Hepatocellular neoplasms – current issues and role of special stains

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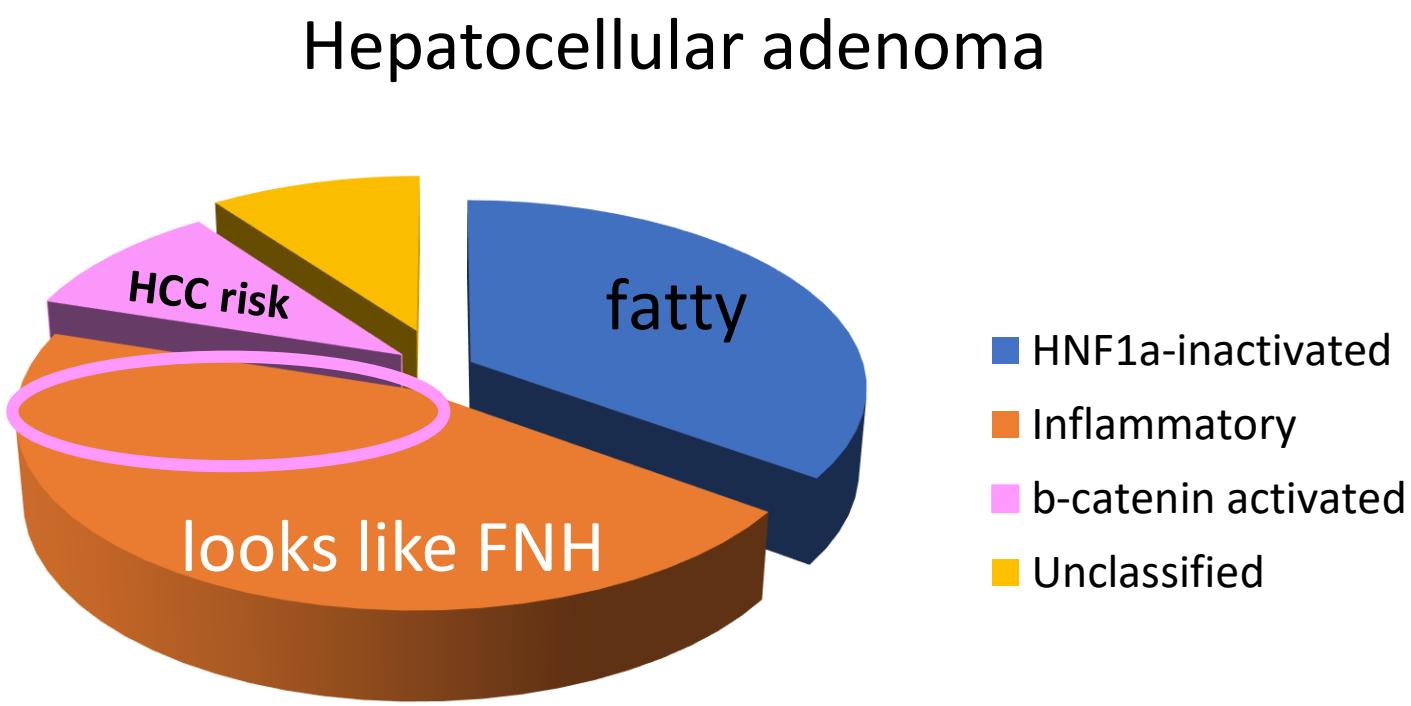
Envoi SPECIALIST PATHOLOGISTS *inside insights*

Outline

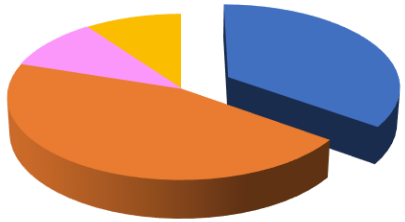
- hepatocellular adenoma
- borderline lesions (HUMP)
- hepatocellular carcinoma
  - steatohepatitic HCC
  - biphenotypic tumours
  - macrotrabecular HCC
  - (early HCC)

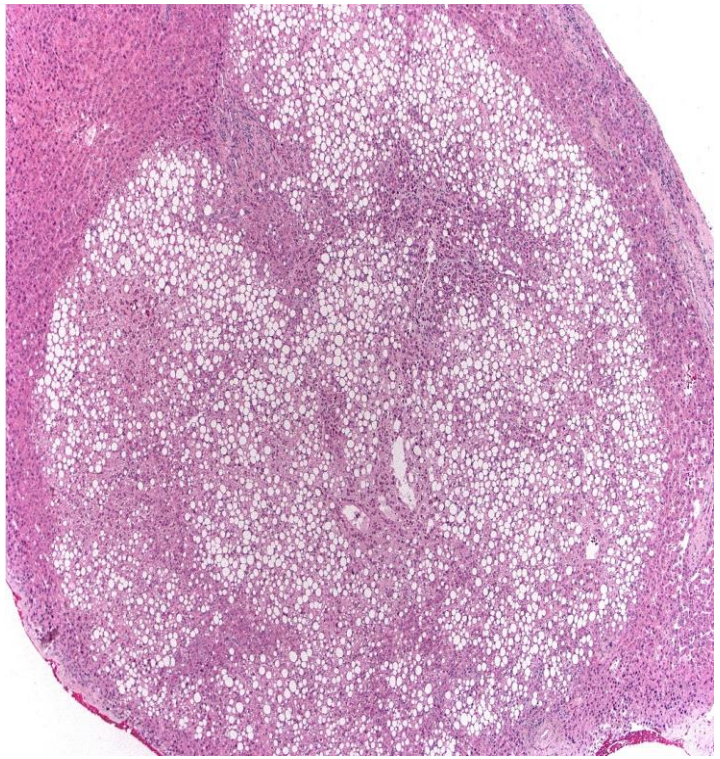
Hepatocellular adenoma is a heterogeneous group



Bioulac-Sage P et al. Semin Diagn Pathol 2017
Bioulac-Sage P et al. Semin Liver Dis 2011; 31:91

#1. HNF1α–inactivated





- 90% are sporadic inactivation
  (10% inherited = MODY type 3)

- steatosis prominent
- usual type in adenomatosis (>10 lesions)

- L-FABP IHC (this is activated by HNF1α)
- >> *loss* of normal staining occurs

- malignant change extremely rare

#1. HNF1α–inactivated

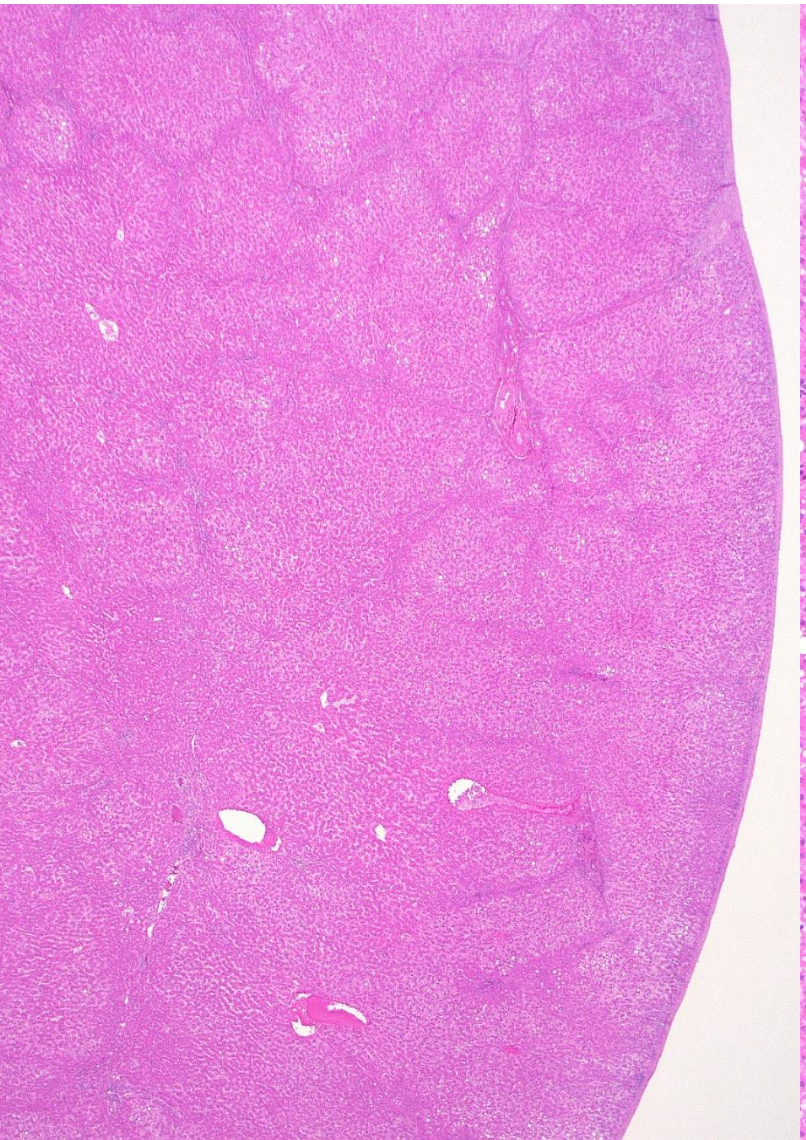
Table 2

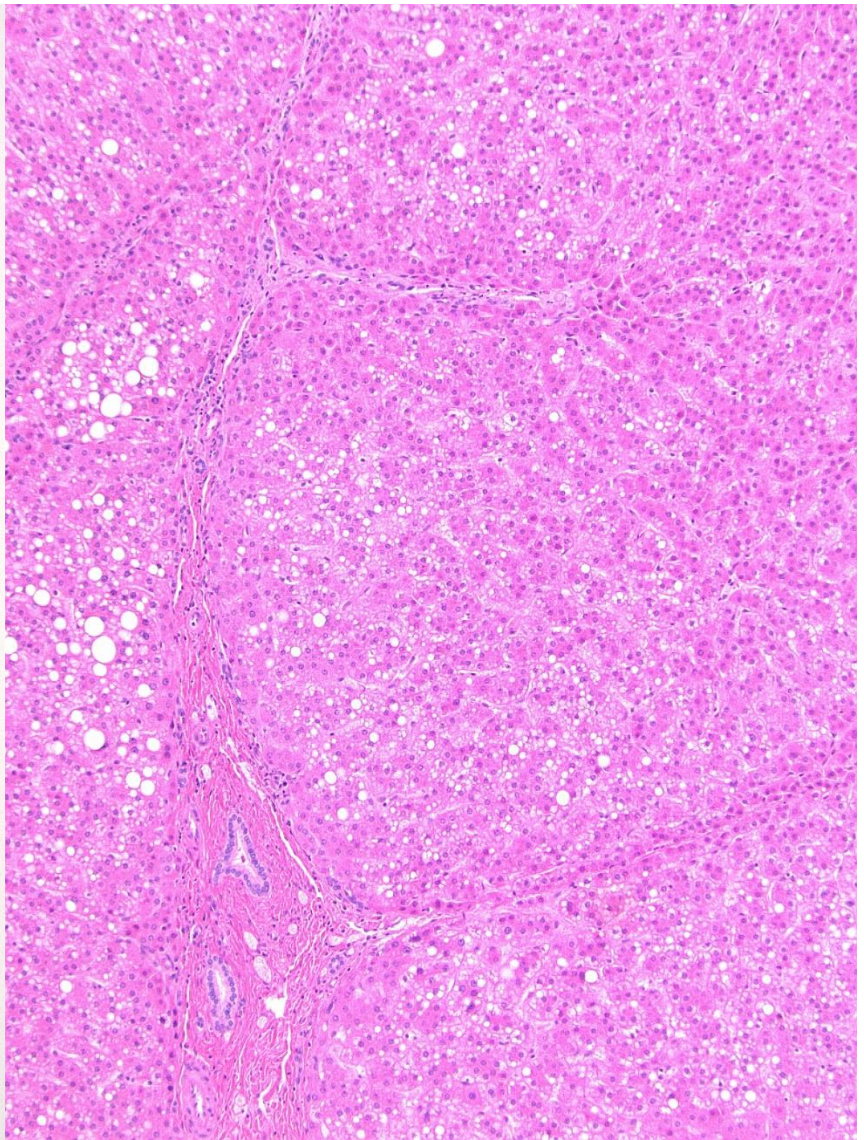
Patterns of steatosis in HCA

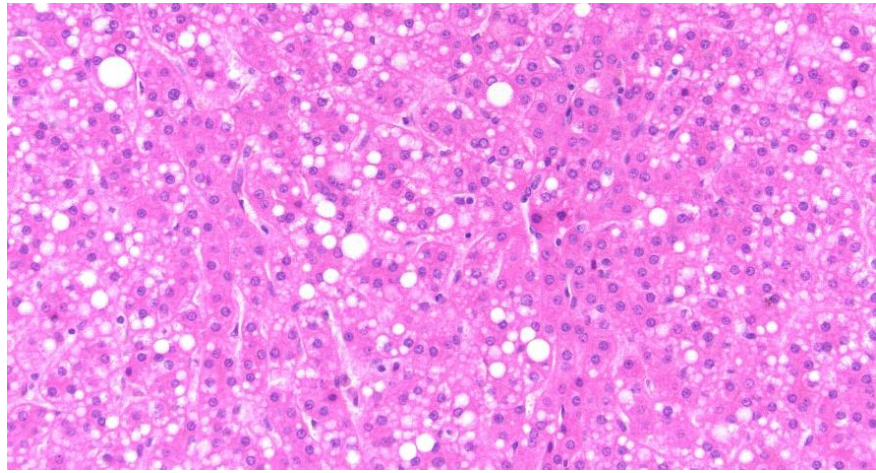
	HCA subtype					
Steatosis score	H-HCA	I-HCA	BI-HCA	B-HCA	U-HCA	Total
0	4	44	9	3	7	67
1+	14	11	1	1	3	28
2+	12	4	1	0	1	18
3+	4	2	0	0	0	6
Adjacent liver (any steatosis)	4	30	2	1	4	
Total	34	61	11	4	11	121

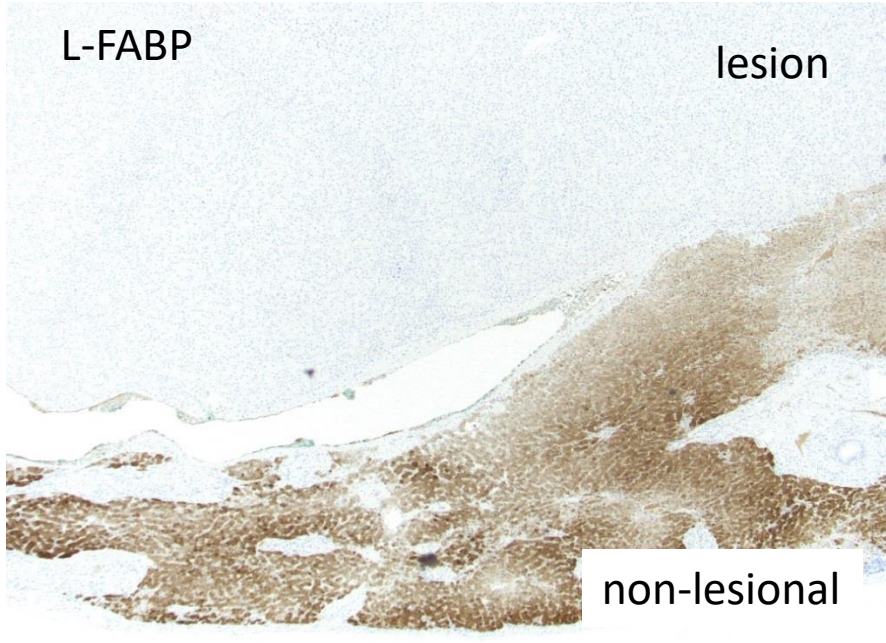
Miller G et al. Pathology 2018; 50:593

#1. HNF1α–inactivated

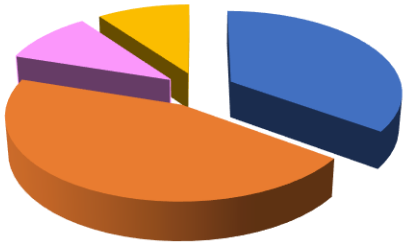


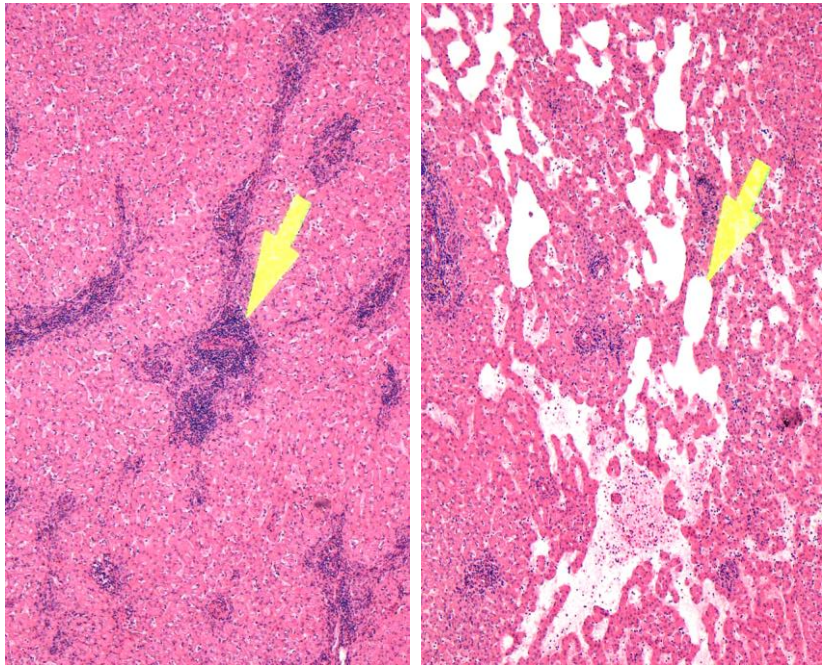






#2. Inflammatory adenoma





- different radiology (may mimic FNH)
- increased in overweight (*fatty outside*)

- >> detect with Amyloid A & CRP IHC

- overlap histology with FNH
  - telangiectatic sinusoids ("atoll sign")
  - some ductules
  - variable inflammation

Luamonier et al. Hepatol 2008; 48:808
Ronot et al. Hepatol 2011; 53:1182
Agarwal et al. AJR 2014; 203:W408-14

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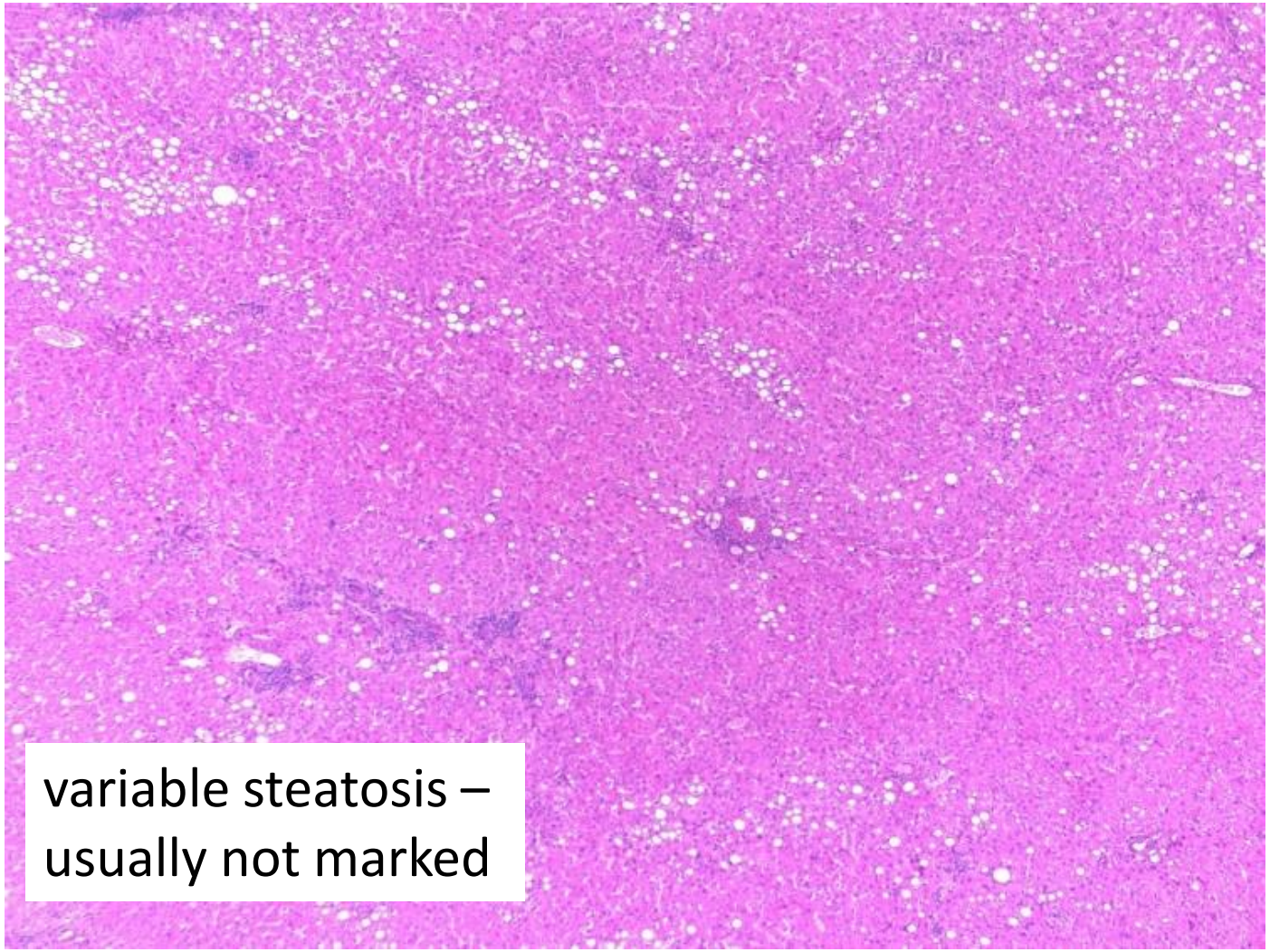

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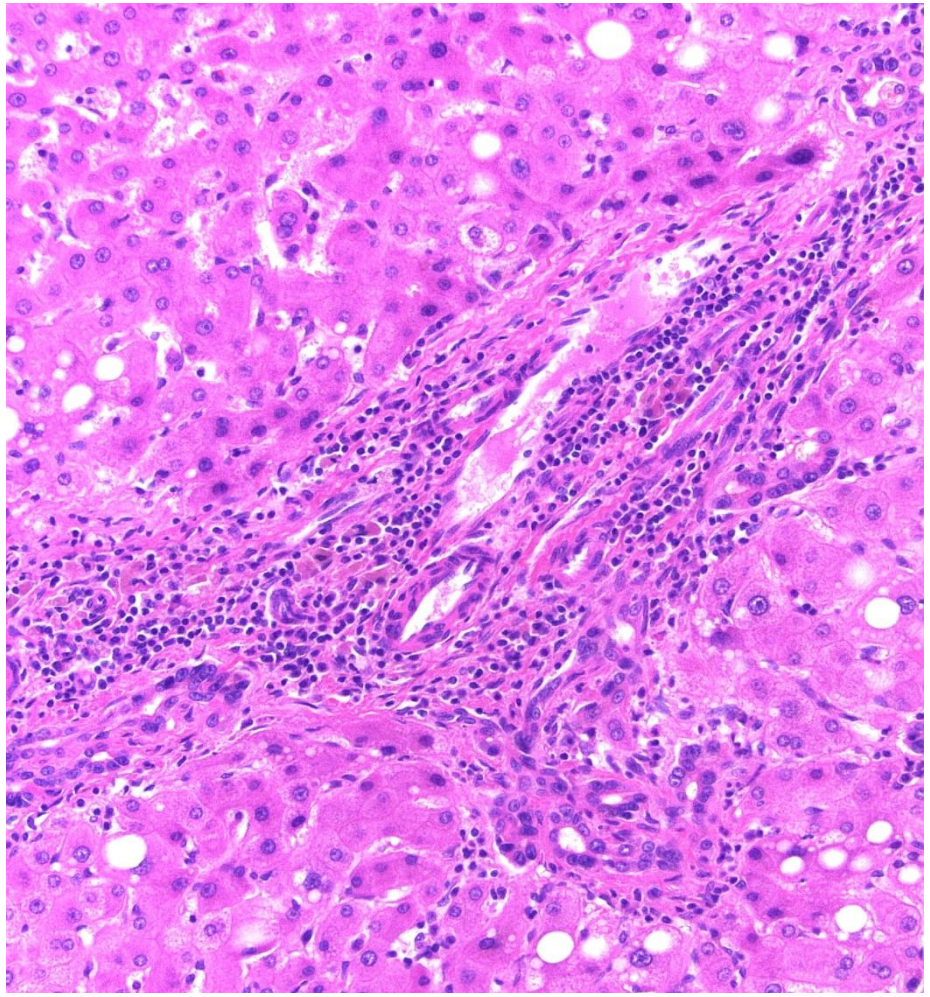
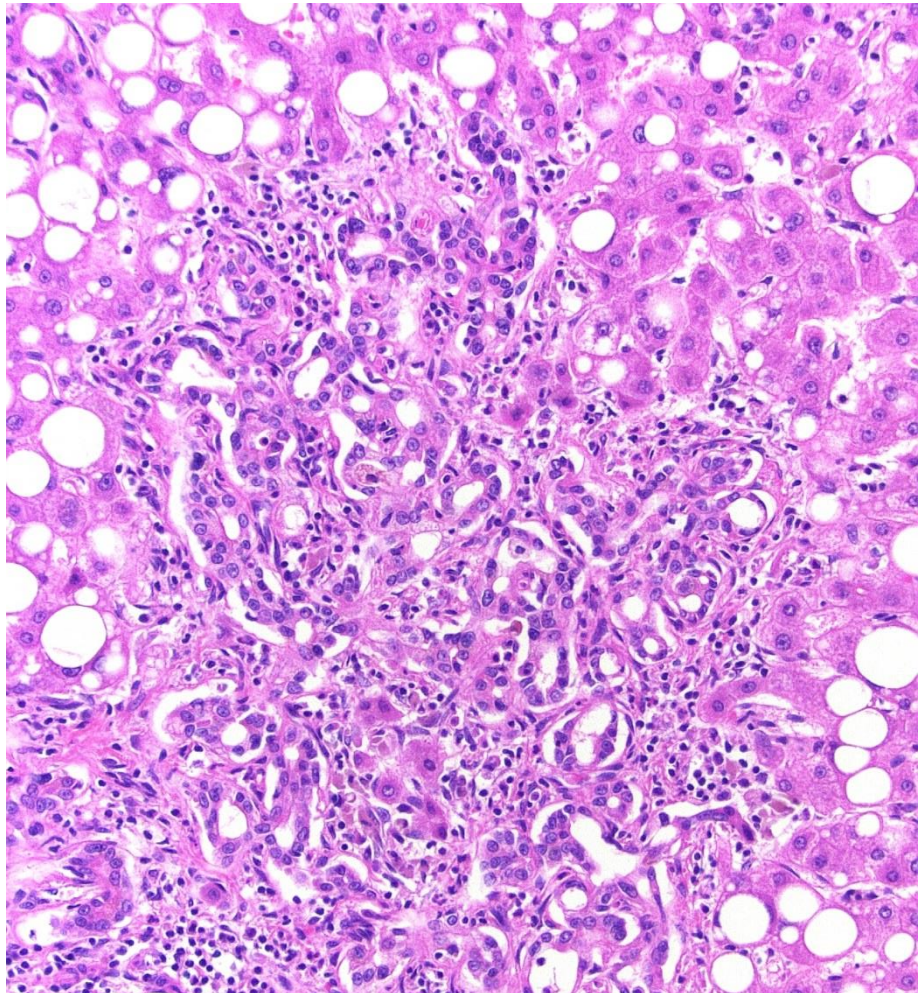
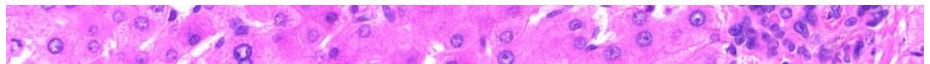
I-HCA - DR may be prominent

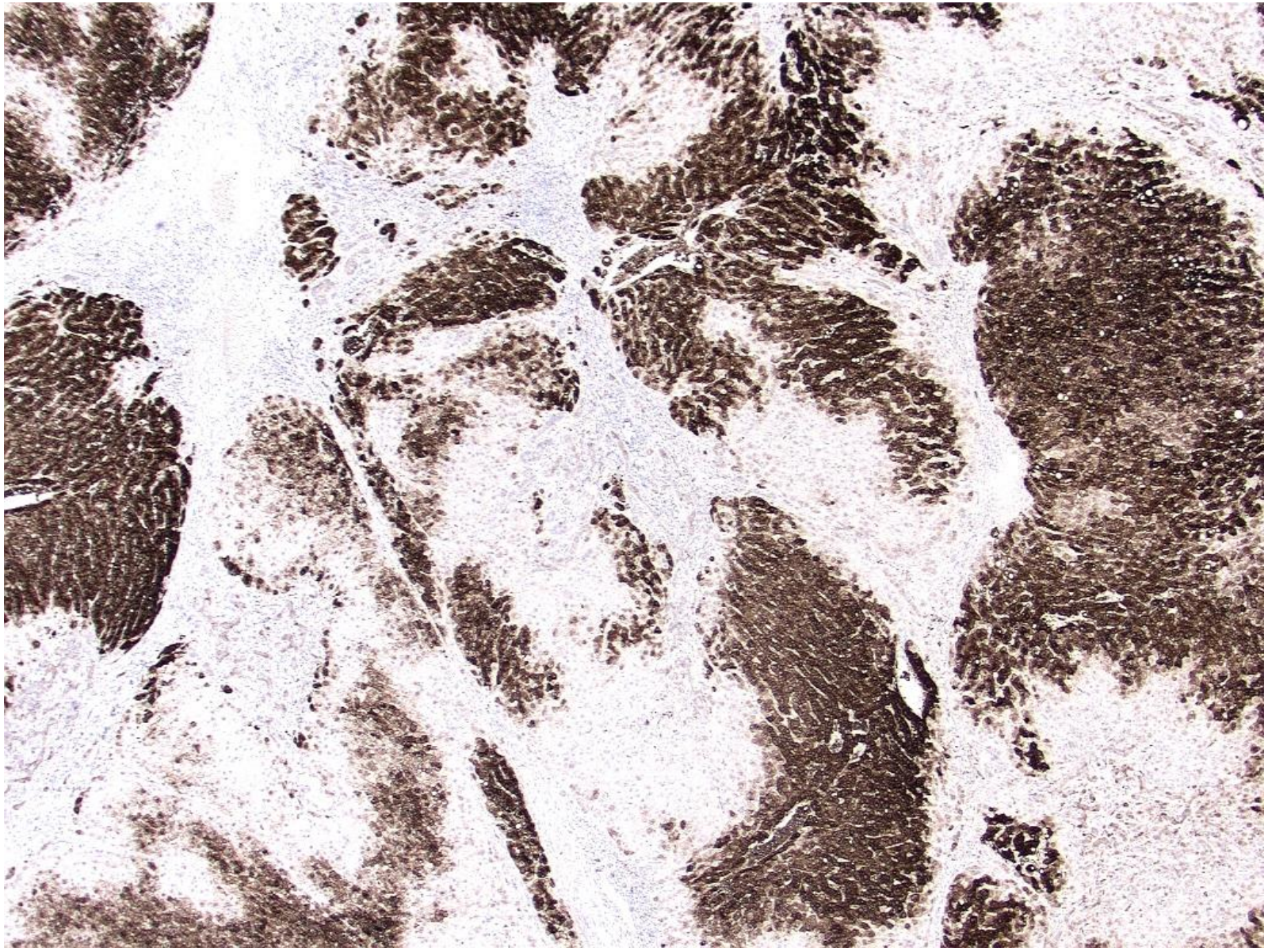




- rare cases difficult to distinguish I-HCA from FNH Joseph et al. Mod Pathol 2014; 27:62
- DR and fibrosis common in remodelled areas particularly



Map-like pattern of GS staining in FNH



glutamine synthetase in FNH

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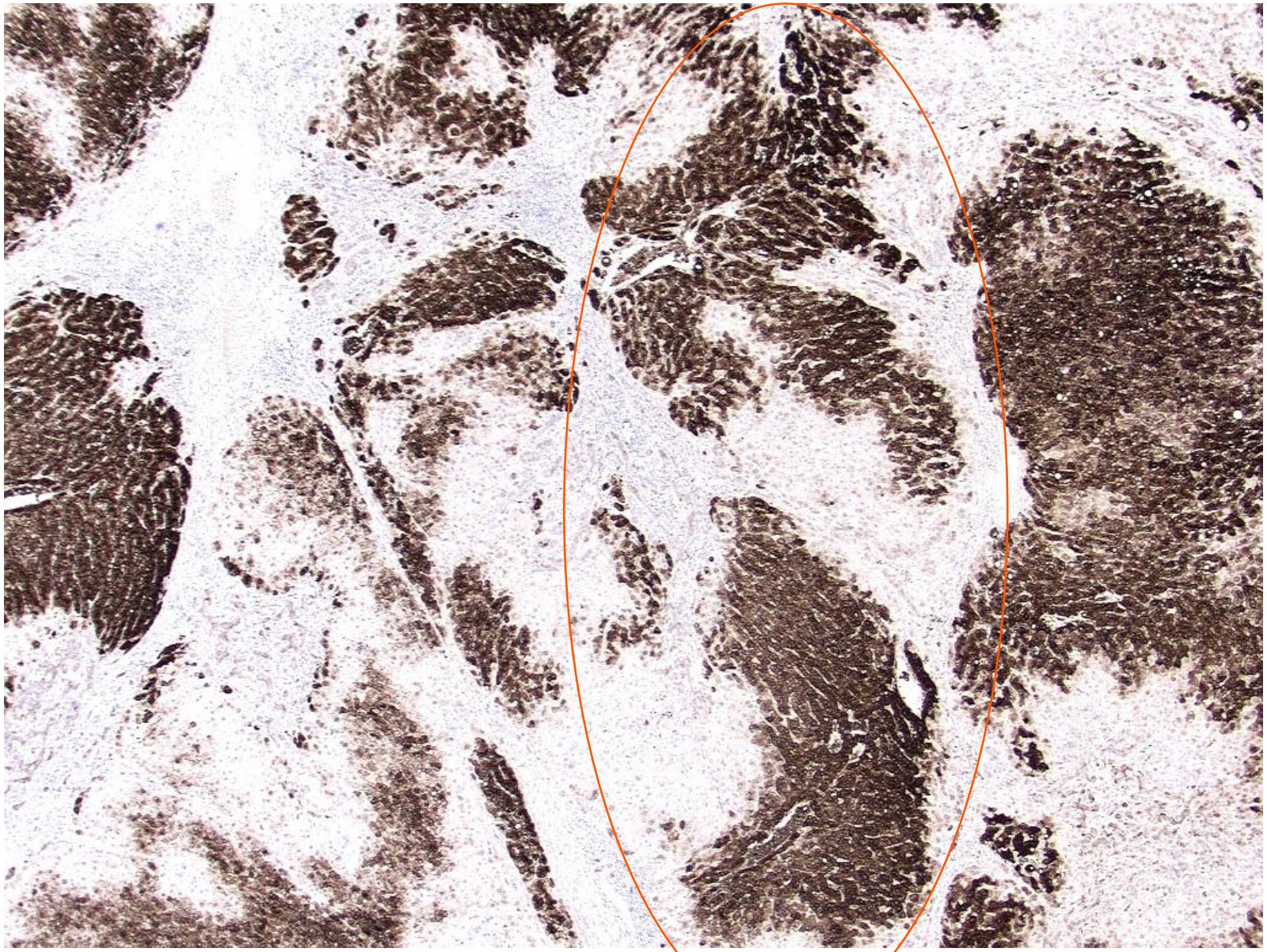
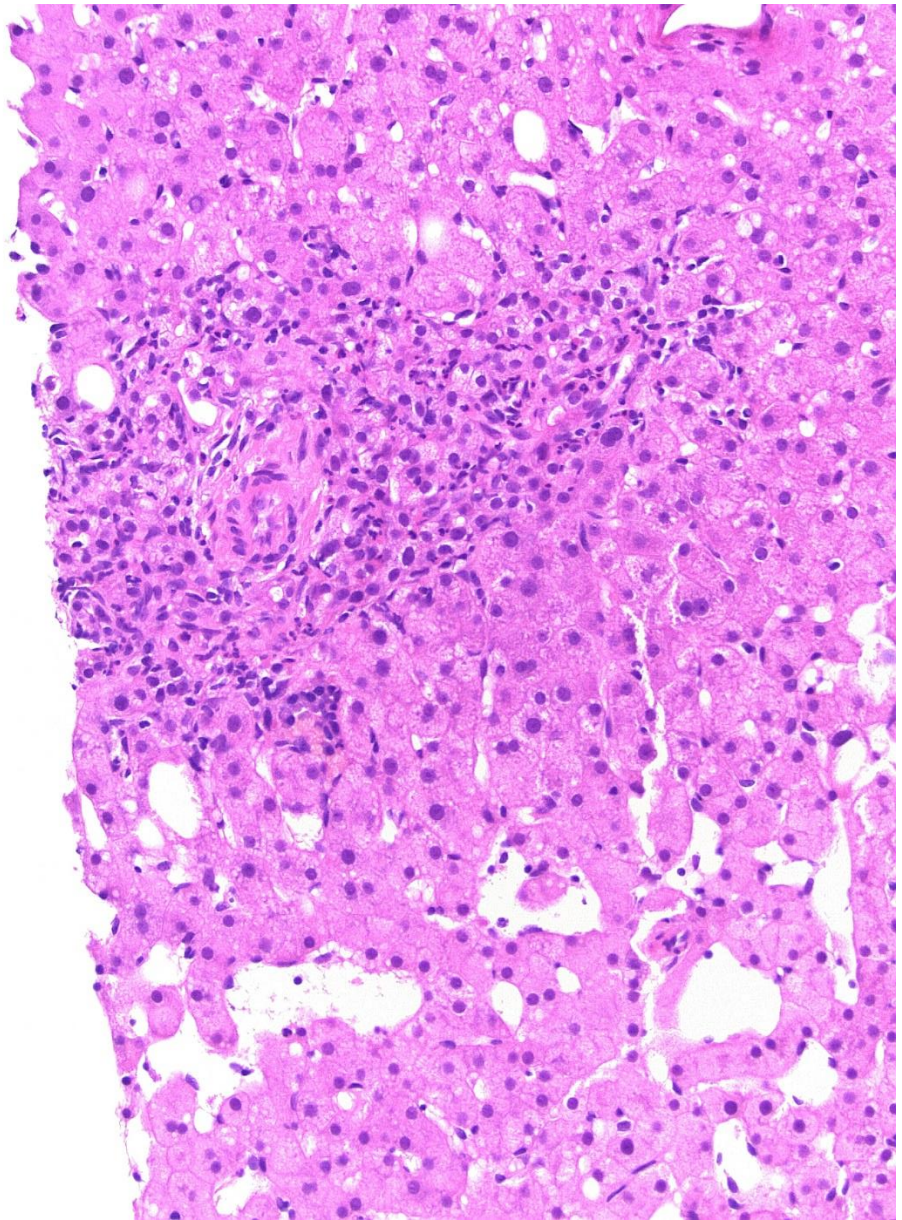
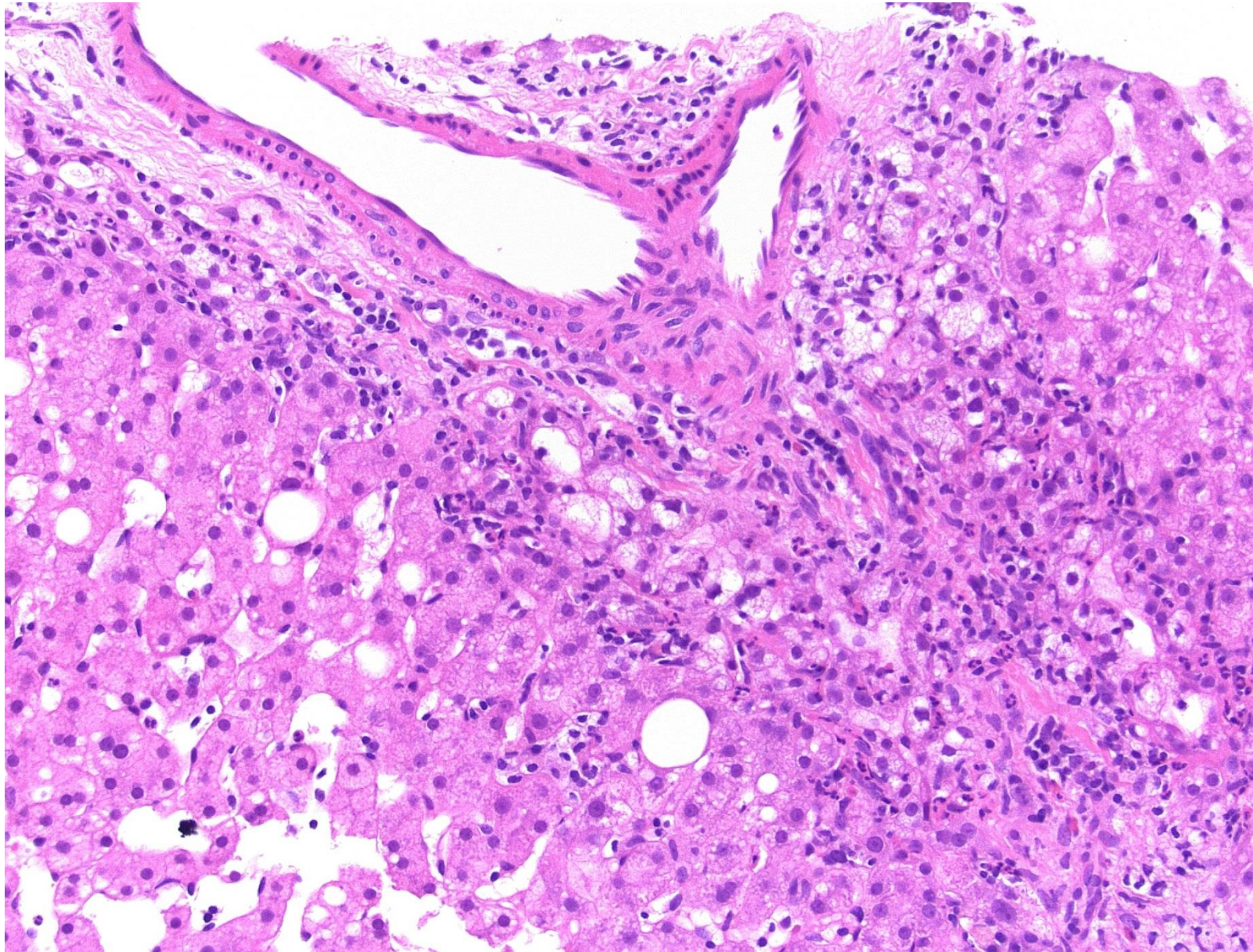


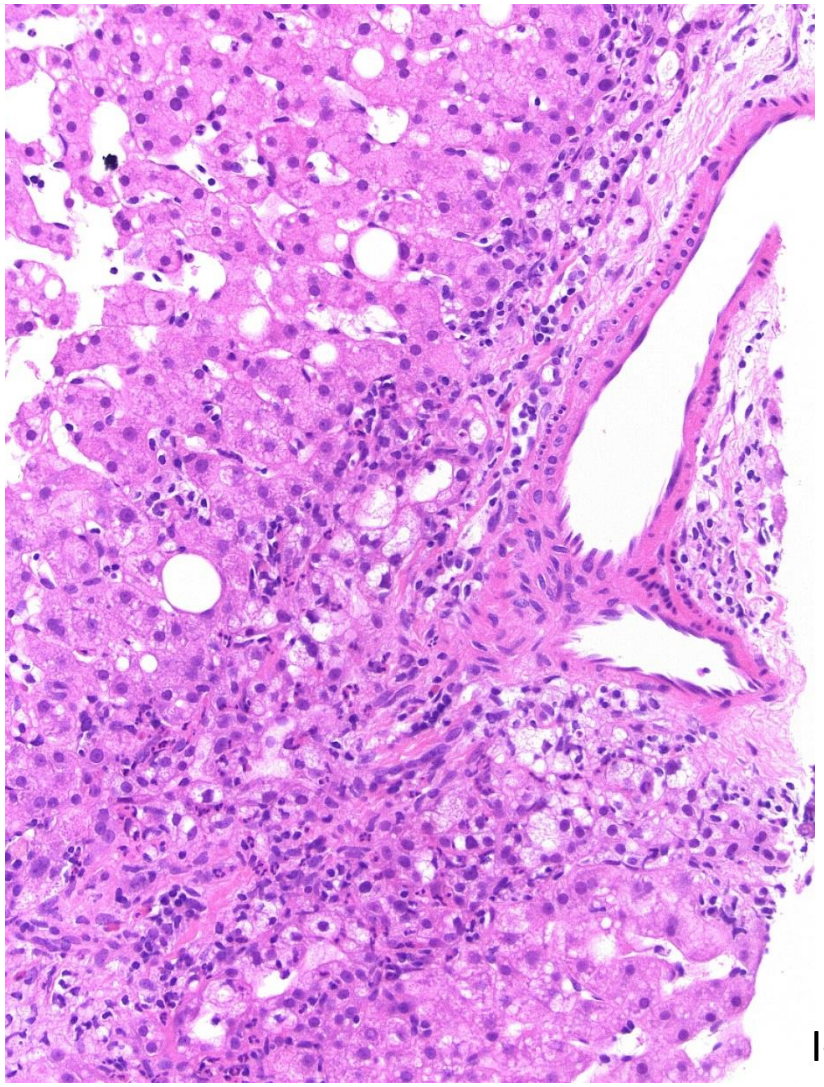
glutamine synthetase in FNH

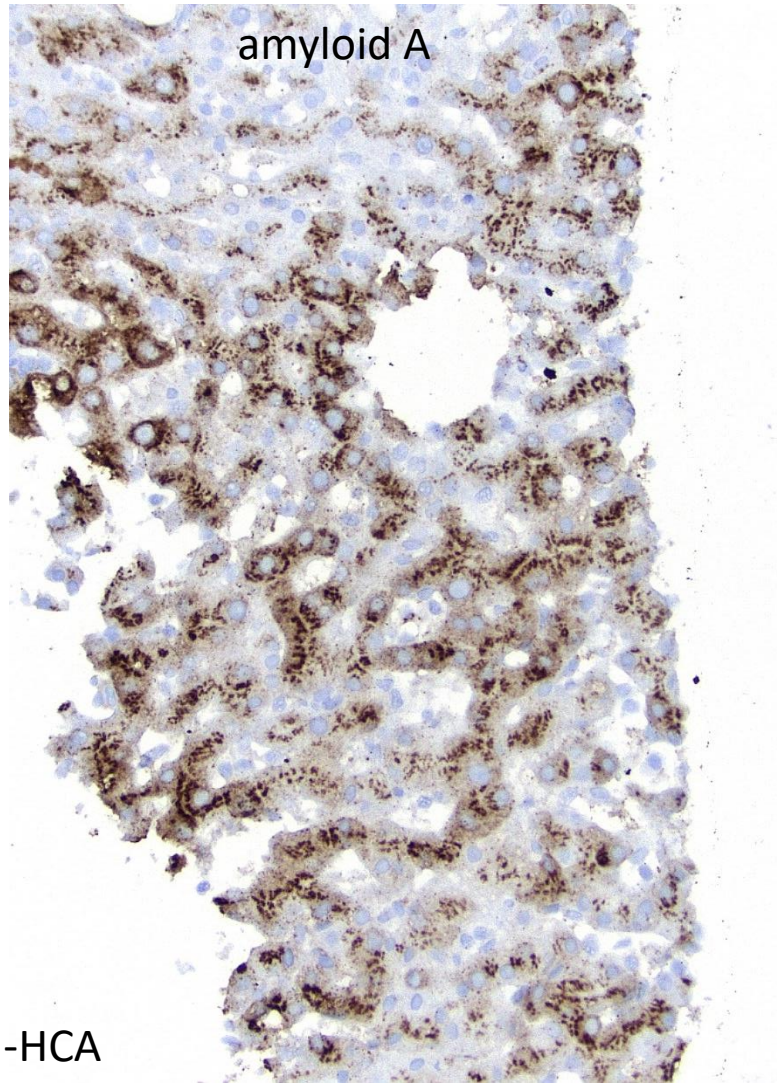
Inflammatory HCA vs FNH - consult





Inflammatory HCA vs FNH - consult

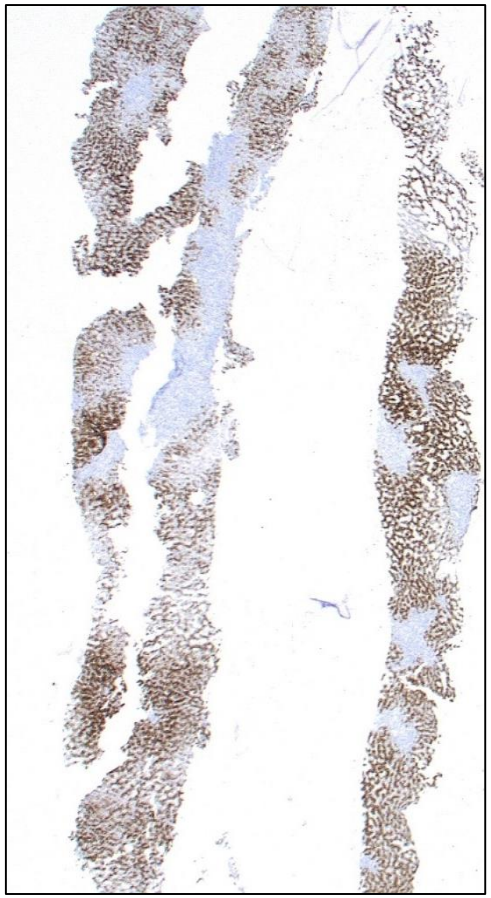




I-HCA

GS

amyloid A



IHC pitfalls - always include NT if possible

- HCC can lose LFABP or gain SAA/CRP
  - they are not specific for adenoma

Bioulac-Sage et al. Clin Res Hepatol Gastroenterol 2014; 38:245
Miller G et al. Pathology 2018; 50:593

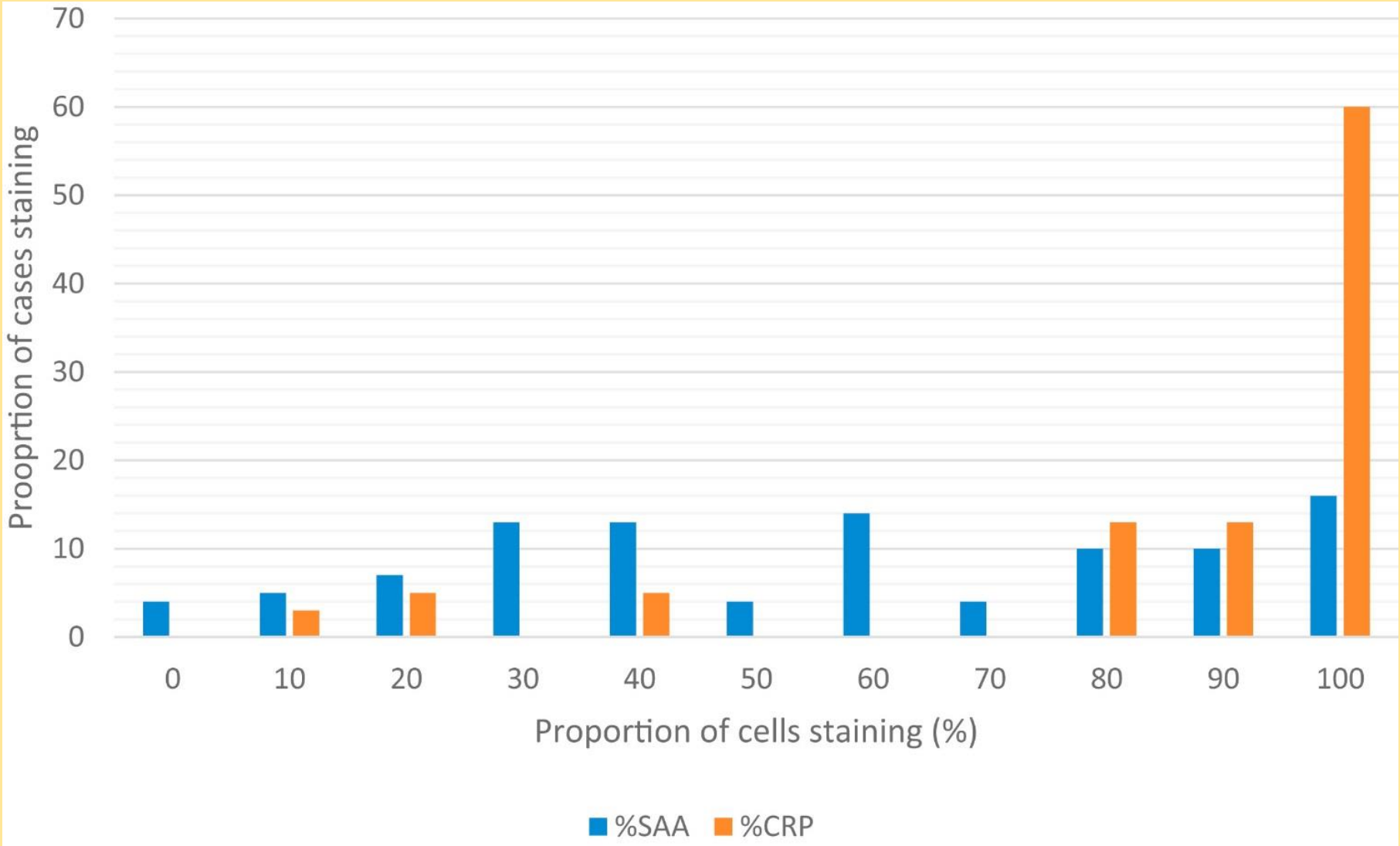
IHC pitfalls - always include NT if possible

- HCC can lose LFABP or gain SAA/CRP
  - they are not specific for adenoma

- SAA can be focal (>25%); occurs in 17% FNH (check GS)
- SAA can increase from inflammation (check NT liver)
- CRP more sensitive for I-HCA but can be non-specific
  - bleeding, embolisation, inflammatory syndrome
  - if adjacent NT liver positive, ignore result
- addition of CRP with SAA increases pickup of I-HCA (together detect 90%)

Bioulac-Sage et al. Clin Res Hepatol Gastroenterol 2014; 38:245
Miller G et al. Pathology 2018; 50:593

Fig. 2

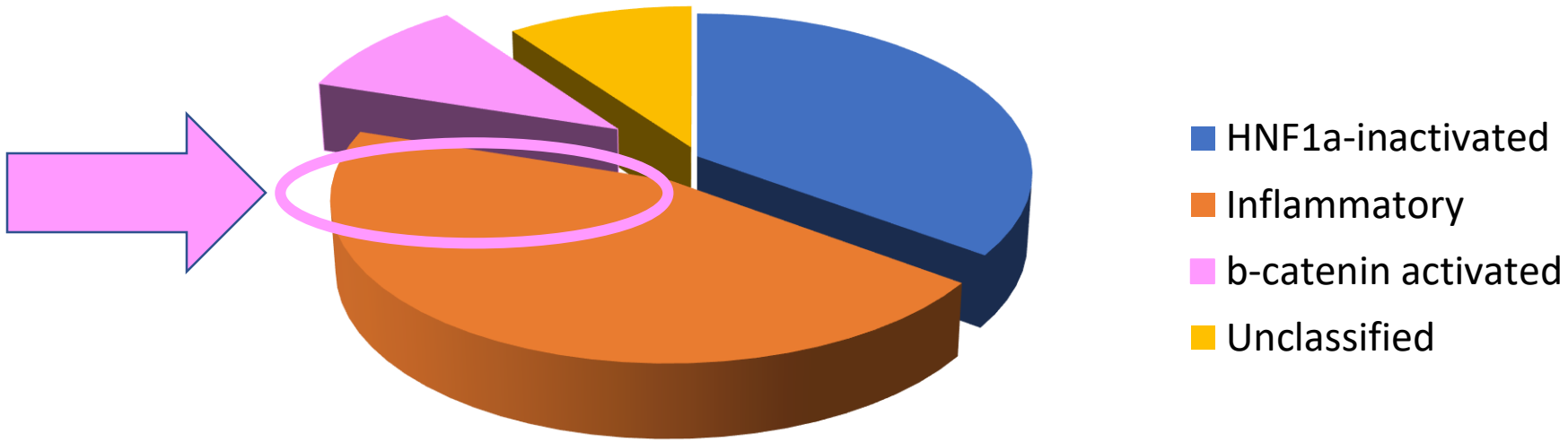


Pathology 2018 50, 593-599DOI: (10.1016/j.pathol.2018.05.003)

Frequency HCA subtypes

	H-HCA	I-HCA	I+B-HCA	B-HCA	U-HCA
France	35	40	7	10	10
UCSF	29	32	3	0	36
Rotterdam	19	56	7	7	11
Brisbane	29	50	9	3	9

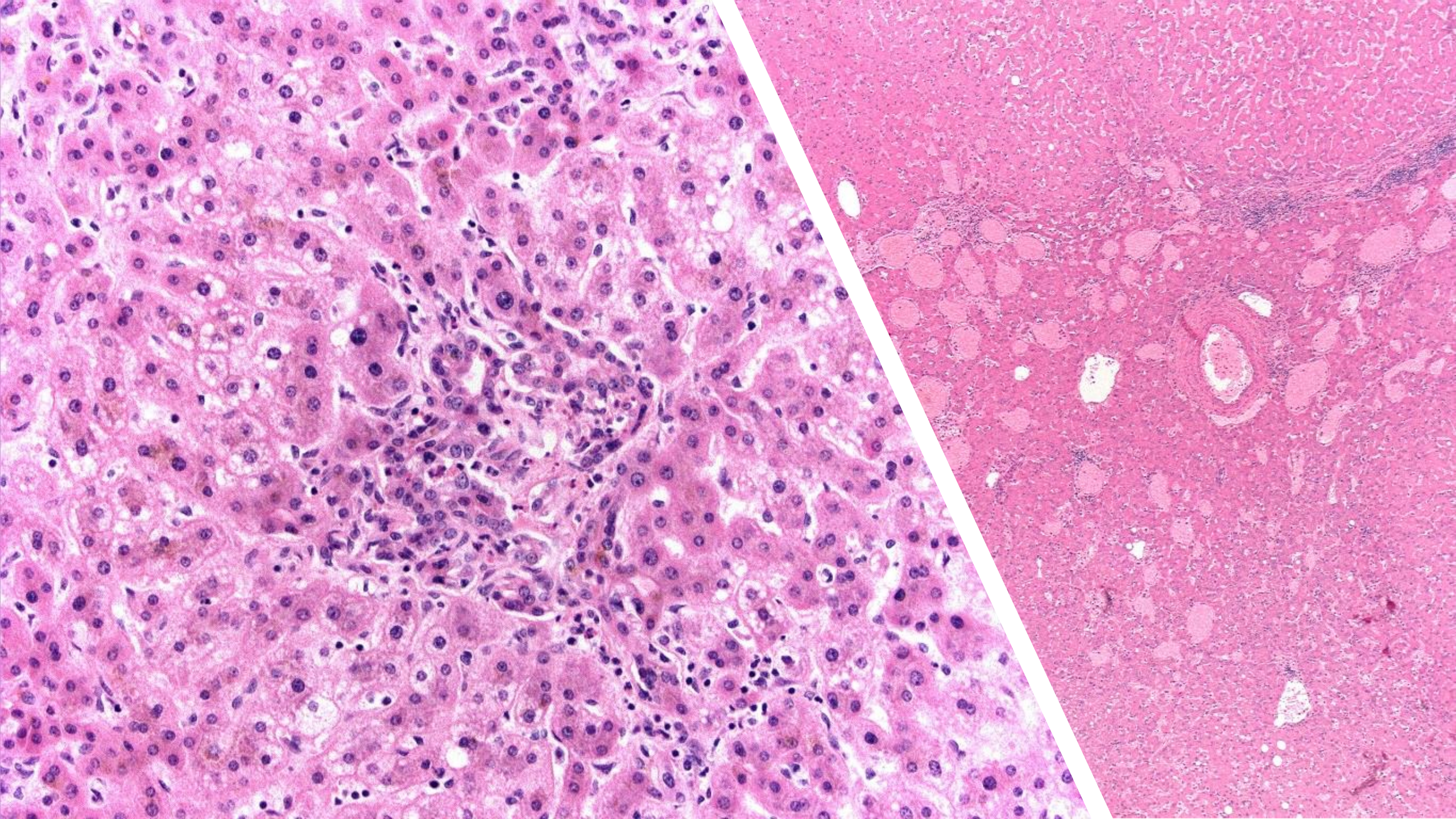
Combined I-HCA with β-catenin mutation (BI-HCA)



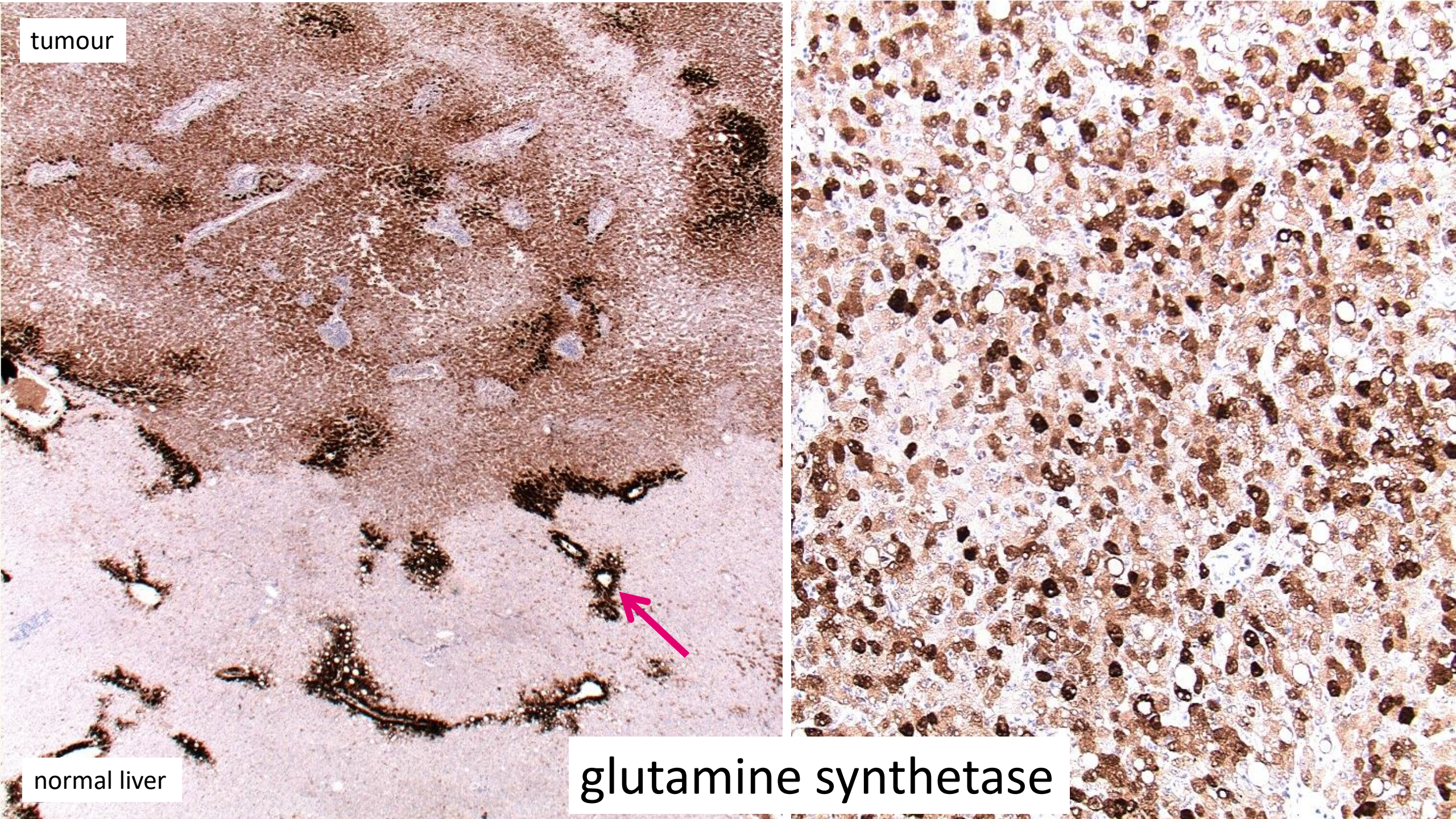
Case – hepatocellular adenoma

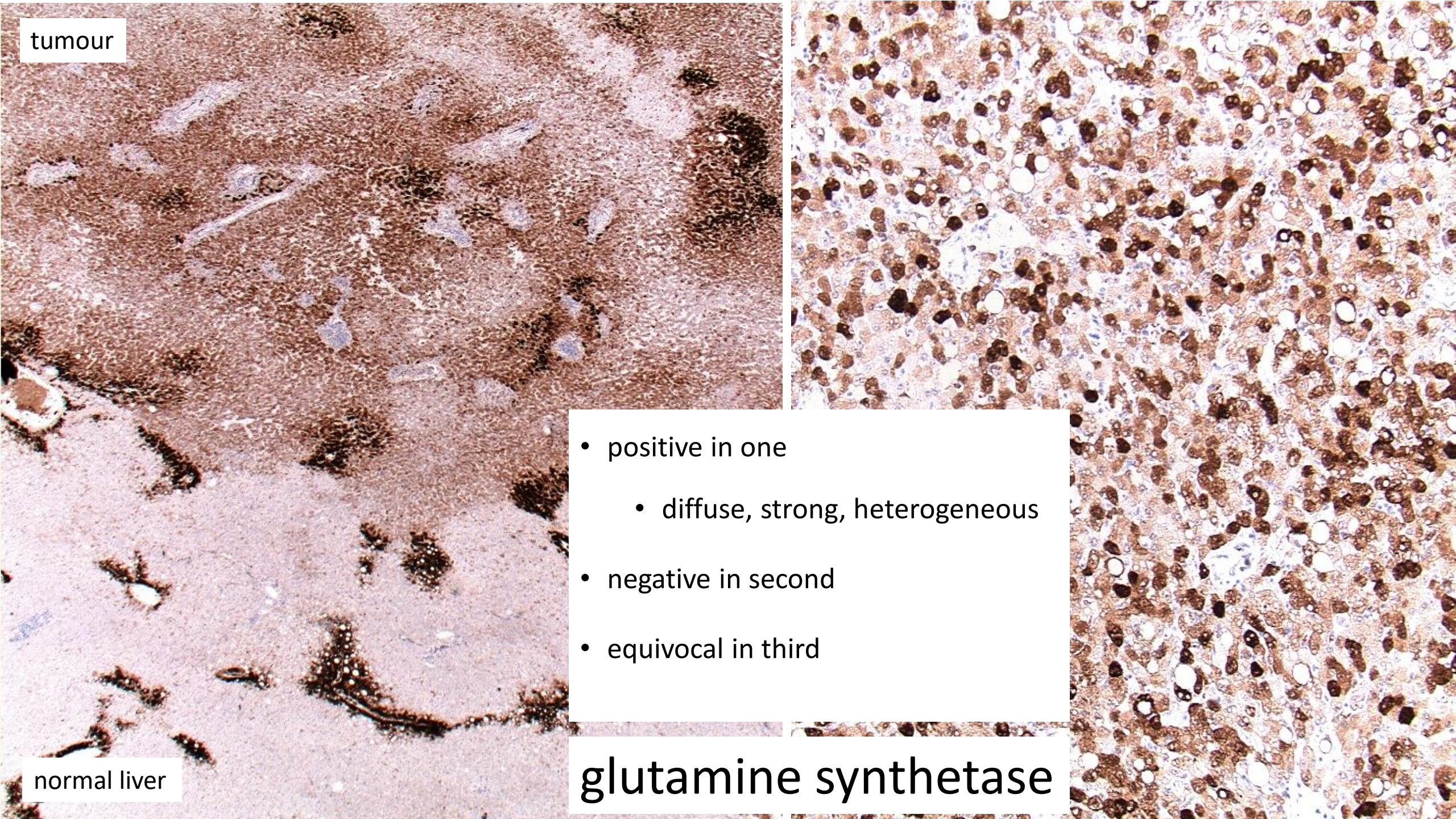
- consultation

- 35 yr female
- 3 lesions in liver - resected




amyloid A


tumour
normal liver
glutamine synthetase



- positive in one
  - diffuse, strong, heterogeneous
- negative in second
- equivocal in third

glutamine synthetase

Hepatocellular adenoma

Inflammatory HCA with β-catenin activation (c/w exon 3 mutation)

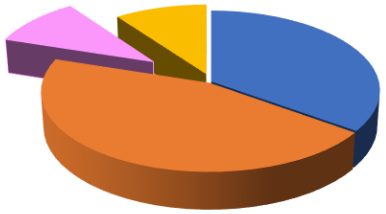
Unclassified HCA

- Unclassified HCA (10%)
  - some have found activation of sonic hedgehog
  - (fusion *INHBE* and *GLI1*) – 4%
    - prostaglandin D synthase IHC marks these

  - one group suggested expression with ASS1 IHC
  - may be downstream in Shh pathway
  - BUT, also expressed in other HCA types if haemorrhage

Nault JC et al. Gastroenterol 2017; 152:880
Nault JC et al. Hepatol 2018; 68:964
Henriet E et al. Hepatol 2017: 66:2016

#4. β-catenin-activated adenoma



- activating mutation of β-catenin (exon 3)

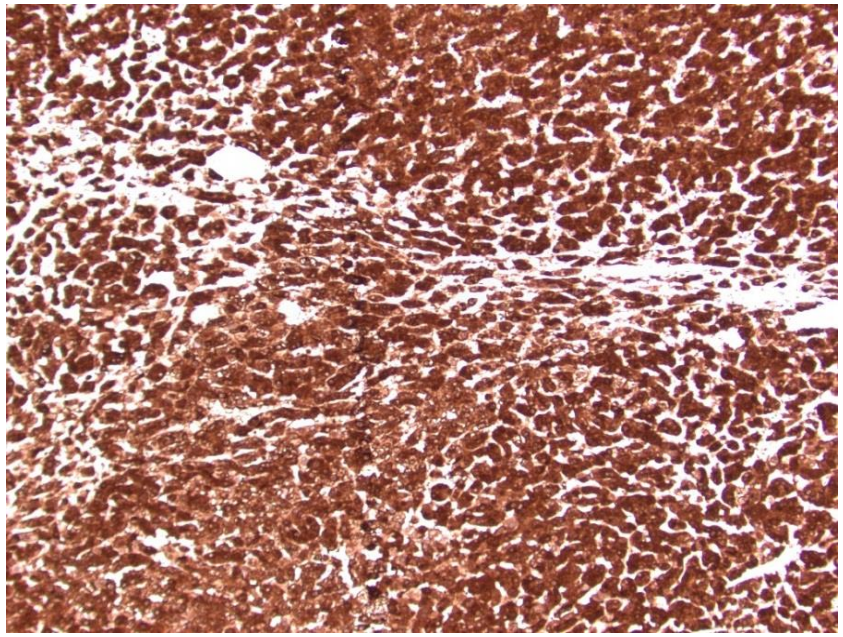
- often have atypia
- no fat
- increased risk of malignant transformation

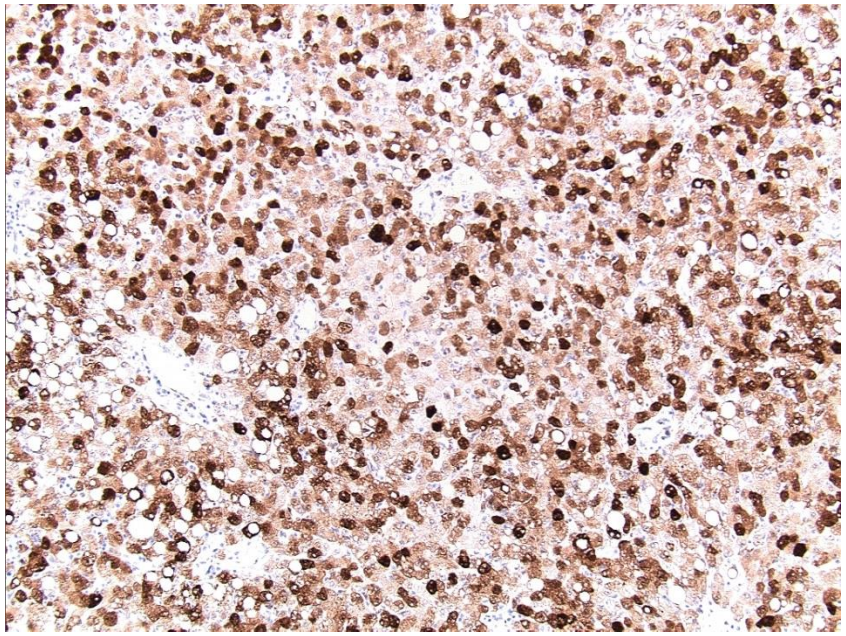
GS - exon 3 patterns (diffuse ie. >50%)

diffuse homogeneous

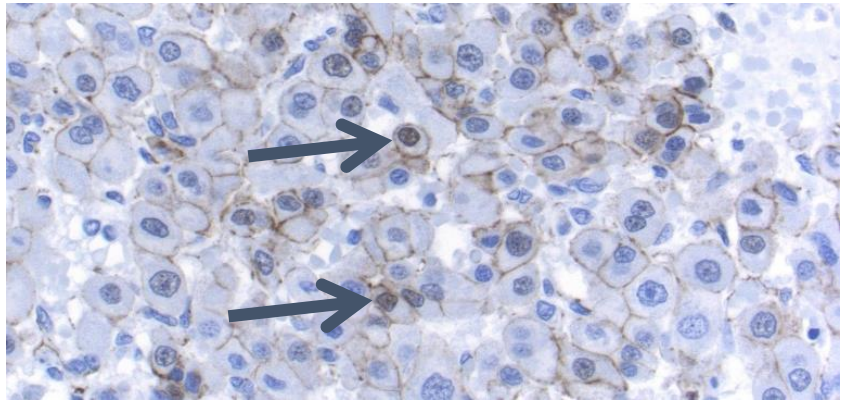
diffuse heterogeneous

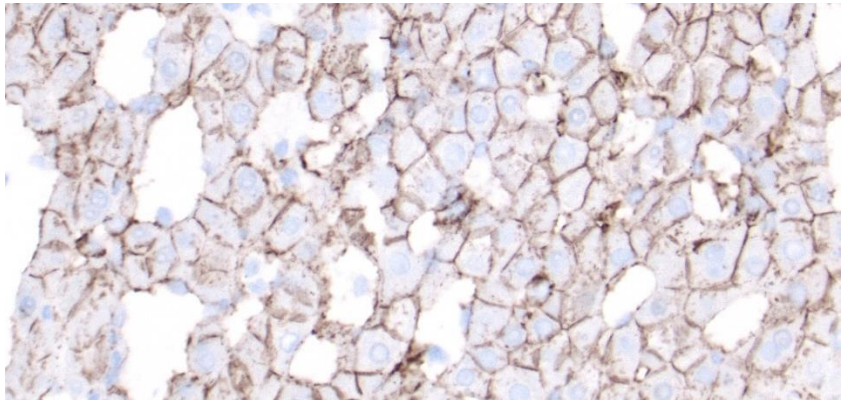
GS



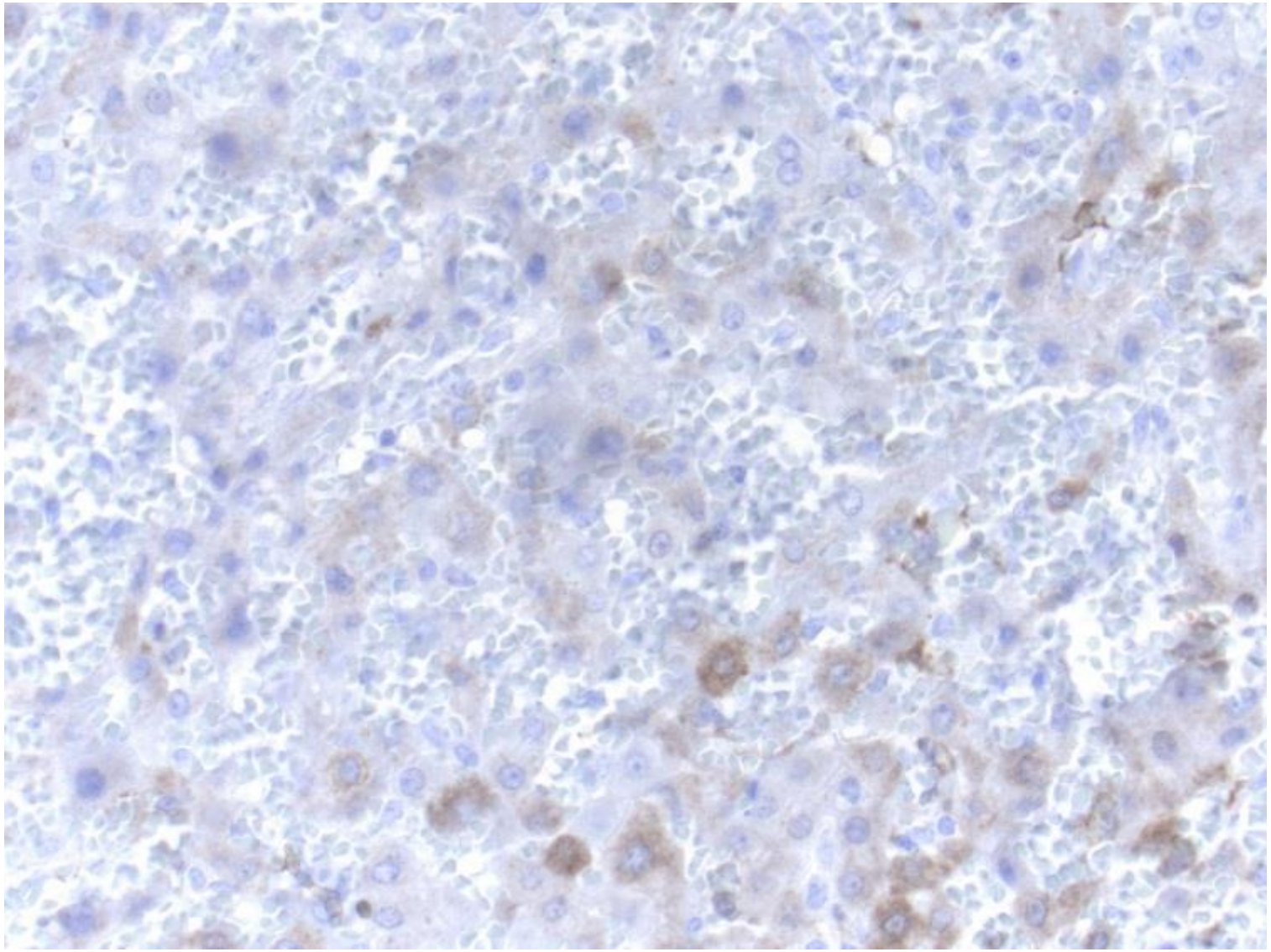


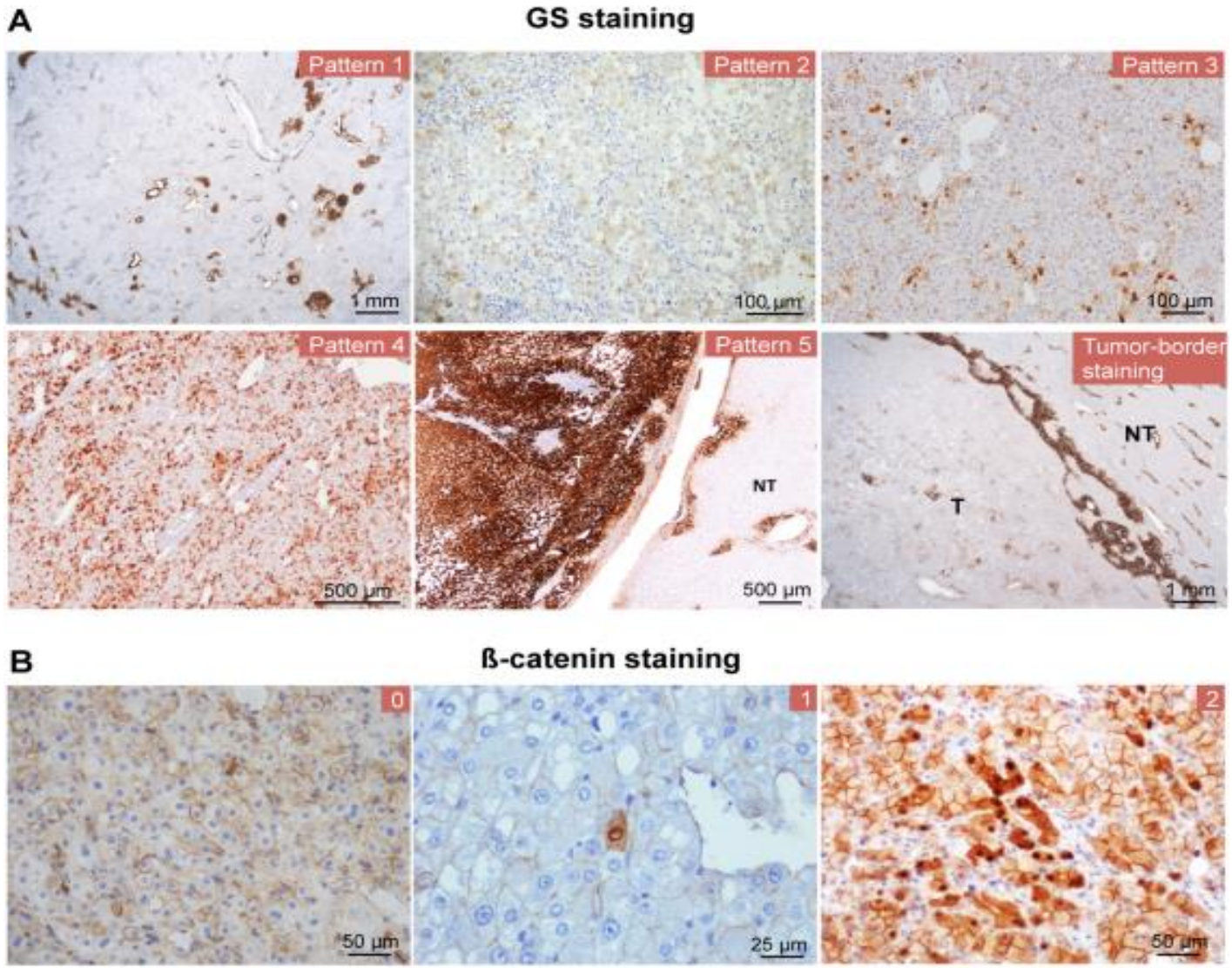
β-cat





GS – “non-specific” pattern (exon 7/8)





Rebouissou S et al. Hepatology 2016; 64:2047

Table 2
Beta-catenin mutations in hepatic adenomas

Mutation	Activation of Wnt Pathway Downstream Targets	Beta-Catenin Nuclear Accumulation	Glutamine Synthetase	Risk of Hepatocellular Carcinoma
Large exon 3 deletions	Strong	>1% of nuclei	Strong and diffuse	High
Exon 3 deletion D32–37	Strong	>1% of nuclei	Strong and diffuse	high
Exon 3, T41	Moderate	>1% of nuclei	Strong and diffuse	High
Exon 3, S45	Weak	Absent to rare positive nuclei	Moderate to strong, patchy	Low
Exon 7, K335	Weak	Absent	Weak, patchy, or perivenular	Low
Exon 8, N387	Weak	Absent	Weak, patchy, or perivenular	Low

Torbenson M. Surg Pathol Clin 2018; 11(2):351-66

IHC issues - always include NT if possible

- glutamine synthetase and β-catenin
- several patterns now recognised for GS

>> strong diffuse (>50%), homogeneous or heterogeneous

(exon 3 mutation – associated with increased HCC)

Rebouissou S et al. Hepatology 2016; 64:2047

IHC issues - always include NT if possible

- glutamine synthetase and β-catenin
- several patterns now recognised for GS

>> strong diffuse (>50%), homogeneous or heterogeneous

(exon 3 mutation – associated with increased HCC)

>> focal with edge staining

(exon 7/8 mutation – no increase in HCC risk)

- in a perfect world these would be sent for genetic analysis

Rebouissou S et al. Hepatology 2016; 64:2047

Other subtypes & variants

- Androgen adenomas often show atypia, cholestasis, pseudoglands, β-catenin +

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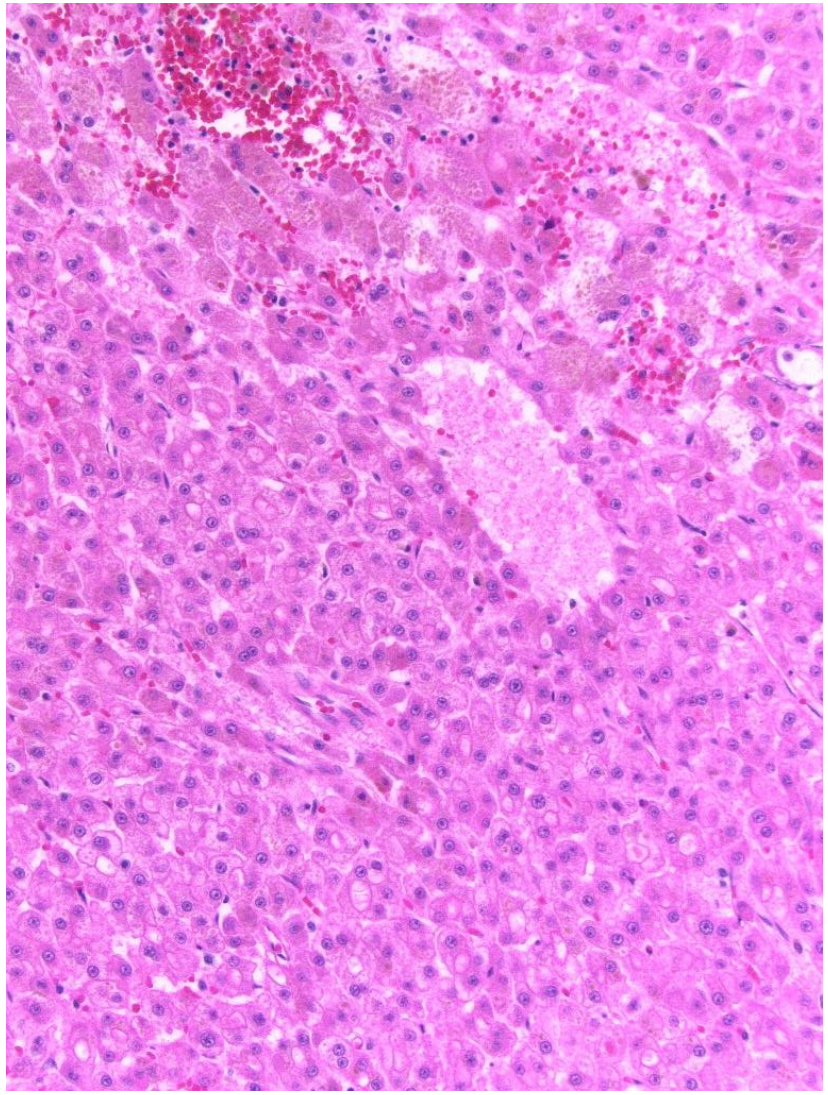
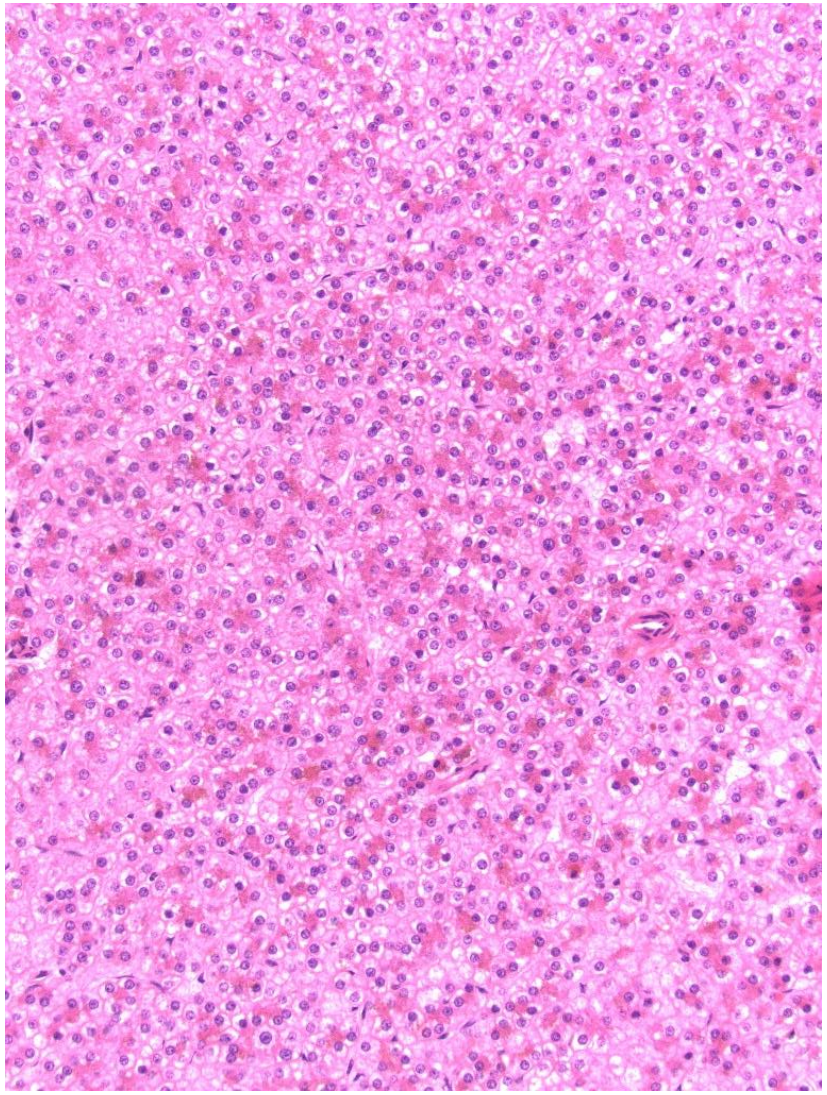
- Androgen adenomas often show atypia, cholestasis, pseudoglands, β-catenin +
- Pigmented adenomas also associated with β-catenin activation and malignancy

Mounajjed T et al. Mod Pathol 2015; 28:1265

Pigmented HCA – heterogeneous group





Other subtypes & variants

- Androgen adenomas often show atypia, cholestasis, pseudoglands, β-catenin +
- Pigmented adenomas also associated with β-catenin activation and malignancy
- Myxoid HCA are very rare but appear to have increased malignancy

Salaria SN et al AJSP 2015; 39:318
Torbenson M. Surg Pathol Clin 2018; 11:351

HCA – Clinical outcomes

- obesity (& alcohol) driving newer cases
- if multiple, ~30% have >1 subtype

- 14% bleeding (>5cm)
- more bleeding with I-HCA & U-HCA

- 3% malignant change (definite HCC nodule)
- 7% borderline between HCA and HCC

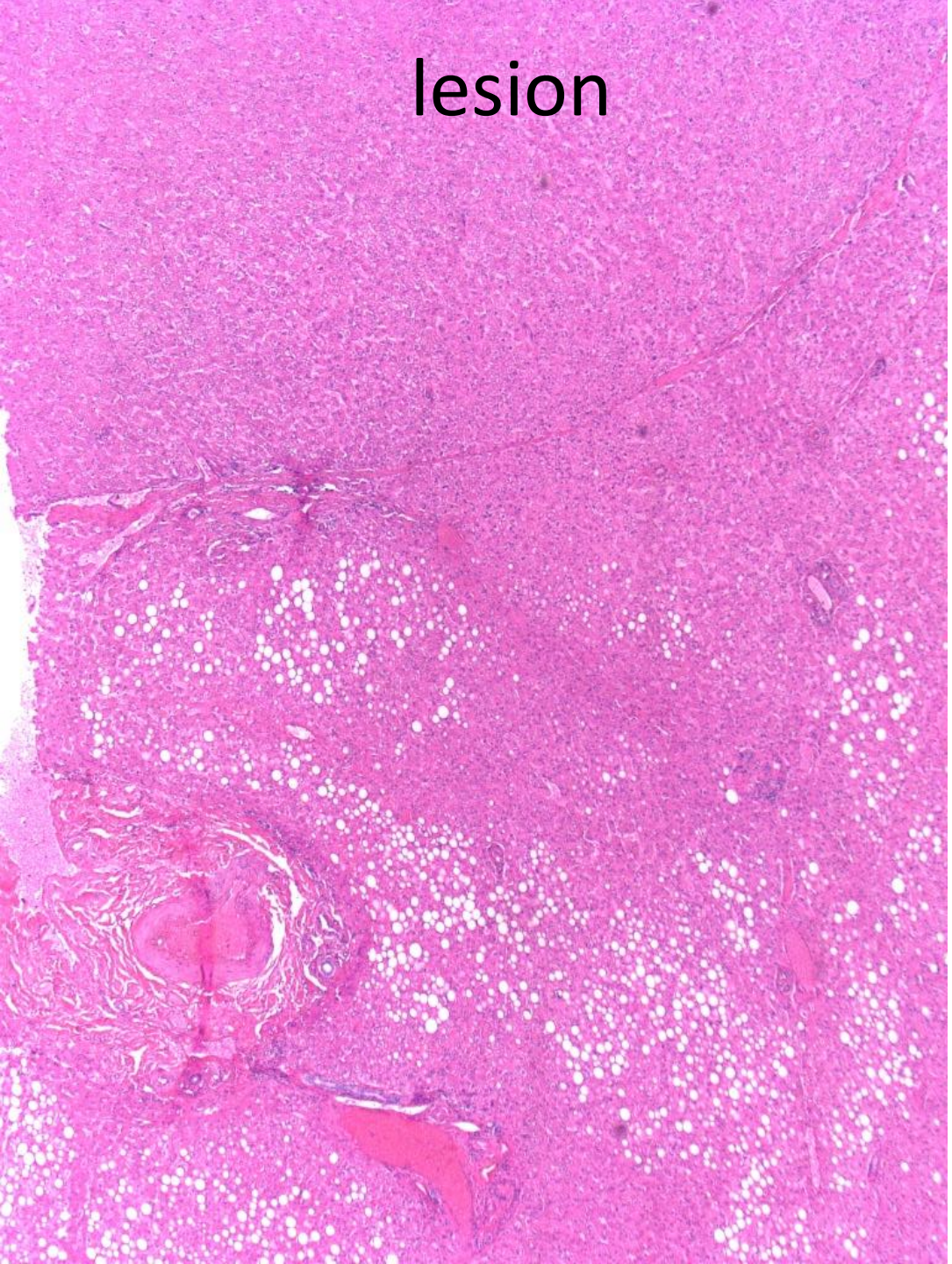
Nault JC et al. Gastroenterol 2017; 152:880
Castain C et al. Histopathol 2014; 64:890

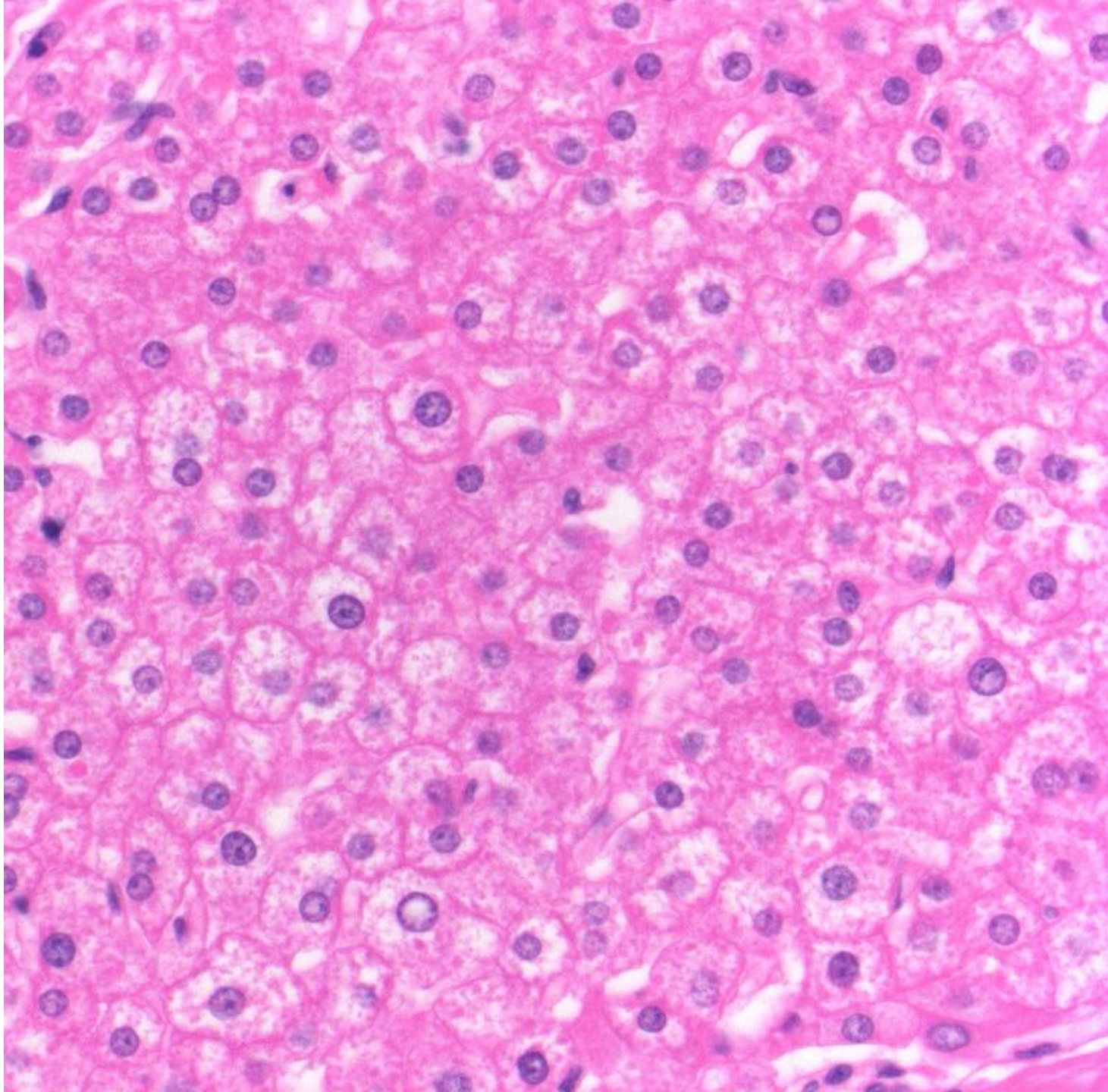
Borderline tumours

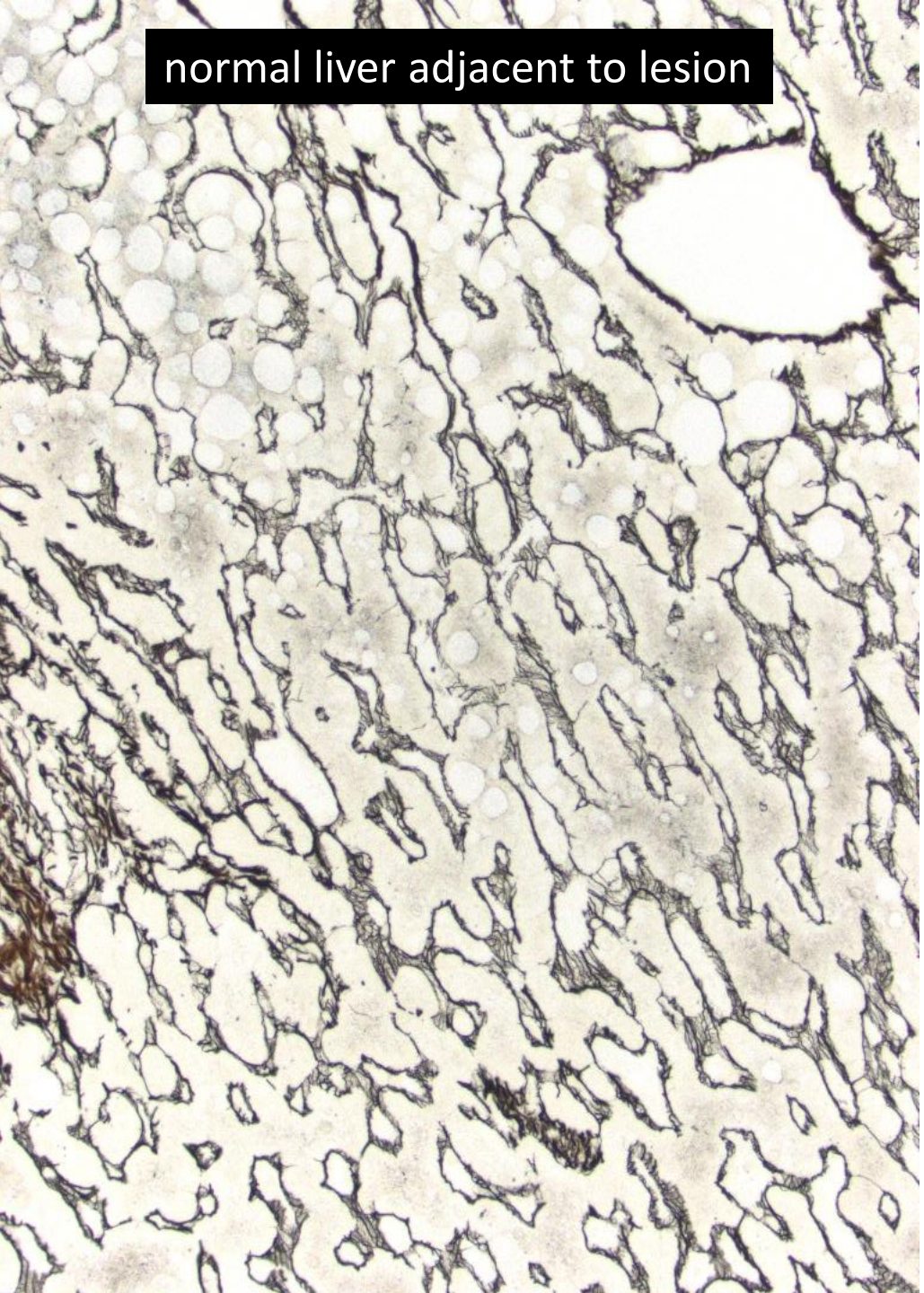
- 30yo female - epigastric pain
- multiple lesions (consistent with HCA) in both lobes
- 8cm pedunculated lesion segment 2
- >> evidence of internal haemorrhage / necrosis.

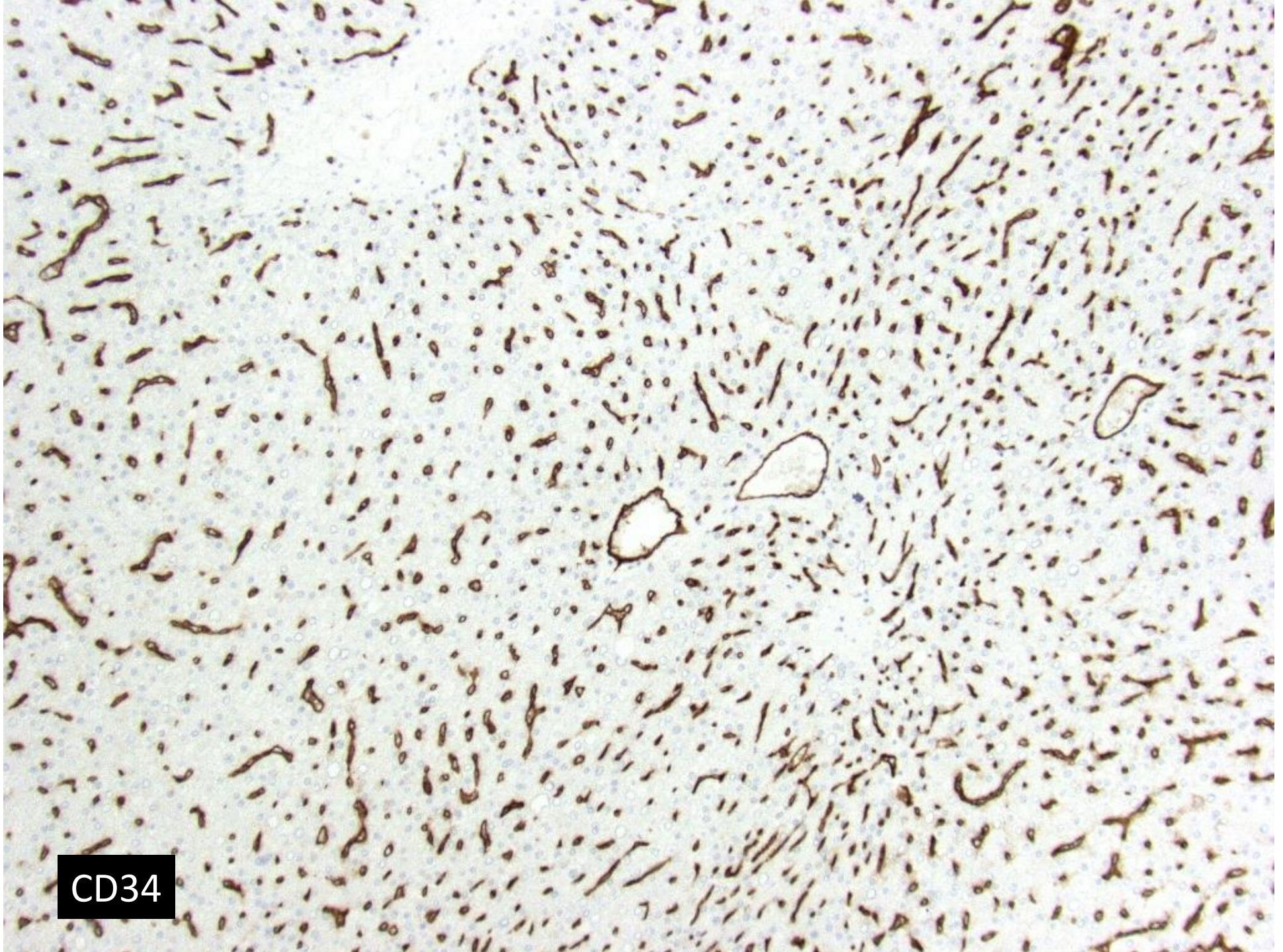

mm
cm
1
2
3
4


mm
cm
1
2
3
4

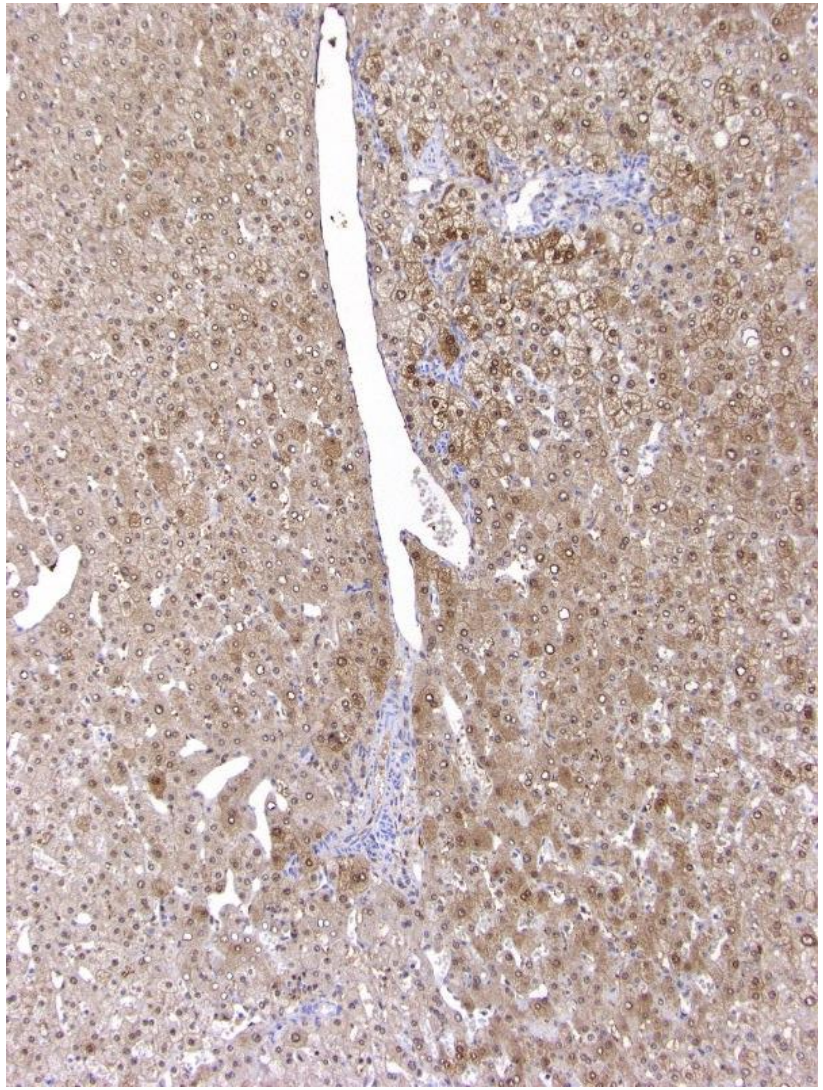

lesion



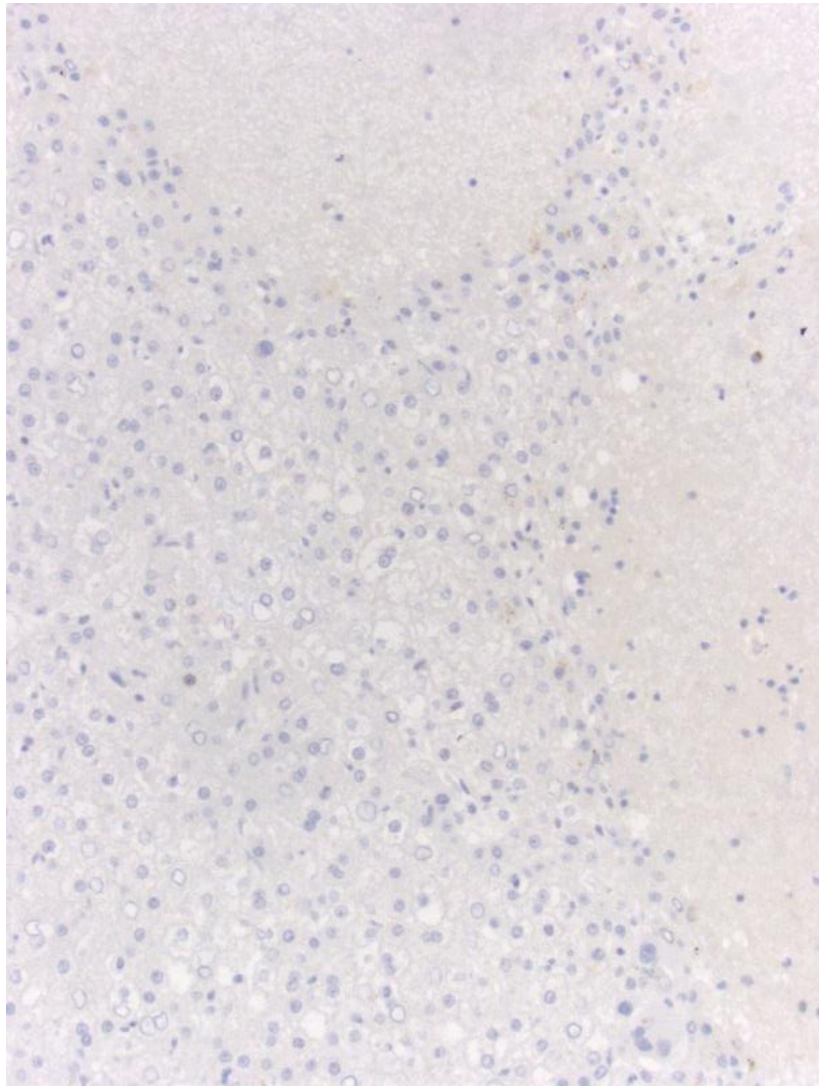

normal liver adjacent to lesion


CD34

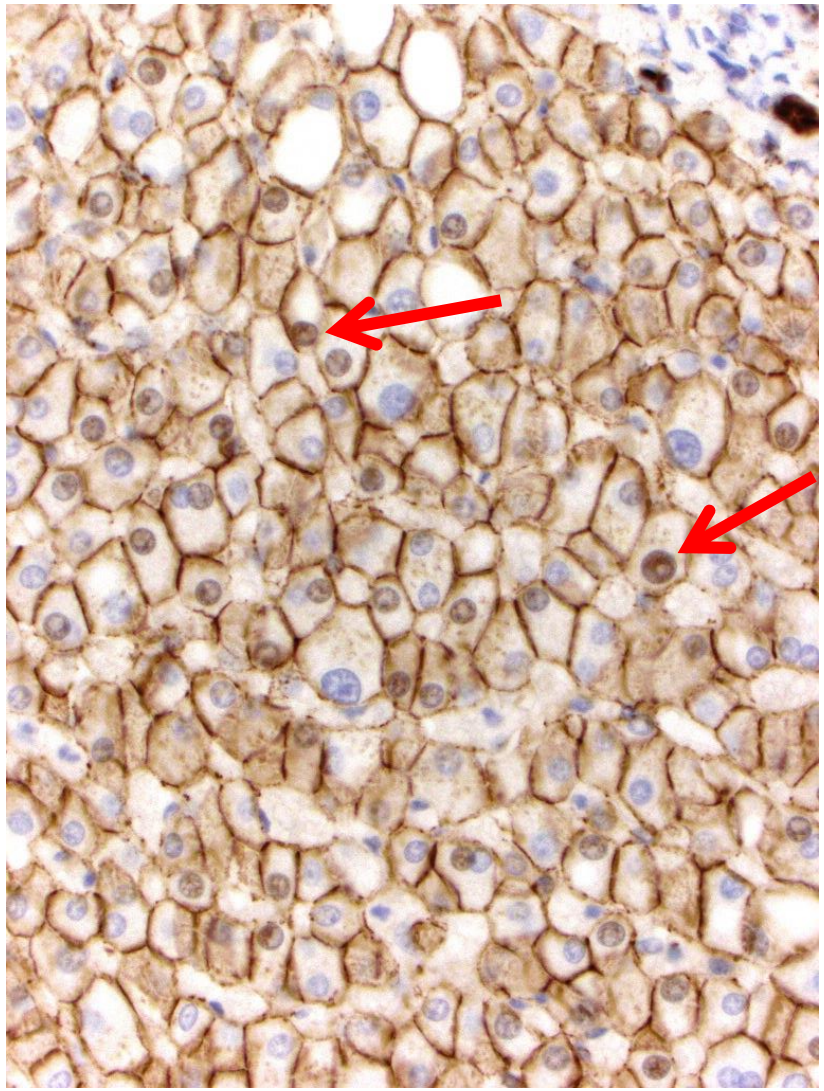
Ifabp



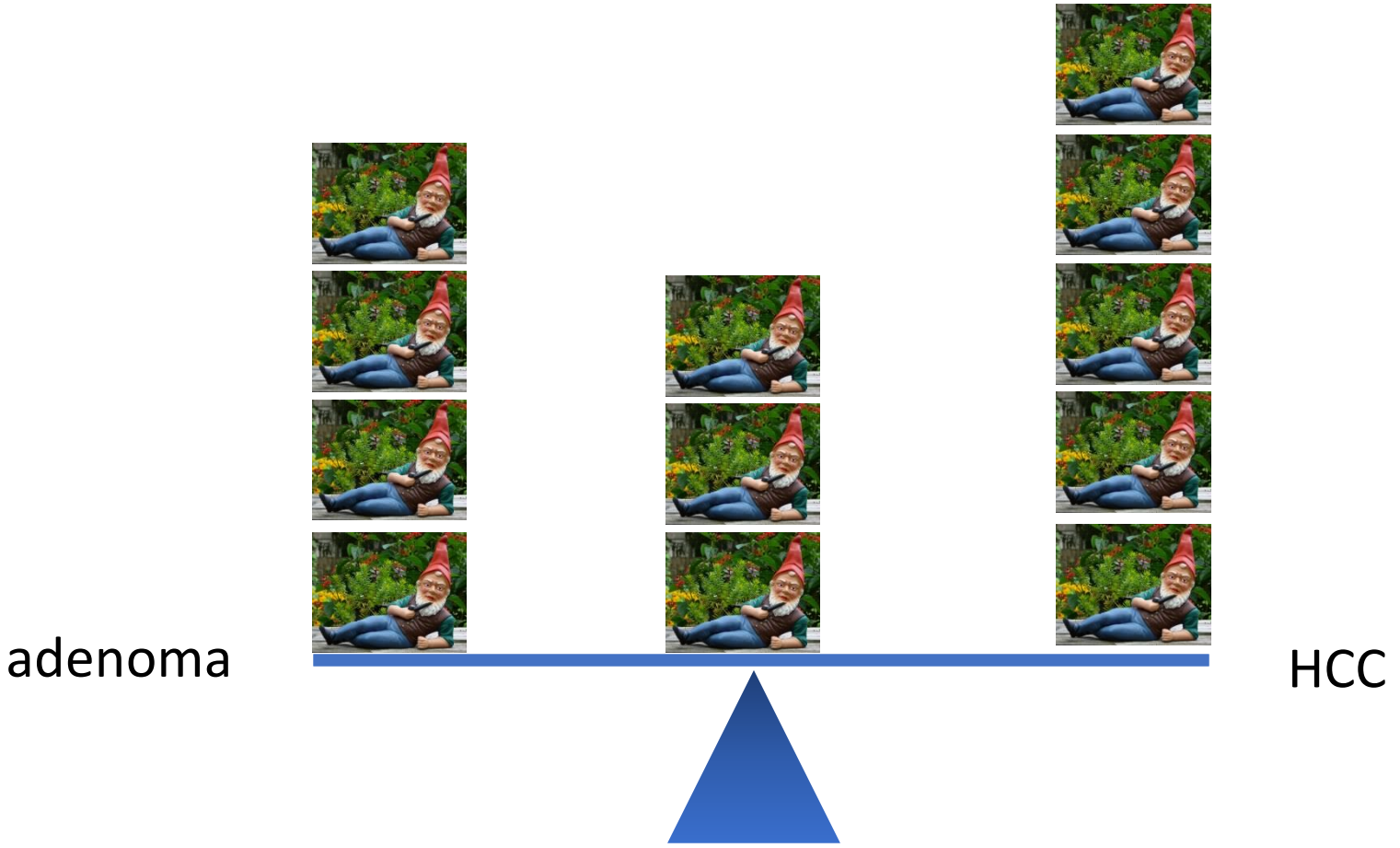
SAA



b-catenin



Experts' diagnoses



Bedossa P et al. Hum Pathol 2014; 45:658

Borderline lesions described

Genotype–Phenotype Correlation in Hepatocellular Adenoma: New Classification and Relationship With HCC

Jessica Zucman-Rossi,[1] Emmanuelle Jeannot,[1] Jeanne Tran Van Nhieu,[2] Jean-Yves Scoazec,[3] Catherine Guettier,[4] Sandra Rebouissou,[1] Yannick Bacq,[5] Emmanuelle Leteurtre,[6] Valérie Paradis,[7] Sophie Michalak,[8] Dominique Wendum,[9] Laurence Chiche,[10] Monique Fabre,[11] Lucille Mellottee,[1] Christophe Laurent,[12] Christian Partensky,[3] Denis Castaing,[4] Elie Serge Zafrani,[2] Pierre Laurent-Puig,[13] Charles Balabaud,[12,14] and Paulette Bioulac-Sage[14,15]

- 96 lesions
- 6 of 96 “borderline” between adenoma and HCC

Zucman-Rossi et al. Hepatol 2006; 43:515

Borderline lesions

- recently recognised as a problem
- liver study group reviewed lesions
  >> often equal split over HCA vs HCC
- Evason et al 2013

Human Pathology (2013) 44, 750–758

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Original contribution

Atypical hepatocellular adenoma–like neoplasms with β-catenin activation show cytogenetic alterations similar to well-differentiated hepatocellular carcinomas☆,☆☆

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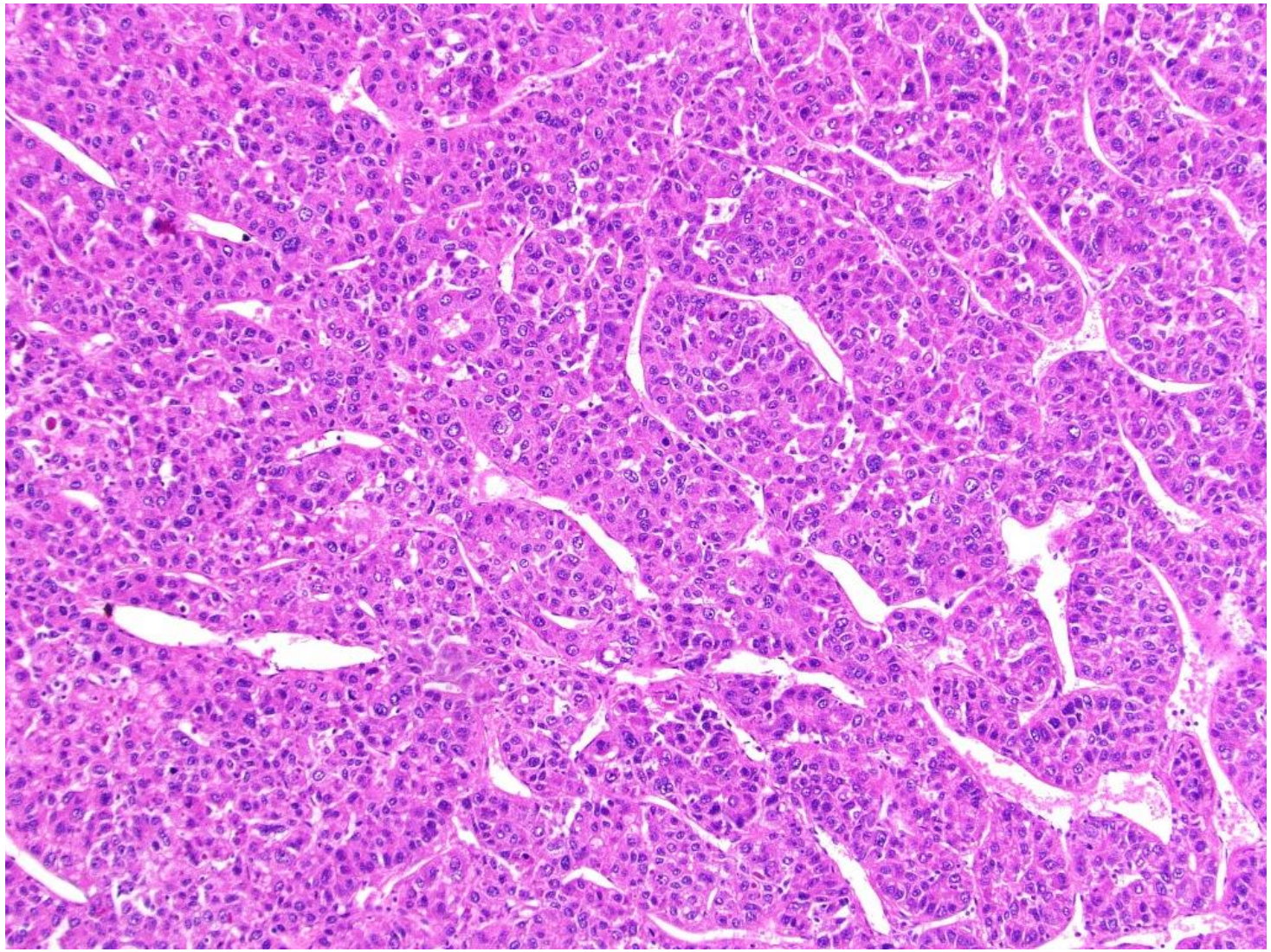
Borderline lesions

- remain incompletely understood
- since 2013 they have probably been OVERDIAGNOSED

- *various names have been used*
- borderline lesion
- hepatocellular neoplasm of uncertain malignant potential (HUMP)
- atypical hepatocellular neoplasm (AHN)

Choi W-T & Kakar S. Adv Anat Pathol 2018; 25:254
- an excellent review

If focal HCC, diagnose HCC ex HCA



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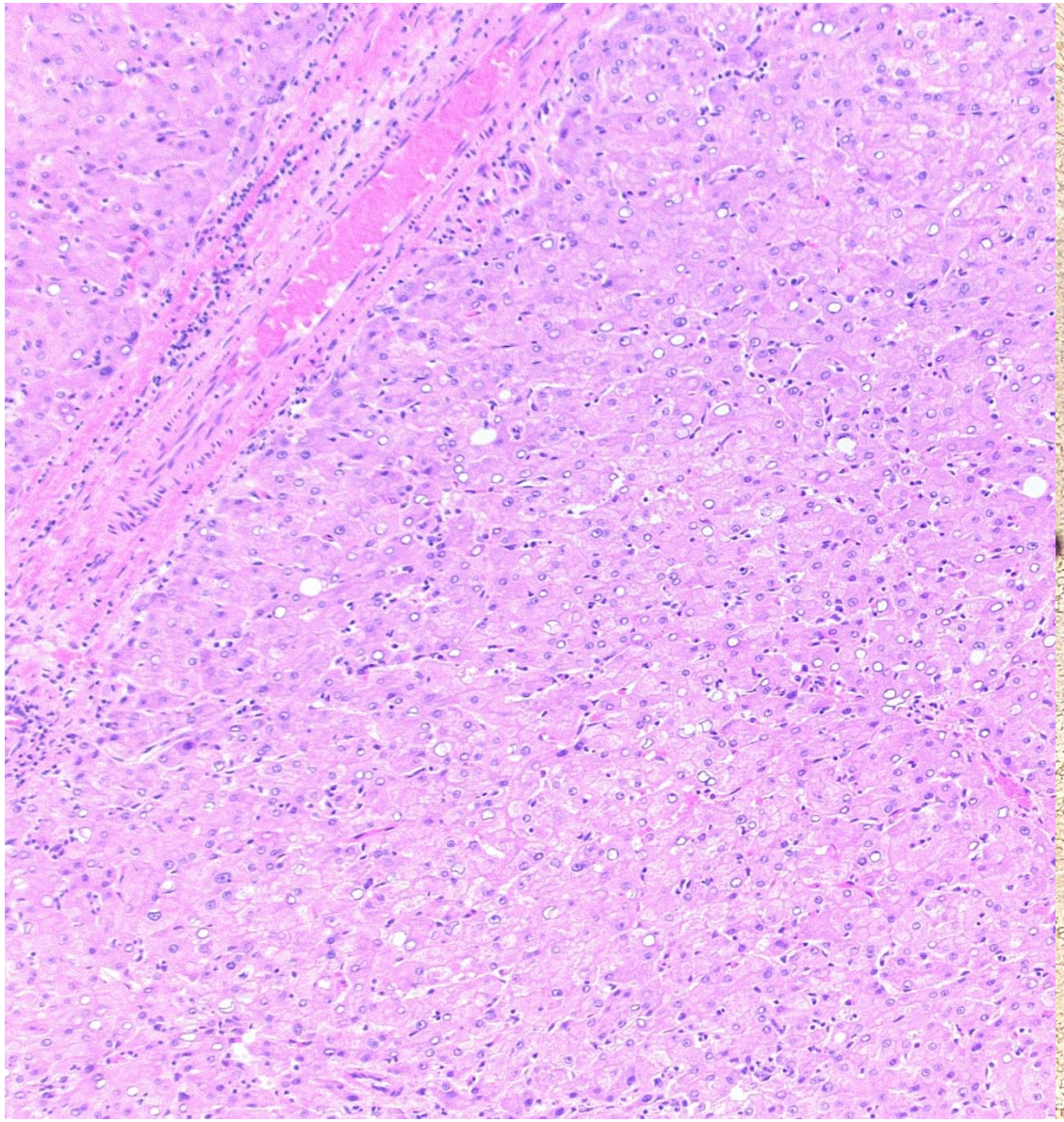
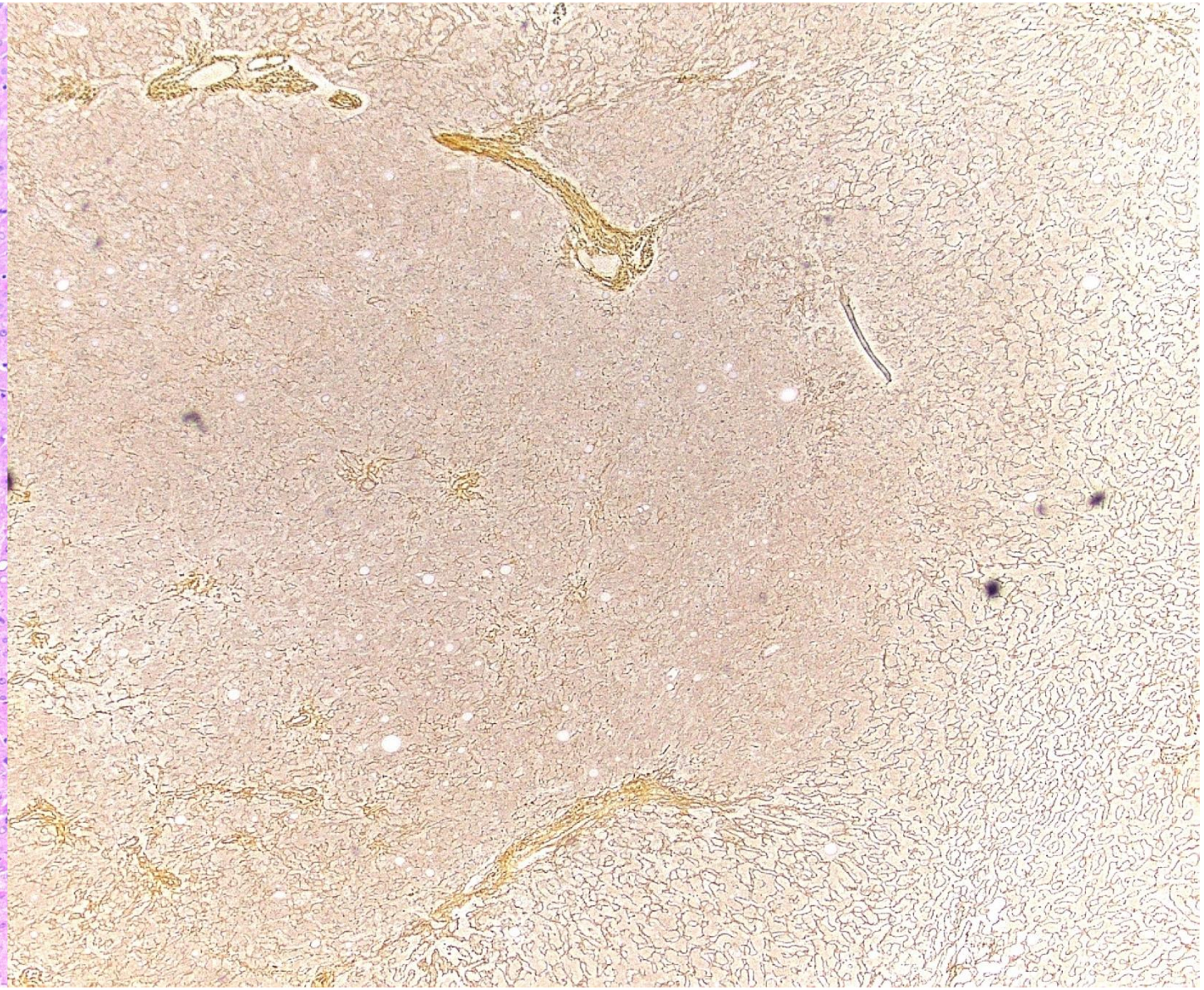




AHN / HUMP

1. Morphological

- arbitrary <5% atypical features (cytological or architectural)
- unifocal reticulin loss, acini, small cell change

2. Clinical

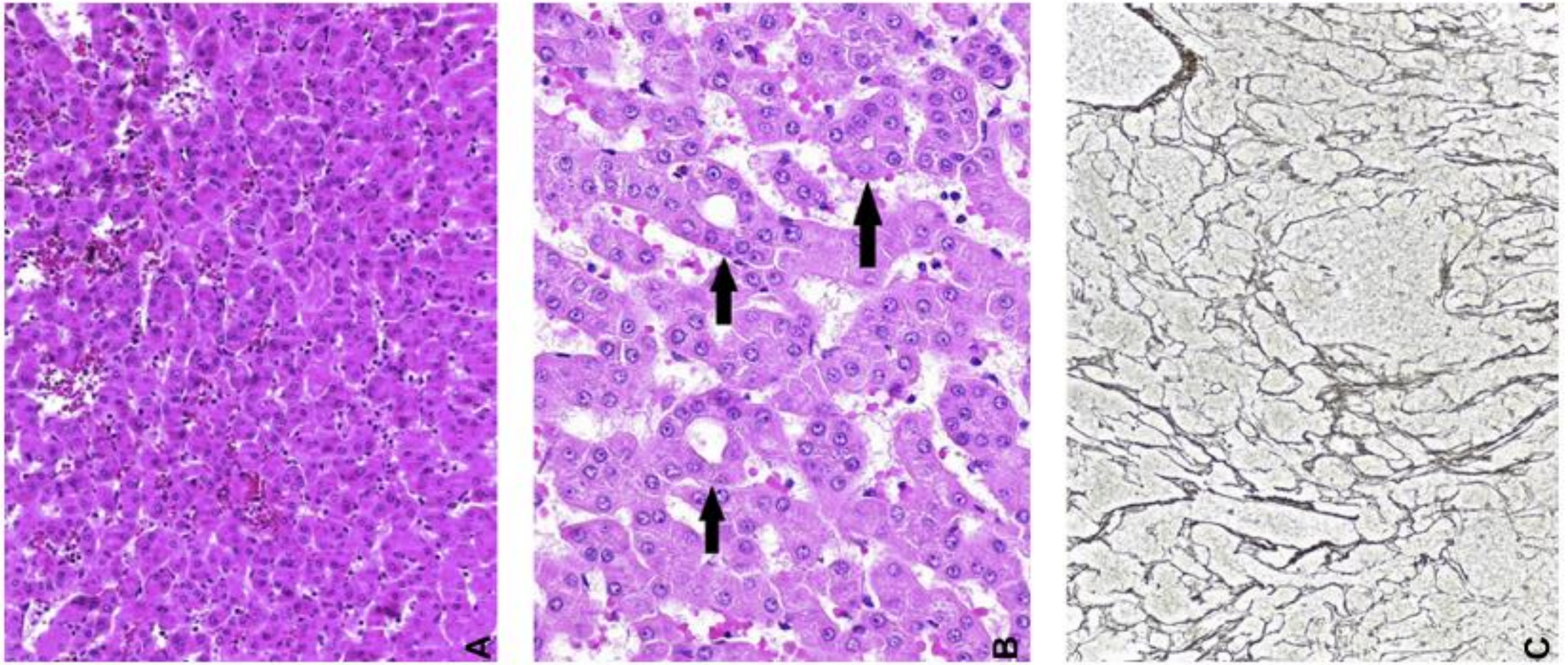
- female >50yo
- males

3. β-catenin-activated tumours

- cytological atypia common

Choi W-T & Kakar S. Adv Anat Pathol 2018; 25:254

AHN / HUMP

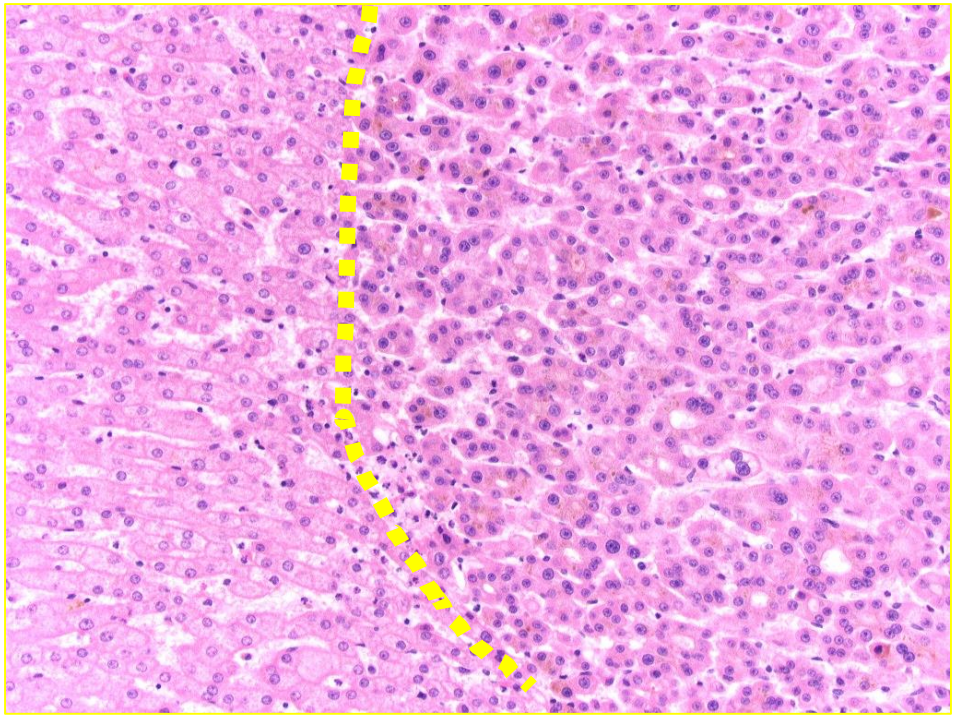


Miller G et al. Pathology 2018; 50:593

Anabolic steroids - tumours

- usually 5-15 years
- can be within 2 years
- usually but not always regress with cessation

- metastasis has been described (Fanconi's)



AHN / HUMP

- whatever the name, more study needed
- these are rare lesions (<1 per year)
- must carefully assess re HCA or HCC
  - must apply routine and IHC stains (reticulin and HCA typing panel, etc)

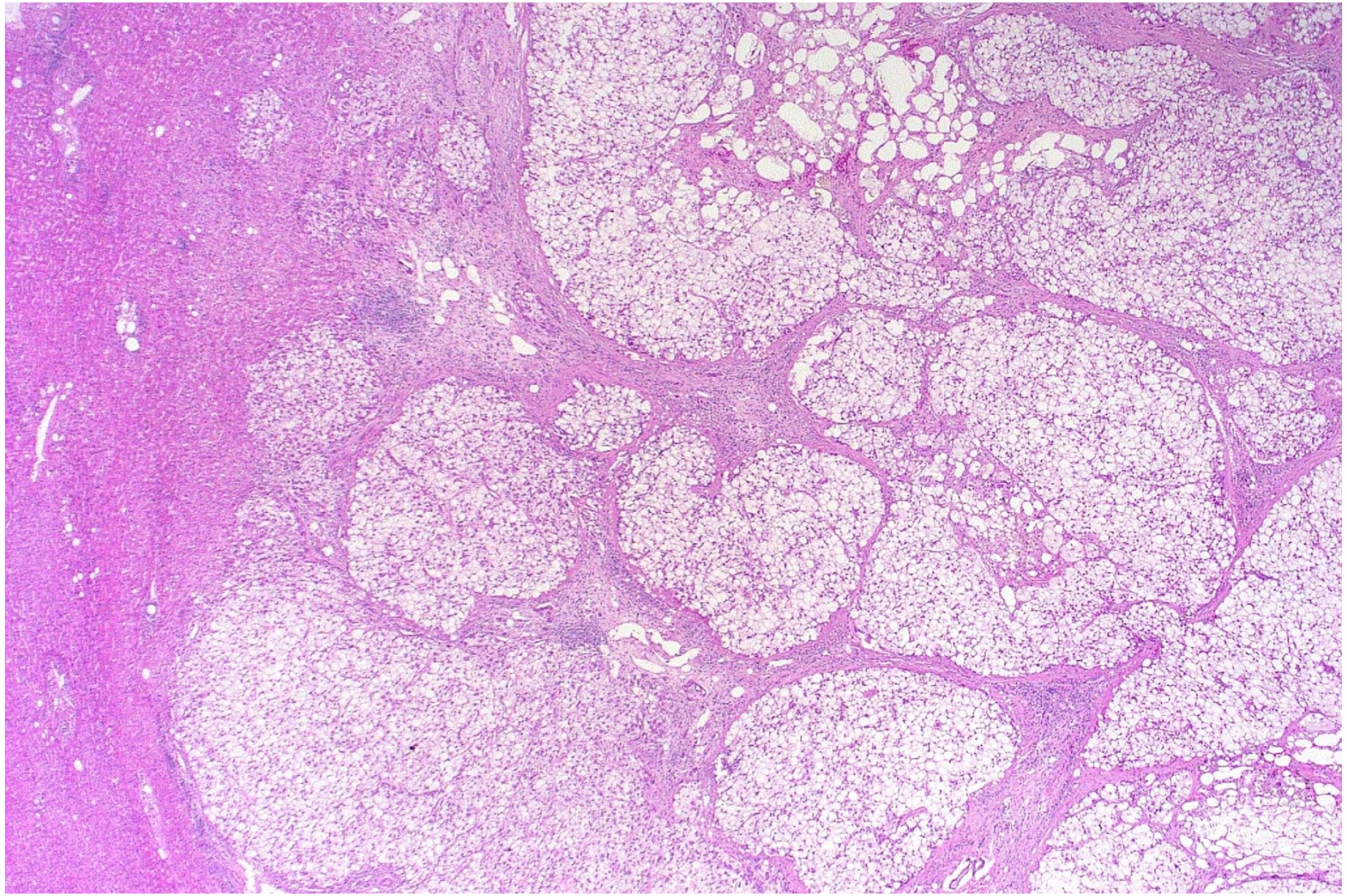
- *"the exact nature of these lesions remains unclear but early studies suggest a progressive course in up to 5-10% of cases"*

HCC variants including combined HCC-CCa

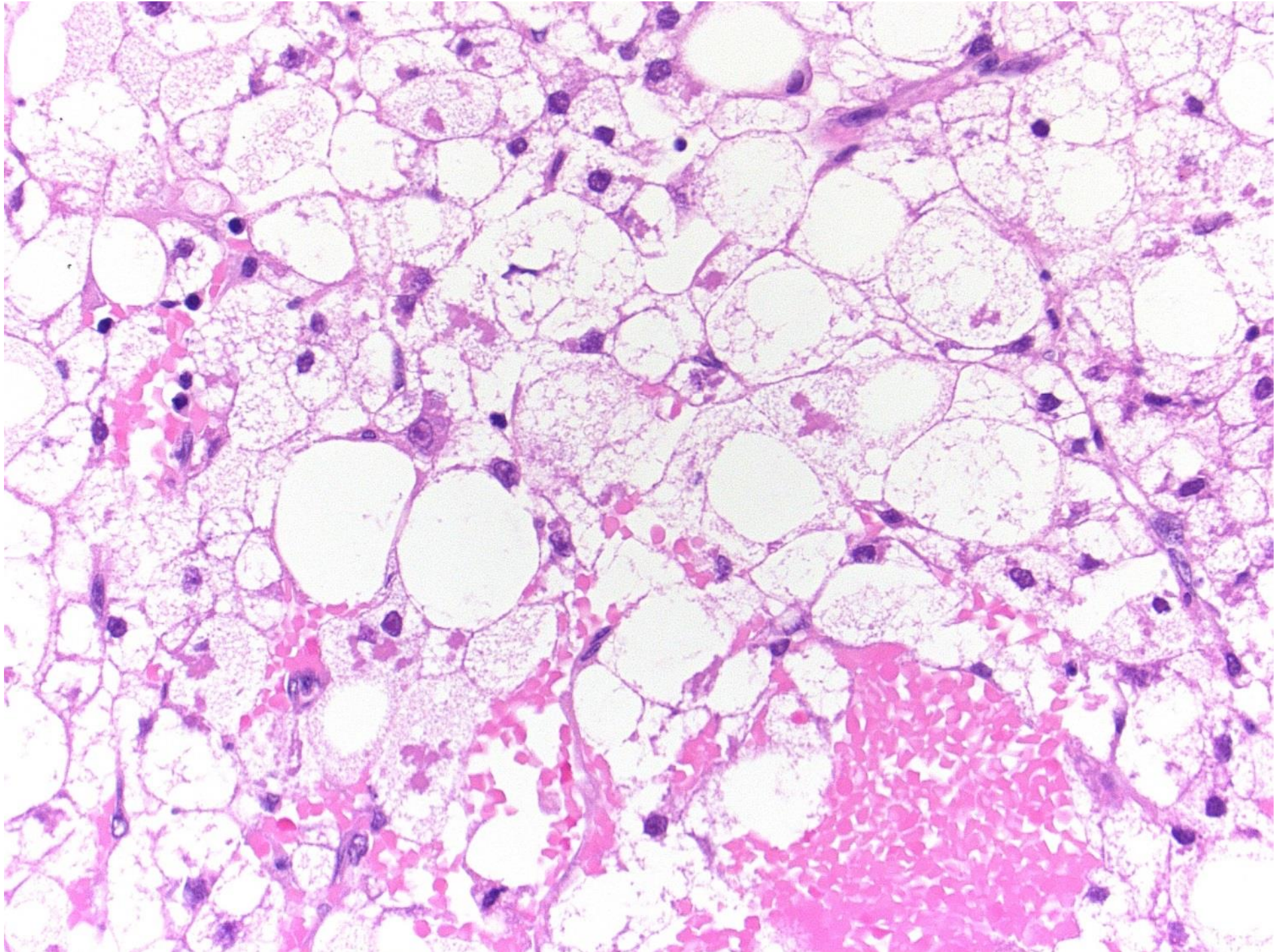
HCC variants

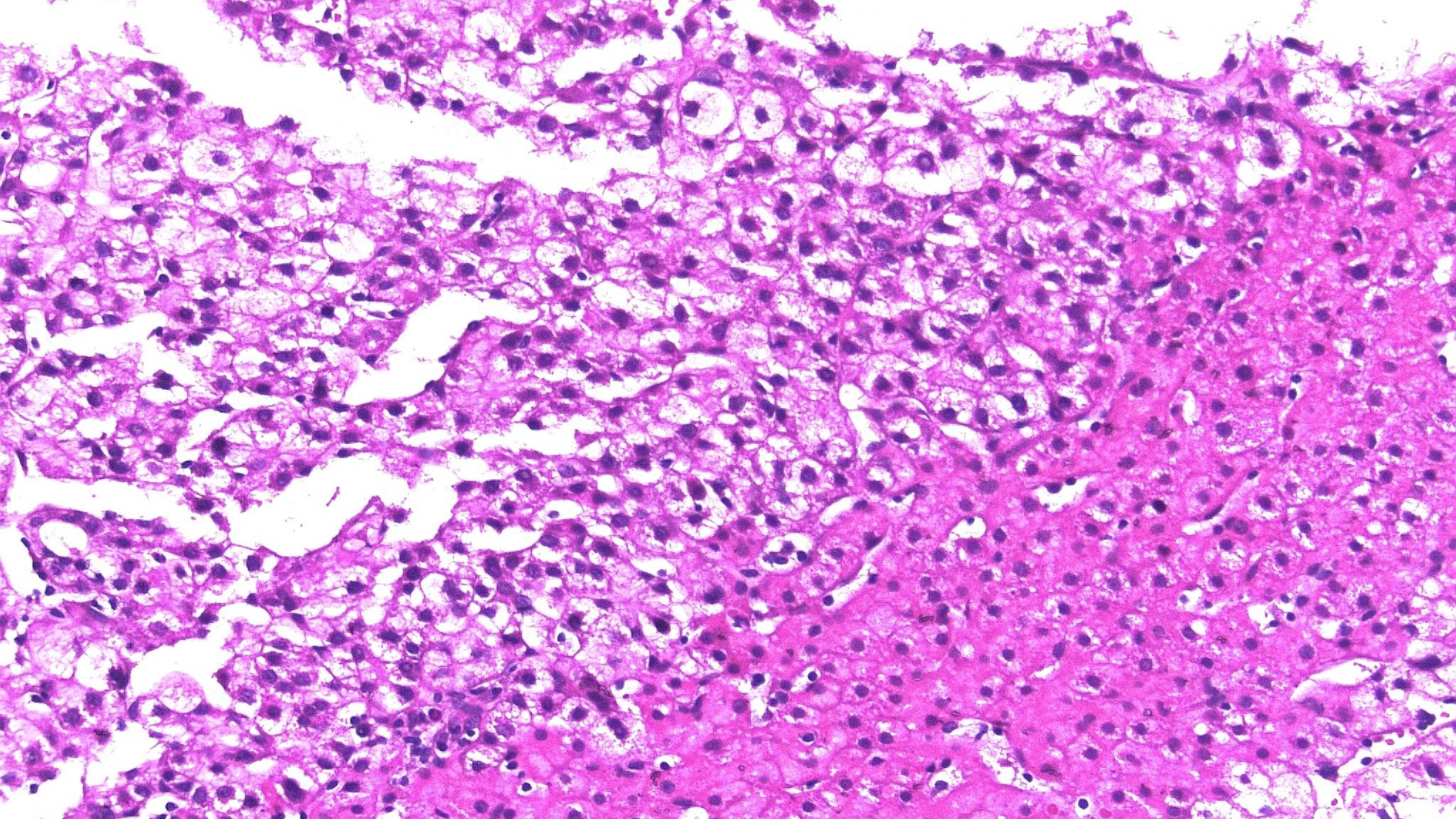
- Steatohepatitic HCC
- Combined HCC-cholangiocarcinoma
- Macrotrabecular massive HCC

Steatohepatitic HCC

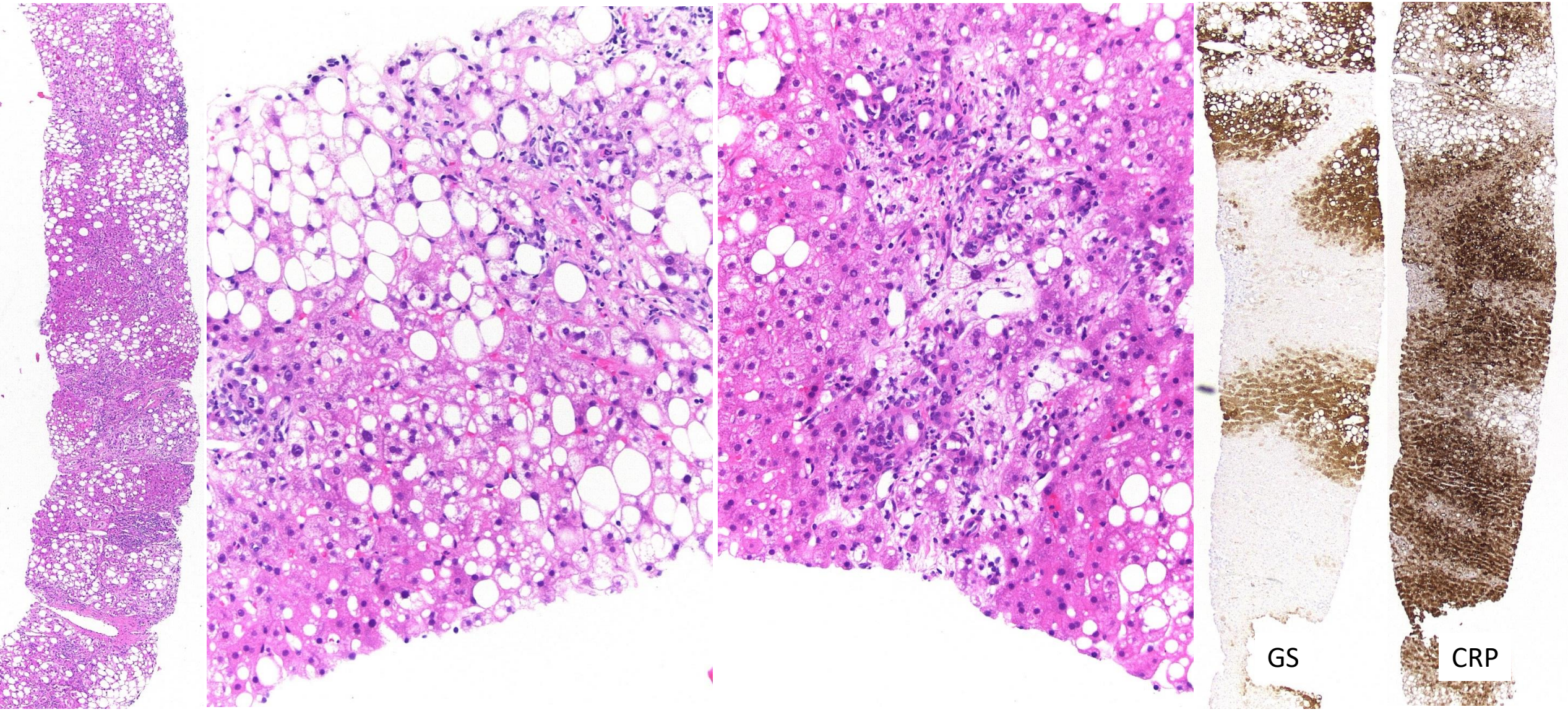


Salamao M et al. Am J Surg Pathol 2010; 34:1630





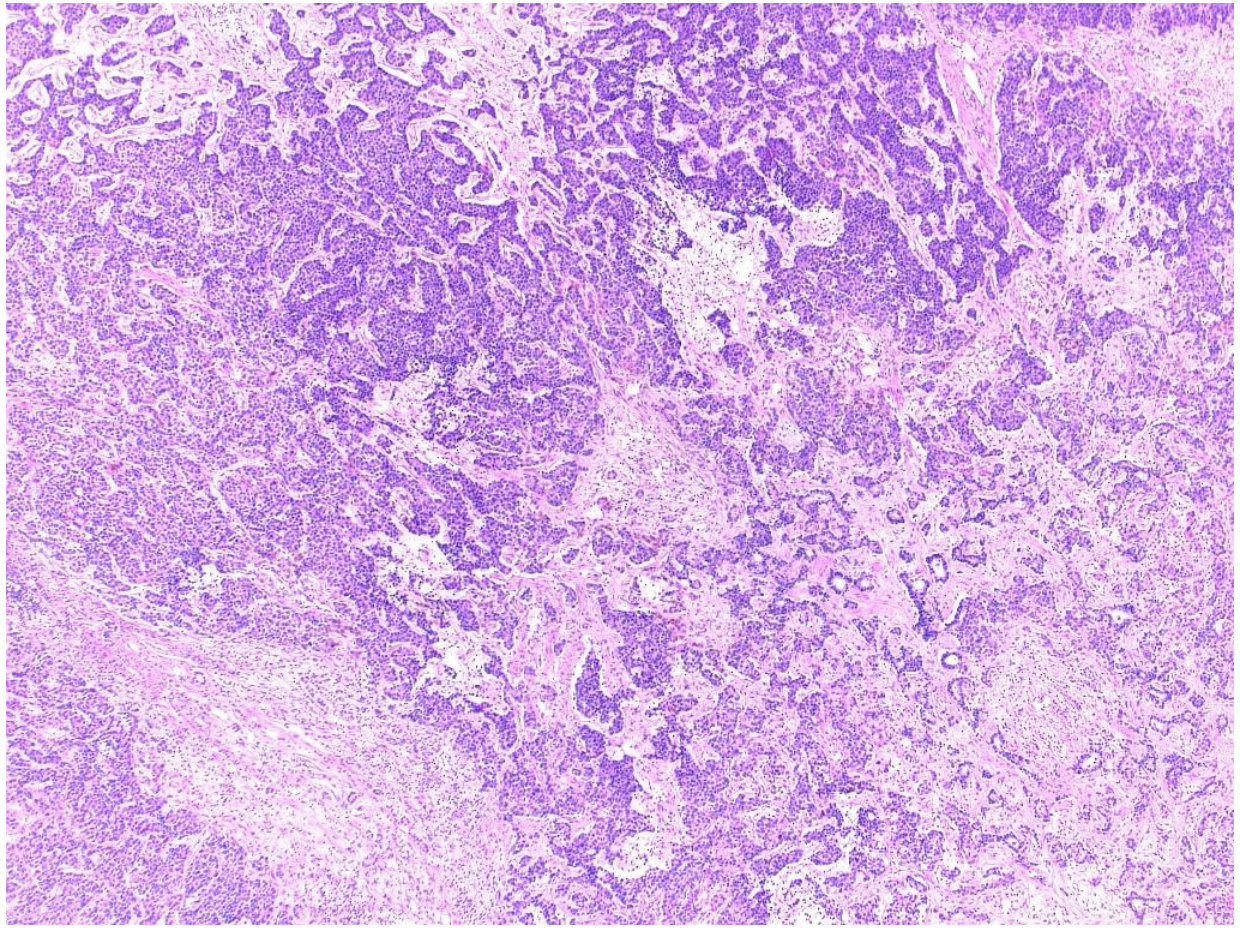
Also be aware of steatohepatitic FNH

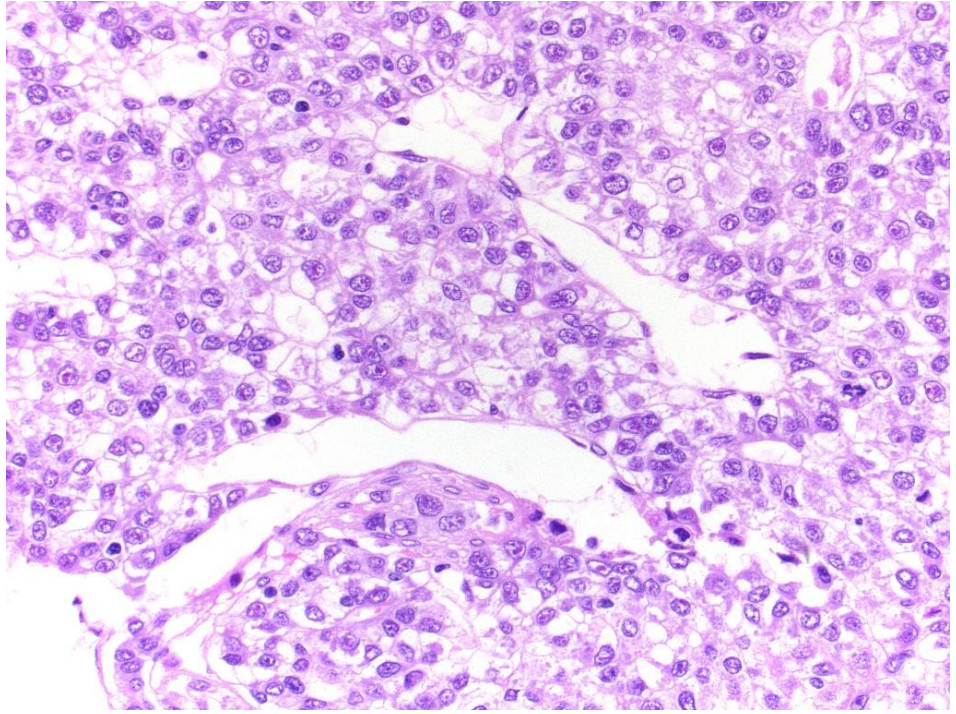
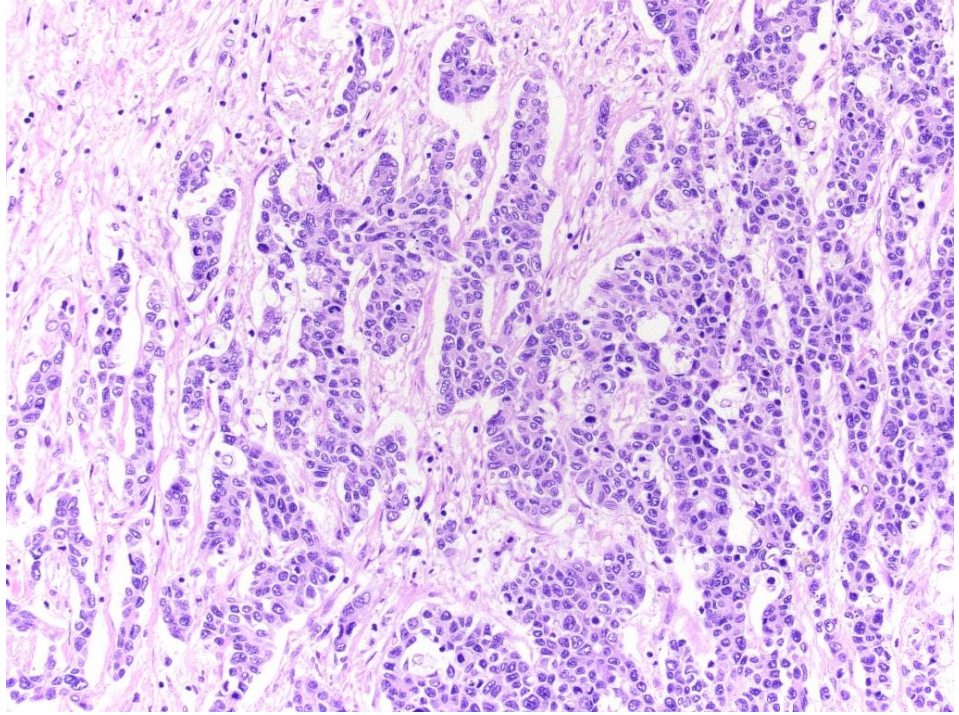
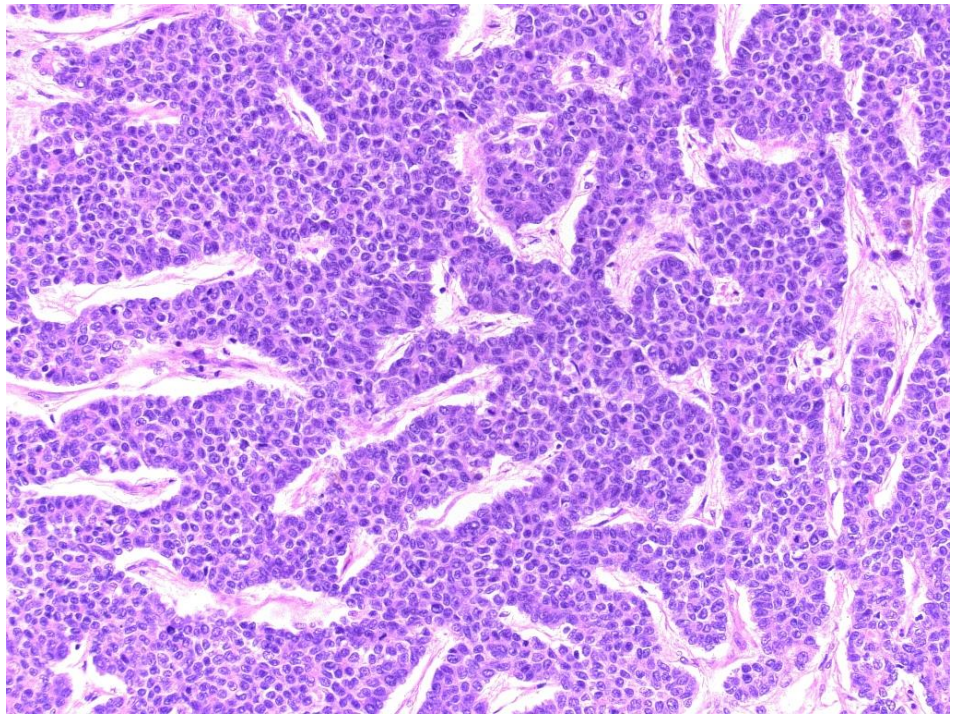
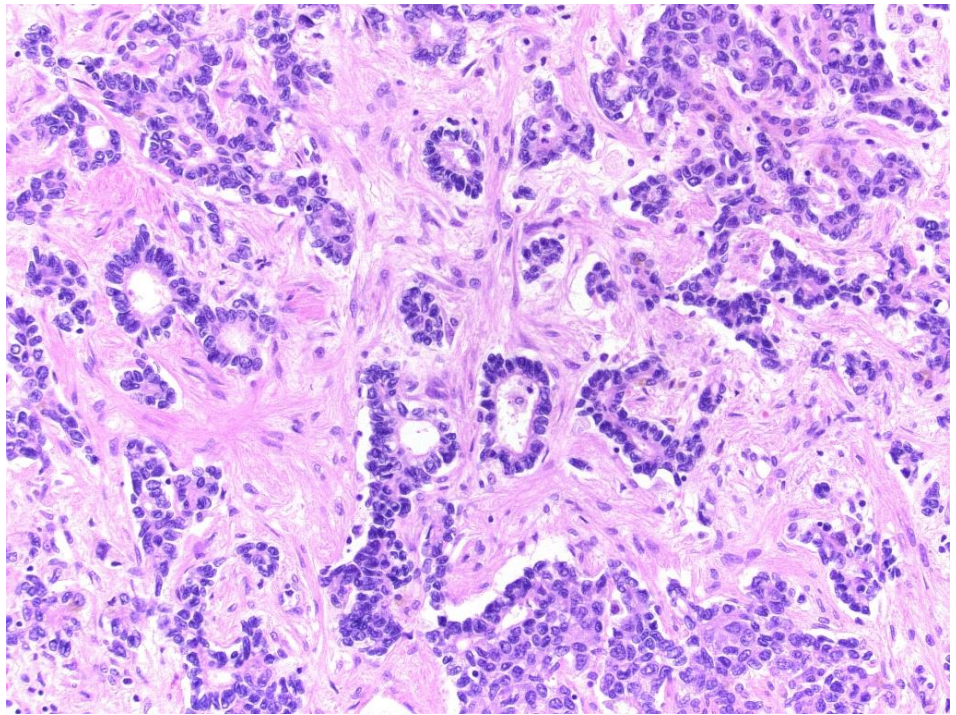


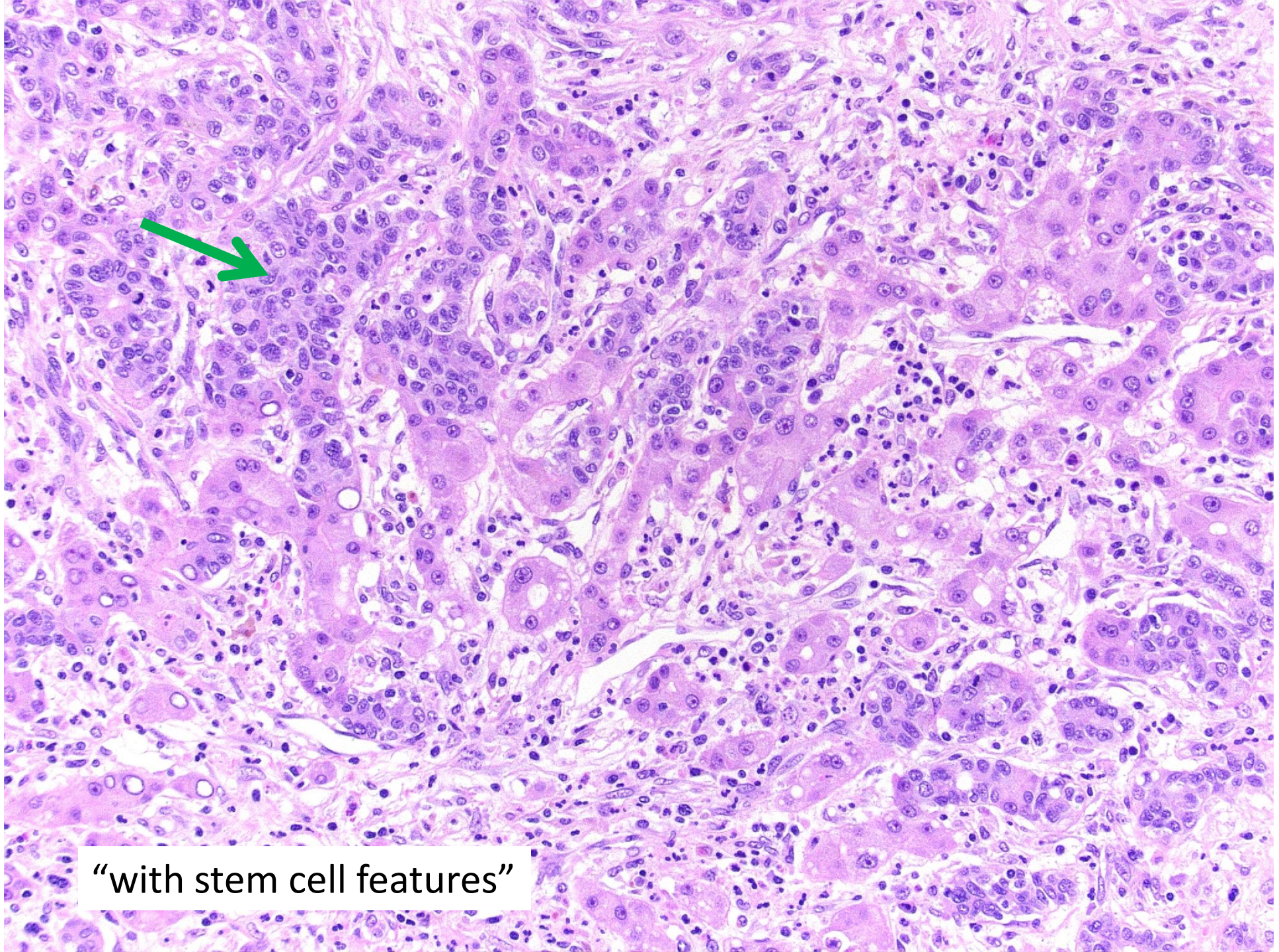
Combined tumours

Case – Combined HCC-CC

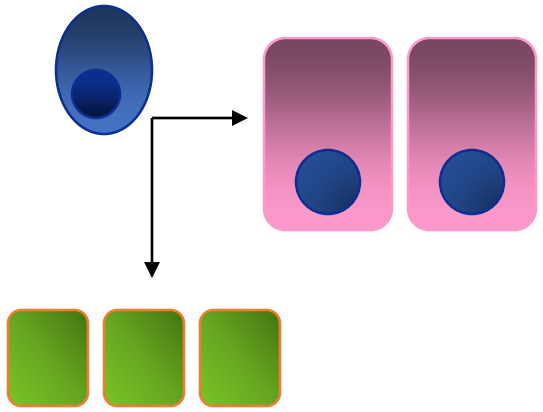
- F 55 - chronic HCV (non-cirrhotic)



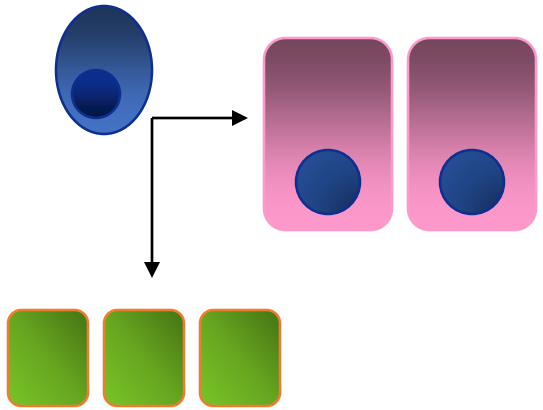





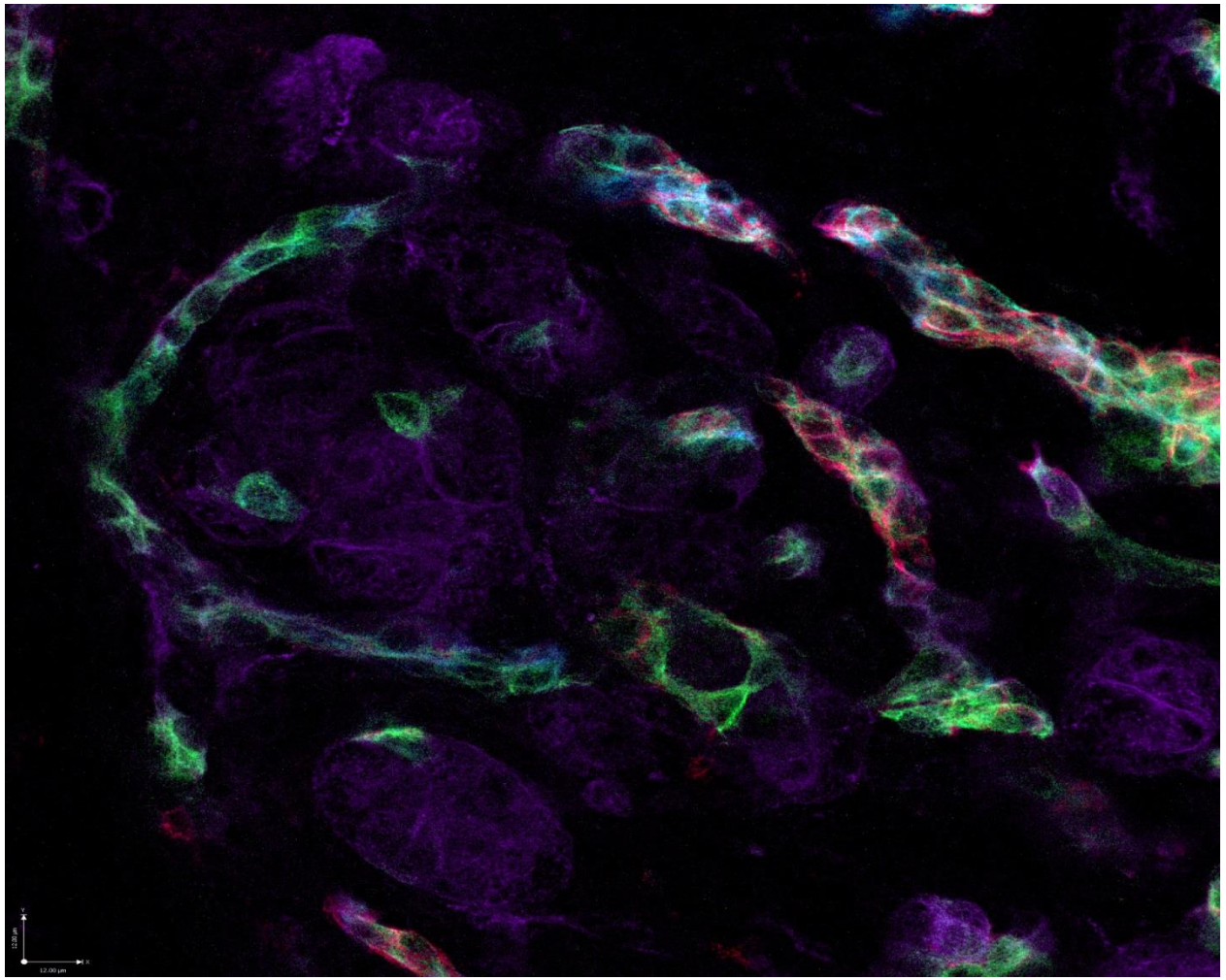

“with stem cell features”

Stemness and combined tumours



Stemness and combined tumours





Purple	Ker18
Blue	Ker19
Red	NCAM
Green	Ker7

Courtesy Dr Emelia Prakoso

HCC with “stemness” markers

- more extensive stem cell/biliary marker expression *= a spectrum*
- HCC + stem cell markers

Tang KH et al. Hepatol 2012; 55:807
Kim H & Park YN. Dig Dis 2014; 32:778
Chan AW et al. Histopathol 2014; 64:935

HCC with “stemness” markers

- more extensive stem cell/biliary marker expression = *a spectrum*

- HCC + stem cell markers
- HCC + stem cell markers + fibrosis

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HCC with “stemness” markers

- more extensive stem cell/biliary marker expression = *a spectrum*

- HCC + stem cell markers
- HCC + stem cell markers + fibrosis

- HCC + stem cell markers +(fibrosis) + stem cell/biliary histology

Tang KH et al. Hepatol 2012; 55:807
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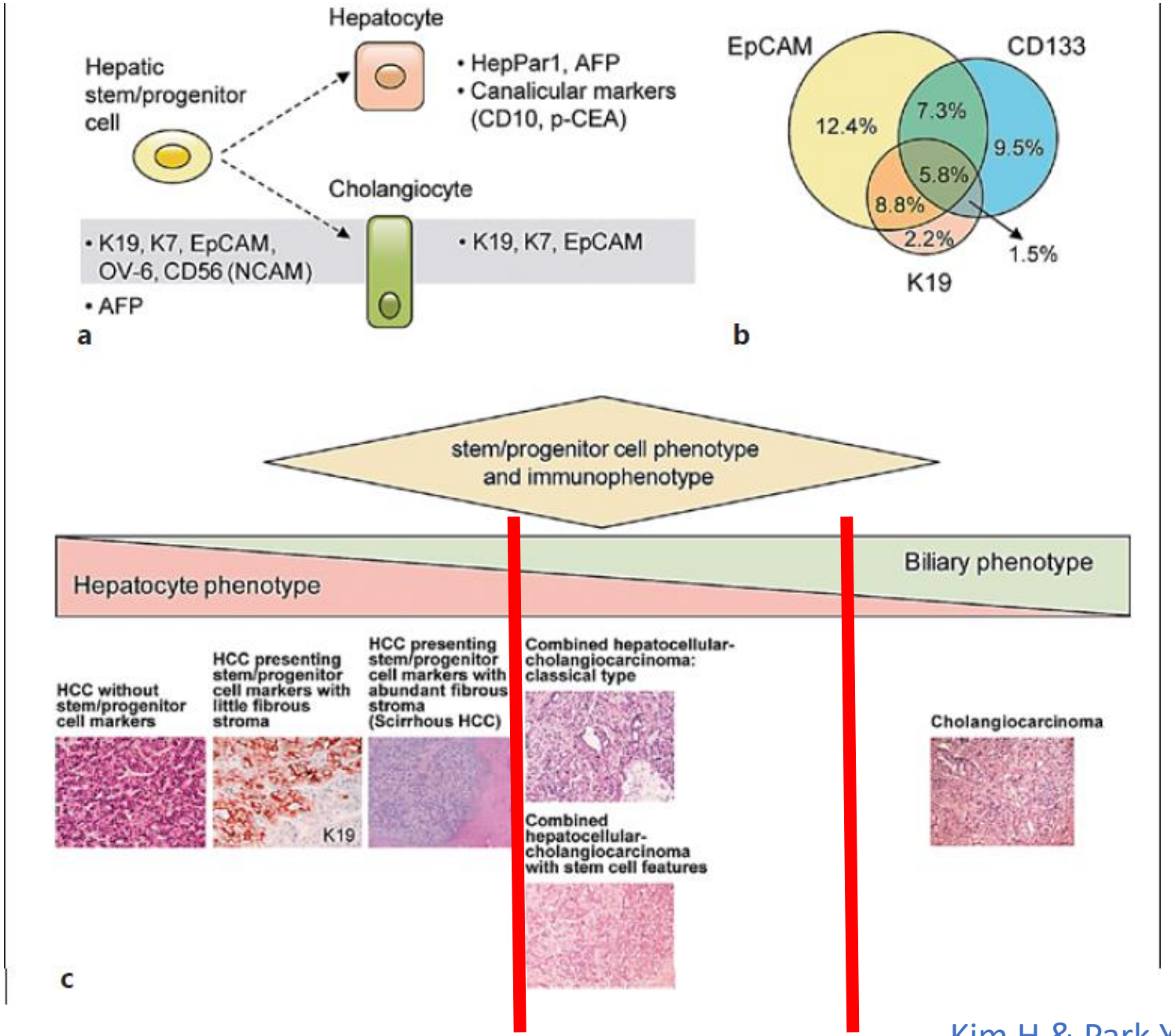
Why bother?

immunohistochemical "stemness/biliary" phenotype can indicate more aggressive HCC

morphological "stem cell" pattern can alert to a combined HCC-CCa

- combined HCC-CCa are more aggressive
- different metastatic patterns
  - (HCC - venous vs CCa - nodal)
- some stem cell patterns are subtle (look benign)
- helps to understand patterns seen



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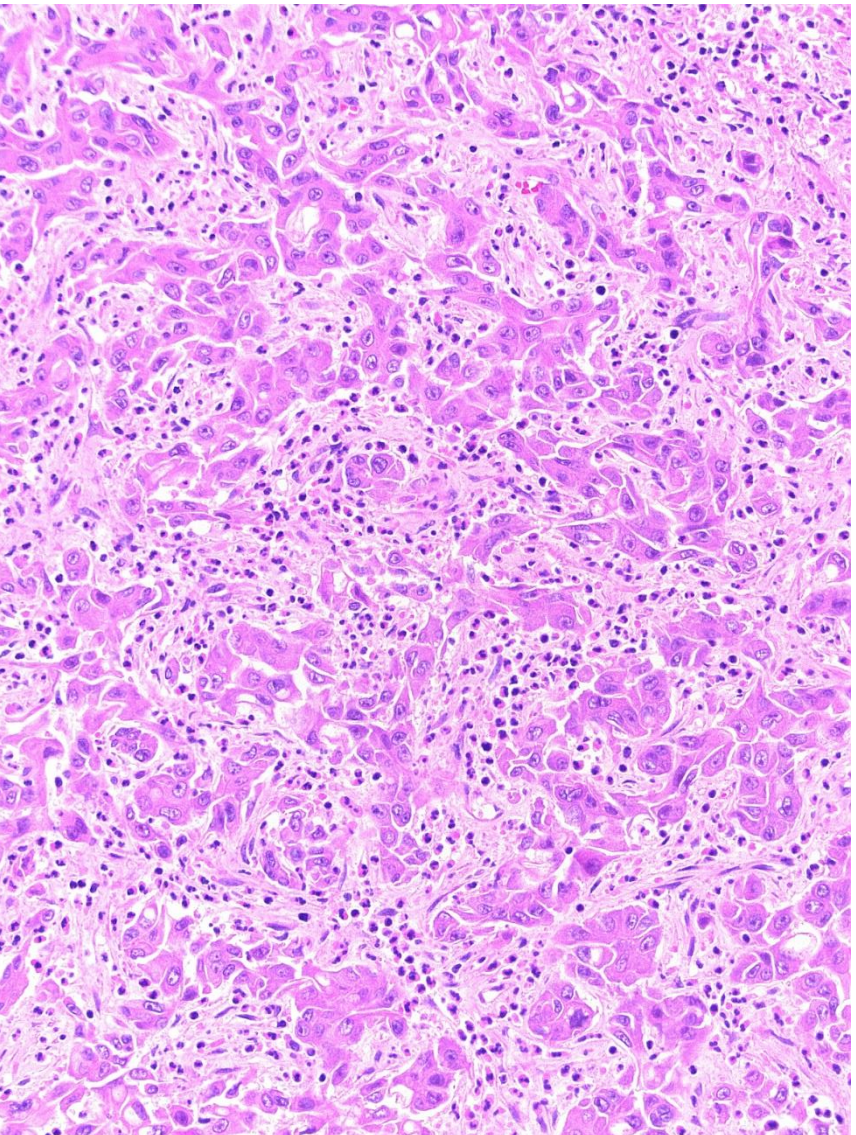
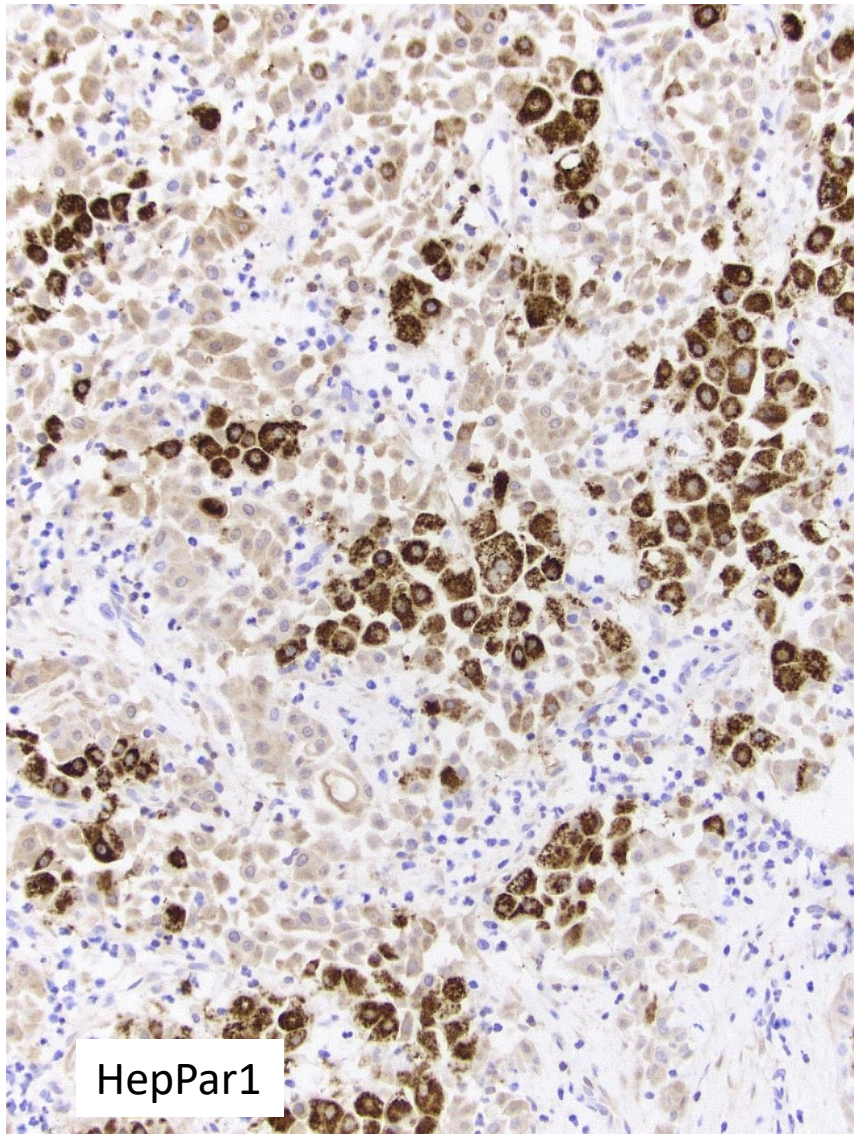
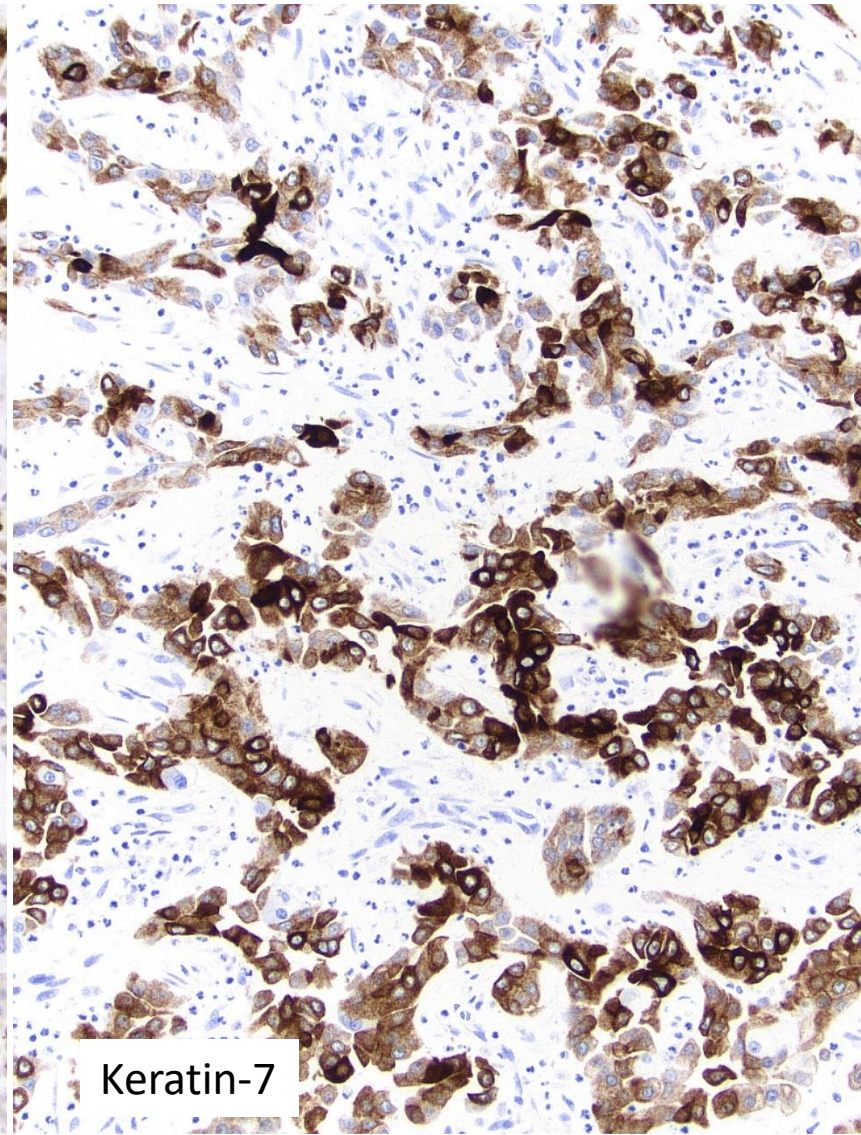
Scirrhous HCC







Stem cell IHC in HCC

- 10-20% HCC (markers like K19, EpCAM) – but no morphological CCa
- worse prognosis & increased lymphovascular invasion

Kim H et al. Hepatol 2011; 54:1707
Seok JY et al. Hepatol 2012; 55:1775
Chan AW et al. Histopathol 2014; 64:935
Kim H & Park YN. Dig Dis 2014; 32:778

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HEPATOLOGY, VOL. 68, NO. 1, 2018

cHCC-CCA: Consensus Terminology for Primary Liver Carcinomas With Both Hepatocytic and Cholangiocytic Differentation

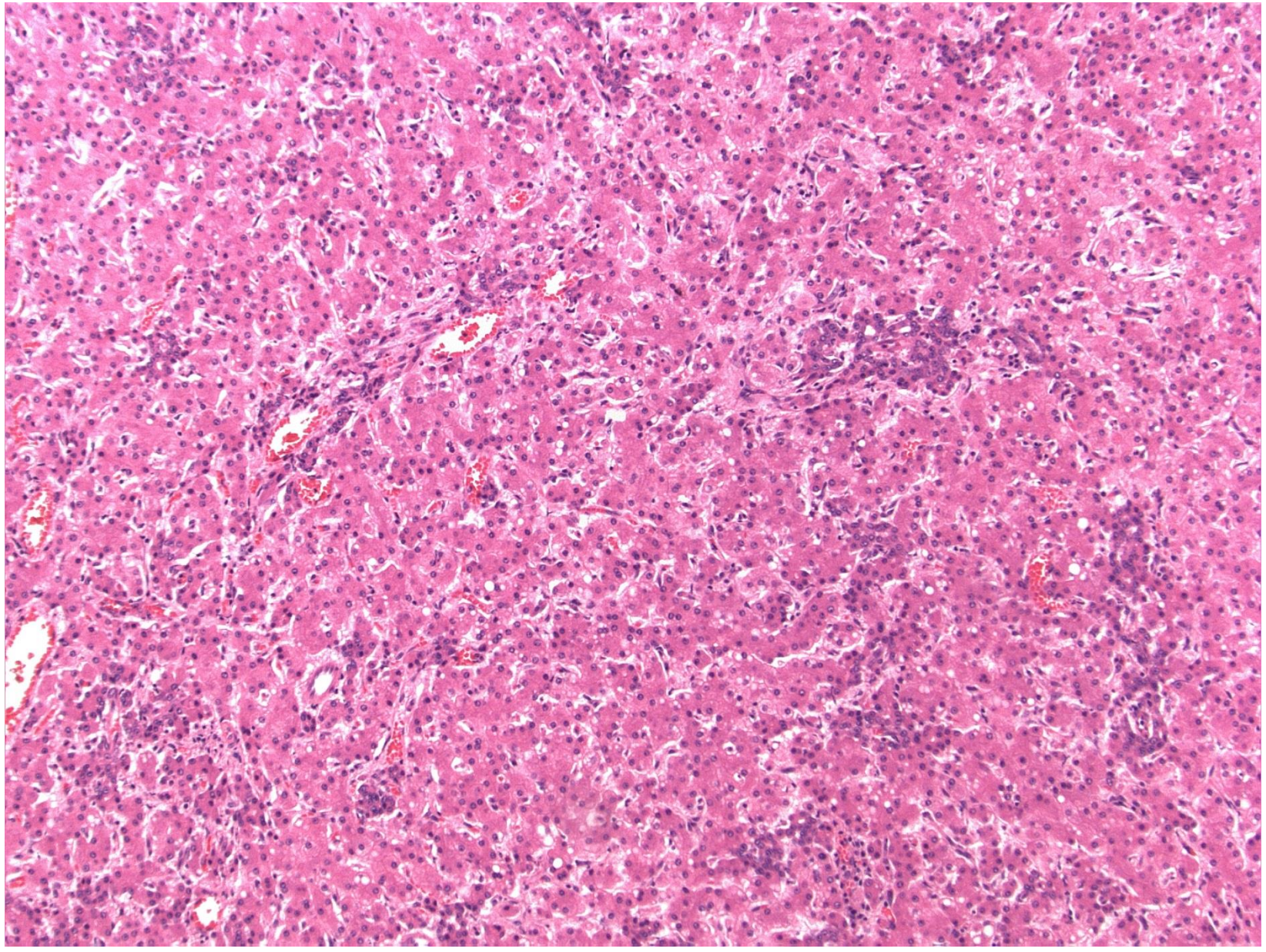
Elizabeth Brunt,[1] Shinichi Aishima,[2] Pierre-Alain Clavien,[3] Kathryn Fowler,[4] Zachary Goodman,[5] Gregory Gores,[6] Annette Gouw,[7] Alex Kagen,[8] David Klimstra,[9] Mina Komuta,[10] Fukuo Kondo,[11] Rebecca Miksad,[12] Masayuki Nakano,[13] Yasuni Nakanuma,[14] Irene Ng,[15] Valerie Paradis,[16] Young Nyun Park,[17] Alberto Quaglia,[18] Massimo Roncalli,[19] Tania Roskams,[20] Michiie Sakamoto,[21] Romil Saxena,[22] Christine Sempoux,[23] Claude Sirlin,[24] Ashley Stueck,[25] Swan Thung,[26] W.M.S. Tsui,[27] Xin-Wei Wang,[28] Aileen Wee,[29] Hirohisa Yano,[30] Matthew Yeh,[31] Yoh Zen,[32] Jessica Zucman-Rossi,[33] and Neil Theise[34]

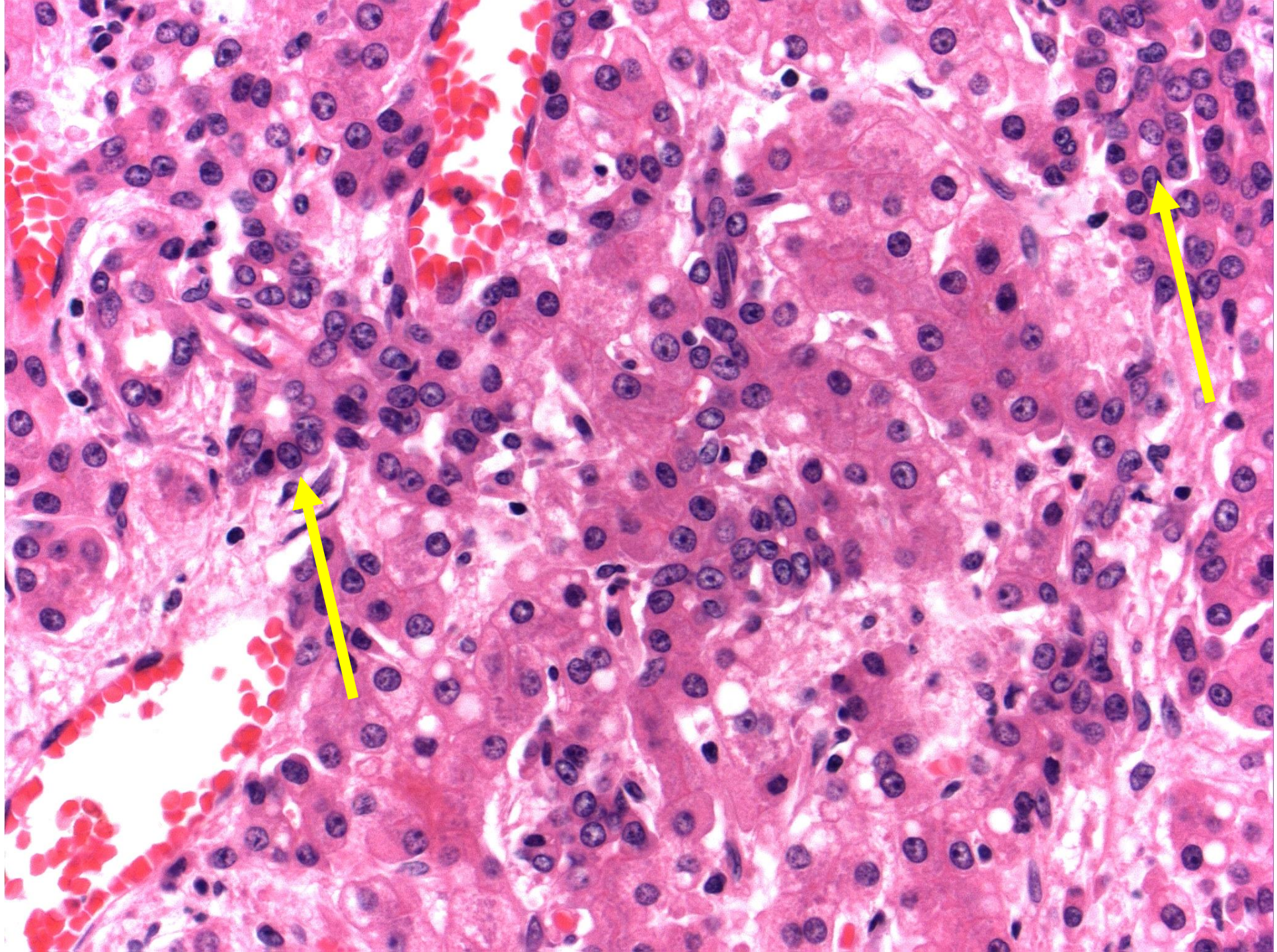
Combined HCC-CCa

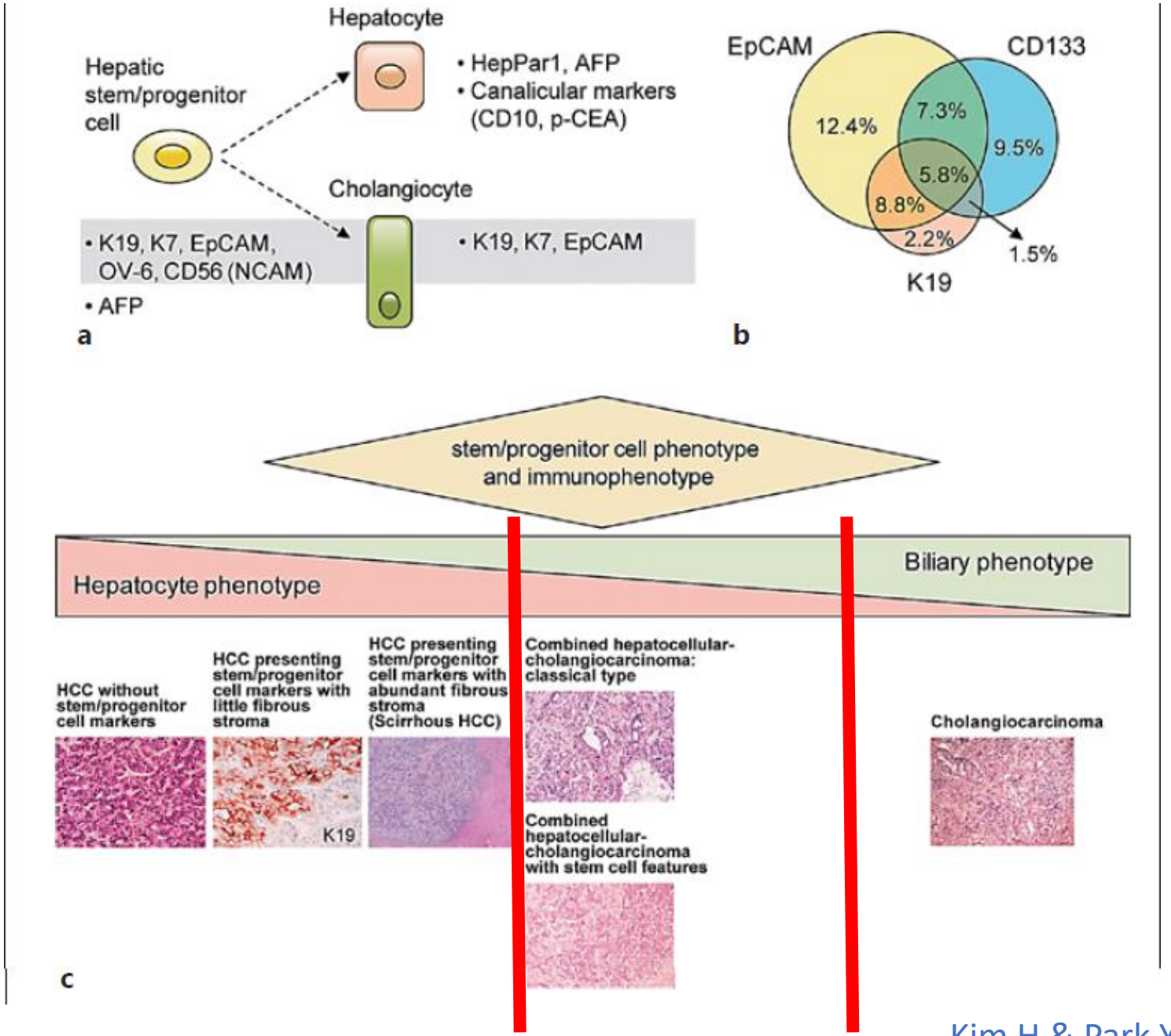
- based on morphology (not just immunostaining)
- components of both HCC and CCa present
  - IHC may be helpful to confirm and discriminate
- there may be “stem cell features”
  - cells intermediate in size or closely resembling ductular reaction
  - correspond to stem cell patterns in WHO 2010 (patterns can be mixed)
  - stem cell features may also be seen in HCC or CCa (eg CCa + CLC)

Brunt et al. Hepatology 2018; 68:113

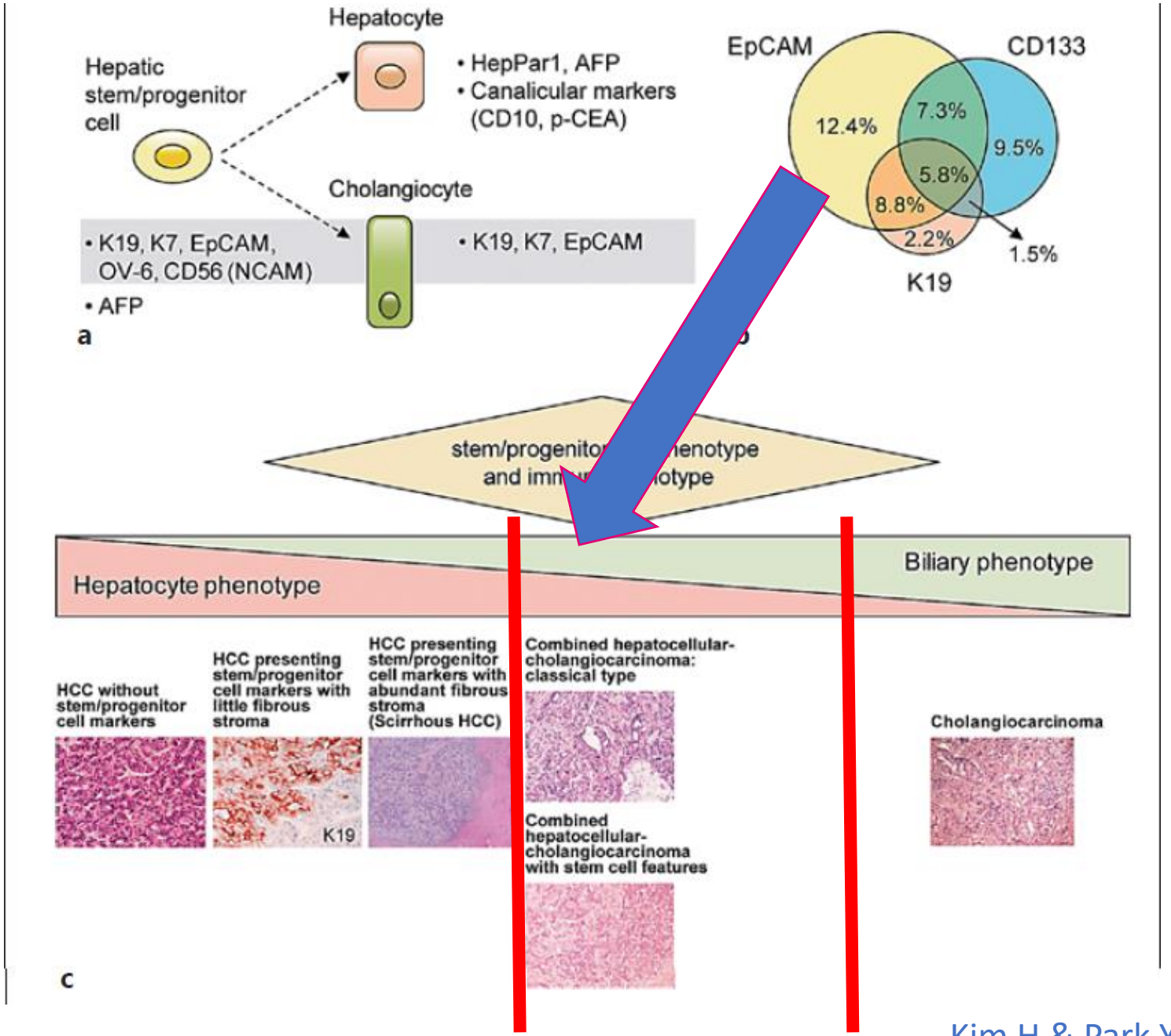
An example







Kim H & Park YN. Dig Dis 2014; 32:778



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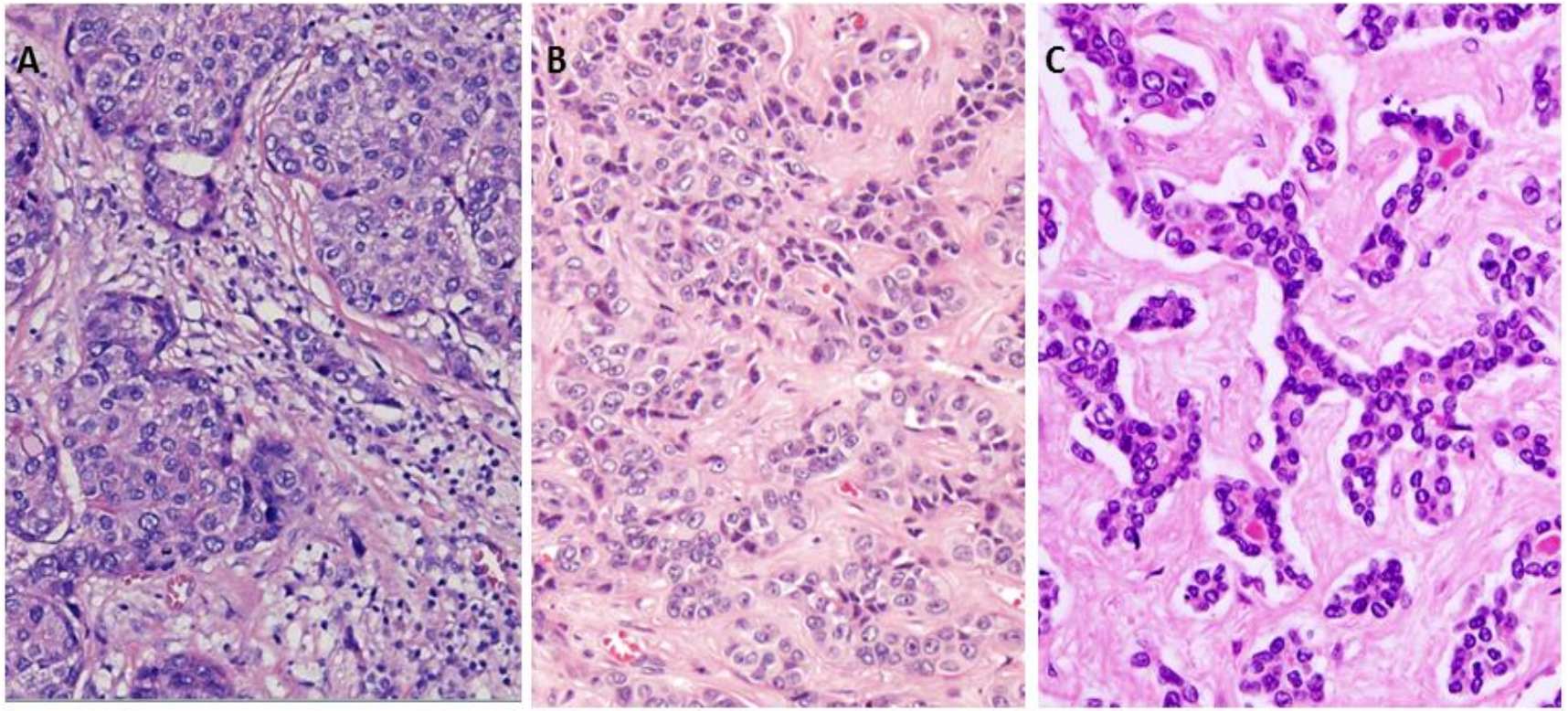
Stem cell patterns (can co-exist)



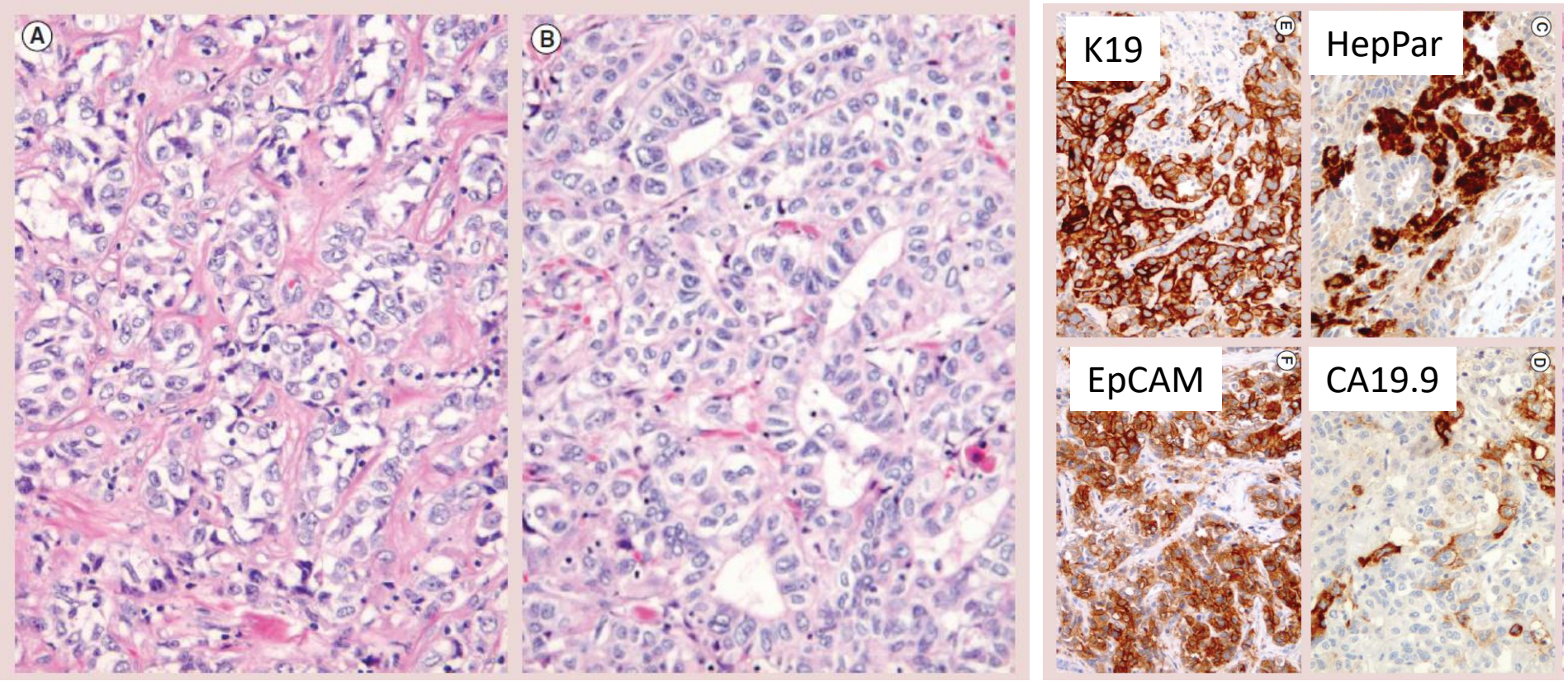
Classical

Intermediate cell

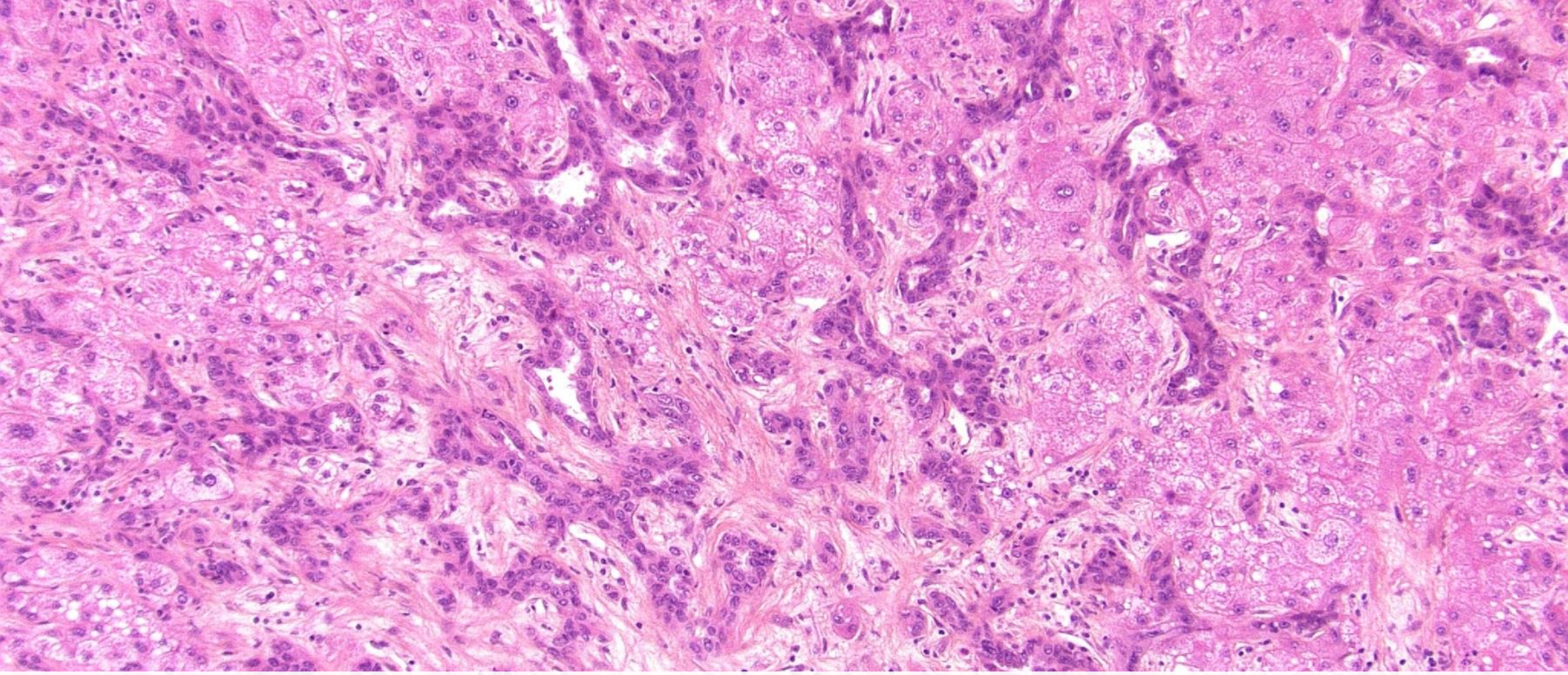
Cholangiolocellular (CLC)

Courtesy Dr E Brunt (St Louis) & Dr N Theise (NY)

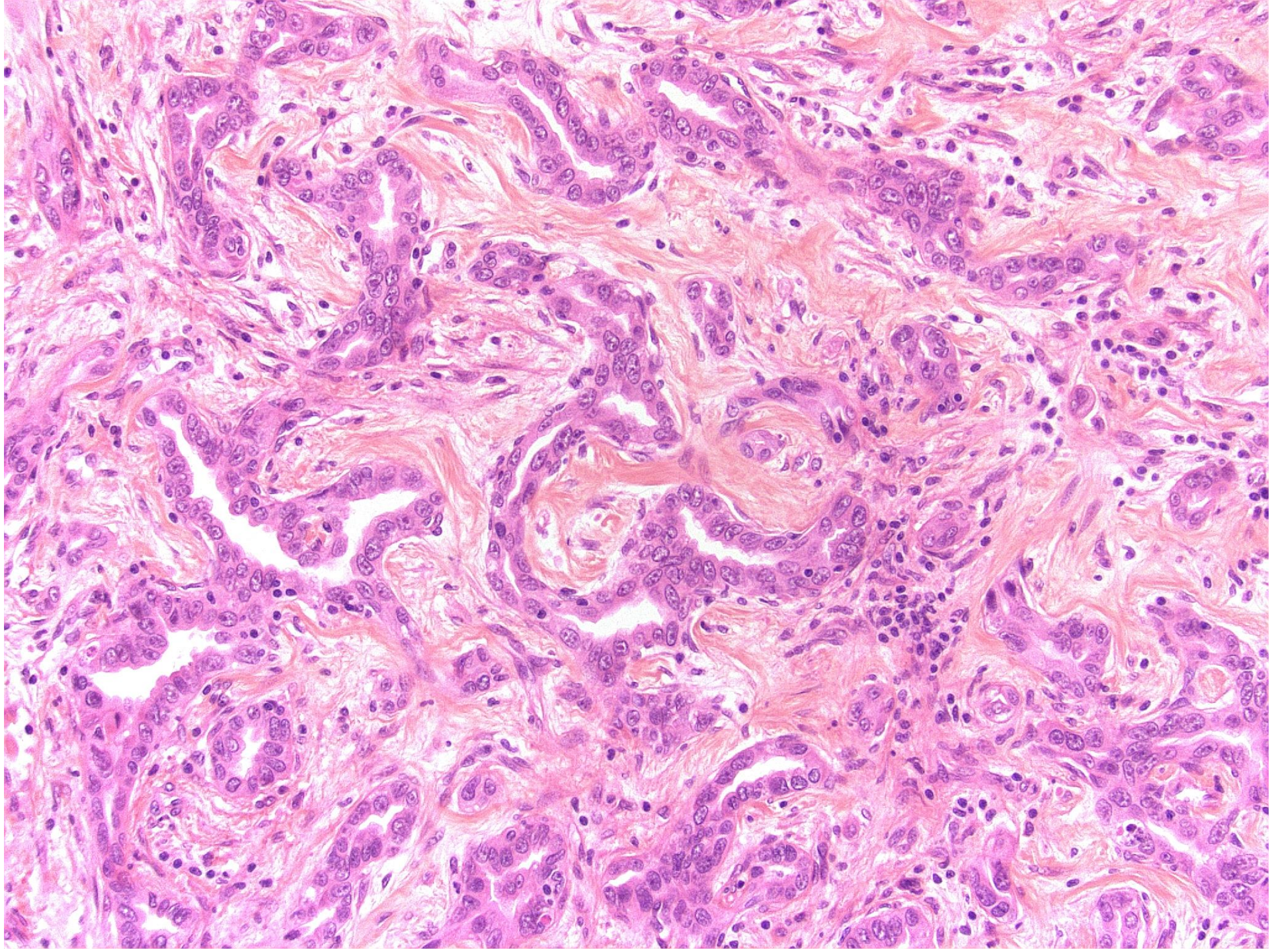
Intermediate cell pattern



Brunt et al. Hepat Oncol 2015; 2:255

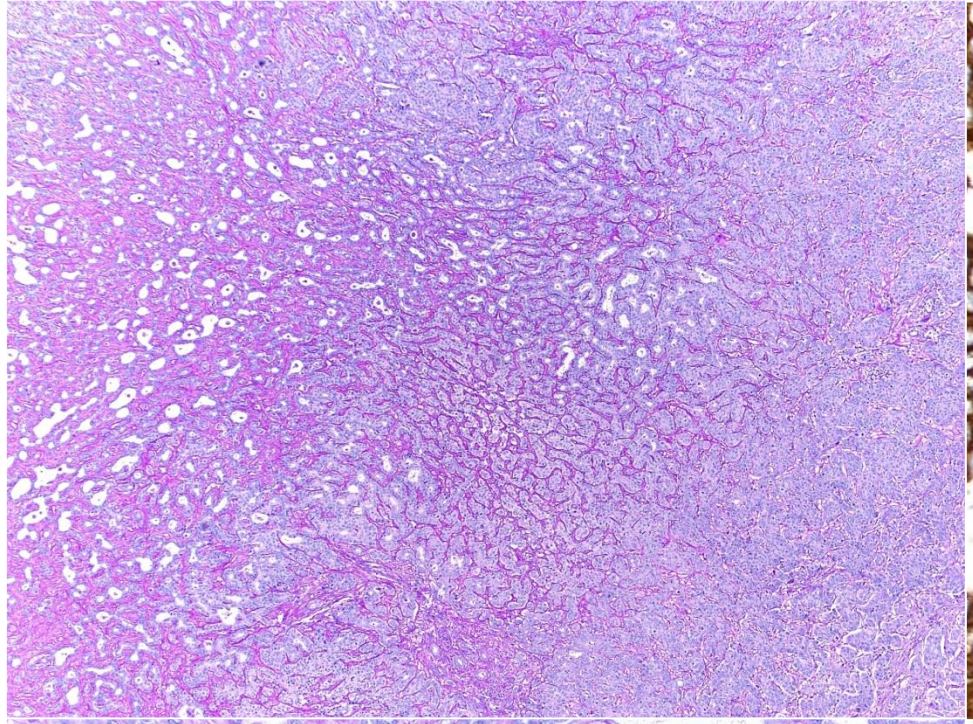


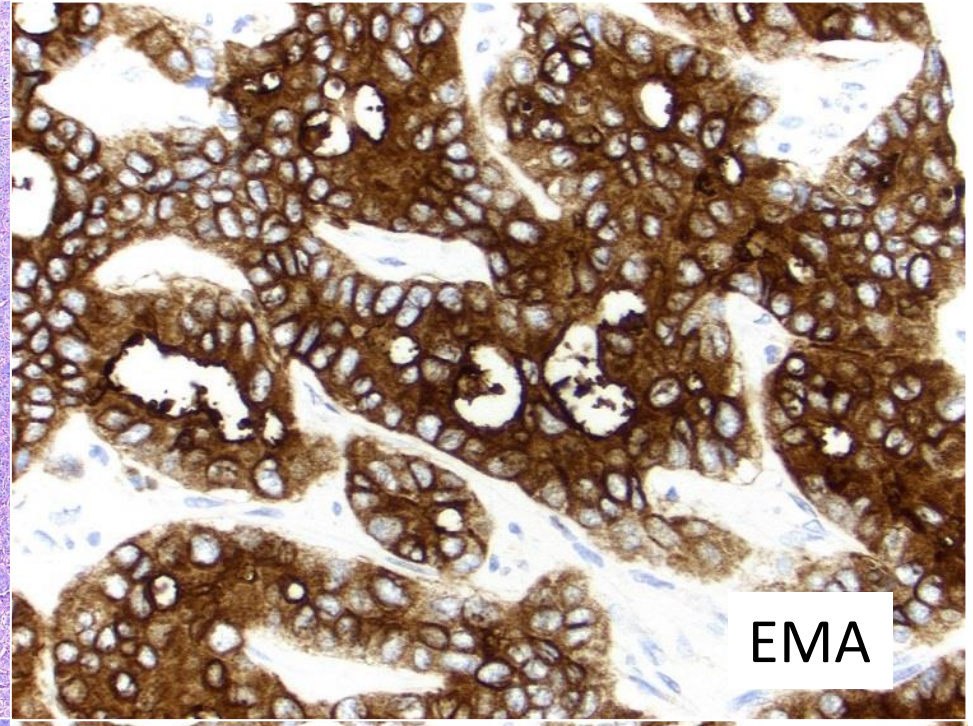
Cholangiolocarcinoma (CLC)



Cholangiolocarcinoma (CLC)

- as pure tumour (>90% of the tumour)
- with combined HCC – CCa
- with intrahepatic CCa

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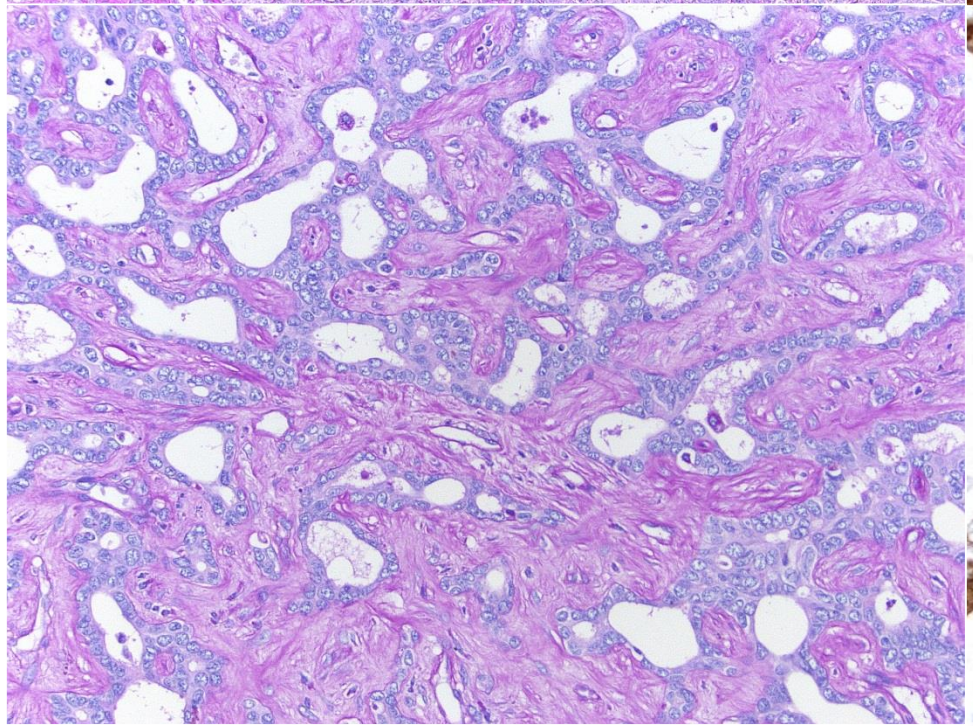

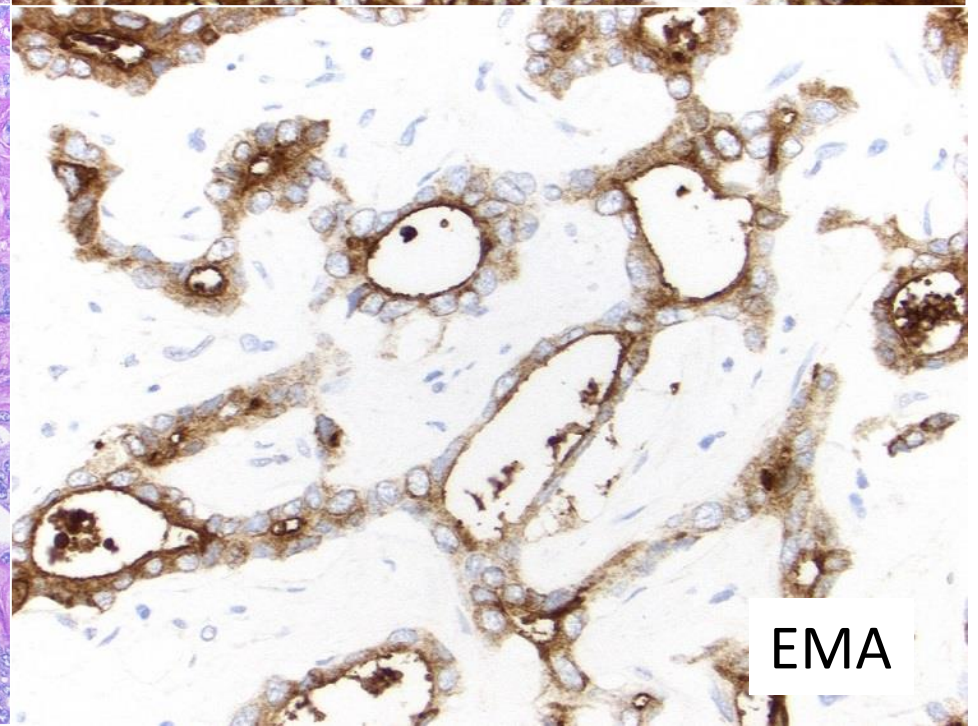
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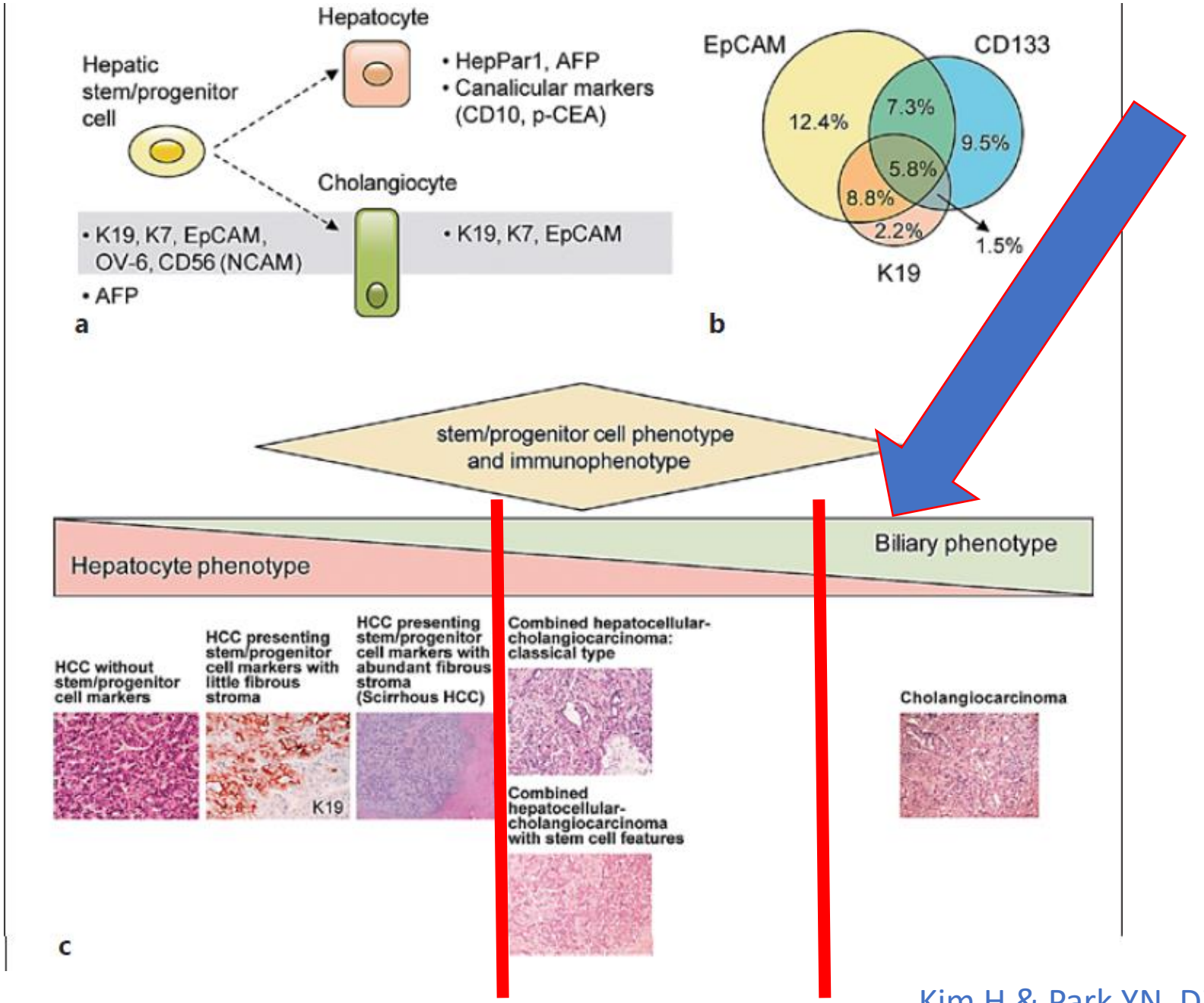


Kim H & Park YN. Dig Dis 2014; 32:778

Combined HCC-CCa

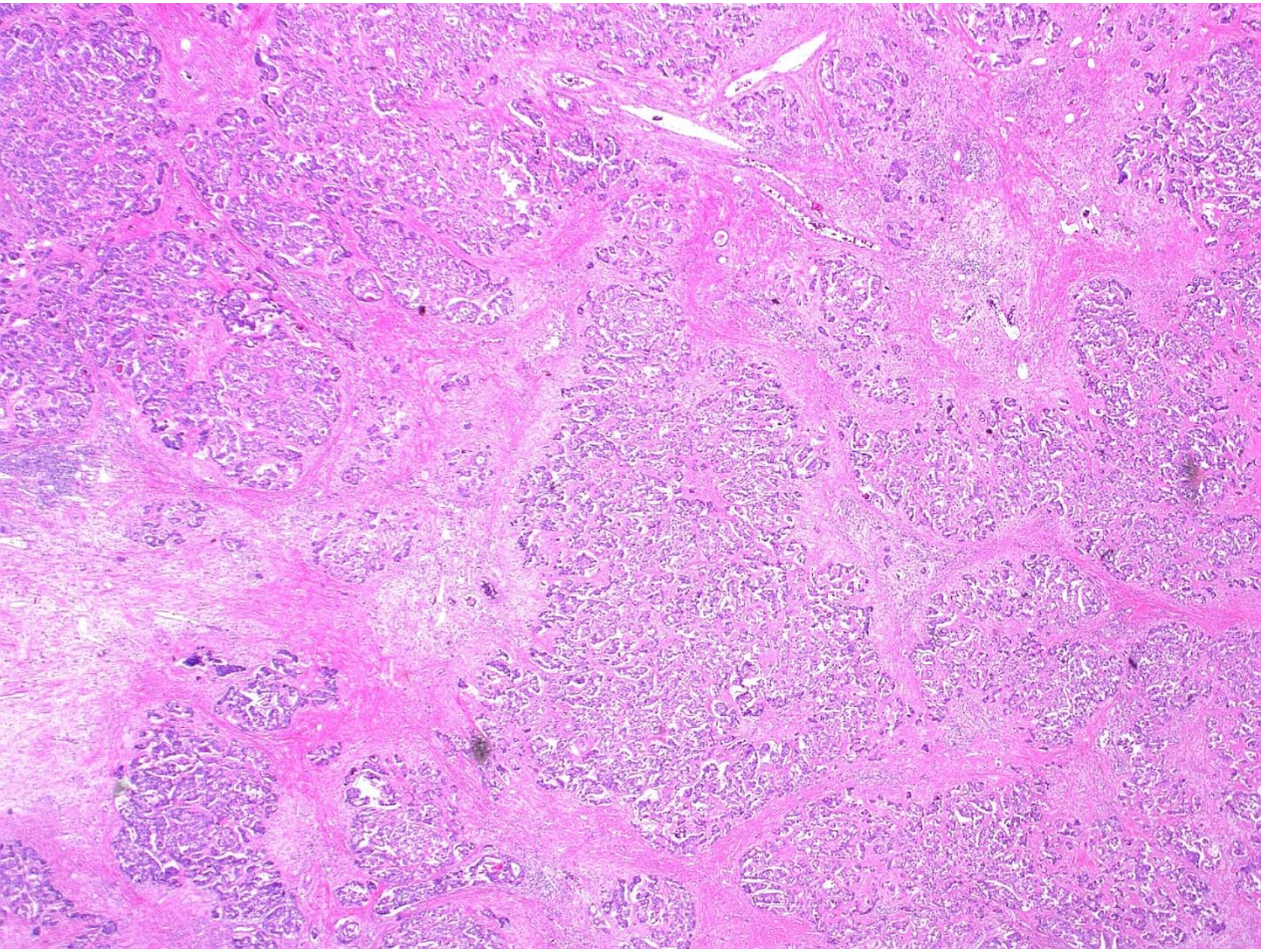
- must see morphology of HCC & CC
- +/- stem cell morphological patterns
- intermediate cell and CLC can occur as pure tumours (if >90%)

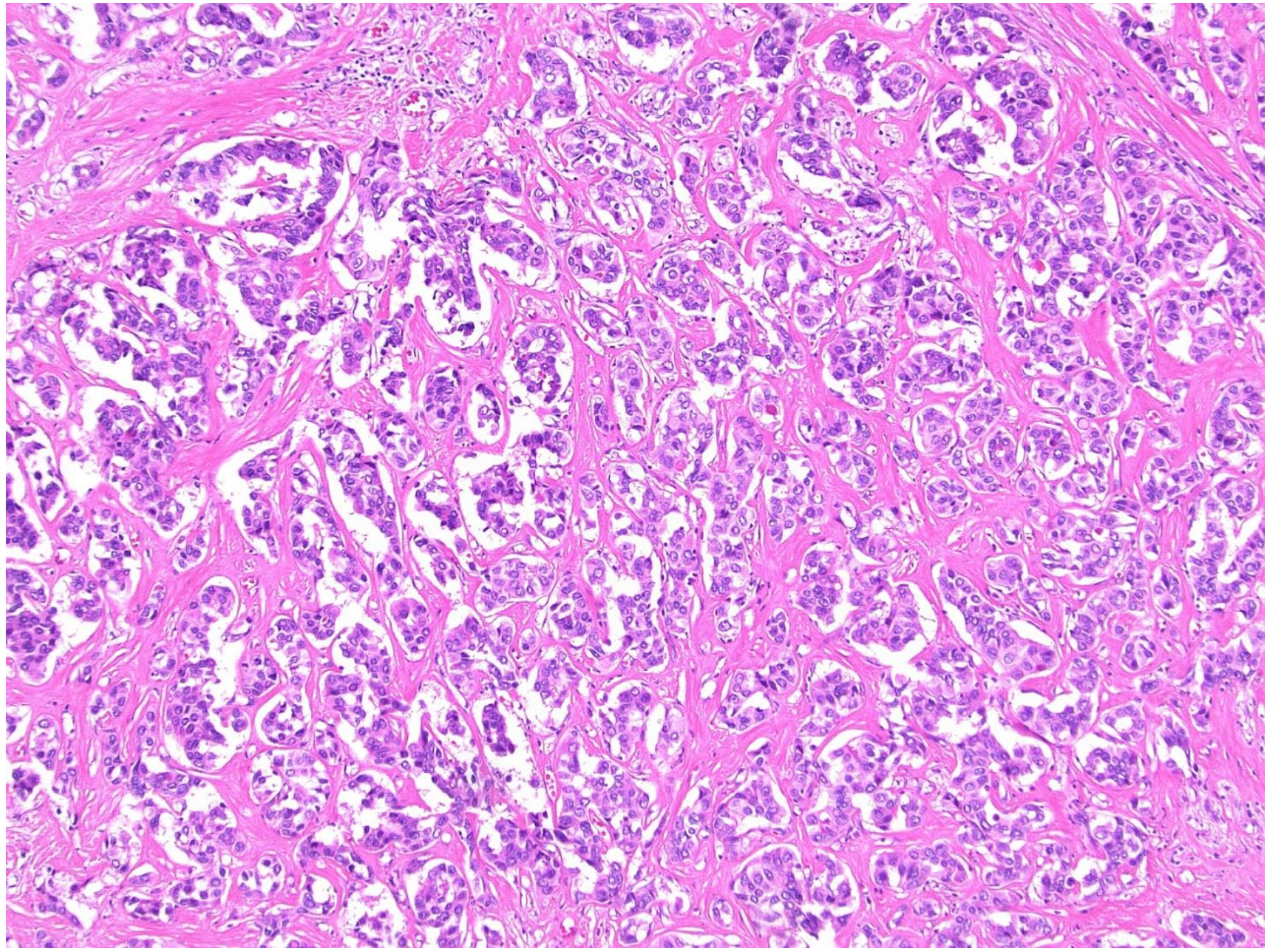
- increased after TACE
  - up to 35% (vs 0/40 HCC without TACE)
  - CD133 (35%), EpCAM (30%), K19 (20%)

Brunt EM et al. Hepat Oncol 2015; 2:255
Zen C et al. Liver Transpl 2011; 17:943
Brunt et al. Hepatol 2018; 68:113

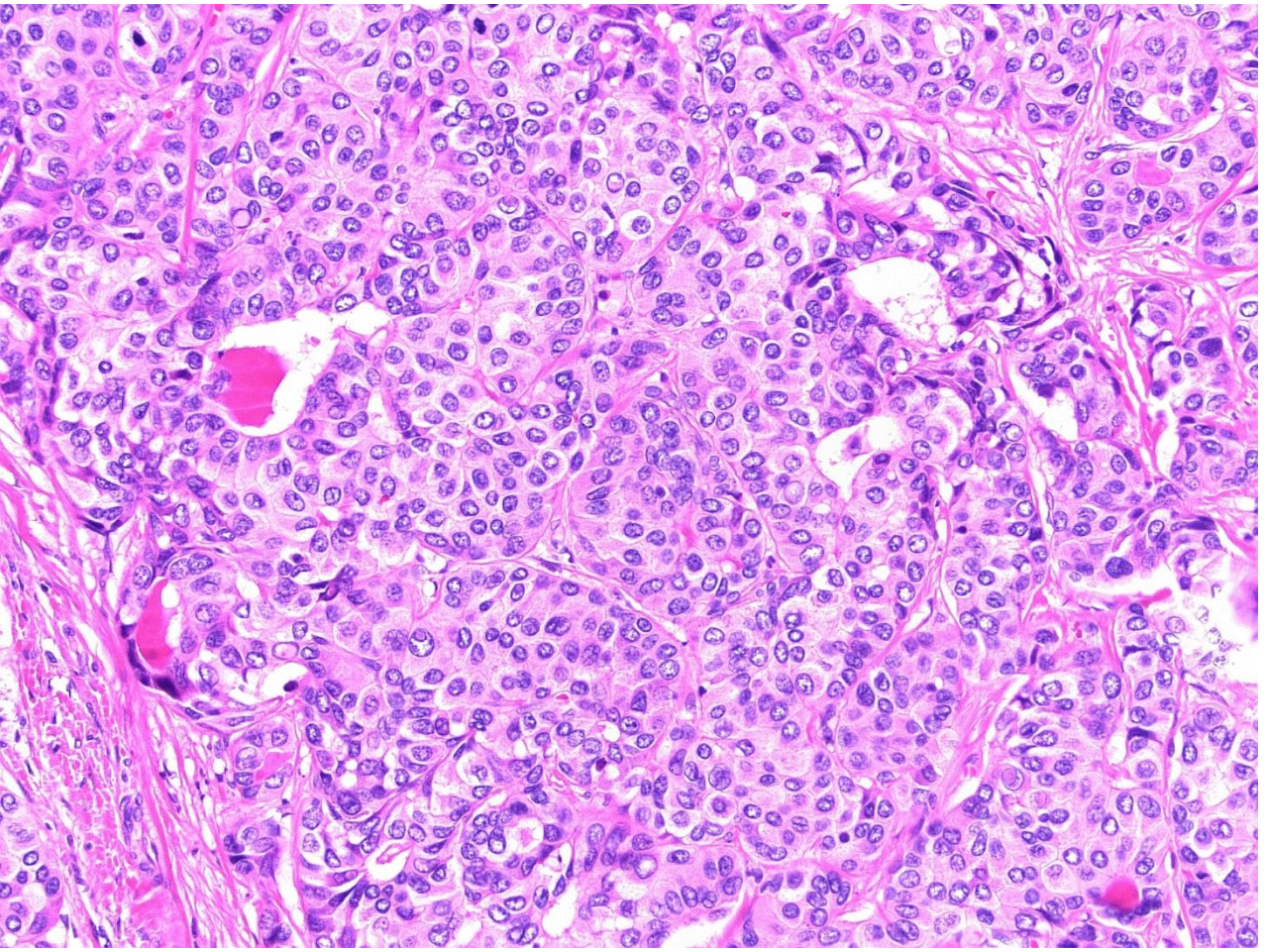
Using IHC in HCC-CCA – an example

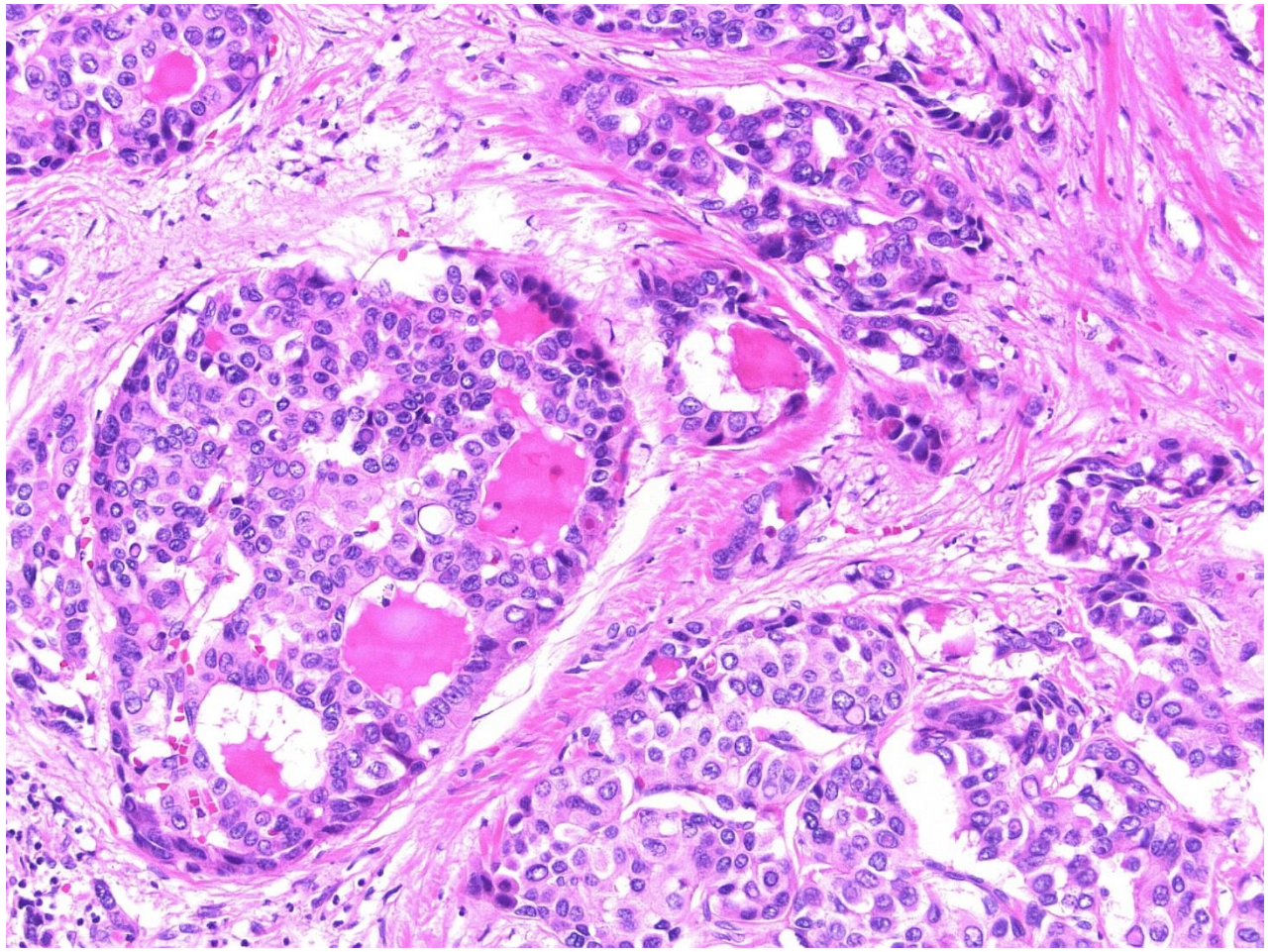
Nests of cells with prominent stroma





HCC-like cytology, glands/pseudoglands





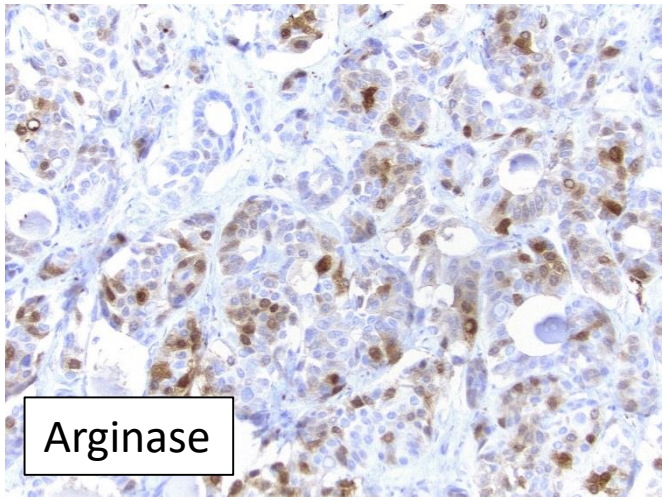
Differential diagnoses

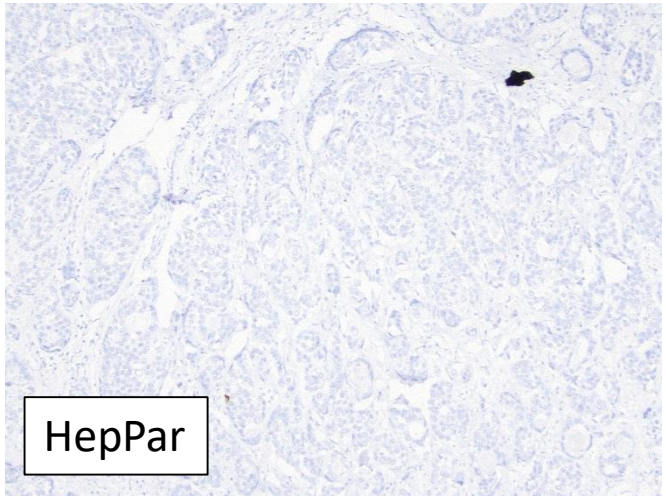
- HCC
- Scirrhous HCC
- Cholangiocarcinoma
- Combined HCC-CCa
  +/- stem cell features

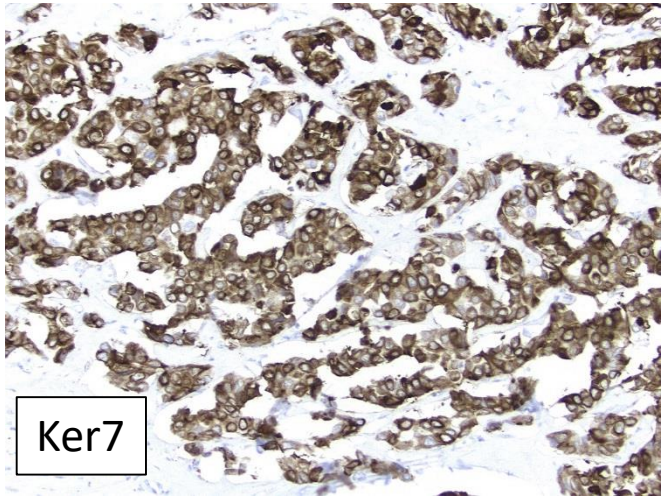
Differential diagnoses - IHC

- HCC — HepPar1, Arginase
- Scirrhous HCC — above + Ker-7
- Cholangiocarcinoma — (PAS-d mucin), Ker-7, MUC1, CEA
- Combined HCC-CCa — mixed morphologies + IHC
  +/- stem cell features — EpCAM, CD56, MUC1 (luminal)

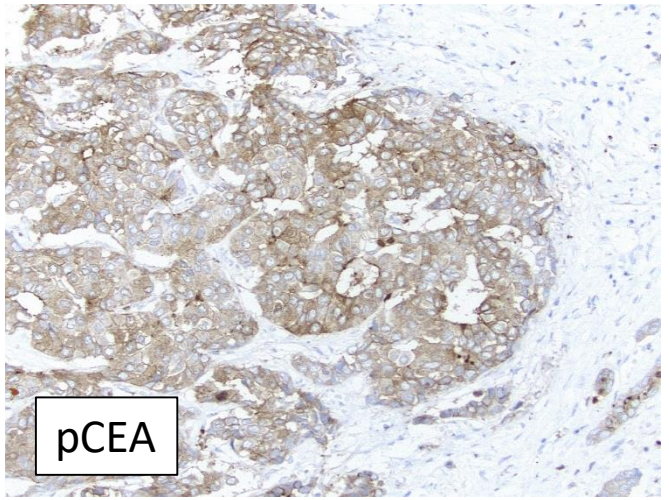
HCC

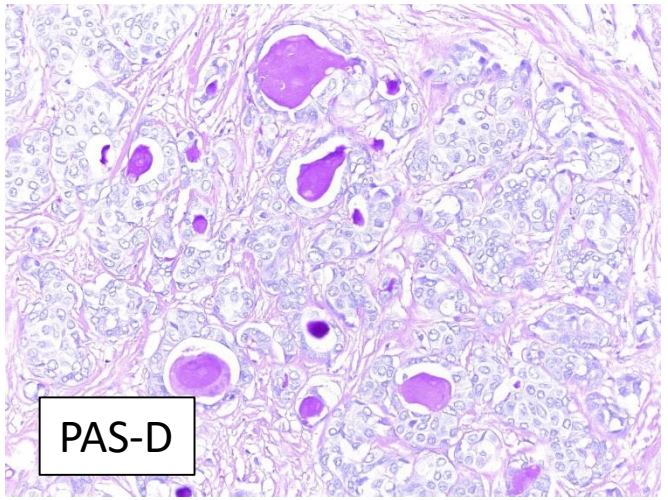


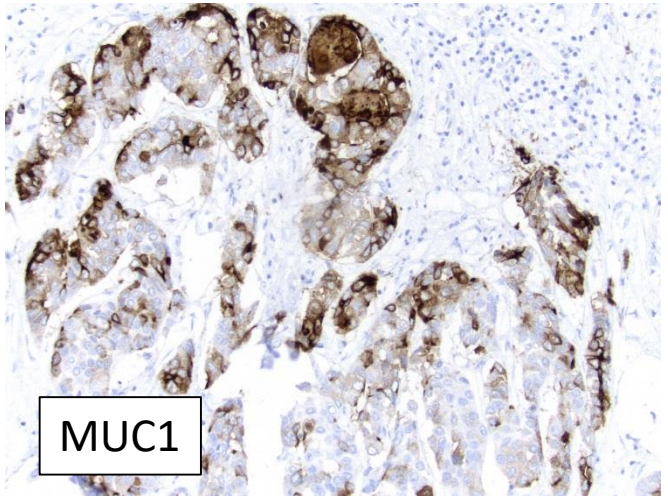




CCa







Macrotrabecular-massive HCC

HEPATOLOGY

AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

HEPATOLOGY, VOL. 68, NO. 1, 2018

Macrotrabecular-Massive Hepatocellular Carcinoma: A Distinctive Histological Subtype With Clinical Relevance

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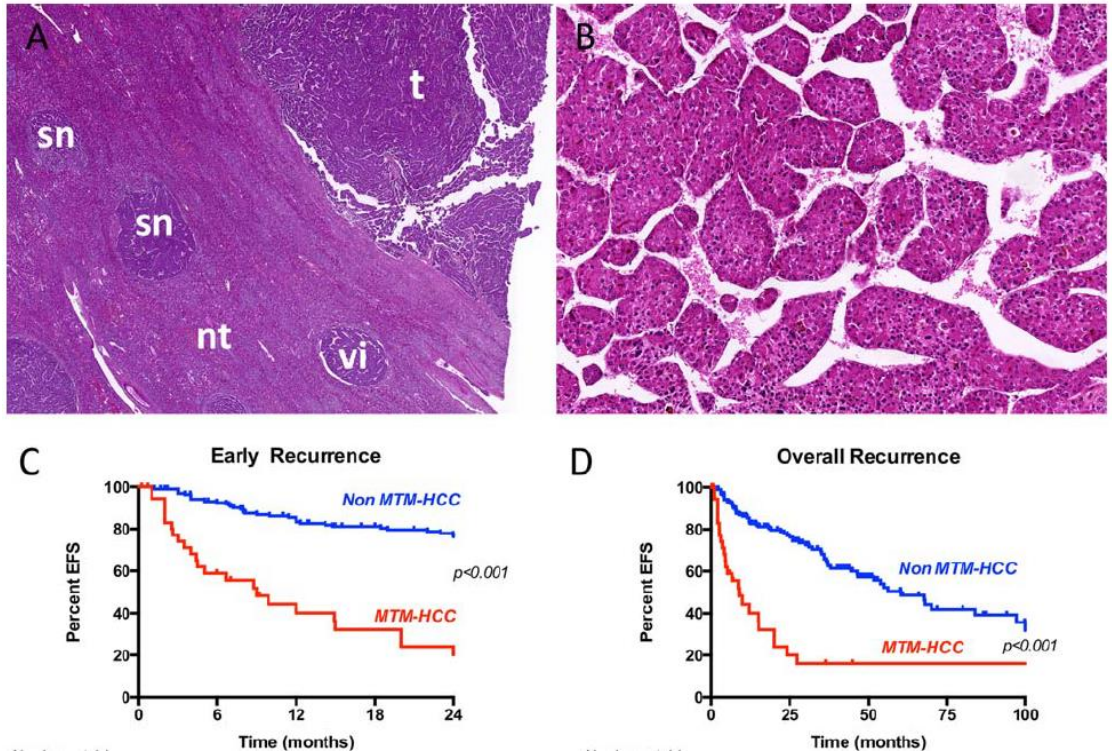
Macrotrabecular-massive HCC

- macrotrabecular architecture
  - >6-9 cells thick trabeculae
  - >50% of tumour
- about 1/4 of resected HCC
- worse survival, increased vascular invasion



Ziol M et al. Hepatol 2018; 68:103
Kleiner DE. Hepatol 2018; 68:13

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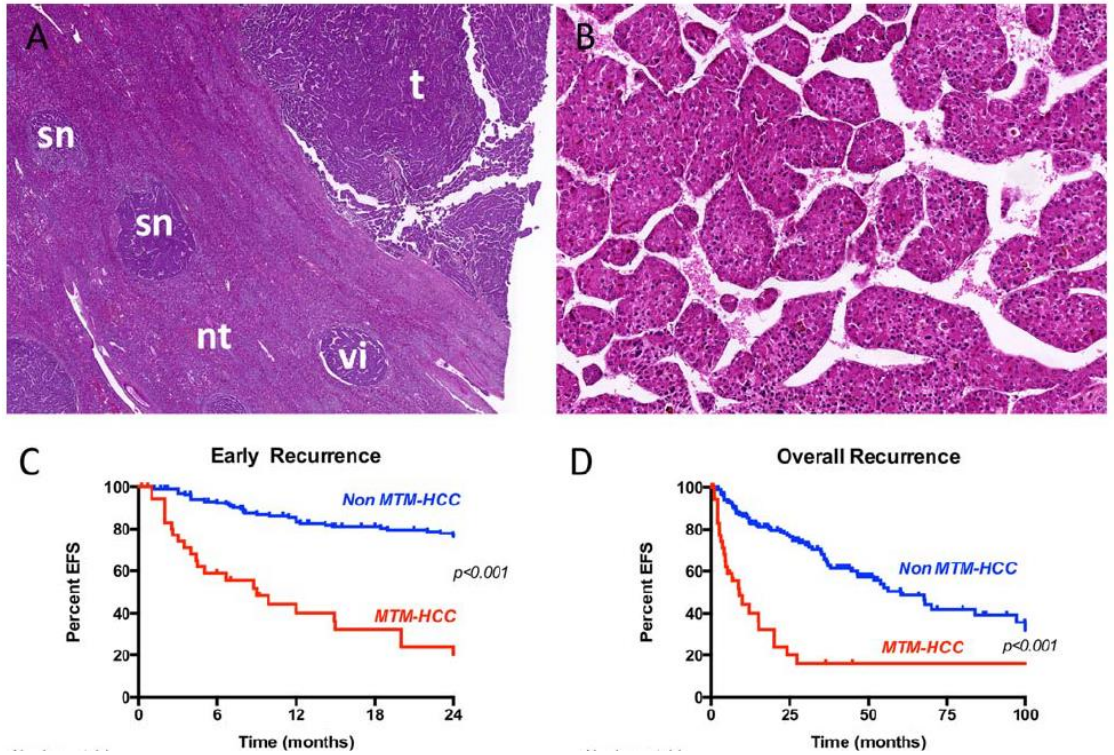


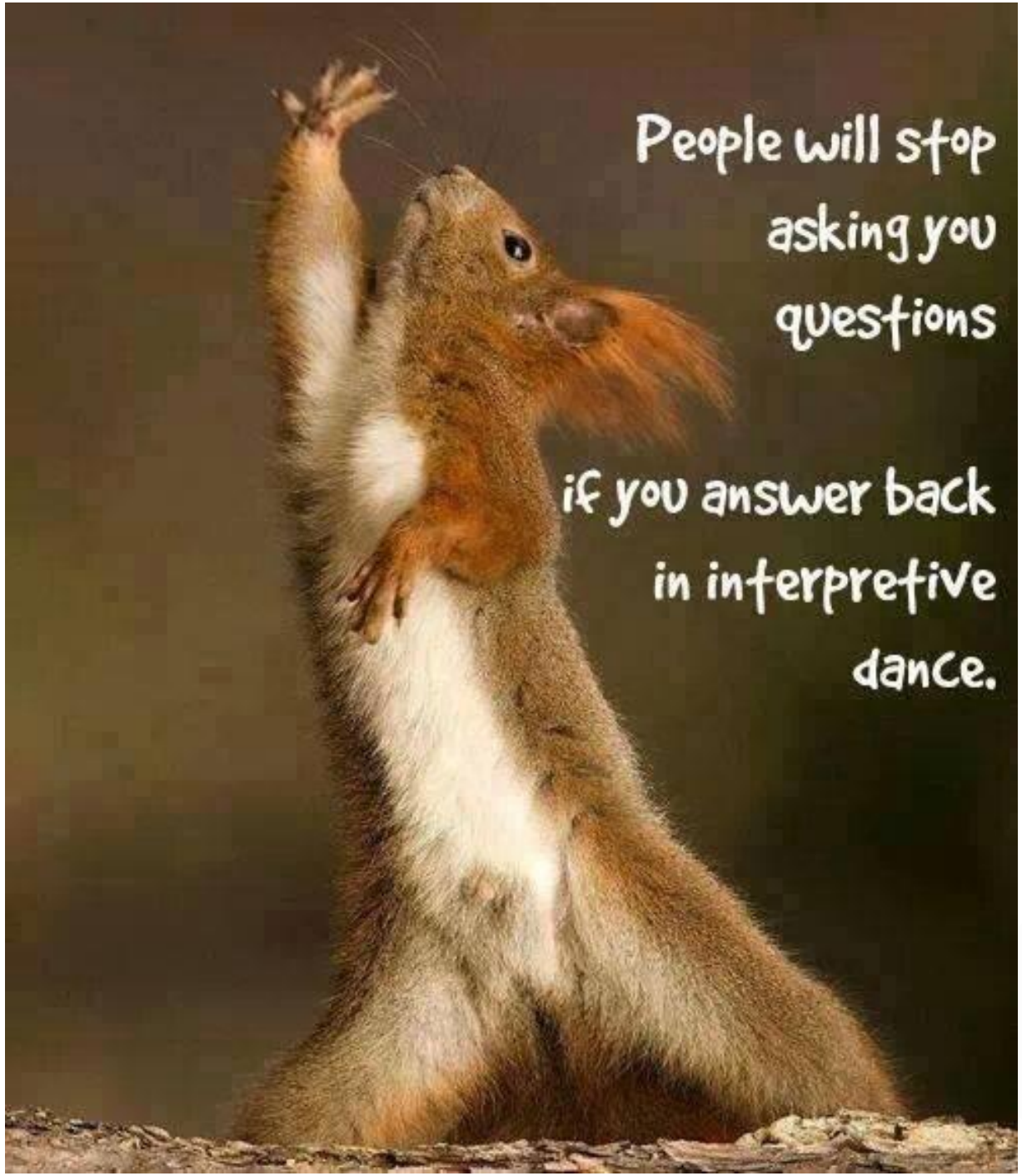
- subgroup with specific genetic mutations
  - *YAP* oncogene activation
  - EpCAM and K19 expression, activated angiogenesis
  - correspond to K19+ HCC

Ziol M et al. Hepatol 2018; 68:103
Kleiner DE. Hepatol 2018; 68:13

Summary

- hepatocellular adenomas are heterogeneous
  - IHC plays a key role but pitfalls exist
  - some types have increased bleeding or malignancy
  - atypical lesions are recognised but not fully understood

- HCC variants, combined HCC-CCa and stemness are recognised and subject of continuing studies


People will stop
asking you
questions
if you answer back
in interpretive
dance.

Case – Early HCC

★ Practice point

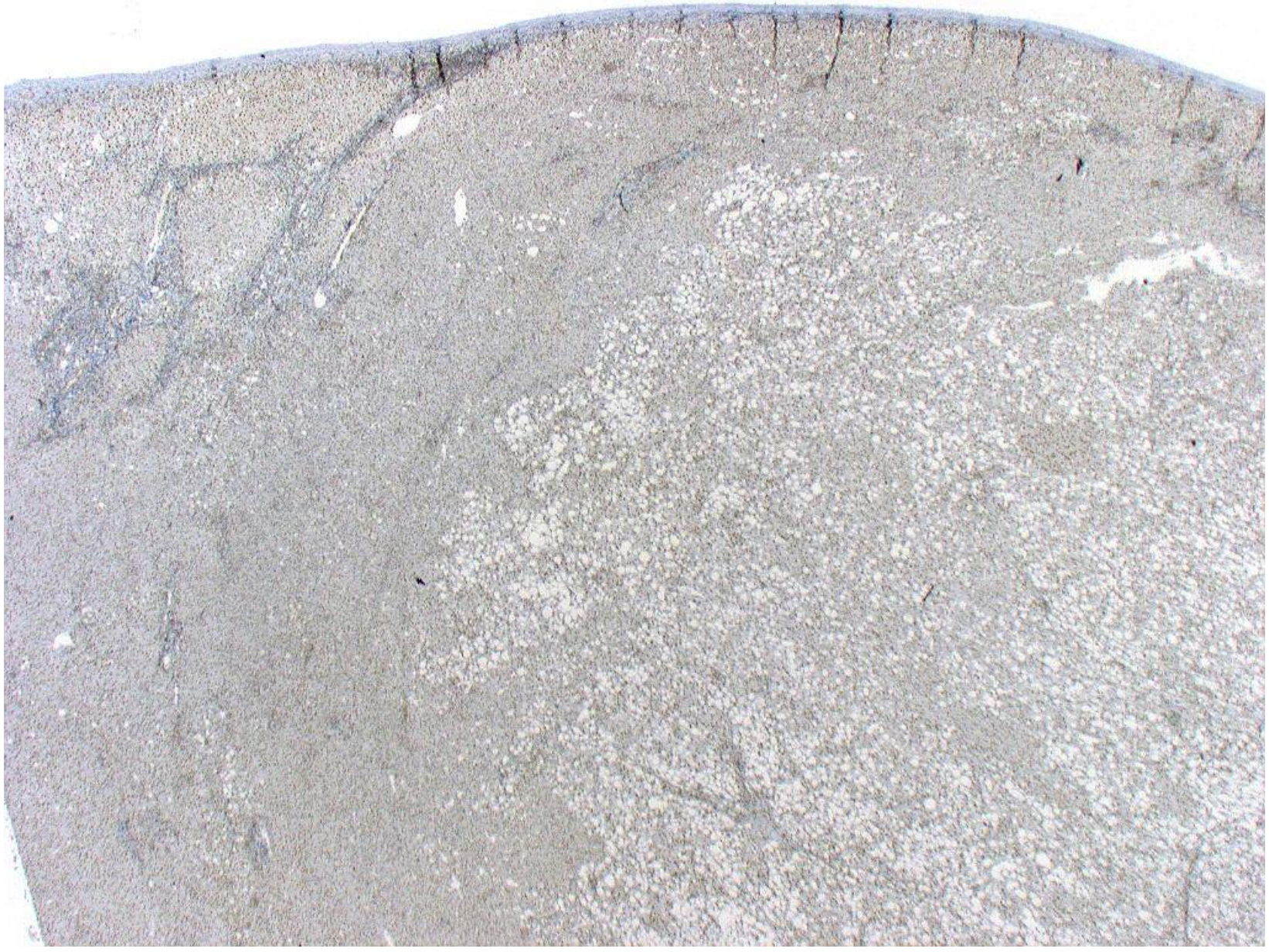
- Differential diagnosis of hepatocellular nodules
  - ➢dependent on clinical context
  - ➢cirrhosis/CLD vs normal liver

- Cirrhosis
  - dysplastic nodule vs early HCC vs progressed HCC

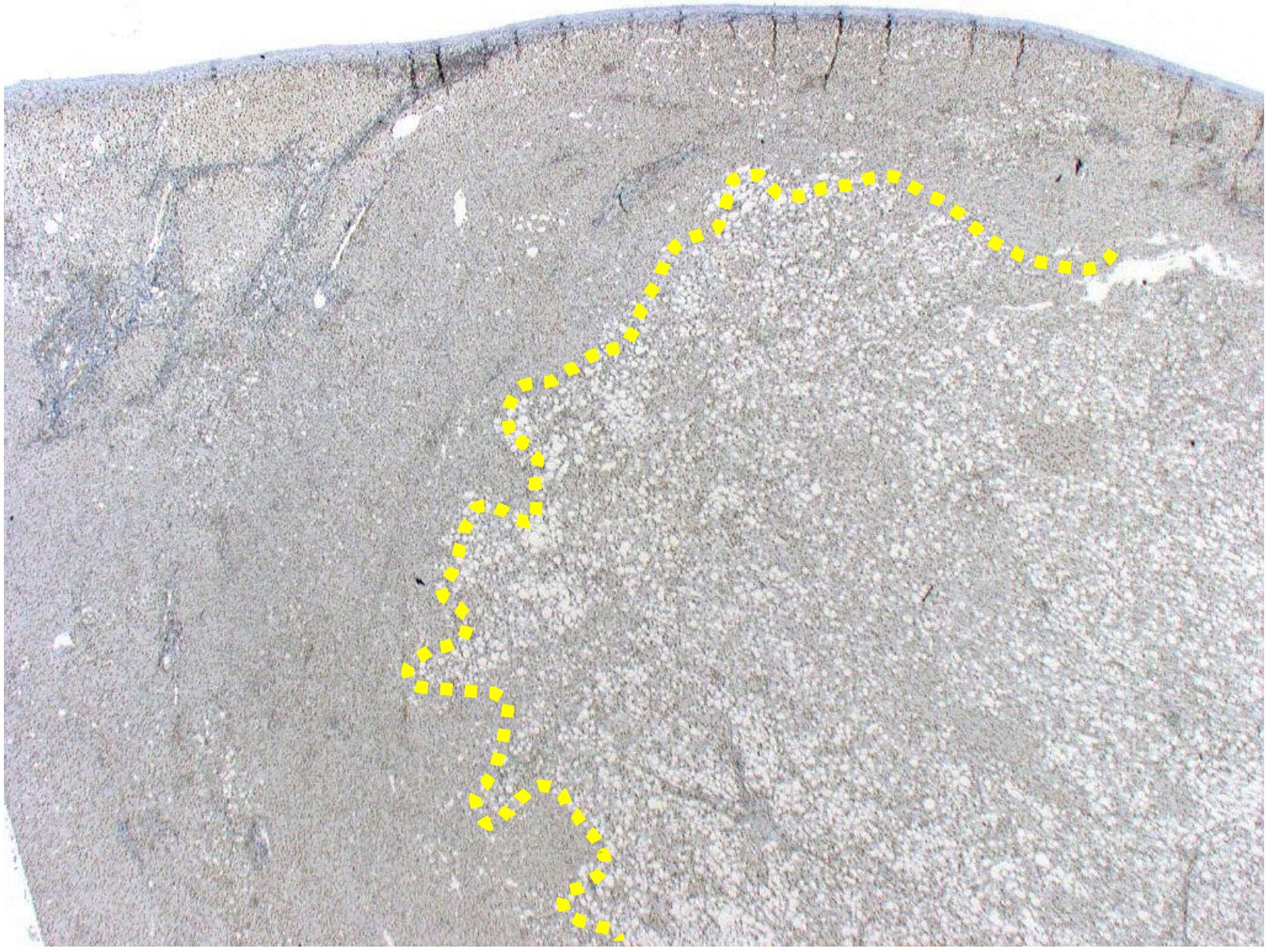
- Normal liver
  - FNH vs adenoma vs HCC

Early HCC (cirrhotic liver)

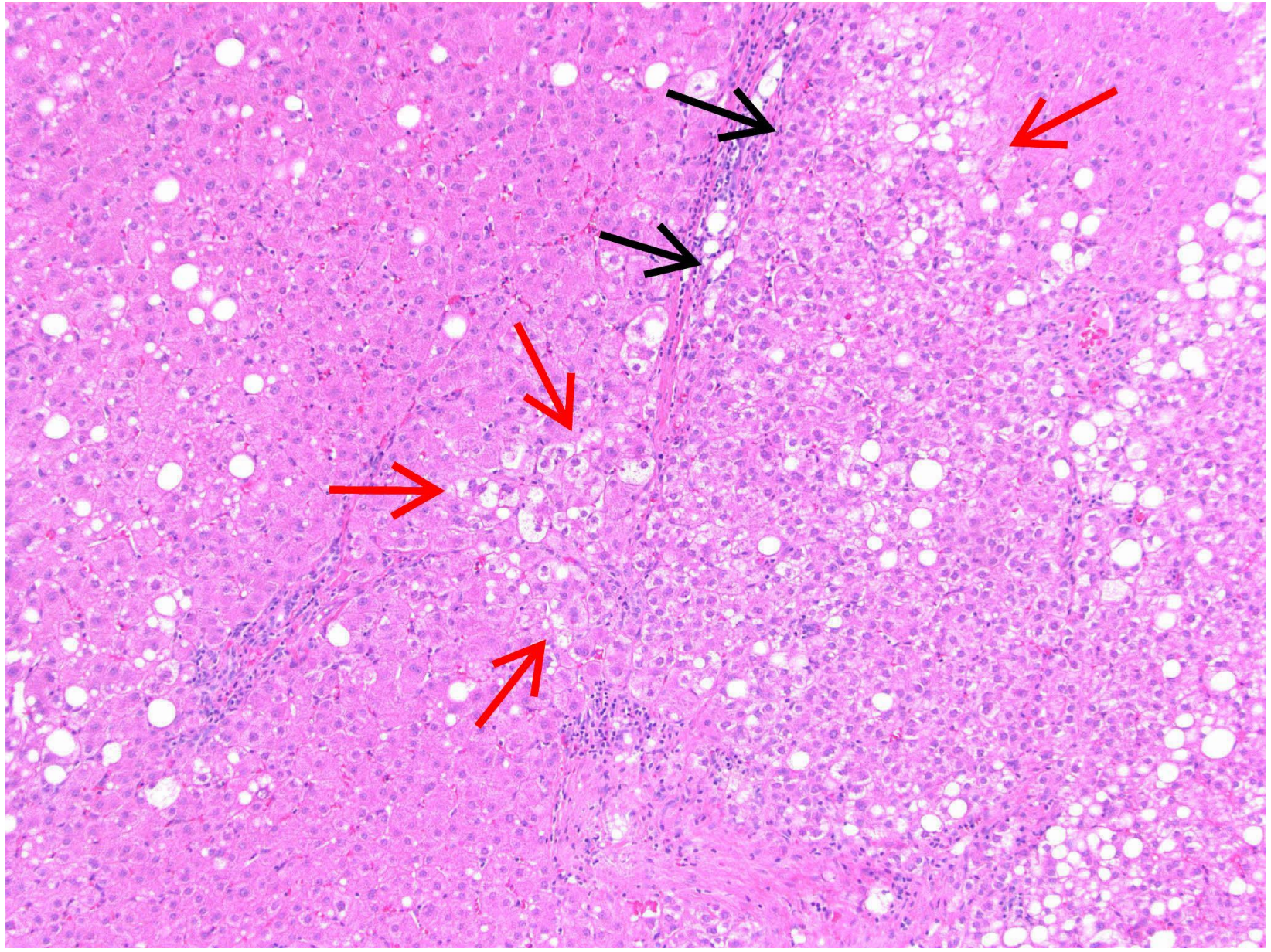
Early HCC



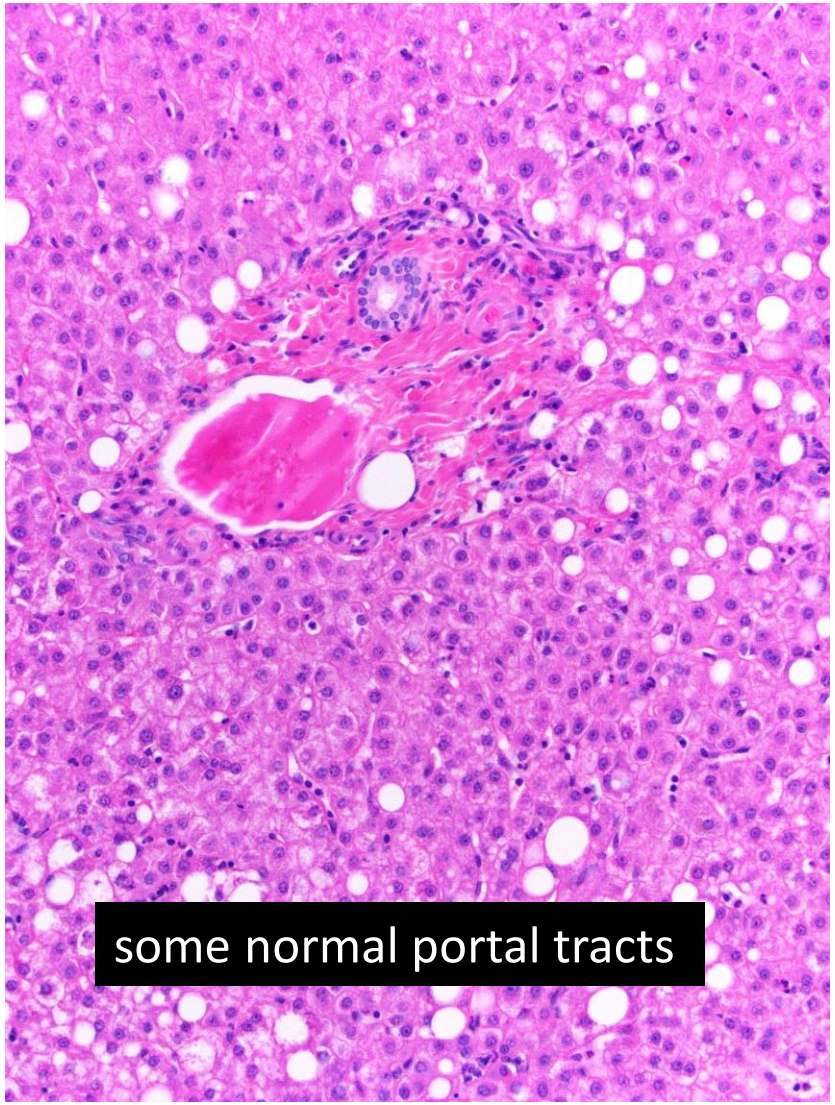
Early HCC

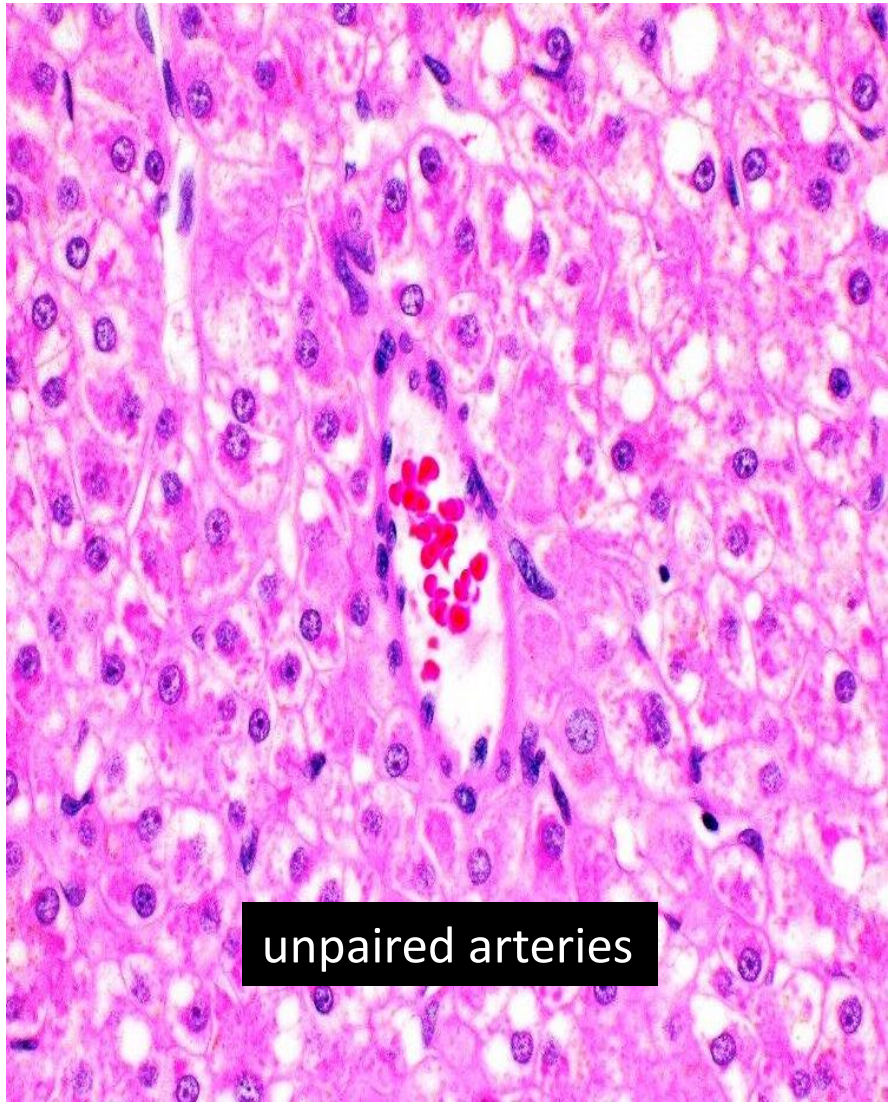


Early HCC



Early HCC





Early HCC

- vaguely nodular with indistinct margin

- Grade 1 (40% steatotic)
- some portal tracts, with partial arterialisation
- subtle infiltration (portal tracts & parenchyma)

- was poorly recognised by Western pathologists

Roncalli M. Dig Liver Dis. 2010 Jul;42 Suppl 3:S228

Consensus classification - nodules in cirrhotic livers

- Large regenerative nodule – LRN
- Low-grade dysplastic nodule – LGDN

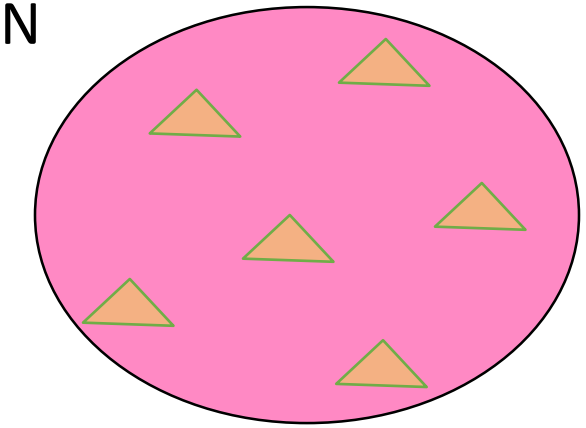
- High-grade dysplastic nodule – HGDN

- HCC – early
- HCC – progressed

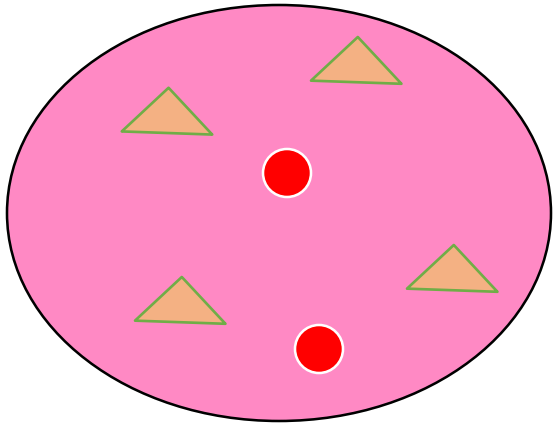
Intnl Consensus Group. Hepatol 2009; 49(2):658-64

Consensus classification

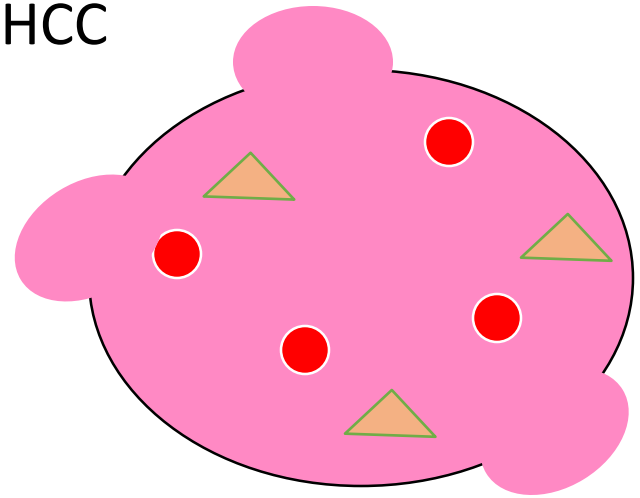
LRN/
LGDN



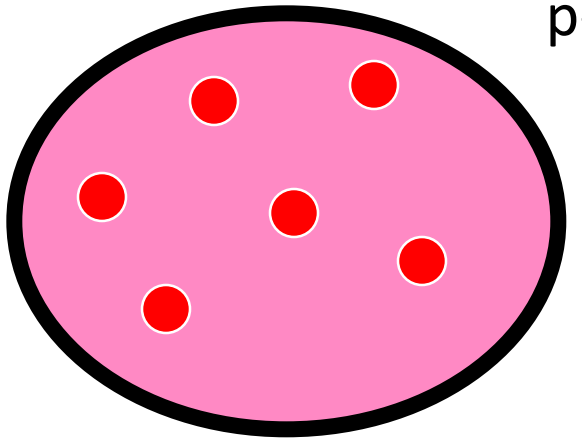
HGDN



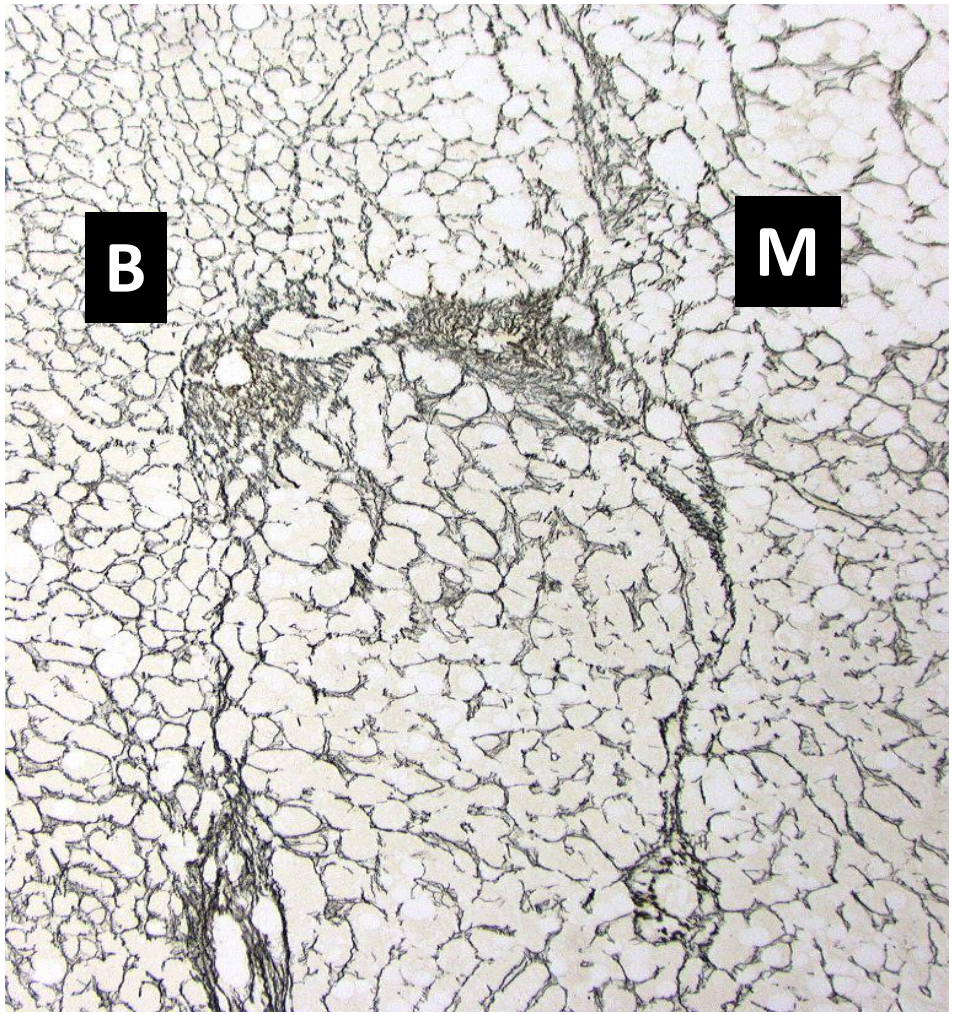
e-HCC



p-HCC



Early HCC – histology aids



- glypican 3
- HSP 70
- glutamine synthetase

- if 2/3 positive = HCC
- up to 70% sensitivity
- core bx *not cytology*

Progressed HCC

- easily recognisable

- moderately differentiated (Grade 2 or 3)
- no portal tracts, fully arterialised
- often a capsule
- venous invasion in 25% small lesions

- origin = early HCC *or*
  = microscopic dysplasia

Progressed HCC

HCC – early vs progressed

	Early HCC	Progressed HCC
Size	<2cm	any
Growth	replacing	expansile
Steatosis	40%	no
Grade	G1	G2-3
Arterialisation	incomplete	complete
Vascular invasion	no	+/-
5 year survival	89%	48%