MOLECULAR PROFILING IN HEPATOBILIARY TUMORS

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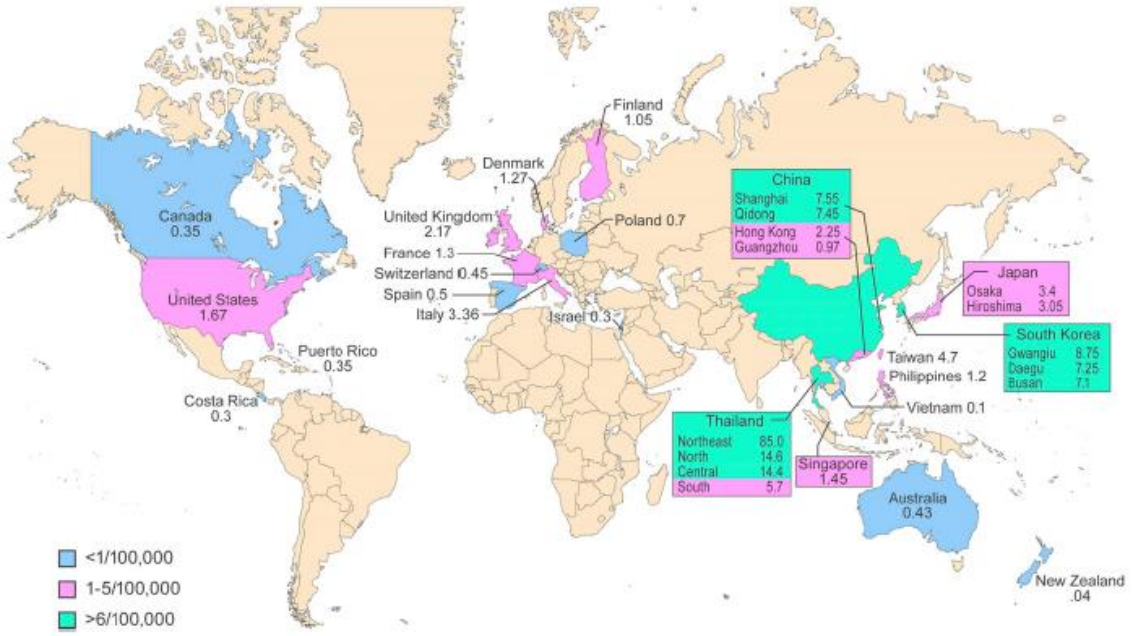
Hepatobiliary Tumors: Introduction

Incidence of Cholangiocarcinoma



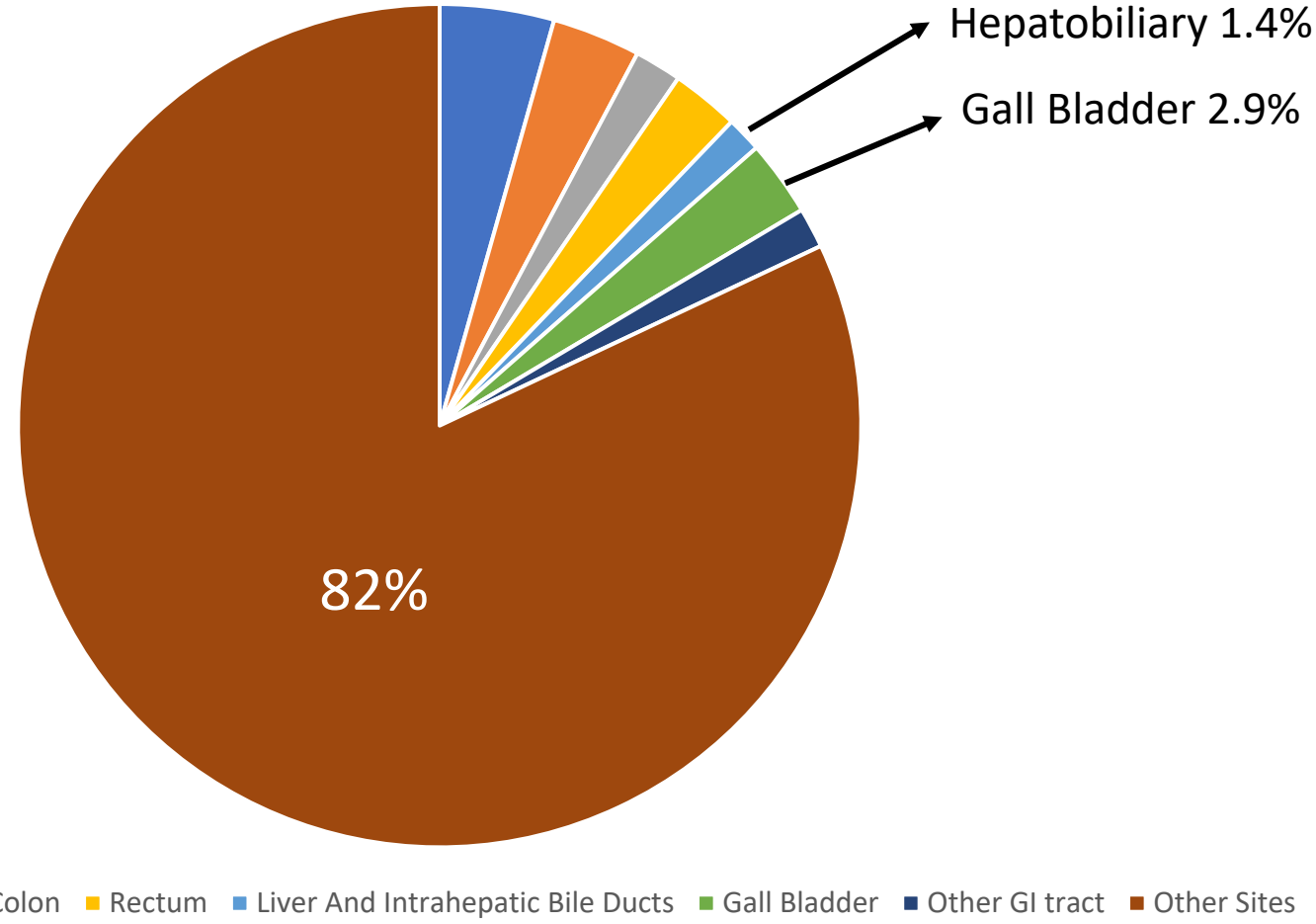
Incidence of Gall Bladder

- *The incidence of cholangiocarcinoma is modest in the western world, between 0.35 to 2 per 100,000 annually; however, in China and Thailand, the incidence can be up to 40 times the rate observed in the United Kingdom and, thus, poses significant public health questions.*
- *The incidence of gallbladder cancer tends to be closely associated with its primary etiology, cholelithiasis. As such, the incidence is uniform for most of the Western world, however, disease clusters are found in northern india, Japan, and the Andes region*

Bridgewater et al. DOI: 10.14694/EDBK_160831

Hepatobiliary Tumors: Introduction

A Report of the Hospital Based Cancer Registries, 2021
National Cancer registry programme



https://ncdirindia.org/All_Reports/HBCR_2021/resources/HBCR_2021_Ch2.pdf

Hepatocellular Carcinoma: Etiology

- **Infections with hepatitis B virus and hepatitis C virus - 75%**
- Chronic alcohol consumption
- Non-alcoholic fatty liver disease (NAFLD)
- Other metabolic disorders have become particularly relevant in Western countries due to a sharp increase in prevalence and a high number of HCCs without underlying cirrhosis.
- Aflatoxin
- Other etiologic considerations:

  Autoimmune chronic active hepatitis, cryptogenic cirrhosis, and metabolic diseases. Metabolic diseases include hemochromatosis (iron accumulation), Wilson disease (copper accumulation), α1-Antitrypsin deficiency, tyrosinemia, porphyria cutanea tarda, glycogenesis types 1 and 3, citrullinemia, and orotic acid urea. In children, congenital cholestatic syndrome (Alagille syndrome) is associated with a familial type of HCC.

Hepato-Biliary Tract Tumors
Molecular profiling

Genomics

Epigenomics

Proteomics

Metabolomics

Downstream alterations in pertinent molecular signalling pathways

The principal objective of this research is to integrate these new omic data with clinicopathologic features of HCC and biliary tract tumors in order to discover new diagnostic tools, improve treatment options, and implement effective prevention strategies

DeVita, Hellman, and Rosenberg's Cancer Principles and Practice of Oncology. 11th Edition

HEPATOCARCINOGENESIS

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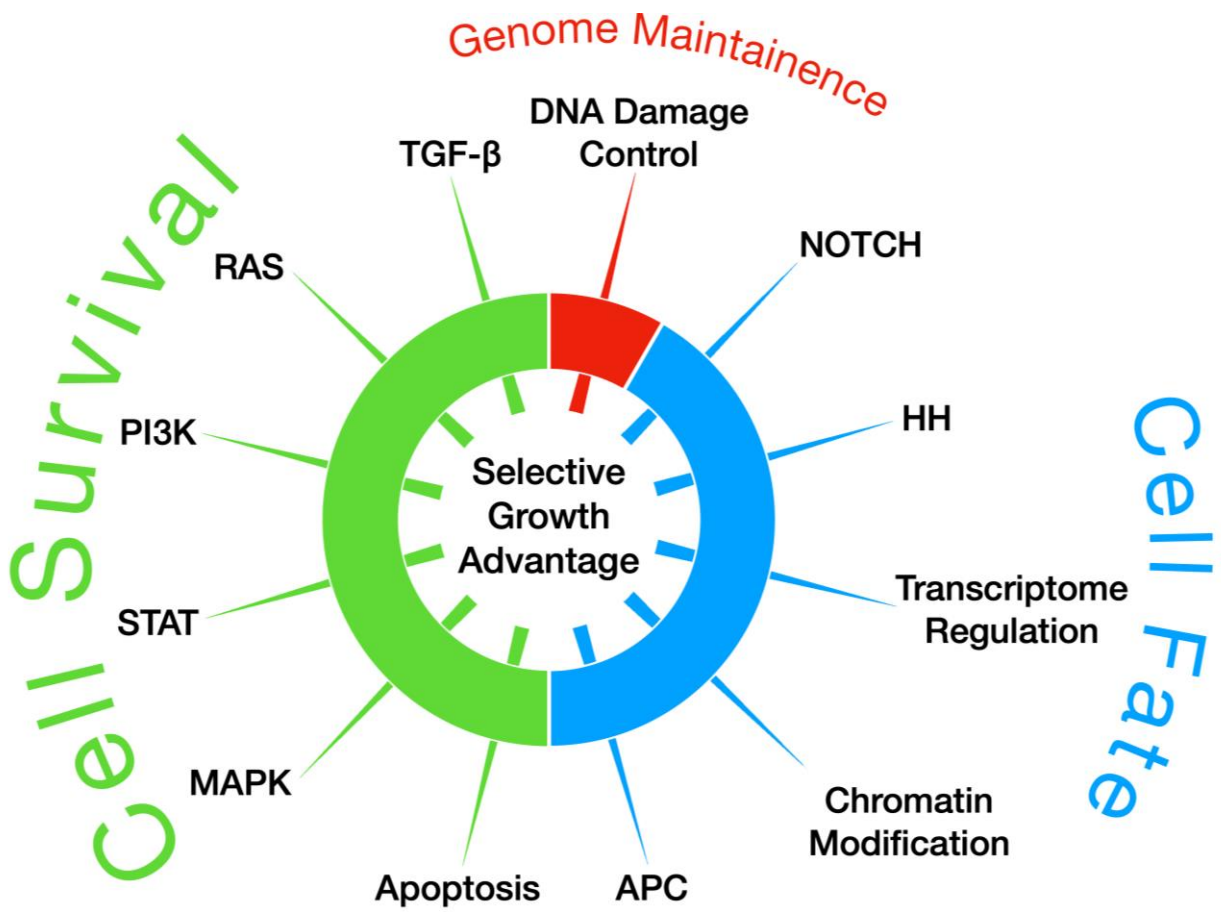

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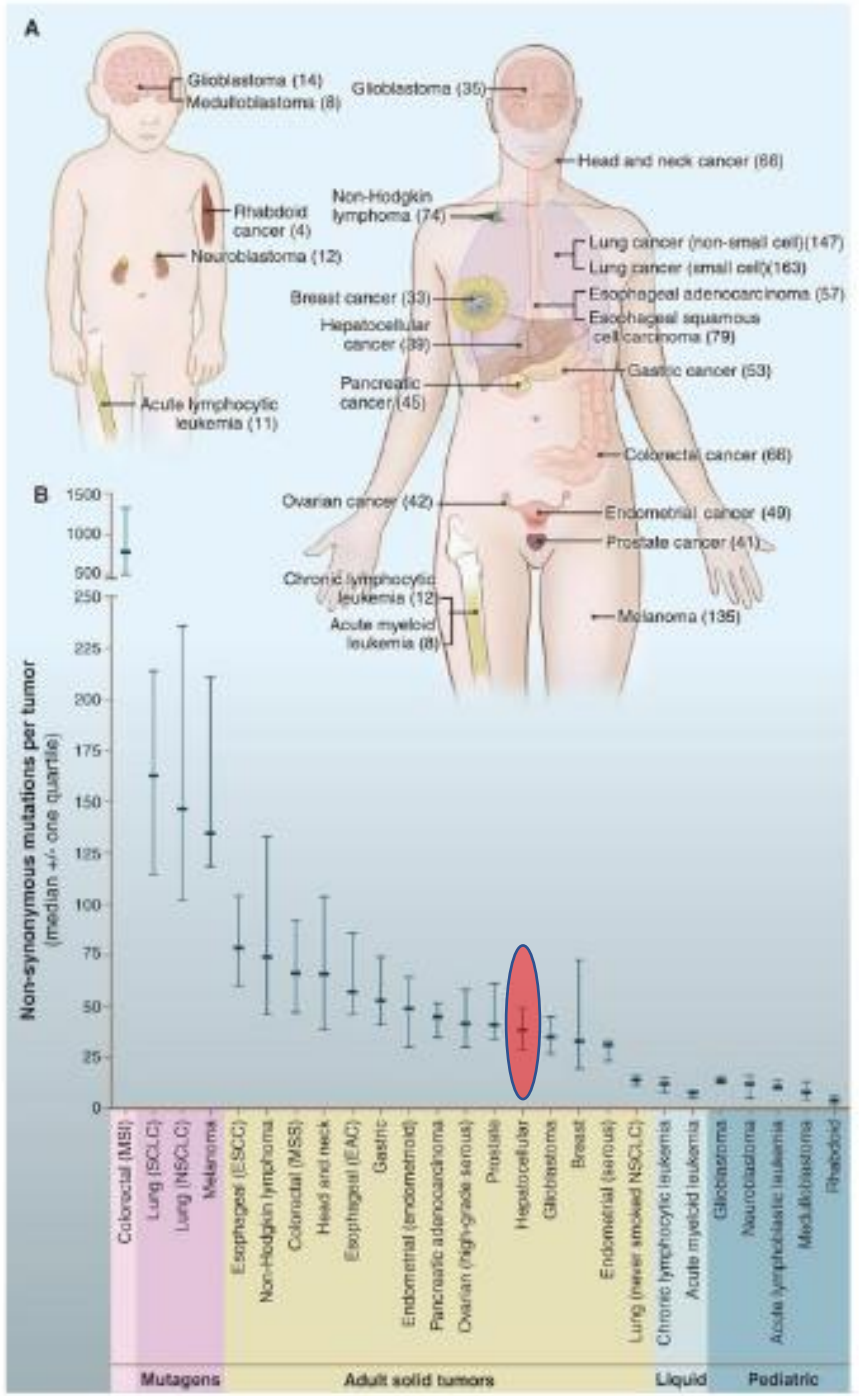


Vogelstein et al. DOI: 10.1126/science.1235122

Hepatocellular Carcinoma - Genomics

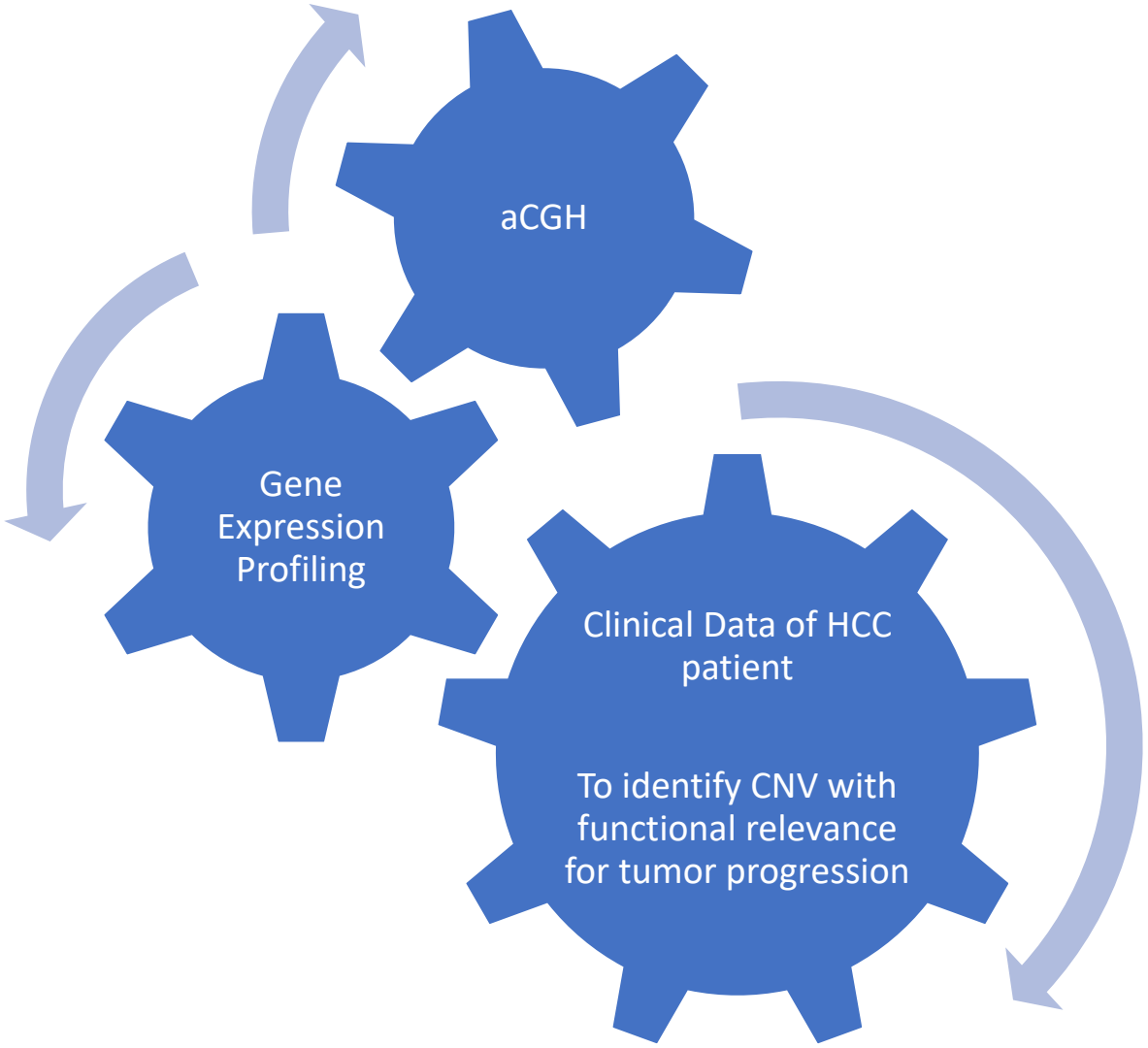
- Amplifications:
  - 1q (57.1%)
  - 8q (46.6%)
  - 6p (22.3%)
  - 7q (22.2%).
- Losses:
  - 8p (38%)
  - 16q (35.9%)
  - 4q (34.3%)
  - 17p (32.4%)
  - 13q (26.2%)

Preneoplastic dysplastic nodules (DNs)

Gain of 1q appears to be an early event in DN development, possibly predisposing affected cells to acquisition of additional chromosomal aberrations

"However, whereas these studies revealed interesting mechanistic clues for hepatocarcinogenesis, the substantial molecular Diversity of alterations in these loci remains a major obstacle and the functional validation of individual genes and the identification of driver genes remains challenging".

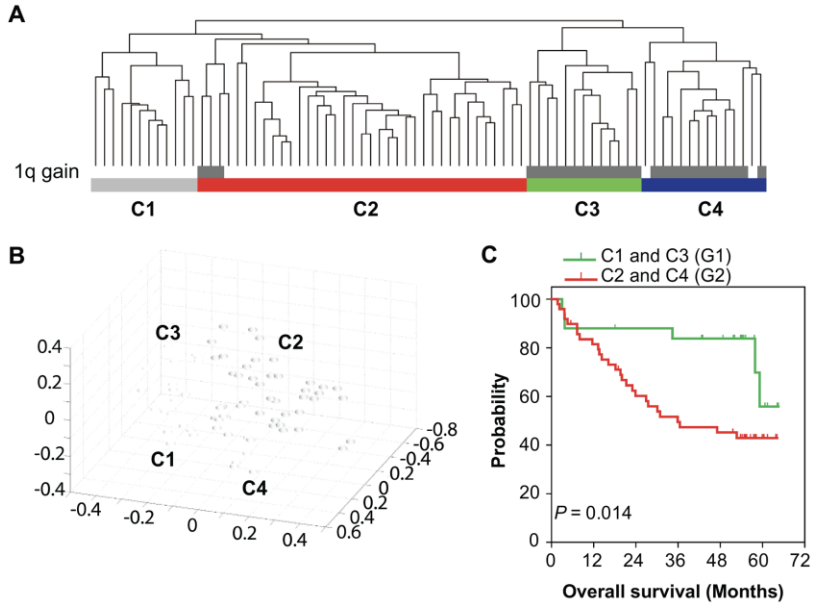
HCC– Integrative approach



The investigation was restricted to genes that showed

1. Recurrent CNVs
2. Correlation of the CNVs and the transcriptome
3. A selective association to patient's outcome to distinguish "drivers" from passengers.

10-gene signature as a molecular predictor of patient survival



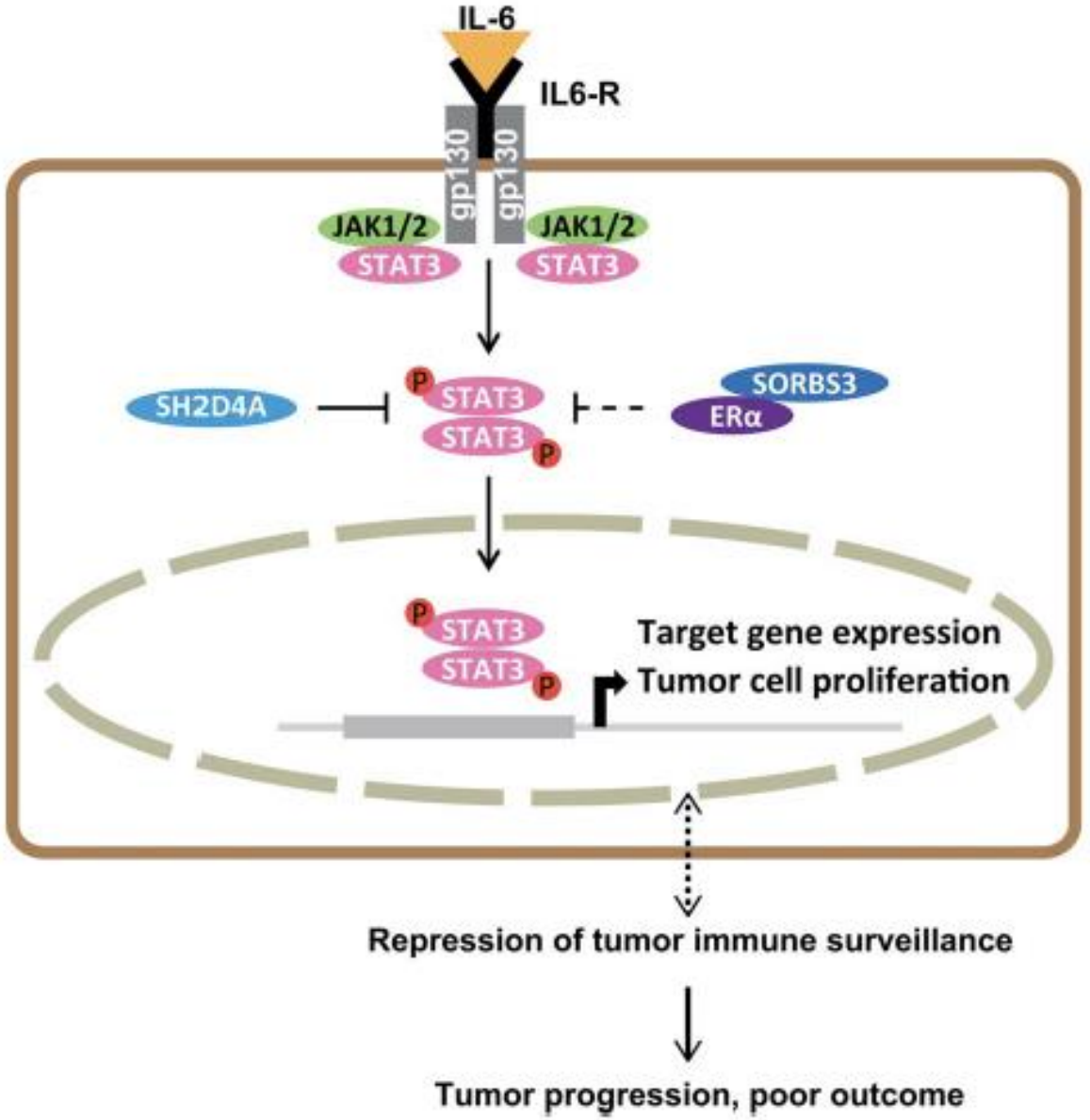
Roessler et al. DOI: 10.1053/j.gastro.2011.12.039

HCC– Integrative approach

Gene expression profile of patients with chromosome 8p loss correlates with increased IL-6 Signaling.

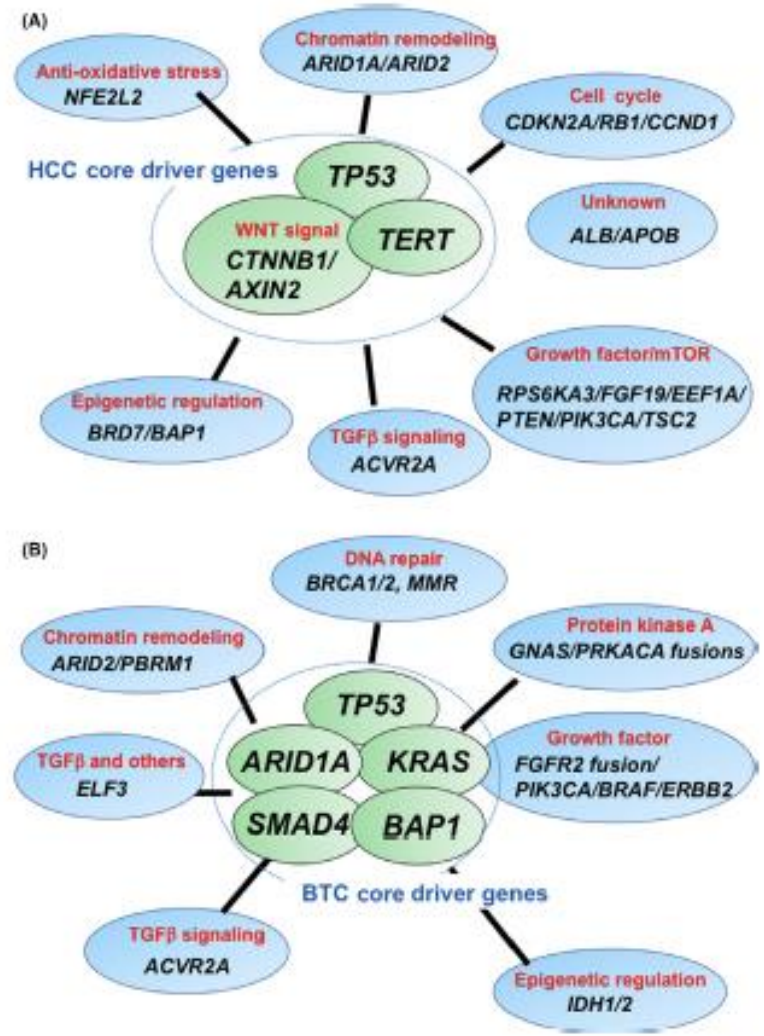
Modulation of the chromosome 8p tumor-suppressor genes SH2D4A and SORBS3 were associated with cell growth and clonogenicity in liver cancer

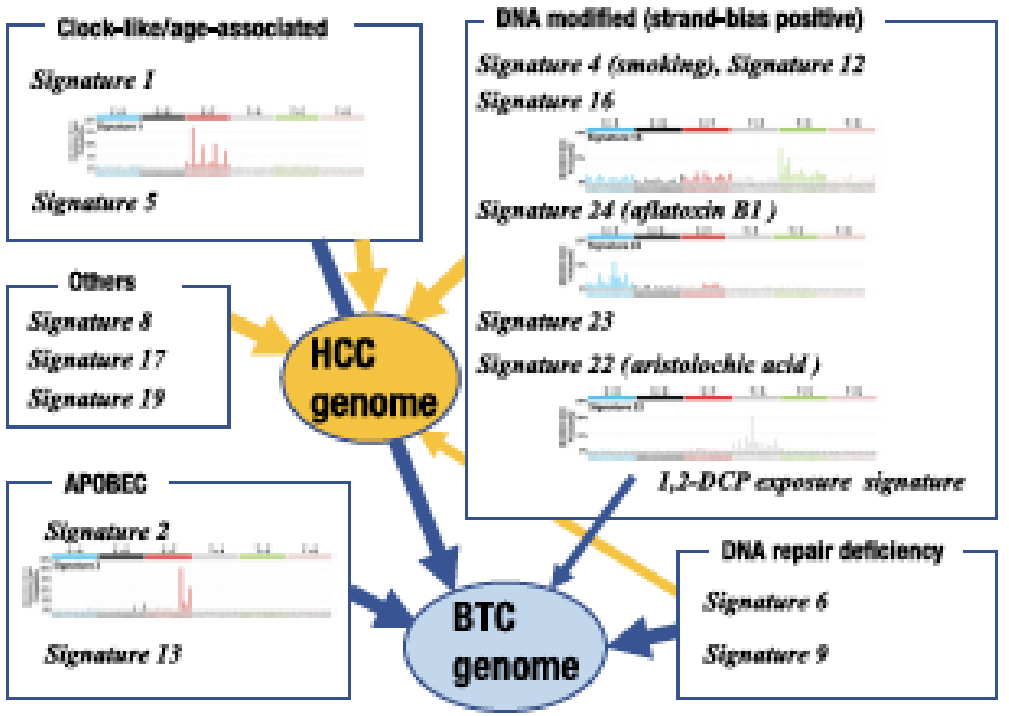
Both tumor suppressors cooperatively inhibited STAT3 signaling and, thus, providing a molecular basis for inhibition of STAT3-mediated IL-6 signaling in HCC cancer



Ploeger et al. 10.1002/hep.28684

Hepato-Biliary cancer: Landscape of Major Driver Genes

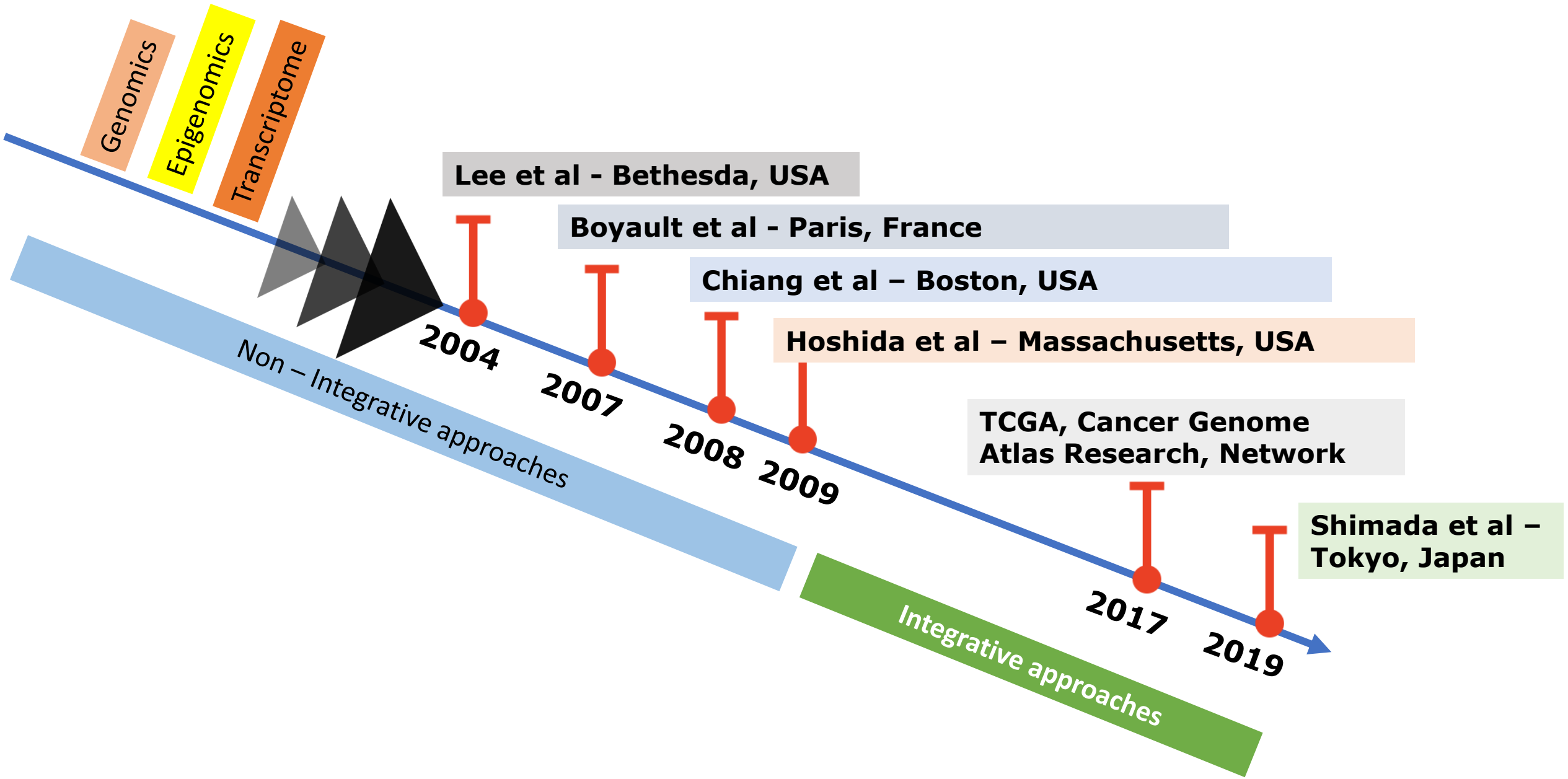




Integrating genome data (mutation, copy number, fusion gene, and mutational signature) with transcriptome, epigenome, proteome, and metabolome data will contribute to identifying unique molecular subtypes in cancer.

Shibata et al. DOI: 10.1111/cas.13582

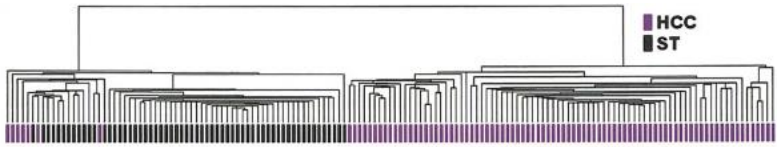
MOLECULAR CLASSIFICATION OF HCC



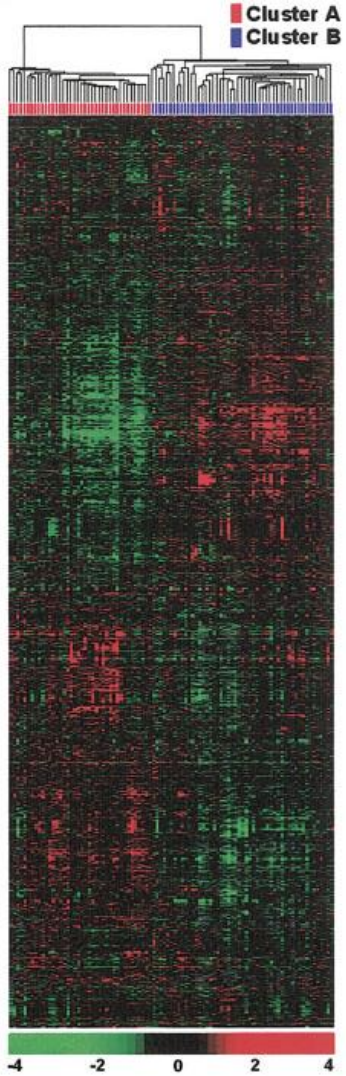
MOLECULAR CLASSIFICATION OF HCC

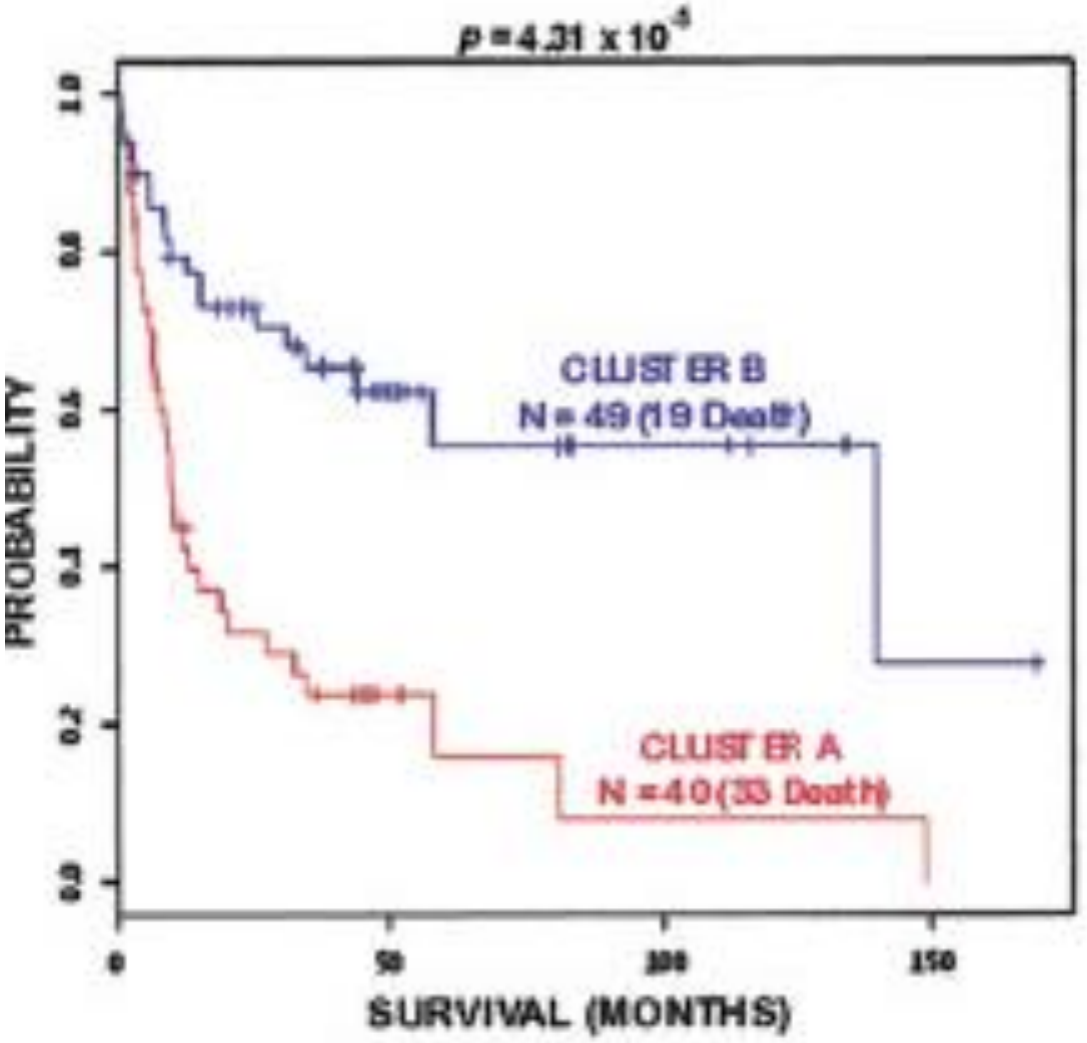
Lee Classification

Gene expression profiles in 91 human primary HCC and 60 matched nontumor surrounding tissues (STs) using DNA microarrays was characterized.



Genes with an expression ratio that has at least a twofold difference relative to the reference in at least 9 tumors were selected for hierarchical analysis (4,187 gene features).



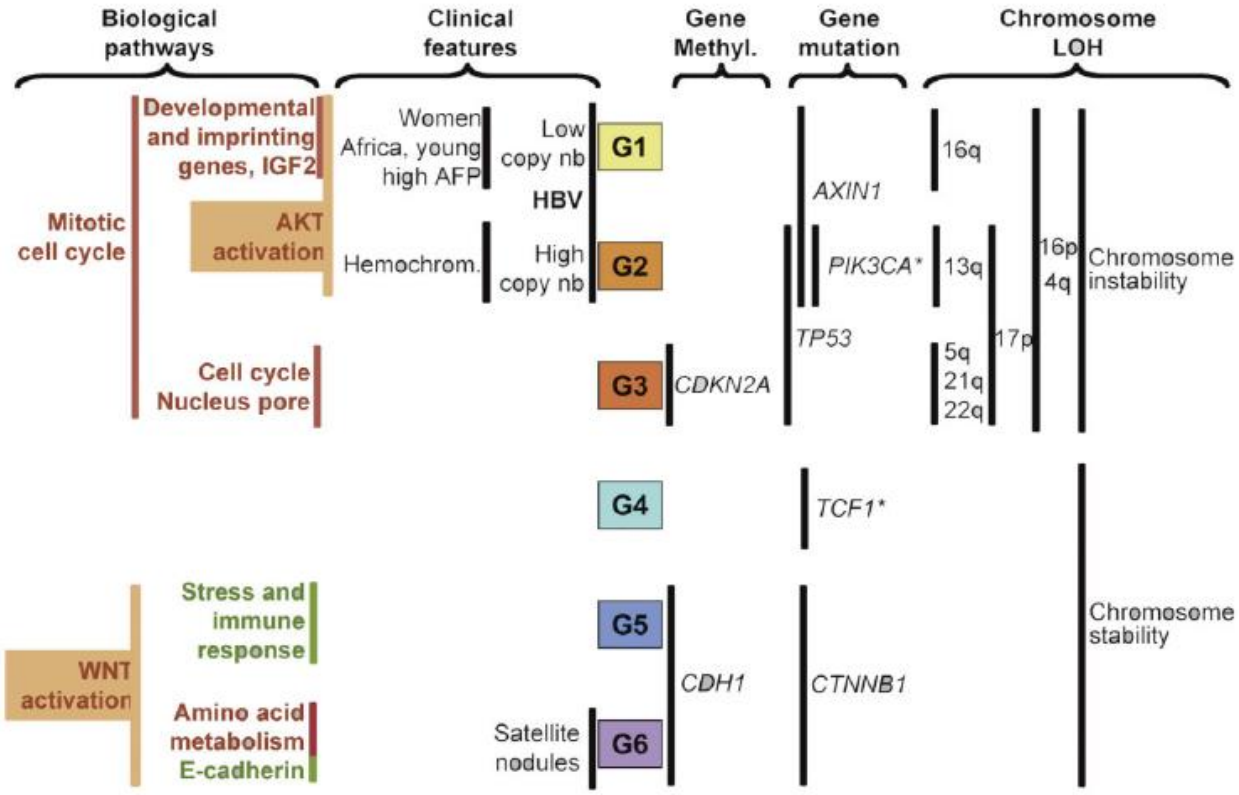


Lee et al - Bethesda, USA DOI 10.1002/hep.20375

MOLECULAR CLASSIFICATION OF HCC

Boyault Classification

- Schematization of the different HCC subgroups defined by transcriptome analysis with their related clinical and genetic pathways.
- G1 to G6 are the subgroups of HCCs defined by transcriptome analysis. Vertical lines indicate significantly associated features.
- Red and green primarily indicate over and under expressed genes, respectively, in that particular functional category.

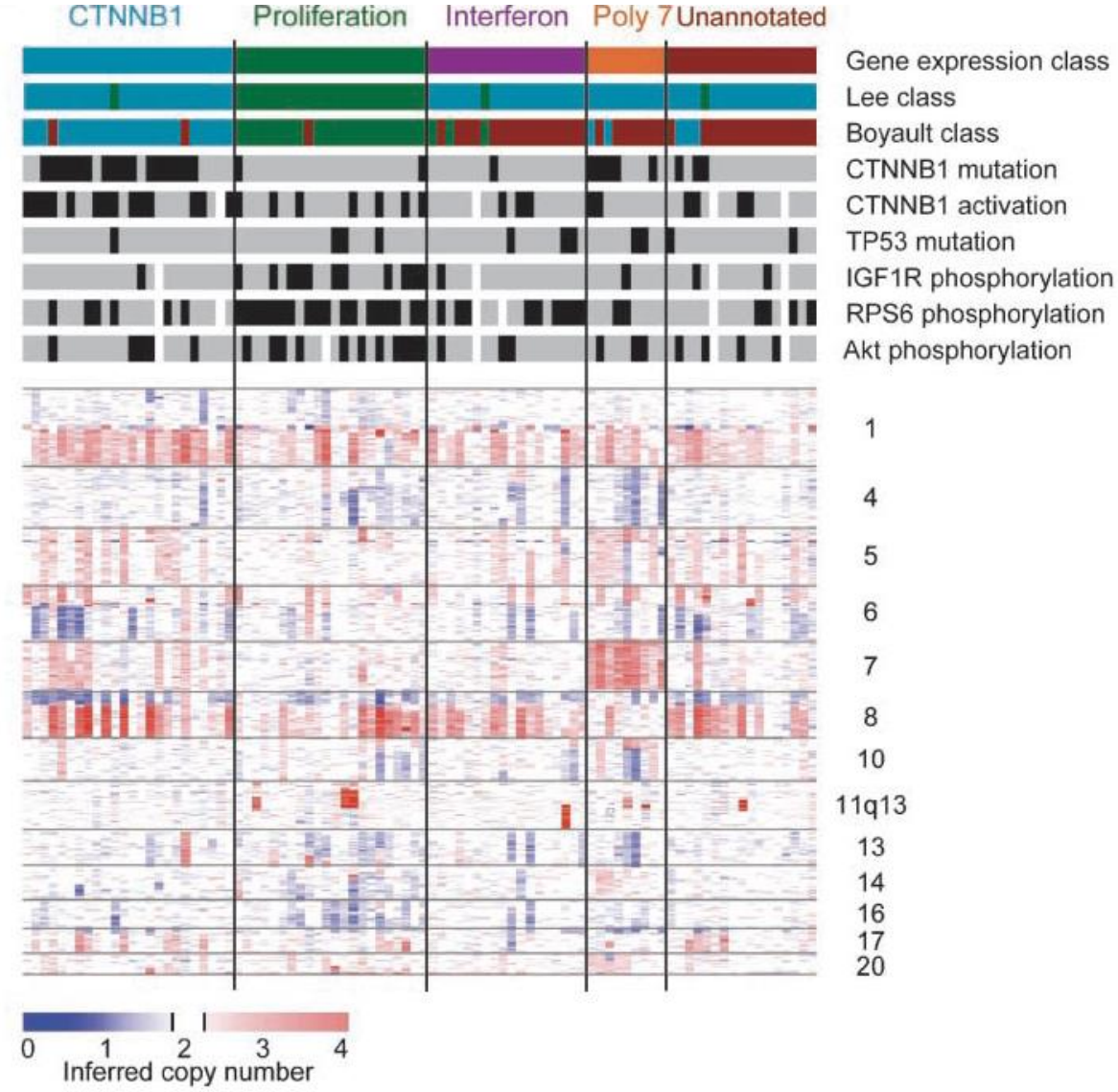


Boyault et al - Paris, France, DOI 10.1002/hep.21467

MOLECULAR CLASSIFICATION OF HCC

Chiang et al – Boston, USA

- To characterize the molecular heterogeneity of hepatocellular carcinomas, gene expression profiles were measured in 91 tumors with oligonucleotide microarrays.
- Five gene expression classes were obtained from unsupervised classification with consensus hierarchical clustering, which considered 32 different parameter combinations

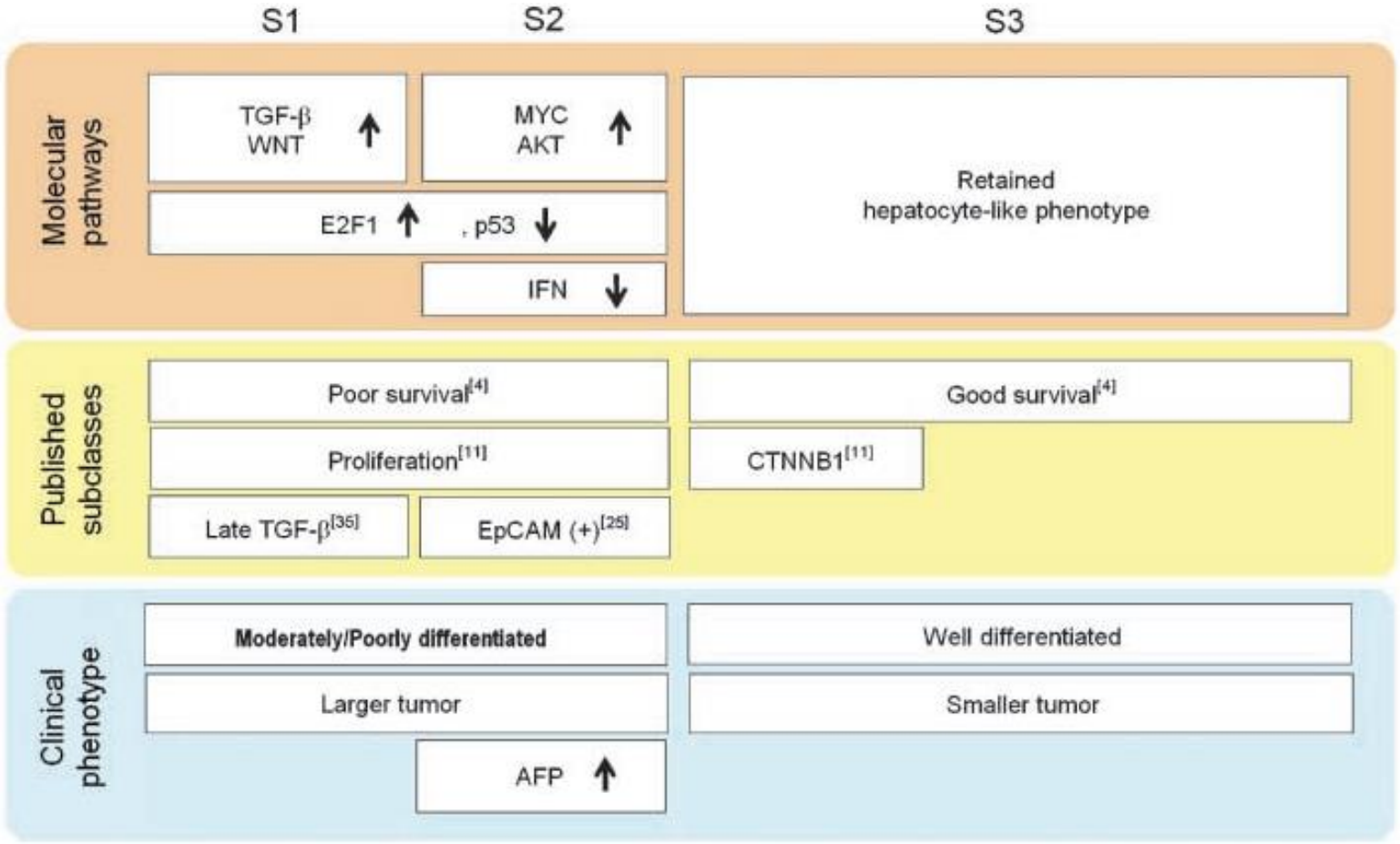


Chiang et al – Boston, USA , DOI: 10.1158/0008-5472.CAN-08-0742

MOLECULAR CLASSIFICATION OF HCC

Hoshida et al – Massachusetts, USA

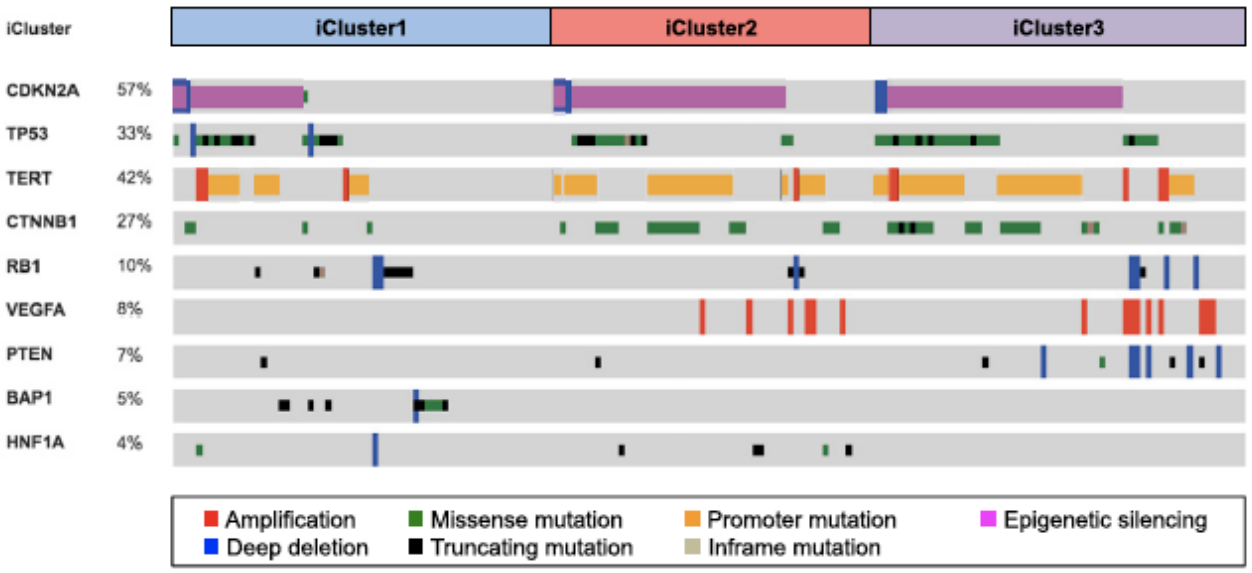
- *Three subclasses are detected with statistical significance*
- *These subclasses are associated with clinical parameters.*
- *Also these subclasses are associated with biological mechanism known to be operative in the pathogenesis of HCC.*

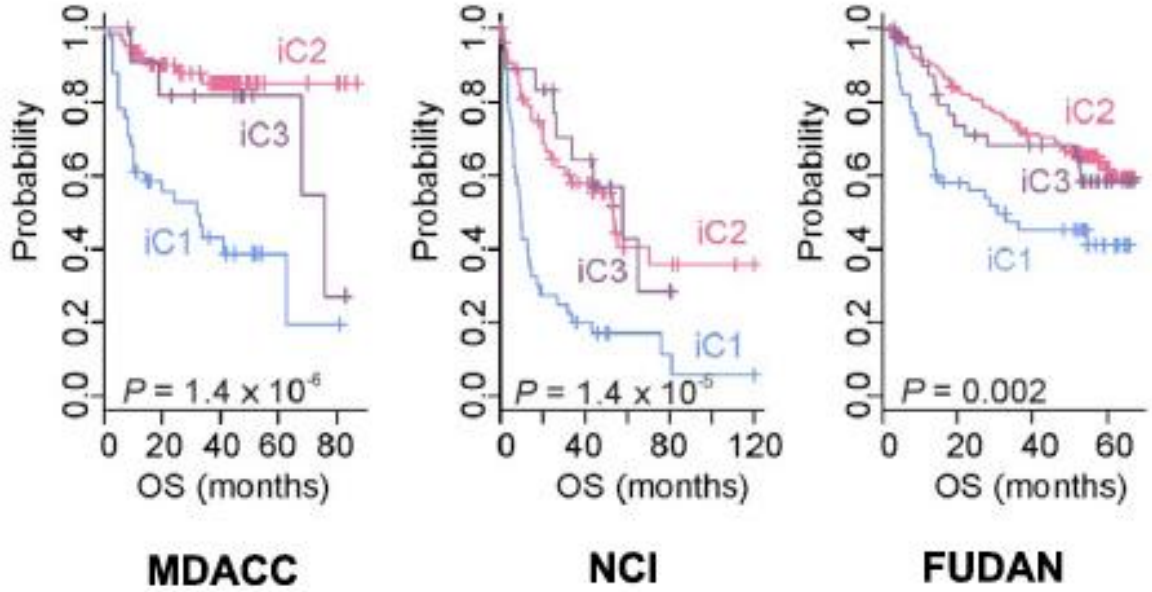


Hoshida et al – DOI: 10.1158/0008-5472.CAN-09-1089

MOLECULAR CLASSIFICATION OF HCC

TCGA, Cancer Genome Atlas Research, Network





Unsupervised clustering of data from five platforms (DNA copy number, DNA methylation, mRNA expression, miRNA expression and RPPA) resulted in a collection of discordant subgroupings specific to each data platform. To reconcile these disparate data types, author used a joint multivariate regression approach to simultaneously cluster data from the five platforms.

TCGA, Cancer Genome Atlas Research, Network, DOI: 10.1016/j.cell.2017.05.046

MOLECULAR CLASSIFICATION OF HCC

Shimada et al. Tokyo Medical and Dental University, Tokyo, Japan 2019

Several studies have challenged categorizing HCC by mutation, DNA methylation and expression profiles, but the links between the molecular and clinicopathological traits have not been fully unveiled.

Curative Resection of HCC, 2006-2013
Control: Adjacent Liver tissue of Colorectal Mets

938 Genes Selected

Integrative analysis for the TGCA study. 726 genes were matched to the 938 genes

For Genome Analysis 33 pairs of HCC and adjacent liver controls were selected

The whole exome sequencing was done

Gene set enrichment analysis and the aggregate score

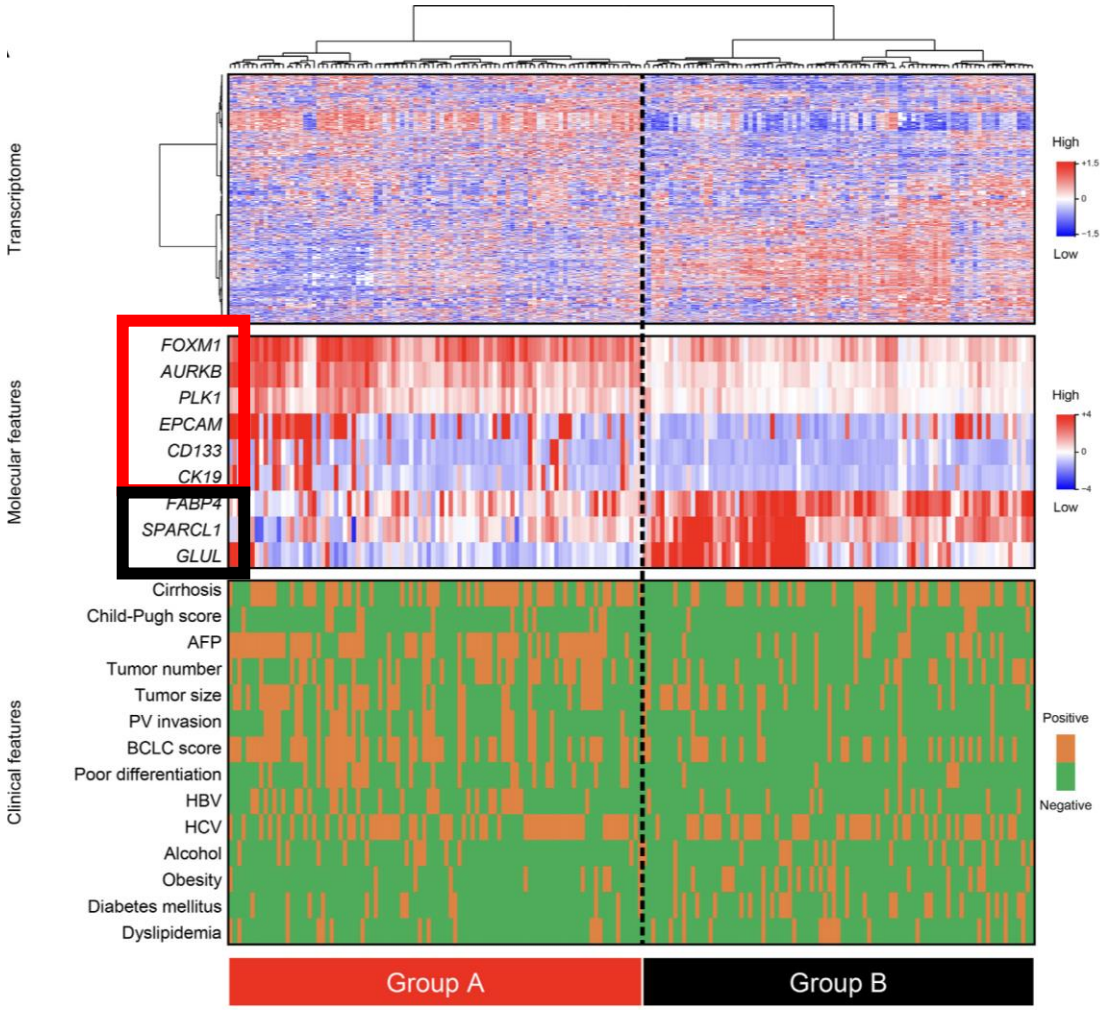
1. Hallmark gene sets
2. Chemical and genetics perturbations
3. Biological process
4. Immune-related gene sets

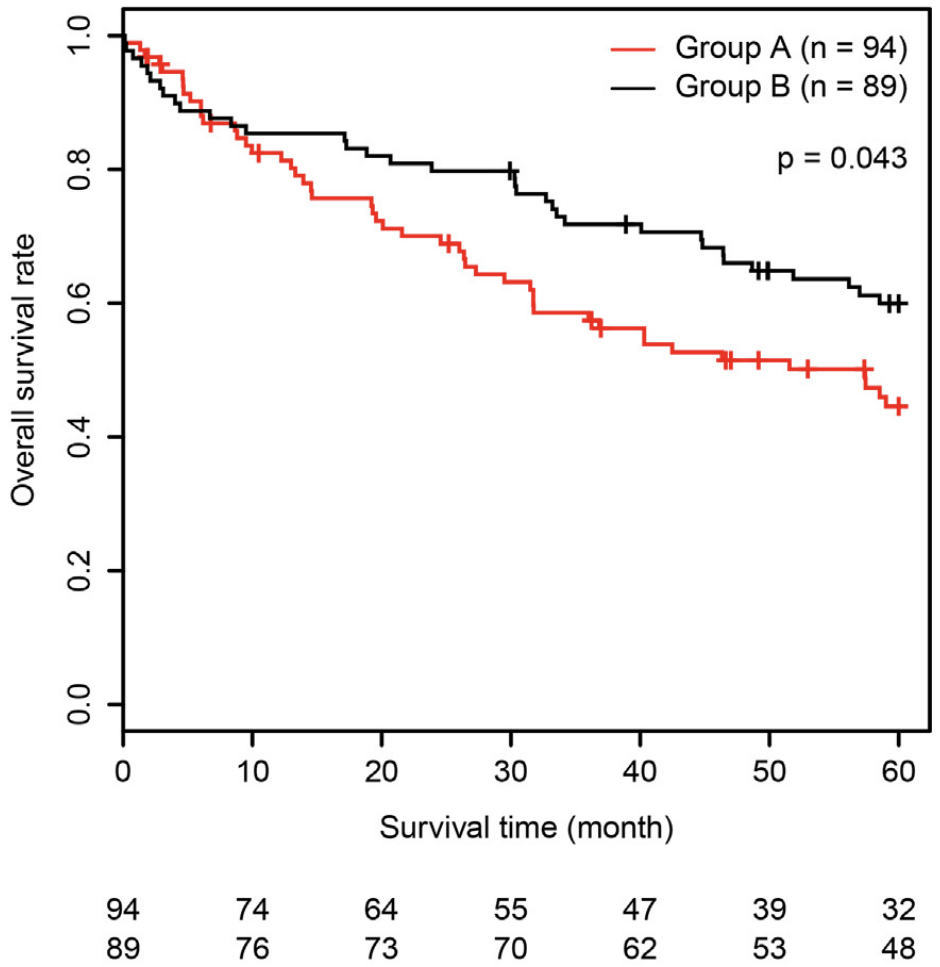
For Methylation Analysis 29 pairs of HCC and adjacent liver controls were selected

12 genes were extracted

Shimada et al, https://doi.org/10.1016/j.ebiom.2018.12.058

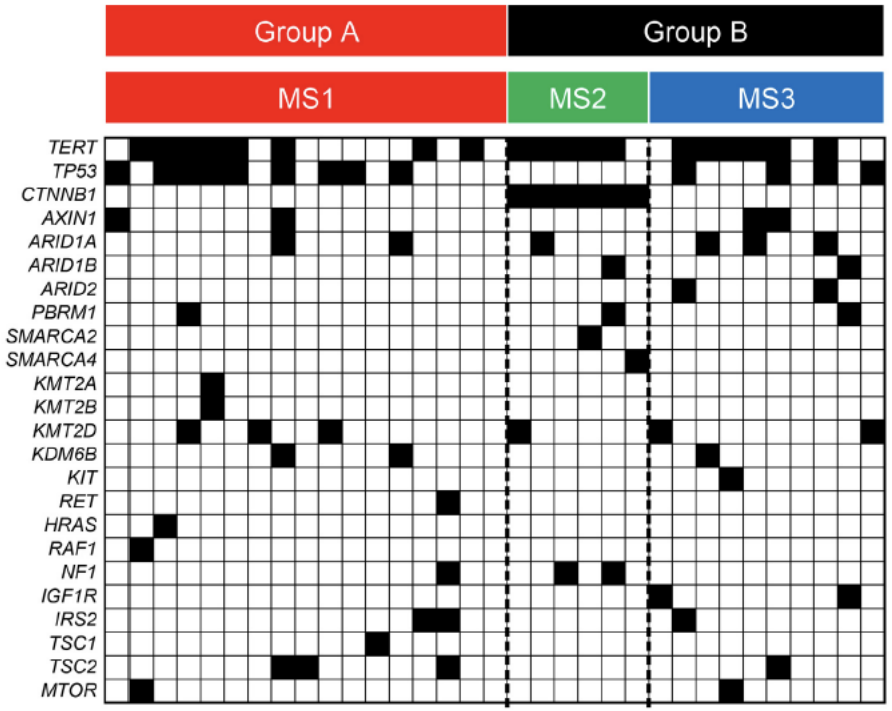
MOLECULAR CLASSIFICATION OF HCC

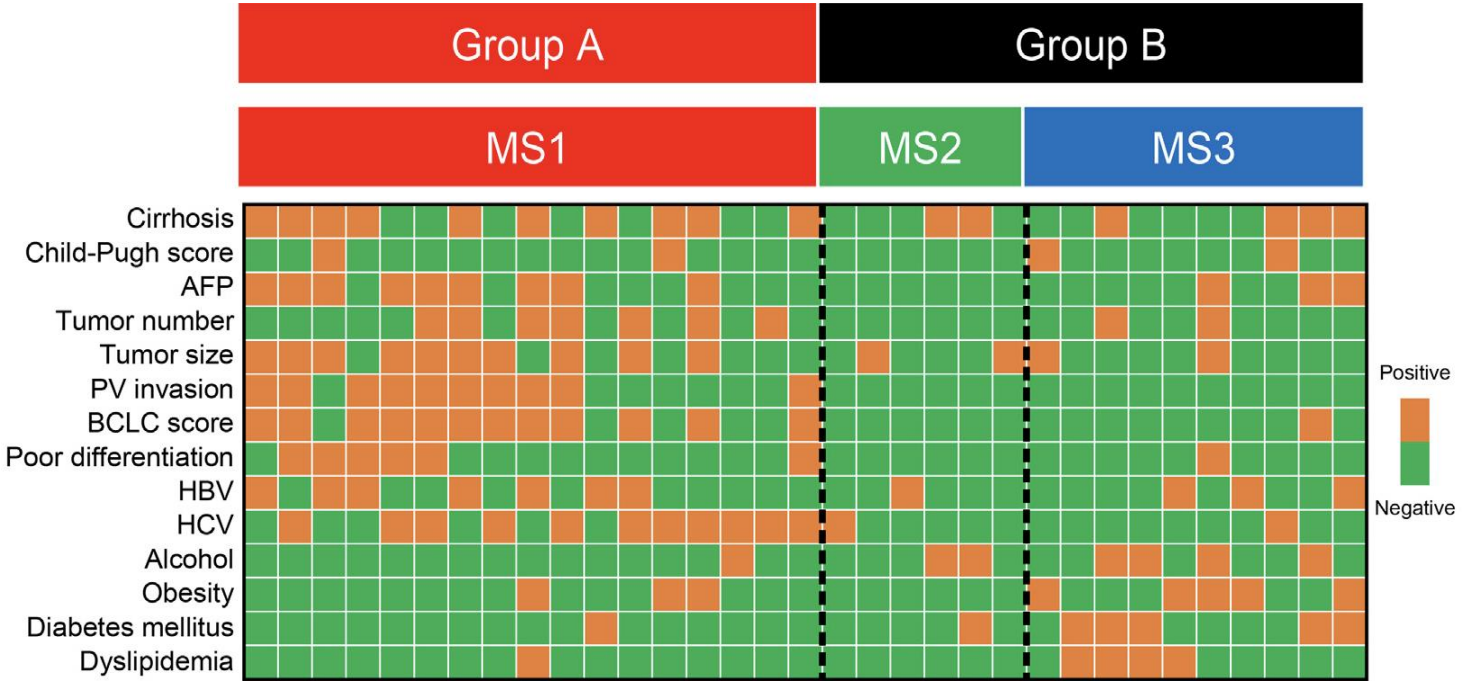




Shimada et al, https://doi.org/10.1016/j.ebiom.2018.12.058

MOLECULAR CLASSIFICATION OF HCC



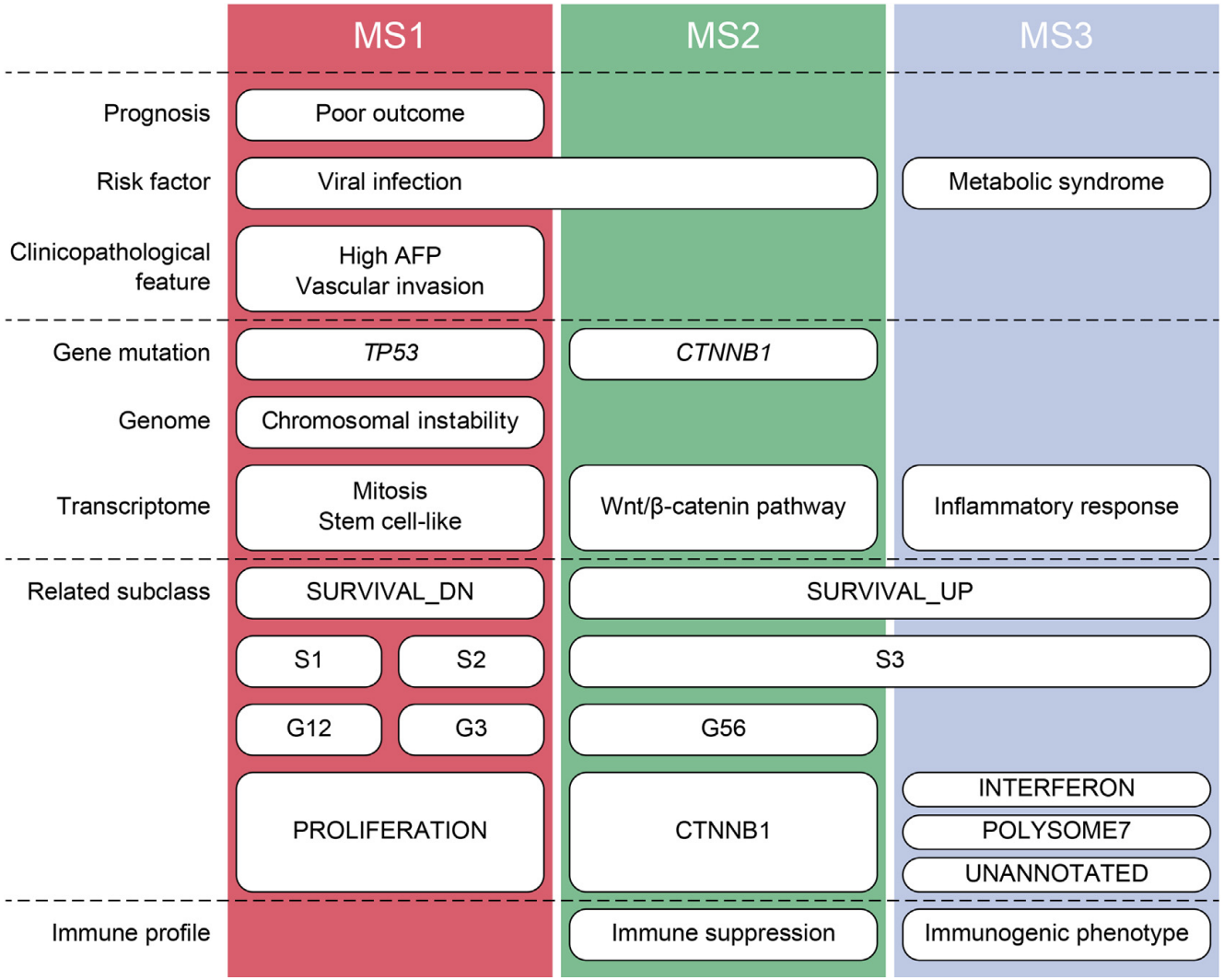


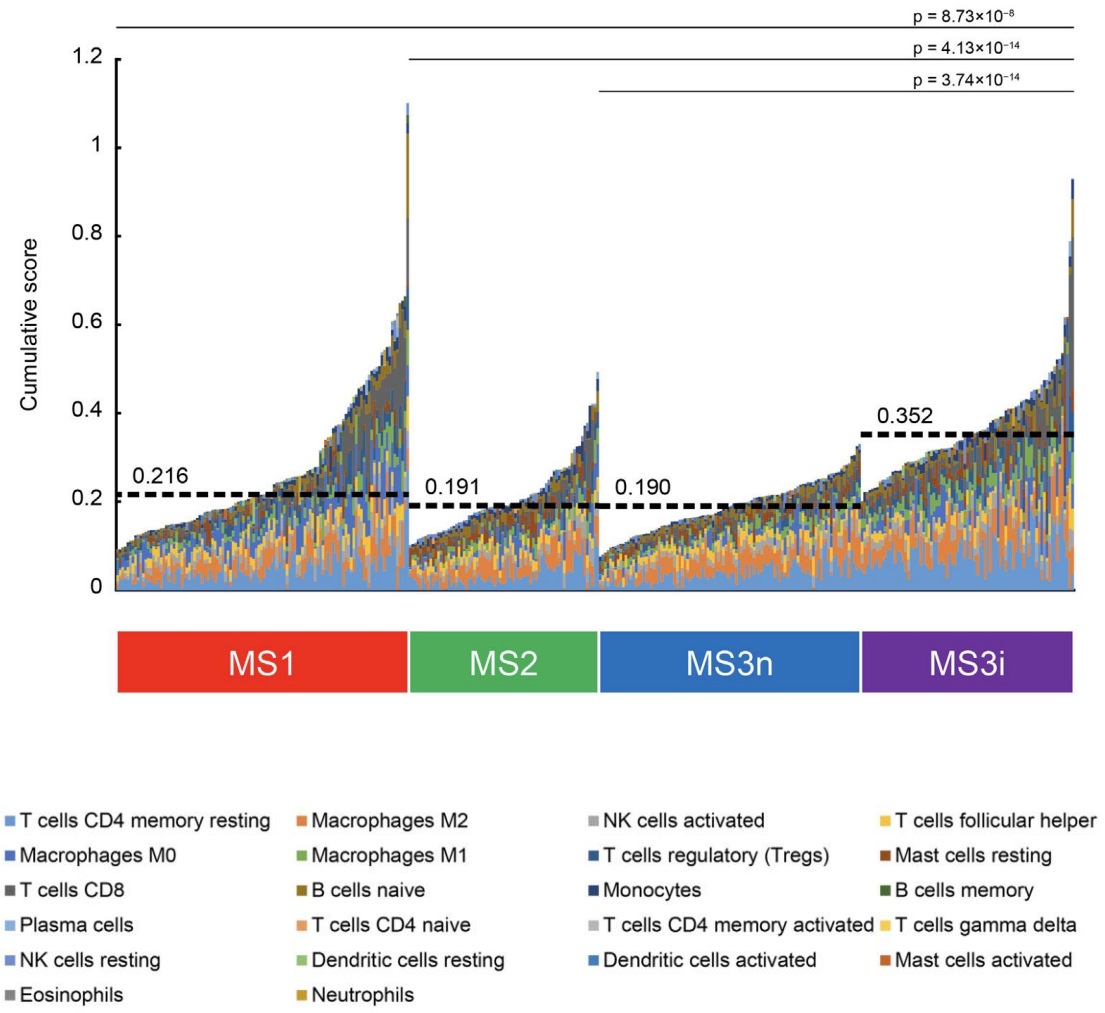
Authors highlighted somatic mutations of CTNNB1 observed only in Group B for two reasons; CTNNB1 ranked as one of the top genes differentially mutated between Group A and B; active mutation of CTNNB1 is frequently detected and well-known as a driver in HCC

So re-categorization was done into three molecular subtypes (MS); the MS1 was equal to Group A, and the MS2 andMS3 were Group B with or without CTNNB1mutations, respectively.

Shimada et al, https://doi.org/10.1016/j.ebiom.2018.12.058

MOLECULAR CLASSIFICATION OF HCC





Shimada et al, https://doi.org/10.1016/j.ebiom.2018.12.058

MOLECULAR CLASSIFICATION OF HCC

Summary

- **Proliferative Subtype (MS1)**
- **Non-Proliferative Subtype (MS2 with CTNB1 mutation and MS3)**
- **Immune signature discovered significant accumulation of HCC with enhanced inflammatory response in the MS3, and further divided this subtype into immunogenic and non-immunogenic subclasses (MS3i and MS3n), resulting in favorable prognosis of MS3i.**
- **TheMS3, the non-proliferative subtype without CTNNB1 mutation, was intimately linked with metabolic risk factors such as diabetes and obesity.**

MOLECULAR TARGETS - HCC

NCCN Guidelines Version 2.2022, Hepatocellular Carcinoma

PRINCIPLES OF SYSTEMIC THERAPY

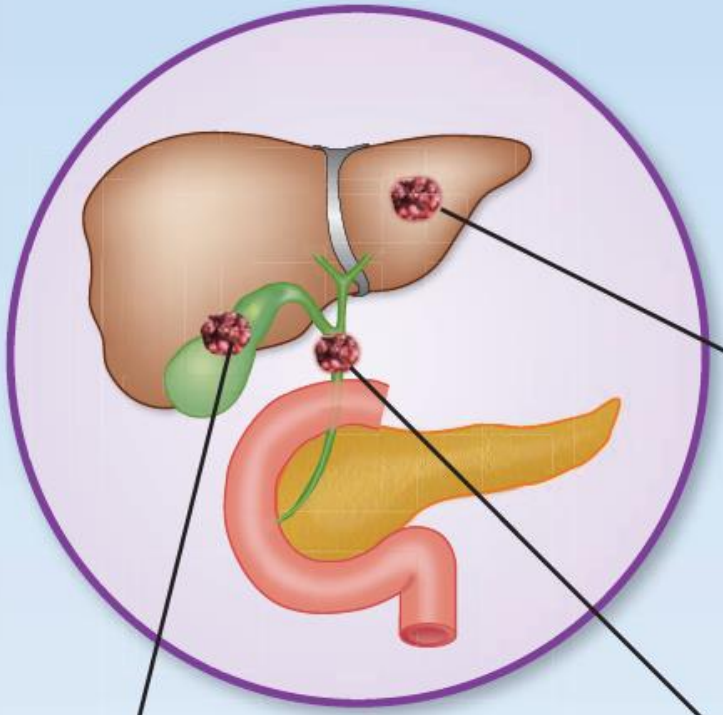
First-Line Systemic Therapy

Preferred Regimens	Other Recommended Regimens	Useful in Certain Circumstances
• Atezolizumab + bevacizumab (Child-Pugh Class A only) (category 1)[a,b,c,1]	• Sorafenib (Child-Pugh Class A) ([category 1] or B7)[d,e,2,3] • Lenvatinib (Child-Pugh Class A only)[4,5] (category 1)[6] • Durvalumab[6] • Pembrolizumab[7] (category 2B)	• Nivolumab[b,8] (if ineligible for tyrosine kinase inhibitors [TKIs] or other anti-angiogenic agents) (Child-Pugh Class A or B) (category 2B)

Subsequent-Line Therapy[f] if Disease Progression[g]

Options	Other Recommended Regimens	Useful in Certain Circumstances
• Regorafenib (Child-Pugh Class A only) (category 1)[h,9] • Cabozantinib (Child-Pugh Class A only) (category 1)[i,10] • Ramucirumab (AFP ≥400 ng/mL and Child-Pugh Class A only) (category 1)[i,11] • Lenvatinib (Child-Pugh Class A only) • Sorafenib (Child-Pugh Class A or B7)[d,e]	• Nivolumab + ipilimumab (Child-Pugh Class A only)[b,i,12] • Pembrolizumab (Child-Pugh Class A only) (category 2B)[b,j,k,13-15]	• Nivolumab (Child-Pugh Class B only)[b,j,16-19] (category 2B) • Dostarlimab-gxly[b,j,l,20,21] for MSI-H/dMMR tumors (category 2B)

Biliary Tract Cancer (BTC)



Biliary tract cancer

- >90% of cases are adenocarcinoma
- Level 1 evidence for adjuvant chemotherapy: capecitabine
- Palliative 1st-line chemotherapy: cisplatin/gemcitabine
- No 2nd-line palliative chemotherapy with a demonstrated survival benefit over active symptom control
- Median overall survival: ~12 months

Intrahepatic cholangiocarcinoma

- Risk factors: primary sclerosing cholangitis, cirrhosis, *Opisthorchis viverrini or Clonorchis sinensis*, obesity, diabetes, chronic hepatitis B and C, hepatolithiasis, Lynch syndrome, biliary papillomatosis, biliary duct morphologic anomalies
- Typically presents as incidental hepatic lesion(s)
- Radioembolization or radiation can be considered for liver-predominant disease

Gallbladder cancer

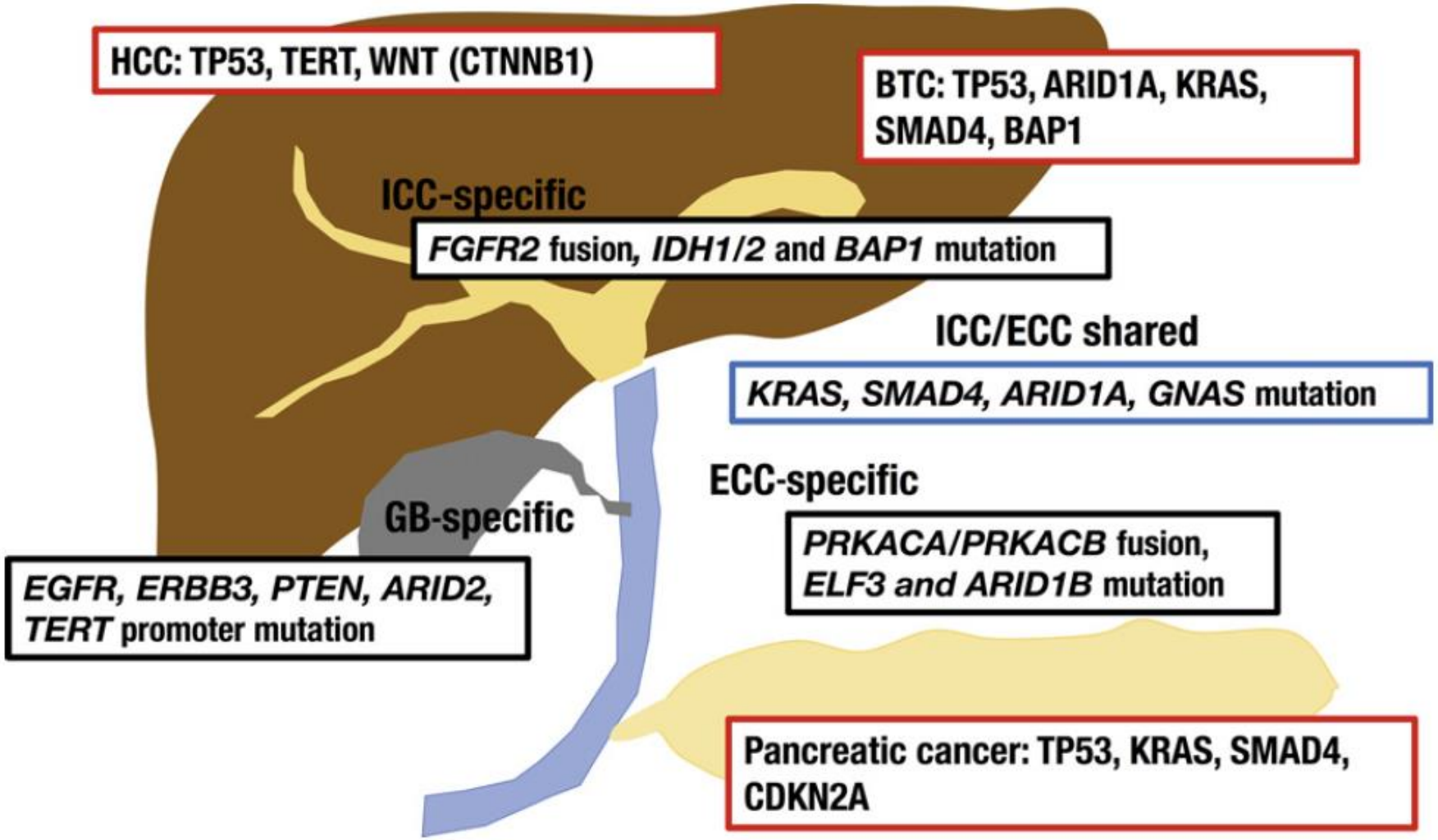
- Females > males
- Risk factors: gallstones, gallbladder polyps, chronic cholecystitis, *Salmonella typhi*, obesity, diabetes
- Typically presents as an incidental finding following cholecystectomy (localized stage) or with abdominal pain (advanced stage)

Extrahepatic cholangiocarcinoma

- Males > females
- Risk factors: primary sclerosing cholangitis, gallstones, Lynch syndrome, *Opisthorchis viverrini or Clonorchis sinensis,* bile duct morphologic anomalies
- Typically presents with obstructive jaundice

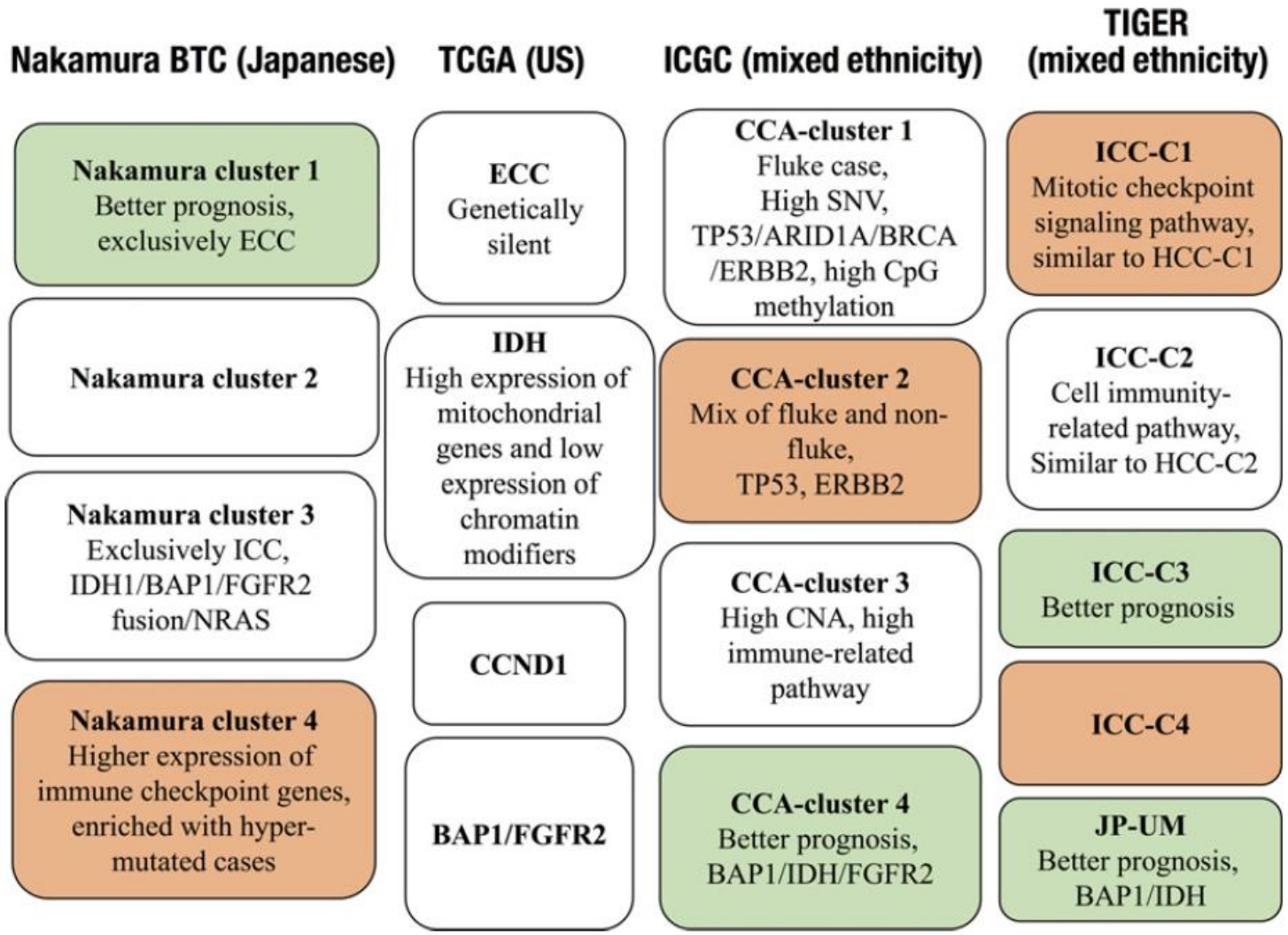
Valle et al 2017, Doi: 10.1158/2159-8290.CD-17-0245

MOLECULAR CLASSIFICATION OF BTC



Shibata et al, 2017 DOI: 10.1111/cas.13582

MOLECULAR CLASSIFICATION OF BTC

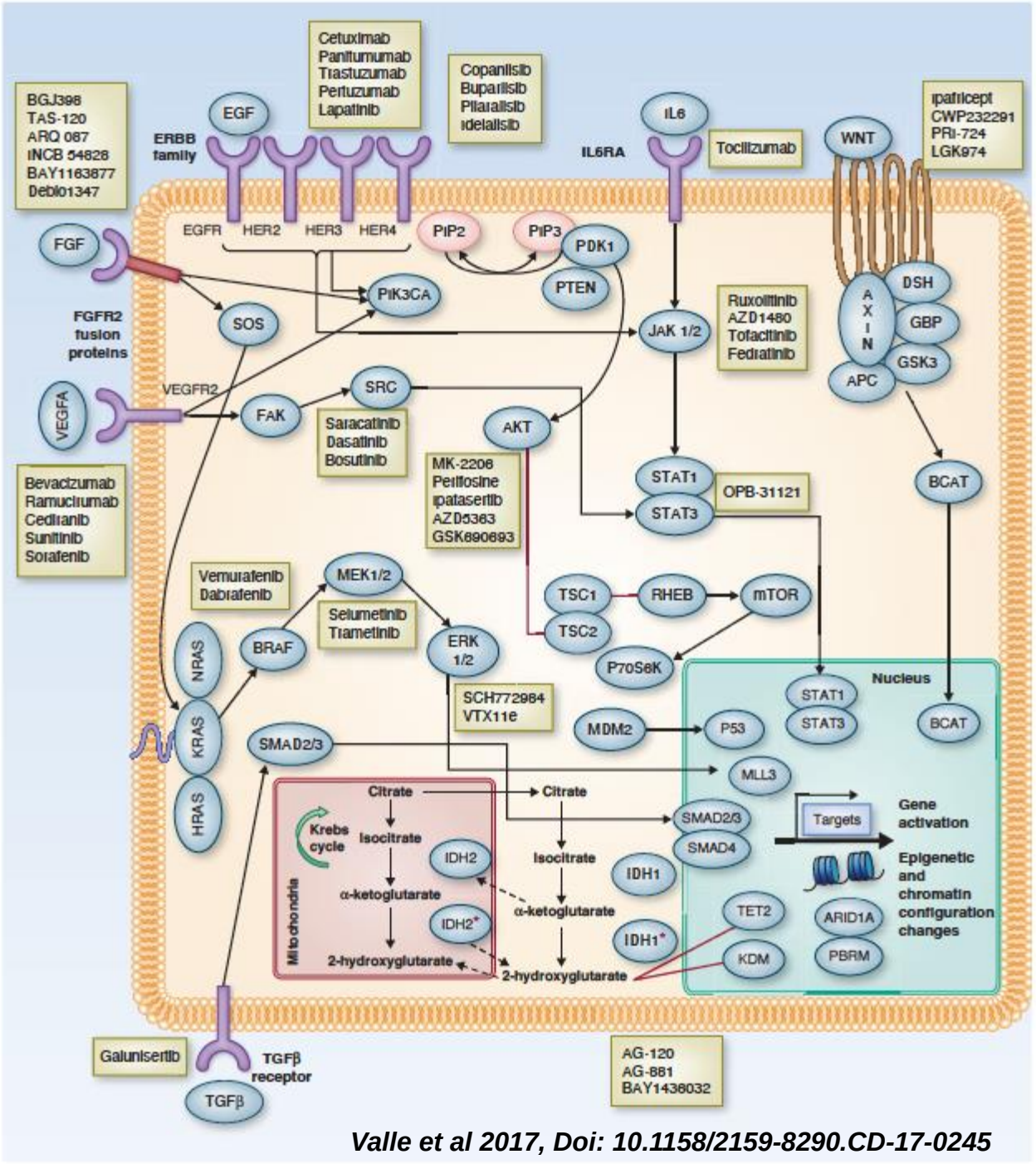


Shibata et al, 2017 DOI: 10.1111/cas.13582

MOLECULAR TARGETS - BTC

Useful in Certain Circumstances

- For *NTRK* gene fusion-positive tumors:
  - Entrectinib[6-8]
  - Larotrectinib[9]
- For MSI-H/dMMR tumors:
  - Pembrolizumab[e,f,h,10,11]
  - Dostarlimab-gxly[f,h,i,17,18] (category 2B)
- For TMB-H tumors:
  - Pembrolizumab[e,f,h,19]
- For *BRAF*-V600E mutated tumors
  - Dabrafenib + trametinib[20,21]
- For CCA with *FGFR2* fusions or rearrangements:
  - Pemigatinib[22]
  - Infigratinib[23]
- For CCA with *IDH1* mutations
  - Ivosidenib[24,25]
- For *RET* fusion-positive tumors:
  - Pralsetinib (category 2B)[12]
- For HER2-positive tumors:
  - Trastuzumab[j] + pertuzumab[26]
- Nivolumab[f,h,27] (category 2B)
- Lenvatinib + pembrolizumab[f,h,28] (category 2B)



NCCN Guidelines Version 2.2022, Biliary Tract Cancer

Valle et al 2017, Doi: 10.1158/2159-8290.CD-17-0245

CONCLUSION

- Although current signatures accurately classify HCCs according to their natural biology, they are unable to predict the response to currently used therapies.
- Based on the exciting results of recent studies and the advent of NGS technologies that offer unprecedented depths and resolution, it seems reasonable to predict that genomic technologies will play an increasingly important role in clinical oncology.
- The immediate focus undoubtedly will be on incorporating these whole-genomic technologies into clinical trials.