

Precursor lesions progressing toward HCC, and its different subtypes

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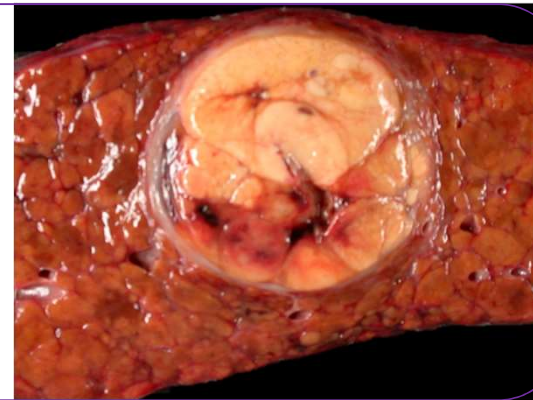
valerie.paradis@aphp.fr; <https://fhu-mosaic.com/>



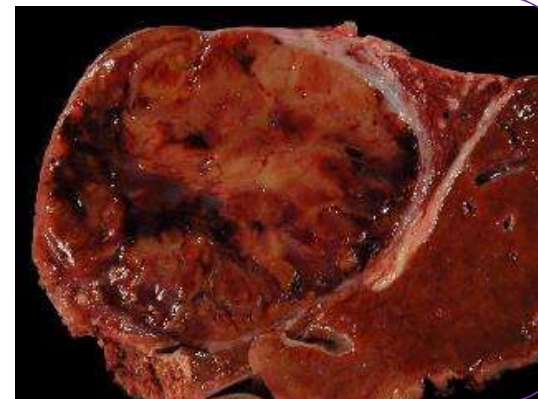
Hepatocellular carcinoma (HCC) development

2 clinical settings

- ① Chronic liver diseases (85%)
≠ risk factors
(VHC, VHB, Alcohol, NAFLD, ...)



- ② ~ Normal liver (15%)

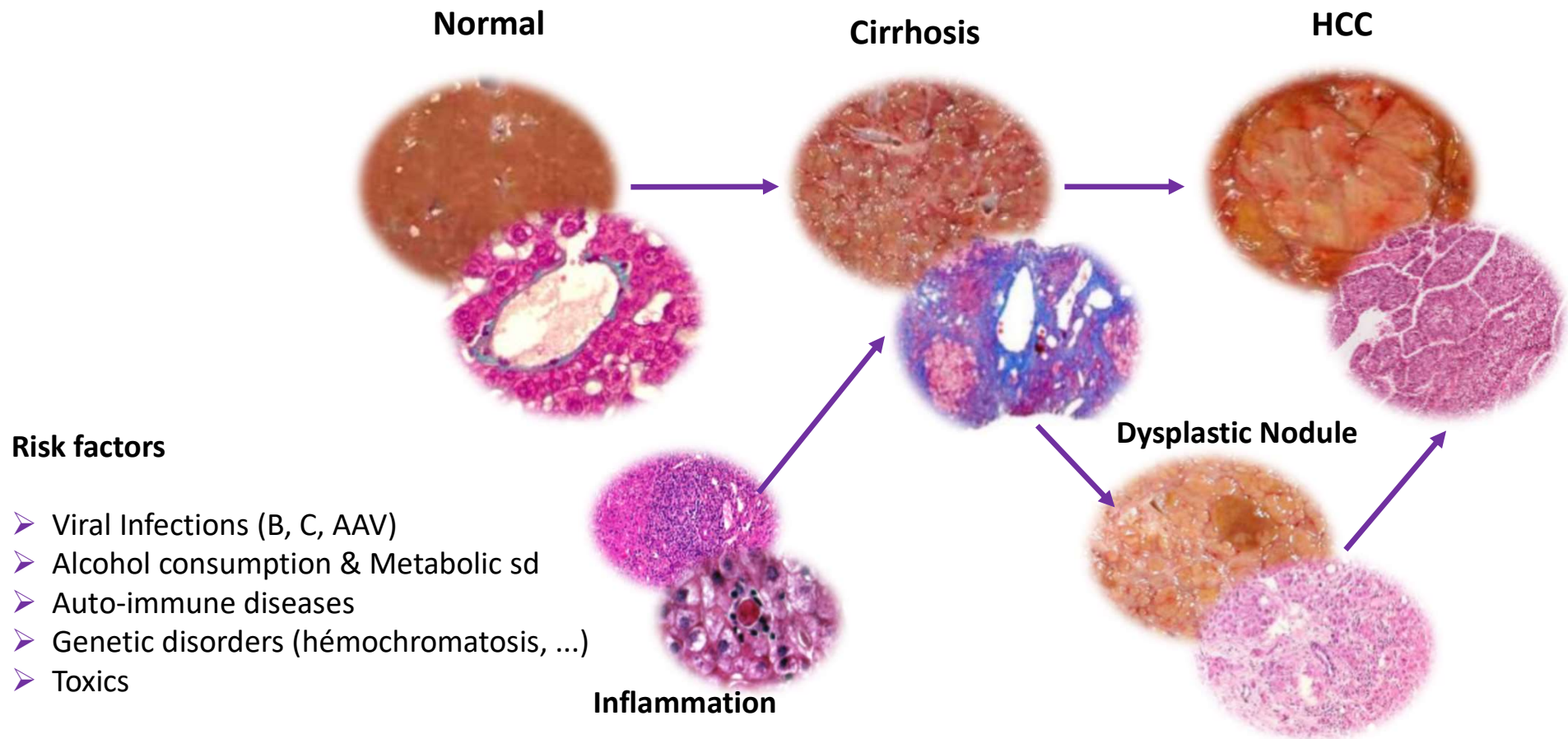


① **CHRONIC LIVER DISEASES**

EPITHELIAL PRECURSOR LESIONS: DYSPLASTIC NODULES

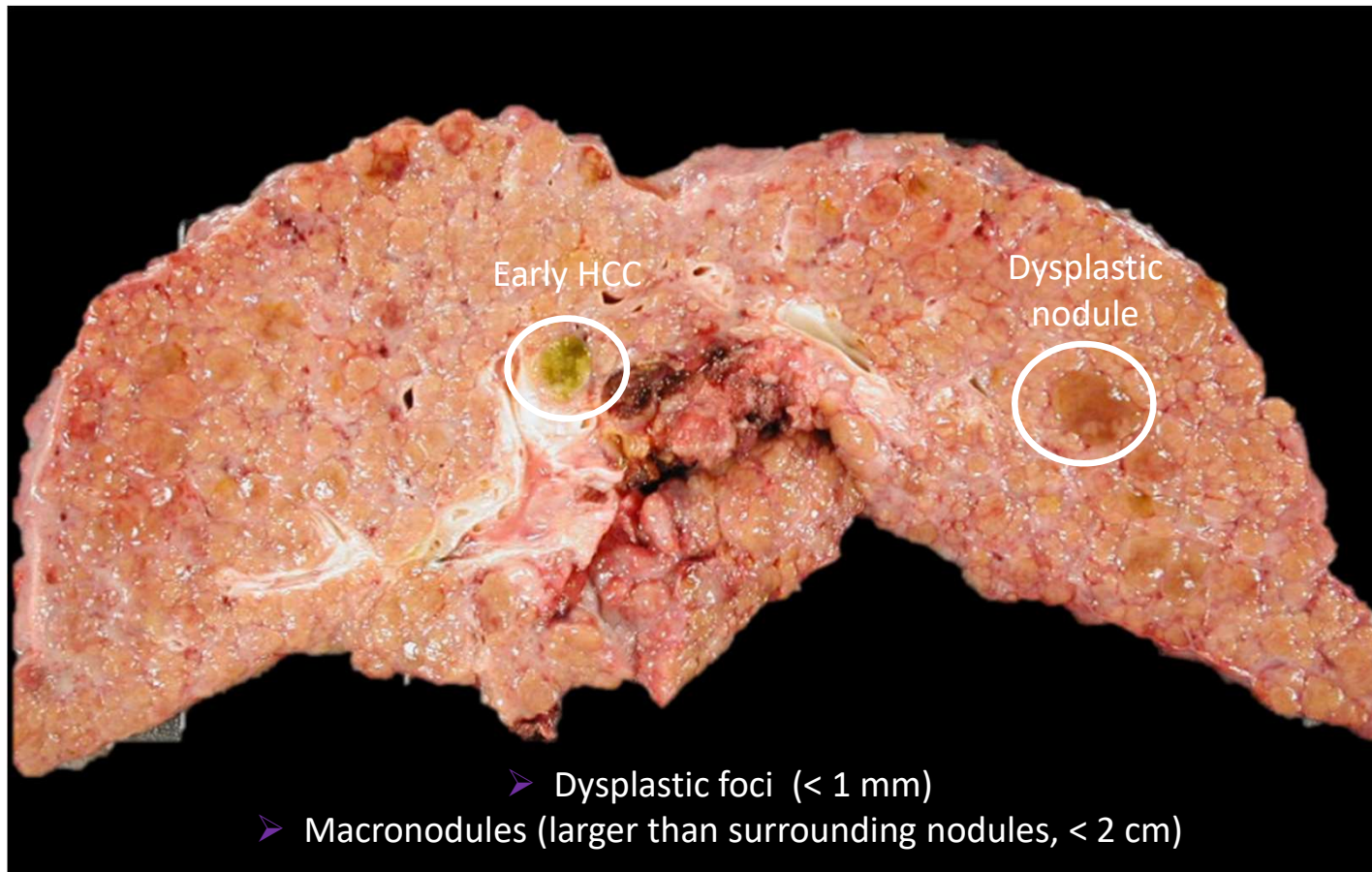
Liver Carcinogenesis

A multistep process initiated by chronic inflammation

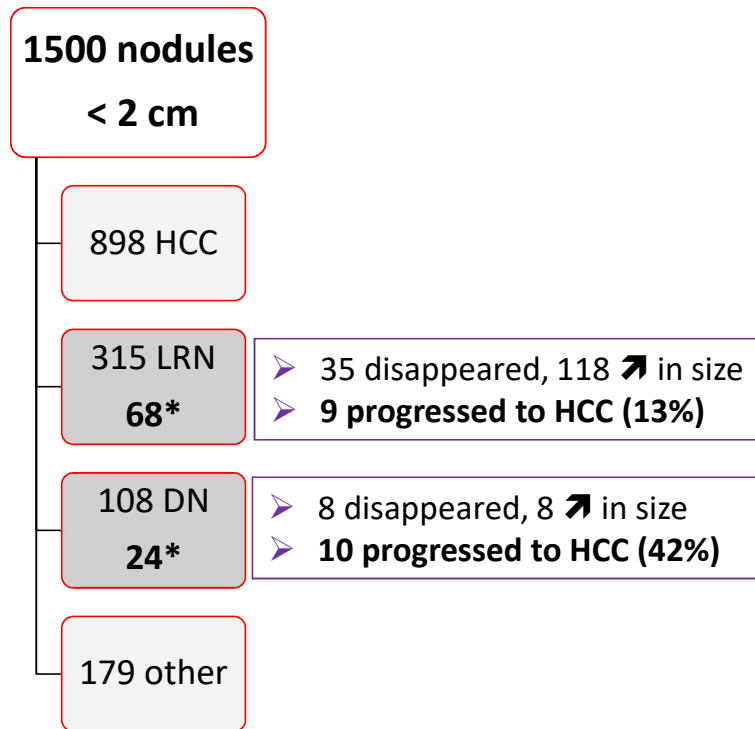


Cirrhosis: A Preneoplastic Condition

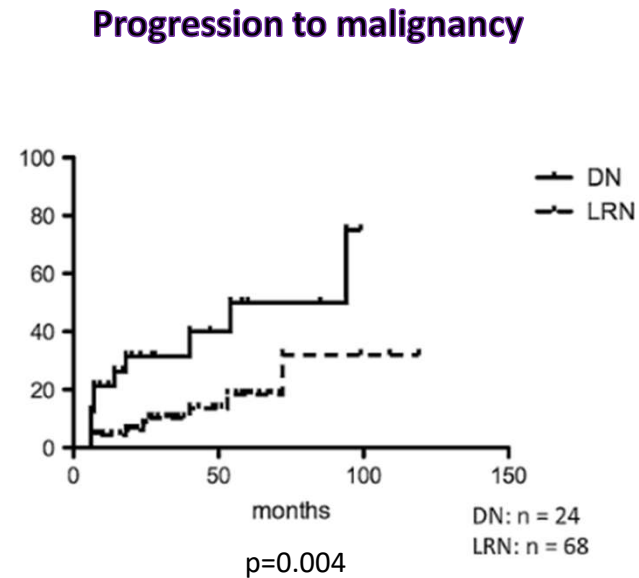
A mixture of poly- and monoclonal nodules



Natural history of large regenerative nodules and dysplastic nodules in liver cirrhosis: 28-year follow-up study

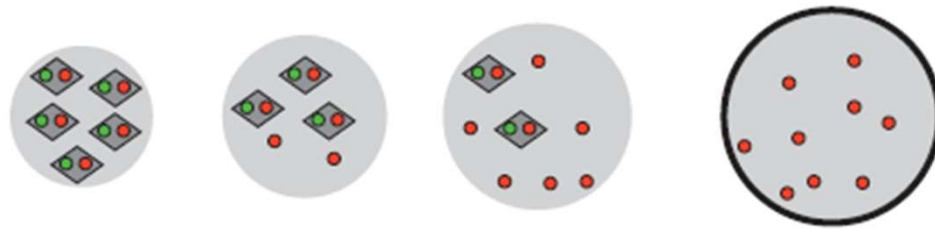


* Followed > 6 months



Pathologic Diagnosis of Early Hepatocellular Carcinoma: A Report of the International Consensus Group for Hepatocellular Neoplasia

Hepatocellular nodules < 2 cm



IWP Classification	Low grade Dysplastic nodule	High grade Dysplastic nodule	Well-Diff HCC	Mod-diff HCC
Pathological features	Gross & microscopic features			
Imaging features	Arterial & portal vein supply			
Biological behaviour	Premalignant nodules		Malignant nodules	
Progression risk to HCC @ 2 y	<20%	≈ 40%	-	

International Consensus Group for Hepatocellular Neoplasia (Hepatology 2009)

Pathologic diagnosis of small hepatocellular nodules

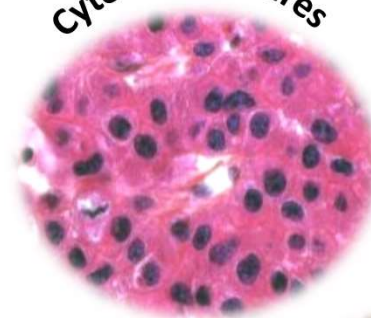
« Combined features »



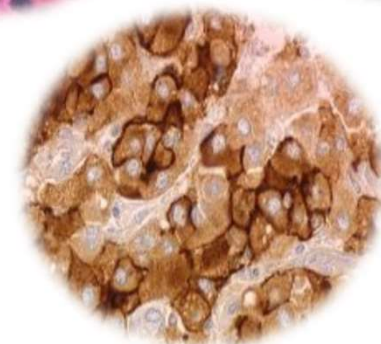
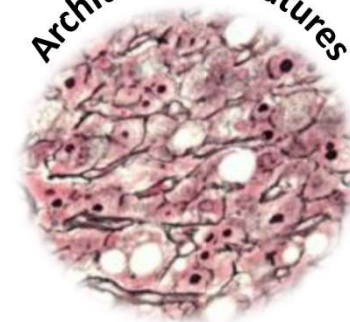
Table 2. Important Pathologic Features of Hepatocellular Nodules

	LRN	LGDN	HGDN	WD HCC
Cytologic features				
Small cell change	-	-	+	+
Large cell change	±	±	±	-
Clonelike foci (clear, fatty)	-	±	+	+
Architectural features				
Plate thickening ≥3 cells	-	-	-	+
Increased cell density compared with surroundings	-	-	1.3 to 2 times	>2 times
Pseudoglands	-	-	±	+
Nodule-in-nodule	-	-	-	±
Portal tract	+	+	+	±
Unpaired arteries and capillarized sinusoids ^a	-	±	±	+
Stromal invasion ^b	-	-	-	+
Reticulin framework	+	+	+	±
Tumor markers				
Glypican-3	-	-	± (9%)	+
Heat shock protein 70	-	-	± (5%)	+
Glutamine synthetase	-	-	± (13%)	+
Positive in at least 2 of above 3 markers	-	-	-	+

Cytologic features



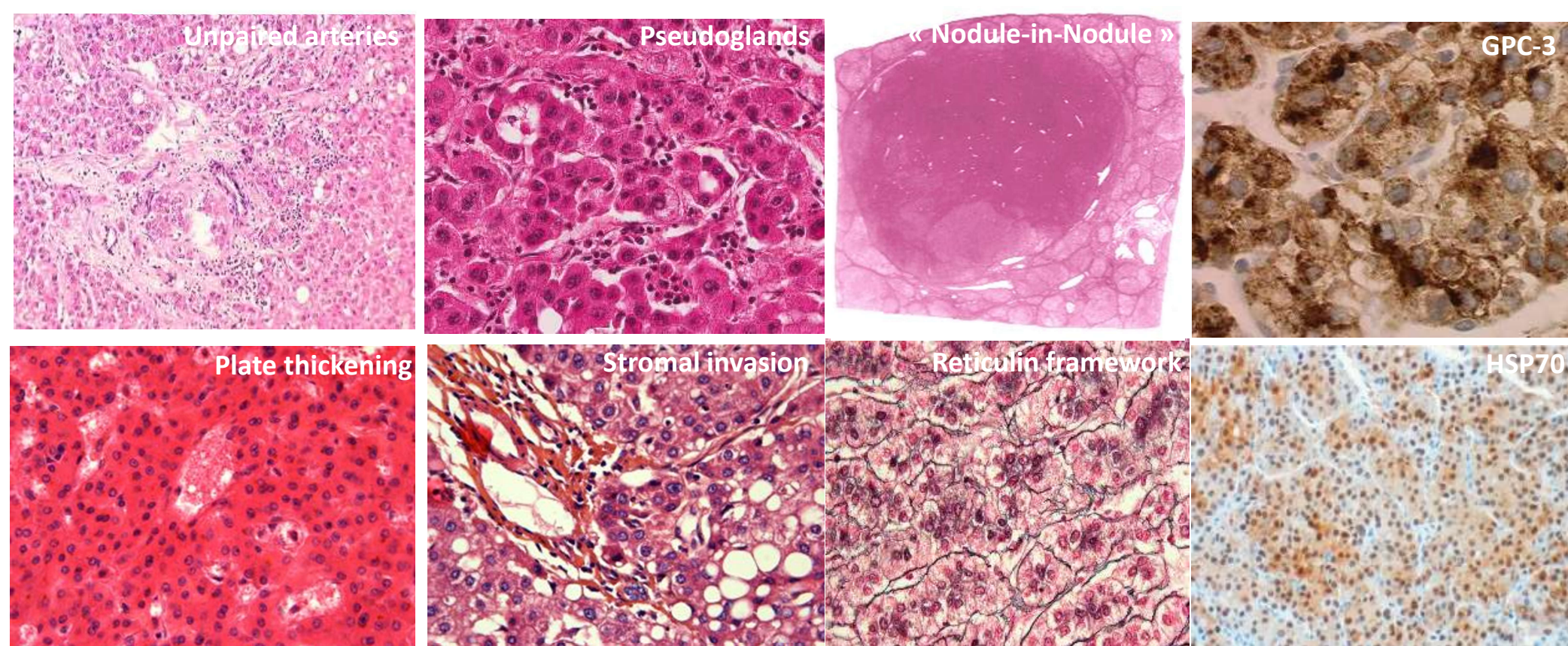
Architectural features



Glypican-3

Di Tommaso L J Hepatol 2009; Park NY Arch Pathol Lab Med 2011

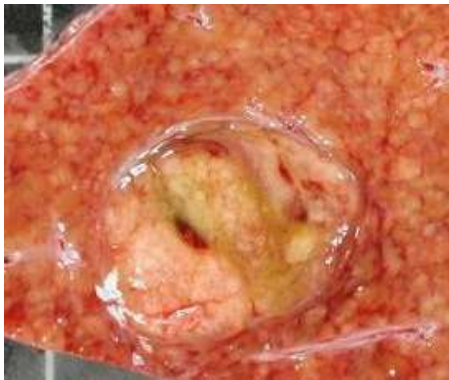
Differential diagnosis « HGDN vs early HCC »



« The panel (GPC3, HSP70 & GS) only slightly increases the diagnostic accuracy in an expert setting »

Tremosini S Gut 2012

Diagnostic Value of HSP70, Glypican 3, and Glutamine Synthetase in Hepatocellular Nodules in Cirrhosis

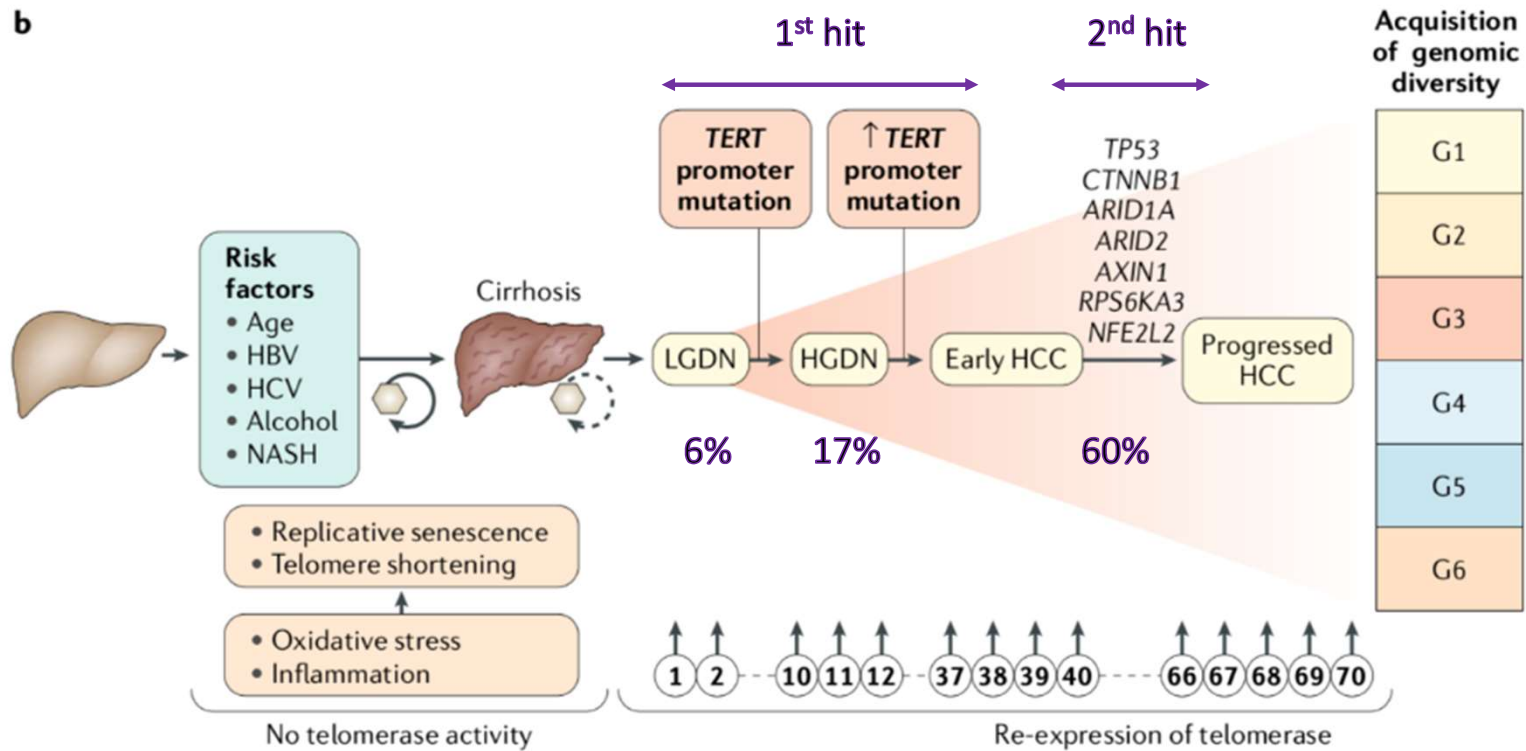


Phenotype	eHCC-G1 (n = 32)	HGDN (n = 22)
HSP70-/GPC3-/GS-	1 (3.1%)	16 (72.73%)
HSP70-/GPC3-/GS+	3 (9.4%)	3 (13.64%)
HSP70+/GPC3-/GS-	3 (9.4%)	1 (4.54%)
HSP70+/GPC3+/GS-	5 (15.6%)	0
HSP70-/GPC3+/GS-	2 (6.2%)	2 (9.09%)
HSP70-/GPC3+/GS+	1 (3.1%)	0
HSP70+/GPC3-/GS+	3 (9.4%)	0
HSP70+/GPC3+/GS+	14 (43.7%)	0

- HGDN are phenotypically distinct from early HCC
- Panel is useful in differential and positive diagnosis of early HCC vs HGDN

Liver Carcinogenesis

A sequential molecular process

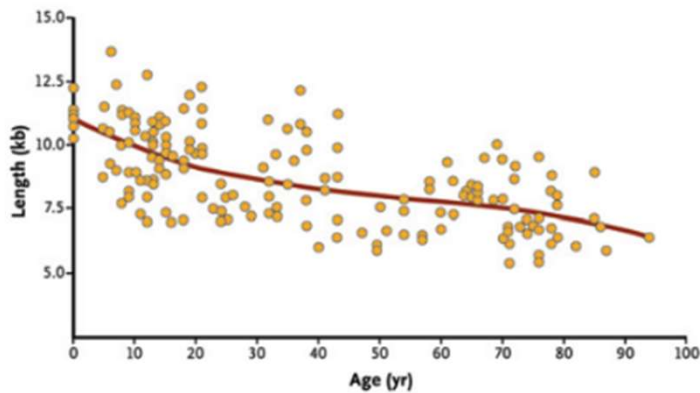


Liver Carcinogenesis

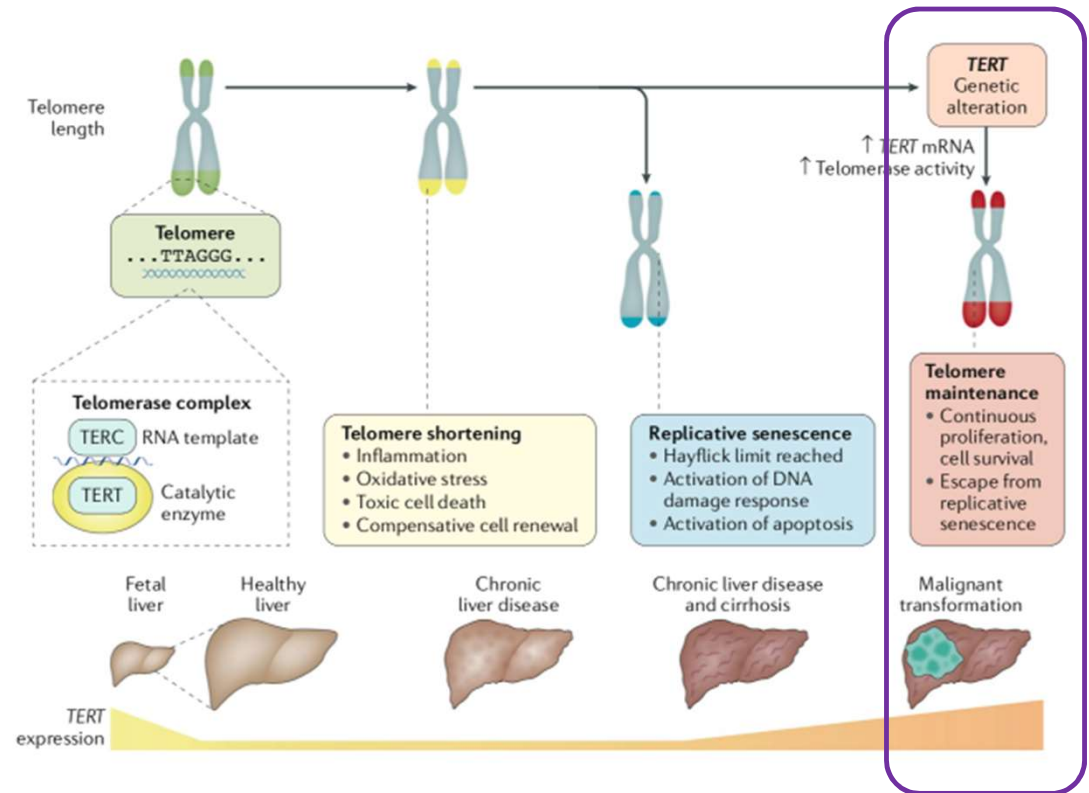
From Replicative Senescence to Immortality

➤ Replicative Senescence

Physiological mechanism related to progressive shortening of telomers

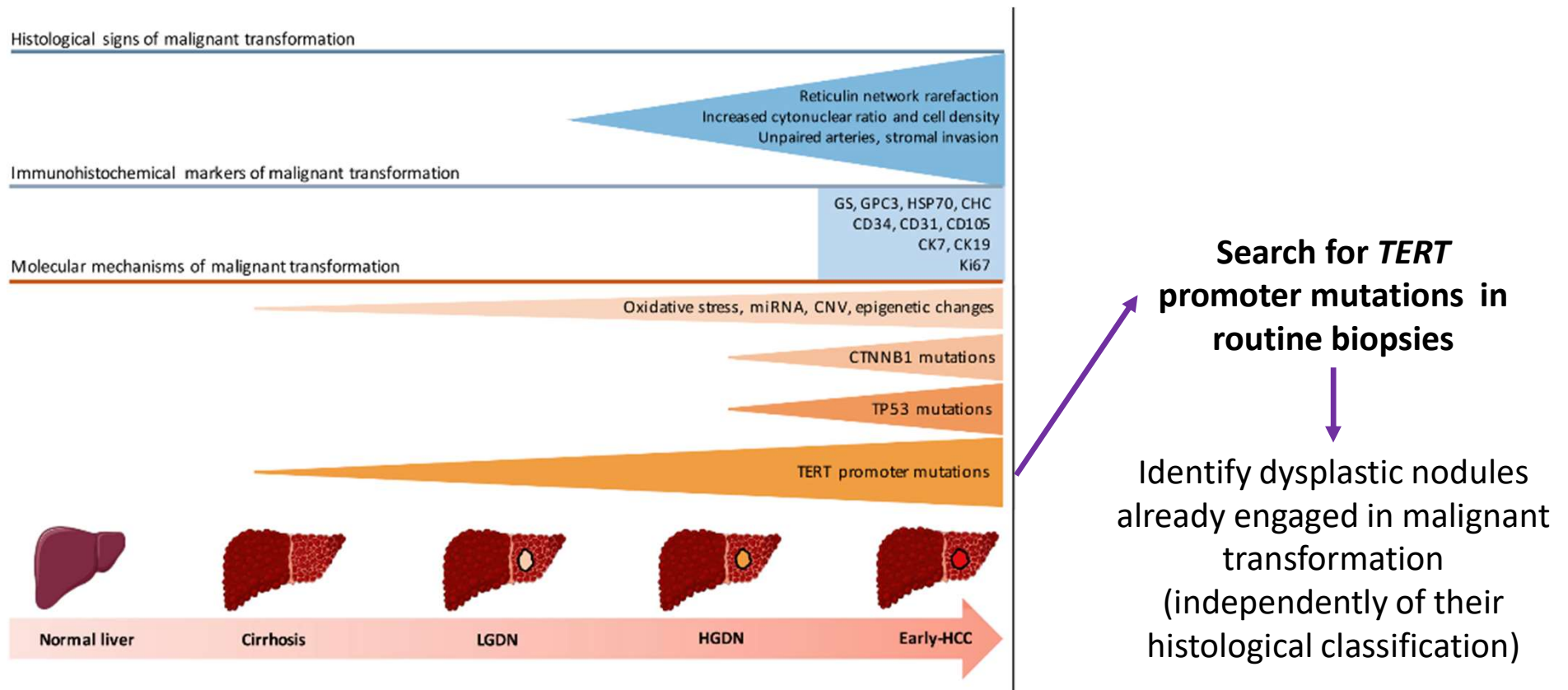


Calado RT NEJM 2009

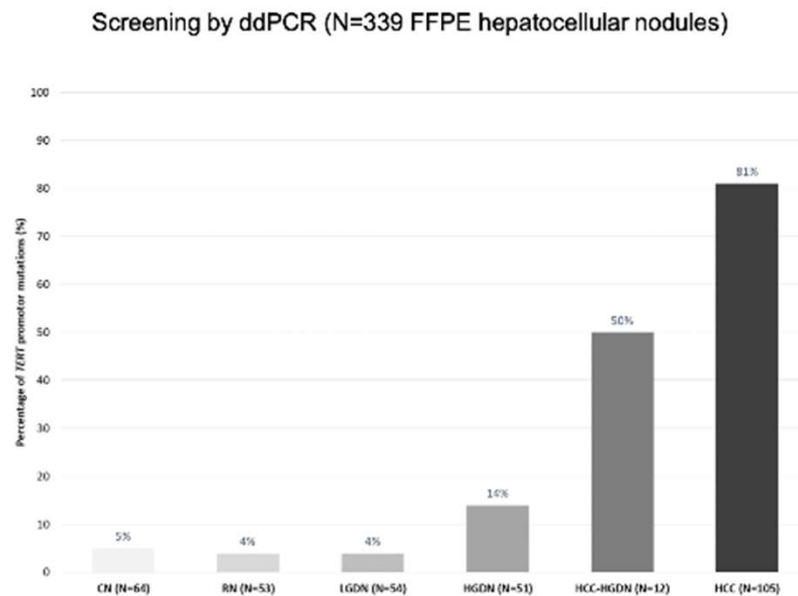


Nault JC Nat Rev Gastroenterology & Hepato 2019

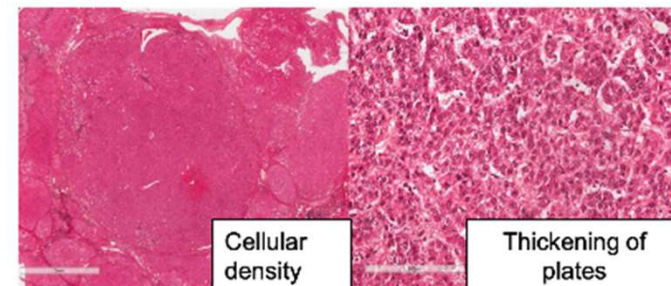
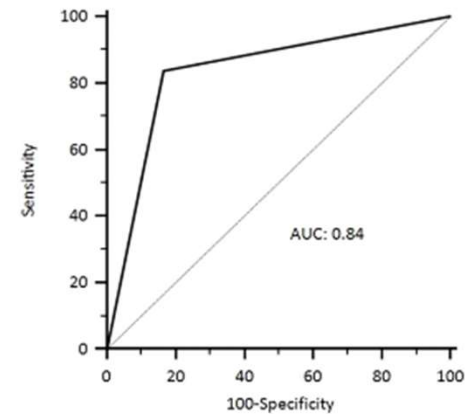
TERT promoter is the gatekeeper events leading to transformation on HCC



Surrogate markers of *TERT* promoter mutations for clinical use



Histological prediction of p*TERT* mutations



Beaufriere A (submitted)

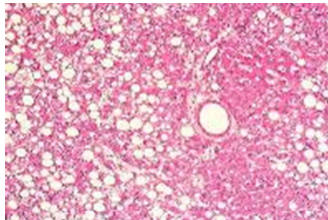
② **NORMAL LIVER**

EPITHELIAL PRECURSOR LESIONS: HEPATOCELLULAR ADENOMA

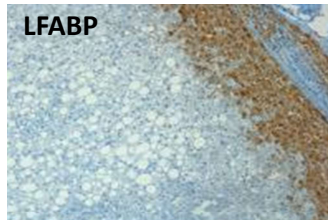
Pathomolecular HCA subtypes

(Morphological & immunophenotypical features)

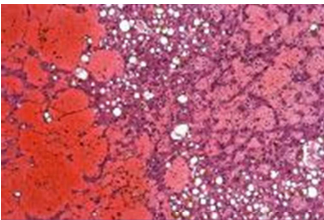
HNF1A mutated



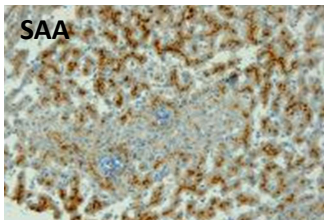
LFABP



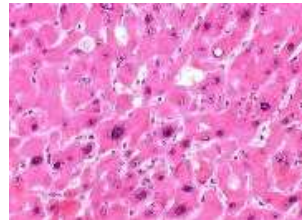
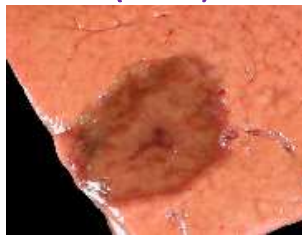
Inflammatory



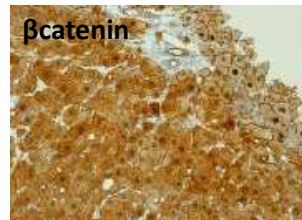
SAA



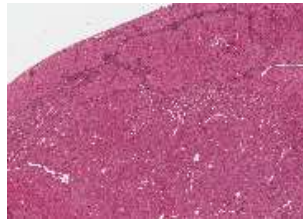
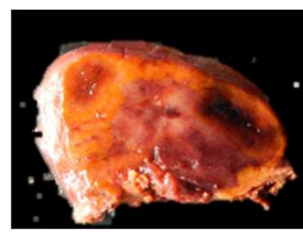
B-catenin activation
(exon 3)



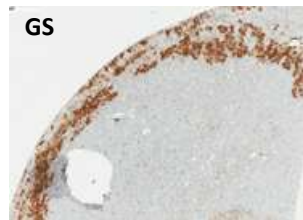
β catenin



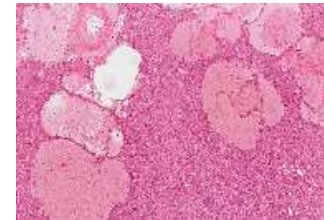
Bcatenin activation
(exon 7/8)



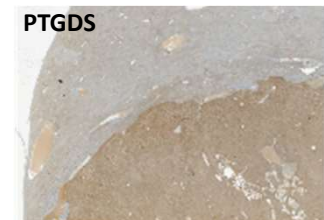
GS

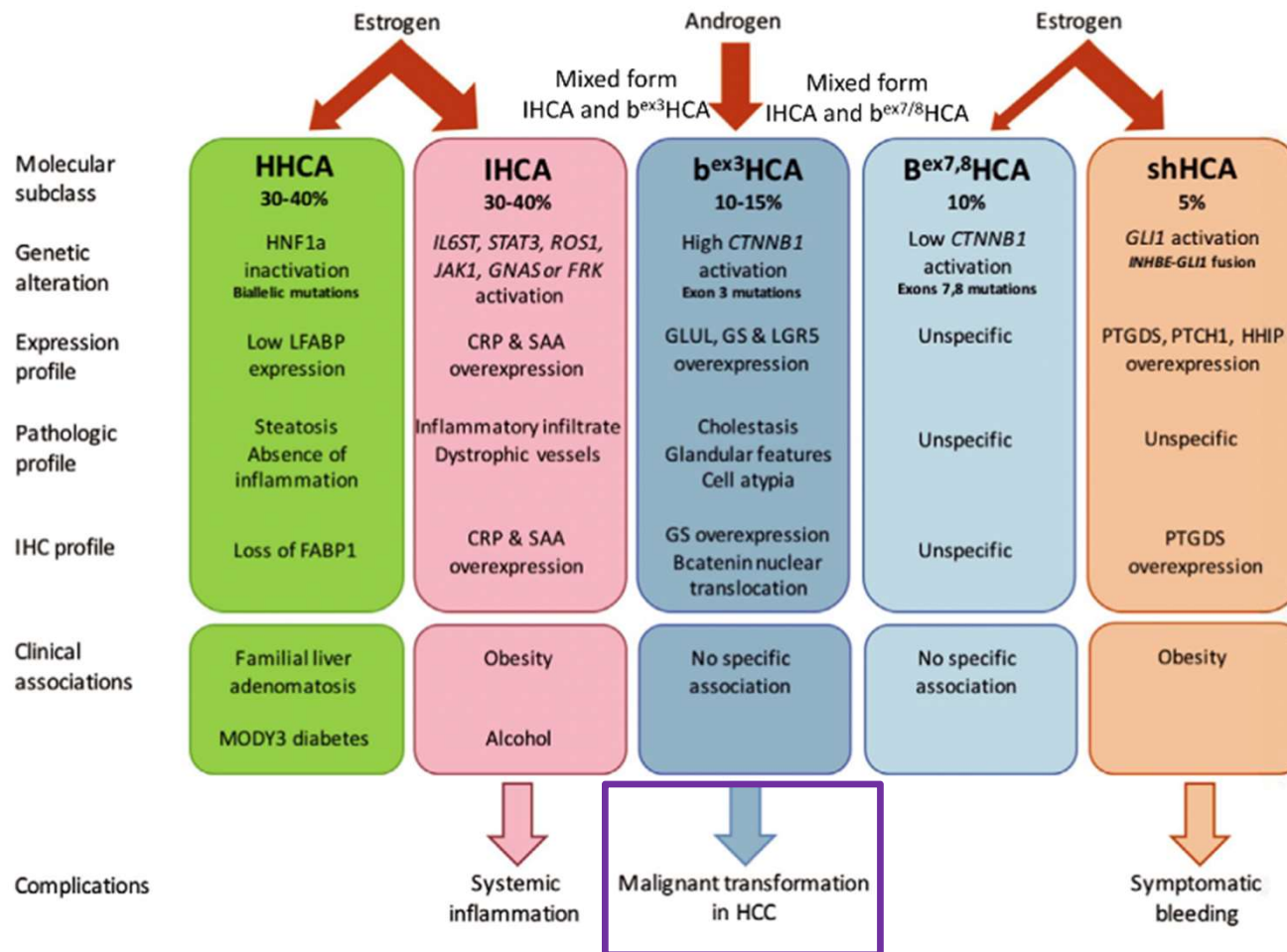


Sonic Hedhehog
activation



PTGDS

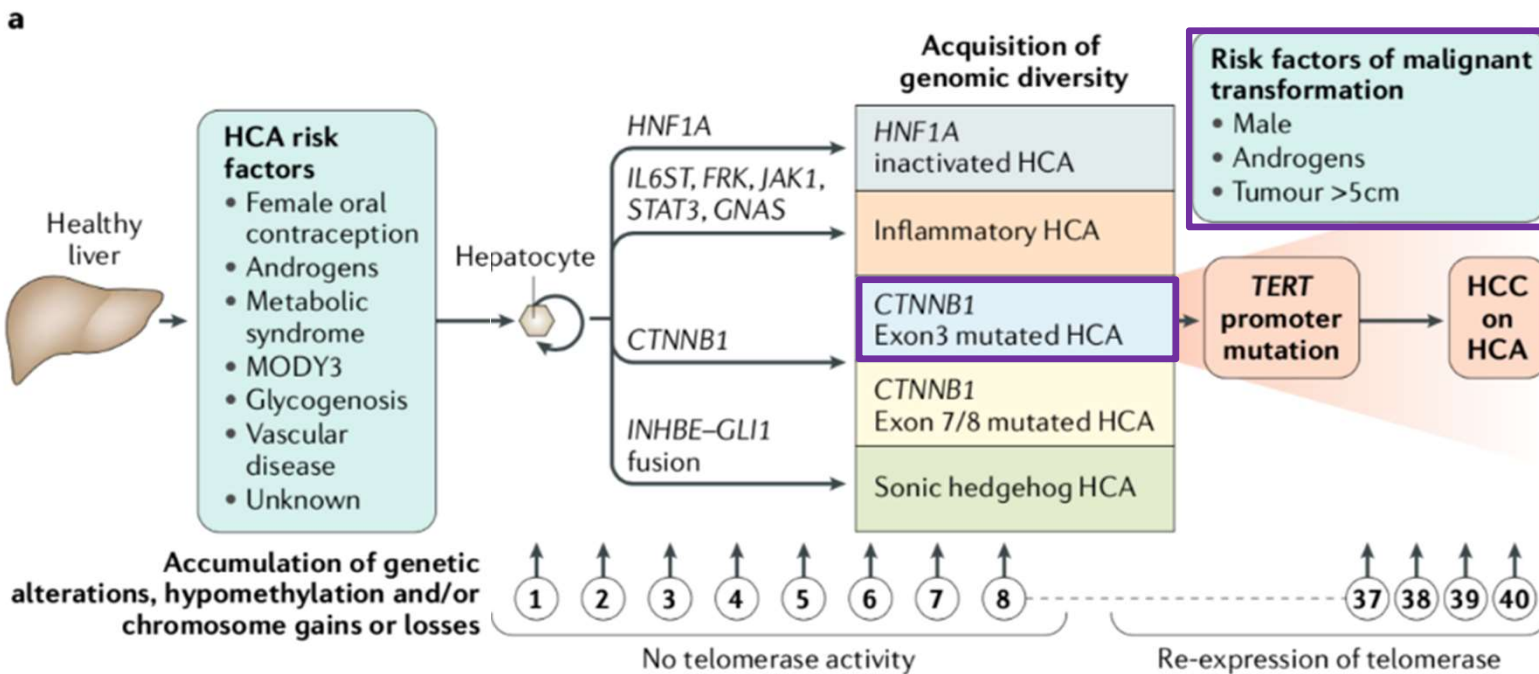


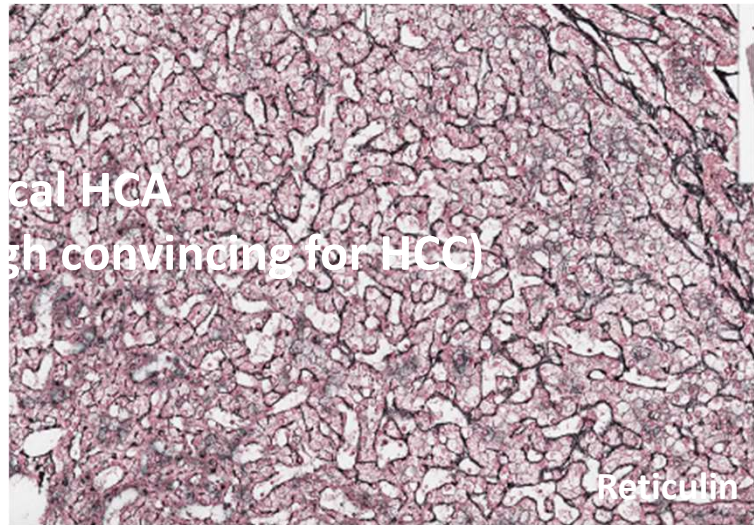
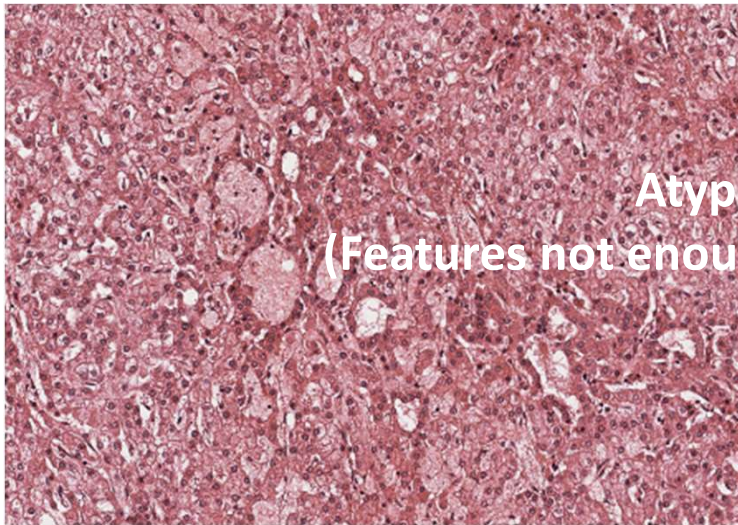
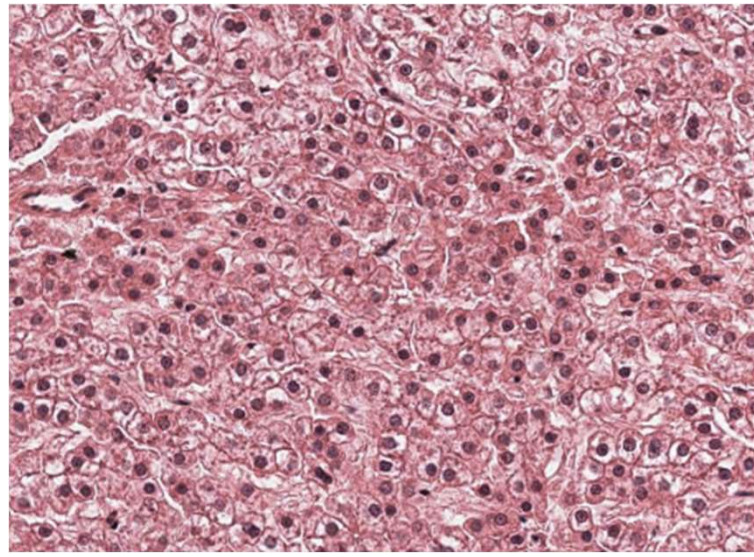
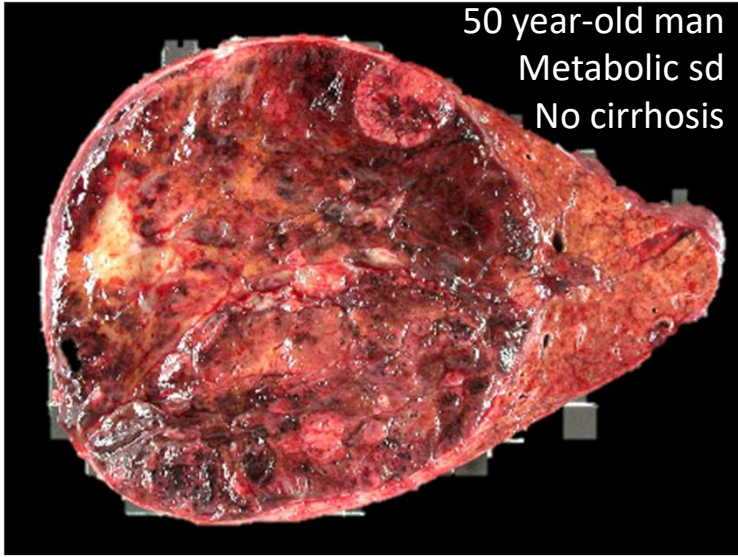


Desjonqueres E Liver Int 2021

From HCA to HCC

Involvement of *TERT* promoter mutations





Atypical (Borderline) Lesions

No consensual definition

➤ Hepatocellular-like lesions with atypical features

- Clinical (♂ ou ♀ < 15 ou > 50 y)

And / or

- Histological features (small cells, pseudoglandular pattern, abnormal reticulin framework)
- **Genetic alterations (β-catenin alterations in exon 3)**

Kakar S Histopathology 2009, Evason KE Human Pathol 2012, Nault JC Nature Com 2013, Rebouissou S Hepatology 2016

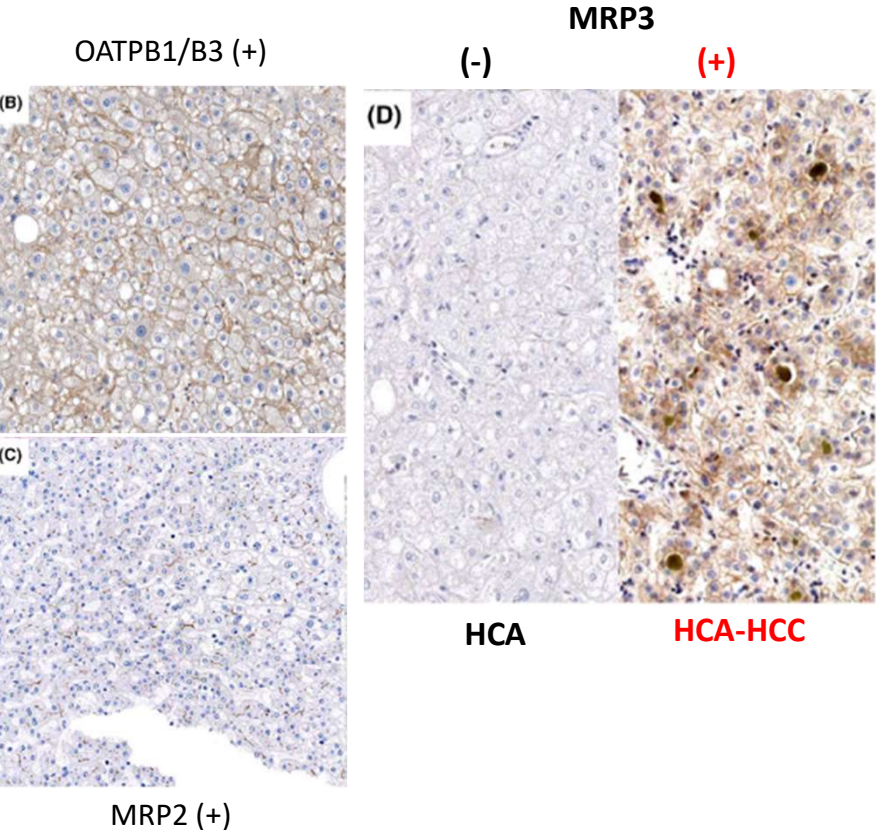
Well-differentiated hepatocellular neoplasm of uncertain malignant potential: proposal for a new diagnostic category

To the Editor:

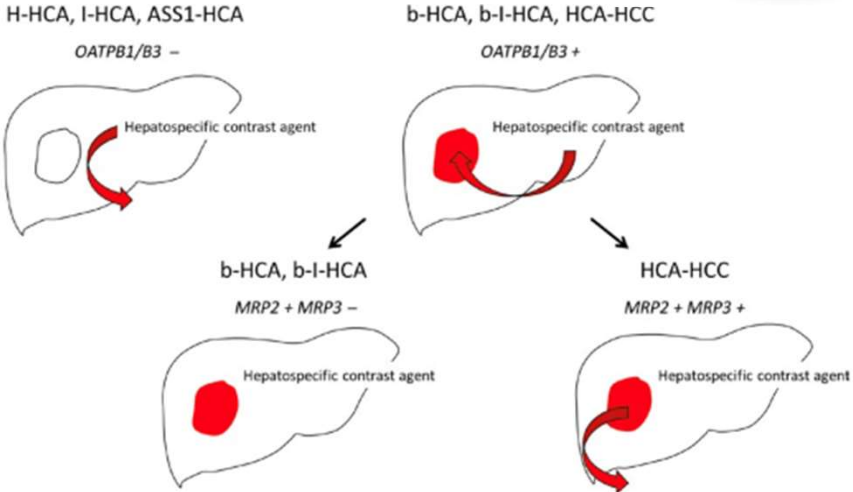
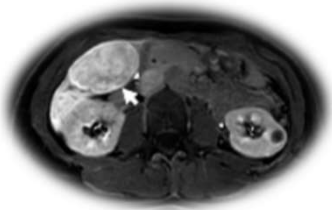
After lengthy discussions, we concluded that the current terminology of hepatocellular neoplasms [4,5] fails to account for lesions that cannot be confidently classified dichotomously as either hepatocellular adenoma or carcinoma. We propose, therefore, that the term well-differentiated hepatocellular neoplasm of uncertain malignant potential (HUMP) would be the best term to use because “atypical hepatocellular neoplasm” or “atypical adenoma” may not adequately convey the necessity for close follow-up. It also permits the single atypical feature of focal reticulin loss to be identified without applying a diagnosis of carcinoma because the cases that we studied all had followed a benign course after resection. More important than the terminology used is the observation that a small subset of well-differentiated hepatic lesions defies classification using currently available methods, even among experienced hepatic pathologists (Table). These lesions

P Bedossa (and The Gnomes) Human Pathol 2014

OATPB1/B3 and MRP3 expression in hepatocellular adenoma predicts Gd-EOB-DTPA uptake and correlates with risk of malignancy



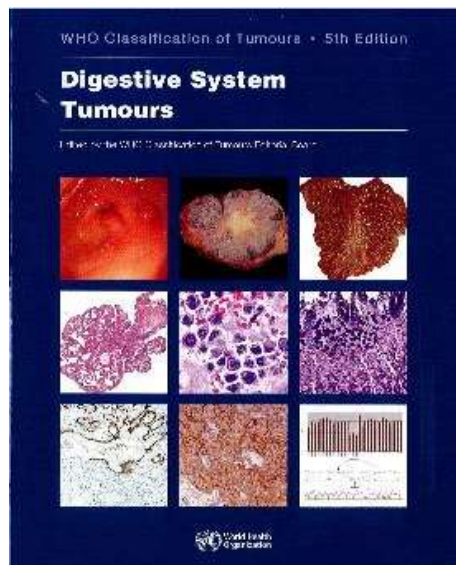
Imaging with hepatobiliary contrast agents



HEPATOCELLULAR CARCINOMA

Pathological classification

WHO classification of tumours of the liver and intrahepatic bile ducts



WHO (5th edition, 2019)

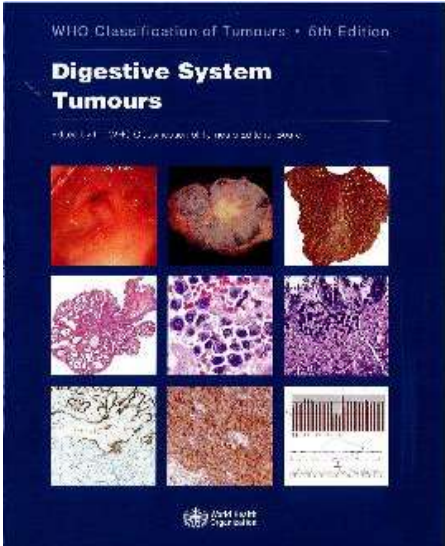
8 ≠ morphological subtypes of HCC

Malignant hepatocellular tumours and precursors

- 8170/3 Hepatocellular carcinoma NOS
- 8171/3 Hepatocellular carcinoma, fibrolamellar
- 8172/3 Hepatocellular carcinoma, scirrhous
- 8174/3 Hepatocellular carcinoma, clear cell type
- Hepatocellular carcinoma, steatohepatitic
- Hepatocellular carcinoma, macrotrabecular massive
- Hepatocellular carcinoma, chromophobe
- Hepatocellular carcinoma, neutrophil-rich
- Hepatocellular carcinoma, lymphocyte-rich
- 8970/3 Hepatoblastoma NOS

Pathological landscape @ the microscope

WHO classification of tumours of the liver and intrahepatic bile ducts

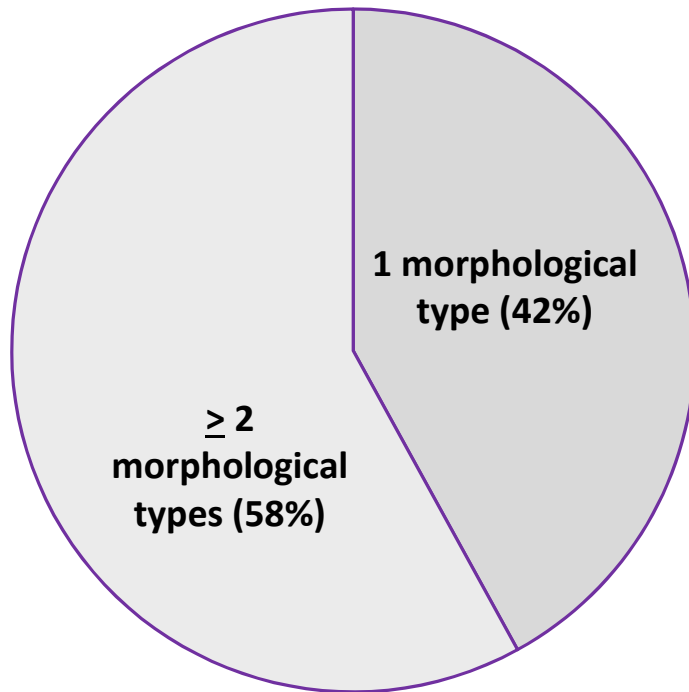


WHO (5th edition, 2019)

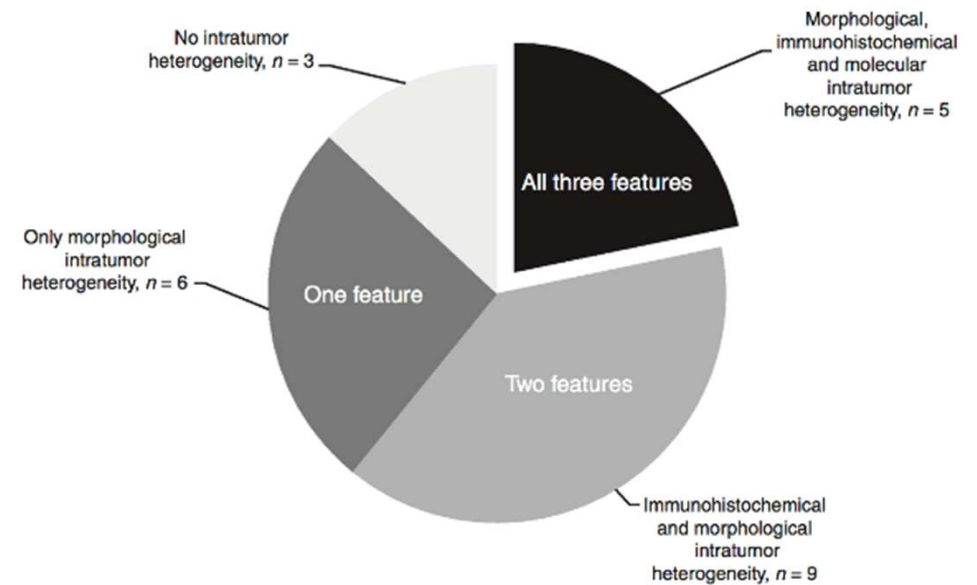
NOS	Macrotrabecular / Massive	Microtrabecular / Pseudoglandular	Scirrhou
Clear cells	Steato-hepatic	Fibrolamellar	Lymphocyte-rich

Intra-tumor heterogeneity @ the microscope

Surgical series (n=414, Beaujon hospital)



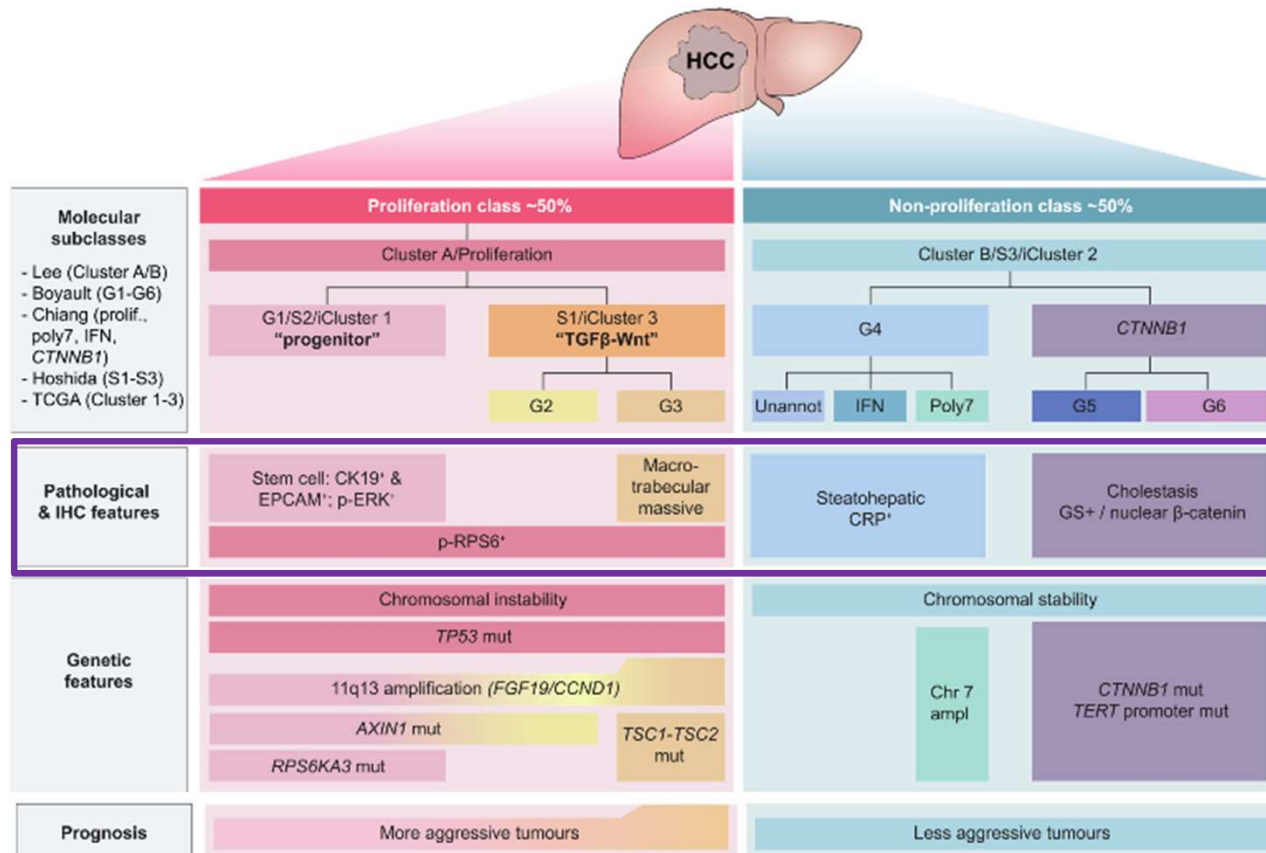
Intra-tumor heterogeneity detectable in 20/23 HCC (87%)



Friemel J et al Clin Cancer Res 2014

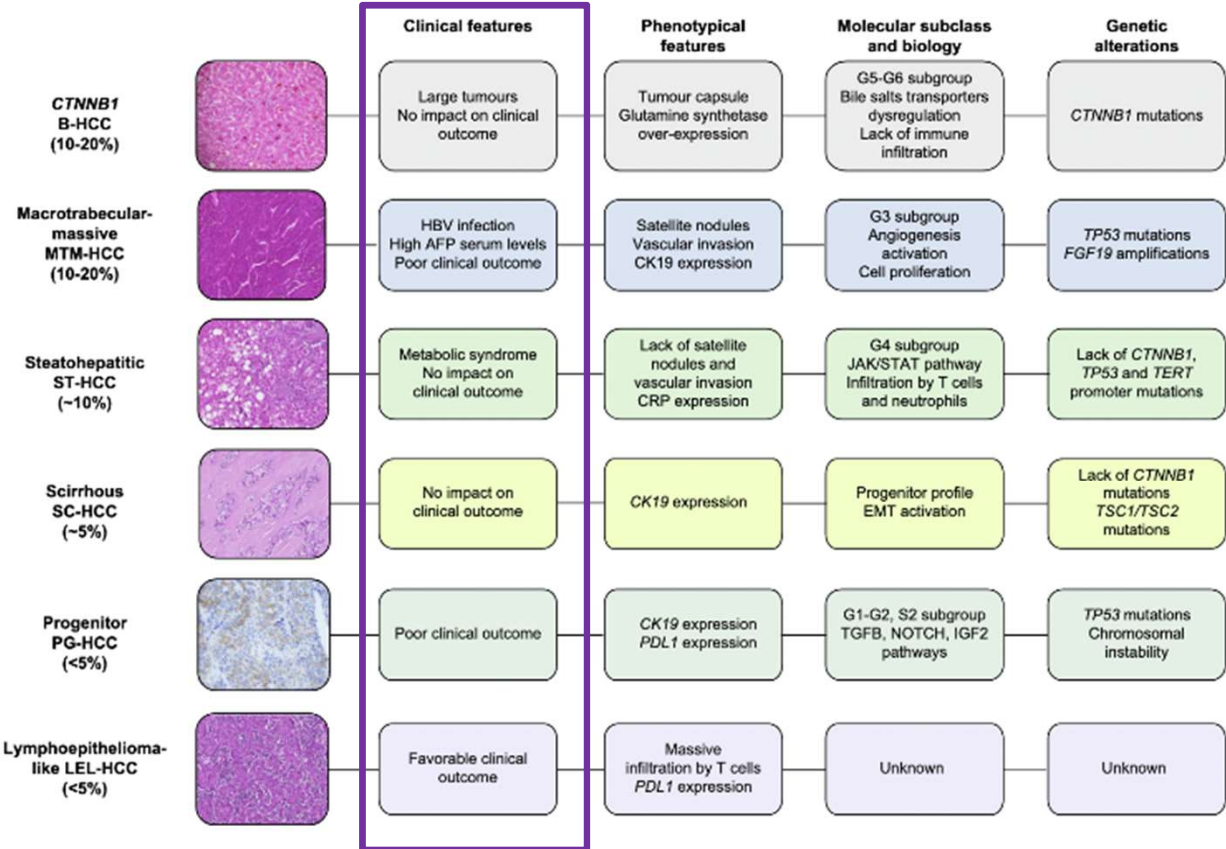
Molecular profiles

2 major transcriptomic classes, several subclasses



From Rebouissou S & Nault JC J Hep 2020

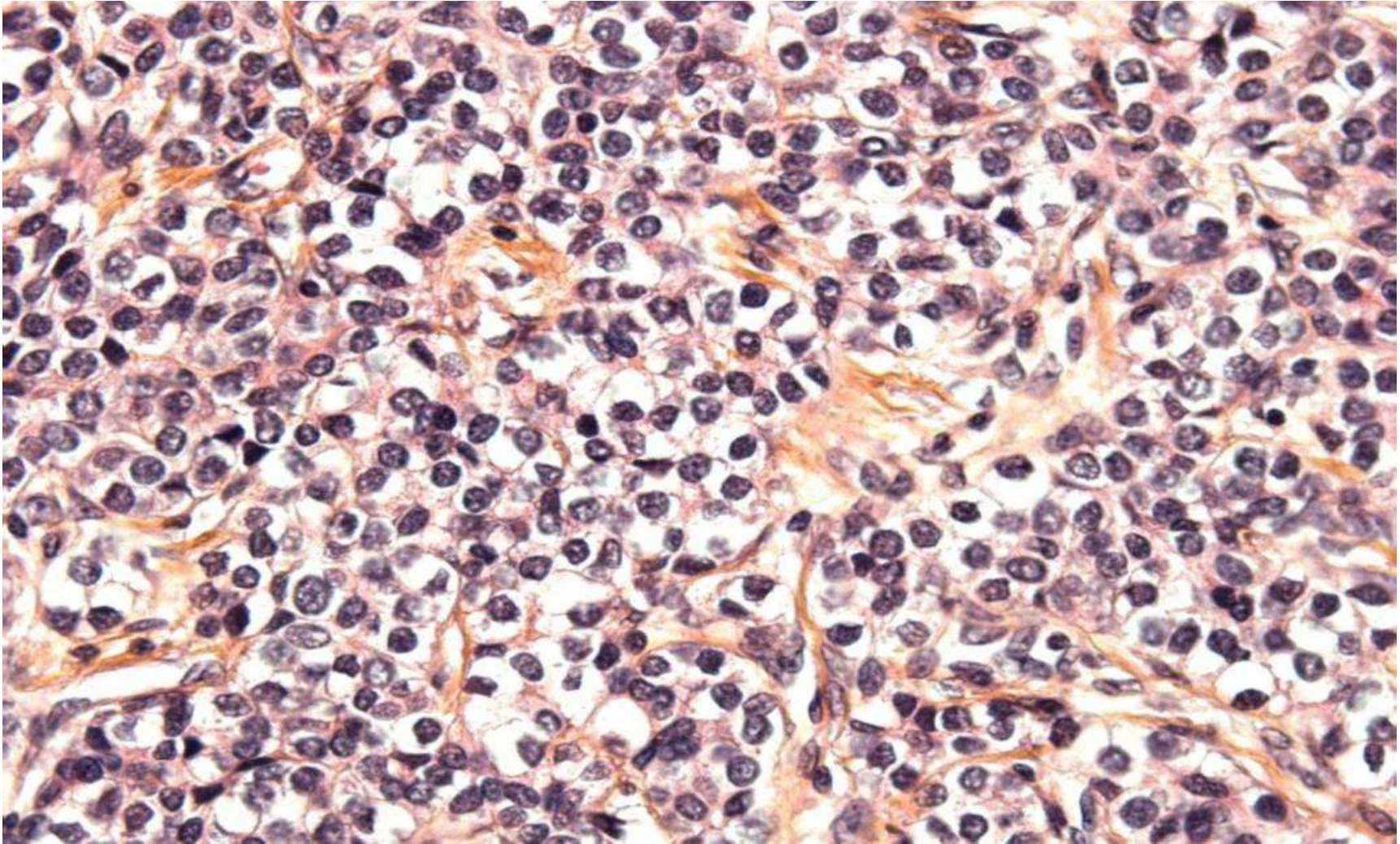
From Molecular to Pathology and Clinics

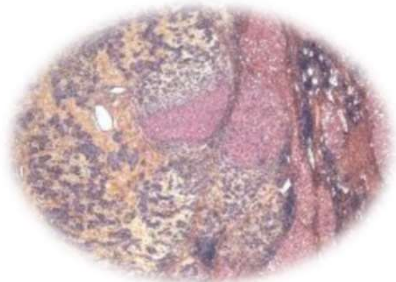


Calderaro J, Ziol M, Paradis V & Zucman-Rossi J J Hepatol 2019

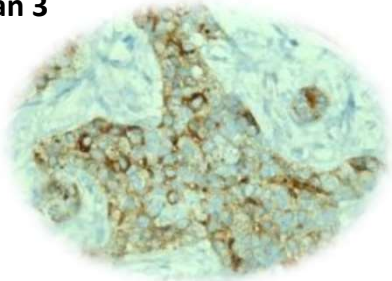


Progenitor phenotype (G1 molecular subclass)

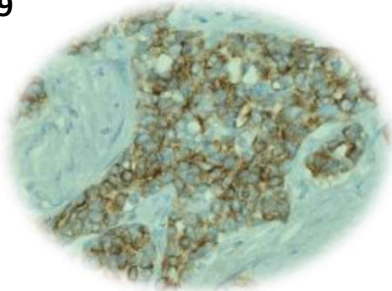




Glypican 3



CK19



Relative frequency : 5 %

Morphological features

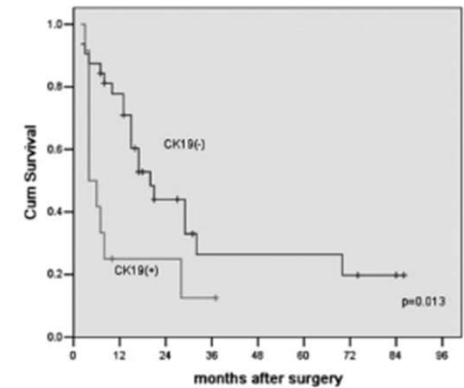
- Marked stromal fibrosis
- **CK19 expression (>5%)**

Molecular features

- *TSC1/TSC2* mutations
- Upregulation of **progenitor / cancer stem cell genes** (CD24, KRT19, THY1, CD133)
- EMT activation

Clinical impact

- Lymph node metastasis & vascular invasion
- [CK19 + HCC]
 - Shorter median survival
 - Independent prognostic factor of OS in HCC with LNM

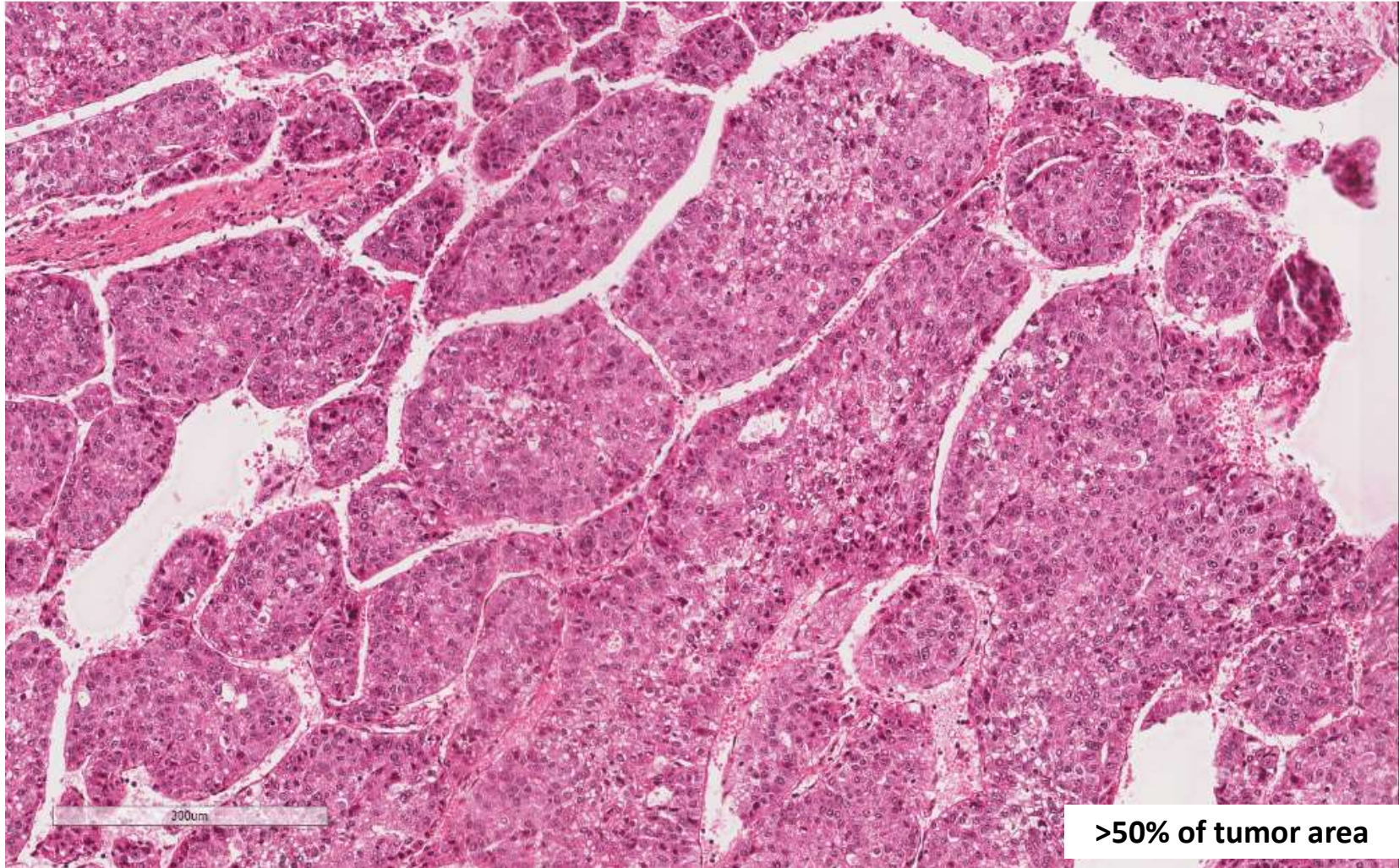


Seok Hepatology 2012, Durnez Histopathology 2006, Zuang P-Y Cancer 2008,

Kim H Hepatology 2011



Macrotrabecular-Massive HCC (MTM-HCC, G3)



>50% of tumor area

Relative frequency (<20%)

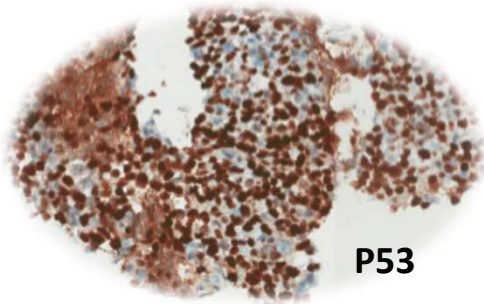
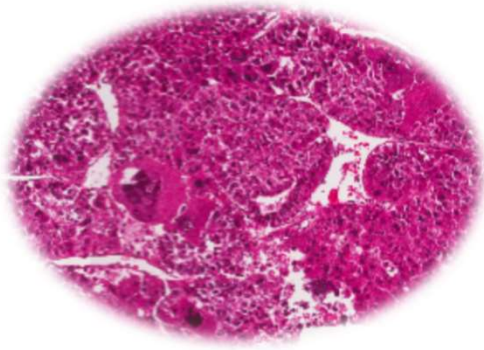
Morphologic features

- Poorly-differentiated tumors
- Macrotrabecular (>10 cells thick) & compact patterns
- Foci of sarcomatous change
- Pleomorphic & multinucleated cells
- Absence of cholestasis

- Vascular invasion

Immunophenotypical features

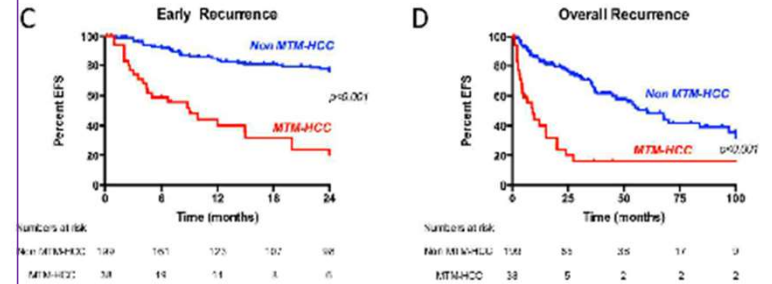
- Progenitor markers (CK19)
- **P53** mutated profile
- **FGF19** amplification



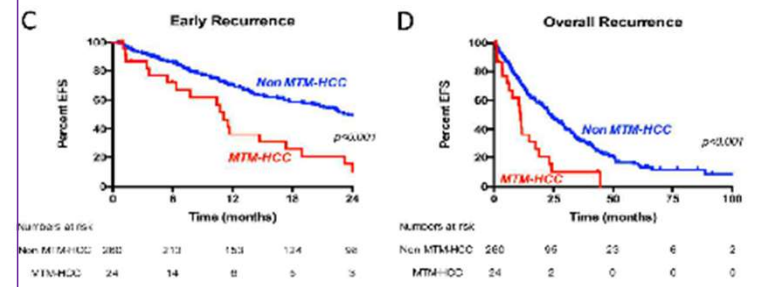
P53

Clinical impact

- 237 HCC (surgical specimens)

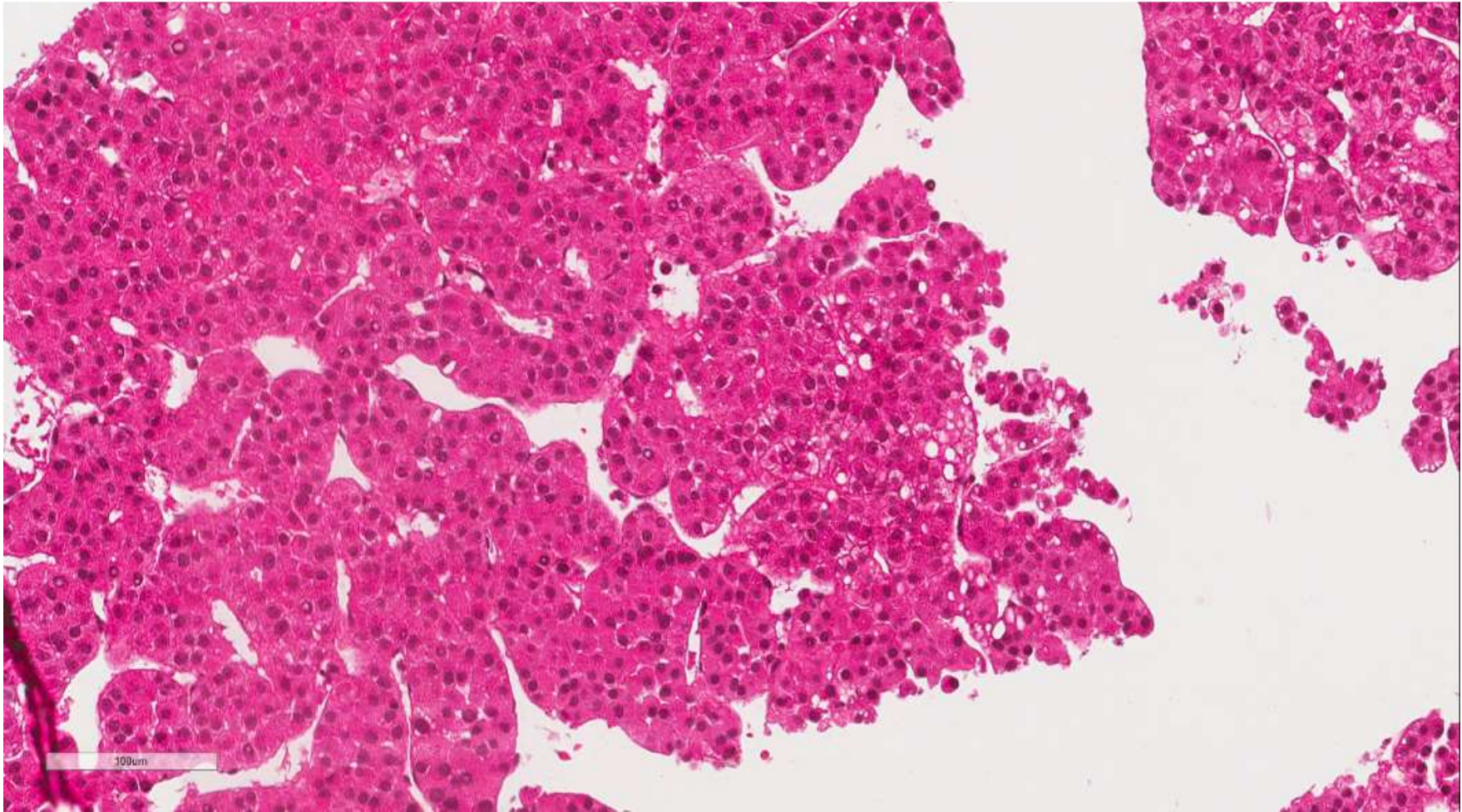


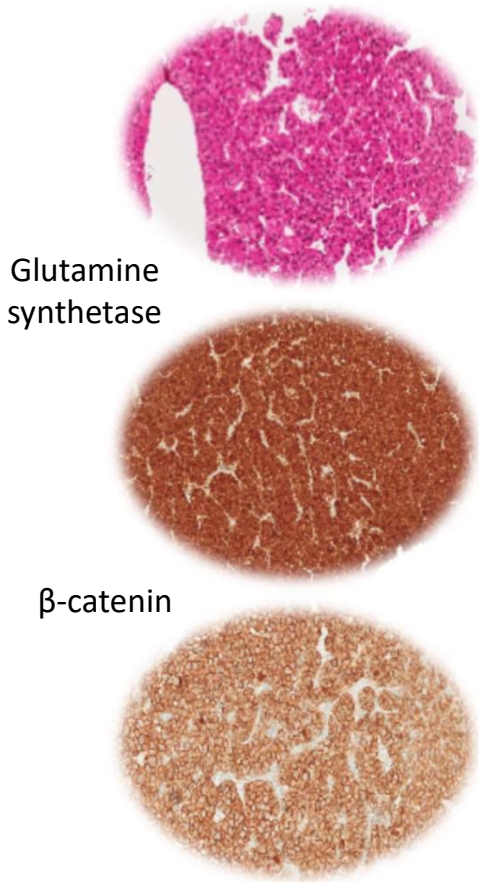
- 284 HCC (Biopsy pre-RFA)





Well-differentiated / Microtrabecular HCC (G5-G6)

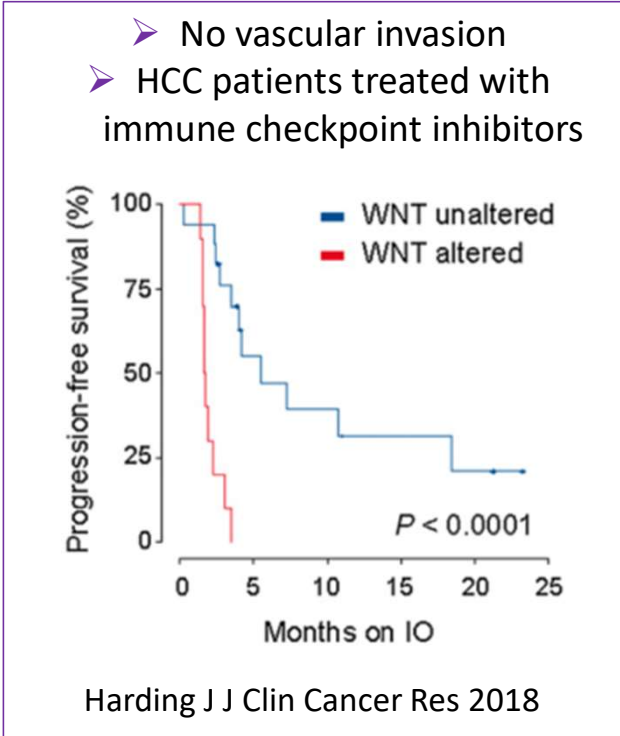




Relative frequency : 20-30%

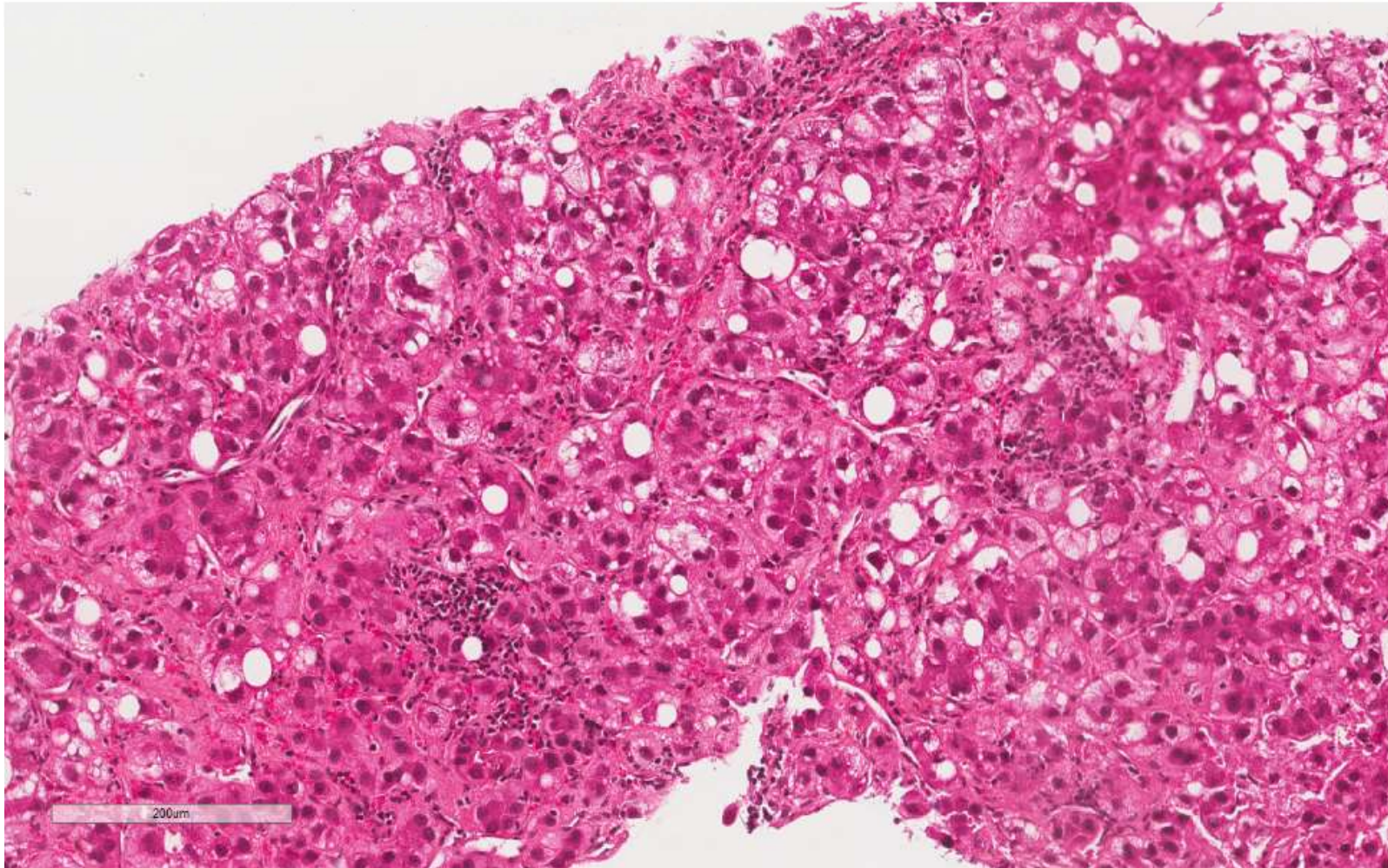
- Morphologic features**
- Well-differentiated tumor
 - Microtrabecular & pseudoglandular patterns
 - Cholestasis
 - Lack of inflammatory infiltrates
- Molecular profiles**
- Wnt/ β -catenin pathway activation
 - Dysregulation bile salt transporters
- Immunophenotypical features**
- GS overexpression
 - Nuclear β -catenin expression

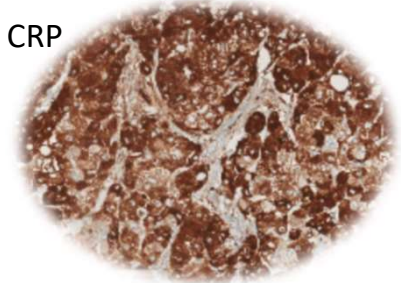
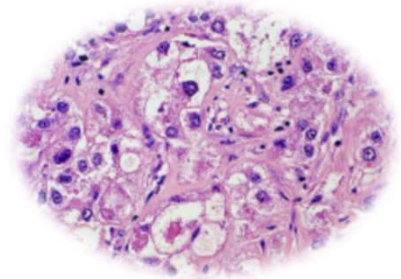
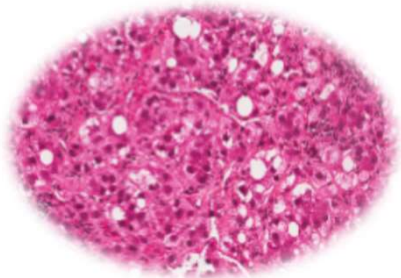
Clinical impact





Steato-hepatitic HCC (G4 molecular subclass)





Relative frequency : 5-25%

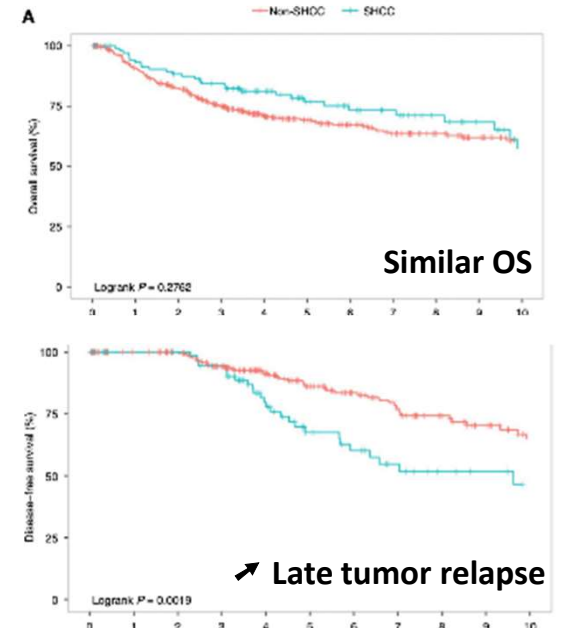
Morphologic features

- Combination of ≠ features [Steatosis, Ballooned cells, Mallory bodies, Fibrosis (trabecular and/or pericellular) & Inflammatory infiltrates]
 - 3 to 5 features
 - Present in > 5 to 50% of tumor area
- **Less aggressive phenotype**
 - Microvascular invasion & satellite nodules less frequent

Salomao M Am J Surg Pathol 2010
& Human Pathol 2012

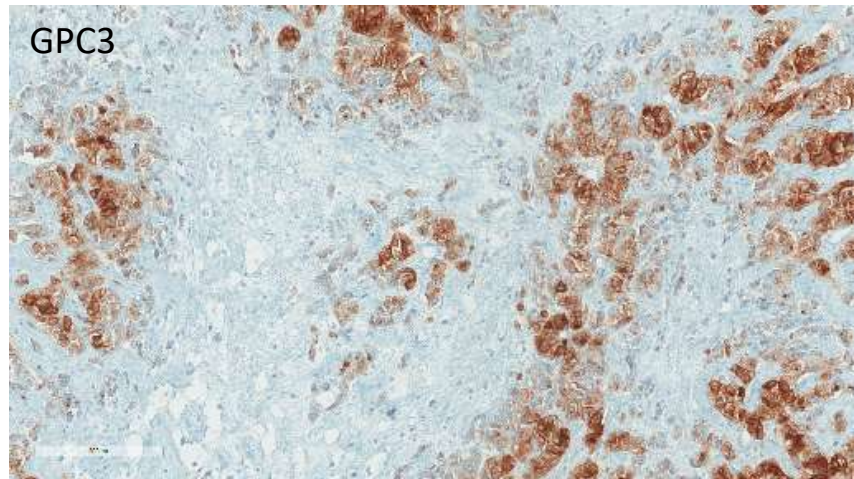
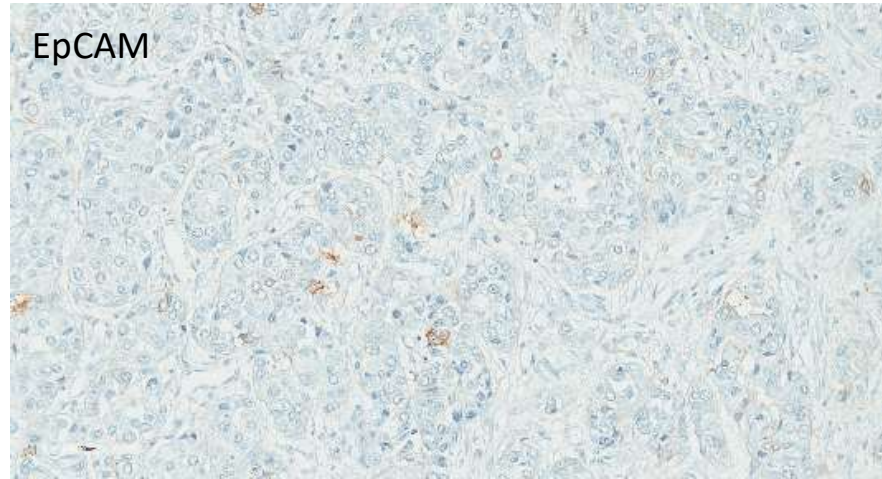
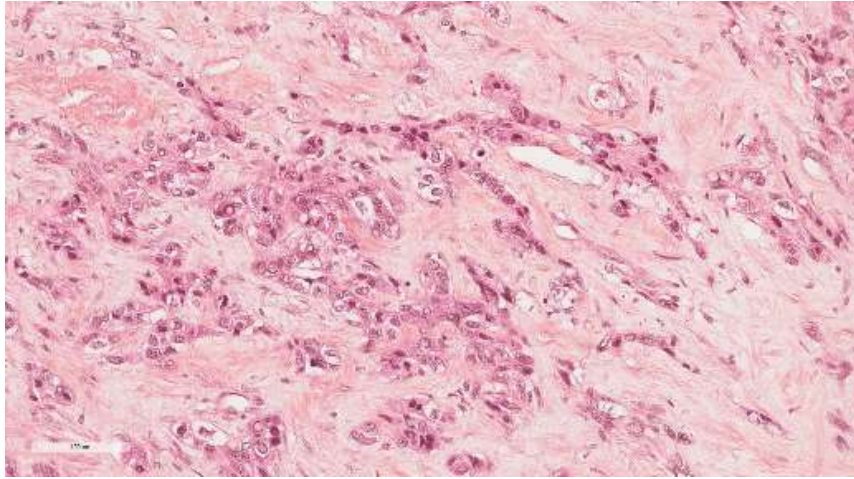
Clinical impact

Surgical series (516 HCC)
Steatotic (21.1%) & SH HCC (12.2%)



Chan AWH Histopathology 2016

Squirrrous HCC



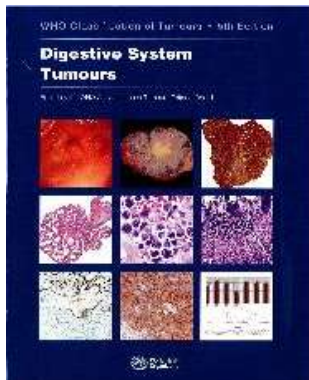
Relative frequency : 5 %

Morphologic features

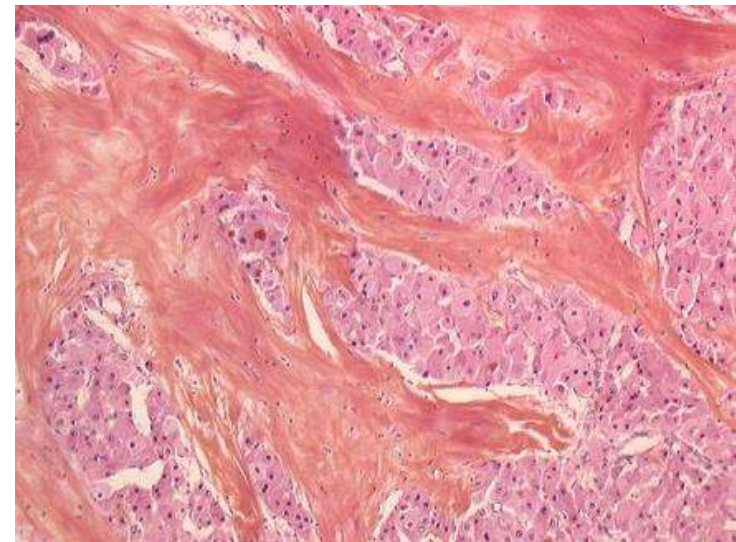
- Marked fibrous stroma involving >50% tumor area
- CK19+, EMT+
- **Molecular features:** TGF signaling activation & *TSC1/2* mutations

Fibrolamellar HCC

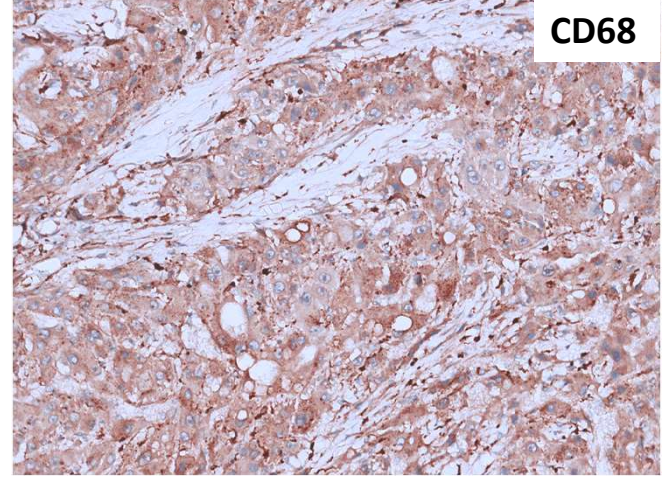
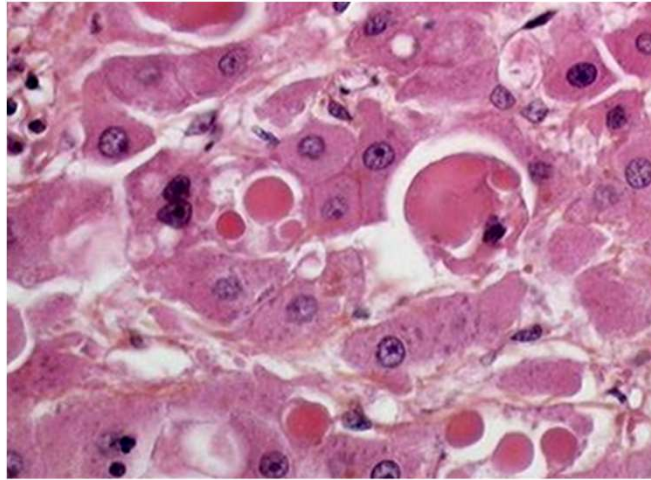
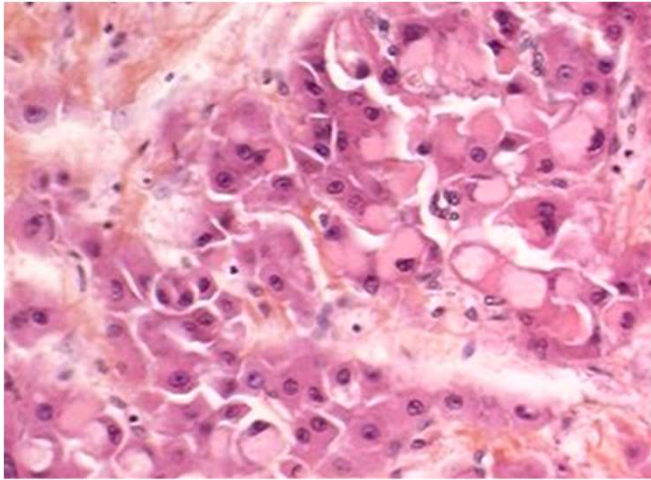
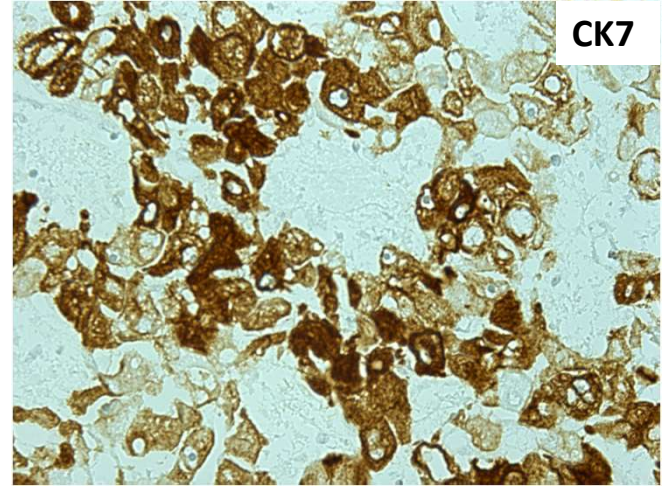
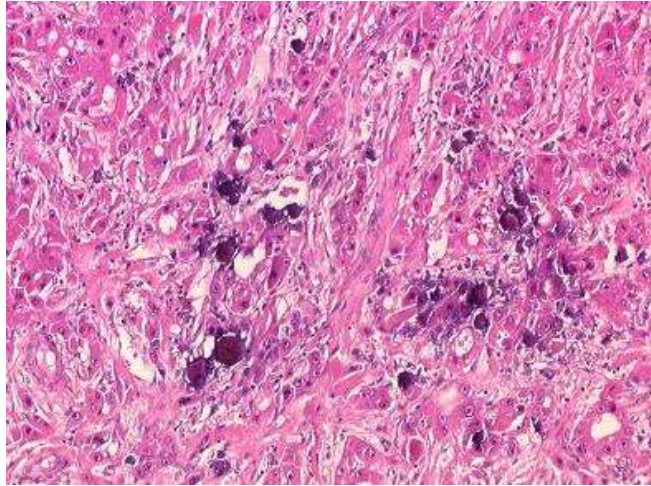
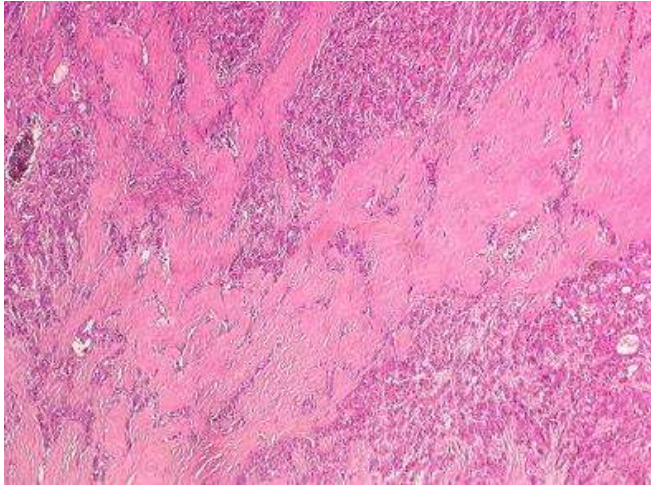
WHO classification of tumours of the liver and intrahepatic bile ducts



- Malignant hepatocellular tumours and precursors**
- 8170/3 Hepatocellular carcinoma NOS
 - 8171/3 → Hepatocellular carcinoma, fibrolamellar
 - 8172/3 Hepatocellular carcinoma, scirrhous
 - 8174/3 Hepatocellular carcinoma, clear cell type
 - Hepatocellular carcinoma, steatohepatic
 - Hepatocellular carcinoma, macrotrabecular massive
 - Hepatocellular carcinoma, chromophobe
 - Hepatocellular carcinoma, neutrophil-rich
 - Hepatocellular carcinoma, lymphocyte-rich
 - 8970/3 Hepatoblastoma NOS



WHO (5th edition, 2019)



Fibrolamellar HCC

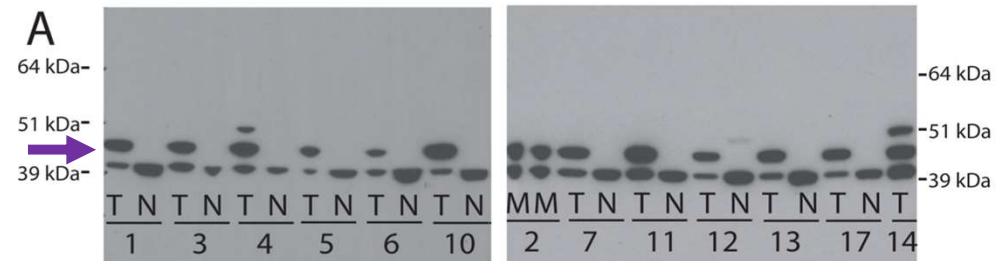
Detection of a Recurrent *DNAJB1-PRKACA* Chimeric Transcript in Fibrolamellar Hepatocellular Carcinoma

Joshua N. Honeyman^{1,2,*}, Elana P. Simon^{1,3,*}, Nicolas Robine^{4,*}, Rachel Chiaroni-Clarke¹, David G. Darcy^{1,2}, Irene Isabel P. Lim^{1,2}, Caroline E. Gleason¹, Jennifer Murphy^{1,2}, Brad R. Rosenberg⁵, Lydia Teegan¹, Constantin N. Takacs¹, Sergio Botero¹, Rachel Belote¹, Soren Germer⁴, Anne-Katrin Emde⁴, Vladimir Vacic⁴, Umesh Bhanot⁶, Michael P. LaQuaglia², and Sanford M. Simon^{1,†}

- Old & rare entity (0.5 – 2%)
- **Adolescents & young adults (M = W)**
- No risk factors
- Normal liver, AFP NI
- Aggressive tumor but better prognosis
 - Lymph node/peritoneal metastasis & extra-hepatic recurrence
 - 80% survival @ 5 y after resection
- HepPar1, CK7 and CD68 co-expression

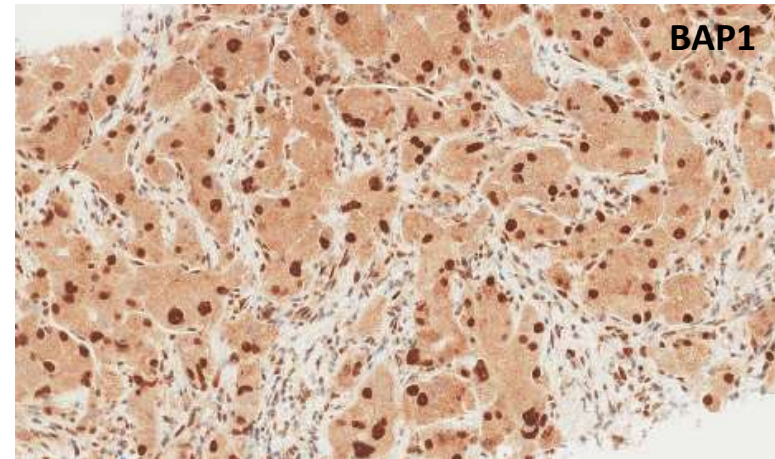
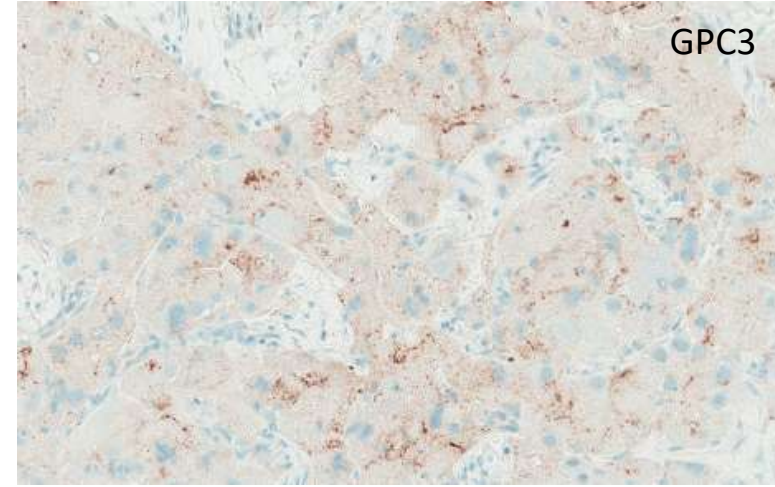
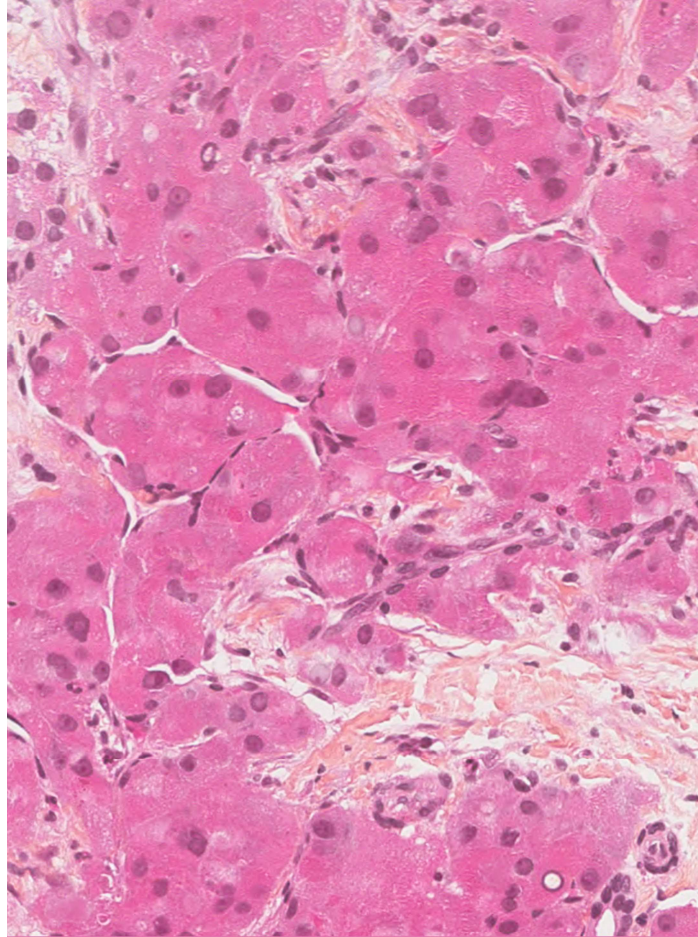
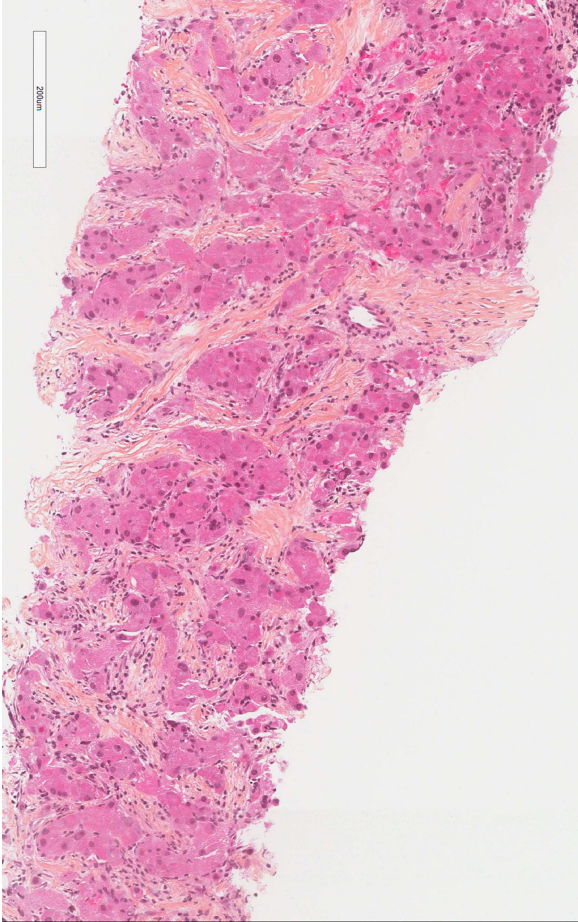
Edmonson J Dis Child 1956

- **Specific driver : *DNAJB1-PRKACA* fusion**
 - Chimeric transcript in 17/17 FL-HCC (≈400Kb deletion on ch 19)
 - Chimeric protein *DNAJB1*-fused *PRKACA* (41 kDa)
 - Retains full PKA activity



Honeyman JN Science 2014

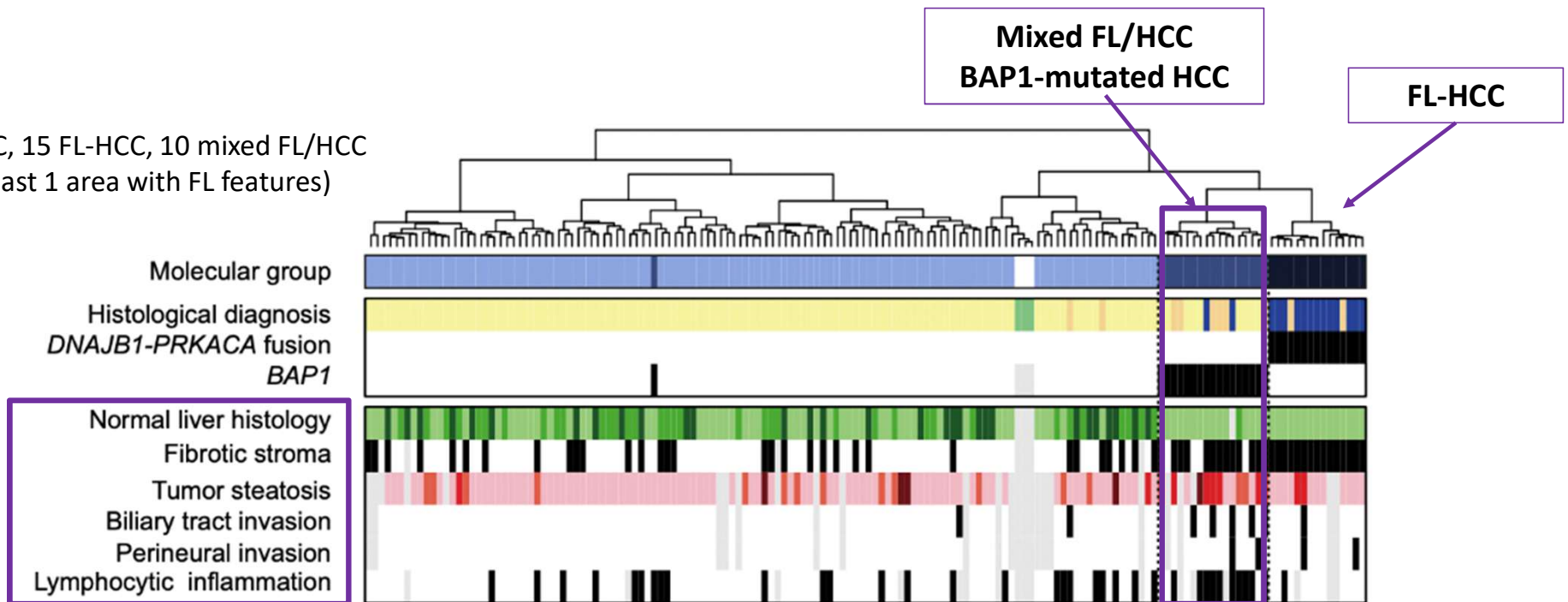
69 year-old man with Metabolic sd (no cirrhosis), Liver nodule 45 mm



***BAP1* mutations define a homogeneous subgroup of hepatocellular carcinoma with fibrolamellar-like features and activated PKA**

- Older patients, > women, no chronic liver diseases, poor prognosis
- Lack of *DNAJB1-PRKACA* fusion, recurrent chromosomal gain (*PRKACA* gene)

126 HCC, 15 FL-HCC, 10 mixed FL/HCC
(at least 1 area with FL features)



Pure and Mixed Fibrolamellar Hepatocellular Carcinomas Differ in Natural History and Prognosis After Complete Surgical Resection

Mixed FL-HCC (n=10) & Pure FL-HCC (n=30)

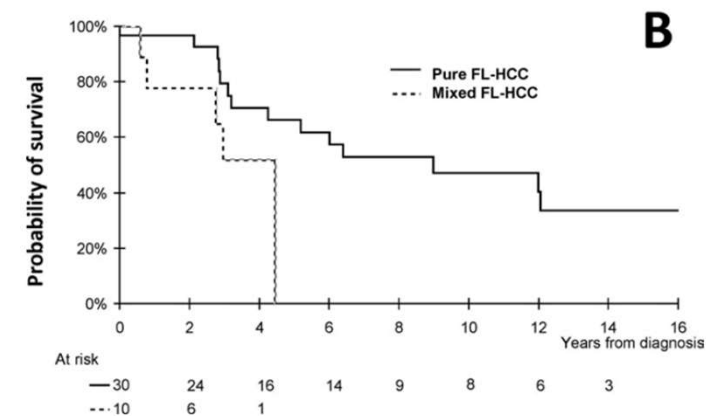
Table 2. Clinicopathological Characteristics of Patients With Mixed FL-HCC

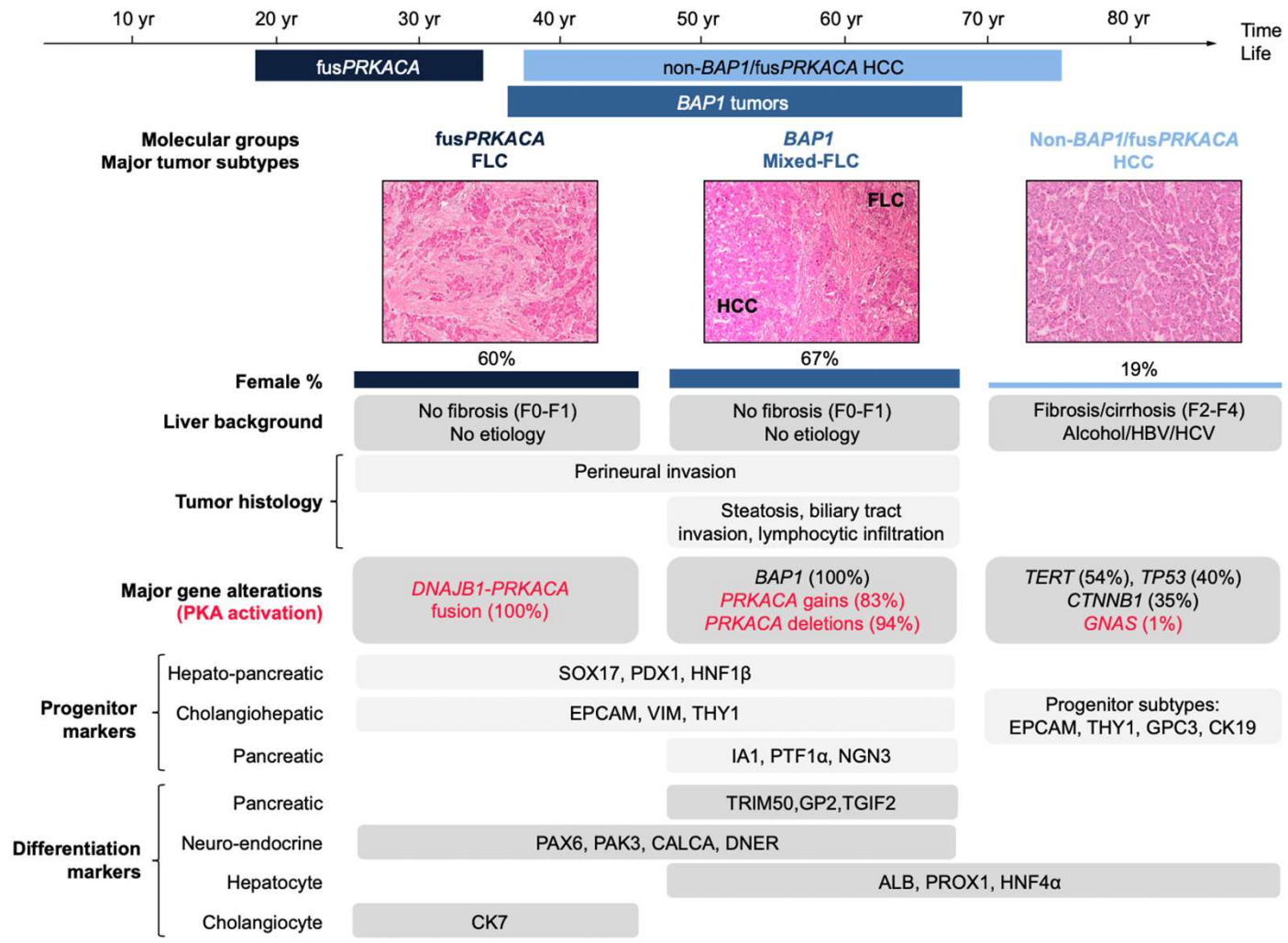
Patient No.	Age, Years	Sex	Size, cm	TNM Stage	History of Viral B Hepatitis	AFP, ng/mL	Microvascular Embolism/Satellite Nodules	% of CFL Component	Adjacent Liver
1	48	Woman	13	T3N0M0	No	25	Yes/no	20%	No cirrhosis; presence of mild centrolobular fibrosis
2	58	Woman	7	T1N0M0	No	4	Yes/no	<20%	Mild macrovesicular steatosis
3	56	Woman	13	T1N0M0	No	6069	Yes/yes	<20%	Normal
4	53	Man	19	T3N0M0	Yes	18,200	No/no	20%	Normal
5	18	Woman	16	T1N0M0	Yes	3	No/yes	30%	Normal
6	43	Woman	13	T1N0M0	No	4	No/yes	<20%	Normal
7	51	Woman	10	T3N0M0	No	16,000	Yes/no	<20%	Normal
8	31	Man	14	T1N0M0	No	5	No/no	20%	Normal
9	42	Man	11	T3N0M0	No	1500	No/no	50%	Normal
10	64	Woman	16	T1N0M0	Yes	3	Yes/No	50%	Normal

Abbreviations: AFP, α -fetoprotein; CFL, fibrolamellar; FL-HCC, fibrolamellar hepatocellular carcinoma.

➤ Compared to FL-HCC: Older patients, AFP ↗ & no lymph node involvement

➤ Mixed FL-HCC: independent prognostic factor



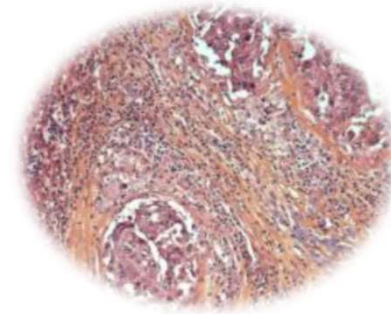
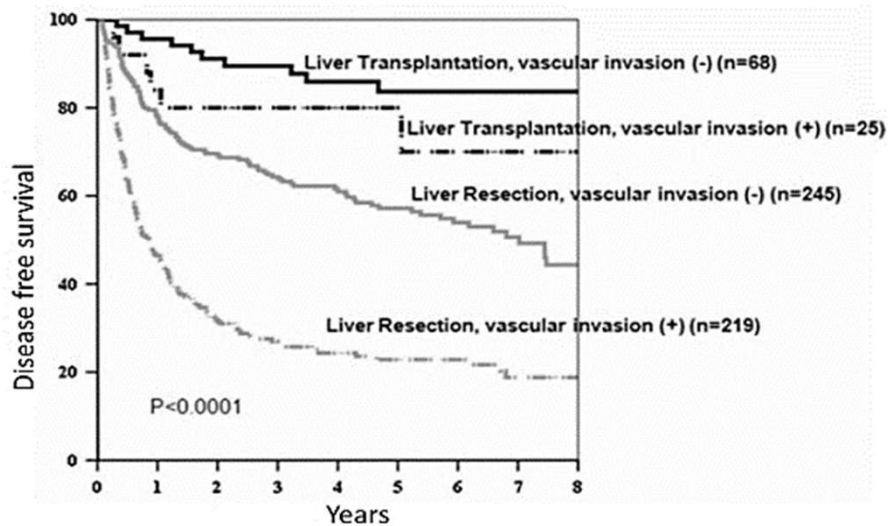


Hirsch T J Hepatol 2020

BEYOND HCC DIAGNOSIS



Microscopic Vascular Invasion



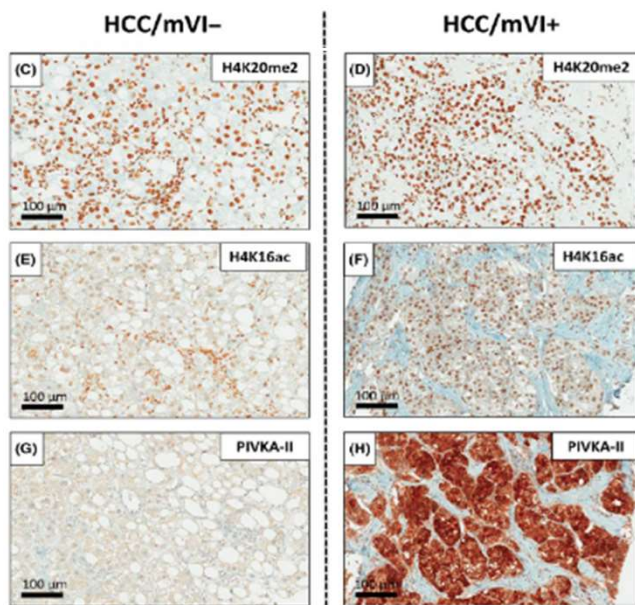
➤ Only assessed on surgical samples

➤ Need for surrogate markers of mVI on biopsy

MicroVascular invasion

A pathological feature too late recognized

Contribution of virtual biopsy to the screening of microvascular invasion in hepatocellular carcinoma: A pilot study

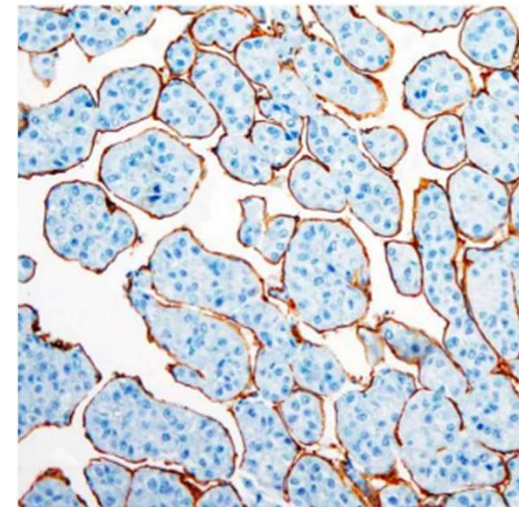


- At least 2 +ve markers: sst 72 %, spe 64%
 - 3 +ve markers: sst 36%, Spe 90%

Poté N et al Hepatology 2013, J Hepatol 2015 & Liver Int 2017

Vessels Encapsulating Tumor Clusters (VETC) Is a Powerful Predictor of Aggressive Hepatocellular Carcinoma

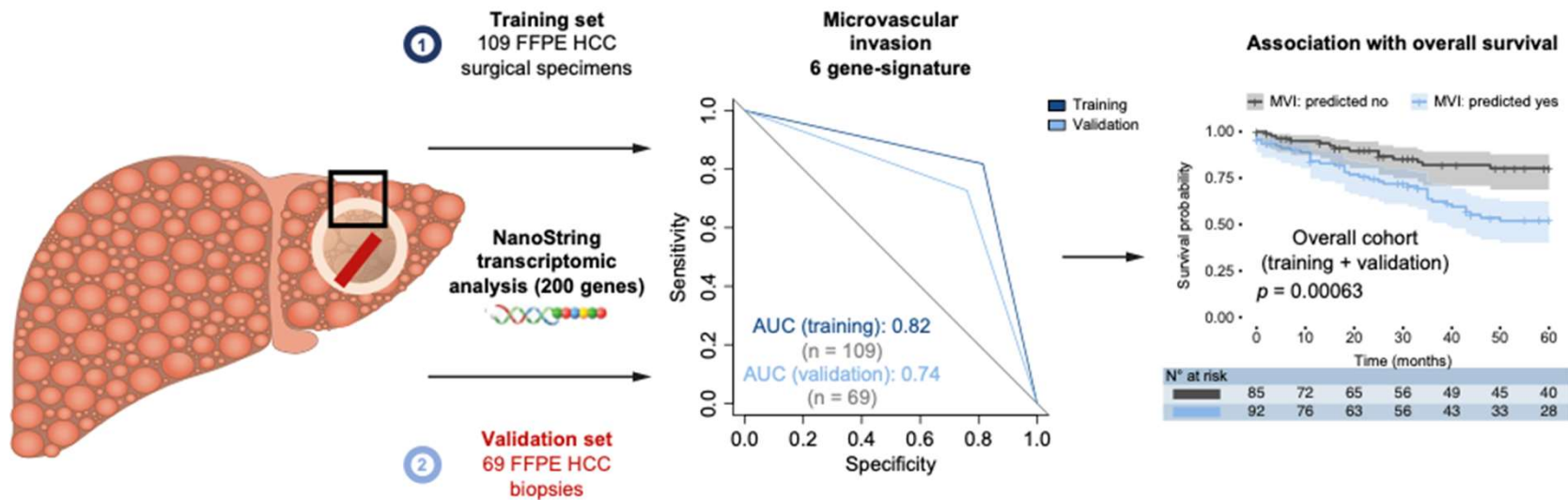
CD34 (>55% of tumor area)



- Correlated with **microvascular invasion**, AFP, tumor size, MTM-HCC

Renne SL Hepatology 2020

Gene expression signature as a surrogate marker of microvascular invasion on routine hepatocellular carcinoma biopsies



(*ROS1*, *UGT2B7*, *FAS*, *ANGPTL7*, *GMNN*, *MKI67*)

Beaufrère A J Hepatol 2021

Conclusions

Epithelial precursor lesions

1. Cirrhosis / Dysplastic nodules and early HCC

- A challenge for radiologists & pathologists
- A pathomolecular diagnosis (cytological, architectural and immunophenotypical features)
- Biopsy nodules and background liver +++

2. Normal liver / Hepatocellular adenomas

- Comprehensive radio-patho-molecular classification
- Risk factors for malignant transformation well-characterized
 - Result in specific management strategy (gender, subtype and size)

3. *TERT* promoter mutations: *A key-player*

- A common molecular pathway associated with malignant potential (cirrhosis & normal liver)
- Need for surrogate markers useful in clinical practice

Conclusions

HCC and its different subtypes

1. HCC molecular landscape

- ≠ subtypes → pathomolecular classification with clinical impact
- New entities (BAP1-mutated HCC)
- Intratumor heterogeneity is a challenge

2. Tumor biopsy

- Increasing interest in clinical practice
- Biopsy tumor & background liver



Inserm U 1149

« From inflammation to cancer in digestive diseases »

V Paradis

- A Couvelard, N Guedj, J Cros, V Rebours, L de Mestier, M Bouattour, R Lebtahi
- A Boumaza, A Hammoutène (Post-doc)
- A Beaufrère, F Cauchy, S Frendi, E Gigante (Doc)
- B Eren (M2)
- S Laouirem, V Priori, M Albuquerque (IE)

- **iMAP platform H Cazier (IE)**

Beaujon hospital

- Pathology (V Paradis)
- Radiology (V Vilgrain)
- Liver Surgery (M Lesurtel)
- Hepatology (F Durand)
- Oncology (M Bouattour)



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