Pathological Society
Understanding Disease — Guiding Therapy

Leeds Pathology 2019

Recent advances in hepatocellular carcinoma

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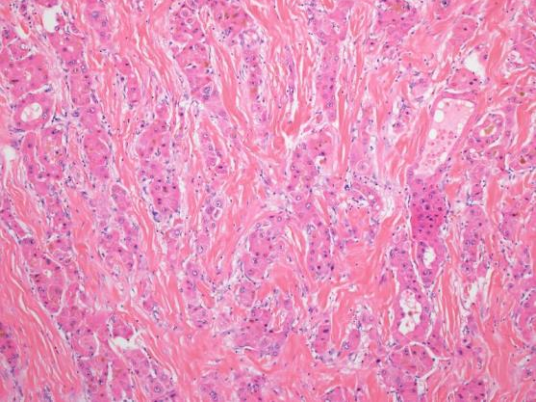
Outline

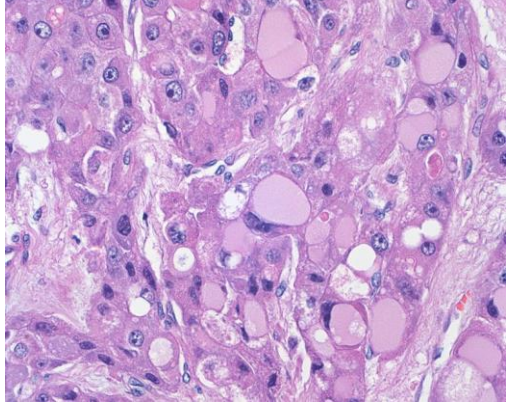
- **Molecular signatures for HCC subtypes**
- **The role of the immune microenvironment**
- **Terminology updates**

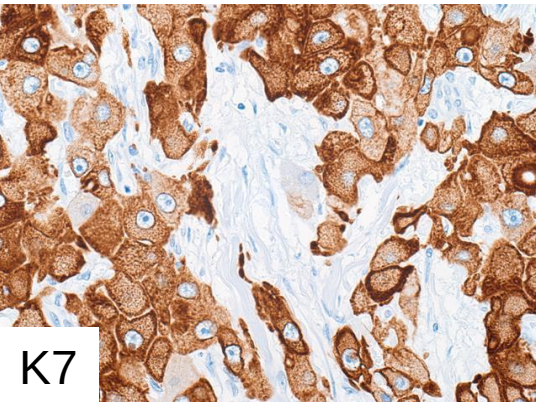
1. Hepatocellular carcinoma - Molecular signatures for HCC subtypes

Histological subtyping of HCC

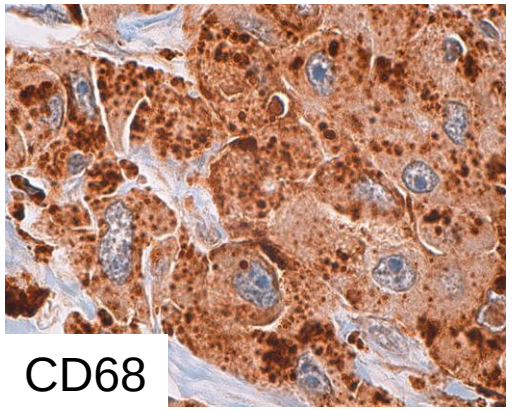
Fibrolamellar HCC





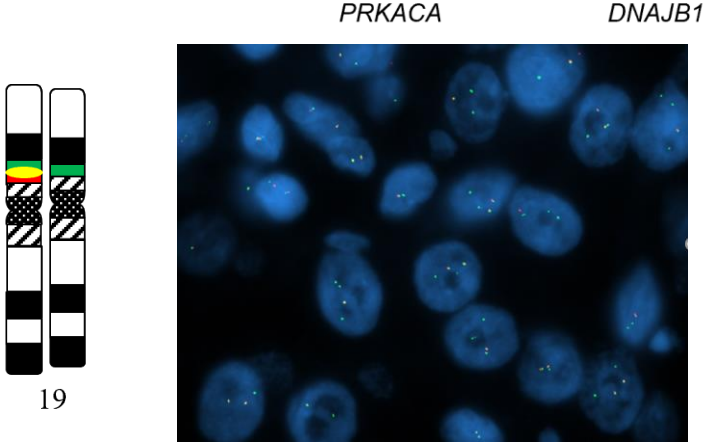


K7



CD68

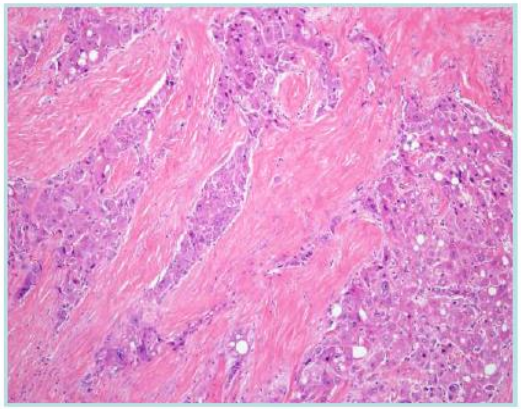
Honeyman, Science 2014
Riehle, Mod Pathol 2015
Graham, Mod Pathol 2015
Torbenson M, Gnomes 2019

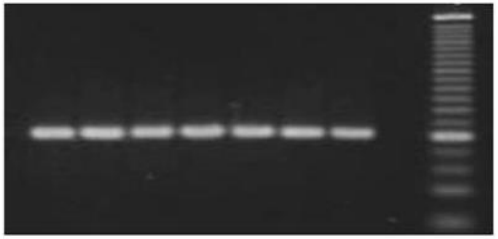


- Unique fusion transcript DNAJB1/PRKACA

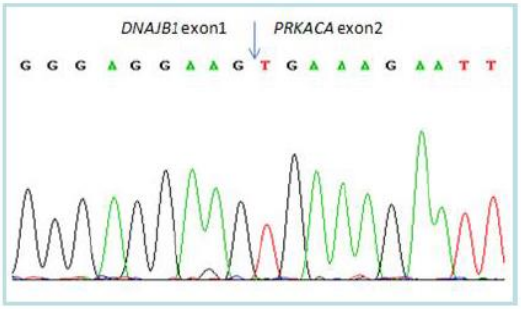
- **Detection of PRKCA rearrangement by FISH: Highly specific for FL-HCC**

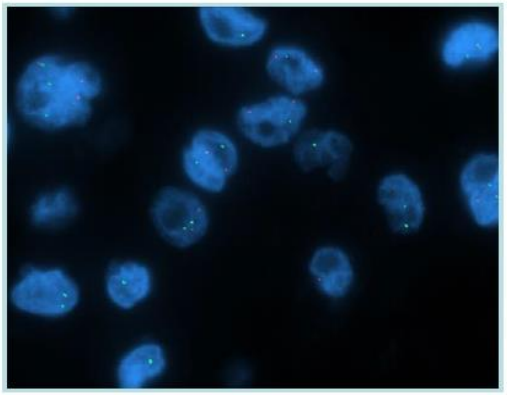
Histological subtyping of HCC





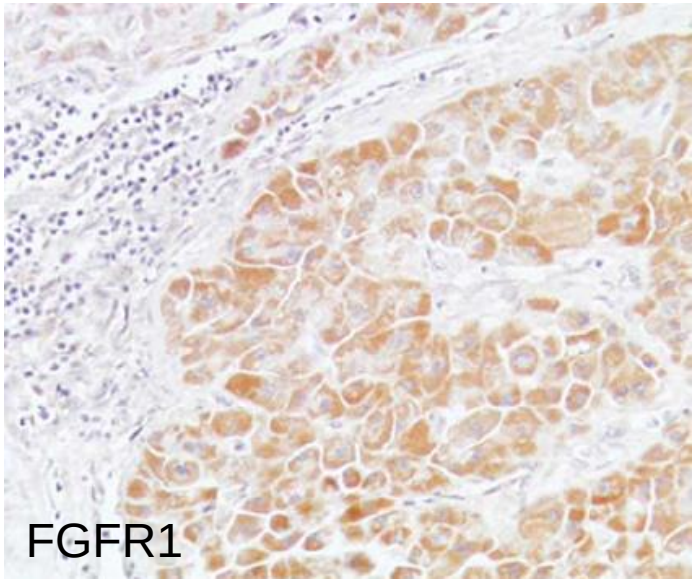
PGK (126 bp)





Fibrolamellar HCC

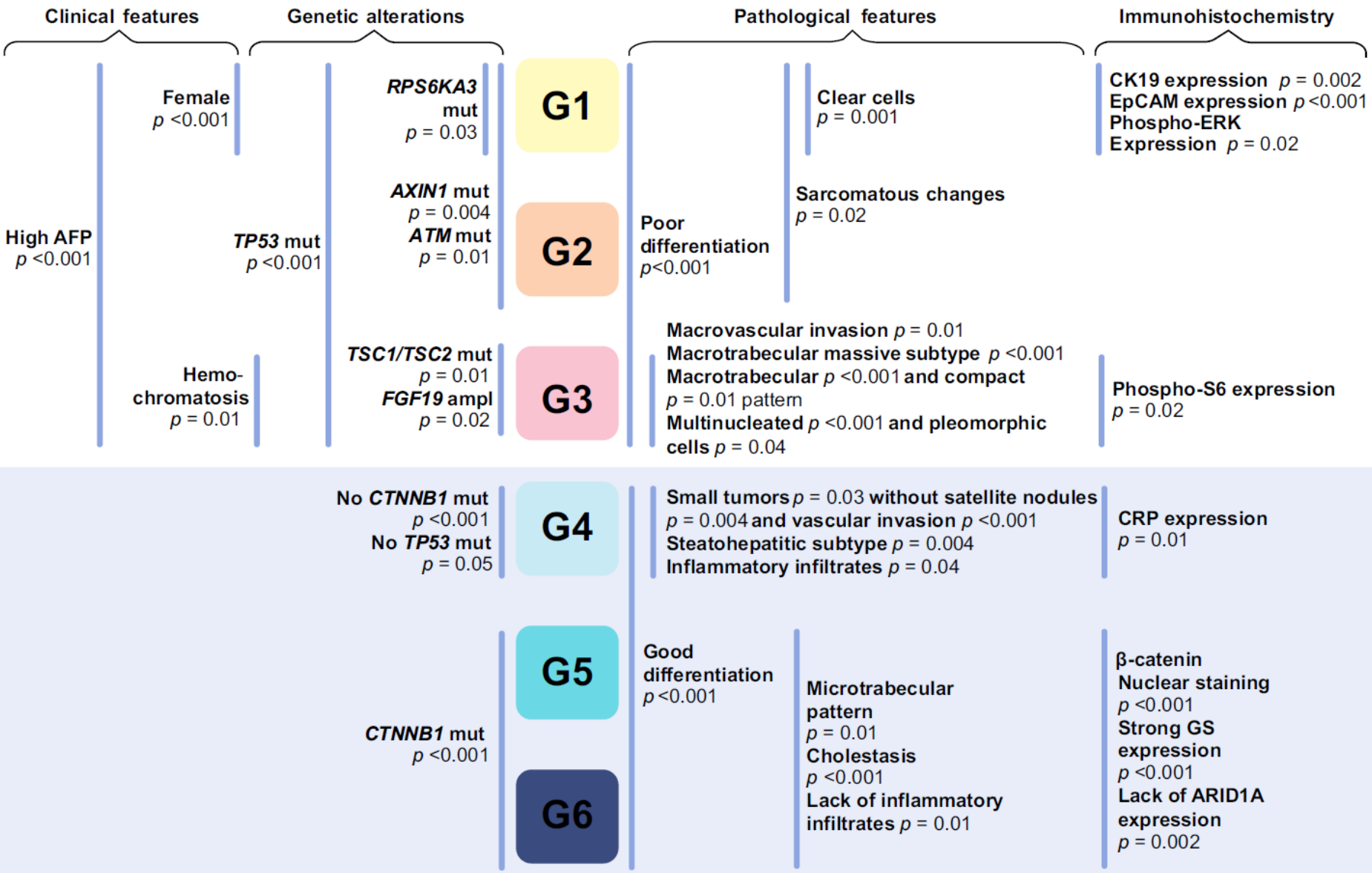
- Unique fusion transcript DNAJB1/PRKACA
- Interaction with FGF pathways
- Overexpression of FGFR1



- **Personalised treatment with FGFR inhibitors ?**

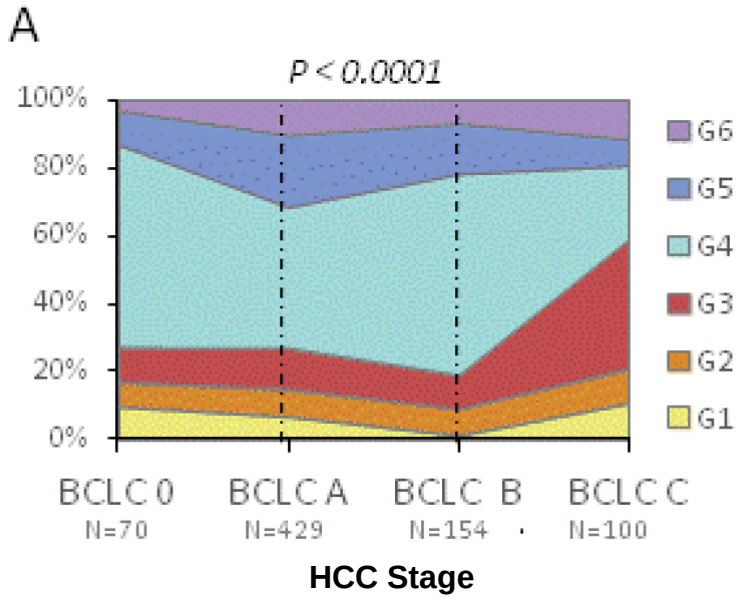
Honeyman, Science 2014
Riehle, Mod Pathol 2015
Graham, Mod Pathol 2015

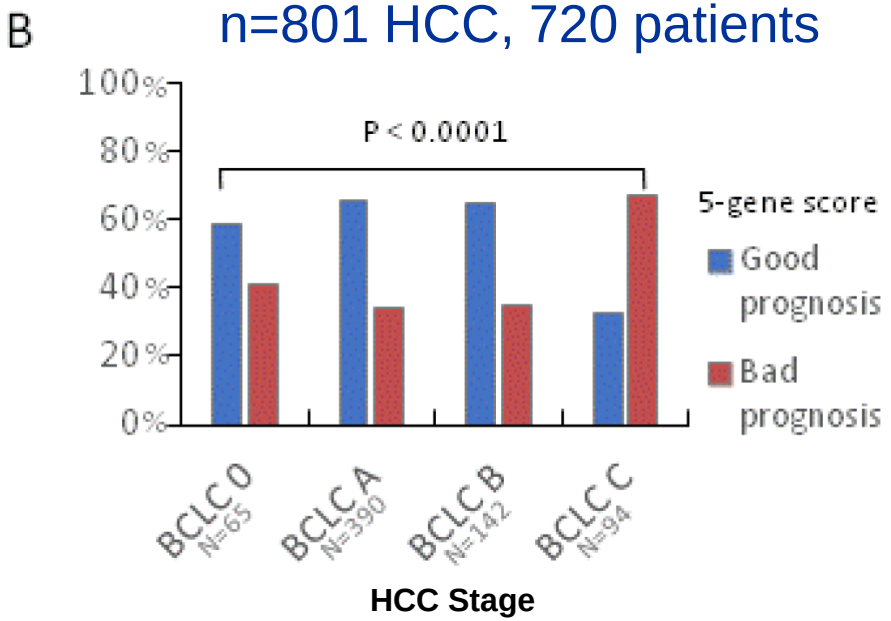
Molecular classification of hepatocellular carcinoma



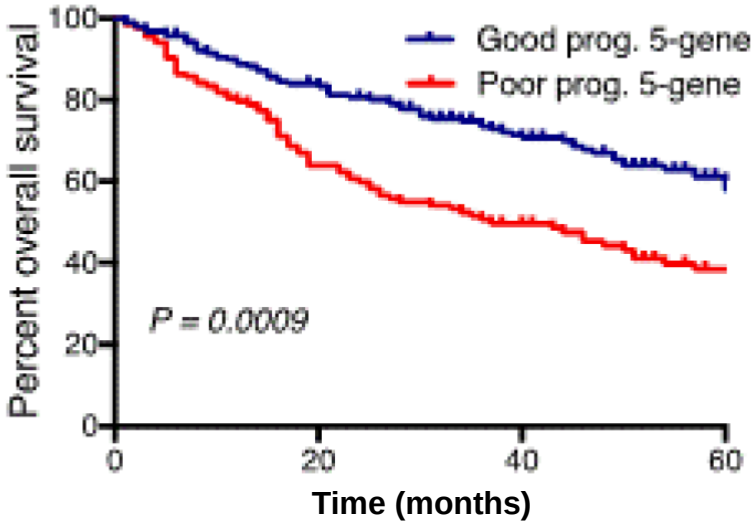
Boyault, Hepatology 2007
Calderaro, J Hepatol 2017

Clinical impact of genomic diversity in HCC





5-gene score (*TAF9*, RAMP3, *HN1*, *KRT19*, *RAN*) predicts survival in resected HCC

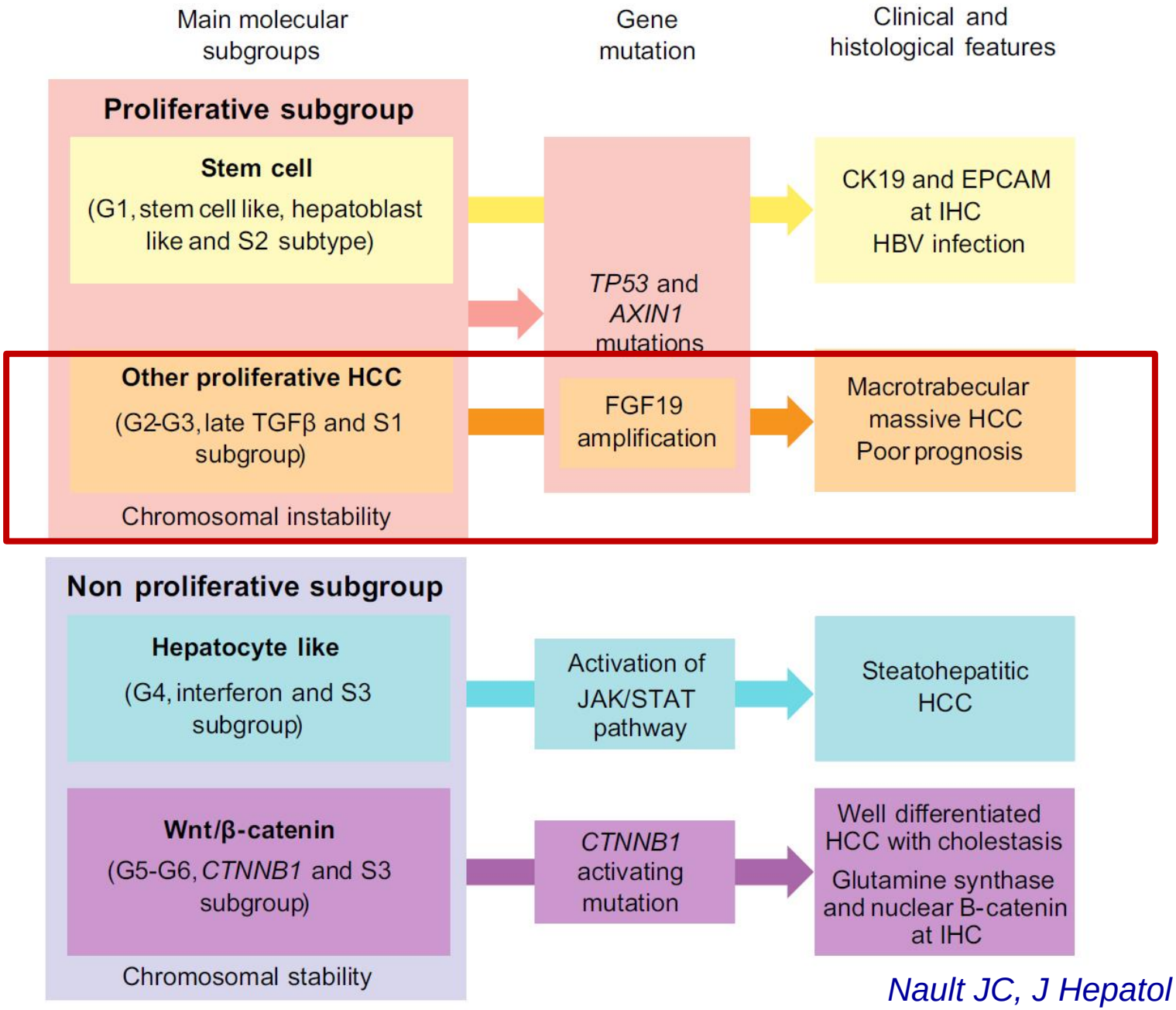


Advanced HCC

- enrichment of G3 transcriptomic class
- poor-prognostic 5-gene score
- increased proliferation/dedifferentiation
- 22% with druggable genetic alterations

Nault JC, Hepatology, June 2019

Molecular classification of hepatocellular carcinoma



Nault JC, J Hepatol 2018

Integrative HCC-Classification

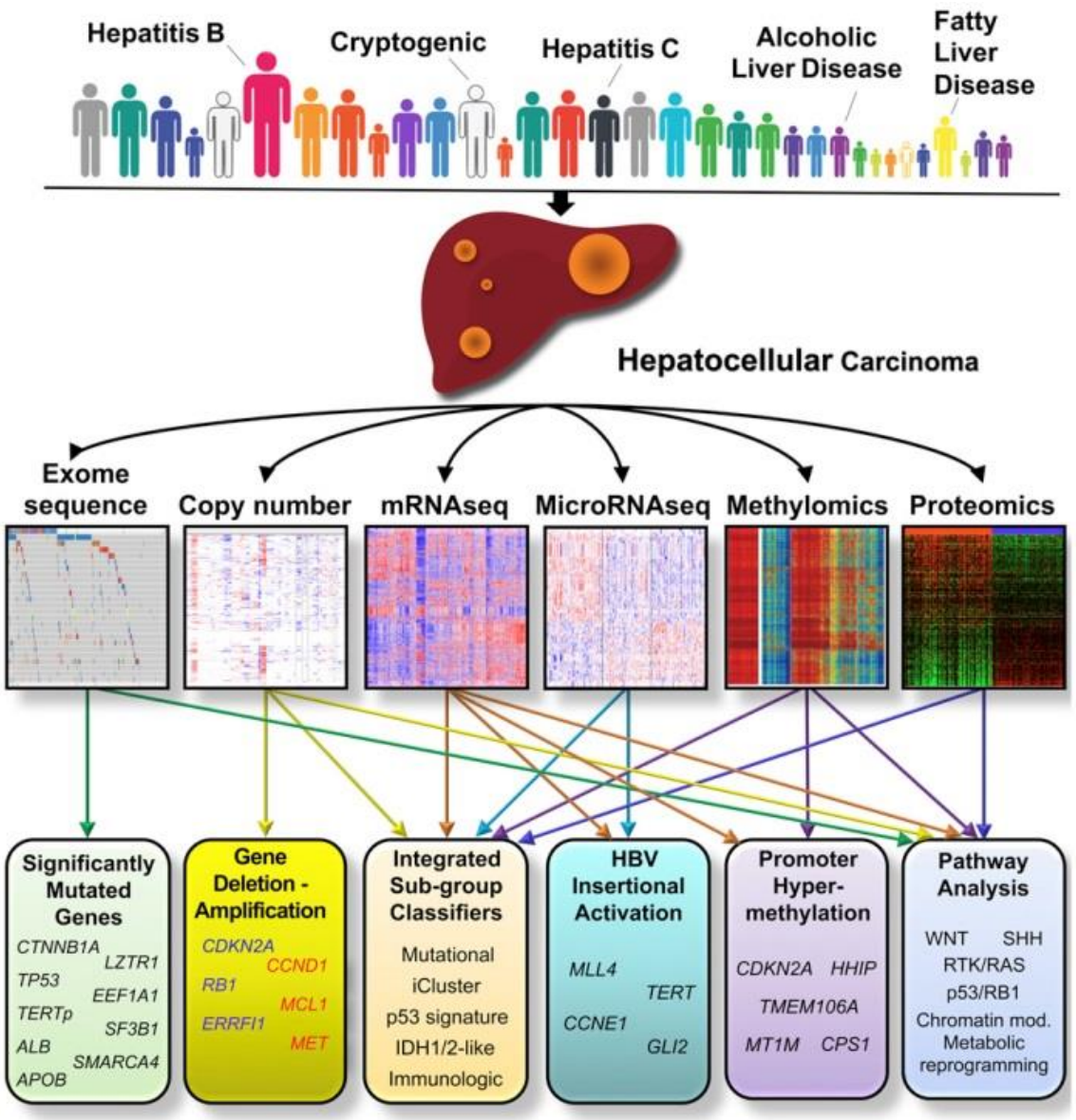
Multiple data platforms
Clinical data

e

Potential therapeutic targets

- WNT signaling
- MDM4, MET, VEGFA,
- MCL1, IDH1, TERT,
- immune checkpoint proteins

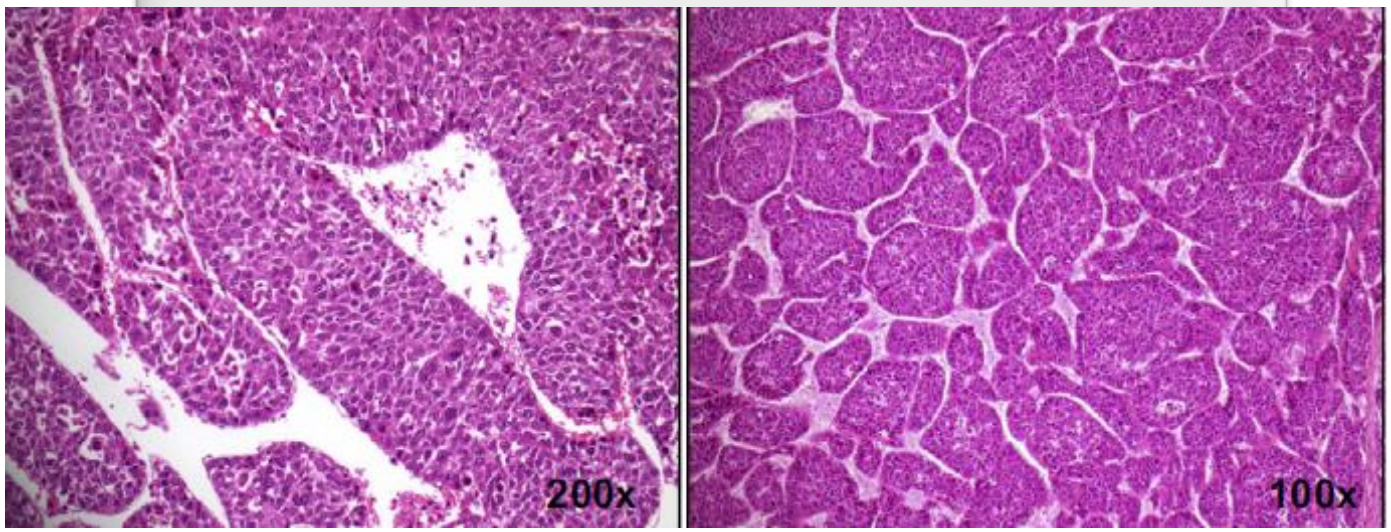
  CTLA-4, PD-1, and PD-L1.



The Cancer Genome Atlas Research Network,
Cell 169: 1327-43, 2017

Novel histological/molecular HCC subtypes

Macrotrabecular Massive HCC



- 5-10% HCC
- Younger patients
- ↑ serum AFP
- Poor prognosis
- 100% vascular invasion

Molecular features

- p53 mutations
- FGF19 amplification
- Activation of angiogenic factors (Ang2, VEGFA)

Calderaro, J Hepatol 2017

HEPATOLOGY, VOL. 68, NO. 1, **2018**

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

Marianne Ziol,[1-3] Nicolas Poté,[4] Giuliana Amaddeo,[5,6] Alexis Laurent,[5,7] Jean-Charles Nault,[2,3,8] Frédéric Oberti,[9] Charlotte Costentin,[6] Sophie Michalak,[10] Mohamed Bouattour,[11] Claire Francoz,[11] Georges Philippe Pageaux,[12] Jeanne Ramos,[13] Thomas Decaens,[14] Alain Luciani,[5,15] Boris Guiu,[16] Valérie Vilgrain,[17] Christophe Aubé,[18] Jonathan Derman,[19] Cécile Charpy,[19] Jessica Zucman-Rossi,[2] Nathalie Barget,[20] Olivier Seror,[21] Nathalie Ganne-Carrié,[3,8] Valérie Paradis,[4] and Julien Calderaro[5,19]

Retrospective study of HCC

237 HCC surgical samples

284 HCC liver biopsies ftom pts treated with resection and RFA

Male 81% HCV (32%), HBV (25%), Alcoholic (23%) & other aetiology

Macrotrabecular architecture in >50% tumour

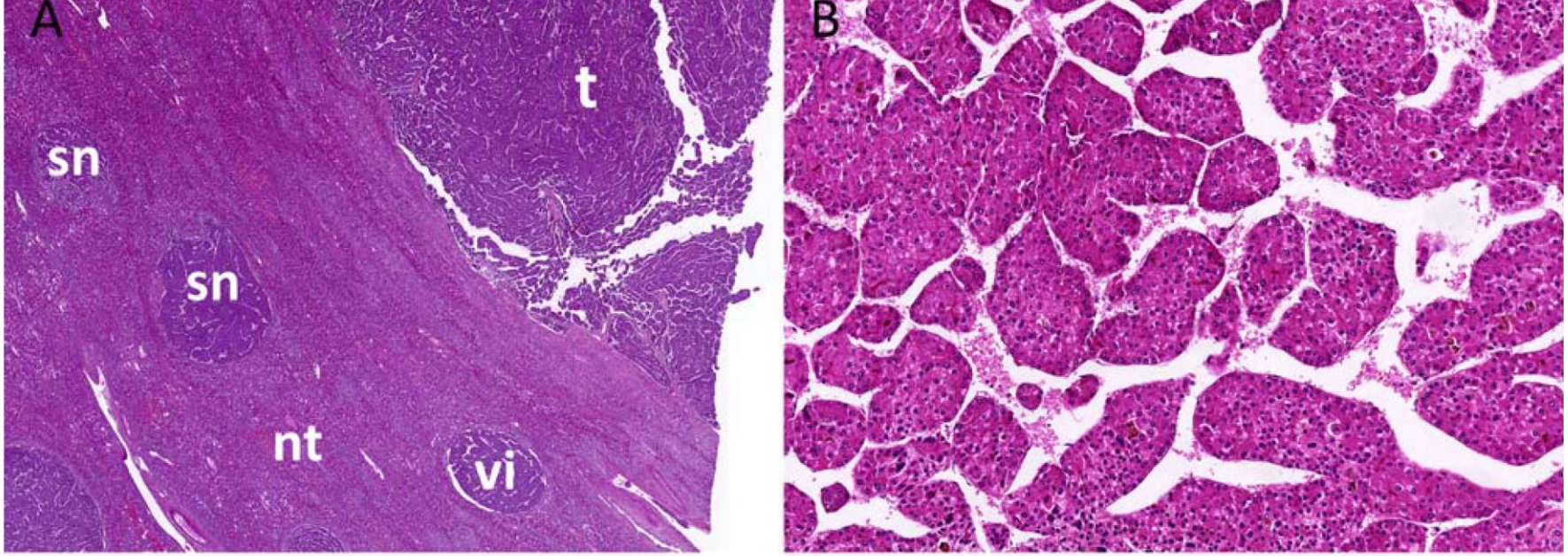
6-cell thick trabeculae

Overall Frequency
12%

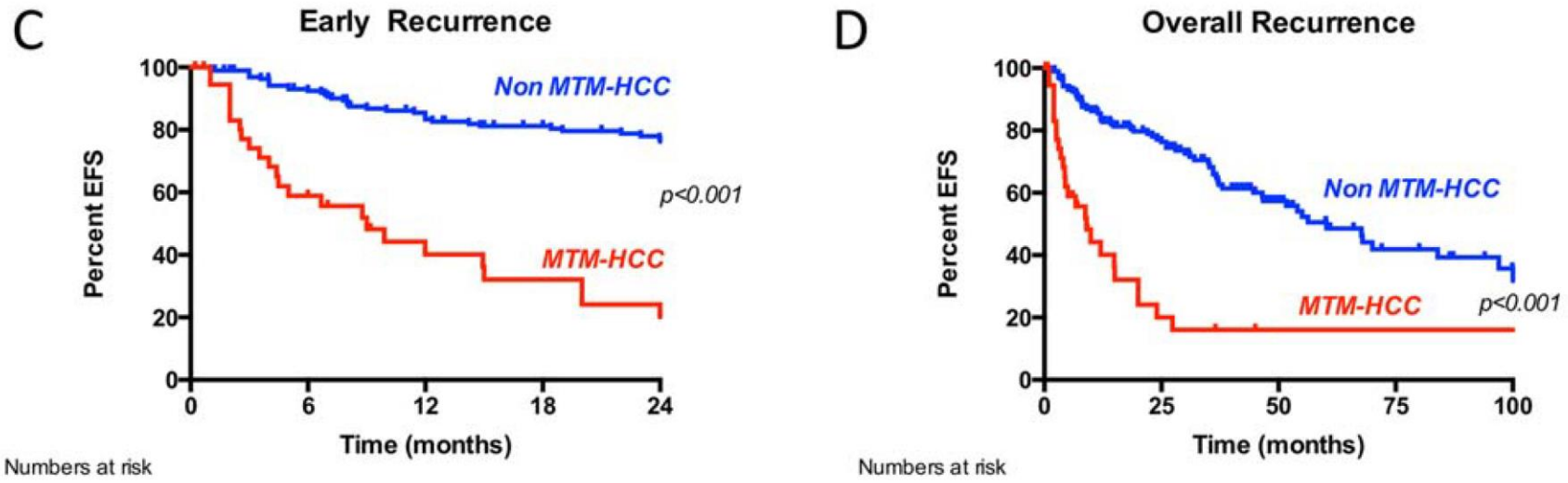
Large size >5 cm

Macrotrabecular architecture correlations:

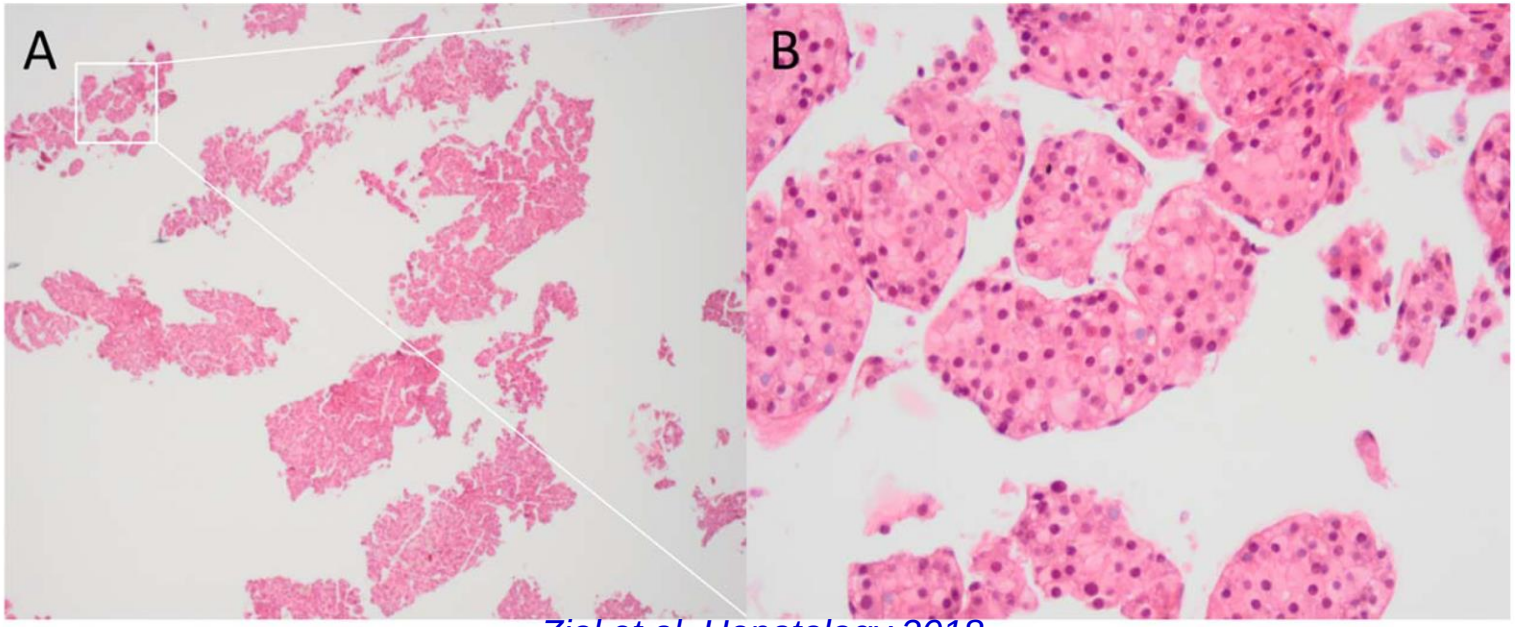
HBV infection, AFP >100 ng/mL, large size and …..



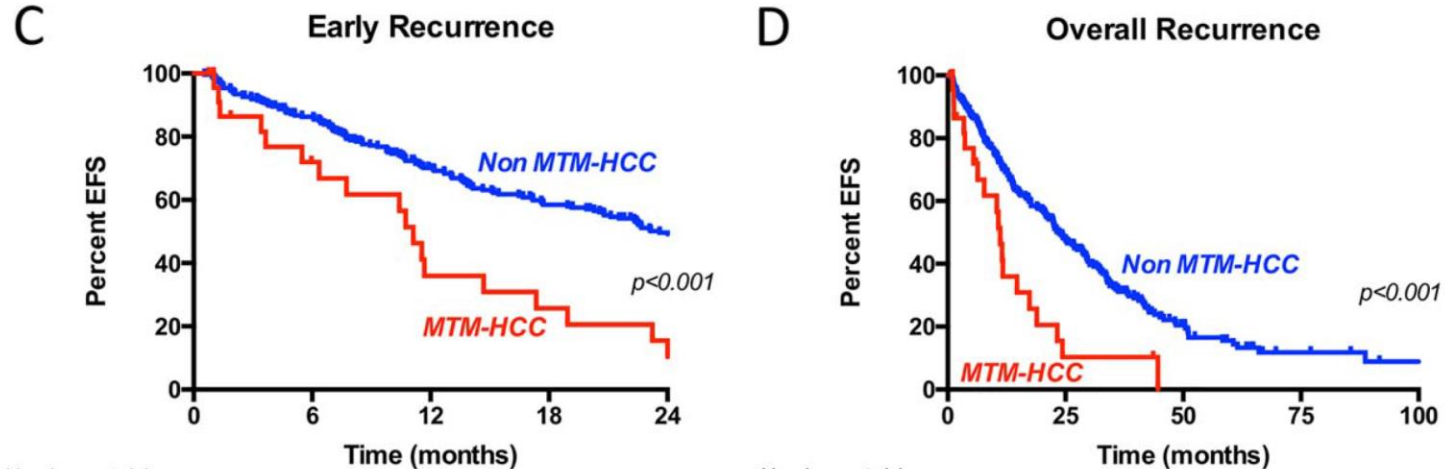
Ziol et al, Hepatology 2018



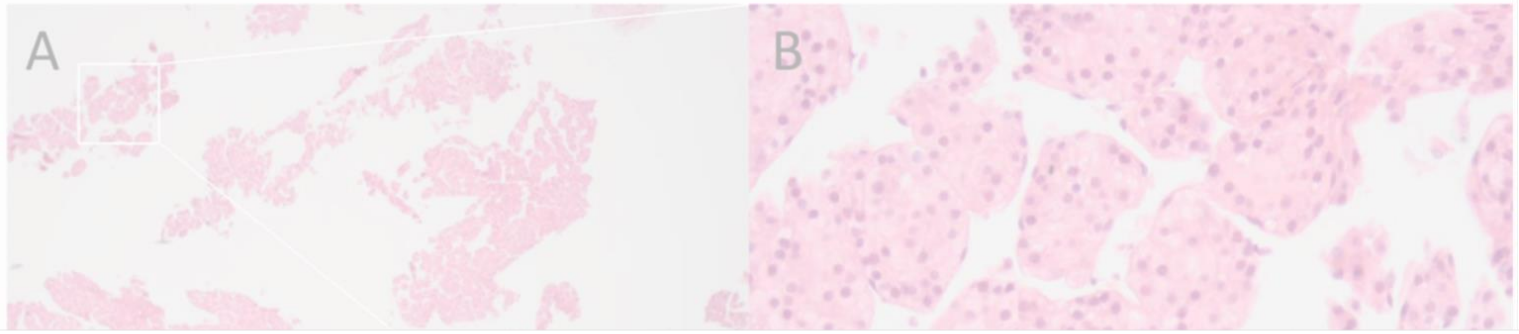
Macrotrabecular massive HCC subtype correlates with early and overall HCC recurrence even in subgroups according to traditional features of tumour aggressiveness



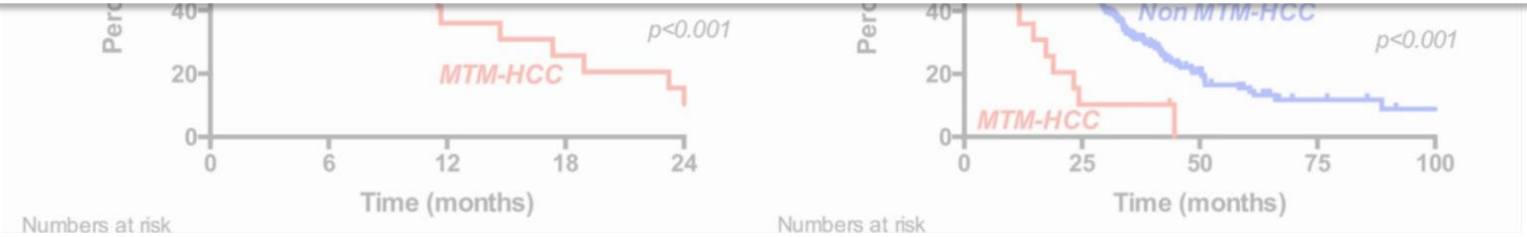
Ziol et al, Hepatology 2018



Macrotrabecular massive HCC subtype correlates with early and overall HCC recurrence in biopsy samples of tumours treated with RFA

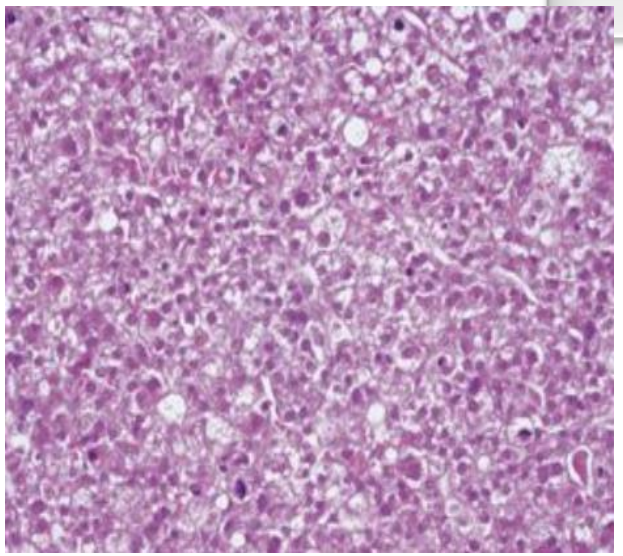


- Identification of the MacroTrabecular Massive (MTM) HCC subtype during pre-treatment workup has strong prognostic implications
- Patients with this MTM-HCC subtype may benefit from adjuvant therapies and/or upfront registration on liver transplant waiting lists after resection or RFA
- Strong argument **pro-liver biopsy in the diagnosis of HCC**

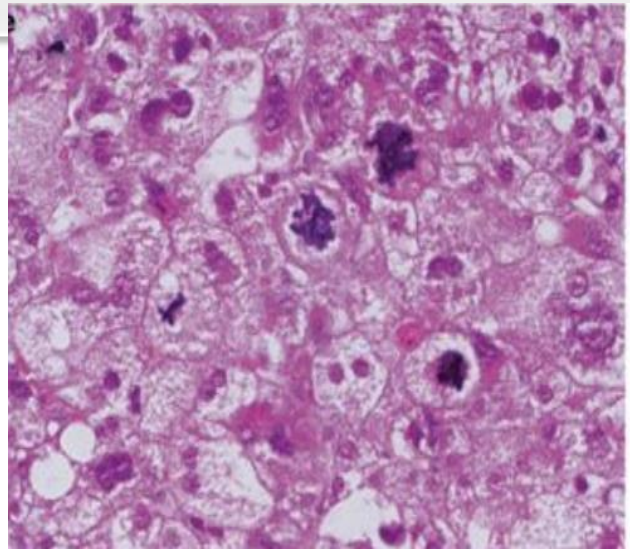


Novel histological/molecular HCC subtypes

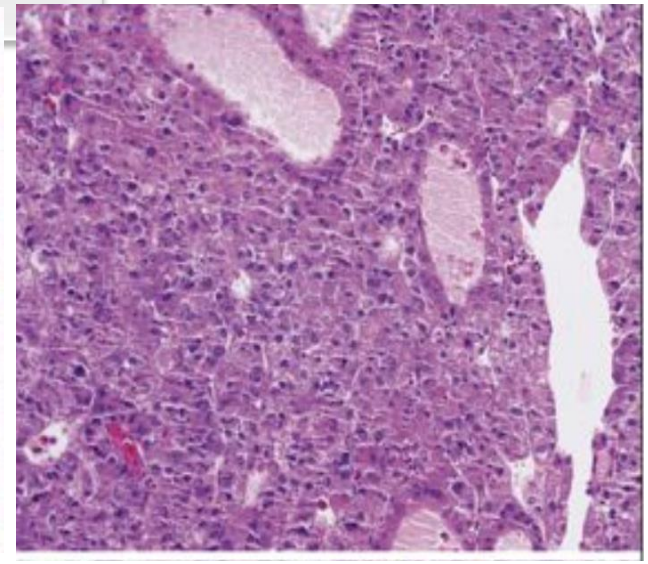
Chromophobe HCC



Chromophobic cytoplasm

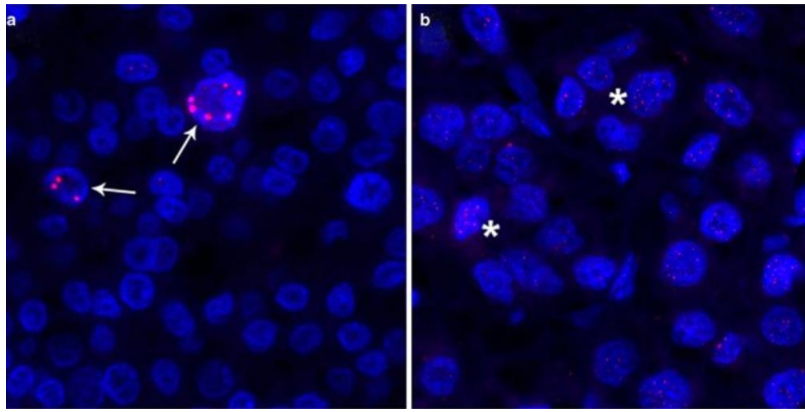


Abrupt focal nuclear anaplasia



Microscopic pseudocysts

- 5% HCC
- HBV-related (50%)
- Alternative lengthening of telomere (ALT) by telomere FISH



Wood, Mod Pathol 2013

2. Hepatocellular carcinoma -

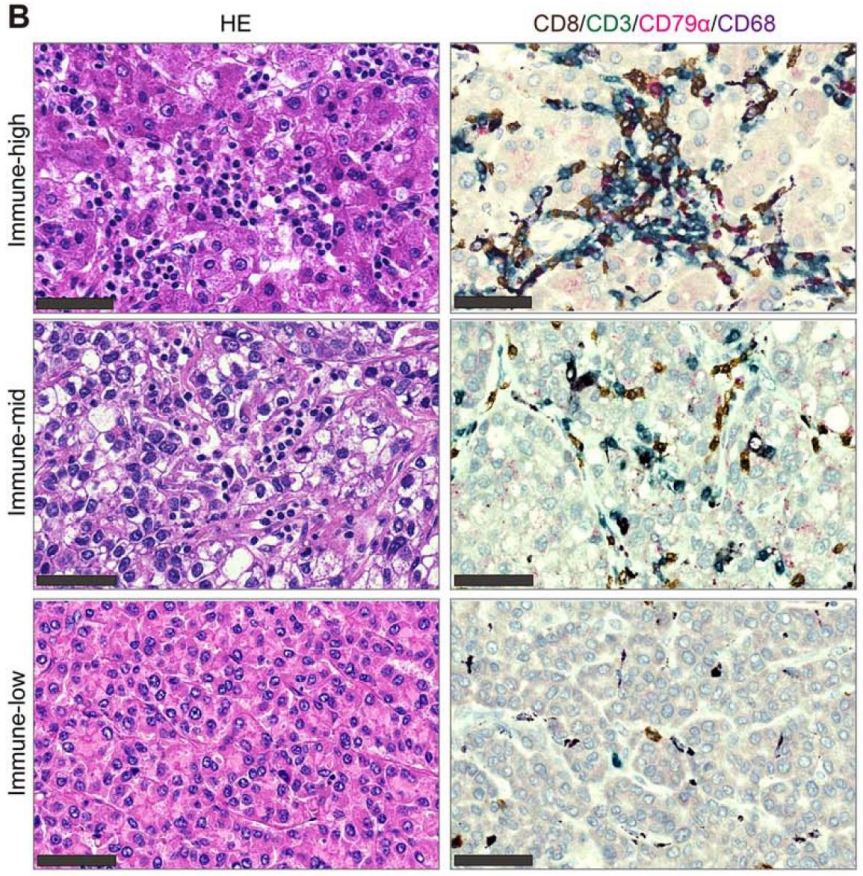
Immune microenvironment

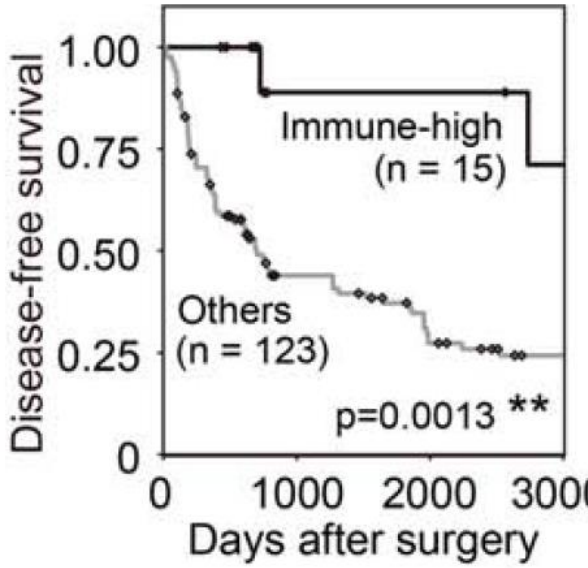
Landscape of Immune Microenvironment in Hepatocellular Carcinoma and Its Additional Impact on Histological and Molecular Classification

HEPATOLOGY, VOL. 68, NO. 3, 2018

Yutaka Kurebayashi,[1] Hidenori Ojima,[1] Hanako Tsujikawa,[1] Naoto Kubota,[1] Junki Maehara,[1,3] Yuta Abe,[2] Minoru Kitago,[2] Masahiro Shinoda,[2] Yuko Kitagawa,[2] and Michiie Sakamoto[1]

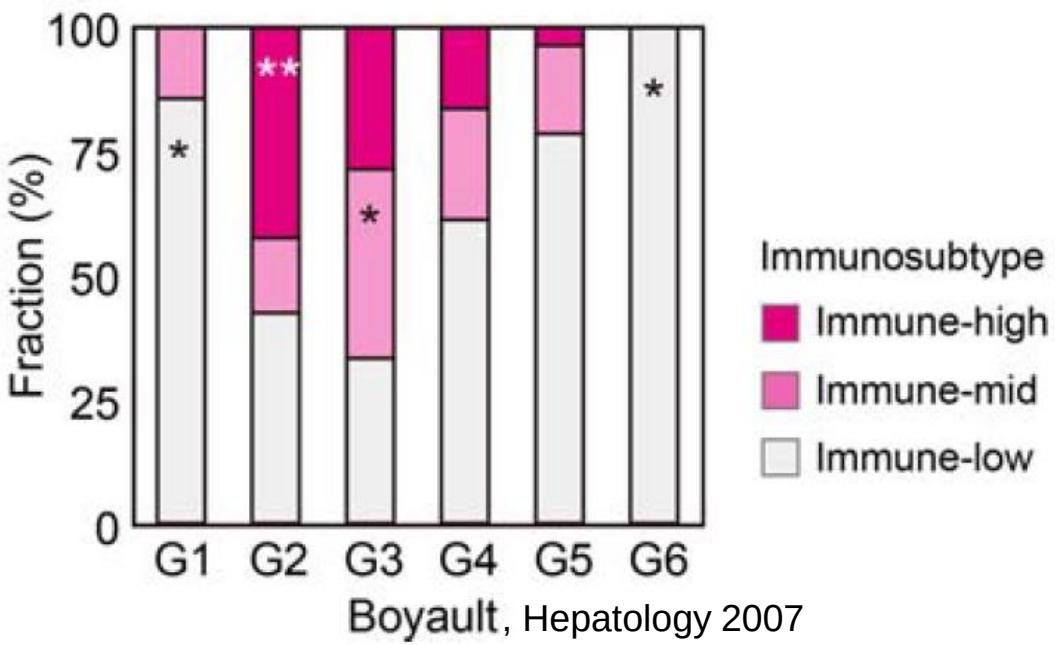
n=158 resected HCC (141 patients)





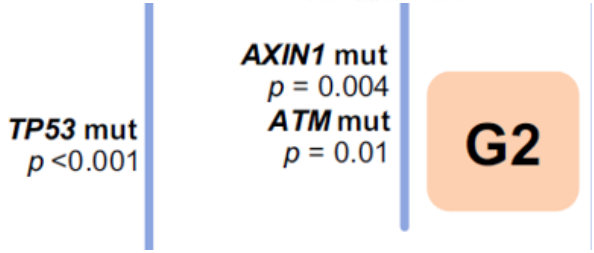
Better disease-free survival in immune-high subtype HCC, high grade

Molecular subtypes of HCC correlate with HCC immuno-subtypes



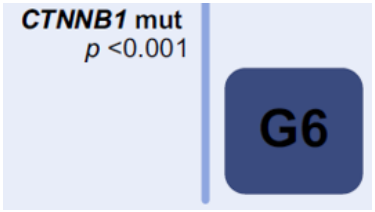
Boyault, Hepatology 2007

Proliferative HCC subgroup



Immune-high subtype

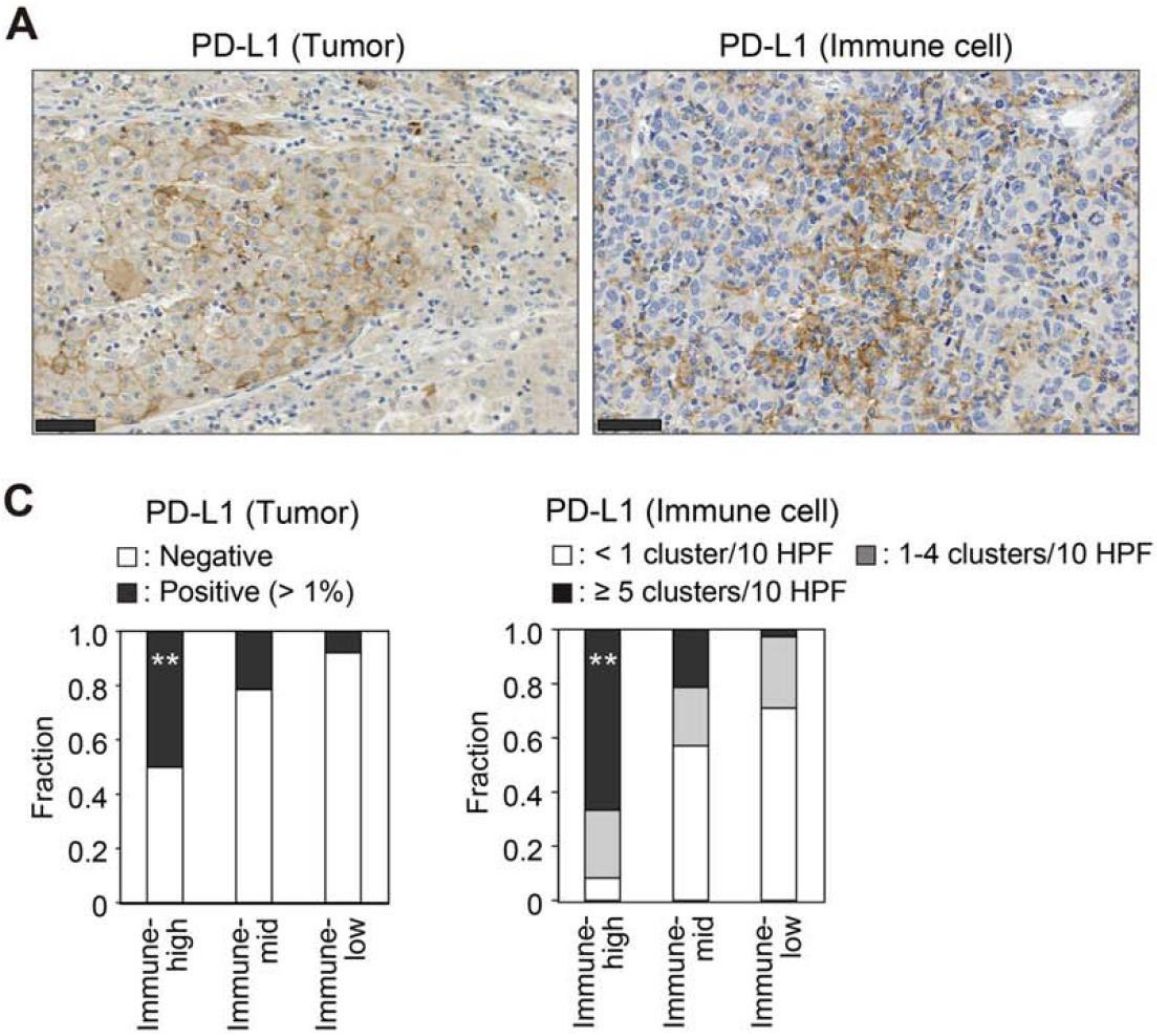
Non-proliferative HCC subgroup



Immune-low subtype

Kurebayashi, Hepatology 2018

PD-L1 in tumour and immune cells and PD-1 in immune cells is expressed in immune-high HCC subtype and indicates better prognosis



Kurebayashi, Hepatology 2018

Intra-tumoral tertiary lymphoid structures are associated with a low risk of early recurrence of hepatocellular carcinoma☆

Julien Calderaro[1,2,3,4,*], Florent Petitprez[4,5], Etienne Becht[4,5], Alexis Laurent[6], Théo Z. Hirsch[7], Benoit Rousseau[2,3,8], Alain Luciani[2,3,9], Giuliana Amaddeo[2,3,10], Jonathan Derman[1], Cécile Charpy[1], Jessica Zucman-Rossi[7,11,12], Wolf Herman Fridman[4,13,†], Catherine Sautès-Fridman[4,13,†]

Journal of Hepatology **2019** vol. 70 | 58–65

n=273 resected HCC

Male 82%, >60-y-o 58%

Aetiology: HBV 29%

Alcohol 27%

HCV 25%

NASH 14%

47% of HCC had TLS

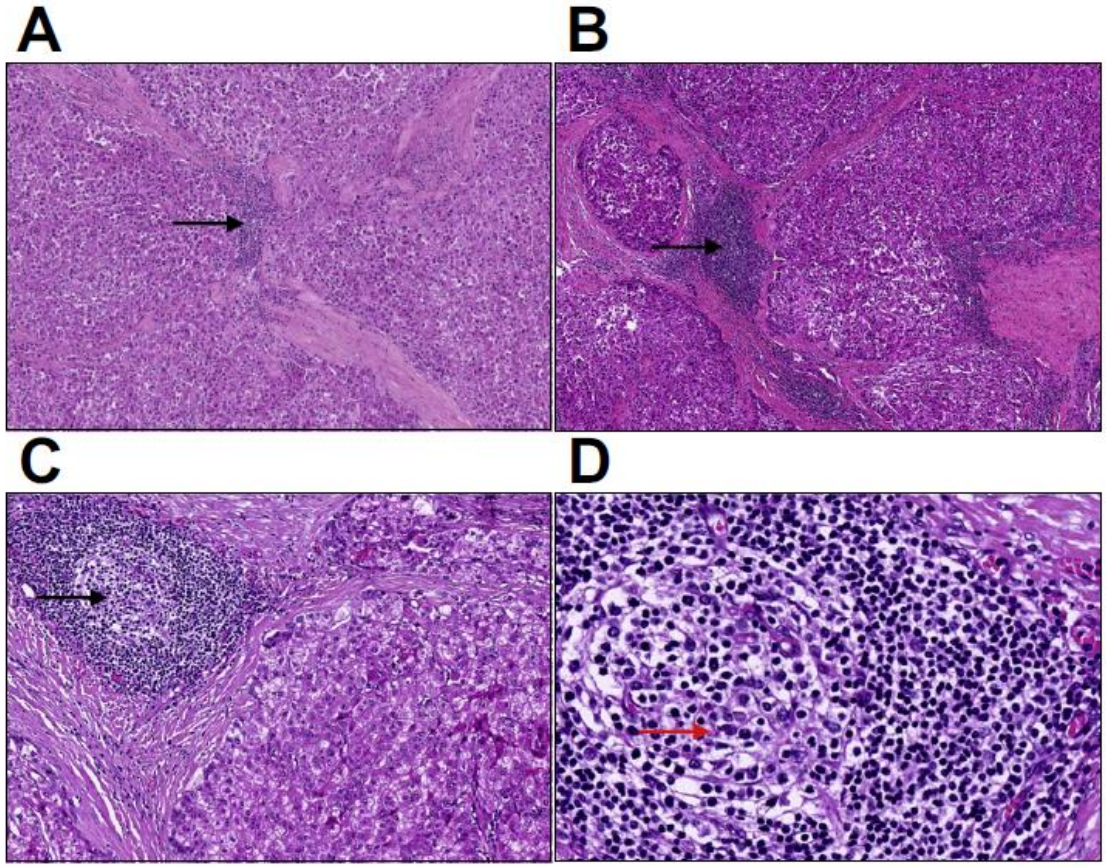
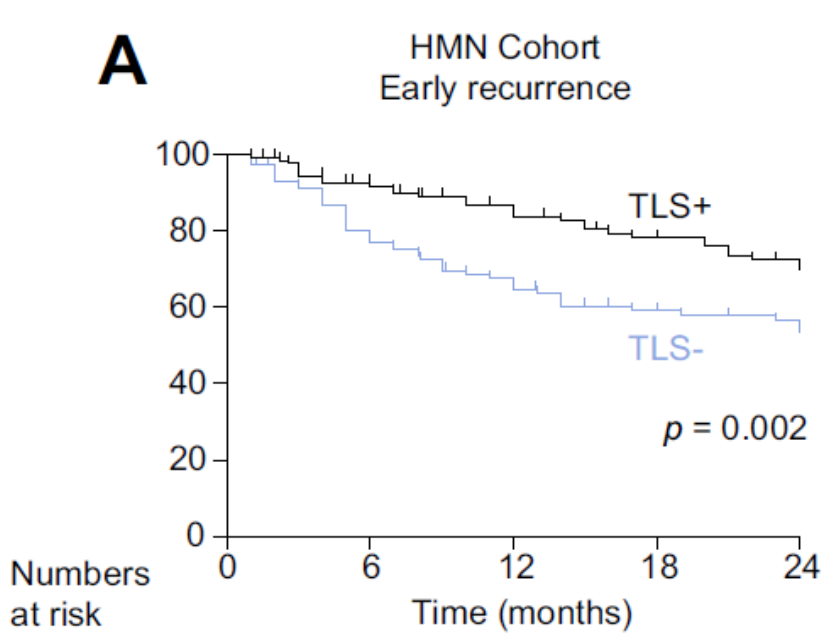
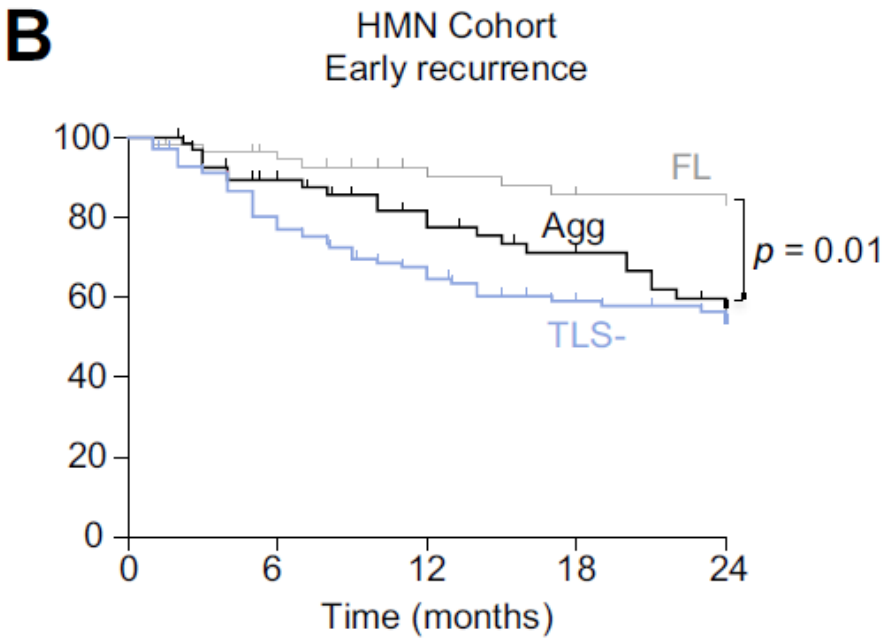


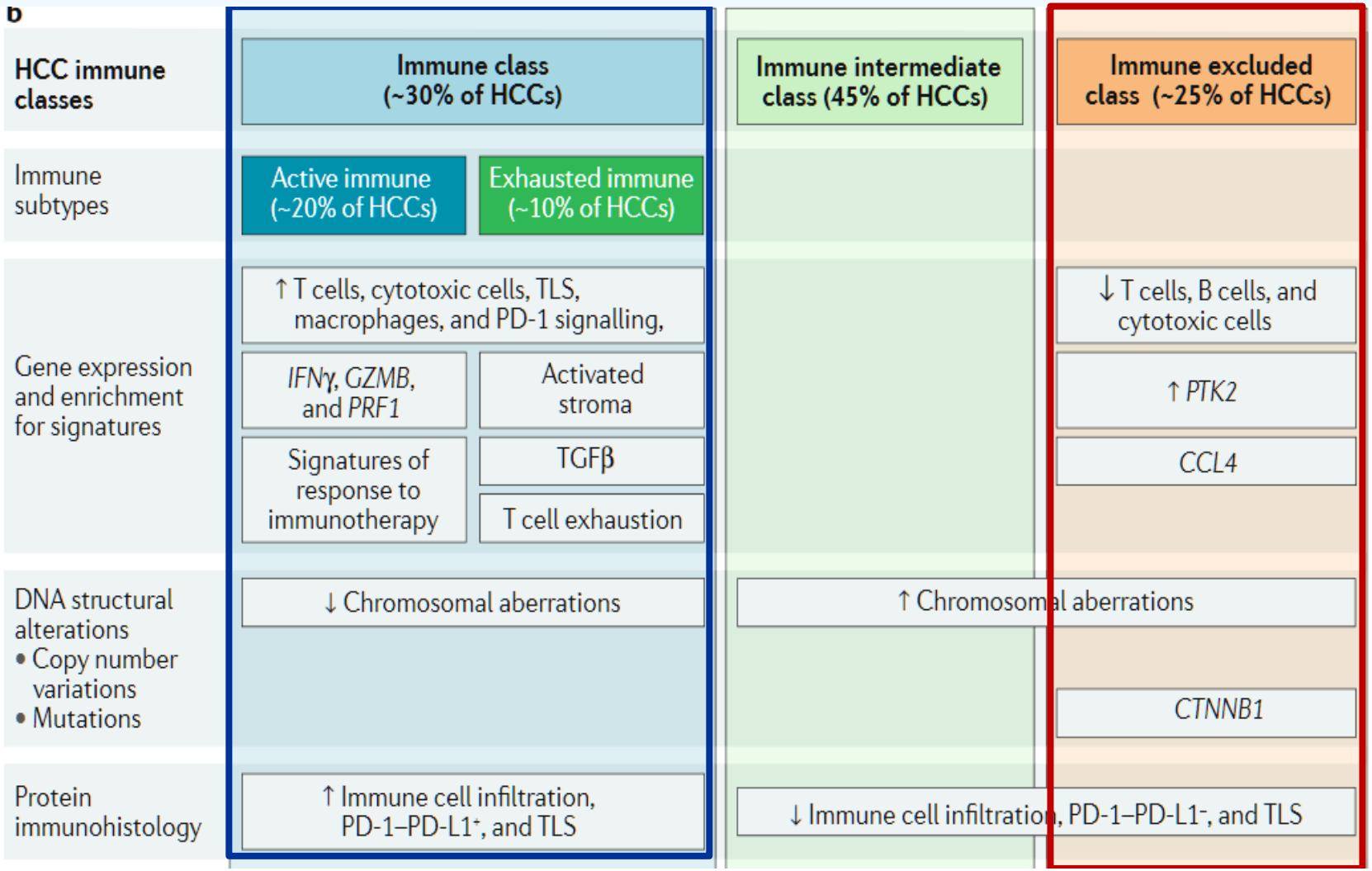
Table 1. Clinical and biological features of the HMN cohort according to the presence of intra-tumoral TLS.

Variables	Available data (n)	n (%)	TLS+ HCC n = 129 (47%)	TLS− HCC n = 144 (53%)	*p* value
Histological and gross features of the tumors					
Tumor size >50 mm	273	147 (54)	59 (46)	88 (61)	0.02
Satellite nodules	273	103 (38)	42 (32)	61 (42)	0.12
Macrovascular invasion	273	53 (19)	21 (16)	32 (22)	0.28
Microvascular invasion	273	136 (50)	56 (43)	80 (55)	0.06
Macrotrabecular-massive subtype	273	44 (16)	9 (7)	35 (24)	<0.001
Poor differentiation	273	47 (17)	22 (17)	25 (17)	0.23
Positive margin	273	21 (8)	10 (8)	11 (8)	1
Cirrhosis	258	97 (37)	47 (36)	50 (35)	0.32



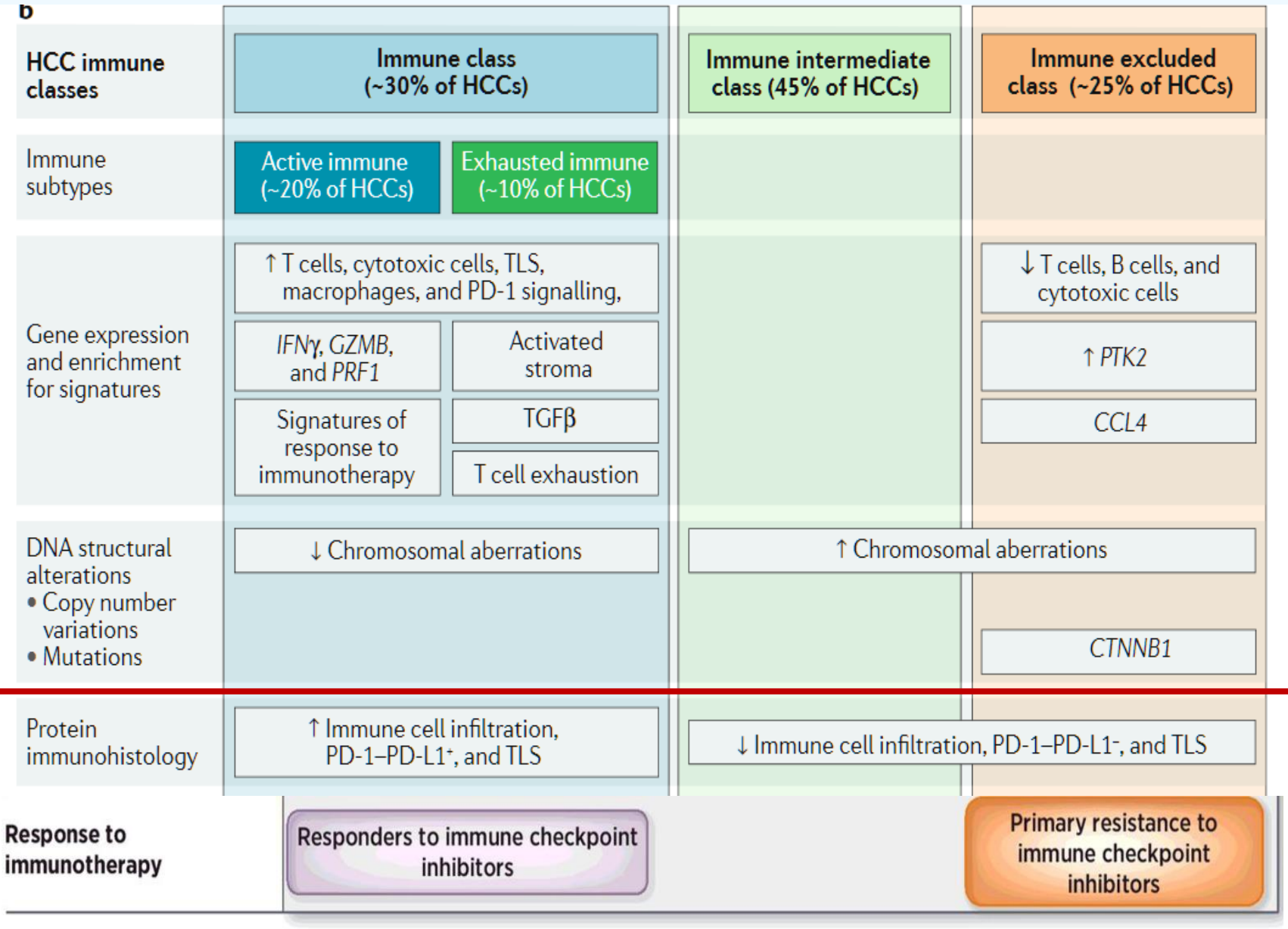


Immune-based classification of HCCs based on the immune status in tumour microenvironment



Llovet, Nat Rev Clin Oncol 2018
Pinyol, Clin Cancer Res 2019

Immune-based classification of HCCs based on the immune status in tumour microenvironment



3. Hepatocellular carcinoma - Combined HCC-CCA

- **2-5% of primary liver carcinomas** cannot be classified as HCC or iCCA
- Combination of HCC and CCA features
- Cirrhotic and non-cirrhotic liver
- Impact on patient management

WHO 2010 Classification of Combined HCC-CC

- **Combined hepatocellular-cholangiocarcinoma, classical type**
  - Has a border between the two types
  - Formerly known as "collision tum
- **Combined hepatocell giocarcinoma,**
  with stem cell fe
  - ***Typical subtyp*** ules with "stem cell" cancer cells at periphery
  - ***Intermediate su***: tumor nodules are comprised of cells that express hepatocyte and biliary markers
  - ***Cholangiolocellular subtype***: tubules, antler-like, in desmoplastic stroma; cells express hepatocyte and biliary markers

Problematic

Theise, Nakashima, Park, Nakanuma in WHO 2010, p225-227

Brunt EM, Laennec Liver Society Meeting 2018

HEPATOLOGY

AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

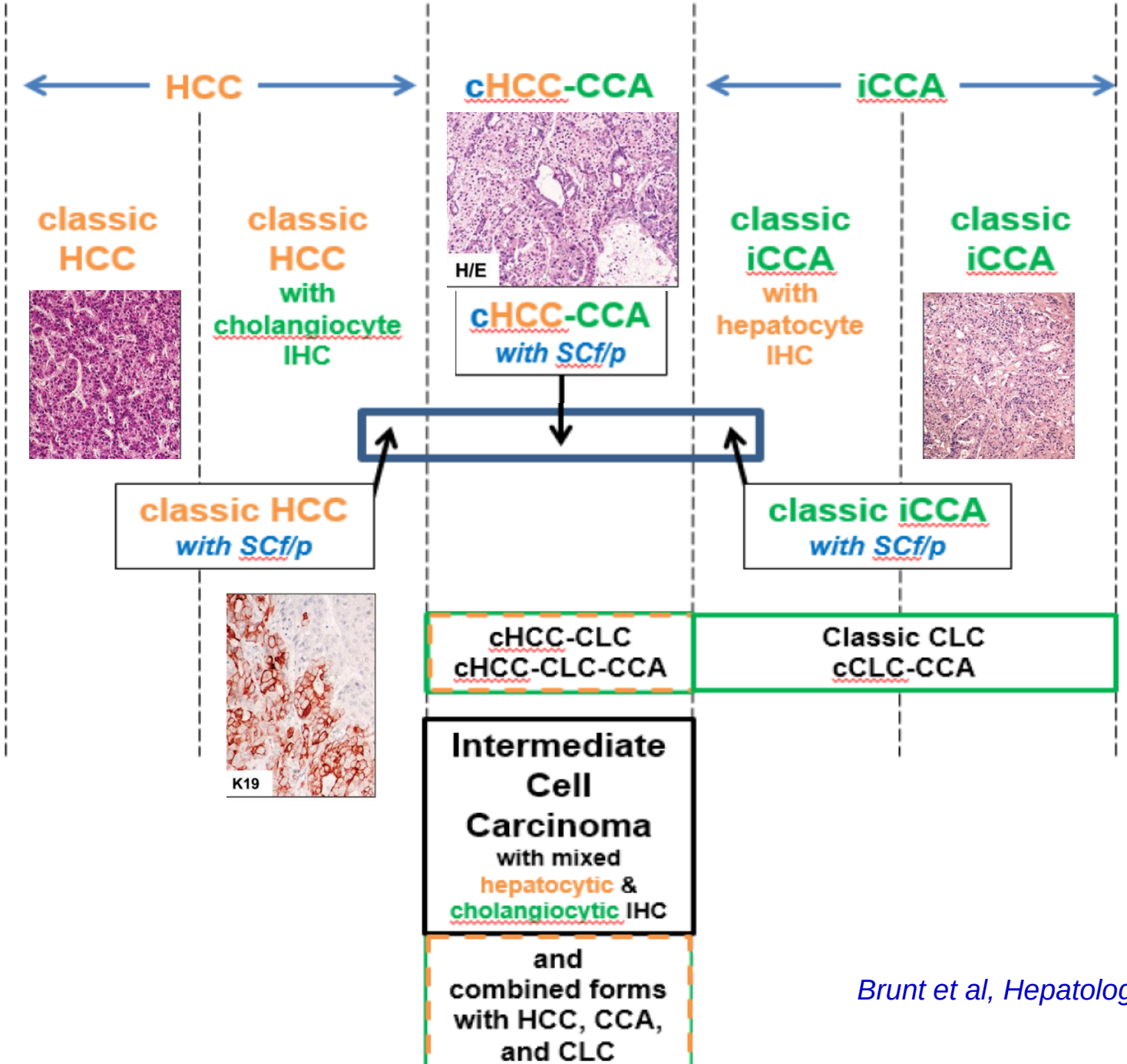
HEPATOLOGY, VOL. 00, NO. 00, 2018

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]

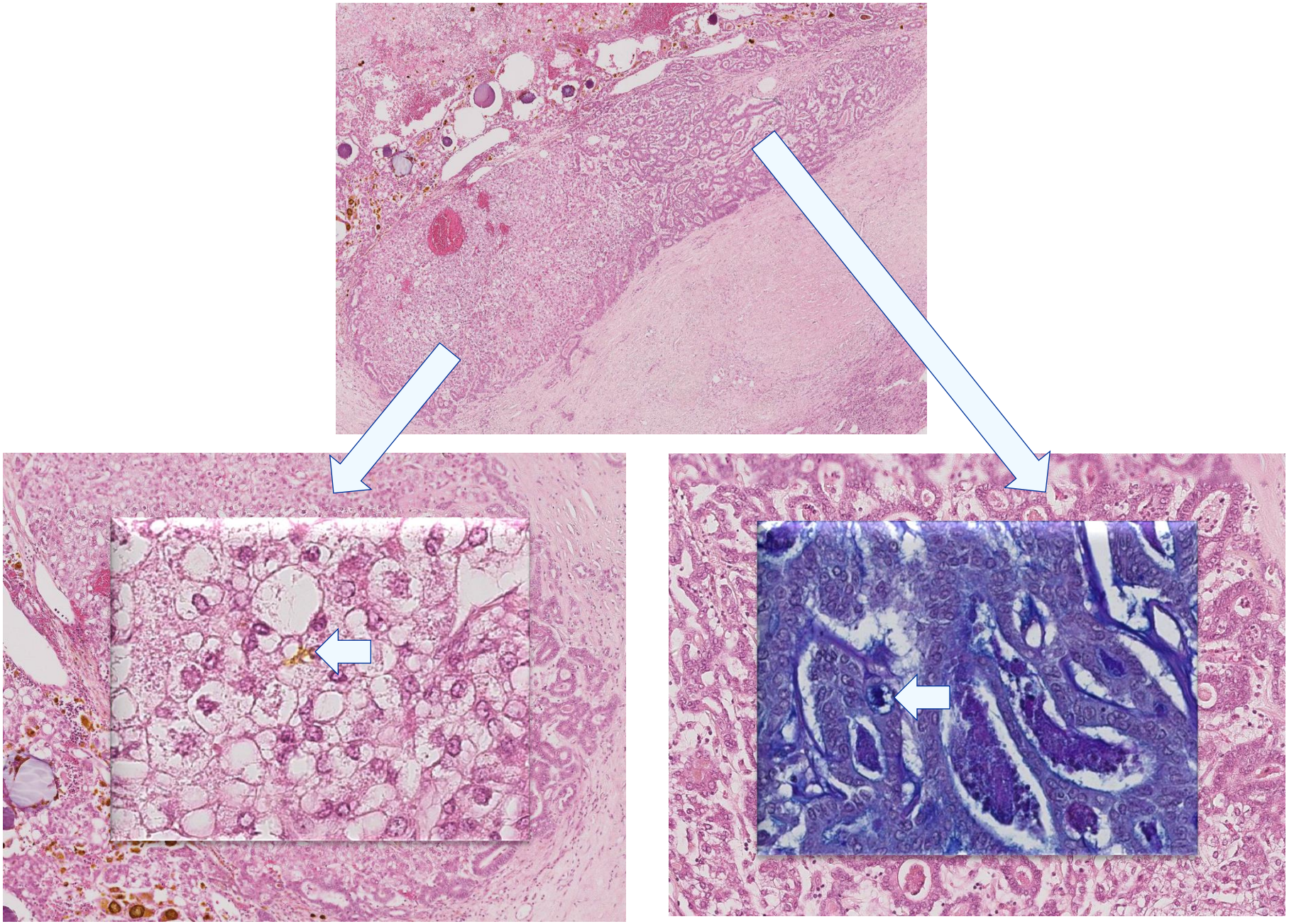
International Multidisciplinary Proposal 2018

Pathology, Radiology, Hepatology, Molecular Biology

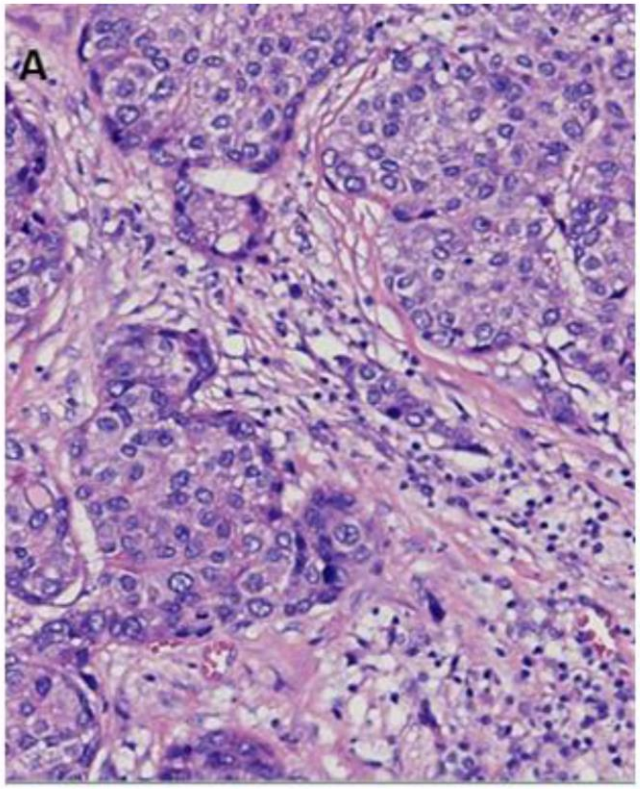


Brunt et al, Hepatology 2018

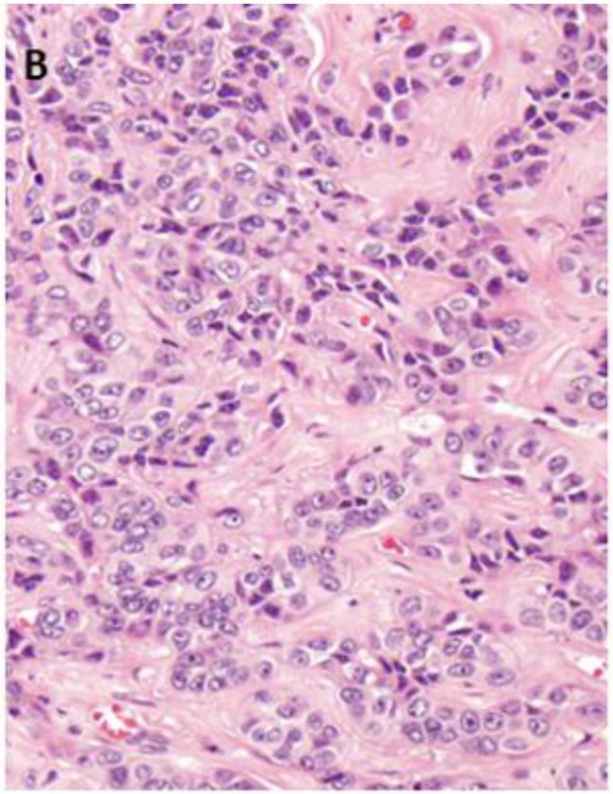
Combined hepatocellular cholangiocarcinoma - cHCC-CCA



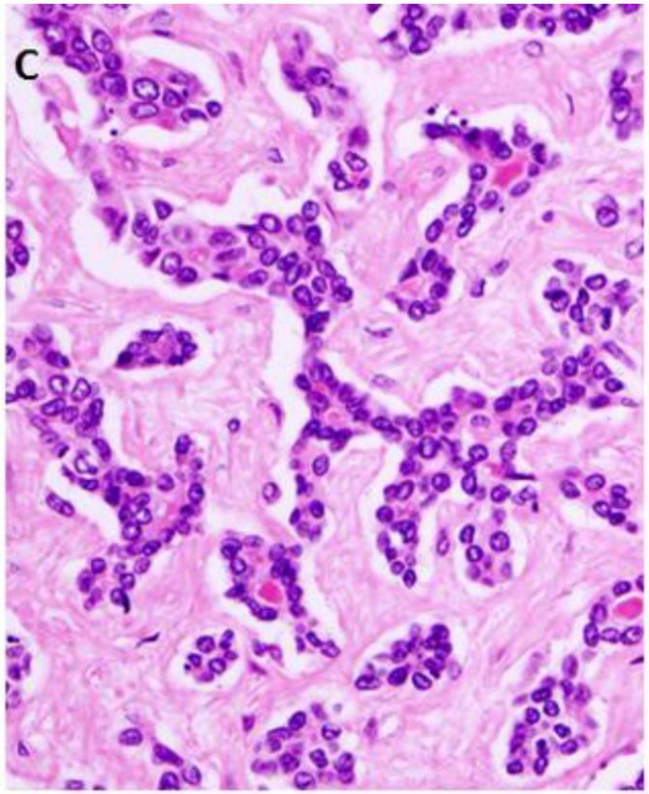
Morphologic variants of “stem/progenitor cell features”



HCC with stem cell/progenitor cell features



Intermediate cell carcinoma



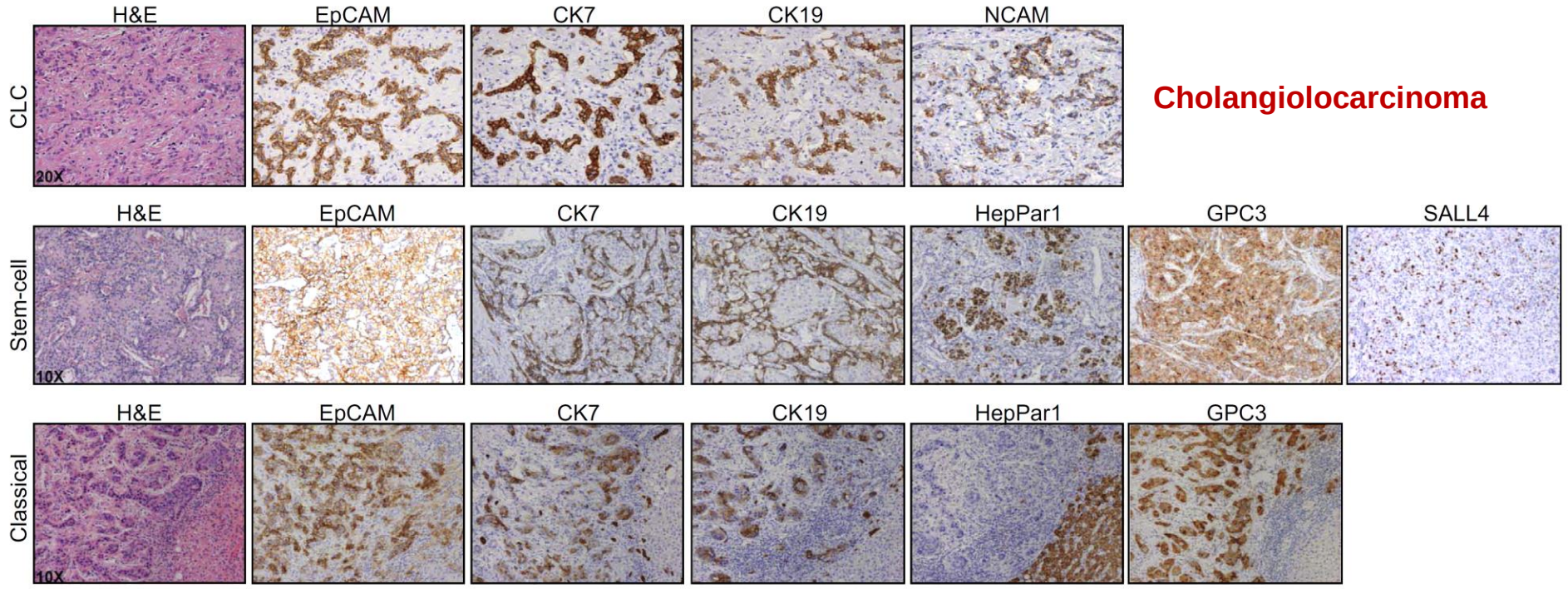
Cholangiolocarcinoma

Brunt et al, Hepatology 2018

Mixed hepatocellular cholangiocarcinoma tumors: Cholangiolocellular carcinoma is a distinct molecular entity

Agrin Moeini[1,2], Daniela Sia[2], Zhongyang Zhang[3,4], Genis Camprecios[2], Ashley Stueck[2], Hui Dong[1], Robert Montal[1], Laura Torrens[1], Iris Martinez-Quetglas[1], M. Isabel Fiel[2], Ke Hao[3,4], Augusto Villanueva[2], Swan N. Thung[2], Myron E. Schwartz[2], Josep M. Llovet[1,2,5,*]

Journal of Hepatology **2017** vol. 66 | 952–961



Cholangiolocarcinoma

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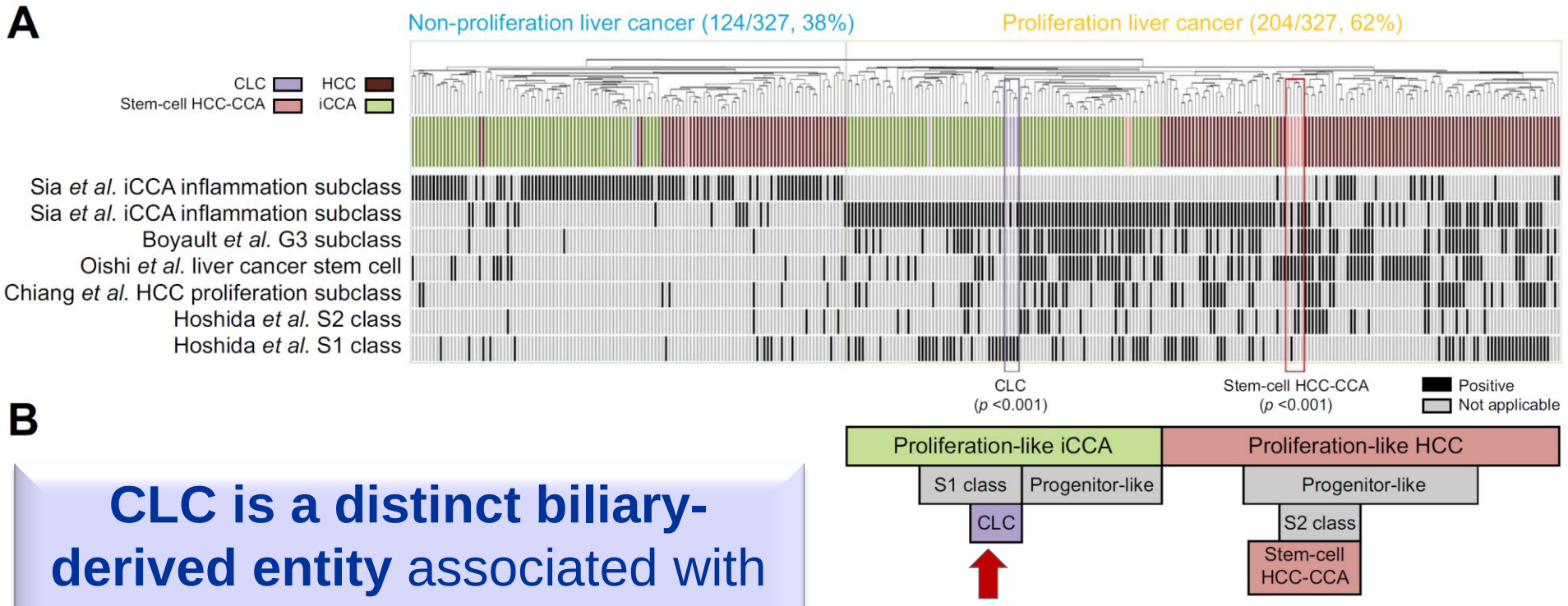
Integrative genomic analysis of HCC-CCA with HCC and iCCA



CLC is a distinct biliary-derived entity associated with chromosomal stability and active TGFβ signaling

Primary Liver Carcinomas With both Hepatocytic and Cholangiocytic Differentation

- **Combined HCC-CCA: cHCC-CCA**
  - Unequivocal presence of HCC and CCA, intermingled or close
  - With or without stem cell/progenitor cell features
  - No Keratin 19+ HCC or iCCA with hepatocytic markers
  - No collision tumours
- **Intermediate Cell Carcinoma**
- **Cholangiolocarcinoma** (CLC)
  - aka cholangiolocellular carcinoma

  This is now considered as subtype of iCCA

cHCC-CCA: Diagnostic considerations

- **Diagnosis of cHCC-CCA relies on routine stains**

  Immunohistochemistry is only supplemental!

- **Stem cell phenotypes/features may exist within cHCC-CCA, and can be noted in a descriptive report**

  No separate subclassification!

cHCC-CCA: Diagnostic considerations

- Diagnosis of cHCC-CCA relies on routine stains
  Immunohistochemistry is only supplemental!

- Stem cell phenotypes/features may exist within
  cHCC-CCA, and can be noted in a descriptive report
  No separate subclassification!

- Radiology of cHCC-CCA to date indicate features between HCC and iCCA, but often not specific for either

  Biopsy confirmation may be indicated

Summary

- **HCC histological subtypes are closely related with specific oncogenic pathways and molecular alterations**

- 15-20% of HCC have identifiable and possibly targetable molecular alterations

- Macrotrabecular massive HCC has ↑ recurrence risk and poor prognosis

Summary

- **Immune subtypes are significant in high grade HCC:**

  ↑ B-cells/plasmacytes/T cells indicate better prognosis

- **Intra-tumoral tertiary lymphoid structures are associated with decreased risk of early HCC after surgery**

- **Wnt/CTNNB1 mutations characterize the immune excluded HCC ⇨ possible biomarker predicting resistance to immune checkpoint inhibitors**

Summary

- **New consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentiation**
  - **Diagnosis based on morphology** not immunohistochemistry
  - Stem cell/progenitor cell features do not define specific histological subtypes of cHCC-CCA
  - Intermediate Cell Carcinoma separate from cHCC-CCA
  - Cholangiolocarcinoma classified as subtype of iCCA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Thank you!