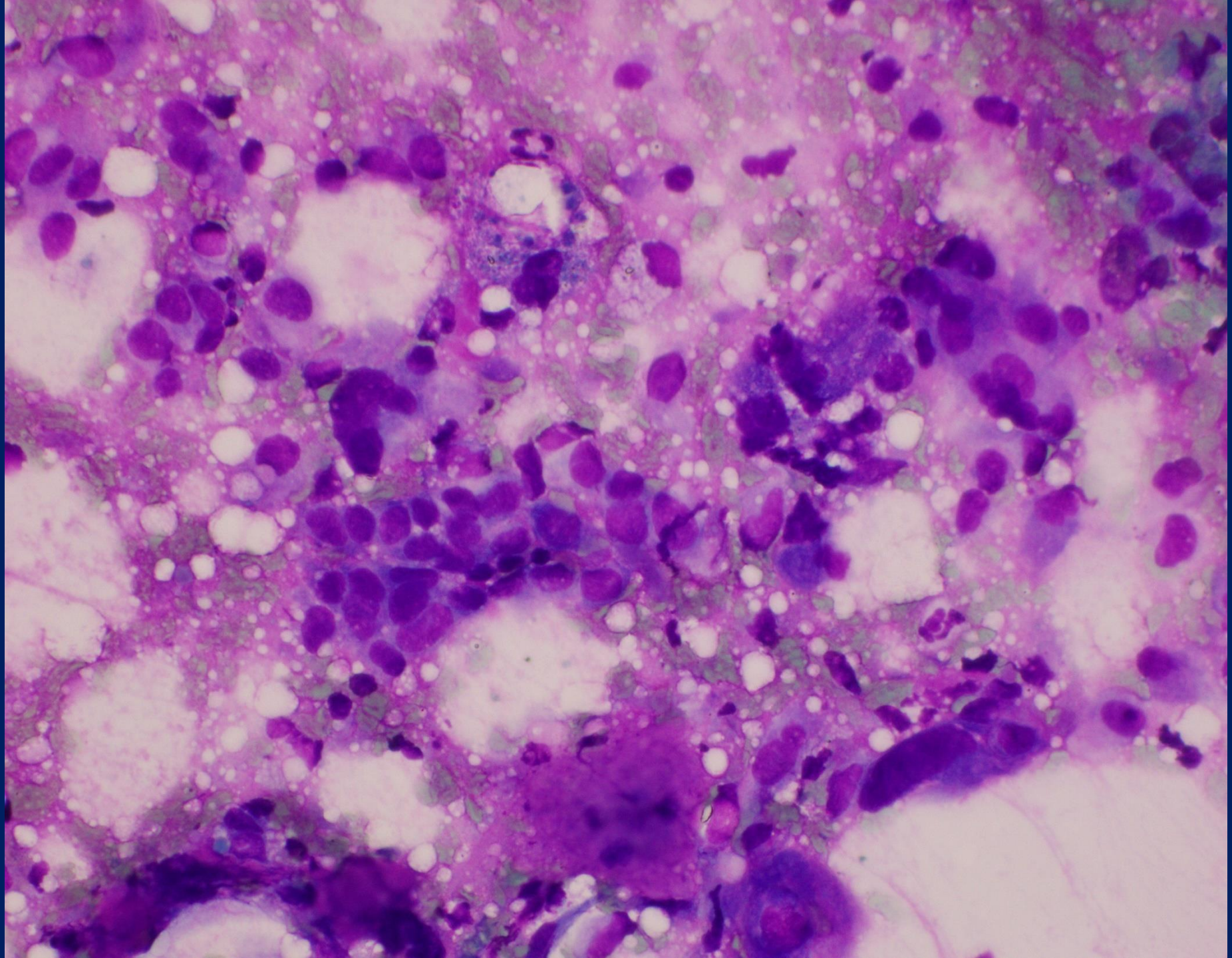
Image from: Law et al. Curr Opin Gastroenterol 2013;59:509-16.

Other Pancreatic Cysts

- Other uncommon pancreatic cysts include:
  - Pseudocysts
  - Cystic degeneration of solid neoplasms
  - Cystic neuroendocrine tumors (PNETs)
  - Cystic pancreatic ductal adenocarcinoma (PDAC)

SOLID PANCREATIC LESIONS


PANCREATIC DUCTAL
ADENOCARCINOMA



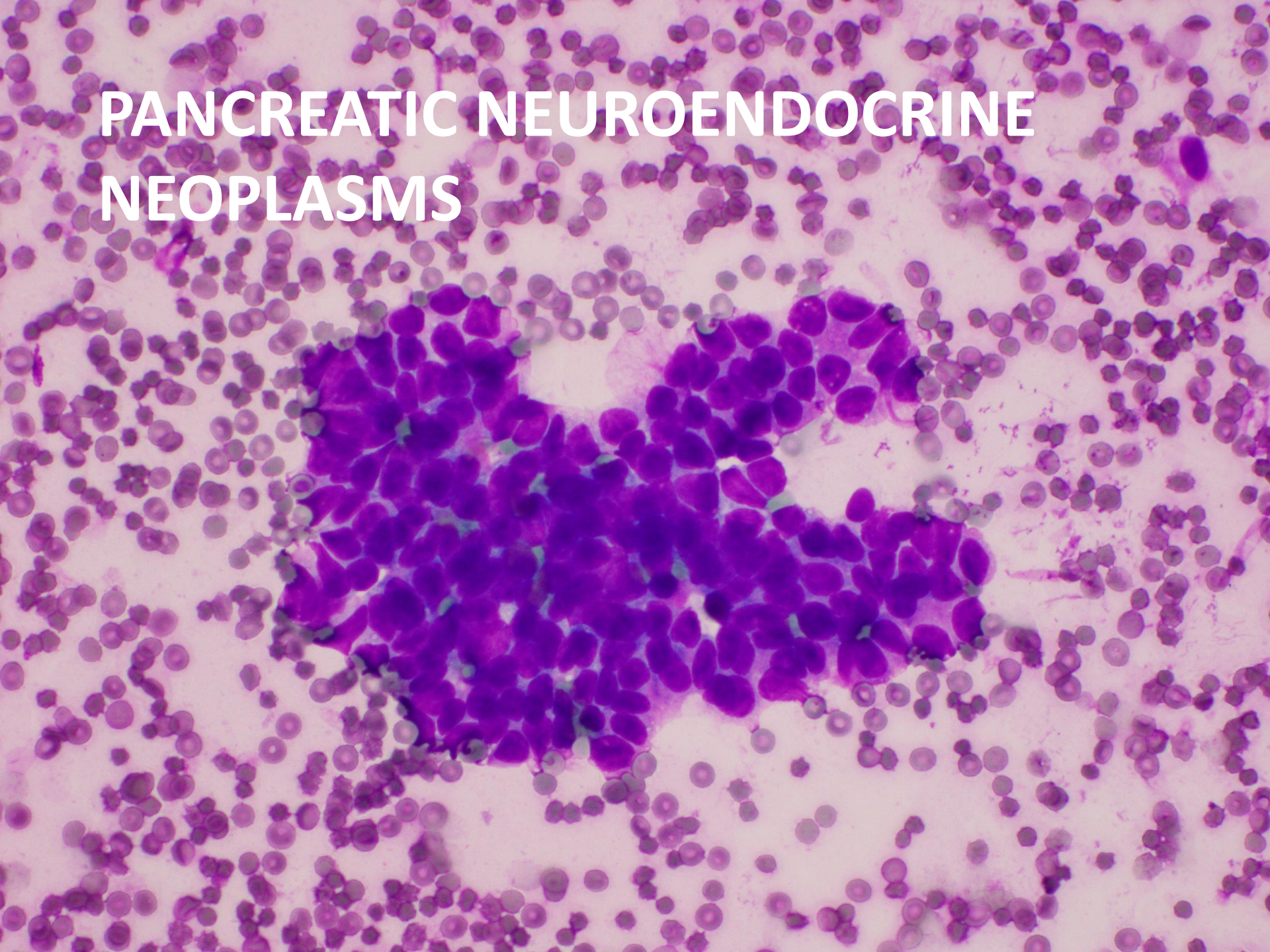
Common Mutations in PDAC

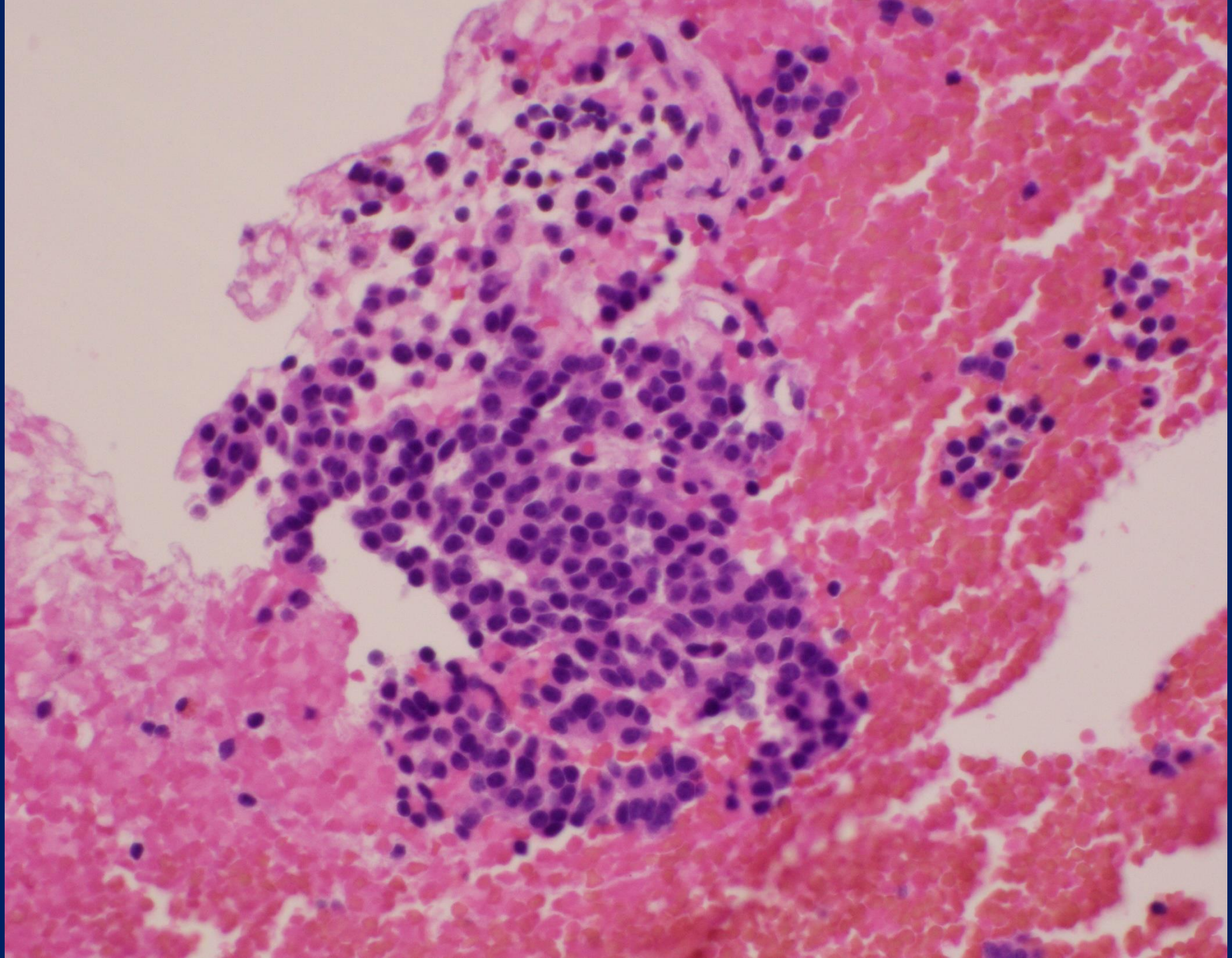
- KRAS, TP53, SMAD4/DPC4 and CDKN2A are the most common mutations in pancreatic ductal adenocarcinomas
- Most cases will show TP53 loss or overexpression pattern by IHC
- About 55% will show loss of SMAD4 by IHC
- PDAC with CDX2 expression (about 1/3 of cases) have been suggested to have shorter OS, compared to those with no CDX2 expression

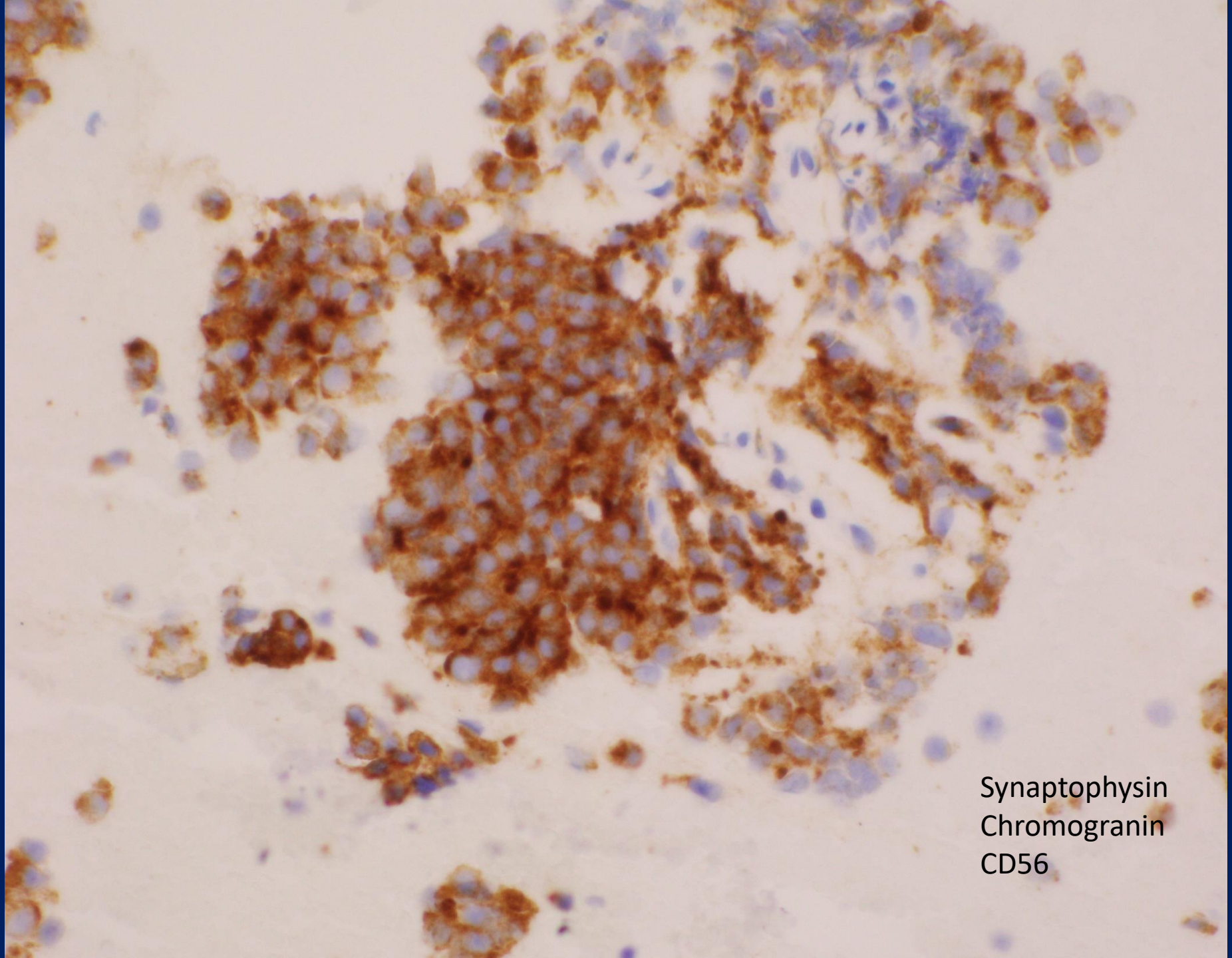
KRAS Mutations in Pancreatic Solid Tumors

- Non-specific
- Found in both low-grade PanIN and other pancreatic neoplasms
- Not useful in distinguishing premaligant neoplasms from PDAC

PANCREATIC NEUROENDOCRINE NEOPLASMS






Synaptophysin
Chromogranin
CD56

Pancreatic Neuroendocrine Tumors (PanNETs)

- 1-2% of all pancreatic lesions
  - Most non-functional
- Can undergo cystic degeneration
- Established NET morphologic criteria apply
  - Including mitotic count/Ki-67
- Express NE markers
  - CD56 the most sensitive by least specific
    - Can be expressed in SPN

(Cystic) Pancreatic Neuroendocrine Tumors (PanNETs)

- Most common mutations occur in DAXX and ATRX genes
  - ~50% of cases
  - IHC stains available as surrogates of molecular testing
- DAXX/ATRX loss is associated with increased risk of metastasis and reduced PFS

(Cystic) Pancreatic Neuroendocrine Tumors (PanNETs)

- MEN1 and/or TSC2 mutations present in a subset of PanNETs
- Mutations in genes in the mTOR pathway
- MEN1 somatic mutations can be seen in up to 45% of sporadic cases
  - Germline MEN1 mutations in familial (MEN1) cases
  - Can also have VHL
- Copy number alterations (CNAs)
  - Associated with decreased DFS and DSS

Pancreatic Neuroendocrine Carcinomas (PanNECs)

- Infrequently associated with MEN1
- Retain DAXX/ATRX
  - Negative for mutations in those genes
- ~95% overexpress p53 by IHC
  - Mutations in TP53 gene
- 60-90% show loss of pRb
  - Mutations of RB1 gene
- 10-40% show loss of p16
  - Mutations of CDKN2A gene

Table 1

Comparison of commonly mutated genes in PanNETs and PDAC[c]

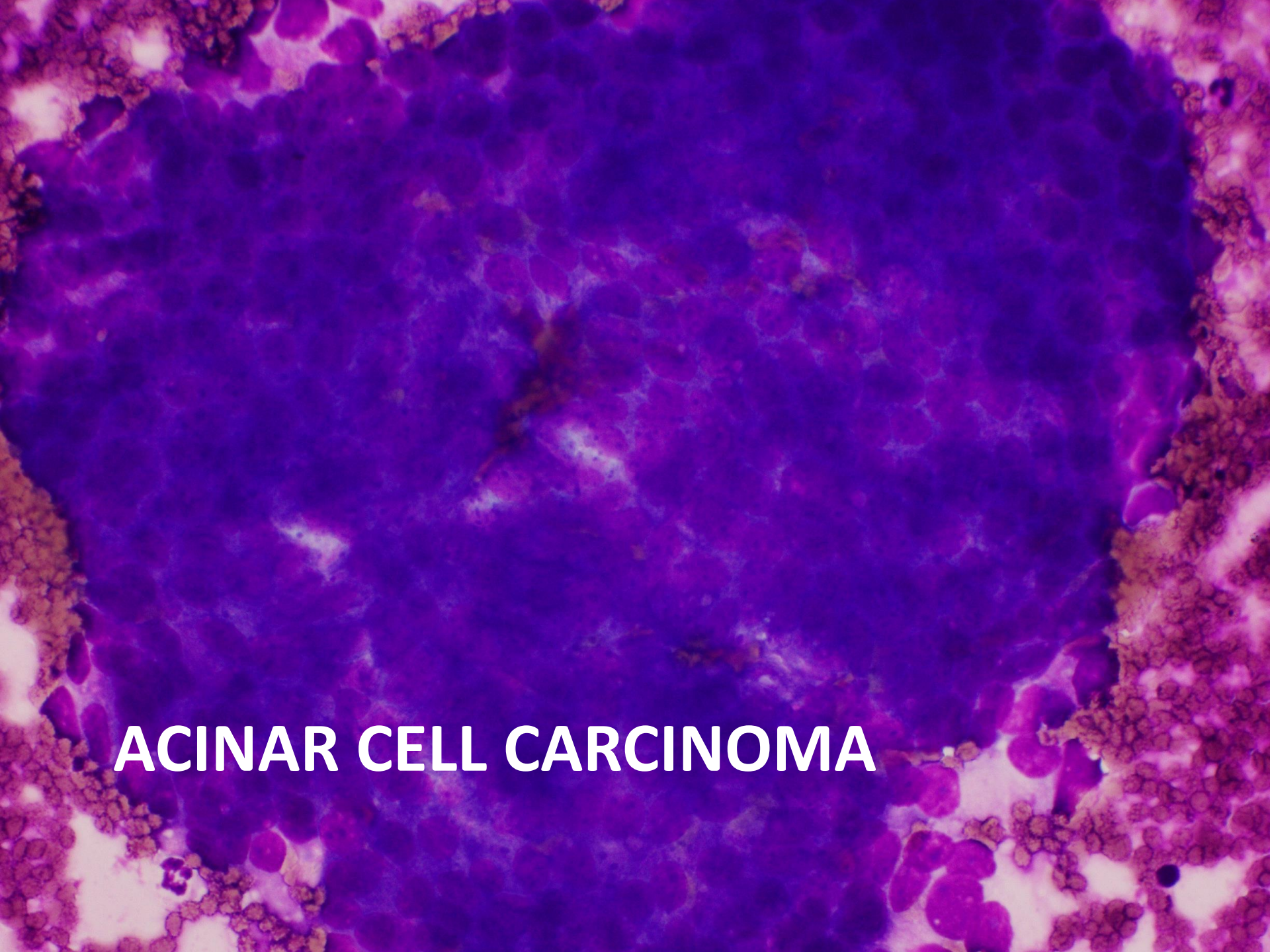
Genes[a]	PanNET	PDAC[b]
MEN1	44%	0%
DAXX, ATRX	43%	0%
Genes in mTOR pathway	15%	0.80%
TP53	3%	85%
KRAS	0%	100%
CDKN2A	0%	25%
TGFBR1, SMAD3, SMAD4	0%	38%

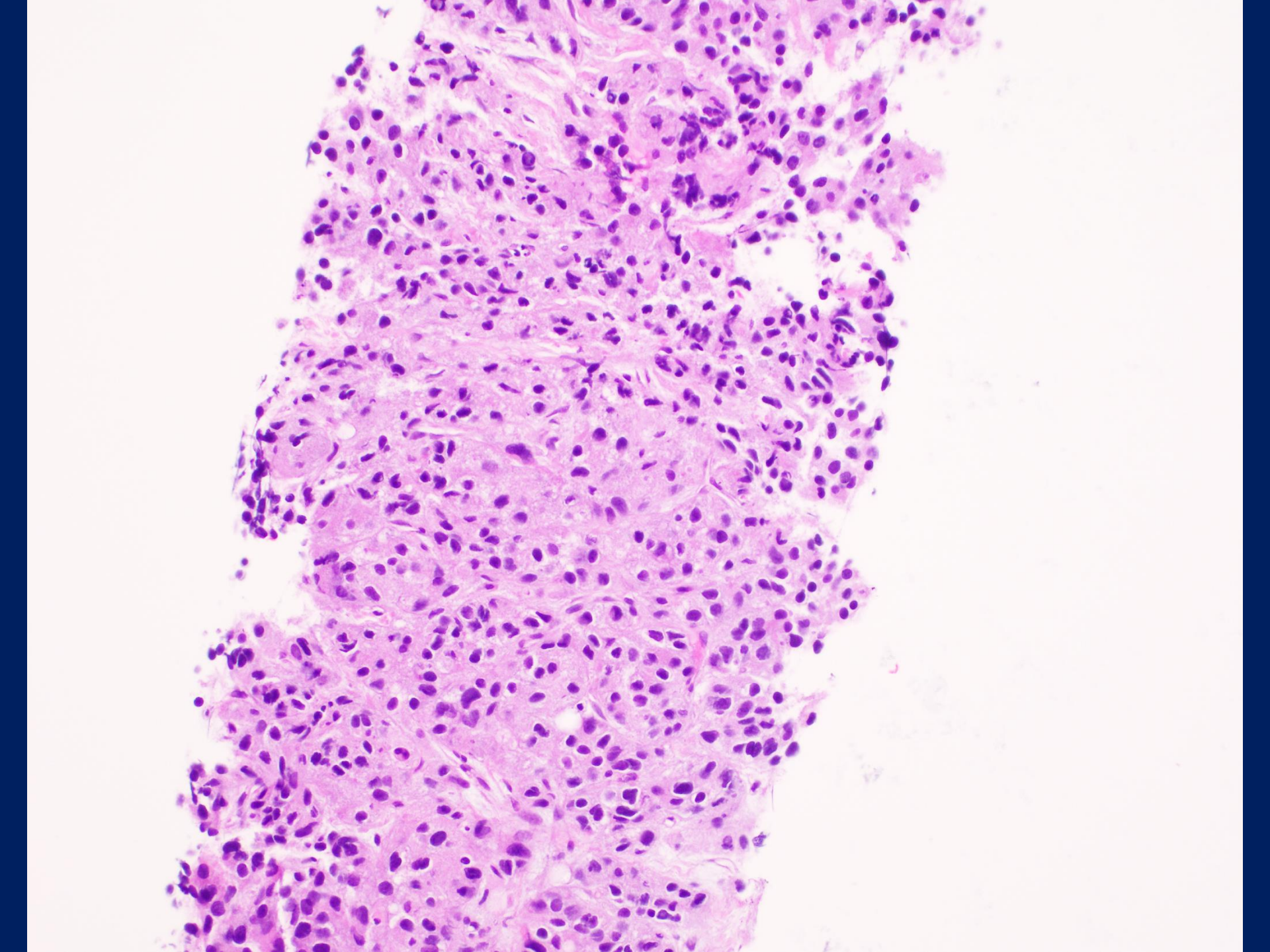
[a] Includes point mutations and indels.

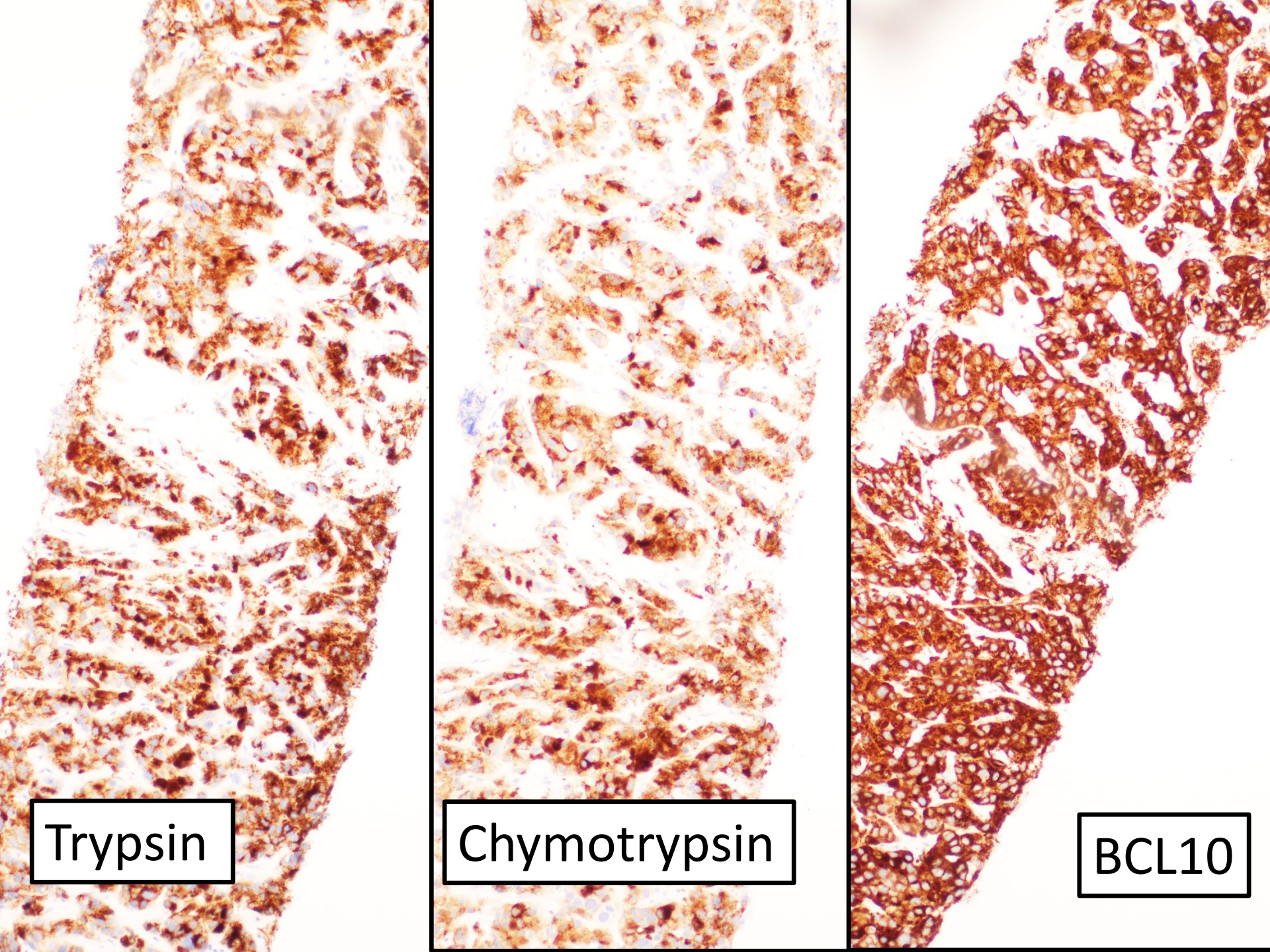
[b] Data from Jones *et al* ., *Science* **321**, 1801 (2008).

[c] Based on 68 PanNETs and 114 PDACs.

From: Science 2014;331:1199–203.


ACINAR CELL CARCINOMA



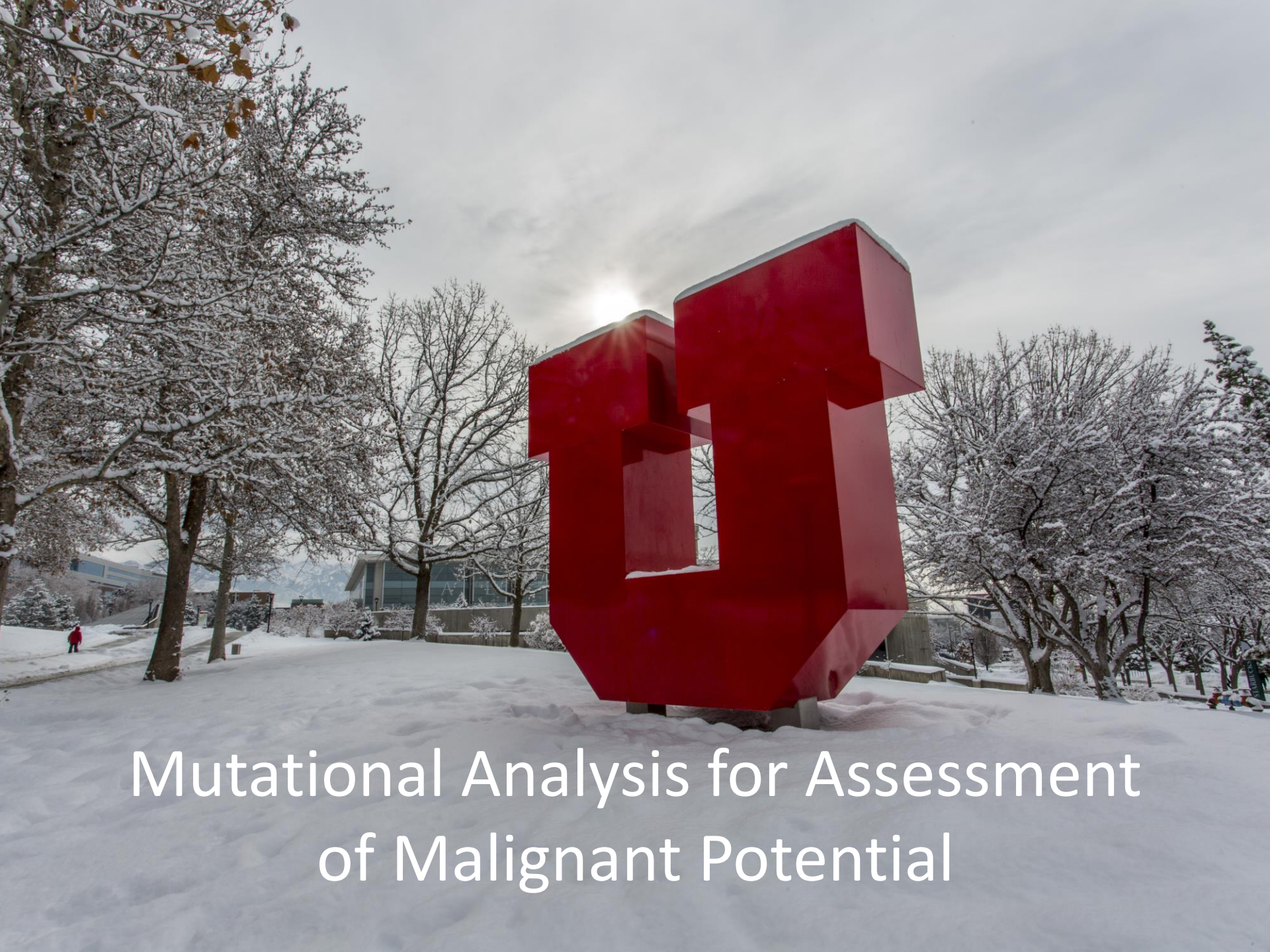

Trypsin
Chymotrypsin
BCL10

Acinar Cell Carcinomas

- <2% of all pancreatic neoplasms
- Enzyme expression by IHC
  - Trypsin, chymotrypsin
- Allelic loss of chromosome 11q
- APC and TP53 mutations
- BRAF fusions (SND1-BRAF most common, up to 23%)
- APC/β-catenin mutations have also been described

Pancreatoblastoma

- <0.5% of all pancreatic neoplasms
- Most common pancreatic malignancy in children
- Multiple lines of differentiation in the tumor

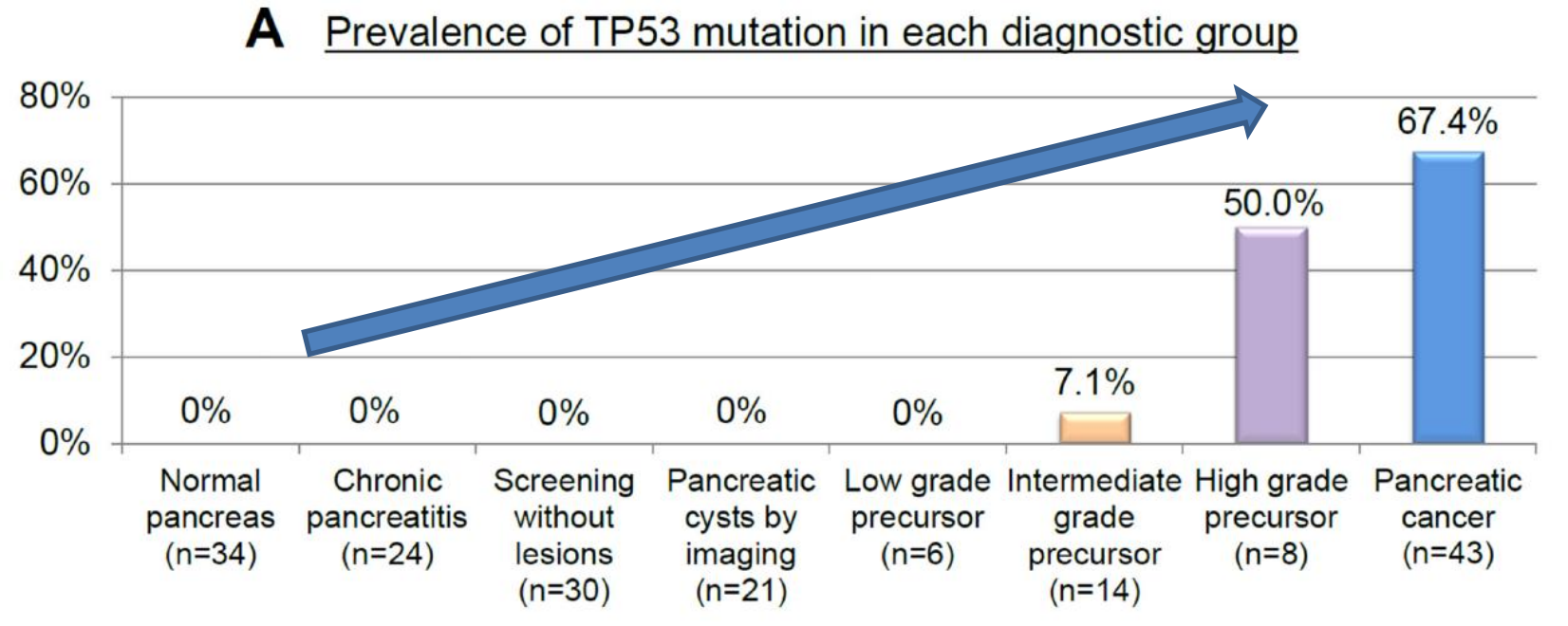


Mutational Analysis for Assessment of Malignant Potential

Table 4 Sensitivities and specificities of molecular testing and other diagnostic modalities based on 102 surgically resected PCs

Parameter	Sensitivity (95% CI)	Specificity (95% CI)
IPMNs		
KRAS and/or *GNAS* mutations	100% (0.92 to 1.00)	96% (0.84 to 0.99)
Presence of multiple cysts	54% (0.40 to 0.67)	72% (0.56 to 0.84)
Increased fluid viscosity	82% (0.69 to 0.91)	80% (0.66 to 0.90)
Elevated CEA*	57% (0.40 to 0.73)	70% (0.53 to 0.83)
IPMNs with advanced neoplasia		
TP53, *PIK3CA* and/or *PTEN* alterations	88% (0.62 to 0.98)	95% (0.88 to 0.98)
KRAS and/or *GNAS* mutations with *TP53*, *PIK3CA* and/or *PTEN* alterations	88% (0.62 to 0.98)	97% (0.89 to 0.99)
GNAS MAF >55% or *TP53*/*PIK3CA*/*PTEN* MAFs at least equal to *KRAS*/*GNAS* MAFs	100% (0.77 to 1.00)	100% (0.95 to 1.00)
Main pancreatic duct dilatation	47% (0.24 to 0.71)	74% (0.63 to 0.83)
Presence of a mural nodule	35% (0.15 to 0.61)	94% (0.86 to 0.98)
Malignant cytopathology†	35% (0.15 to 0.61)	97% (0.91 to 1.00)
IPMNs and MCNs		
KRAS and/or *GNAS* mutations	89% (0.79 to 0.95)	100% (0.88 to 1.00)
Increased fluid viscosity	77% (0.65 to 0.86)	89% (0.73 to 0.96)
Elevated CEA*	57% (0.42 to 0.71)	80% (0.61 to 0.92)
IPMNs and MCNs with advanced neoplasia		
TP53, *PIK3CA* and/or *PTEN* alterations	79% (0.54 to 0.93)	95% (0.88 to 0.98)
KRAS and/or *GNAS* mutations with *TP53*, *PIK3CA* and/or *PTEN* alterations	79% (0.54 to 0.93)	96% (0.89 to 0.99)
GNAS MAF >55% or *TP53*/*PIK3CA*/*PTEN* MAFs at least equal to *KRAS*/*GNAS* MAFs	89% (0.66 to 0.98)	100% (0.95 to 1.00)
Main pancreatic duct dilatation	42% (0.21 to 0.66)	74% (0.63 to 0.82)
Presence of a mural nodule	32% (0.14 to 0.57)	94% (0.86 to 0.98)
Malignant cytopathology†	32% (0.13 to 0.57)	98% (0.91 to 1.00)

From: Singhi AD, et al. Gut 2018;67:2131–41.



From: Clin Gastroenterol Hepatol 2013;11: 719–30.

KRAS Mutation and Oncosuppressor Gene LOH Analysis

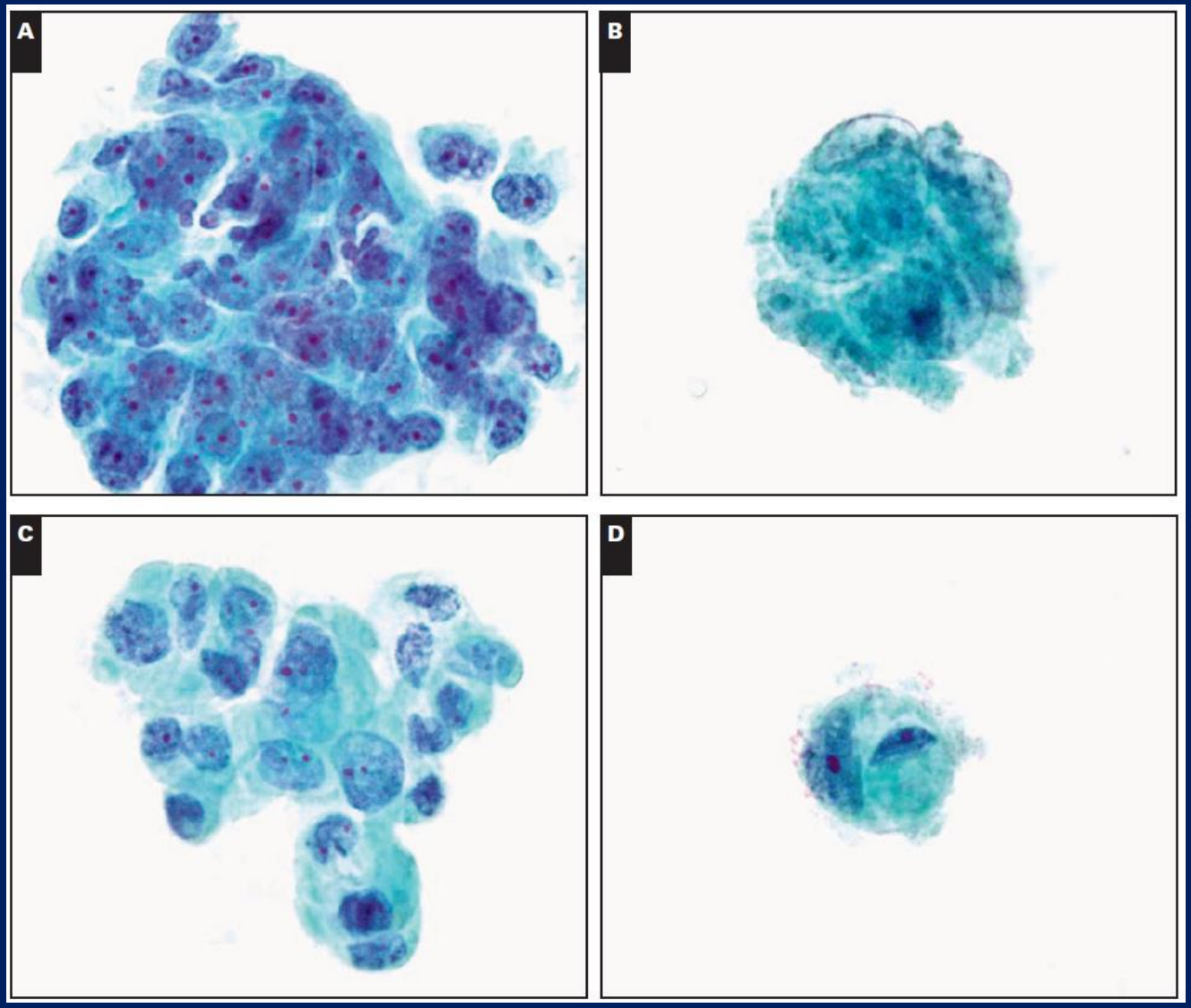
A panel of microsatellite markers has been developed and has been analytically and clinically validated to target common sites for tumor suppressor genes associated with pancreaticobiliary cancers

Accounts for other parameters, including cytomorphology, DNA quantity, radiological findings etc.

Locus	Gene(s)
1p	CMM1, LMYC
3p	VHL, OGG1
5q	MCC, APC
9p	CDKN2A
10q	PTEN, MXI1
17p	TP53
17q	NME1, RNF43
18q	SMAD4, DCC
21q	PSEN2, TFF1
22q	NF2

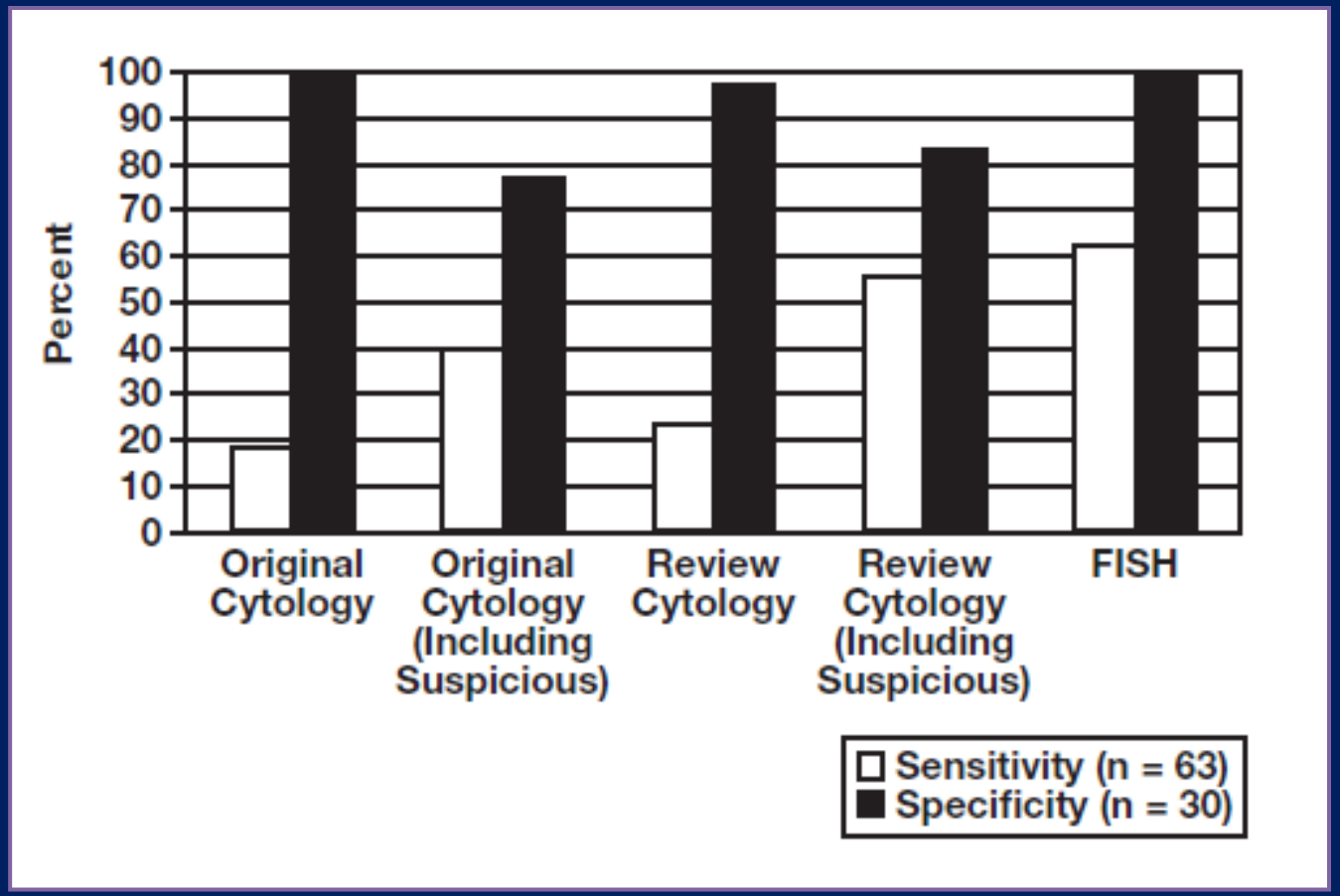
PANCREATICOBILIARY EXFOLIATIVE CYTOLOGY AND BILIARY TUMORS

Morphologic Criteria for Biliary Cytology



From: Fritcher et al., 2011.

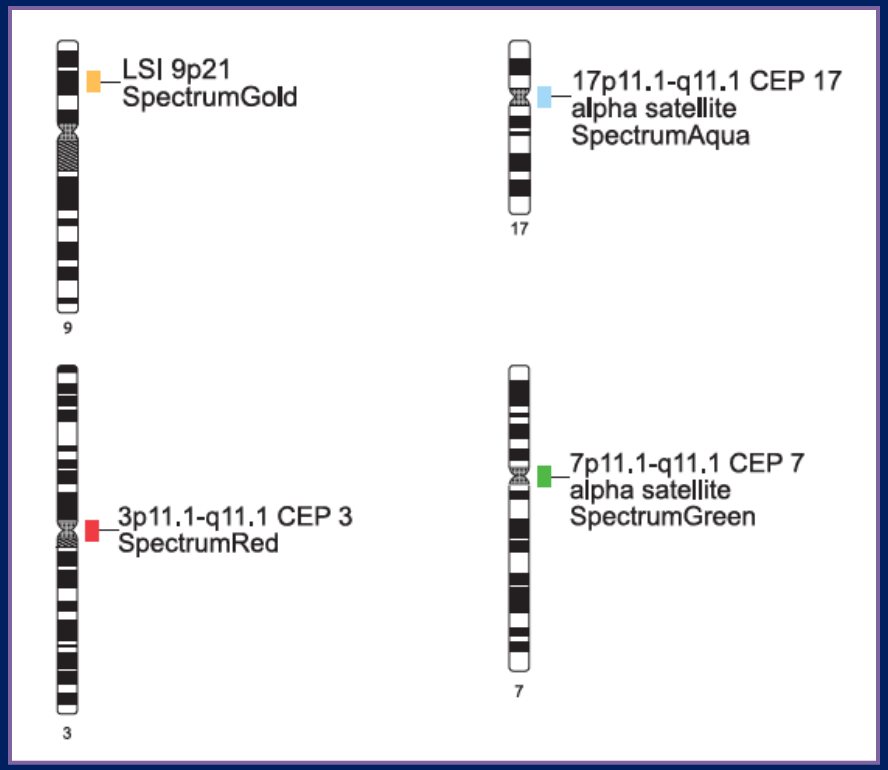
Comparison of Morphology and FISH in CBD Cytology



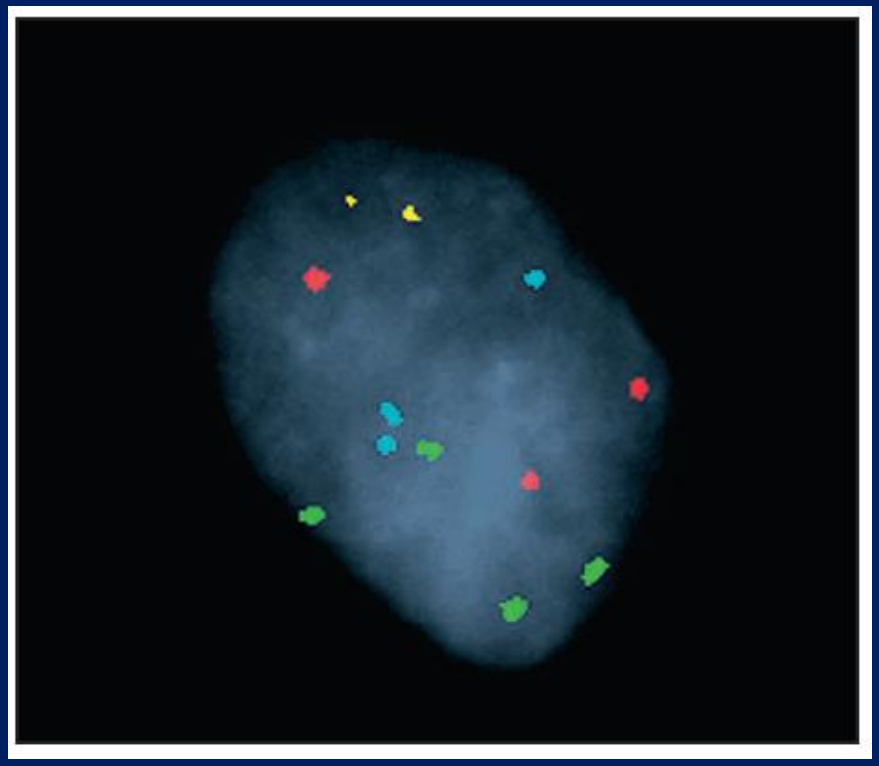
From: Fritcher et al., 2011.

Urovysion

- Loss of 9p21 and chromosome 3, 7 and 17 aneuploidy correlates with urothelial carcinoma

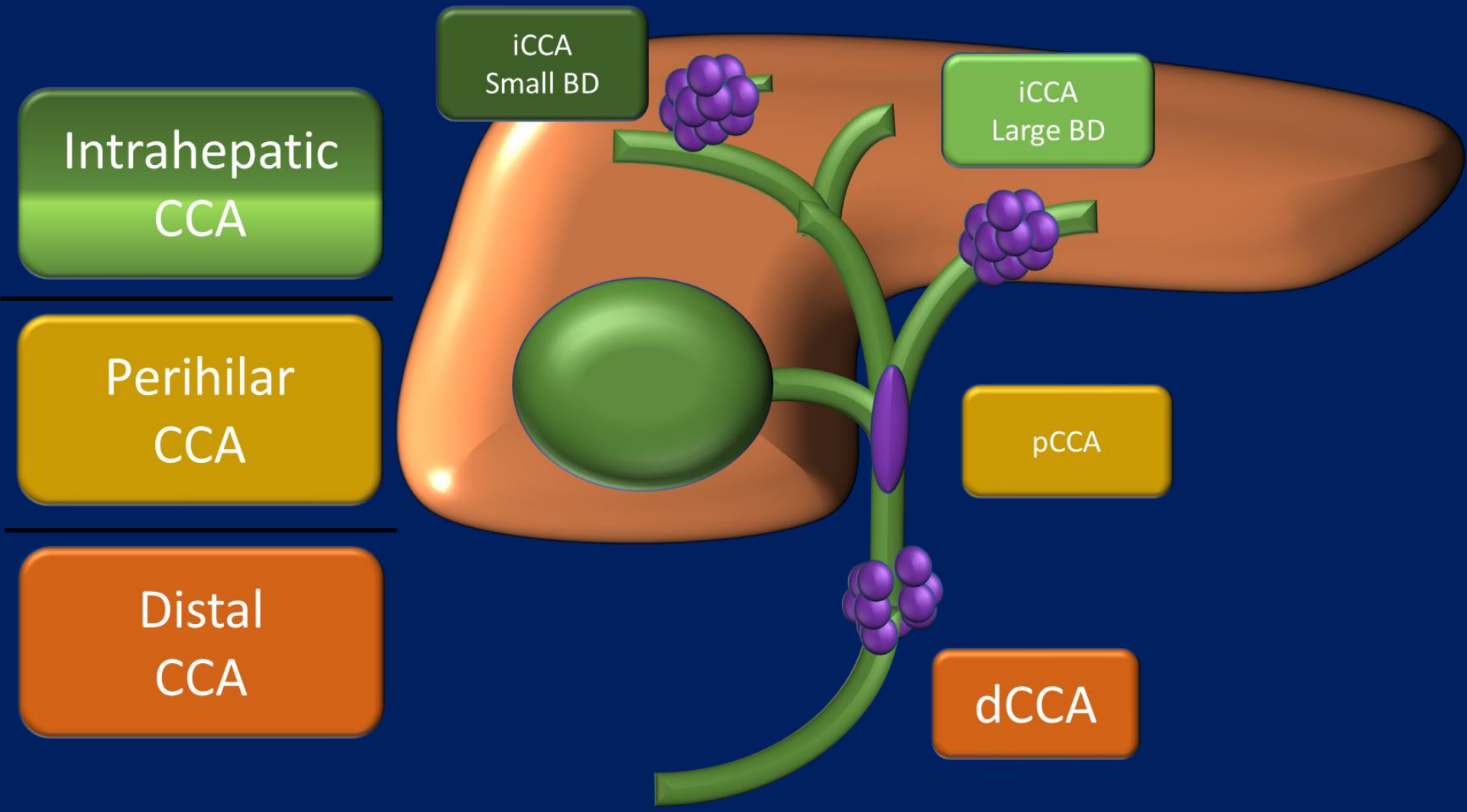


Abbott Molecular, Des Plaines, IL

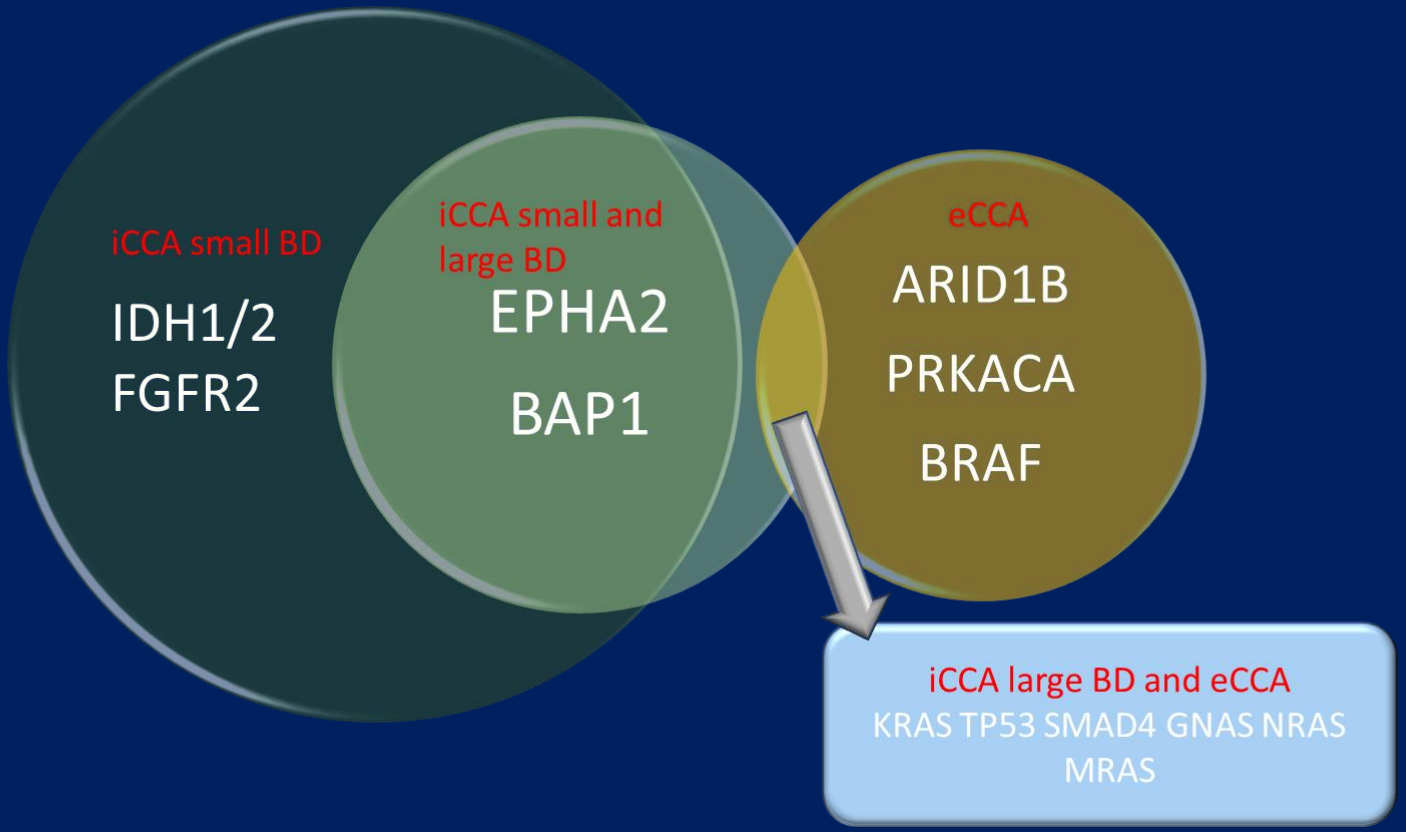


From: Fritcher et al., 2011.

Classification of Cholangiocarcinomas (CCAs)



Common Mutations in Cholangiocarcinomas (CCAs)



Common Mutations in Cholangiocarcinomas (CCAs)

- IDH1/2 mutations in 10-23% of iCCAs
  - Prognostic effect uncertain
- IHD1 mutations in 0.8% of eCCAs
  - Poor prognosis
- FGFR2 fusions in 13-14% of iCCAs
  - Possible favorable prognosis

Clinical Case

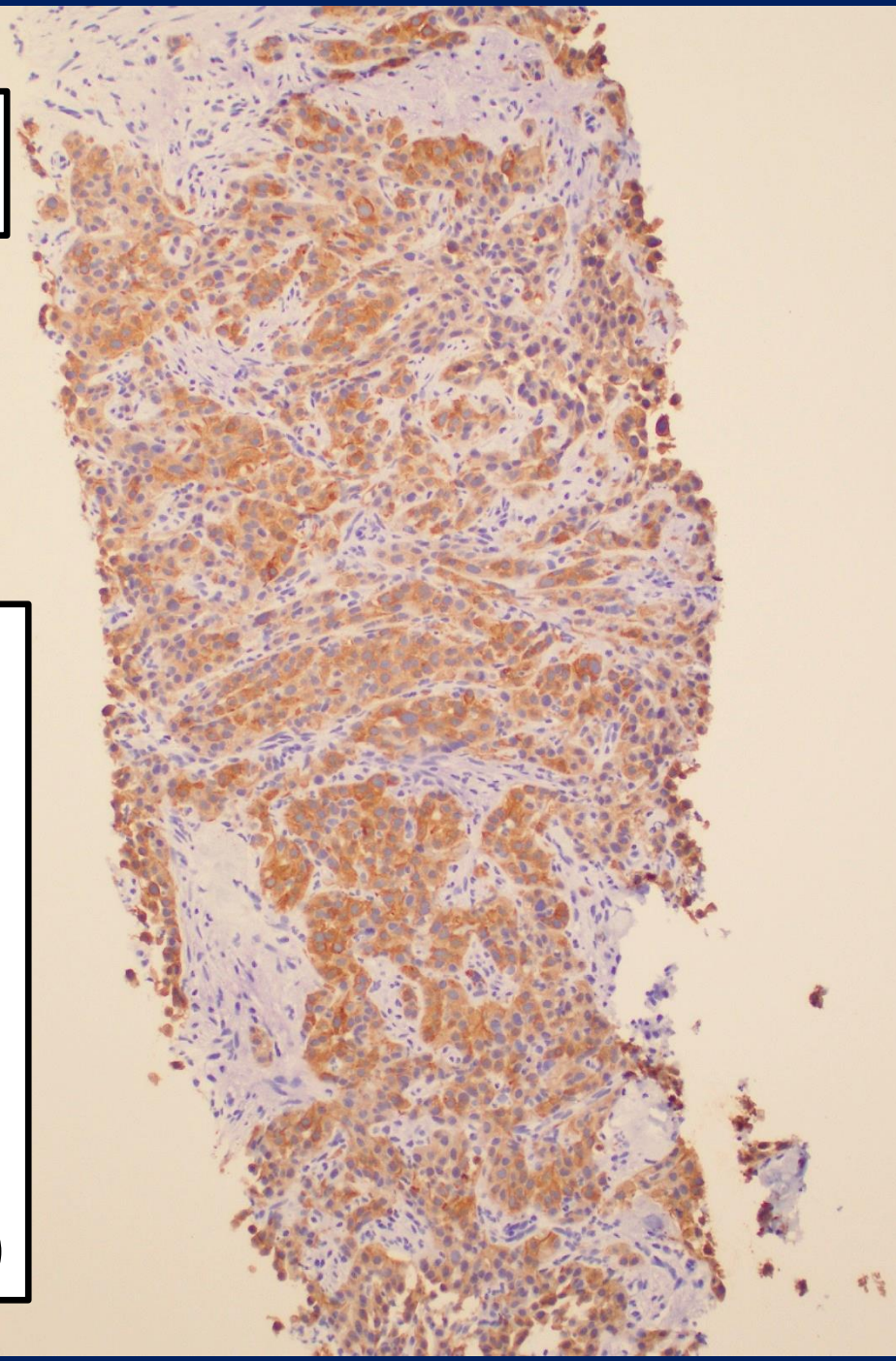
- 75 yo female
- Remote history of breast cancer
- Multiple lung nodudes, liver nodules, mediastinal/abdominal lymphadenopathy

Left Groin LN Core Needle Bx

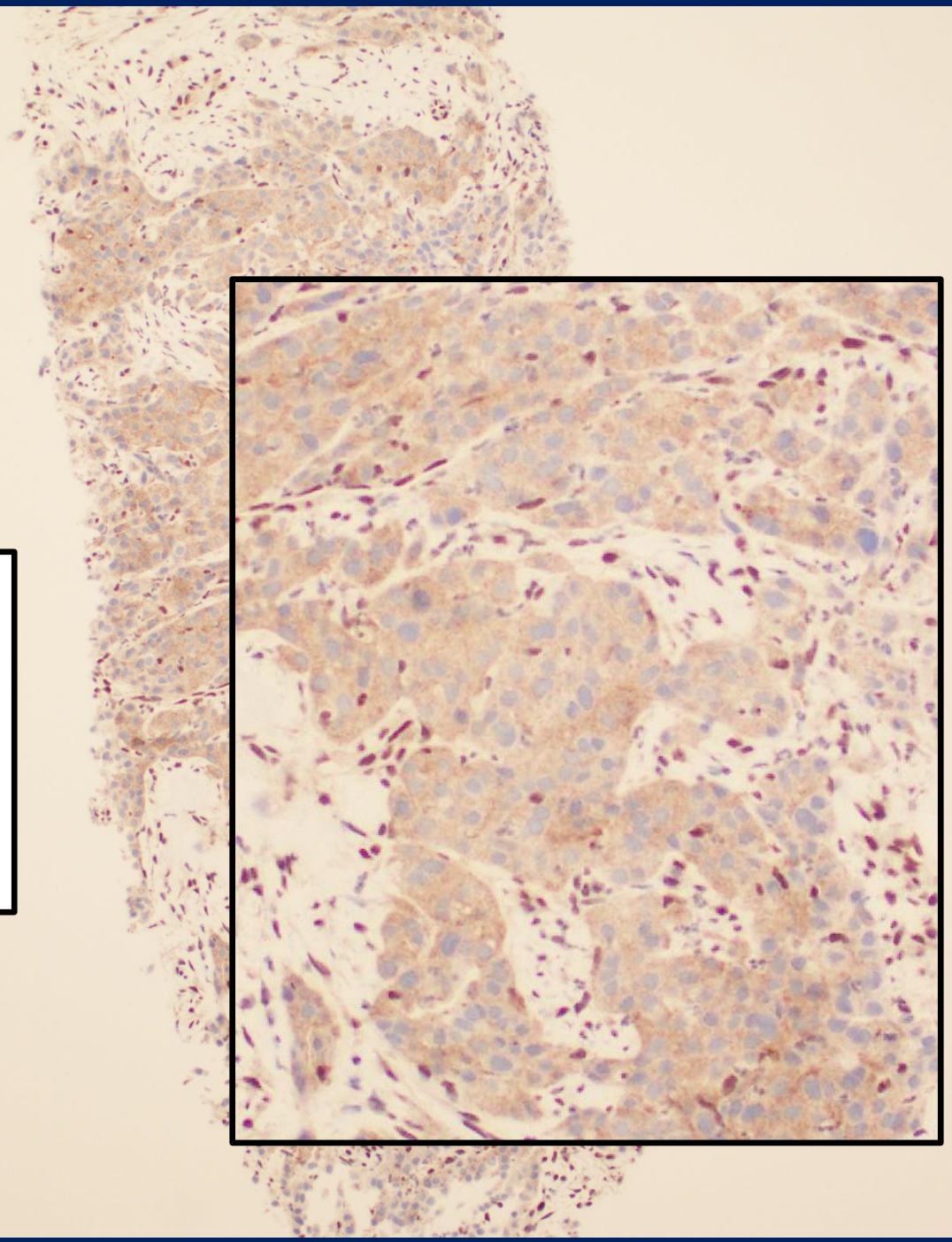
AE1/AE3

Negative for:
CK7
CK20

Negative for:
GATA3
p63
p40
PAX8
CDX2
ARG-1
GLYP-3
Hep-Par-1 (HSA)



BAP-1



Negative for:
calretinin

Positive for:
Ber-EP4 (faint)

Clinical Case

- CT abdomen included muptiple hepatic nodules
  - Hilar nodule
  - Periportal lymphadenopathy
- Noted combination of CK7 negative, CK20 negative and loss of BAP-1 and suggested intrahepatic cholagiocarcinoma as possible primary

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Utilization of Ancillary Studies in the Cytologic Diagnosis of Biliary and Pancreatic Lesions:

The Papanicolaou Society of Cytopathology Guidelines for Pancreatobiliary Cytology

Proposed Ancillary Tests for Solid Pancreatic Lesions

Marker	Purpose	Diagnostic finding	Utility
KRAS mutations	Identification of adenocarcinoma	Mutation present	Insufficient specificity for malignancy to warrant usage
SMAD4	Identification of adenocarcinoma	Mutation present [IHC shows loss of staining]	Supports the diagnosis of adenocarcinoma
FISH	Identification of adenocarcinoma	Presence of copy number abnormalities in CEP3, CEP7, CEP17 and abnormalities of band 9p21 favor malignancy	Most reliable test for confirming adenocarcinoma in conjunction with routine cytology
Mesothelin	Identification of malignancy	Overexpression of mesothelin by IHC	Supports the diagnosis of adenocarcinoma
Loss of heterozygosity	Identification of adenocarcinoma	Losses of chromosome arms 3p, 6Qp and 10pq along with gains of 5q, 12q, 18q, and 20q supports a diagnosis adenocarcinoma	Clinical importance to be determined
microRNAs	Identification of adenocarcinoma	Presence of miRNA including miR-21 and mi-155 supports a diagnosis of adenocarcinoma	Clinical utility to be determined

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Marker	Purpose	Diagnostic finding	Utility
Mucin (mucicarmine, alcian blue ph 2.5)	Identification of mucinous lesions	Positive stain	Diagnostically helpful
Cyst fluid amylase	Identification of pseudocysts and serous cystadenomas	Diagnosis of pseudocyst (level in 1000s, but not <250 U/L) and SCA (low levels, generally <1000 U/L); IPMNs have variable but elevated levels	Differential diagnosis of pancreatic cysts
Cyst fluid CEA	Identification of cystic mucinous lesions	CEA levels above 110 ng/mL support the diagnosis of a mucinous cyst	Distinction between mucinous and nonmucinous cysts
DNA analysis	Separation of benign from malignant cysts	Aneuploid and tetraploid results favor malignancy	Does not significantly improve diagnostic accuracy over routine cytology in majority of studies
KRAS mutations	Identification of mucinous cystic lesions	Presence of *KRAS* mutations supports diagnosis of a mucinous cyst	Distinguishes mucinous from nonmucinous cysts
CA 19-9	Separation of benign from malignant cysts	CA 19-9 level may be elevated in malignant cysts	Not generally useful in the diagnosis of pancreatic cysts
VHL gene mutation	Identification of SCA	Mutation present	Support the diagnosis of SCA
CTNNB1 (beta-catenin) mutation	Identification of SPN	Mutation present	Supports the diagnosis of SPN
GNAS mutation	Identification of IPMN	Mutation present	Supports the diagnosis of IPMN
RNF43 mutations	Identification of cystic mucinous lesions	Mutation present	Distinguishes mucinous from nonmucinous cysts

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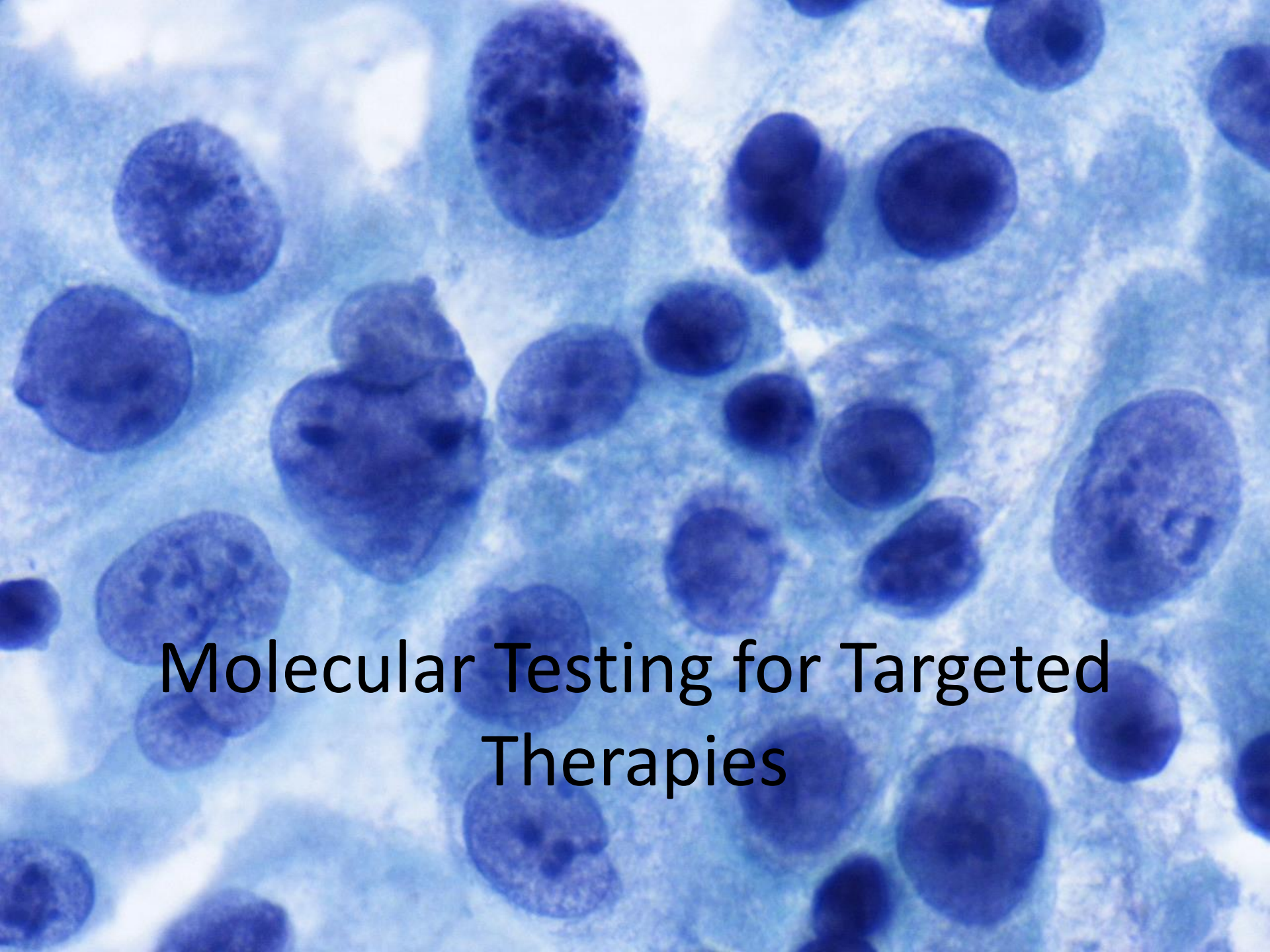
The Papanicolaou Society of Cytopathology Guidelines for Pancreatobiliary Cytology

Lester J. Layfield, M.D.[1,*], **Hormoz Ehya, M.D.**[2], **Armando C. Filie, M.D.**[3], **Ralph H. Hruban, M.D.**[4], **Nirag Jhala, M.D.**[5], **Loren Joseph, M.D.**[6], **Philippe Vielh, M.D., Ph.D.**[7], and **Martha B. Pitman, M.D.**[8]

Proposed Ancillary Testing for Pancreatobiliary Strictures

Marker	Purpose	Diagnostic finding	Utility
Digital image analysis	Separation of benign from malignant strictures	Aneuploid and tetraploid results support malignancy	Does not improve diagnostic accuracy above that achievable by cytology alone
KRAS	Separation of benign from malignant strictures	Mutation present	Insufficient specificity for malignancy to warrant usage
Sequential mutational analysis	Separation of benign from malignant strictures	Loss of heterozygosity	Diagnostic utility to be determined by future studies
FISH	Separation of benign from malignant strictures	Presence of copy number abnormalities in CEP3, CEP7, CEP17, and abnormalities of 9p21 favor malignancy	Diagnostically useful. It is the preferred test to complement routine cytology



Molecular Testing for Targeted Therapies

Targeted Treatment in Cholangiocarcinomas (CCAs)

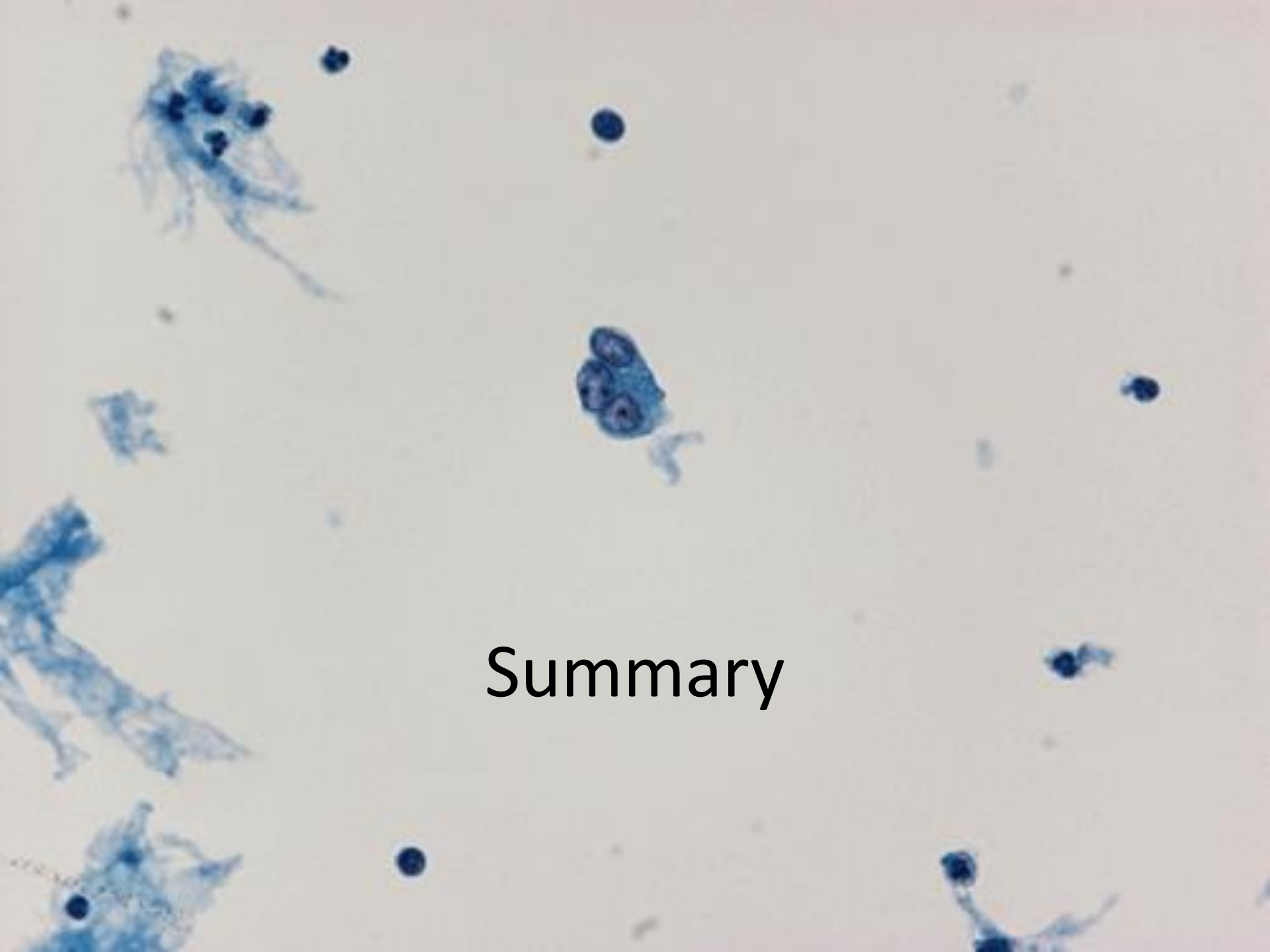
- Phase III trial for IDH1 mutant advanced CCAs showed increased PFS with ivosedinib
- Phase II clinical trials for FGFR2 rearranged CCAs showed promising results (ORR) with pemigatinib and infigratinib
- NTRK fusions in 0.75% of biliary tract tumors
  - Entrectinib
  - Larotrectinib

Targeted Treatment in Cholangiocarcinomas (CCAs)

- BRAF V600E mutations
  - Dabrafenib + trametinib useful in certain clinical scenarios
- HER2 amplifications in up to 18% of eCCAs
  - Possible poor prognosis
- MSI, dMMR and TMB-H CCAs
  - Approved for PD-1 inhibitor treatment
  - Pembrolizumab

Targeted Treatment in Pancreatic Ductal Adenocarcinomas (PDACs)

- NTRK1 (1.8%), NTRK2 (0.4%) and NTRK3 (1.1%) rearrangements are seen in ~3.3% of PDAC
  - Larotrectinib
  - Entrectinib
- MSI, dMMR and TMB-H CCAs
  - Approved for PD-1 inhibitor treatment
  - Pembrolizumab



Summary

Summary

- Pancreatic cystic and solid lesions have characteristic mutational profiles
- Mutational analysis of pancreatic cyst fluid and cytology specimens can help in
  - Resolving differential diagnosis
  - Help establish the presence of dysplasia or carcinoma
  - Especially true in cases of paucicellular specimens and indeterminate morphology
- Accumulation of specific mutation correlates with malignancy or malignancy potential
- CBD cytology specimen diagnosis can be aided by FISH for discovery of polysomy
- Several biliary tumors can show type-specific genetic alterations
- A subset of pancreaticobiliary malignancies have actionable genetic alterations
- Testing can be pretty basic if you know the biology

Thank you
Questions/Comments