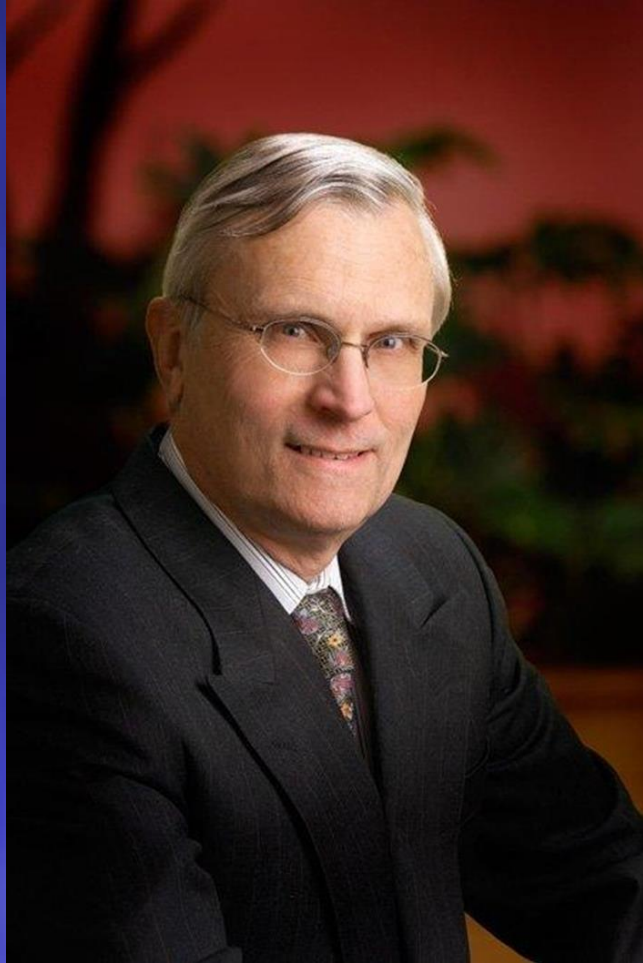


Lester Layfield, MD



- Full Professor at the University of Missouri
- Past President of the Papanicolaou Society of Cytopathology and currently at its Executive Board
- Editor in Chief of Diagnostic Cytopathology.
- Ward Burdick Award for Distinguished Service to Pathology from the ASCP.

Pancreaticobiliary Cytology: Morphology, FISH and NGS

Lester J Layfield, MD

Department of Pathology and Anatomical Sciences

The Clinical Challenge:

- Improve diagnostic sensitivity without reducing specificity
- Identify pre-cancer lesions

Molecular Studies are Valuable Ancillary Methods for Diagnosis of:

- Bile duct and Pancreatic duct brushings
- Pancreatic Cystic Lesions
- Solid Pancreatic Neoplasms

Cytologic Investigation of Bile and Pancreatic Duct Brushings has Good Specificity but a Poor Sensitivity

Sensitivity 44 to 67% (average around 50%)

Multiple Multi-variant Linear Regression Analyses have Identified cytologic Features of Potential Diagnostic Value Including:

- Four-fold or greater variation in Nuclear size
- Nuclear Molding
- Loss of Nuclear Polarity
- Loss of Honeycomb Pattern
- Chromatin Clumping
- Increased Nuclear/Cytoplasmic Ratio

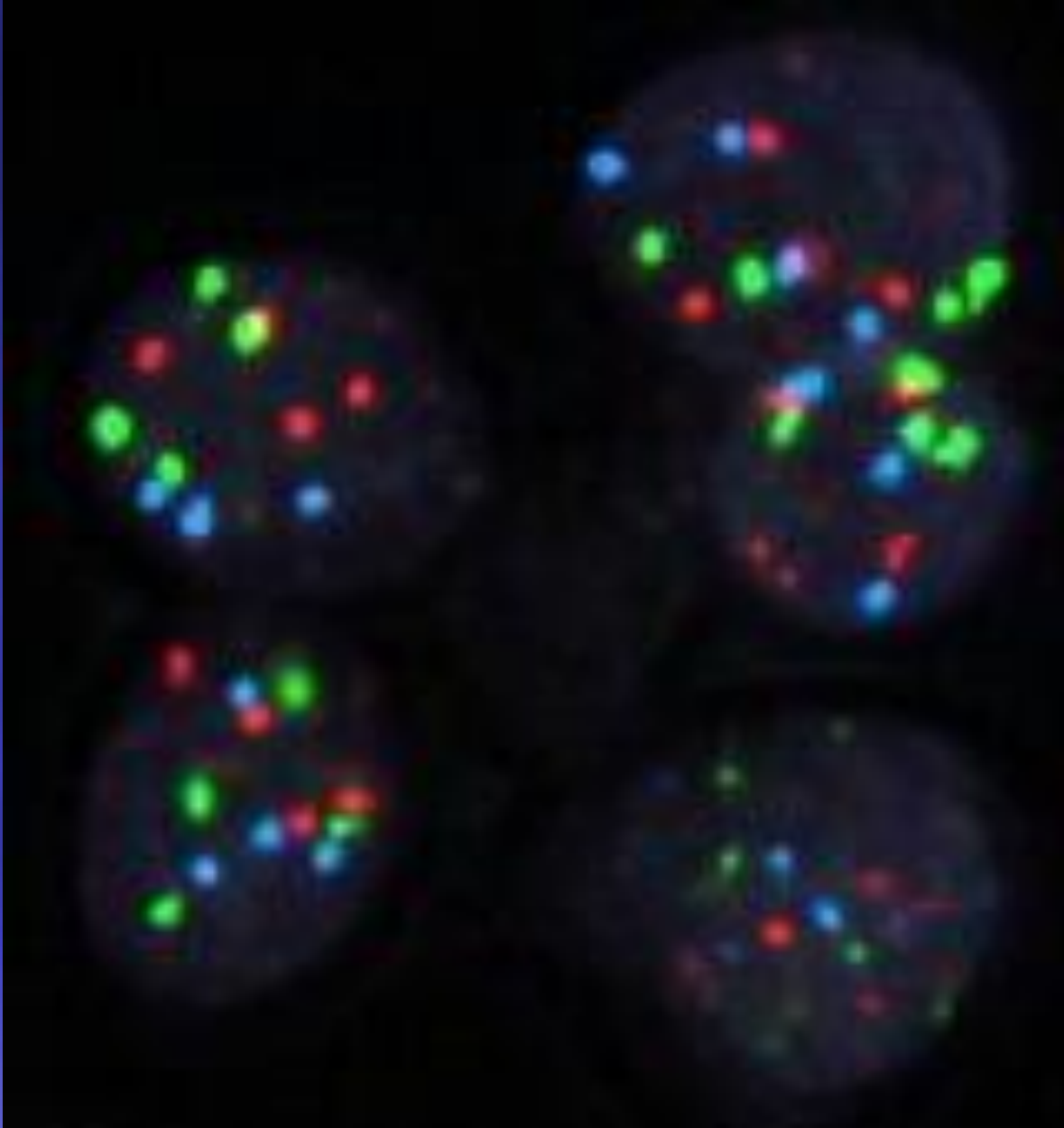
Despite these Criteria,
Sensitivity remains poor

Ancillary Techniques Applied:

- DNA Ploidy Analysis
- KRAS Mutational Analysis
- Fluorescence in-situ Hybridization
- Next Generation Sequencing

*Only the latter two show promise for improved sensitivity

FISH testing most commonly studies specimens for aneuploidy in pericentromeric bands of chromosomes 3 (CEP3), 7 (CEP7), and 17 (CEP17) and chromosomal band 9p21



FISH Testing improves sensitivity to approximately 60% using commercial kit* and up to 90% using alternate probes**

*Vlajnic T Somaini F, Savic S, et al. Cancer Cytopathology 2014; 122 (8): 627-634.

**Boldorini R, Paganotti A, Sartori Metal Pathology 2011; 43 (4): 335-339.

Next Generation Sequencing Performance Improvement?

Sensitivity of NGS for Malignancy has been reported to be as high as 85% when combined with cytology.

-Dudley JC, Zheng Z, McDonald T., et al. Cancer Cytopathology 2016; 122(24): 3838-3847

Issues with NGS Testing

- Expense
- Insufficient studies available to establish it's diagnostic value

Cystic Neoplasms of the Pancreas

Mucinous Cystic Lesions

- Intraductal Papillary Mucinous Neoplasm
- Cystic Mucinous Neoplasm

Serous Cystic Neoplasm

- Serous Cystadenoma

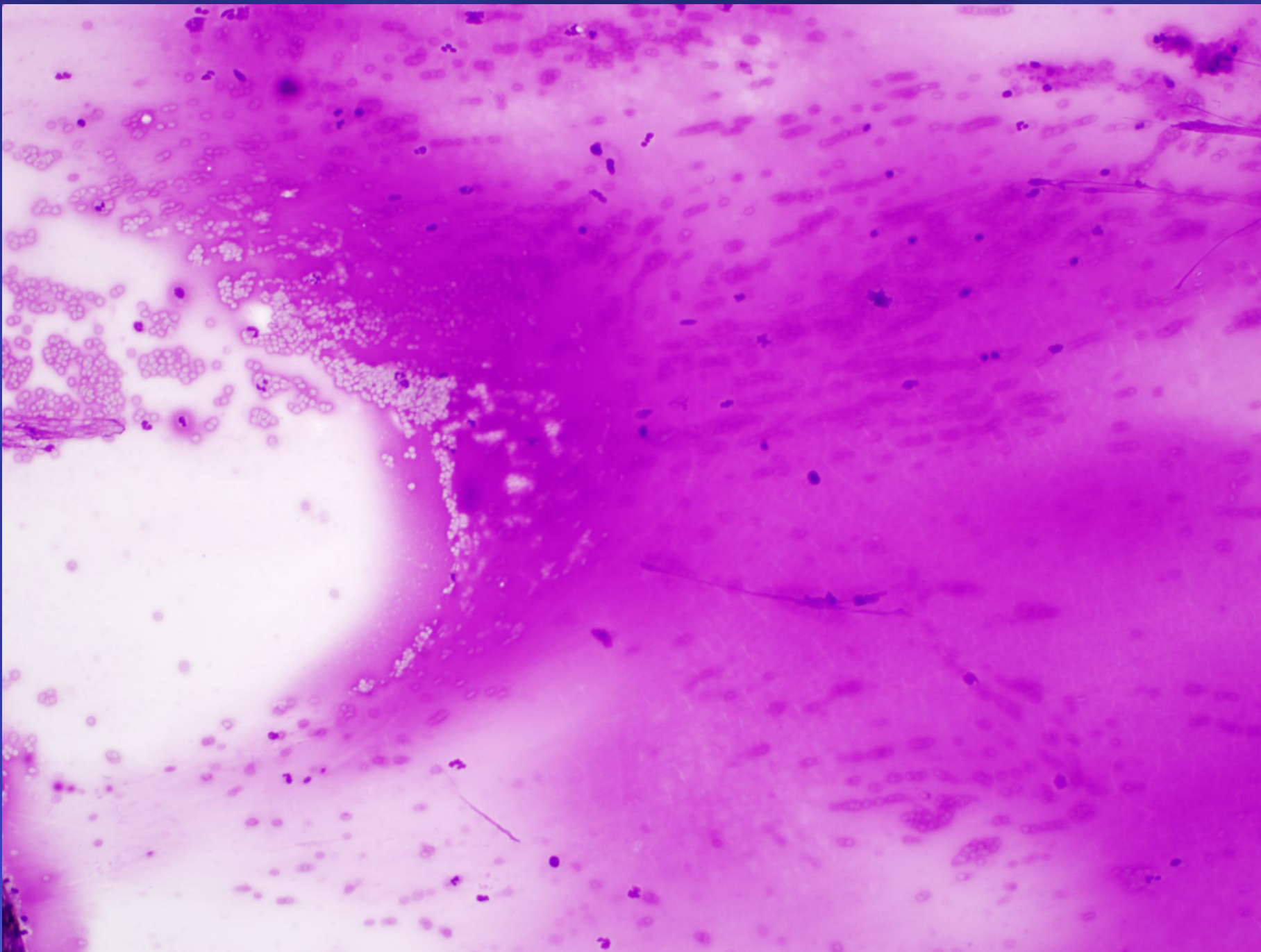
Other Neoplasm which are occasionally cystic

- Islet Cell Tumor
- Solid-Pseudopapillary Neoplasm
- Acinar Cell Carcinoma

Mucinous Cystic Lesions

Must separate from other cystic lesions:

- CEA above 192 ng/ml
- Often grossly mucinous sample



Mutations Confirming Mucinous Nature

KRAS – Both IPMN and Mucinous Cystic
Neoplasm

GNAS -IPMN

Markers predicting malignancy in Mucinous Cystic Lesions:

- SMAD4, CDKN2A, TP53

Next Generation Sequencing helpful in predicting behavior of Mucinous Cystic Lesions by testing for KRAS/GNAS, TP53, SMAD4 Family and CDKN2A

- Rosenbaum MW, Jones M, Dudley JE, et al. Cancer Cytopath 2017; 125: 41-47.
- Jones M, Zheng Z, Wang J, et al. Gastrointest Endosc. 2016; 83(1): 140-148

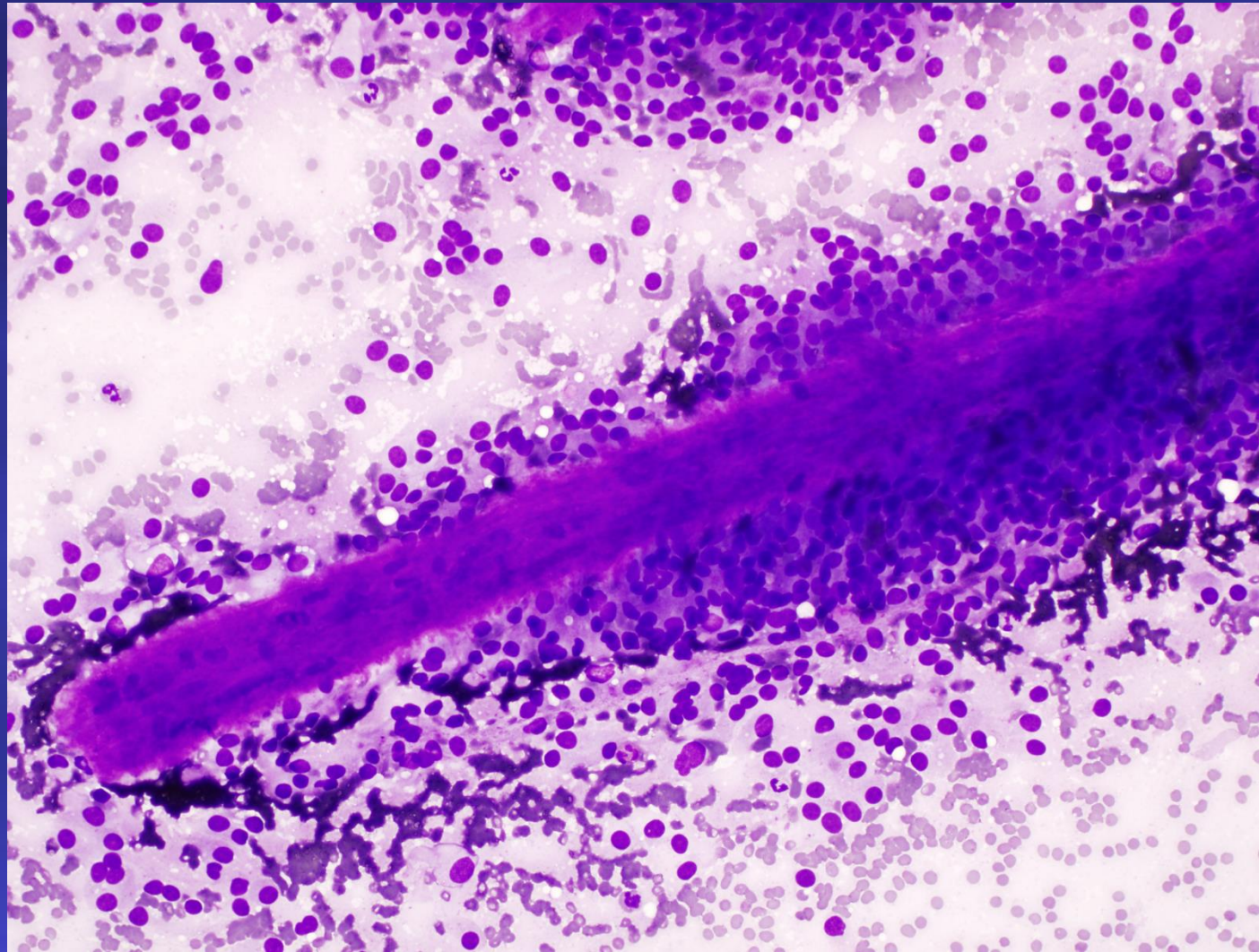
Specific Mutations Associated with Pancreatic Cysts

IPMN – KRAS and GNAS

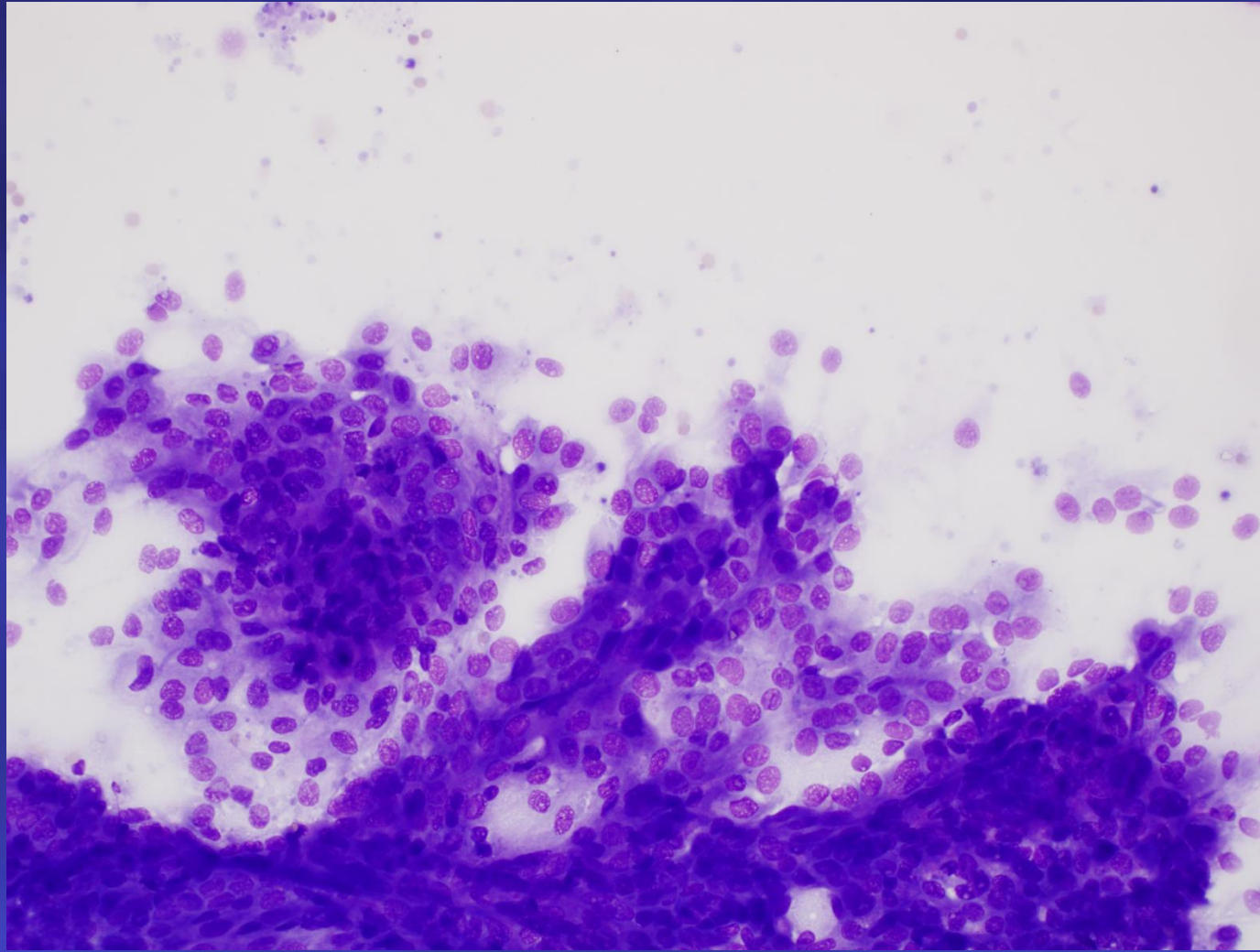
Mucinous Cystic Neoplasm – KRAS

Serous Cyst adenoma – 3p25 (VHL)

Solid and pseudopapillary Neoplasm – CTNNB1 (907)



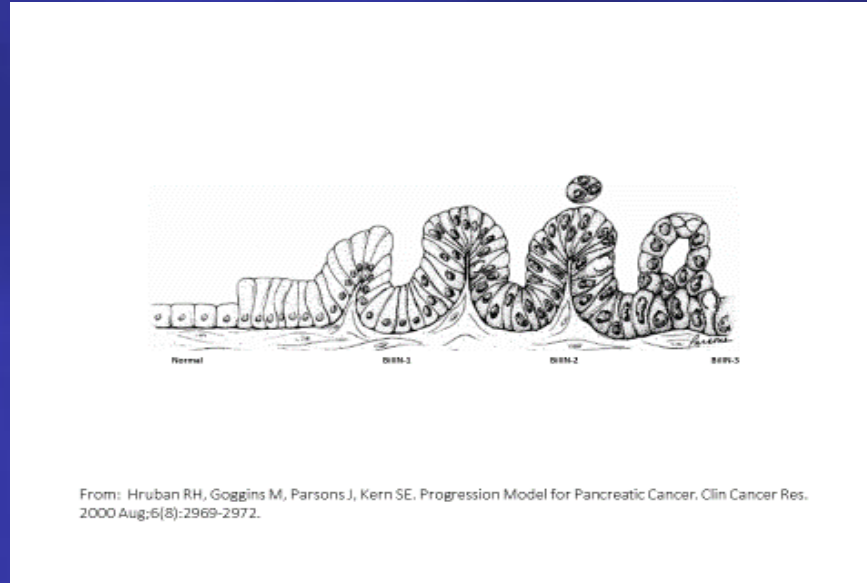
Presence of CTNNB1 mutation confirms diagnosis of
Solid and Pseudopapillary Neoplasm



Presence of both KRAS and GNAS mutations confirms
the presence of IPMN

Ancillary Testing of Cytologic Specimens of Pancreatic Ductal Carcinoma

Progression Model for Pancreatic Ductal Carcinoma



PanIN1a to Invasive ductal carcinoma

KRAS mutations are an early change while
PT53 and others are later changes

A variety of Mutations have been used for confirmation of a diagnosis of Pancreatic ductal carcinomas

- SMAD 4
- KRAS
- TP53
- Wnt/B-catenin

SMAD4 mutations can be studied by immunohistochemistry and molecular diagnostics

- Immunohistochemical SMAD4 loss is associated with pancreatic adenocarcinoma and is associated with metastases and higher stage
- SMAD4 inactivated in 50%/pancreatic carcinomas

The liquid biopsy in diagnosis and surveillance

- NGS testing of pancreatic juice may help assess malignancy risk*
- Pancreatic juice may help assess malignancy progression risk in IPMN's

*Mateos RN, Nakagawa H, Hirono S, et al. Cancer Med. 2019;

*Okada T, Iwano H, Karasuki H, et al. Endosc Int Open 2018; 6(12).

Genes which may be important for prediction risk of pancreatic cancer may include:

- APC
- ATM
- BRAC1 and 2
- CDKN2A
- CFTR
- CHEK2
- MLH1
- MSH2
- NBN
- PALB2
- PALLD
- PRSS1
- SPZNK1
- TP53

These genes can be assessed by NGS testing of pancreatic juice. These genes may be useful in surveillance.

Important Questions for Surveillance by NGS Testing of Pancreatic Juice:

- Who should be tested?
- How often should an at risk patient be tested?
- If a patient tests positive for a “driver” gene how do we intervene?
 - increased frequency of imaging
 - Cytologic study of brushing specimens
 - FNA of any suspicious area
 - Prophylactic surgery