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Use of Molecular Analysis and Immunohistochemistry in the Diagnosis of Hepatocellular and Pancreaticobiliary Tumors

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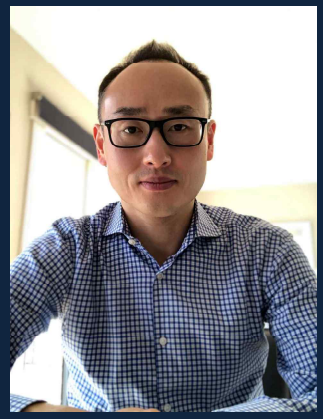

Education:
- UCLA (BS)
- University of Illinois (MD/PhD)
- Residency: University of Washington (AP/CP)
- Fellowship: UCSF (GI/liver)

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Subspecialty: GI/liver pathology

Expertise: Dysplasia, polyps, liver pathology

Outline

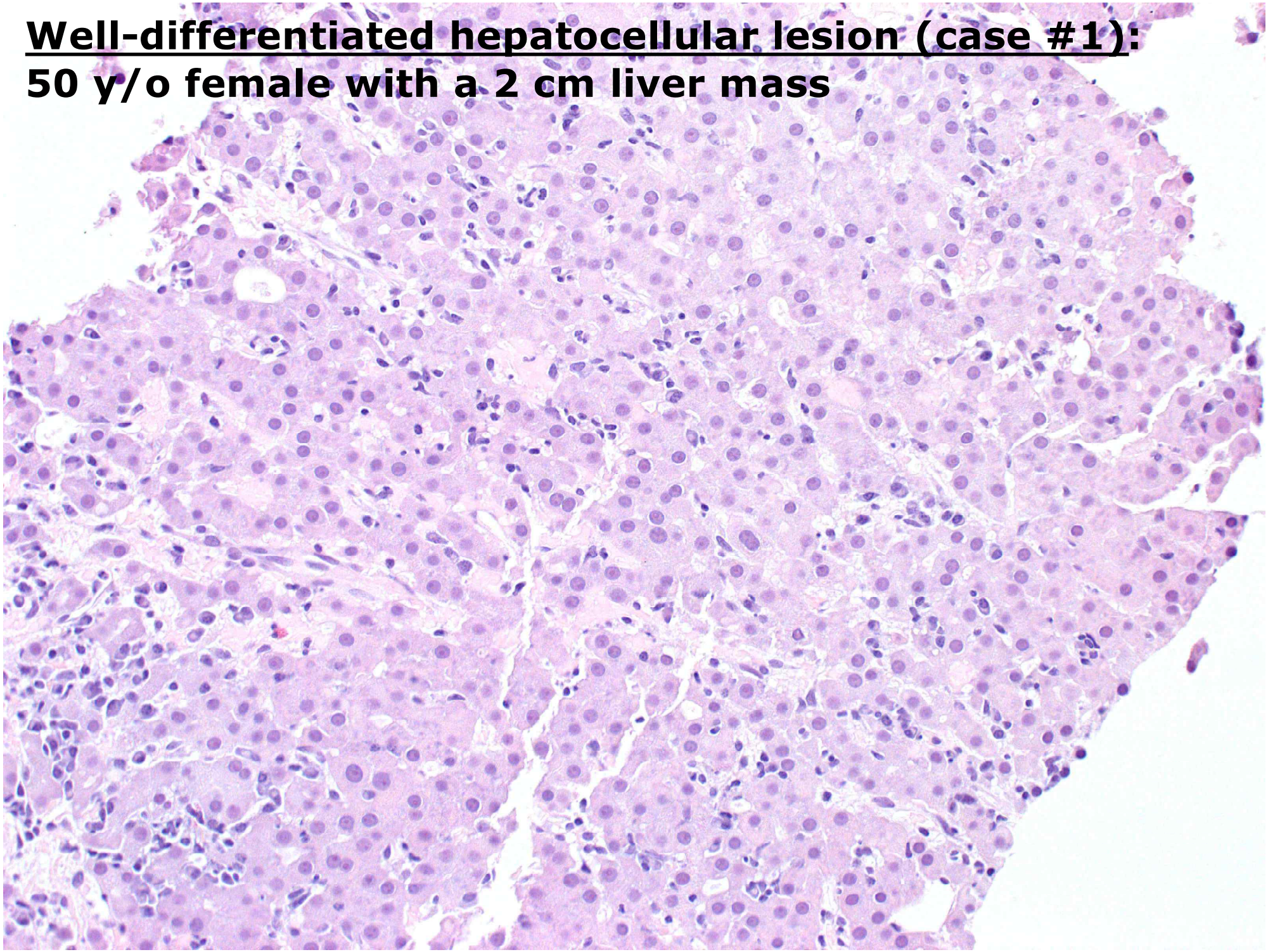
1. Well-differentiated hepatocellular lesion

2. Poorly-differentiated tumor in the liver

3. Pancreatic neuroendocrine neoplasm

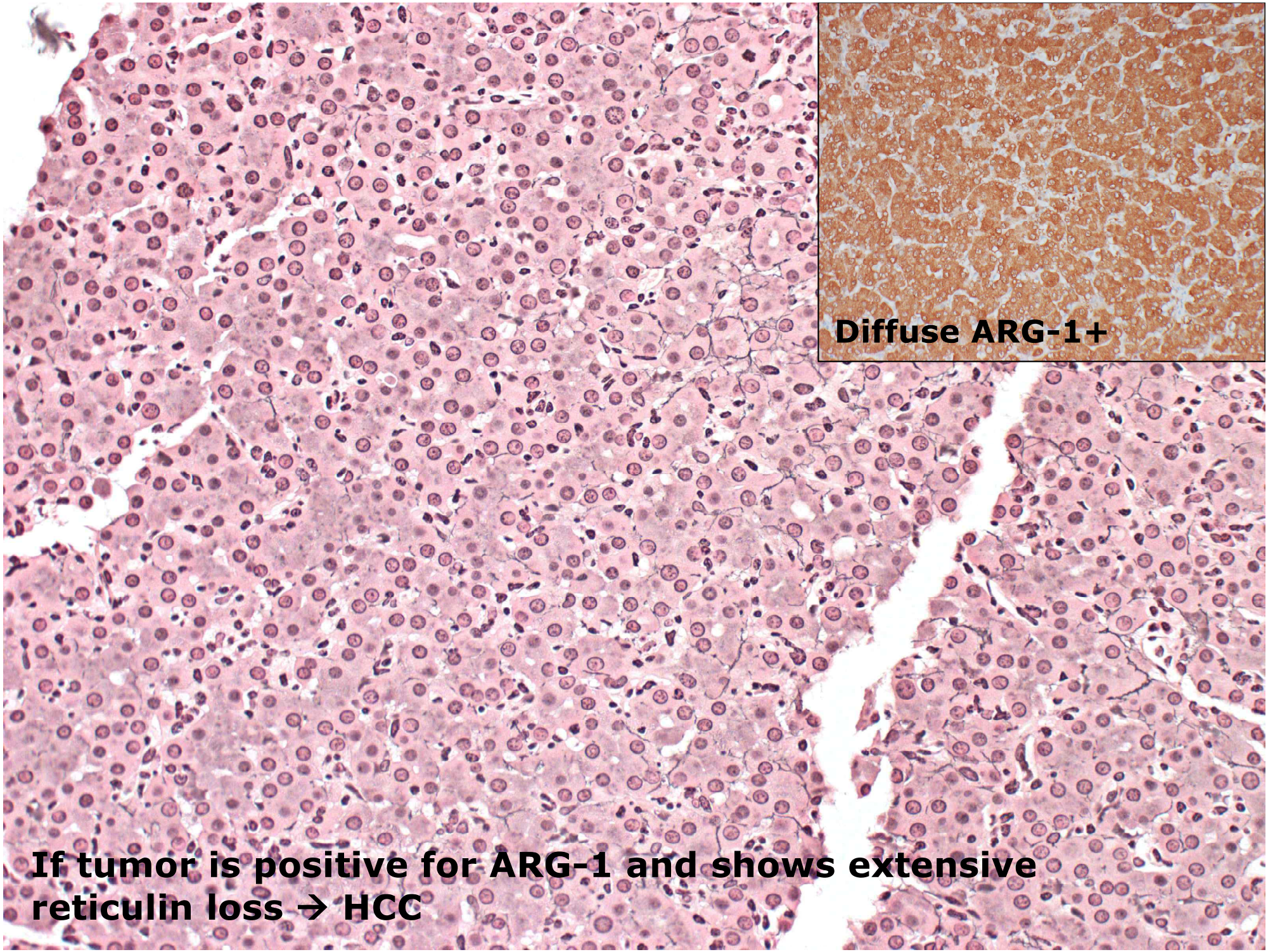
    - Differential diagnosis, including molecular features
    - IHC workup
    - Role of molecular testing

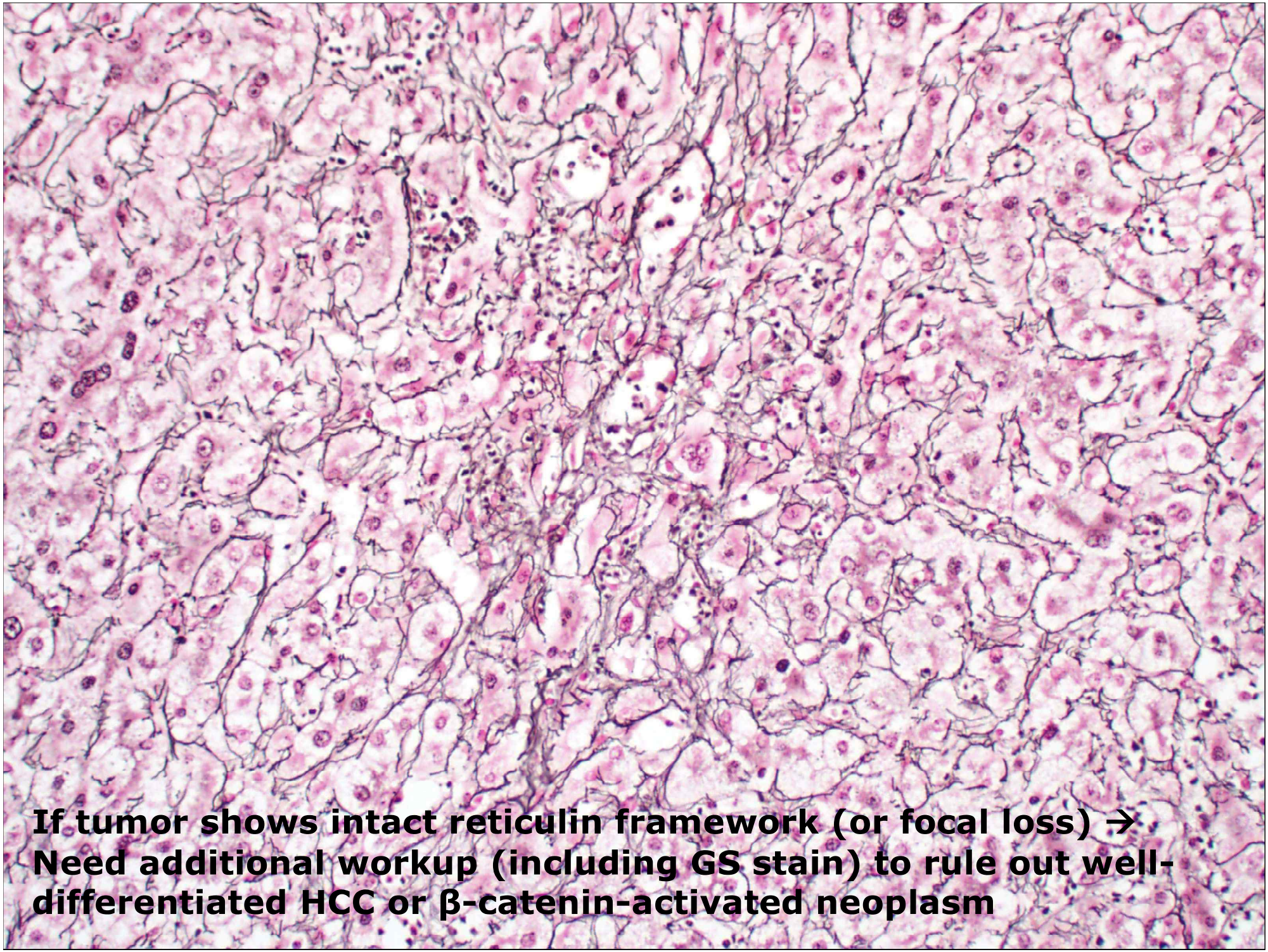
Well-differentiated hepatocellular lesion (case #1):
50 y/o female with a 2 cm liver mass



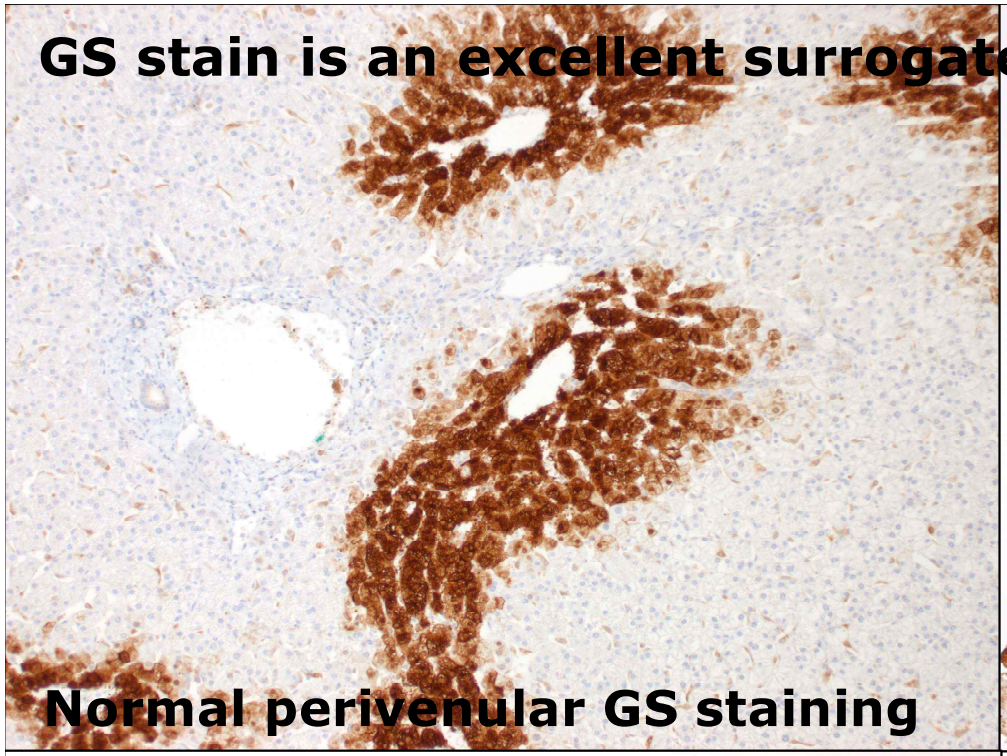
- Differential diagnosis of a well-differentiated hepatocellular lesion:
  - Hepatocellular carcinoma (HCC)
  - Focal nodular hyperplasia (FNH)
  - Hepatocellular adenoma (HCA)
    - Inflammatory HCA
    - Hepatocyte nuclear factor (*HNF*) *1-α*-inactivated HCA
    - β-catenin-activated HCA or neoplasm (high-risk for HCC)
    - Unclassified HCA (no *HNF-1α* or *CTNNB1* mutation)

- Initial IHC workup: A panel of 4 stains, including arginase-1 (ARG-1), glutamine synthetase (GS), serum amyloid acid (SAA), and reticulin

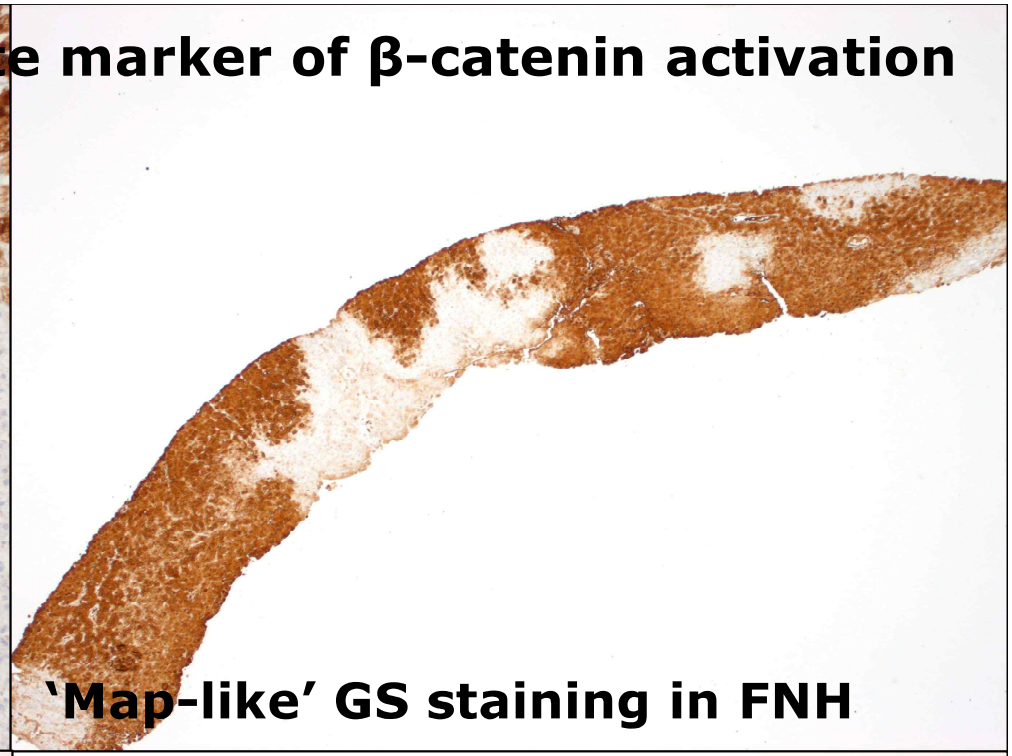

Diffuse ARG-1+
If tumor is positive for ARG-1 and shows extensive reticulin loss → HCC


If tumor shows intact reticulin framework (or focal loss) →
Need additional workup (including GS stain) to rule out well-differentiated HCC or β-catenin-activated neoplasm

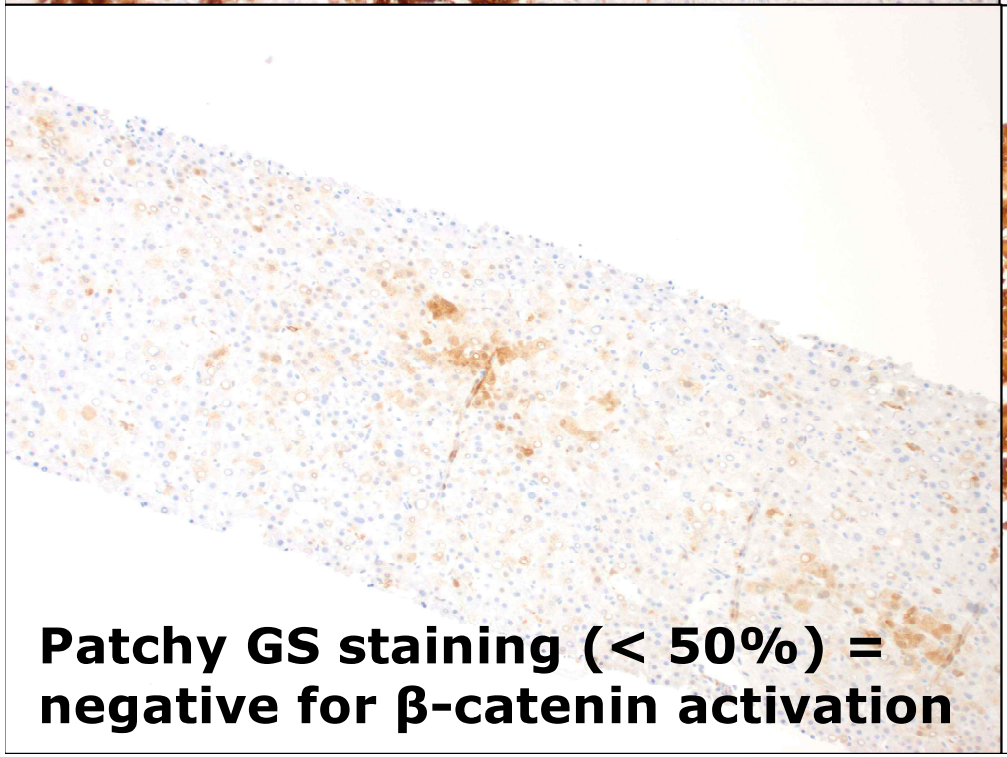
GS stain is an excellent surrogate marker of β-catenin activation



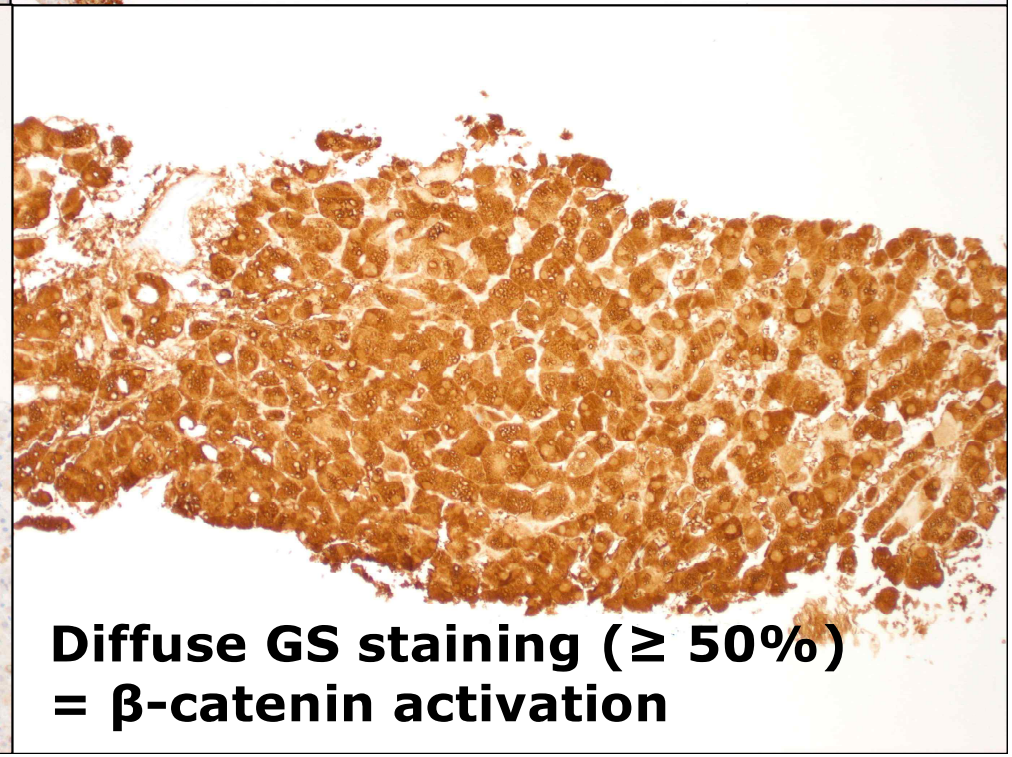
Normal perivenular GS staining



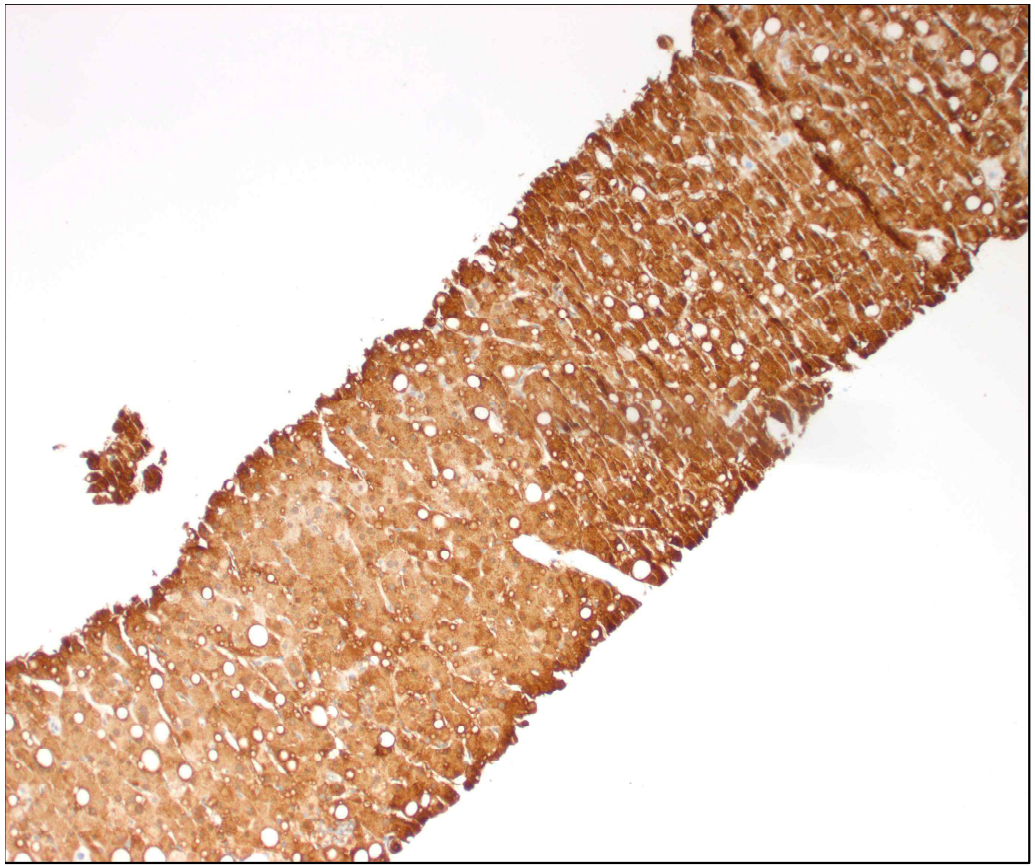
'Map-like' GS staining in FNH



Patchy GS staining (< 50%) = negative for β-catenin activation

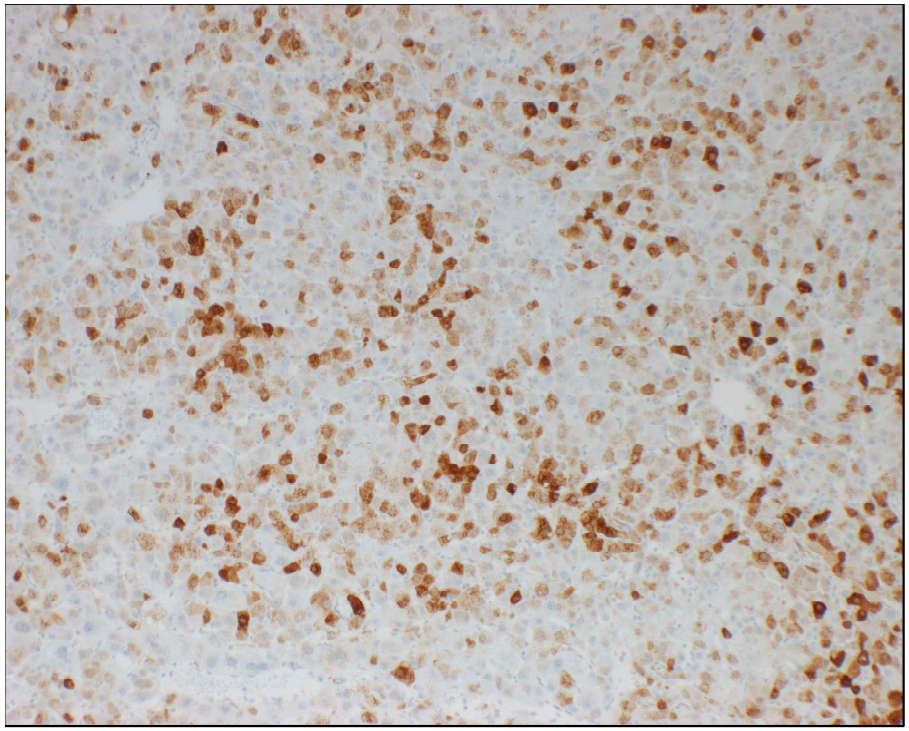


Diffuse GS staining (≥ 50%) = β-catenin activation



Diffuse "homogeneous" GS staining (moderate to strong cytoplasmic staining in ≥ 90% of lesional cells)

- Strongly correlates with high level of β-catenin activation.
- Often due to large in-frame exon 3 deletions of *CTNNB1* gene or point mutations in the β-TrCP-binding domain (D32-S37) that are crucial for β-catenin degradation.
- High-risk feature for concurrent or subsequent HCC.

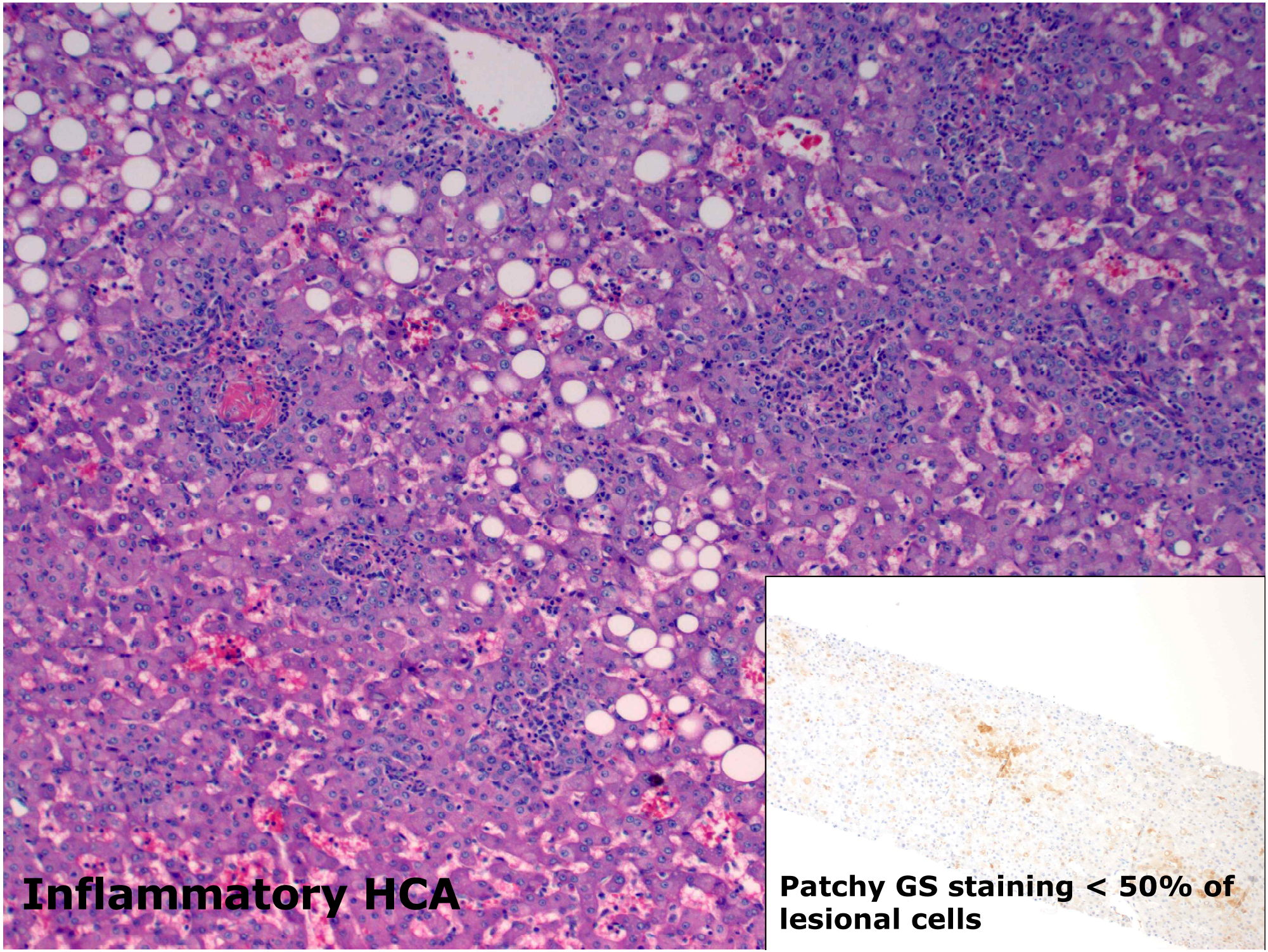


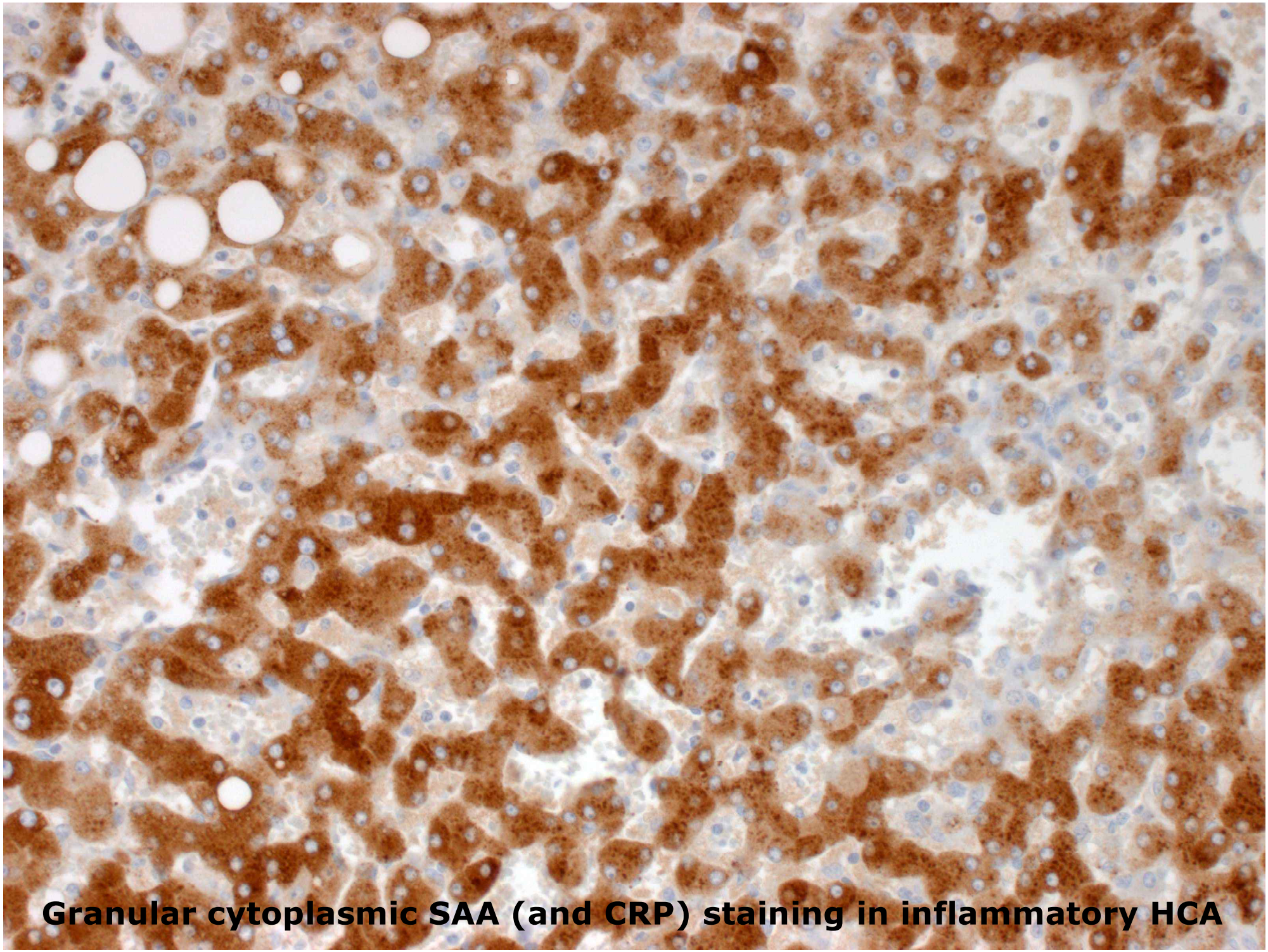
Diffuse "heterogeneous" GS staining (moderate to strong cytoplasmic staining in ≥ 50% but <90% of lesional cells)

- Less strong correlation with β-catenin activation, often related to *CTNNB1* exon 3 point mutations at serine/threonine sites (S45 and T41) or mutations in other Wnt signaling pathway genes (*APC, AXIN1, and AXIN2*).
- Rarely, it is associated with *CTTNB1* exon 7 or 8 mutations, which typically lead to patchy GS staining (< 50% of lesional cells) and weak β-catenin activation (considered no/low risk of HCC).
- Important to confirm the status of β-catenin activation by molecular testing (i.e., NGS) in this setting.

β-catenin-activated HCA/neoplasm

- Comprises 10% of HCA, often occurs in male patients (~40%).
- Most often due to activating mutation/deletion of *CTNNB1* gene, most commonly in exon 3 at serine/threonine sites (S45 and T41) or neighboring amino acids (D32-S37).
- Association with concurrent or subsequent HCC in up to 70%.
- It has been argued that β-catenin-activated neoplasm should be called "atypical hepatocellular neoplasm" rather than HCA (unless mutations are found in exon 7 or 8).


Inflammatory HCA
Patchy GS staining < 50% of lesional cells



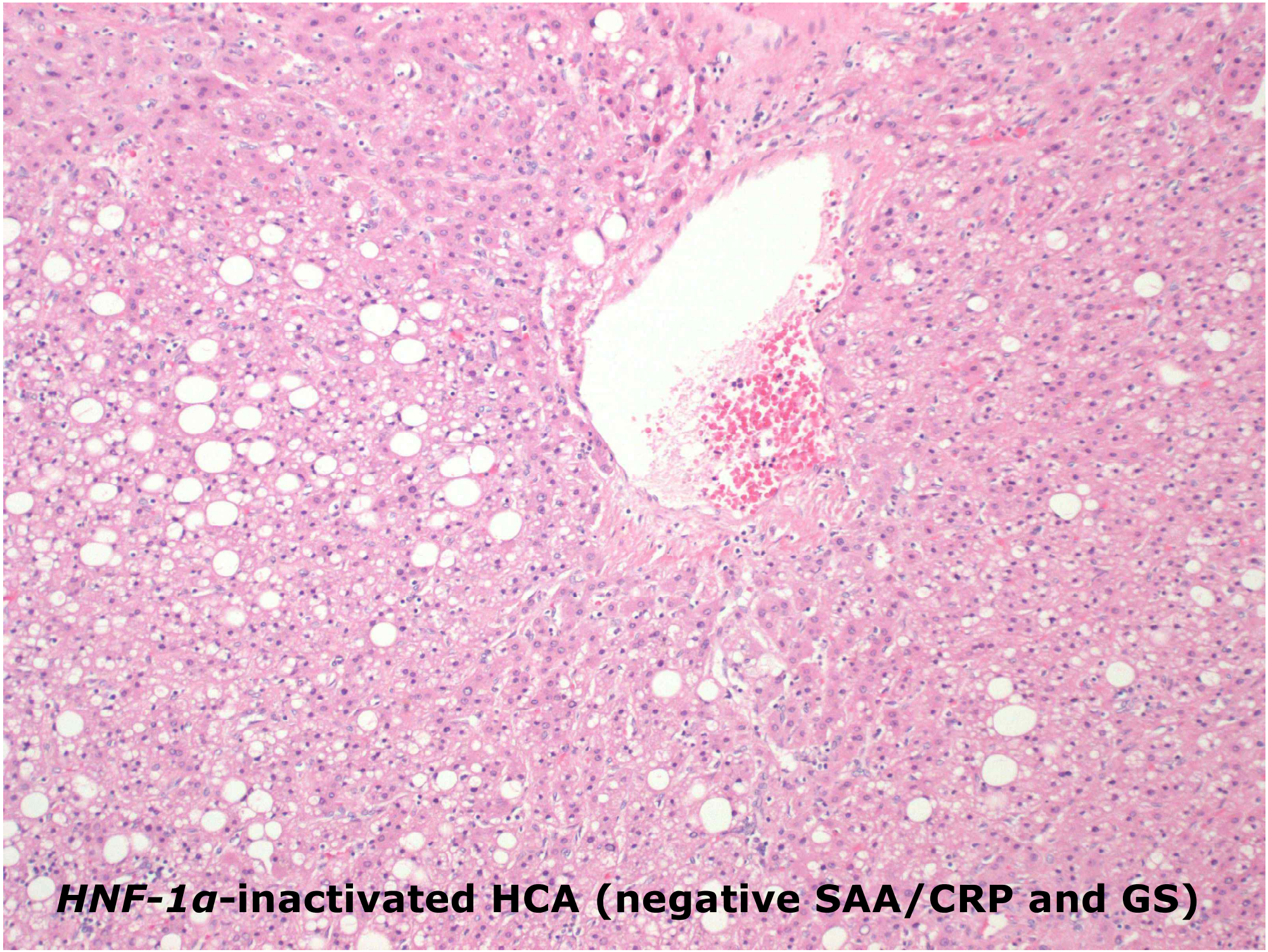
Granular cytoplasmic SAA (and CRP) staining in inflammatory HCA

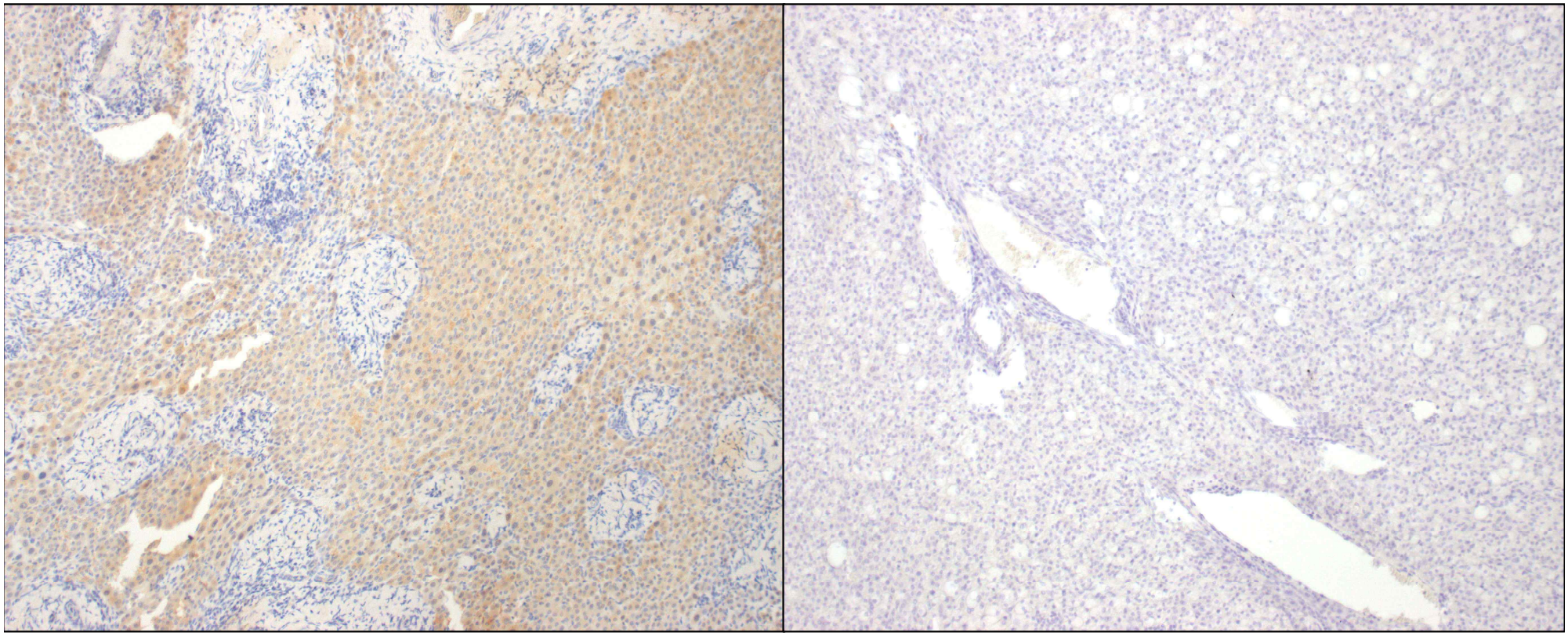
Inflammatory HCA (I-HCA)

- Accounts for 35-40% of HCA, more common in female patients (90%).

- Characterized by recurrent somatic mutations that activate interleukin (IL)-6 signaling pathway, most commonly IL-6 signal transducer gene (*IL6ST)* that encodes the signaling co-receptor gp130 (60%).

- Low risk for HCC, but activation of β-catenin (with diffuse GS staining) occurs in 10-15%, often due to exon 3 *CTNNB1* mutations (referred to as I-HCA with β-catenin activation) → Risk for HCC in that setting is similar to those with β-catenin activation but without inflammatory features.


HNF-1α-inactivated HCA (negative SAA/CRP and GS)



Positive cytoplasmic LFABP staining in normal liver

Loss of LFABP staining in *HNF-1α*-inactivated HCA

HNF 1-α-inactivated HCA (H-HCA)

- Comprises 30-35% of HCA, more common in female patients (90%)
- Characterized by biallelic inactivating mutations of *HNF-1α* gene.
- *HNF-1α* mutation leads to negative regulation of *FABP1* gene, which codes for liver fatty acid binding protein (LFABP).
- Low risk for HCC.

Immunohistochemistry Results	Interpretation
GS: map-like SAA: negative or focally positive	FNH
GS: patchy (not diffuse or map-like) SAA: positive	I-HCA, can obtain CRP if SAA positivity is focal
GS: patchy (not diffuse or map-like) SAA: negative	CRP + → I-HCA LFABP loss → H-HCA
GS: diffuse SAA: negative/positive	β-catenin-activated neoplasm → need additional workup to rule out HCC

Choi WT et al. Adv Anat Pathol. 2018;25(4):254-262.

Situations when molecular testing should be considered

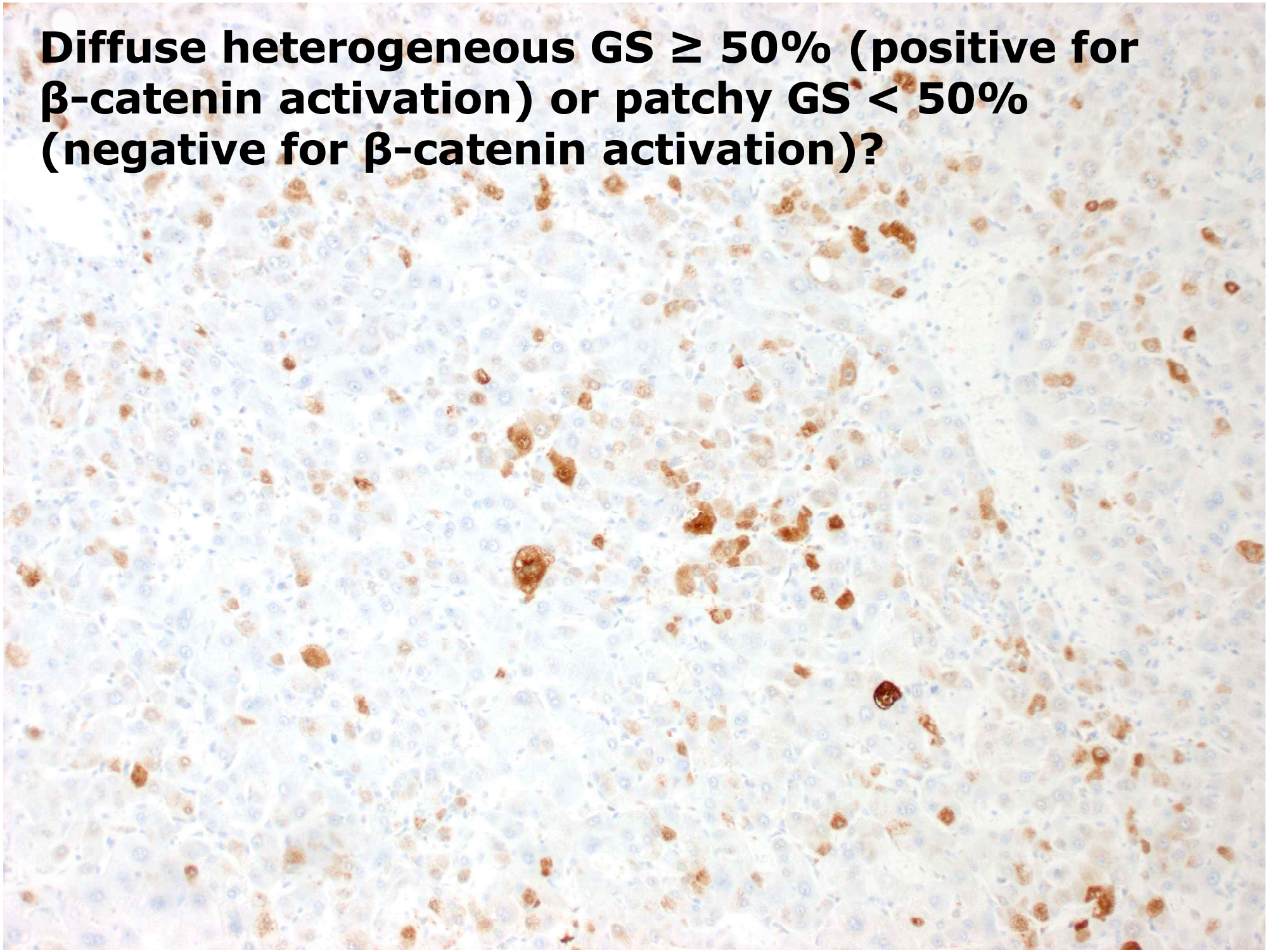
- Indeterminate GS staining (i.e., the status of β-catenin activation cannot be reliably determined based on IHC)

- "Atypical hepatocellular neoplasm (AHN)"

  - Morphologic features of HCA but with β-catenin activation (by diffuse GS staining)

  - Borderline morphologic features with or without β-catenin activation

Diffuse heterogeneous GS ≥ 50% (positive for β-catenin activation) or patchy GS < 50% (negative for β-catenin activation)?



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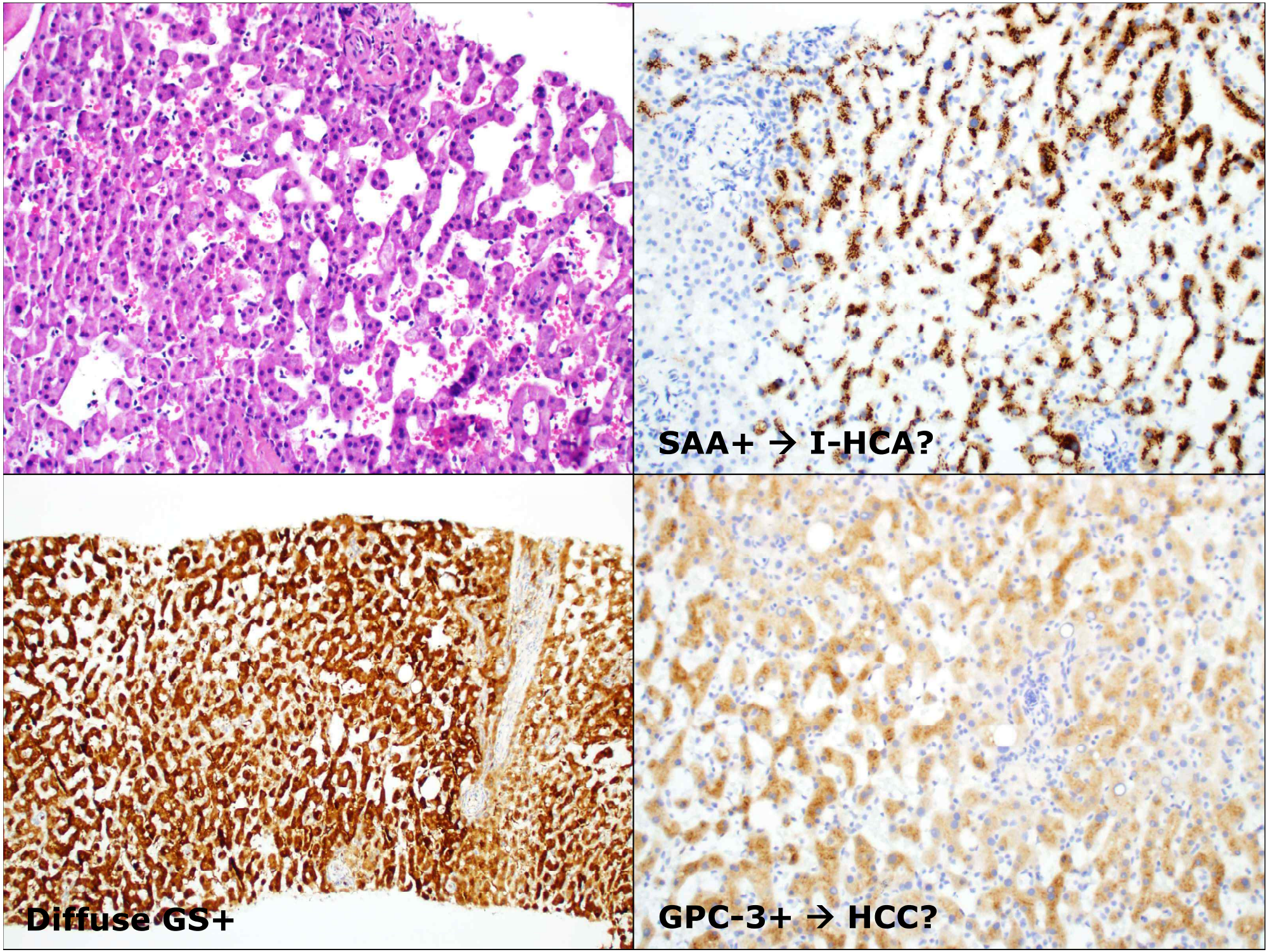
SAA+ → I-HCA?
Diffuse GS+
GPC-3+ → HCC?

Situations when molecular testing should be considered

- Indeterminate GS staining (i.e., the status of β-catenin activation cannot be reliably determined based on IHC)
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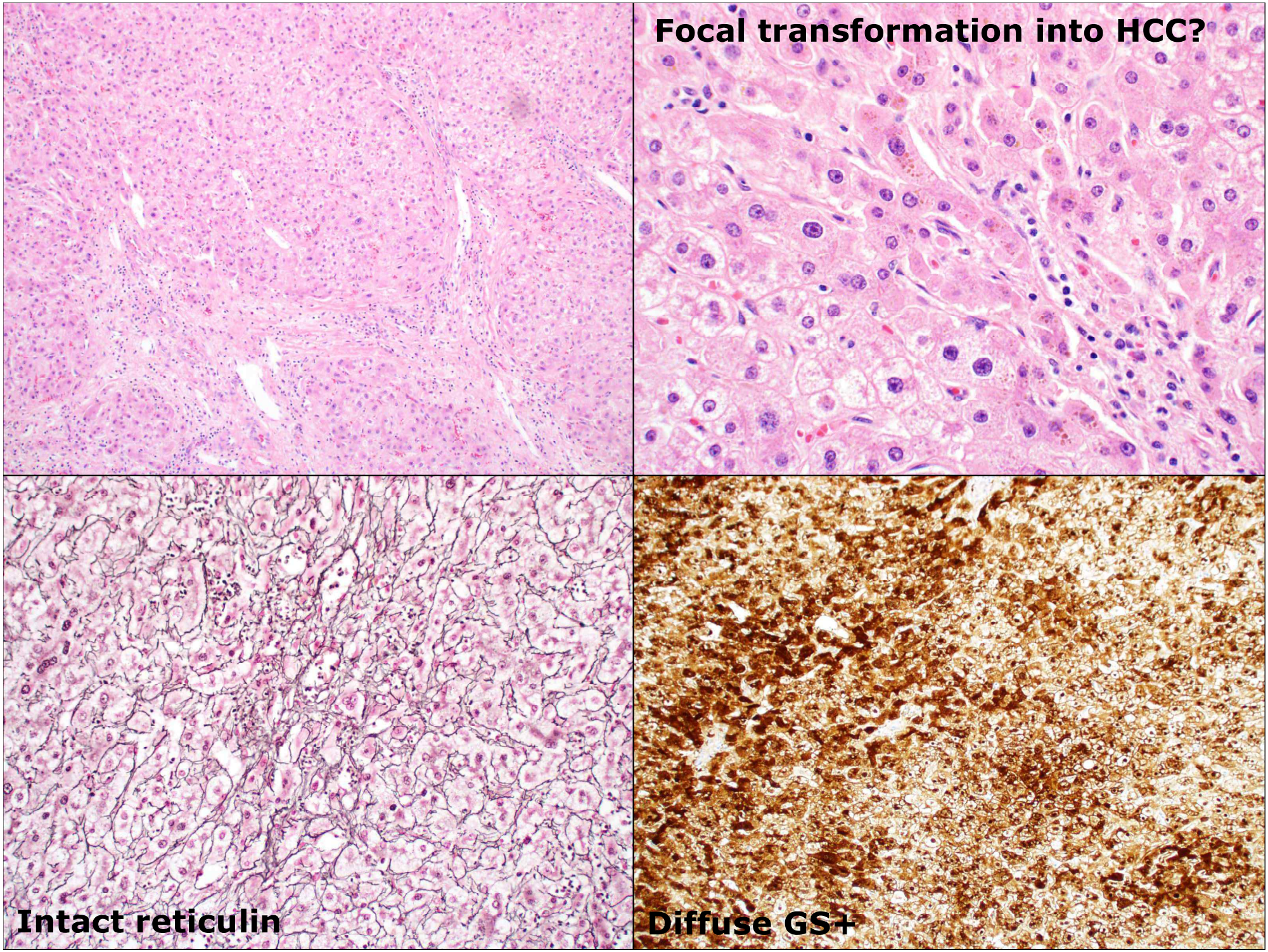
  - Morphologic features of HCA but with β-catenin activation (by diffuse GS staining)
  - Borderline morphologic features with or without β-catenin activation


Focal transformation into HCC?
Intact reticulin
Diffuse GS+

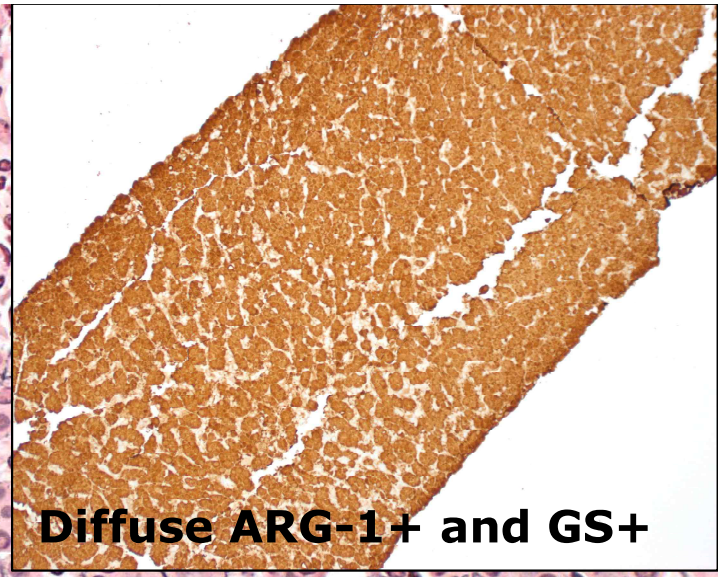
Role of molecular testing in identifying high-risk hepatocellular neoplasms

- *TERT* promoter mutations (characteristic feature of HCC [50-60%]) → AHN/HCC
- FISH to look for characteristic chromosomal gains (including 1q, 7q, and 8q) found in HCC → AHN/HCC
- *CTNNB1* exon 3 mutational analysis → If present, AHN, but treat like HCC
- Mutational analysis in other Wnt signaling pathway components (*APC*, *AXIN1*, and *AXIN2*) → If present, AHN, but treat like HCC

Practical Considerations

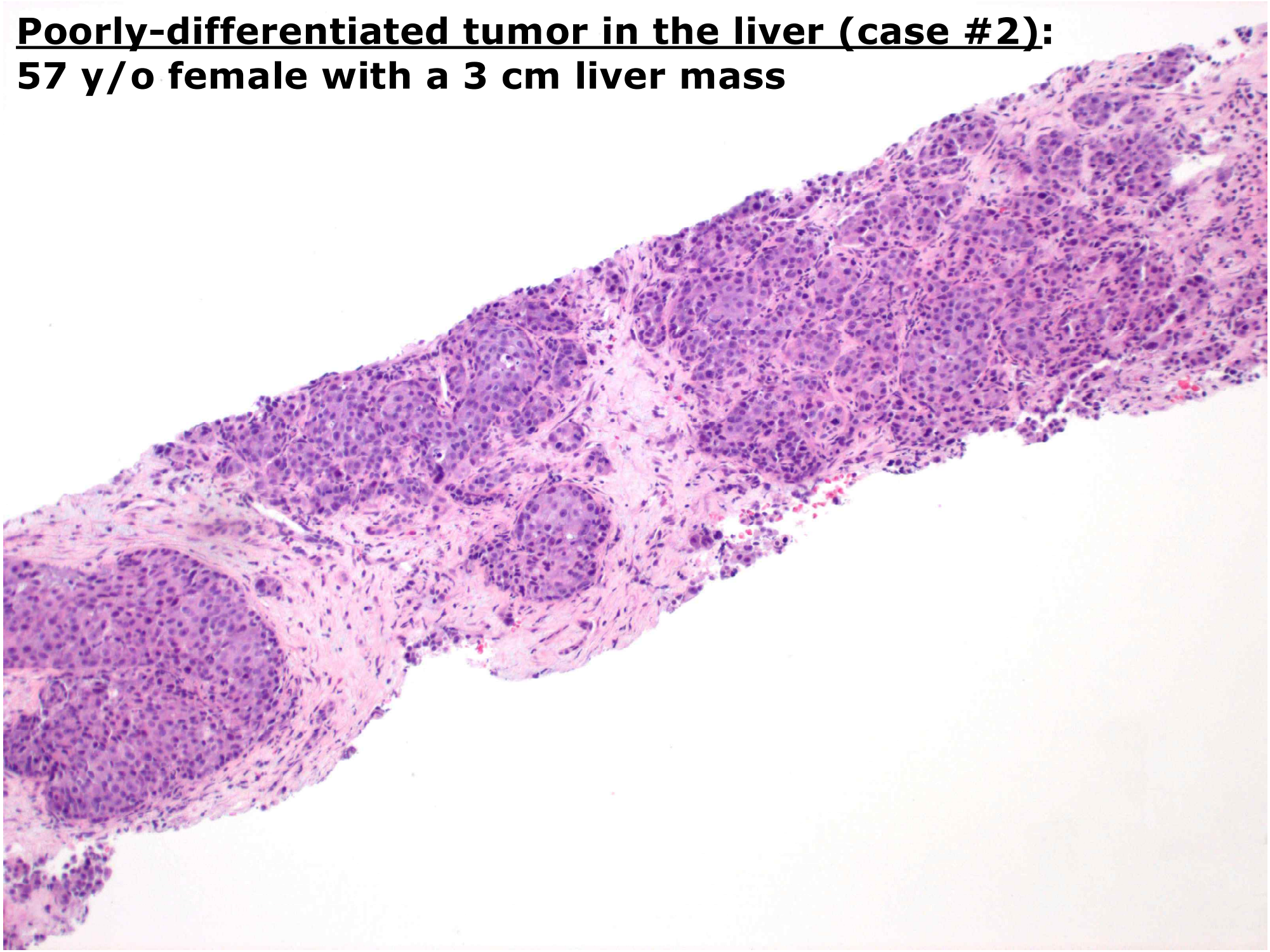
- For large tumors (> 5 cm), resection is recommended due to risk for hemorrhage (20-25%) and/or HCC.
- For small tumors (< 5 cm), a detailed workup (with IHC and/or molecular testing) is necessary to confirm the status of β-catenin activation or to rule out HCC.

Back to Case #1:



Reticulin loss → HCC

<u>Poorly-differentiated tumor in the liver (case #2)</u>:
57 y/o female with a 3 cm liver mass



- Differential diagnosis of poorly-differentiated tumor in the liver:
  - HCC
  - Cholangiocarcinoma (CC)
  - Combined HCC-CC
  - Metastatic tumor

- Initial IHC workup:
  - A panel of 4 stains, including two hepatocellular markers (Arg-1 and GPC-3) and two adenocarcinoma markers (CK19 and MOC-31).

If limited tissue is available, a two-stain approach using Arg-1 and CK19 is recommended for initial evaluation.

- Group 1: Arg-1 positive, CK19 negative
- Group 2: Arg-1 negative, CK19 positive
- Group 3: Arg-1 positive, CK19 positive
- Group 4: Arg-1 negative, CK19 negative

Choi WT et al. Hum Pathol. 2017;63:1-13
Choi WT et al. Gastroenterol Clin N Am. 2017;46(2):311-325.

Group 1: Arg-1 positive, CK19 negative

- In most cases, this pattern establishes the diagnosis of HCC.

- If morphologic features are not typical, staining patterns are weak or focal, or the clinical and imaging data are discordant, additional hepatocellular markers (Hep Par-1 and GPC-3) may be necessary to confirm the diagnosis of HCC.

Choi WT et al. Hum Pathol. 2017;63:1-13
Choi WT et al. Gastroenterol Clin N Am. 2017;46(2):311-325.

Group 2: Arg-1 negative, CK19 positive

- HCC is less likely, and differential diagnosis includes metastatic adenocarcinoma, cholangiocarcinoma, and HCC mimics (NET, RCC).

- Additional immunohistochemistry should be chosen based on morphology and clinical setting, including (1) CK20, CDX-2 for colorectal; (2) CK7, TTF-1, napsin A for lung; (3) PSA, p501s (prostein), PAP, NKX3.1 for prostate; (4) ER, mammaglobin, GATA-3, GCDFP for breast; (5) CK7, ER, WT-1 for ovary; (6) CK7, TTF-1, thyroglobulin for thyroid; (7) DPC-4 loss for pancreas; (8) PAX-2, PAX-8, RCC for RCC; and (9) synaptophysin, chromogranin for NET.

- If HCC is likely based on the clinical and imaging data, Hep Par-1 and GPC-3 can be considered.

Group 3: Arg-1 positive, CK19 positive

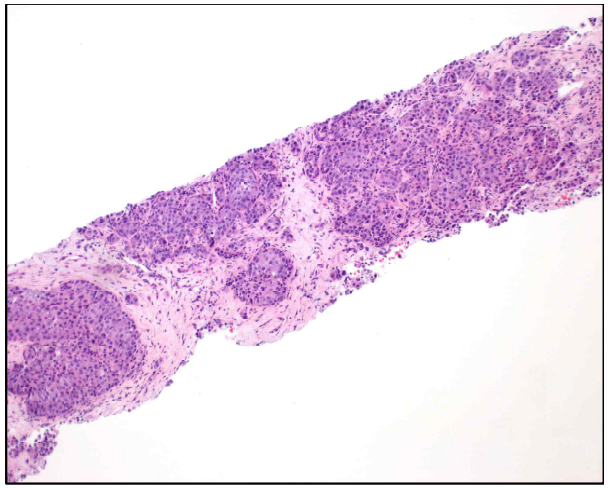
- In most instances, this phenotype represents CK19-positive HCC.
- If morphologically distinct areas of the tumor show positivity for Arg-1 and CK19, the possibility of combined HCC-CC should be considered.
- Rarely, metastatic adenocarcinoma and intrahepatic cholangiocarcinoma may show aberrant Arg-1 staining, but the staining is weak or focal.

Group 4: Arg-1 negative, CK19 negative

- Pancytokeratin-positive:
  - Arg-1-negative HCC (Hep Par-1, GPC-3)
  - CK19-negative adenocarcinoma (site-specific markers)
  - HCC mimics (NET, RCC)
  - Other carcinomas (SCC, urothelial carcinoma)

- Pancytokeratin-negative:
  - Adrenocortical carcinoma (inhibin, Melan-A)
  - Melanoma (SOX-10, S100, HMB-45, Melan-A)
  - Angiomyolipoma (SMA, HMB-45, Melan-A)
  - Epithelioid GIST (c-KIT, DOG-1)
  - Sarcomas with epithelioid morphology

Back to Case #2 (IHC work-up):



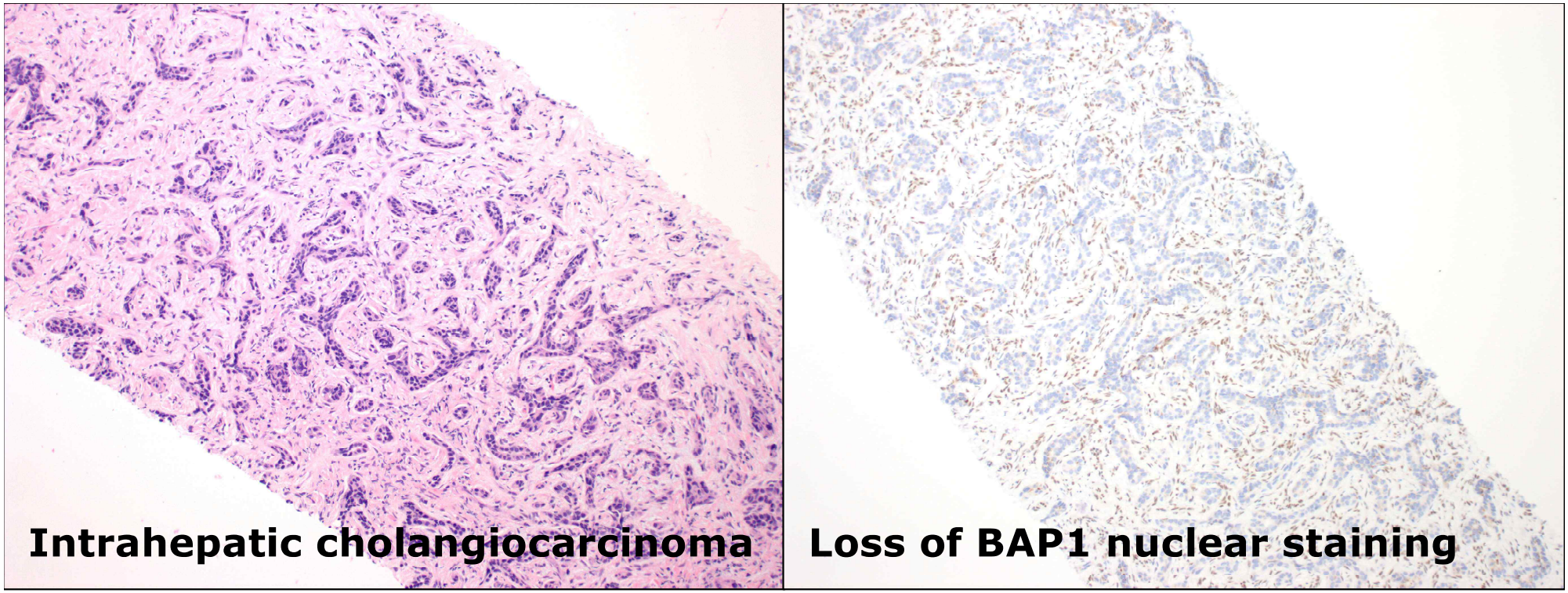
Negative stains:	Positive stains:
Arg-1	CK19
Hep Par-1	CK7
GPC-3	MOC-31
CK20	
CDX-2	
TTF-1	
ER	
Mammaglobin	
GATA-3	
Pax-8	
Synaptophysin	
Chromogranin	
DPC-4 (intact)	

Group 2: Arg-1 negative, CK19 positive

HCC is less likely, and differential diagnosis includes metastatic adenocarcinoma, cholangiocarcinoma, and HCC mimics (NET, RCC).

<u>Diagnosis</u>: Poorly differentiated (adeno)carcinoma; see comment.

COMMENT:
HCC is unlikely, and potential primary sites include pancreaticobiliary (including cholangiocarcinoma) and upper GI (such as stomach). Correlation with clinical and radiological data is recommended.



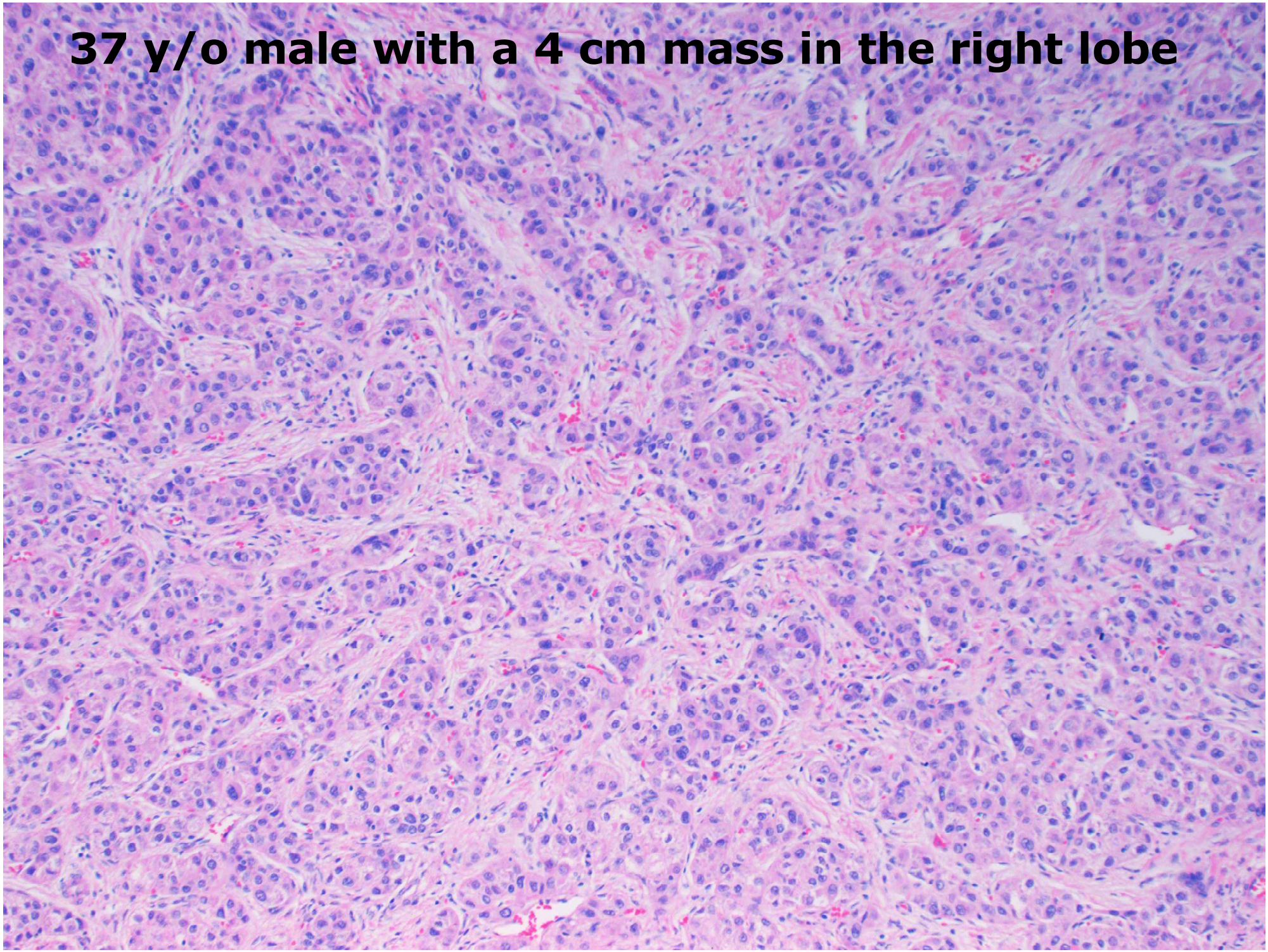
- Loss of BAP1 expression is seen in approximately 7-25% of cases of intrahepatic cholangiocarcinoma, and is related to inactivating mutation of *BAP1*, a gene involved in chromatin remodeling.

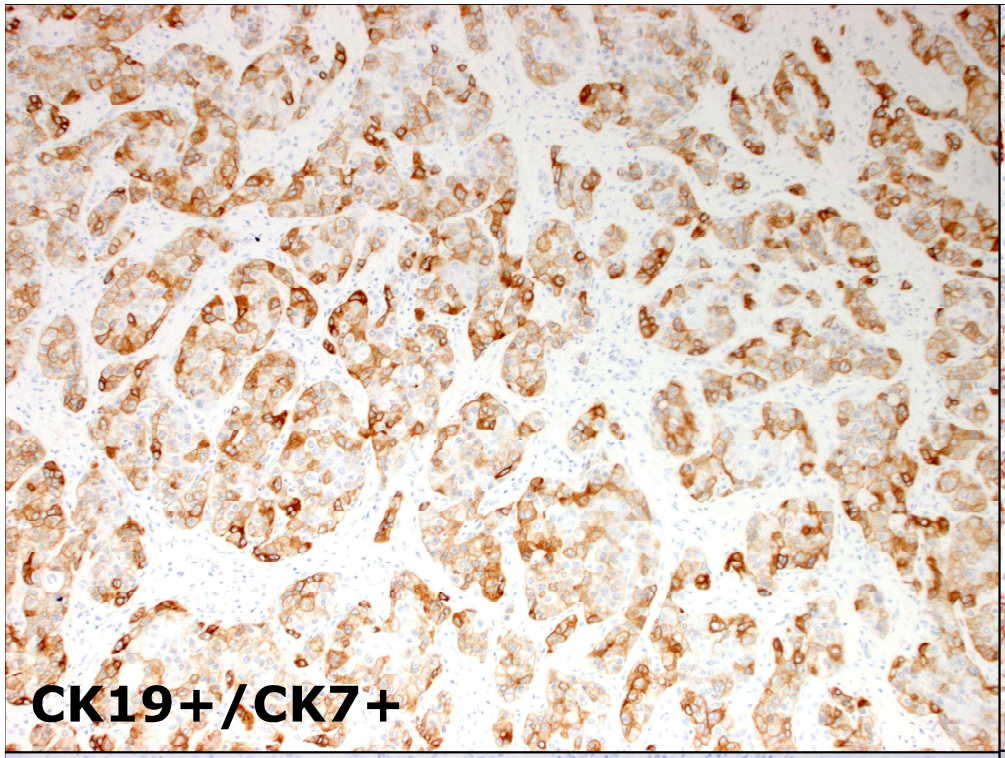
- Loss of BAP1 expression is rare (<1%) in pancreatic ductal adenocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder adenocarcinoma.

- Albumin RNA ISH can be potentially useful in distinguishing intrahepatic cholangiocarcinoma and HCC (> 90%) from pancreatic ductal adenocarcinoma or extrahepatic cholangiocarcinoma (0%).

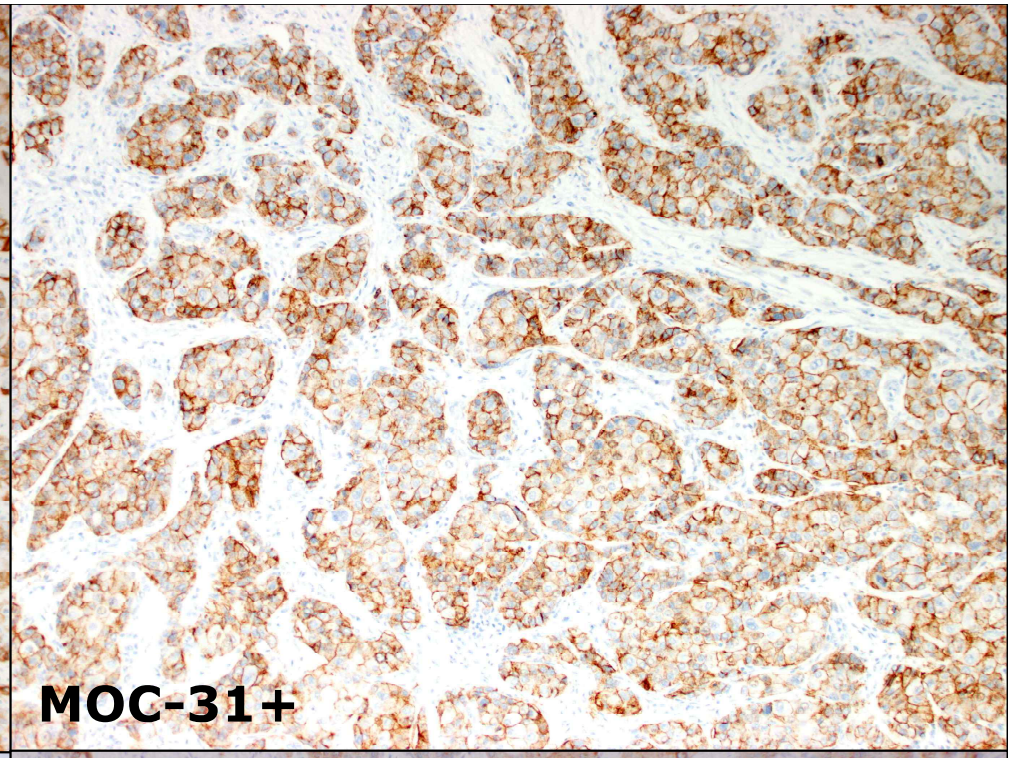
Role of molecular testing

Diagnosis	Molecular alterations
Intrahepatic cholangiocarcinoma (ICC)-specific	*IDH1/2* (5-36%) and *BAP1* mutations; *FGFR2-PPHLN1* fusion (5-45%)
Hepatocellular carcinoma	*TERT* promoter (50-60%), *TP53*, *and* *CTNNB1* mutations
Extrahepatic cholangiocarcinoma (ECC)-specific	*PRKACA/PRKACB* fusion; *ELF3* and *ARID1B* mutations
ICC/ECC shared	*KRAS*, *TP53*, *DPC4/SMAD4*, *ARID1A*, and *GNAS* mutations
Pancreatic ductal adenocarcinoma	*KRAS* (> 90%), *TP53* (75%), *DPC4/SMAD4* (55%), and *CDKN2A/p16* (40%) mutations
Gallbladder adenocarcinoma	*TP53* (> 50%), *CDKN2A/B* (19%), *ARID1A* (13%), *PI3KCA* (10%), and *CTNNB1* (10%) mutations


37 y/o male with a 4 cm mass in the right lobe

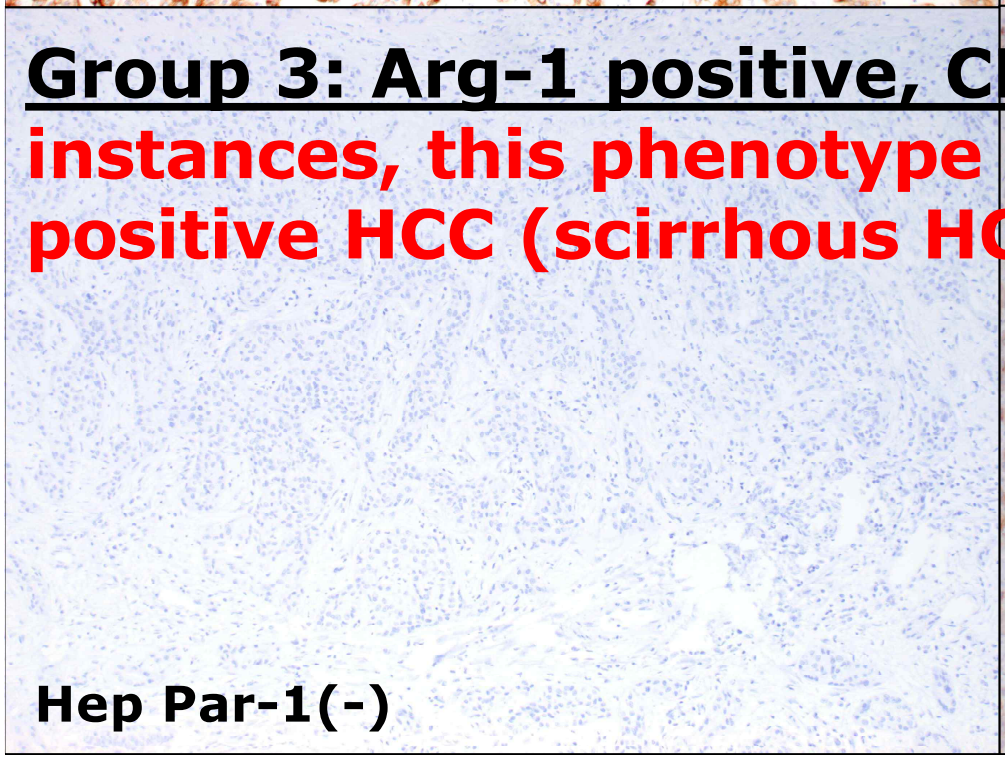


CK19+/CK7+

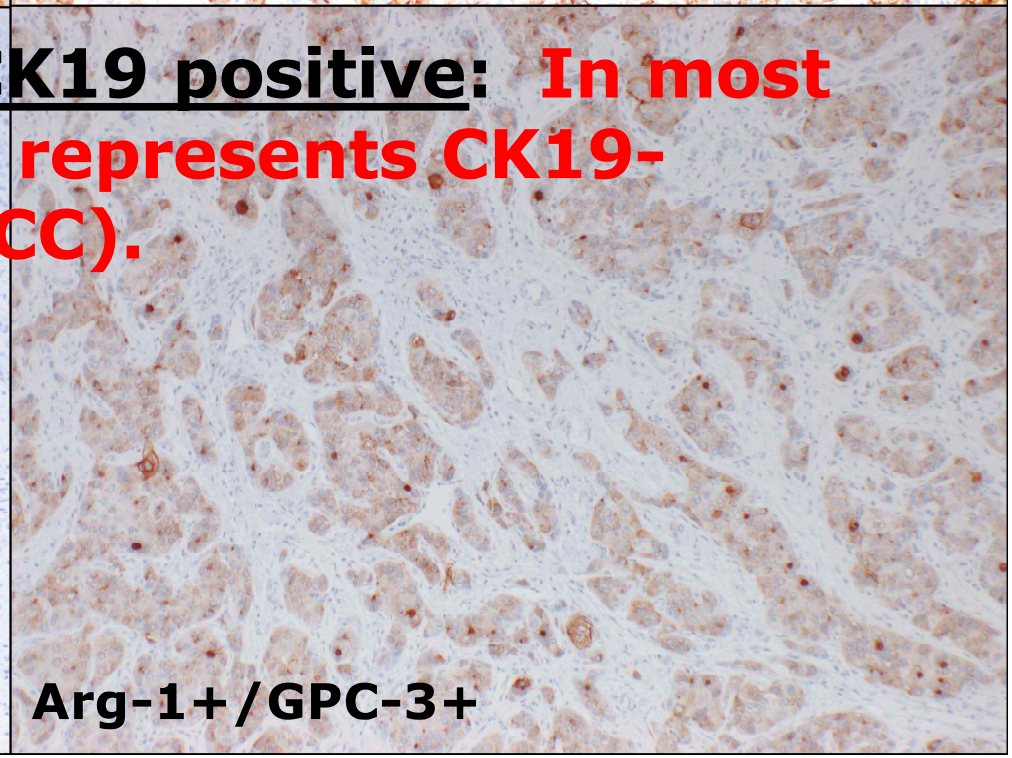


MOC-31+

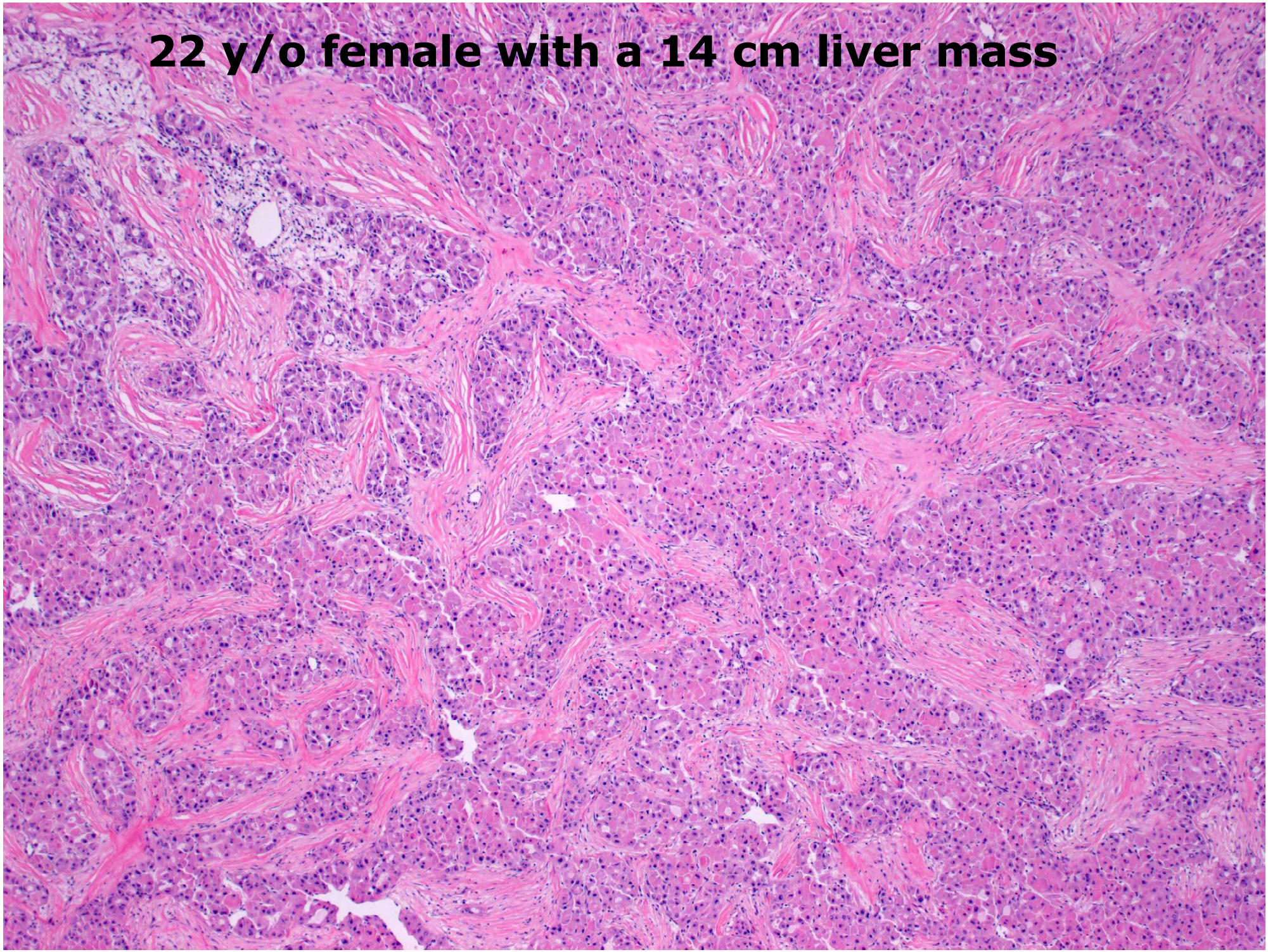
Group 3: Arg-1 positive, CK19 positive: In most instances, this phenotype represents CK19-positive HCC (scirrhous HCC).

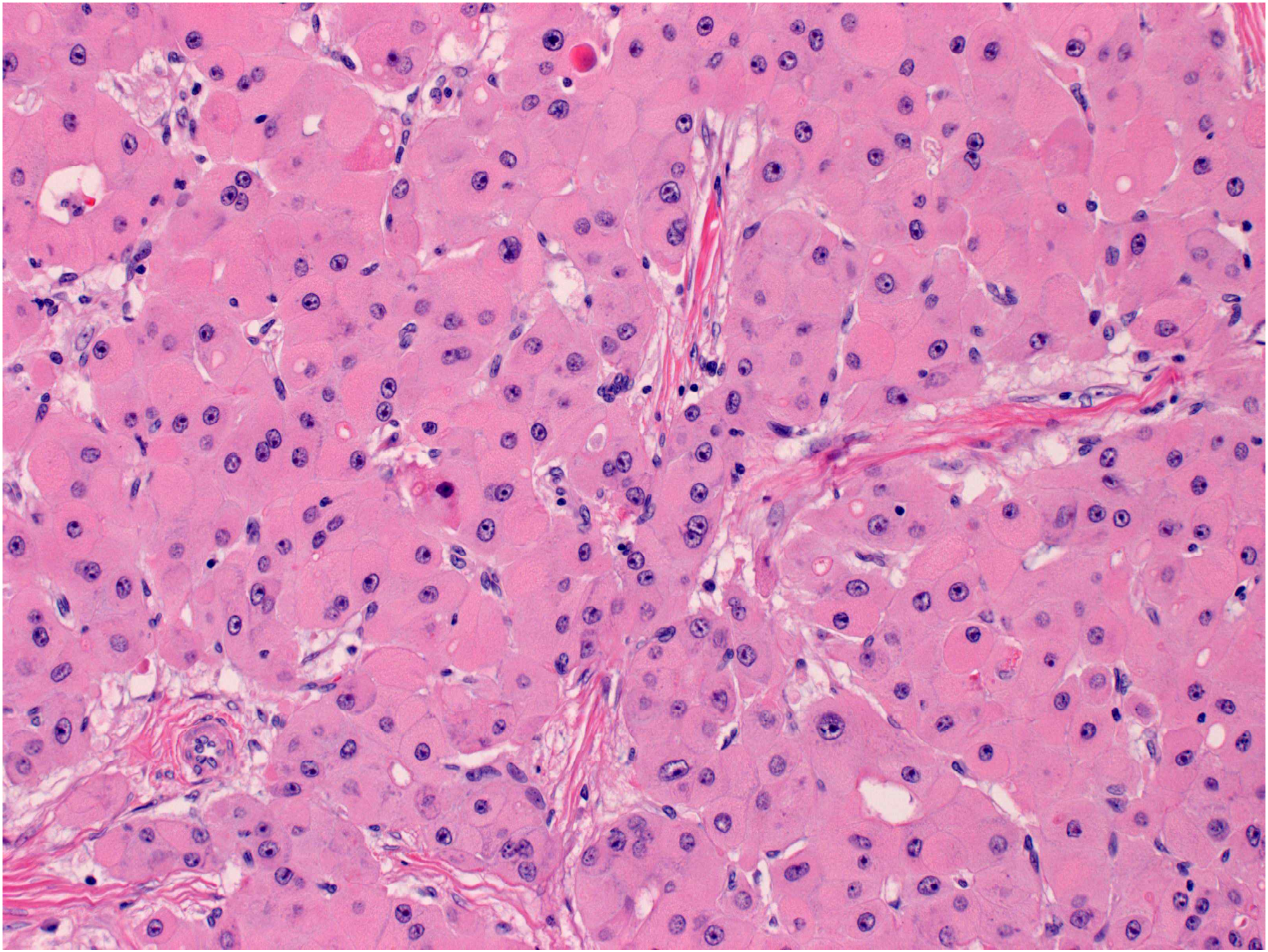


Hep Par-1(-)

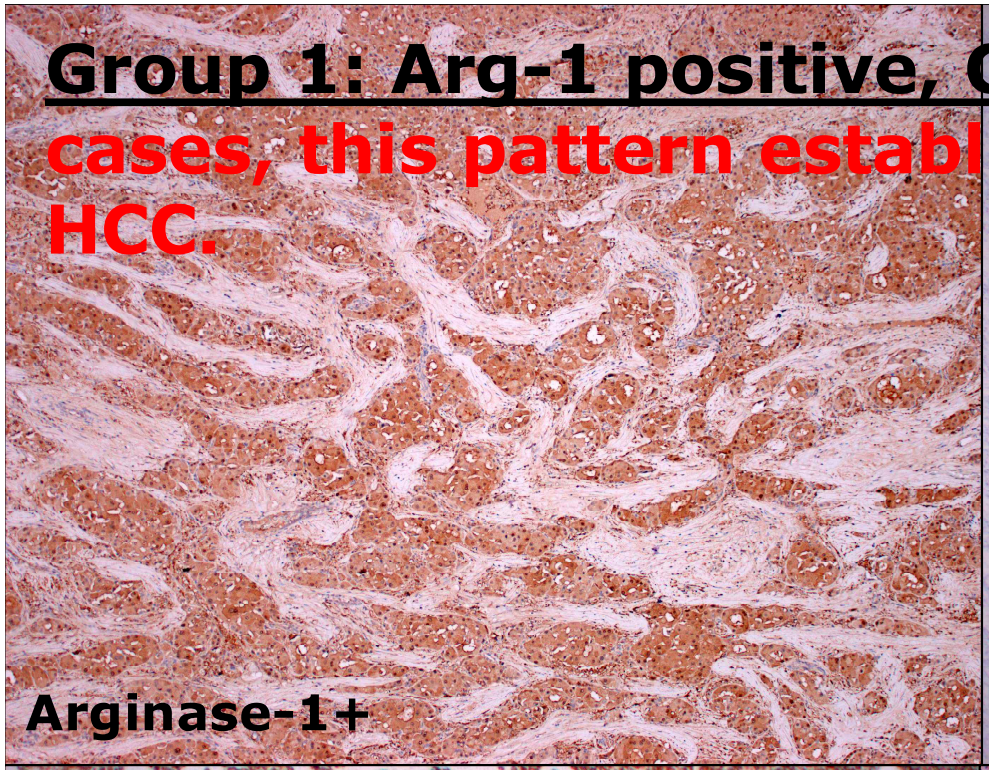


Arg-1+/GPC-3+

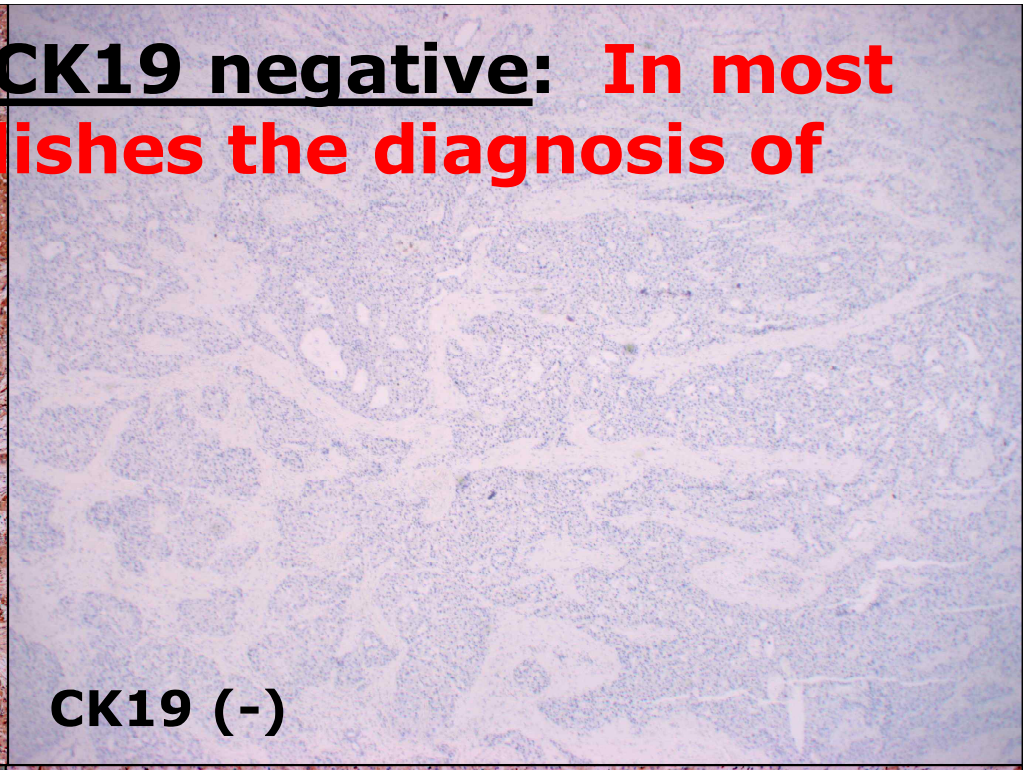

22 y/o female with a 14 cm liver mass



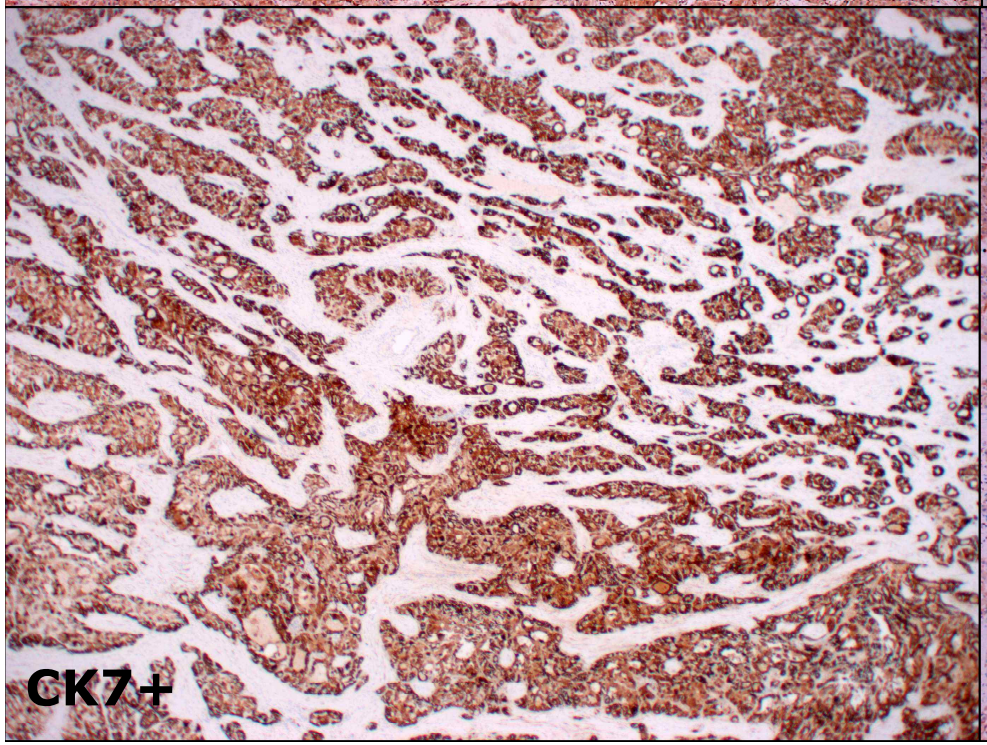
Group 1: Arg-1 positive, CK19 negative: **In most cases, this pattern establishes the diagnosis of HCC.**



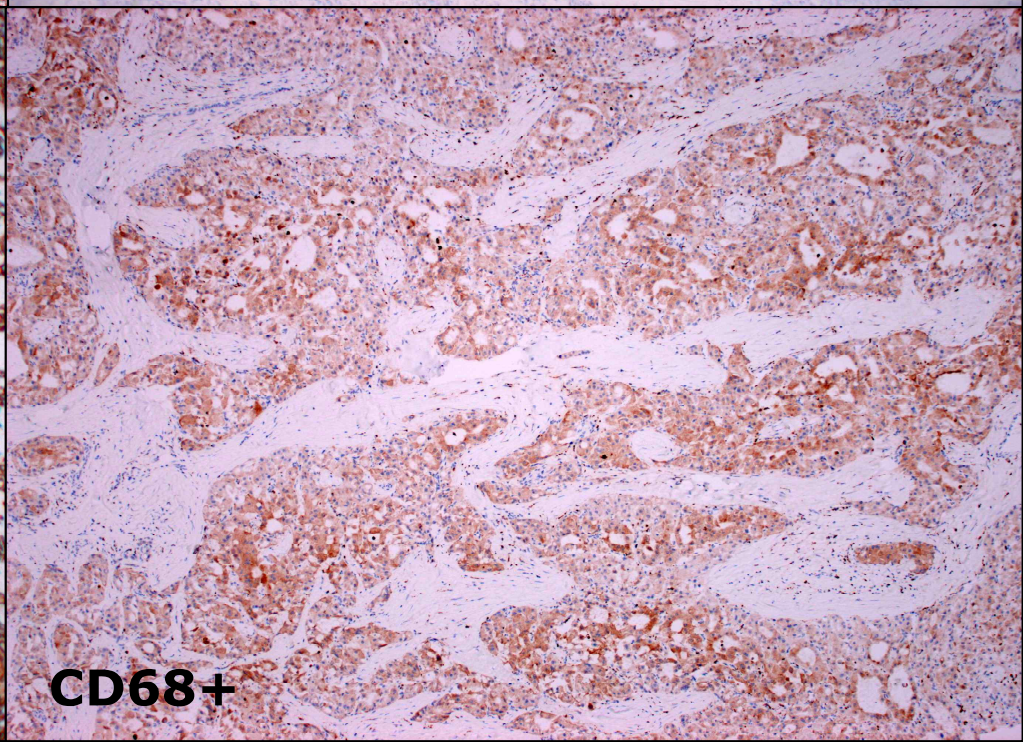
Arginase-1+



CK19 (-)



CK7+



CD68+

Fibrolamellar carcinoma

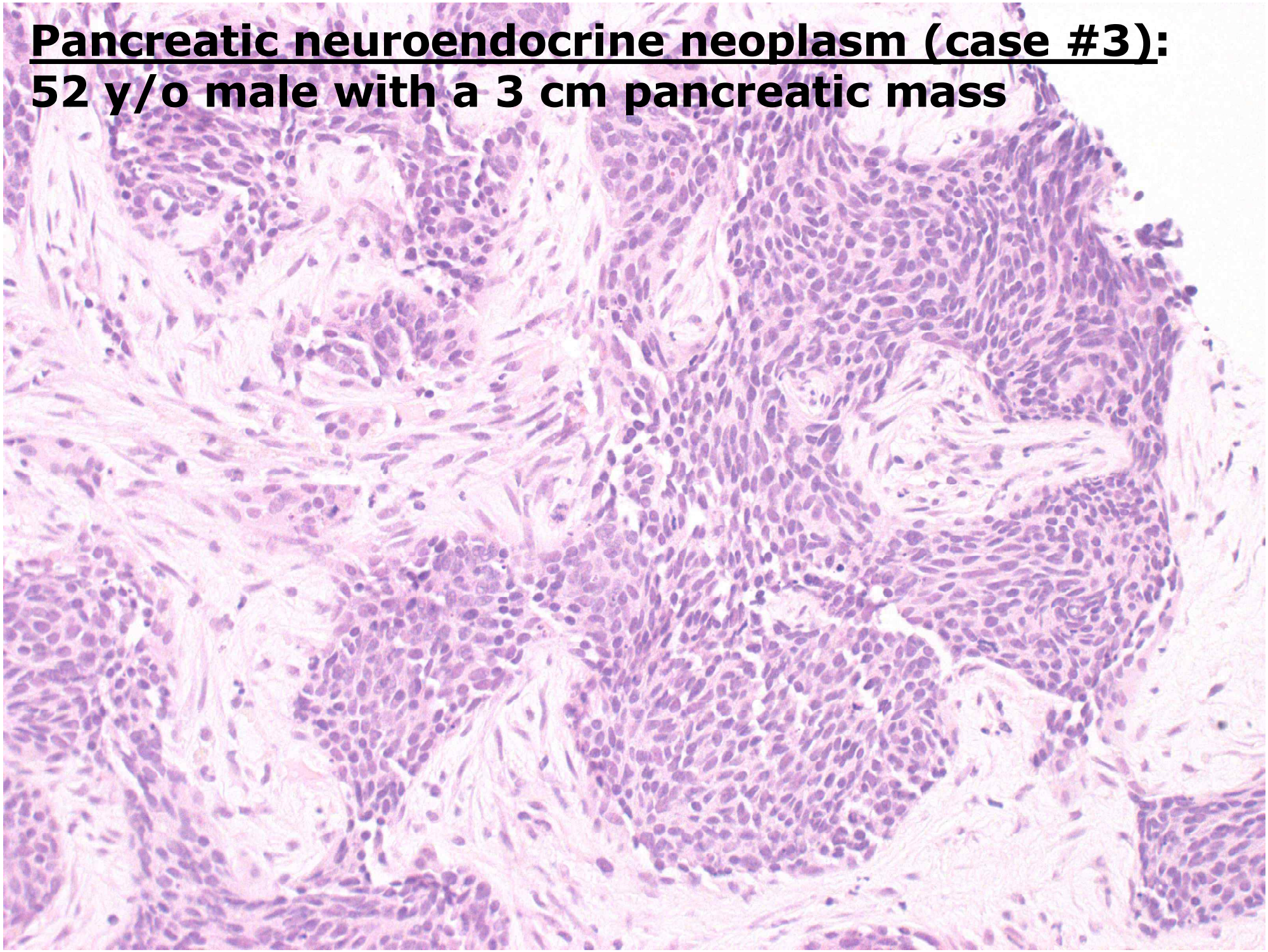
- A disease of young adults in the absence of cirrhosis.

- Management: Aggressive surgical resection often with regional lymph node dissection (median survival of 1 year in the absence of surgical resection)
  - Lymph node metastasis: 50-60% in FLM
  - Classical HCC: < 5%

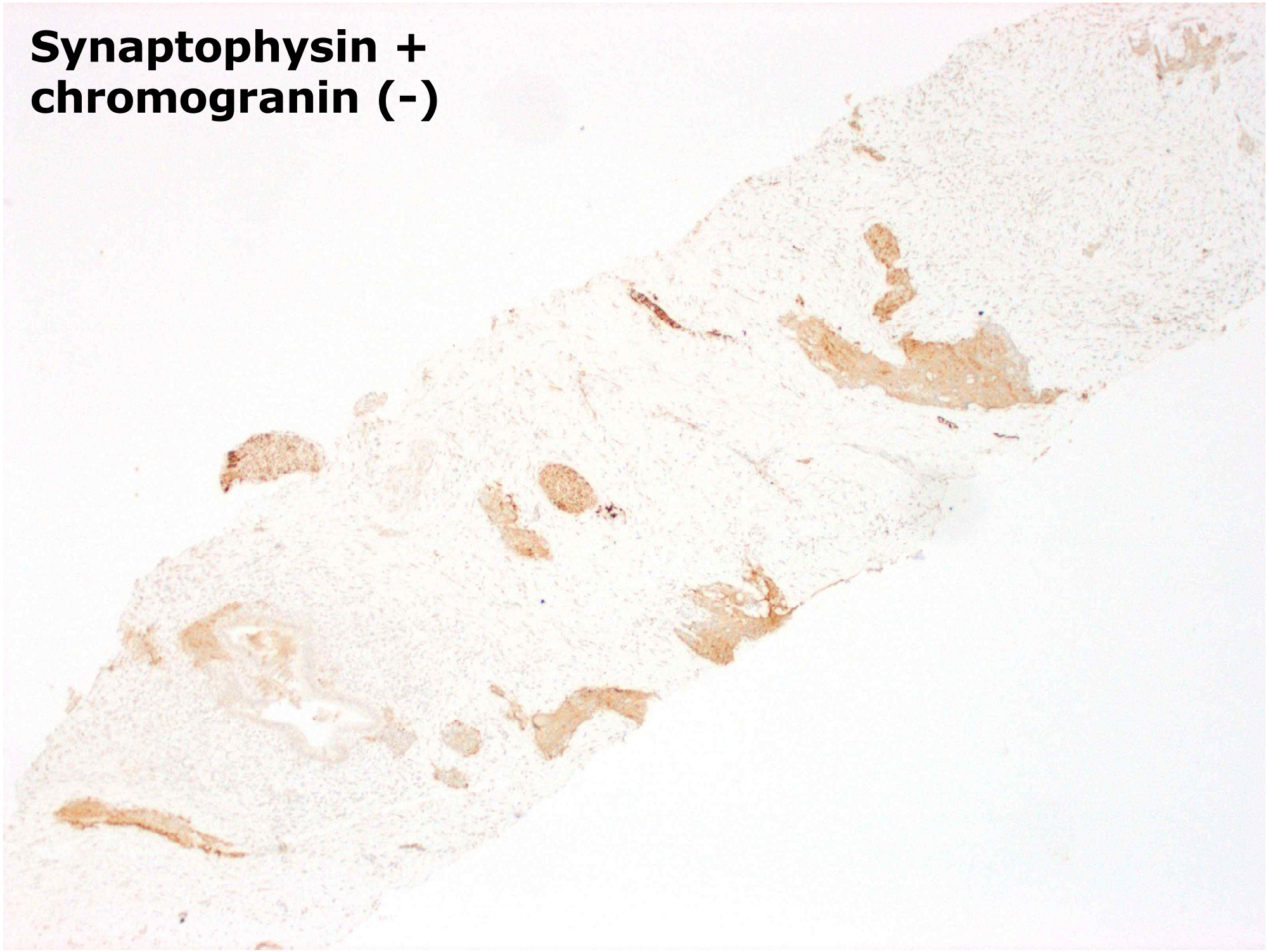
- Thus, it is important to confirm the diagnosis using IHC and/or molecular testing:
  - Nearly all cases are positive for CK7 and CD68.
  - > 80% of cases have *DNAJB1-PRKACA* fusion transcript which can be detected by RT-PCR or FISH.

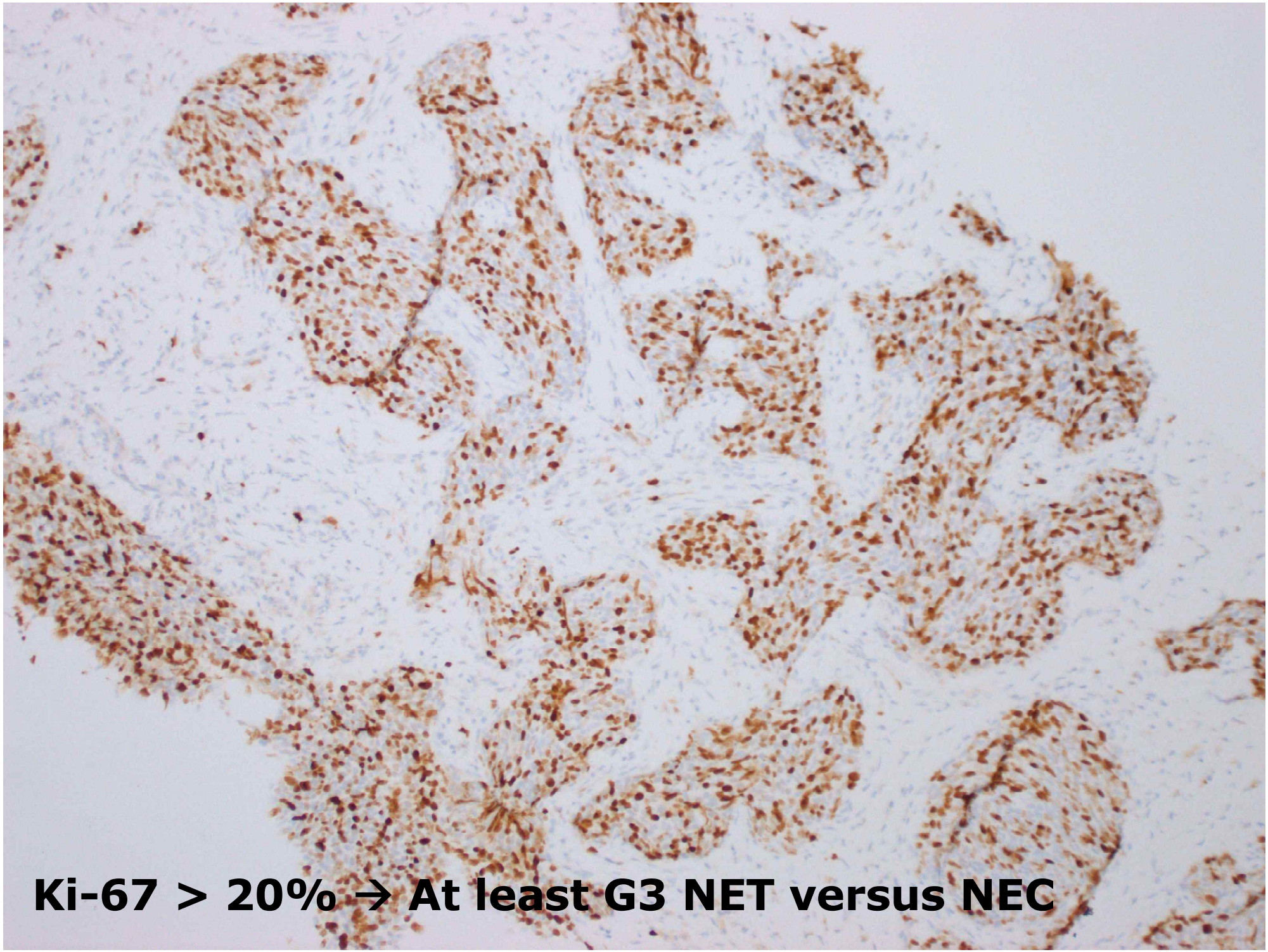
Graham et al. Mod Pathol. 2015;28(6):822-9
Ross et al. Mod Pathol. 2011;24(3):390-5.

<u>Pancreatic neuroendocrine neoplasm (case #3)</u>:
52 y/o male with a 3 cm pancreatic mass



Synaptophysin +
chromogranin (-)




Ki-67 > 20% → At least G3 NET versus NEC

WHO classification of neuroendocrine neoplasm

- Well-differentiated NET:
  - G1 (low-grade): < 2 mitoses/2 mm^2 or Ki-67 < 3%
  - G2 (intermediate-grade): 2-20 mitoses/2 mm^2 or Ki-67 = 3-20%
  - G3 (high-grade): > 20 mitoses/2 mm^2 or Ki-67 > 20%

- Poorly-differentiated NEC (high-grade): > 20 mitoses/2 mm^2 or Ki-67 > 20%

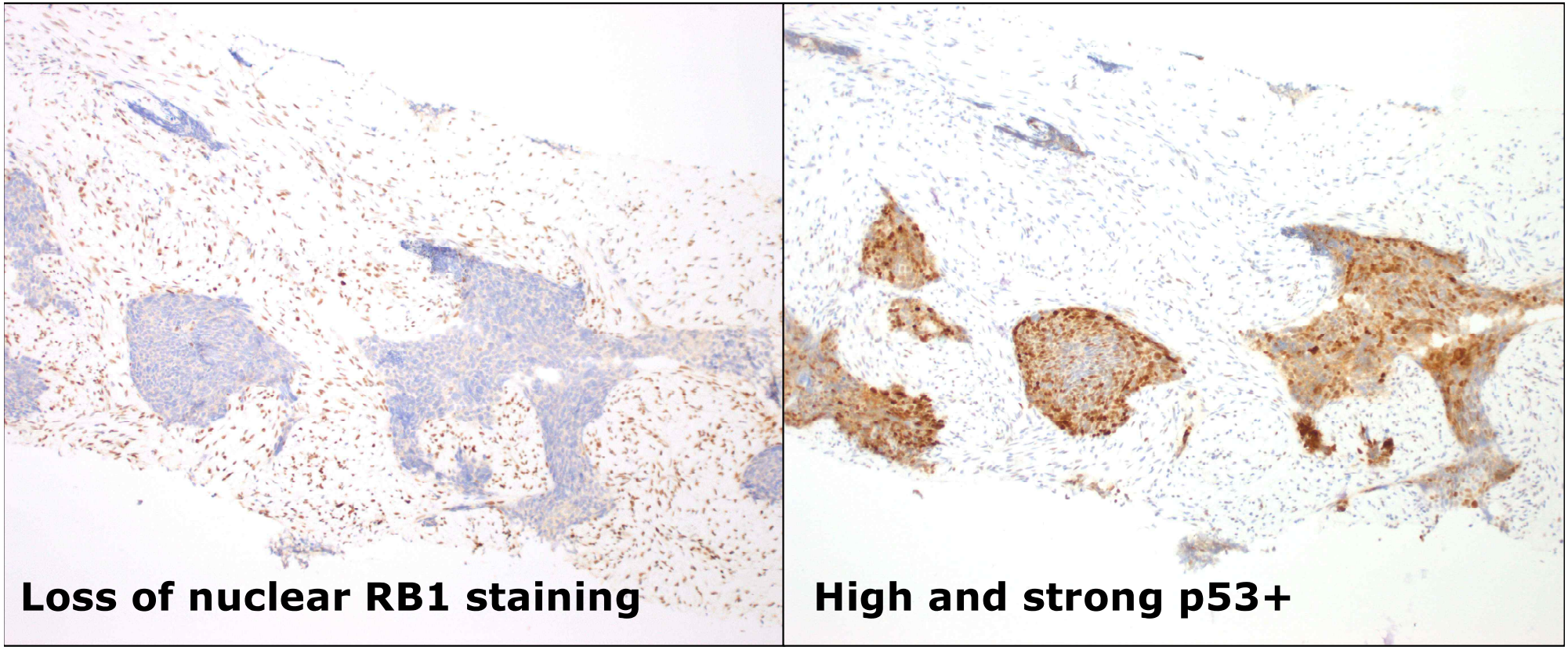
- G3 NET has a worse prognosis than G1/G2 NET but less aggressive than NEC.
  - NEC → Platinum-based chemotherapy.
  - G3 NET → Other regimens used in G1/G2 NETs (including temozolomide- or streptozocin-based chemotherapy)

NEC versus NET: Molecular and Immunohistochemical Features

- NECs often show mutations of cell-cycle regulatory genes, such as *TP53* and *RB1*, whereas chromatin remodeling genes such as *MEN1*, *ATRX*, and *DAXX* are not involved.
  - Often show p53 over-expression and loss of nuclear RB1 staining.

- NETs usually have intact *TP53* and *RB1*.
  - Inactivation of *MEN1* (~40%).
  - Mutation in either *DAXX* or *ATRX* (~40%)
  - Low (< 20% of tumor cells) and weak nuclear p53 expression, and intact nuclear RB1 expression.

Back to Case #3:



NEC (small cell type)

Differential Diagnosis of PanNEC

Diagnosis	Immunophenotype	Molecular alterations
PanNEC	Chromo & synapto (+); p53 (+); RB1 (lost)	*TP53*, *RB1*, *CDKN2A/p16*, *KRAS* mutations
Acinar cell carcinoma	Trypsin & chymotrypsin (+); chromo & synapto (focal+ in 40%)	*BRAF* fusions (23%), including *SND1-BRAF* and *HERPUD1-BRAF; APC* (8%), *CTNNB1* (7%), *TP53* (12-24%), *DPC4/SMAD4*, and *CDKN2A/2B* mutations
Solid pseudopapillary neoplasm	β-catenin & CD10 (+); synapto (focal +); trypsin, chymotrypsin & chromo (-)	*CTNNB1* exon 3 mutation
Ductal adenocarcinoma	DPC4/SMAD4 (lost in 55%); chromo & synapto (- or focal +); acinar markers (-)	*KRAS* (> 90%), *TP53* (75%), *DPC4/SMAD4* (50%), and *CDKN2A/p16* (40%) mutations and/or deletions
Pancreatoblastoma	EMA & β-catenin (+) in squamous nests; trypsin & chymotrypsin (+); chromo & synapto (focal+ in 40%)	Common loss of heterozygosity of 11p; APC/β-catenin pathway alterations (50-80%)

Conclusions

- Although molecular testing is playing an increasingly important role in our practice, immunohistochemistry can serve as a surrogate for molecular testing.

- If initial IHC workup is inconclusive, you should consider molecular testing:
  - AHN (HCC vs. HCA)
  - Poorly-differentiated tumor in the liver (cholangiocarcinoma vs. HCC vs. other tumors)
  - G3 NET vs. NEC vs. other poorly-differentiated pancreatic tumors