



Genomics in HCC

Implications for Diagnosis and Treatment

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Disclosures:

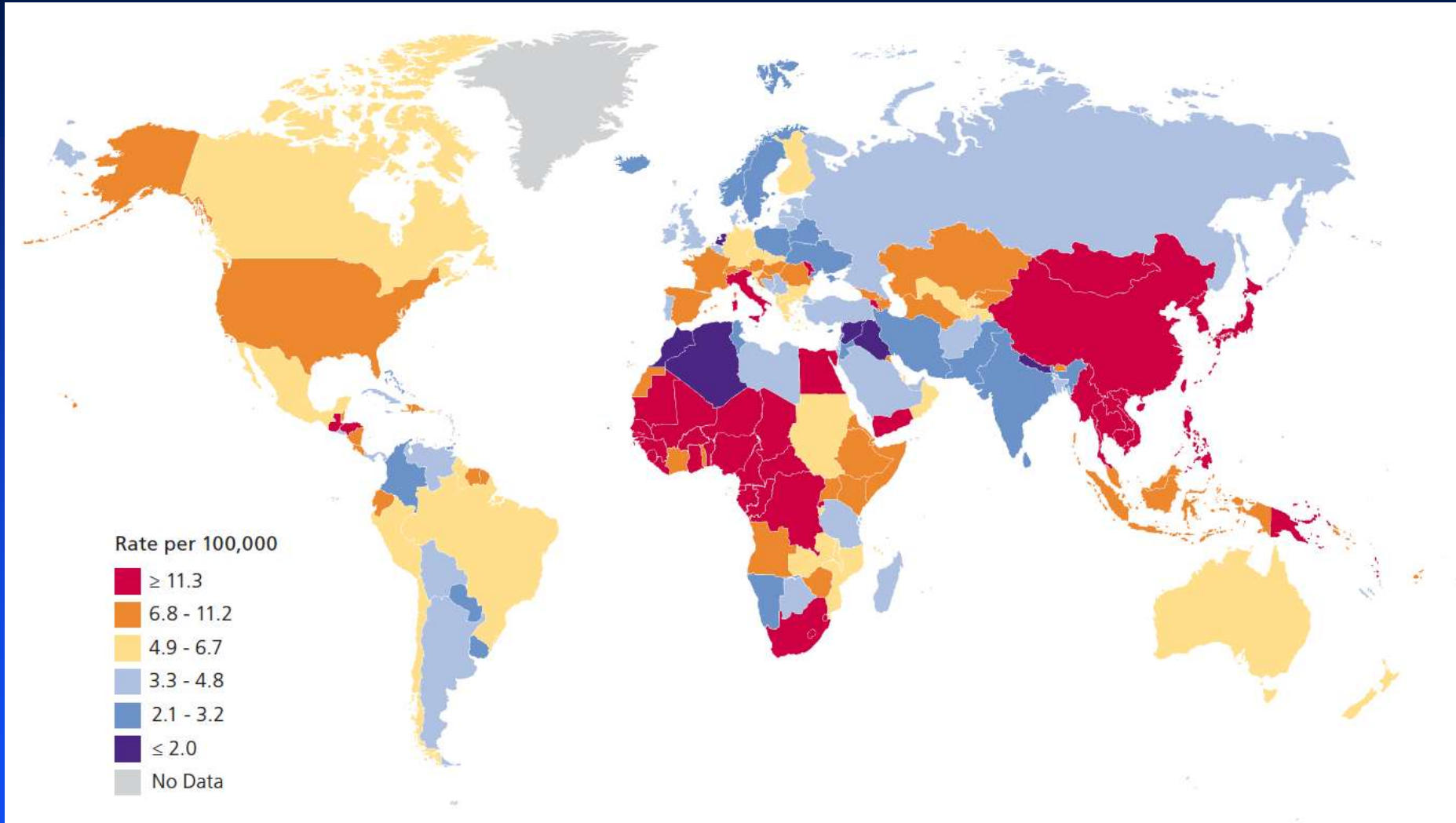
Gilead Sciences - Grant Support
Inova Diagnostics – Grant Support
Wako Diagnostics - Grant Support
BTG – Consulting & Grant Support

Overview

- **Global Epidemiology of HCC**
- **Cancer Hallmarks and Mechanisms – Exhausted Regeneration vs Oncogenic Etiology**
- **Cancer Heterogeneity – Observations and Pitfalls of Molecular Profiling**
- **New Insights from Genetic Testing – SNPs and Next Generation Sequencing**
- **Individualizing Therapy for HCC**
- **Keeping our Eyes on the Prize – Prevention, Early Diagnosis & Therapy**

HCC: The Global Challenge

6th most common cancer, 3rd most common cause of death from cancer



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Hallmarks of Cancer

Independence from Growth Factors

Limitless replication

Resistance to Apoptosis



Angiogenesis

Metastasis & Invasion

Evasion of Tumor Suppression

Expanded Hallmarks of Cancer

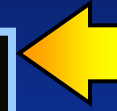
Independence from Growth Factors



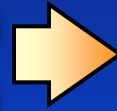
Limitless replication



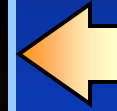
Resistance to Apoptosis



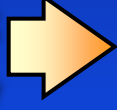
Deregulation of Metabolism



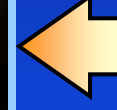
Immune Escape



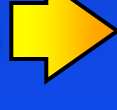
Genomic Instability



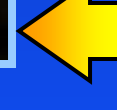
Inflammation



Angiogenesis



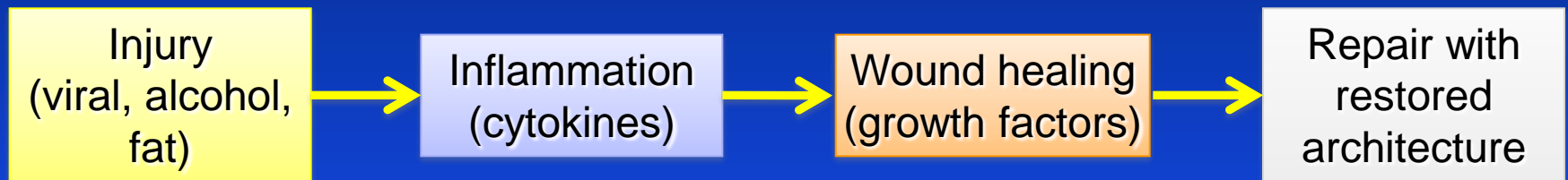
Metastasis & Invasion



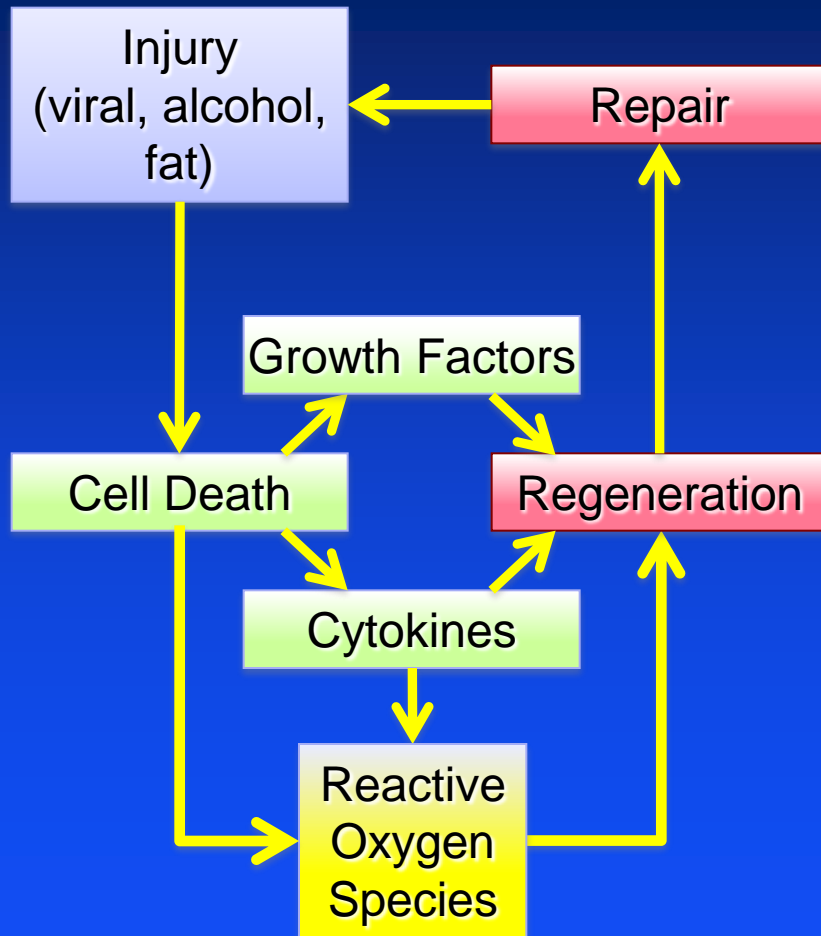
Evasion of Tumor Suppression



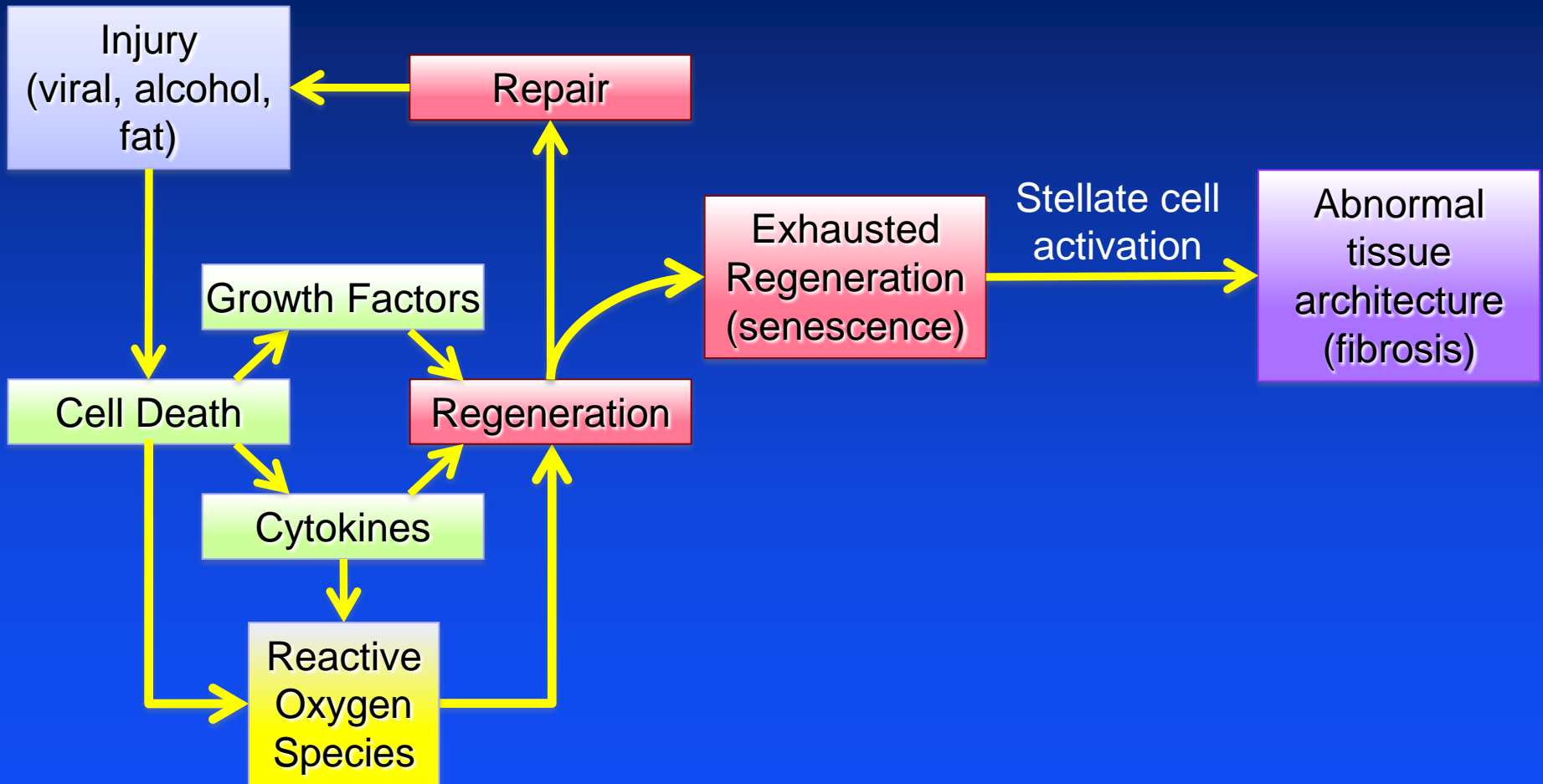
Acute injury induces inflammation and growth factors leading to restoration of normal tissue cytology and architecture



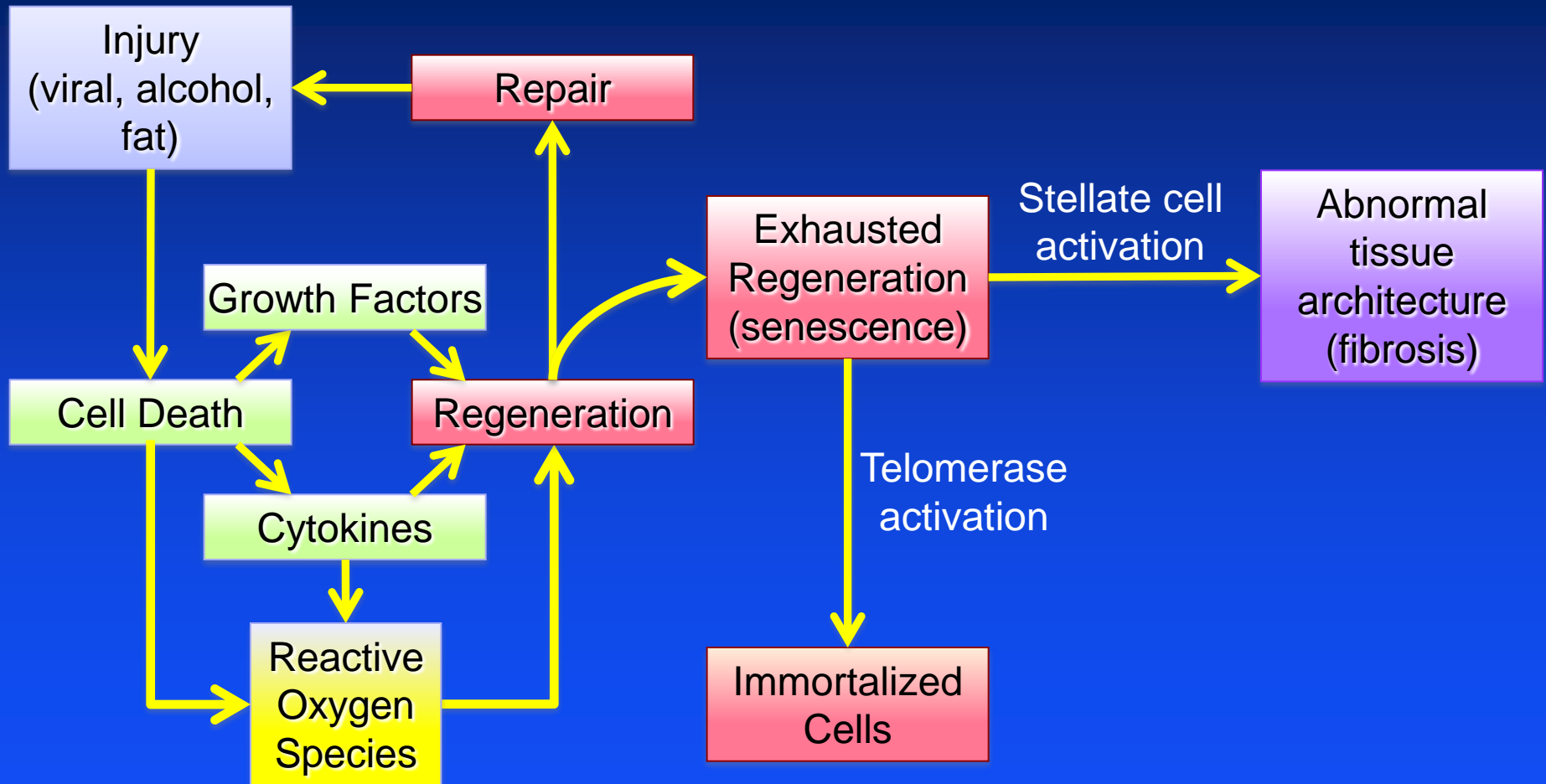
Generation of reactive oxygen species by cytokines is a key pathogenic mechanism



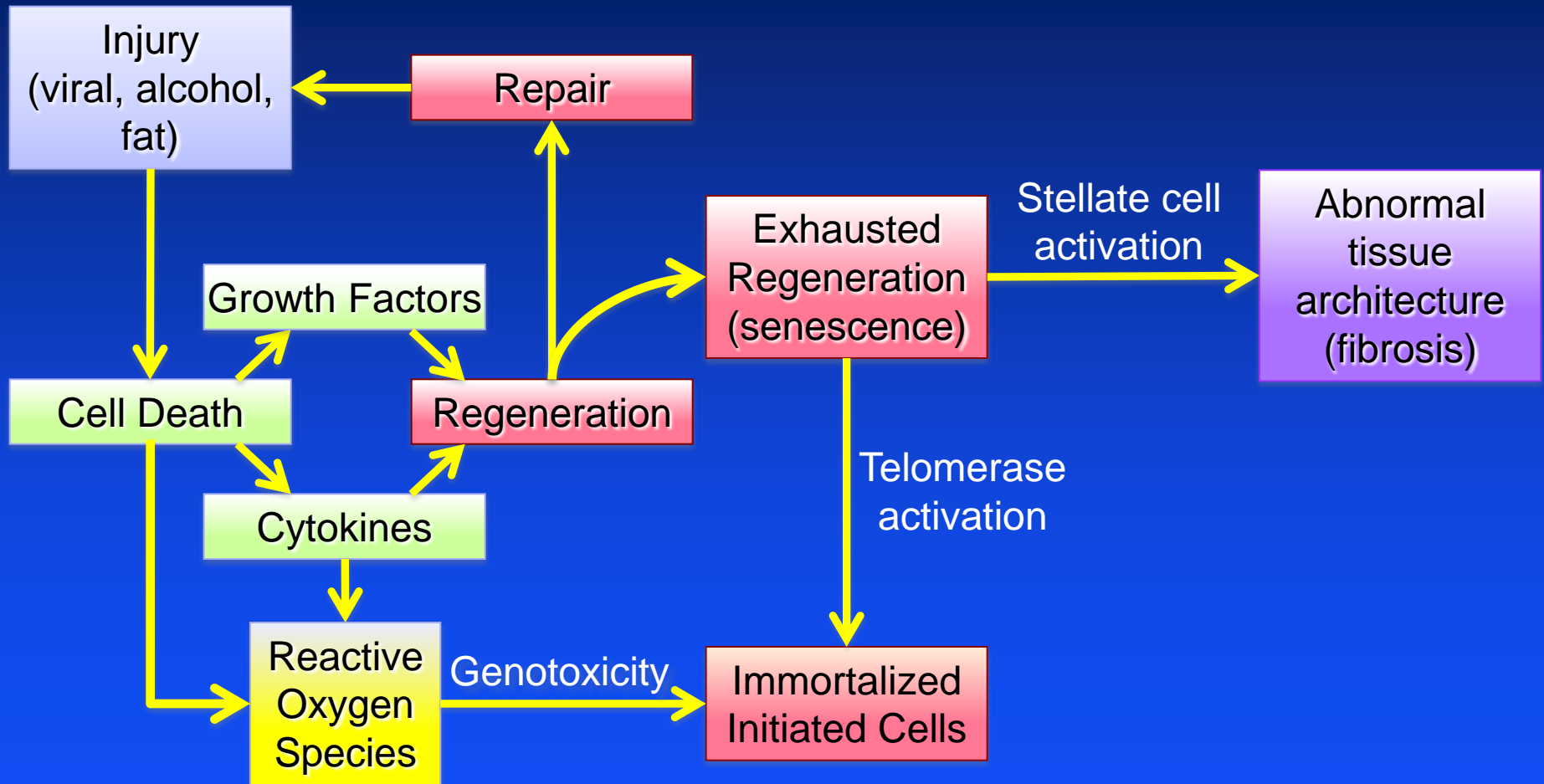
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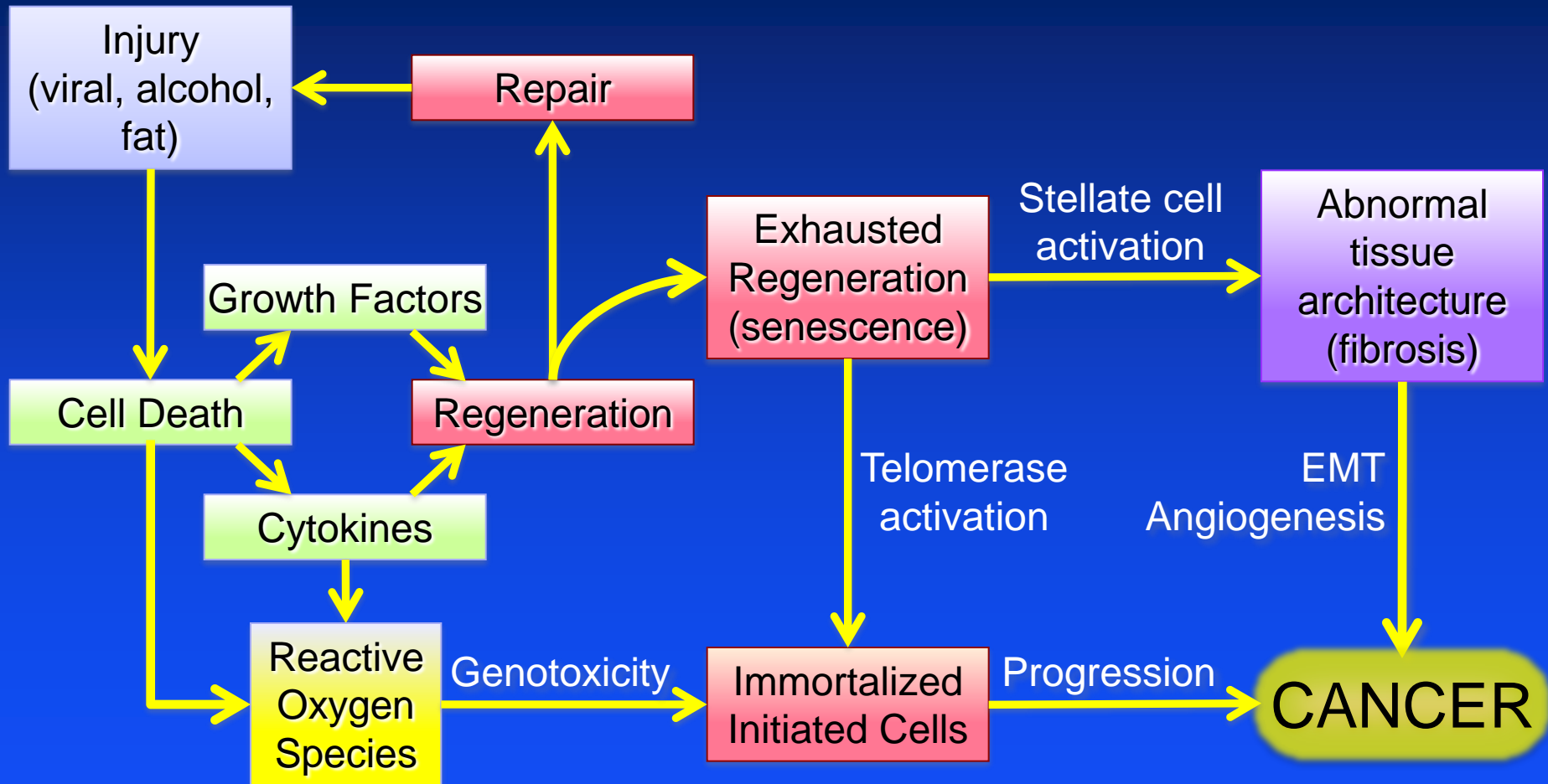
Generation of reactive oxygen species by cytokines is a key pathogenic mechanism



Generation of reactive oxygen species by cytokines is a key pathogenic mechanism

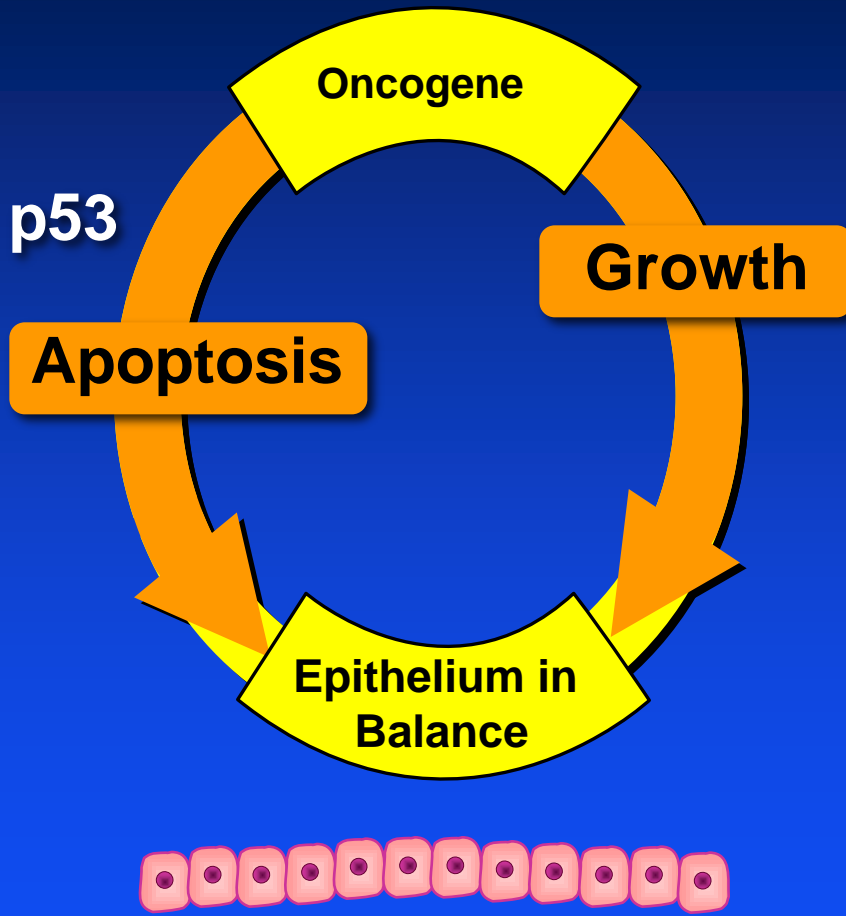


Generation of reactive oxygen species by cytokines is a key pathogenic mechanism

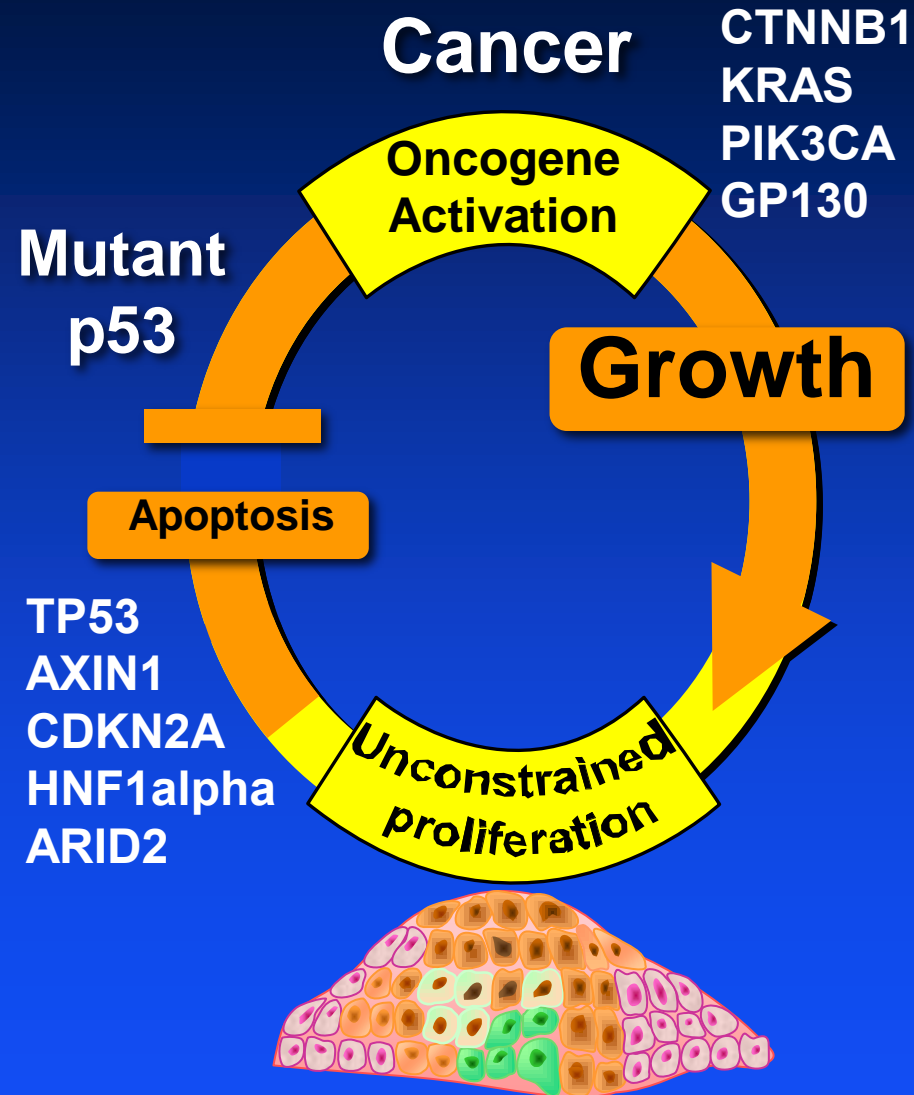


The Cancer Platform Concept

Homeostasis



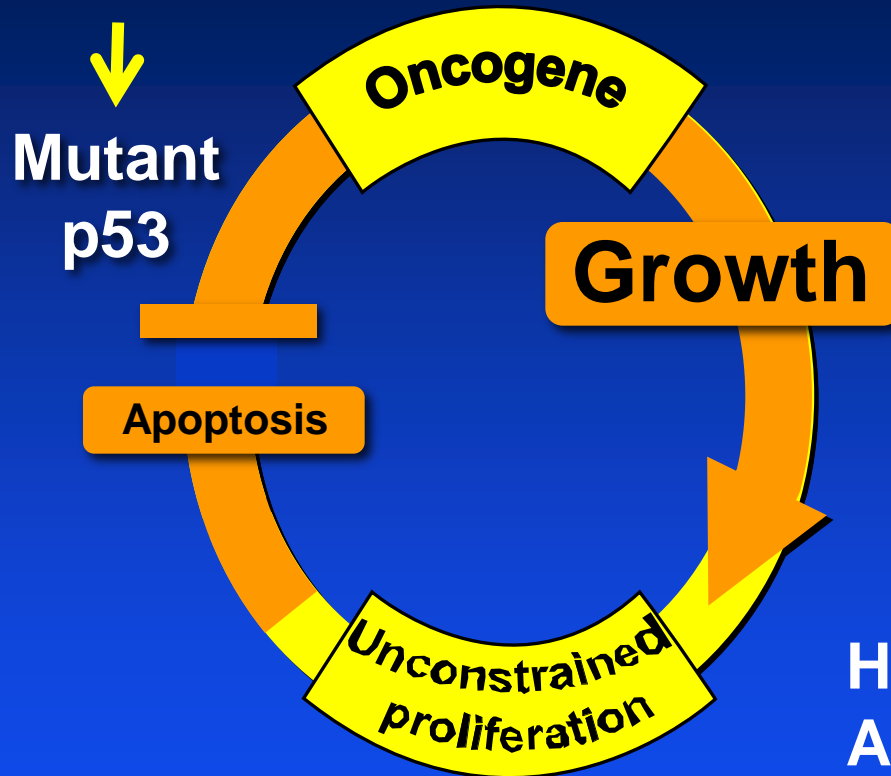
Cancer



The Effects of HBV and Aflatoxin in Liver Carcinogenesis are Synergistic

Aflatoxin

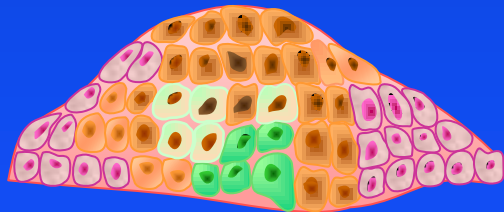
HBx



HBV RR: 7.3 (2.2, 24.4)

AFB1 RR: 3.4 (1.1-10.0)

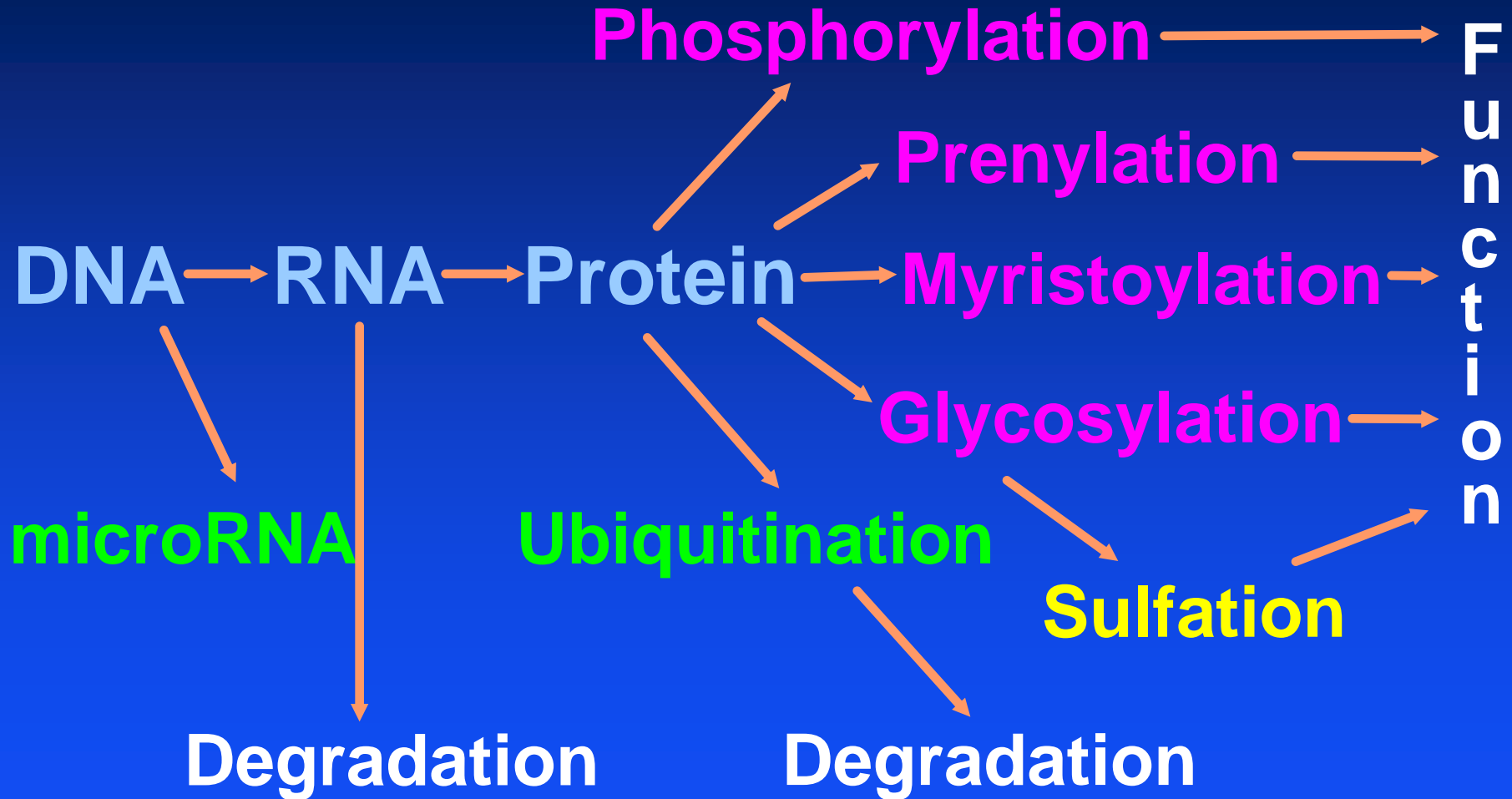
HBV & AFB1: 59.4 (15.6-212)



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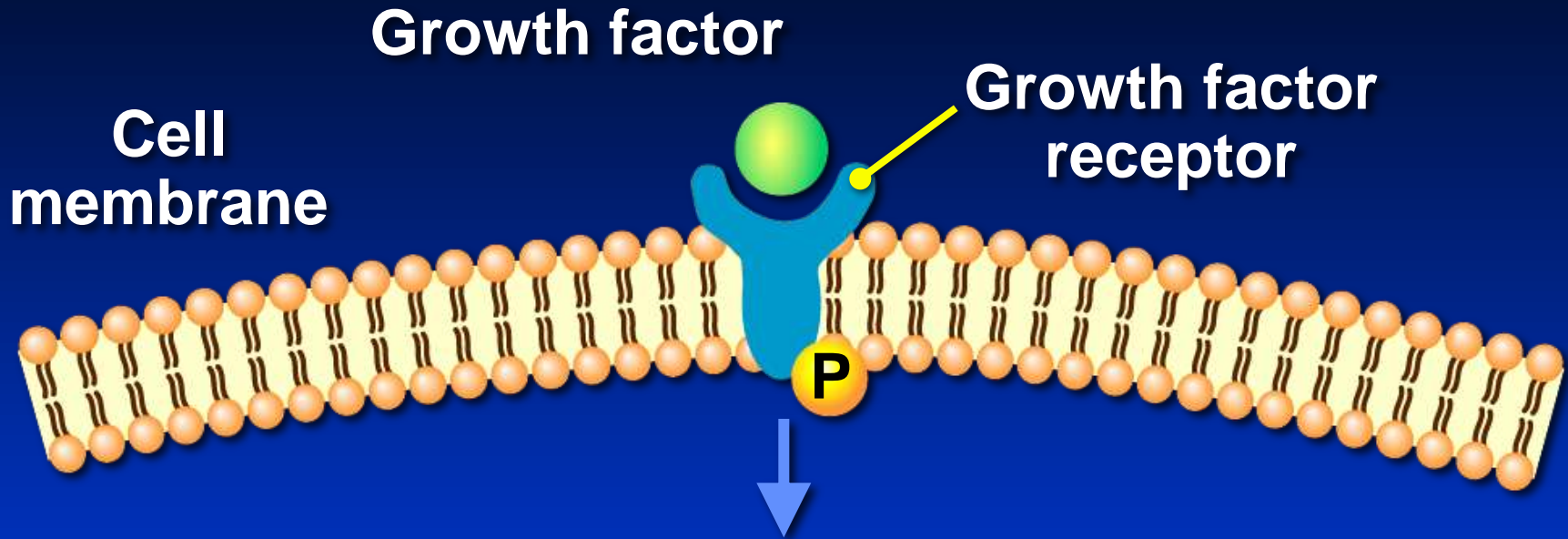
Profiling of Different Molecular Types in Hepatocellular Carcinoma



Molecular Signatures: Different Genetic and Molecular Types

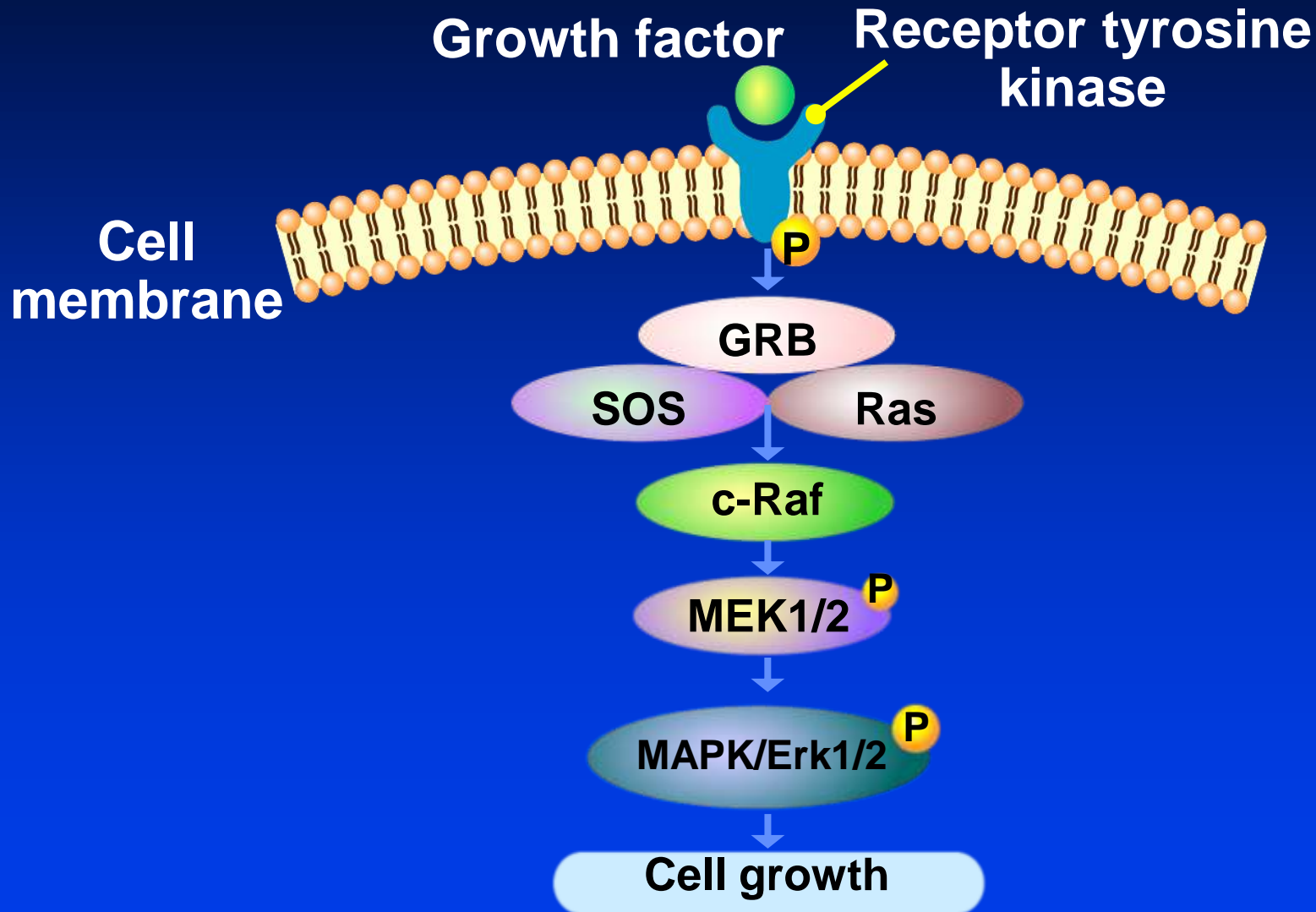
- Allelic imbalance/loss of heterozygosity
- Gene copy number
- Gene mutation
- Gene rearrangements
- Gene methylation
- Messenger RNA expression
- Non-coding RNA expression (miRNA etc)
- Proteomic
- Post translational - Phosphorylation
- Glycomic - N- or O-glycan, sulfation

Growth Factor Signaling

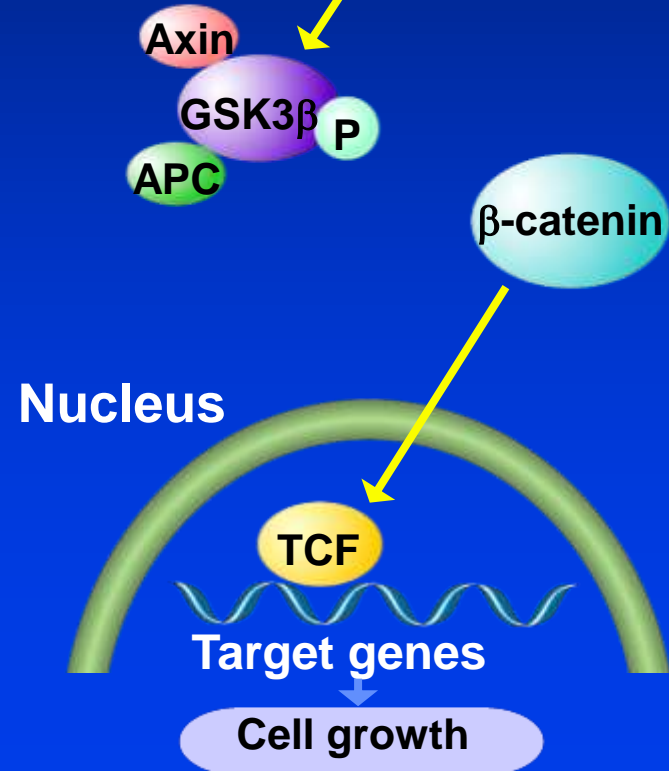
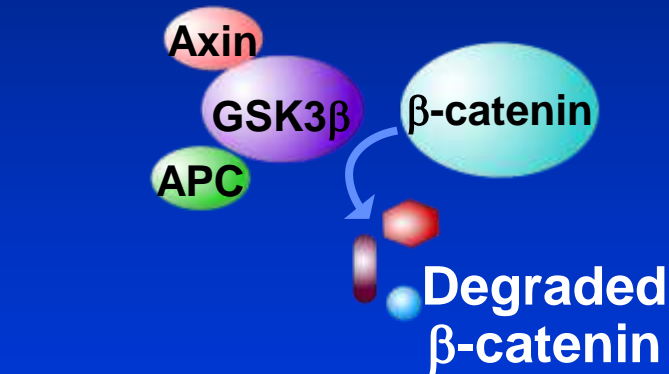
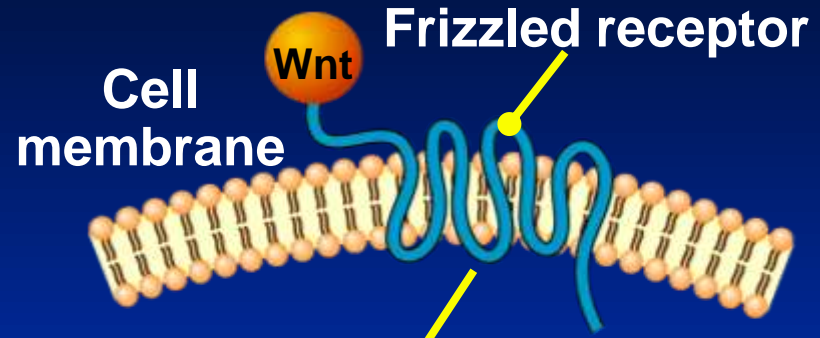
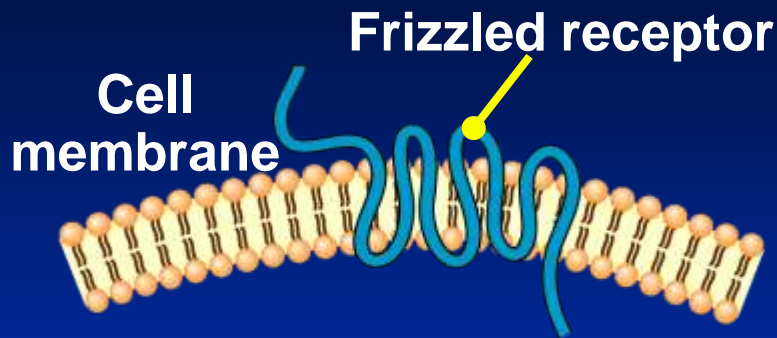


- EGFR
- FGFR
- HGFR (c-MET)
- PDGFR
- VEGFR
- IGFR

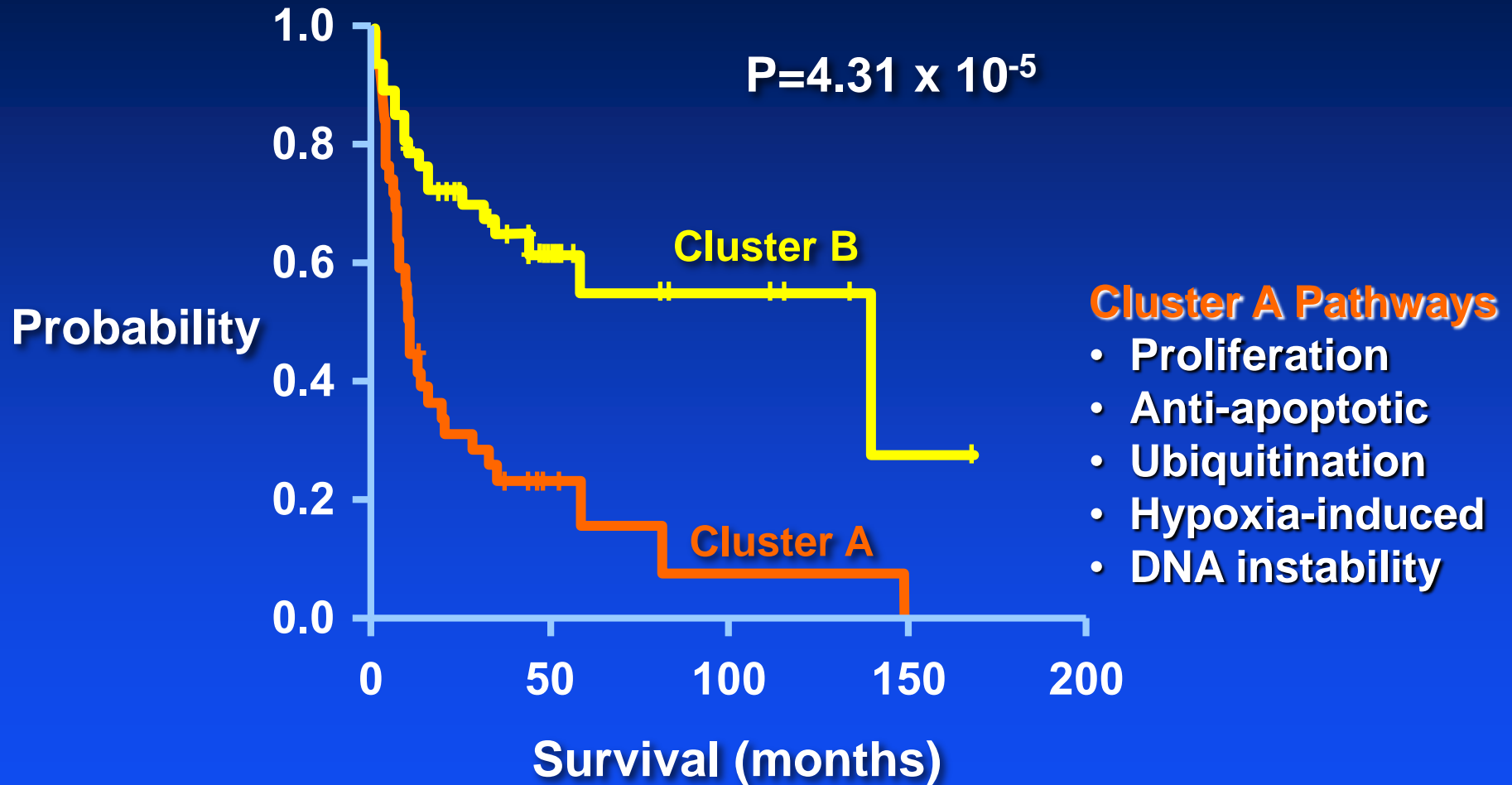
Growth Factor Signaling



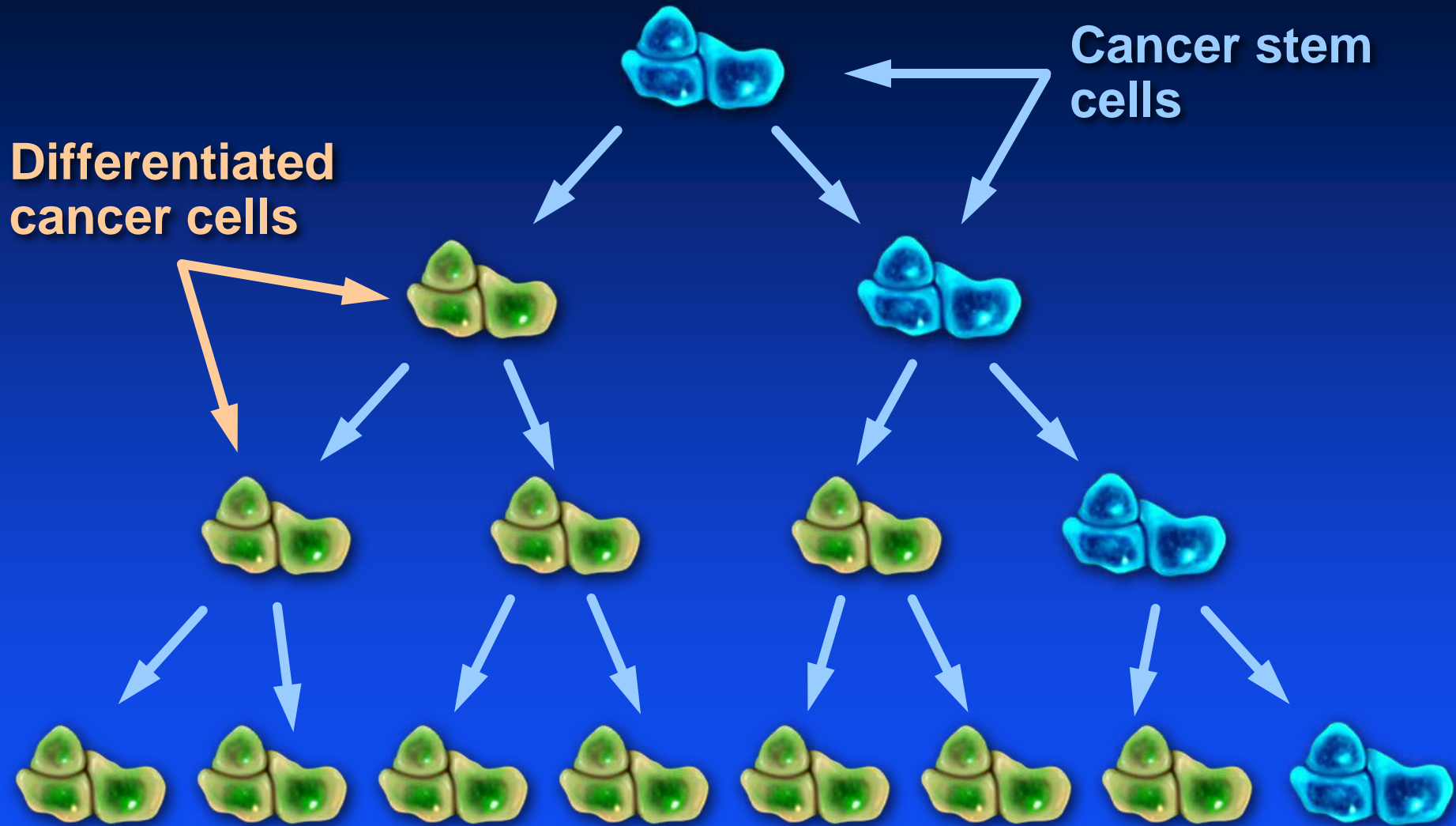
Wnt/ β -catenin Signaling



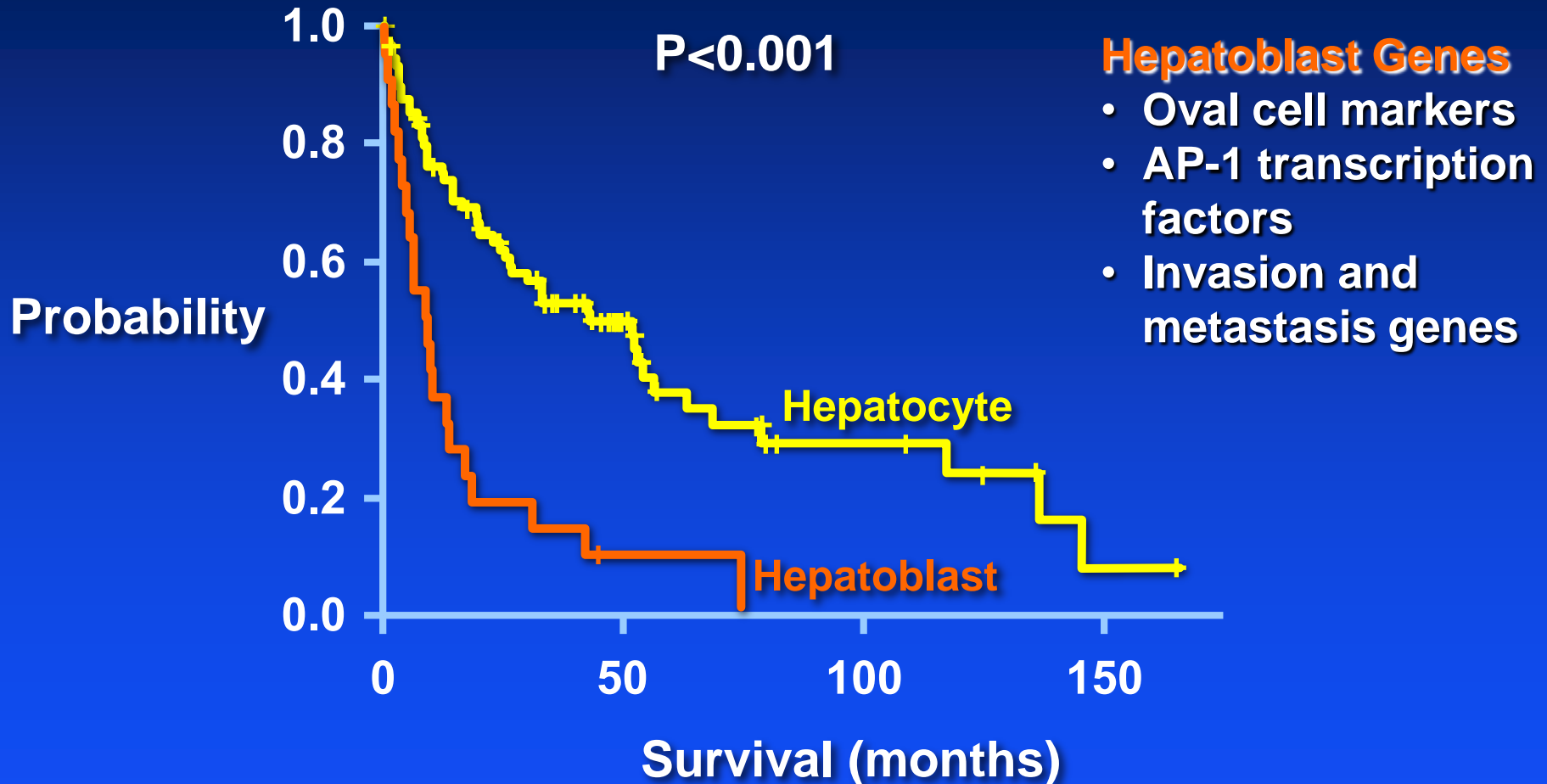
Molecular Signatures: Transcriptome Analysis of 91 (mainly HBV) HCCs



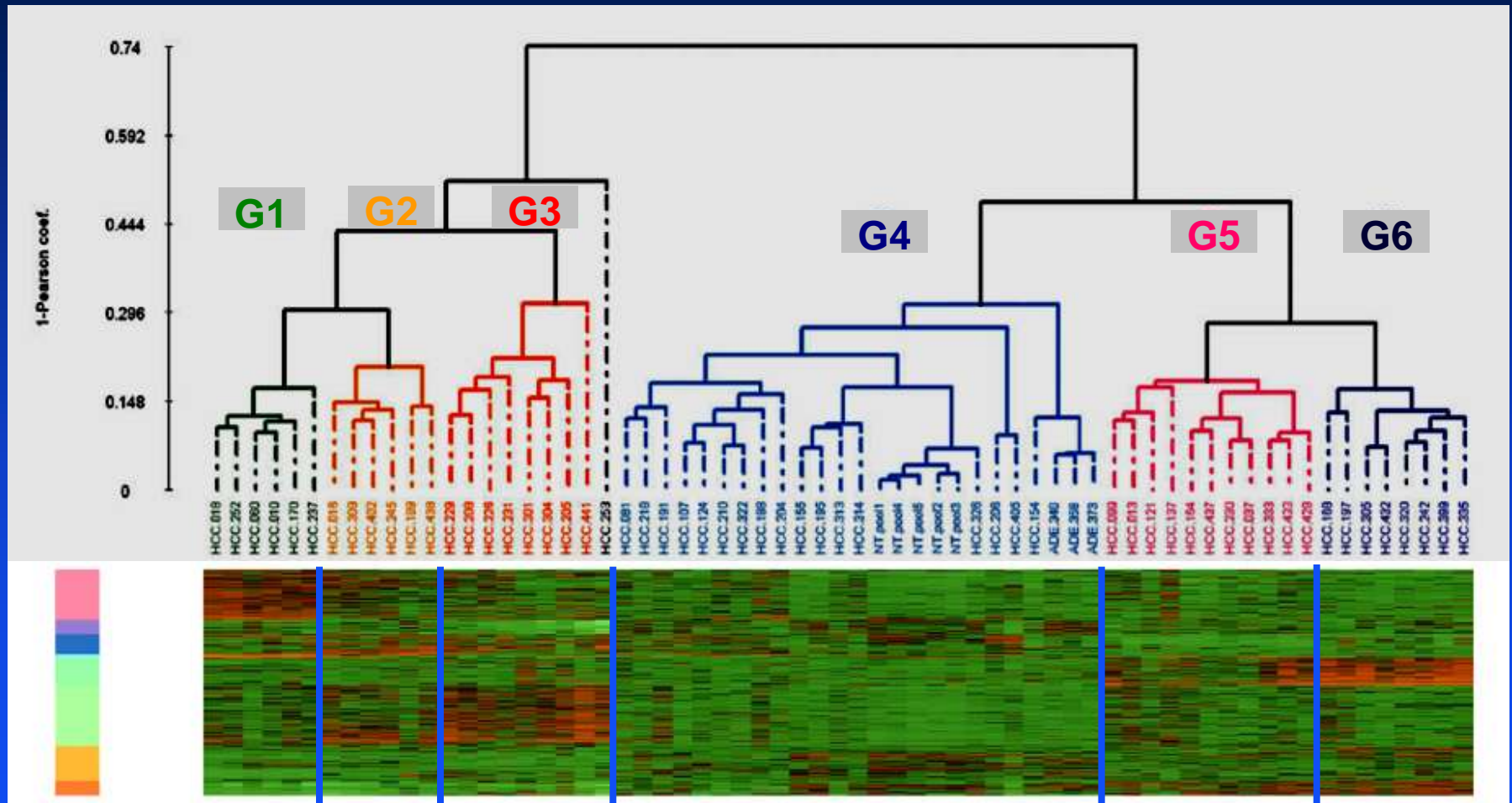
Cancer Stem Cell Concept



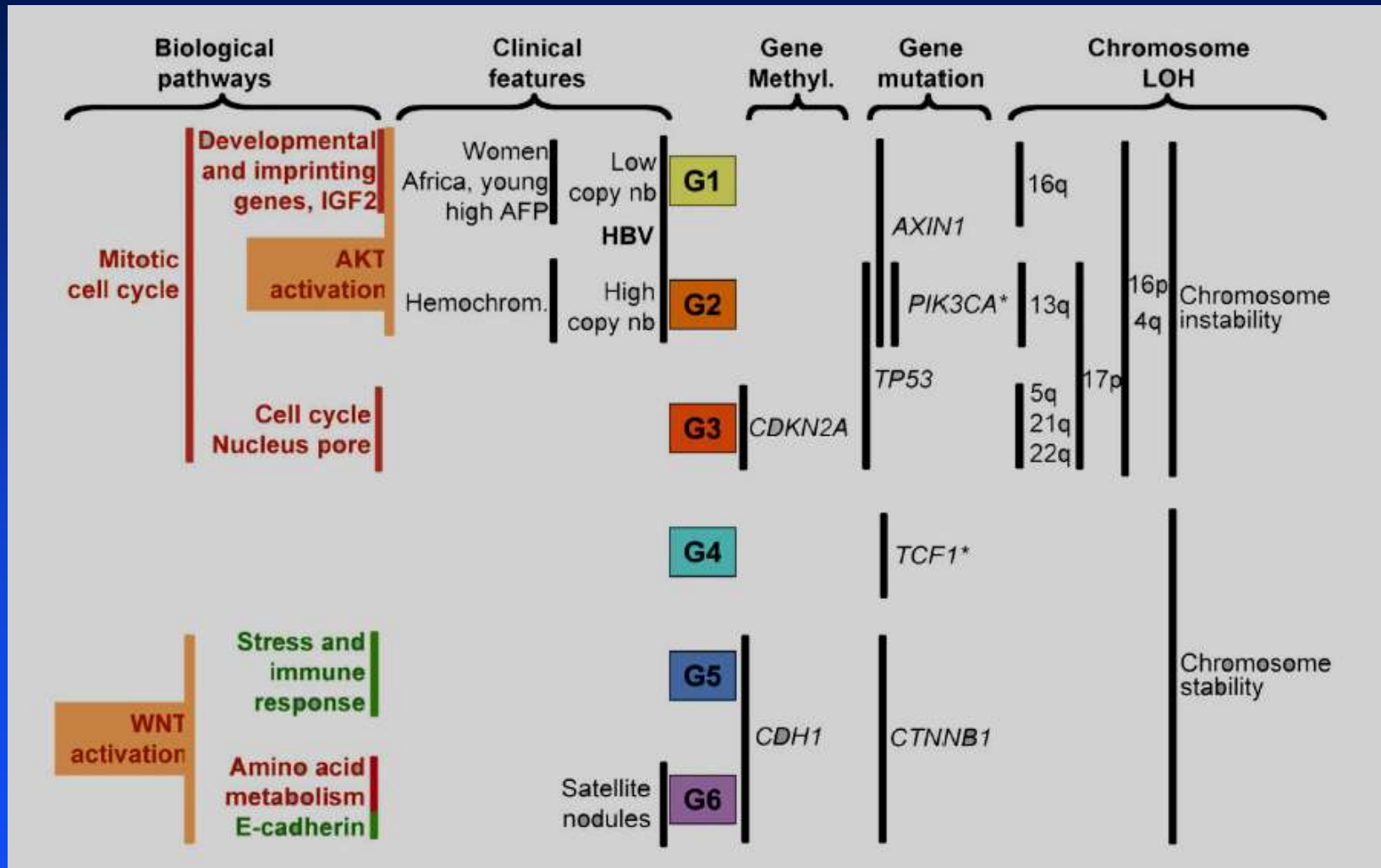
Stem Cell Signature Predicts Survival



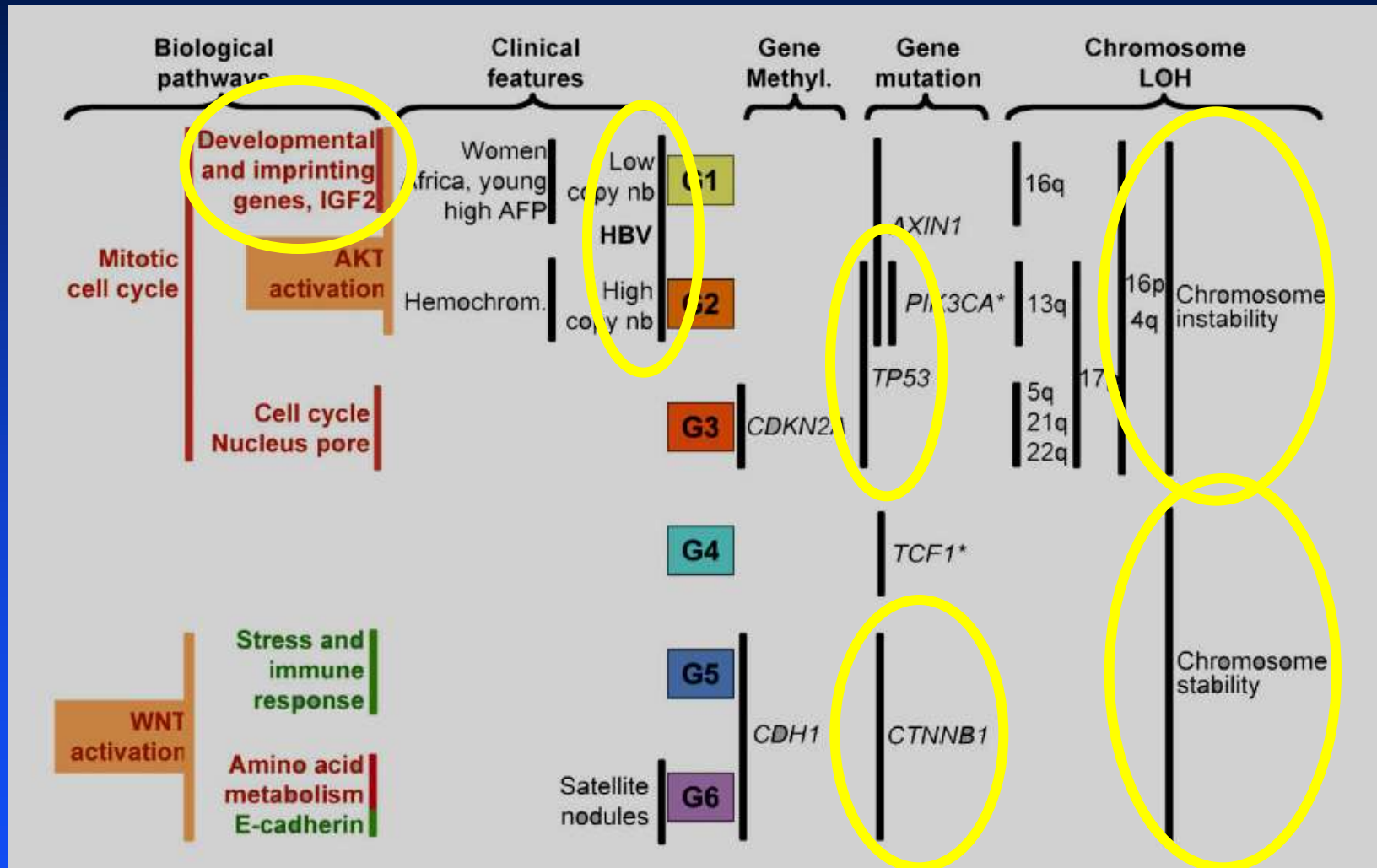
Molecular Signatures: Transcriptome Analysis of 120 Mixed Etiology HCCs



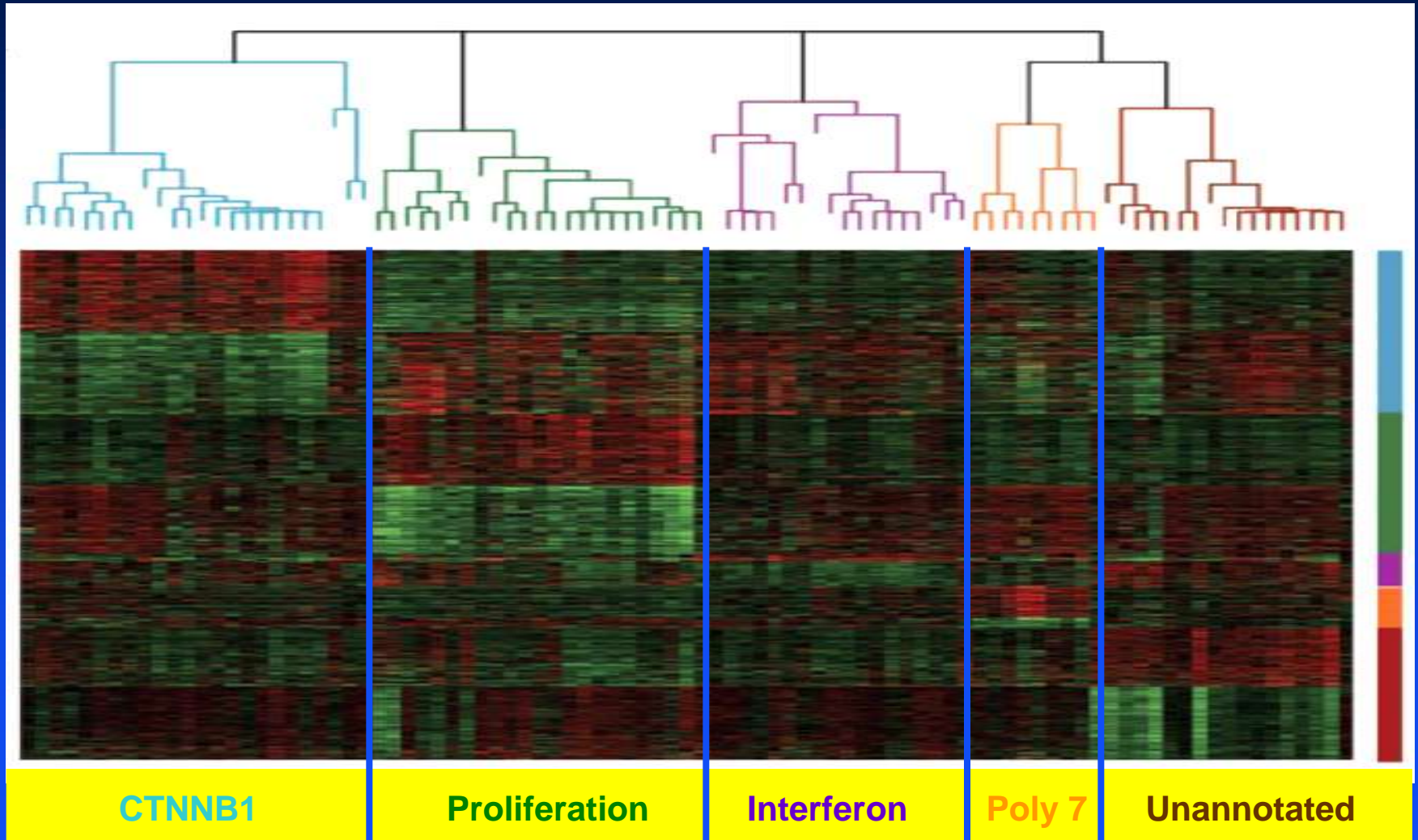
Molecular Signatures: Integrated Gene, Epigenetic, and Transcriptome Analysis



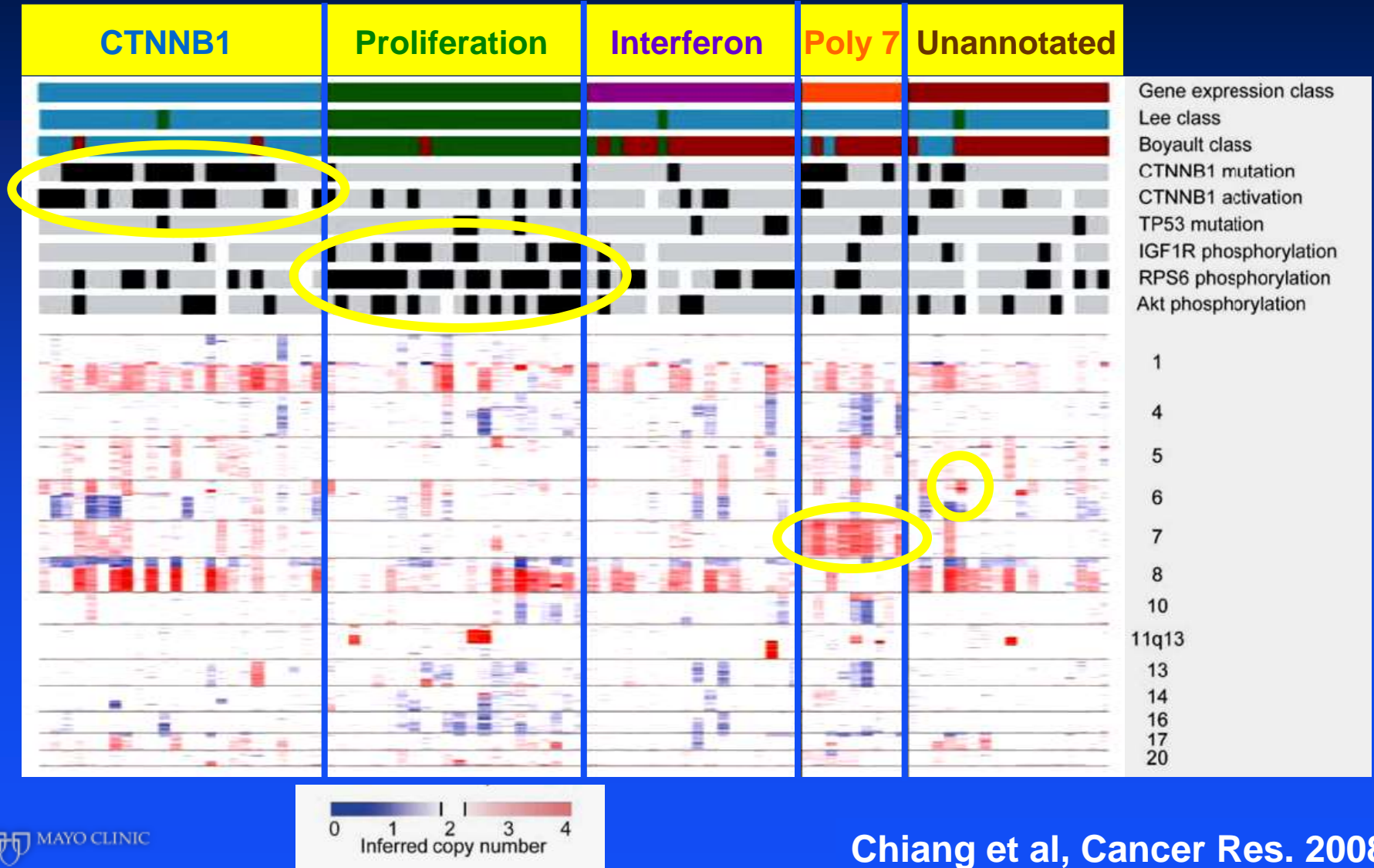
Molecular Signatures: Integrated Gene, Epigenetic, and Transcriptome Analysis



Molecular Signatures: Transcriptome Analysis of 91 HCV-Induced HCCs

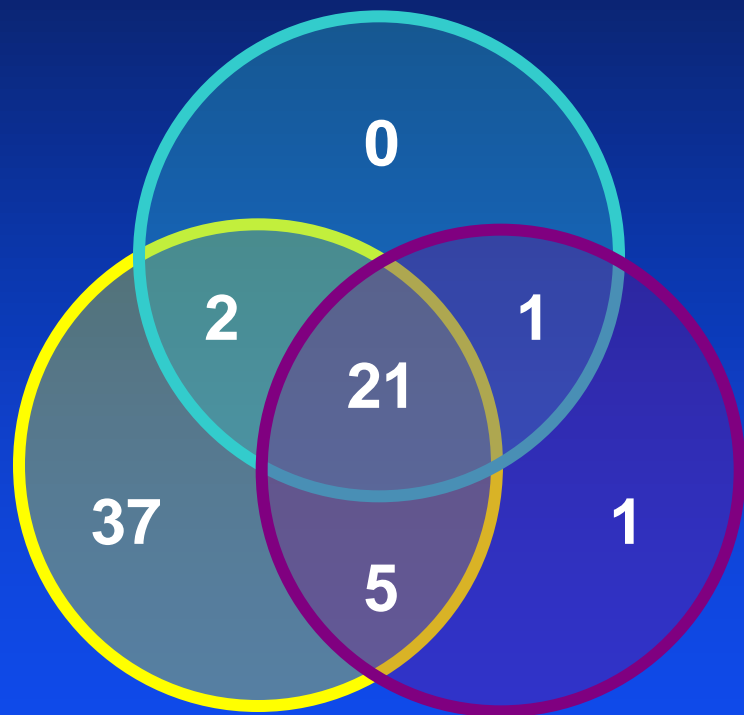


Molecular Signatures: Integrated Copy Number and Transcriptome Analysis



Combining Molecular Signatures From Three Separate Experiments

Chiang et al.
CTNNB1



Lee et al.
Cluster B

Boyault et al.
G5/G6

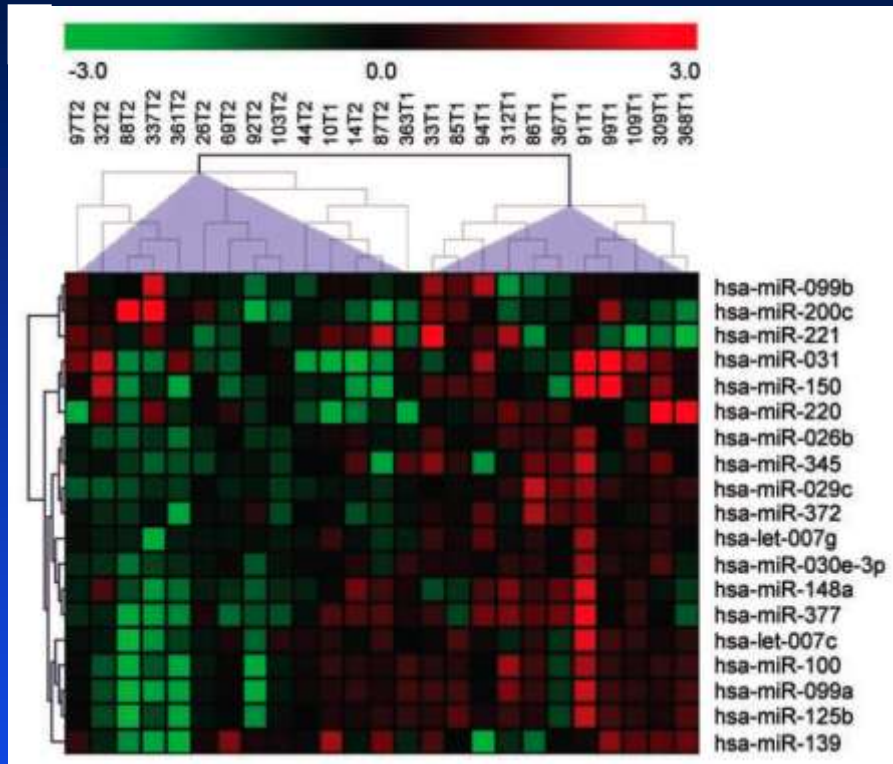
Chiang et al.
Proliferation



Lee et al.
Cluster A

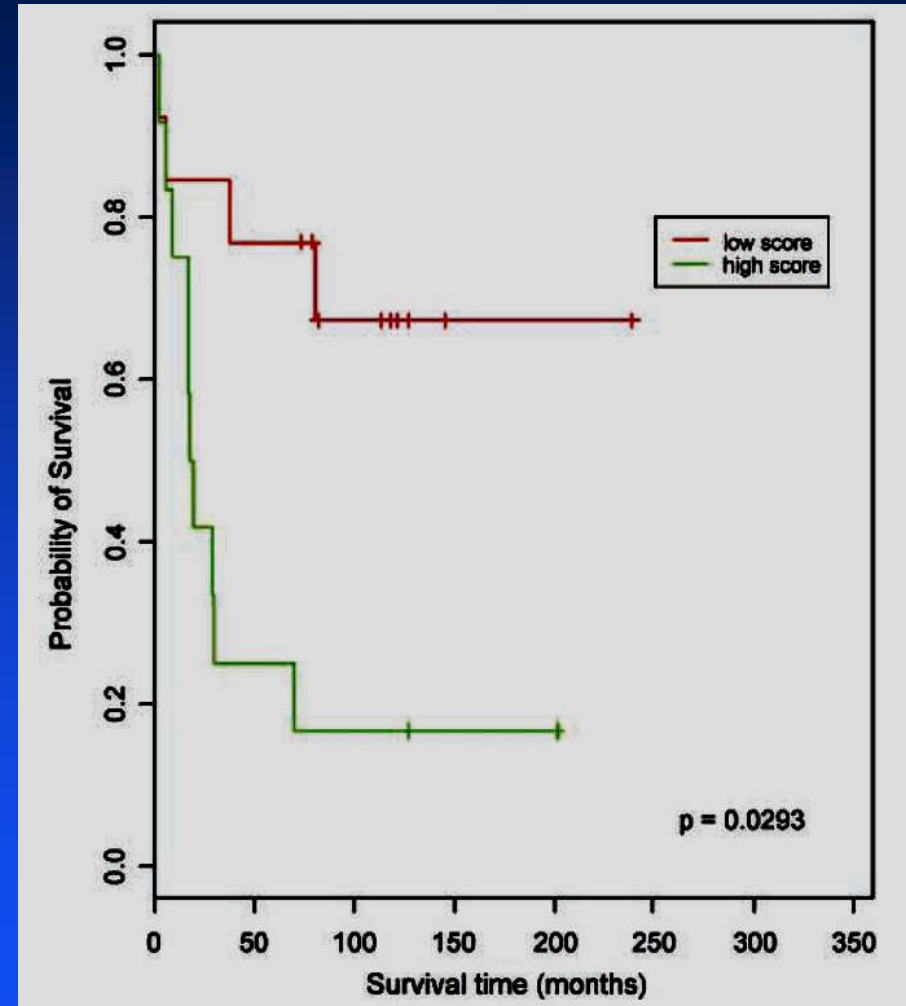
Boyault et al.
G1/G2/G3

19 miRNA Signature Predicts Survival



Target Pathways

- Cell division
- Mitosis
- G1-S cell cycle transition



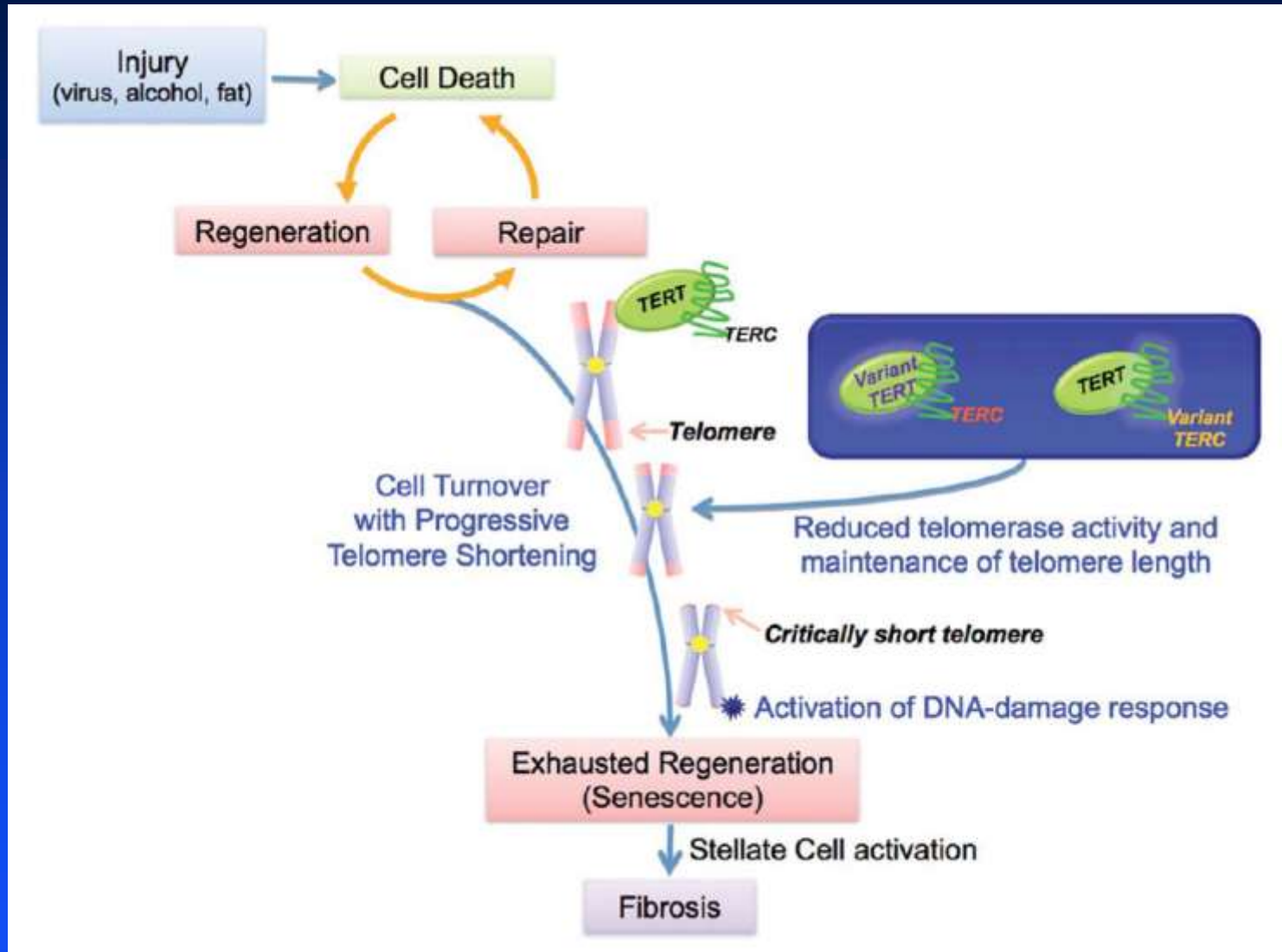
Pitfalls of Molecular Classification Studies of HCC

- Typically use resected specimens - biased
 - Small, resectable tumors
- Adjacent tissue is usually abnormal/cirrhotic
- Significant geographic variability in etiology and pathogenesis of HCC?
- Difficult to establish lead time before diagnosis
- Survival is often substantially modified by therapy, hence residual non-cancerous tissue can be more predictive of outcome

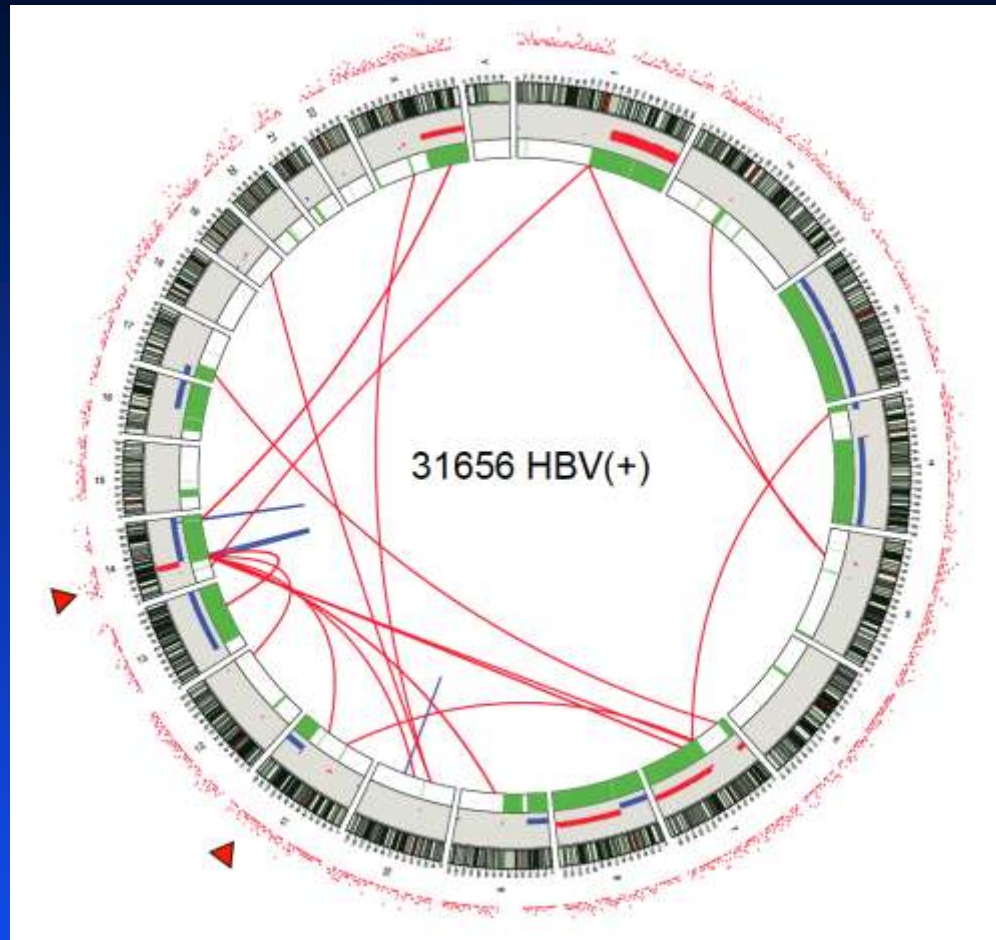
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Germline Telomerase Mutations are Associated with Risk of Cirrhosis



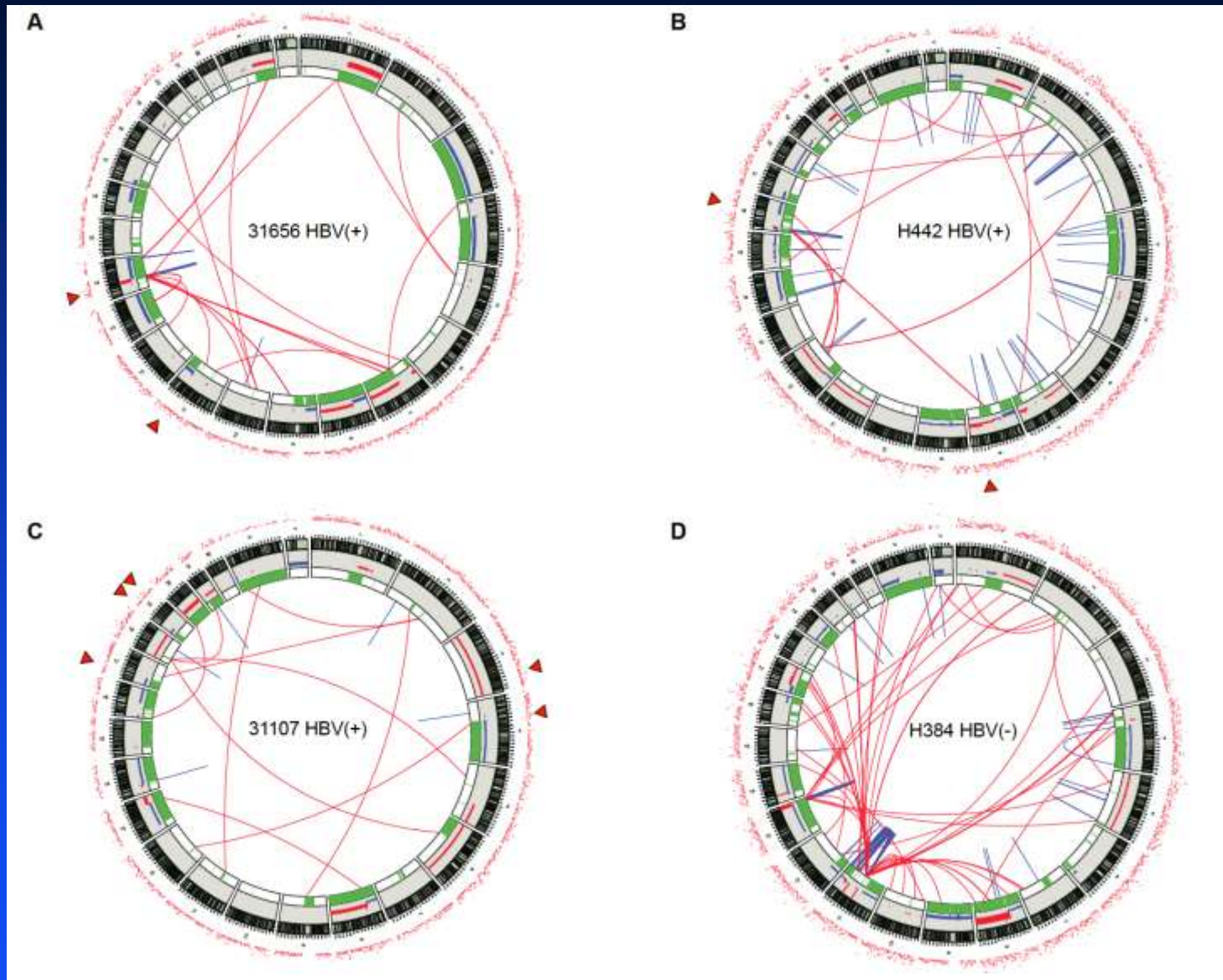
Insights from Next Generation Sequencing



Circos Plots: The new shorthand for the genome

Lines: Somatic structural variation: Red: Inter-, Blue Intrachromosomal
Green Bars: Allelic Imbalance; Bar Plots: Copy Number Gain or Loss
Red Dots: Single nucleotide variations

HCC Tumor Genomes are Heterogeneous



Does HBV Integrate into Random Sites in the Human Genome?

Oncogene (2003) 22, 3813–3820

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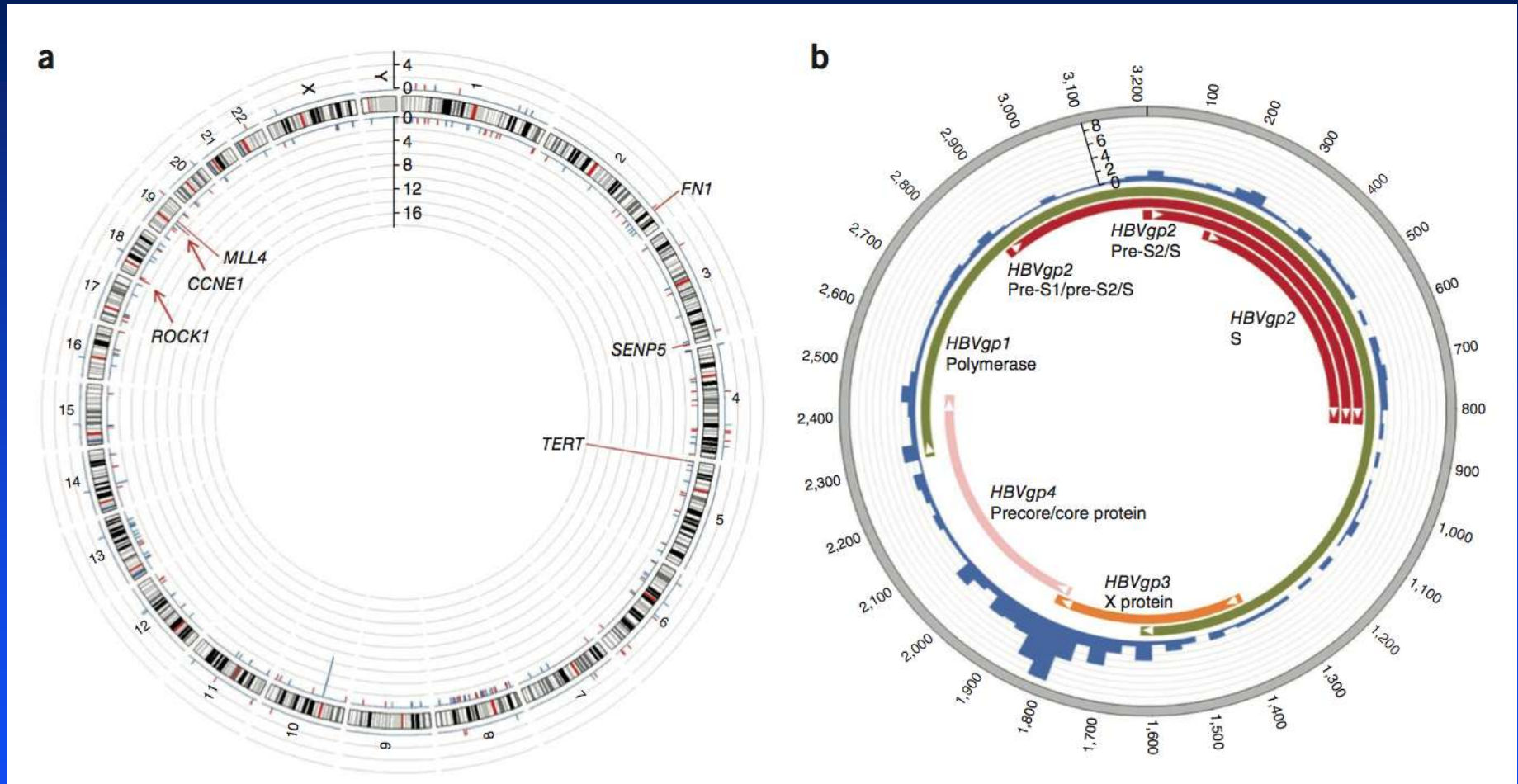
www.nature.com/onc

Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers

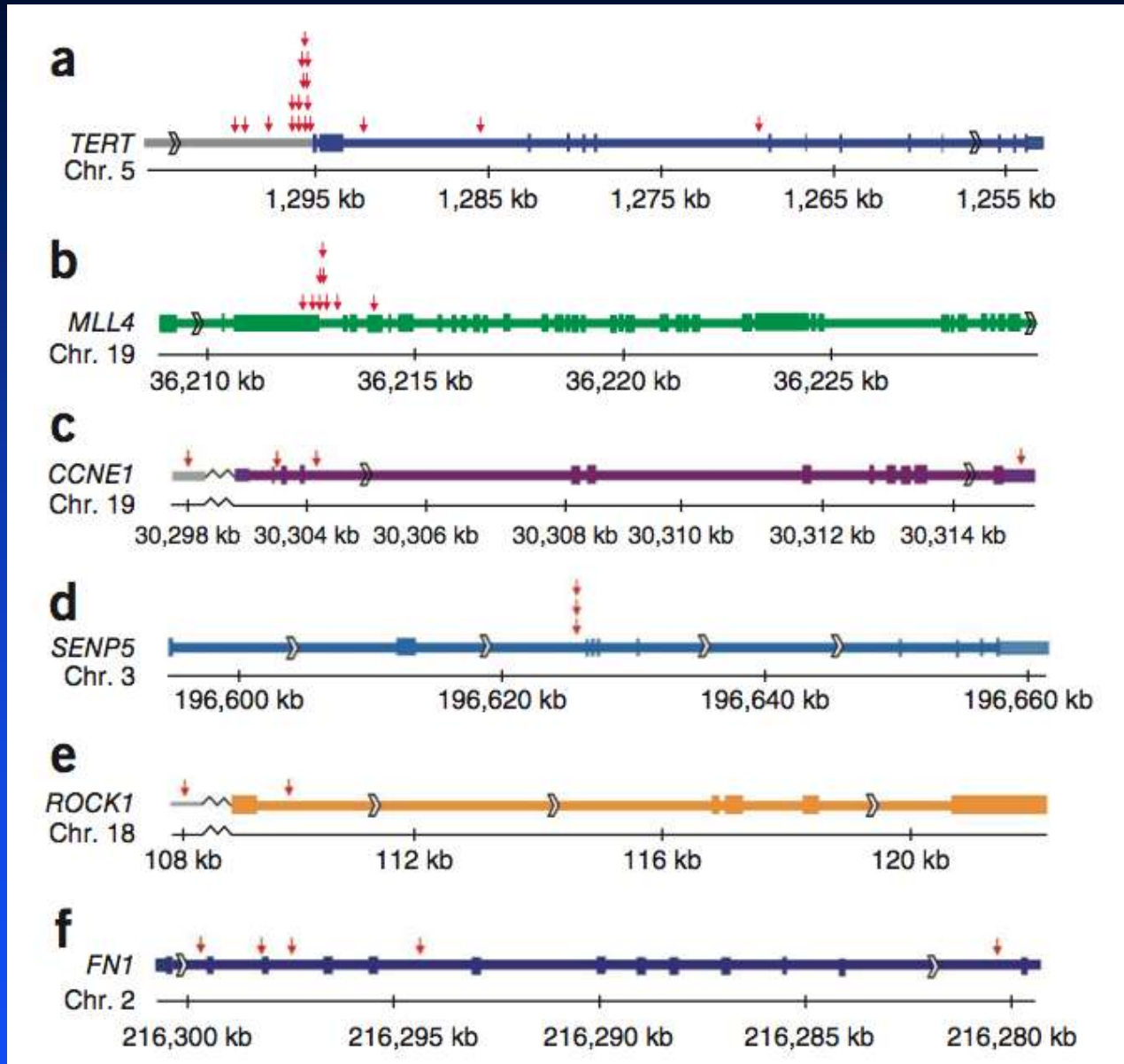
MJ Ferber^{1,2,10}, DP Montoya^{3,10}, C Yu³, I Aderca³, A McGee¹, EC Thorland¹, DM Nagorney⁴, BS Gostout⁵, LJ Burgart⁶, L Boix⁷, J Bruix⁷, BJ McMahon⁸, TH Cheung⁹, TKH Chung⁹, YF Wong⁹, DI Smith¹, and LR Roberts^{*,3}

“Our work supports the hypothesis that the sites of oncogenic viral integration are nonrandom and that genes at the sites of viral integration may play important roles in carcinogenesis.”

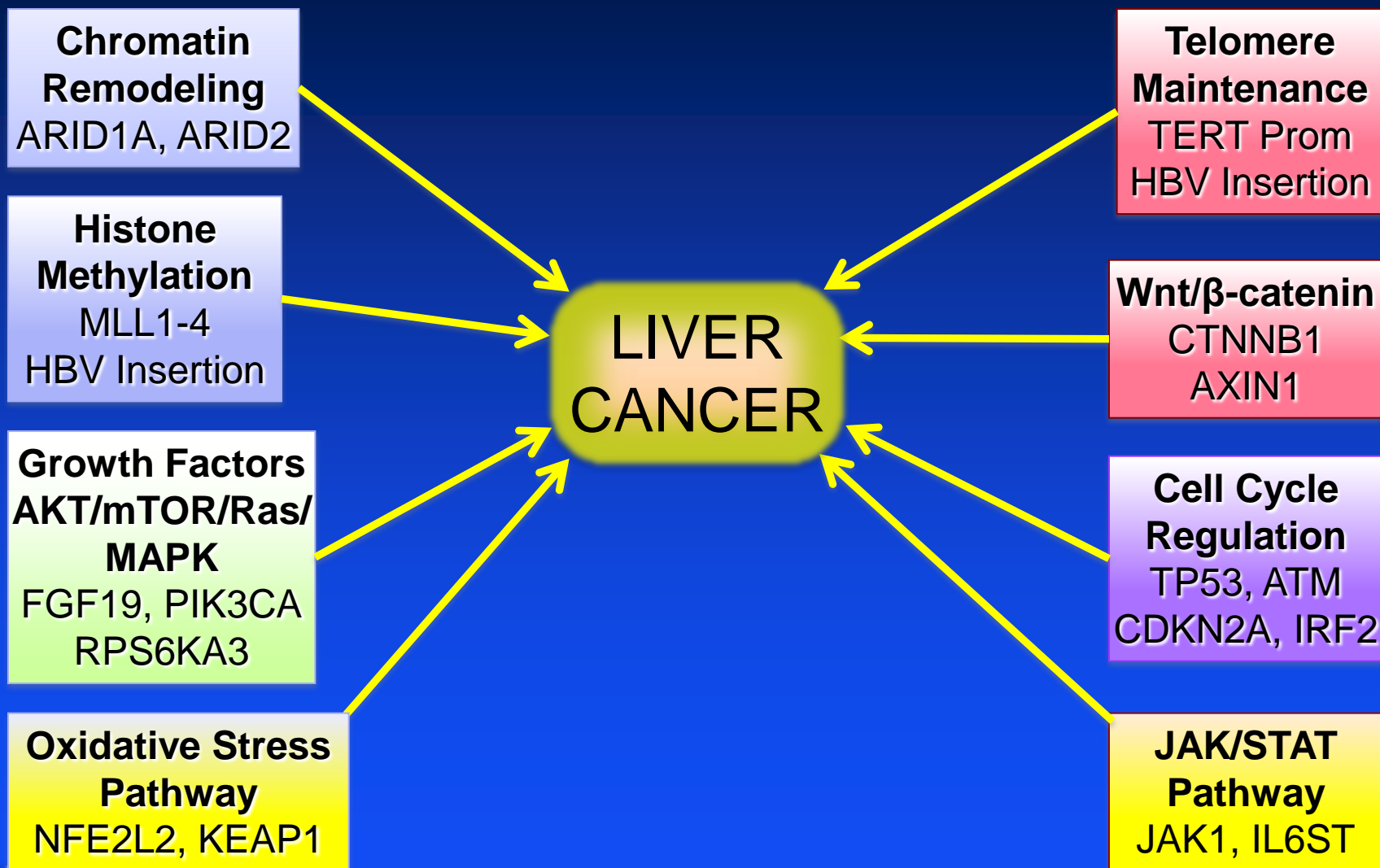
NGS Confirms Recurrent HBV Integrations into the Human Genome in HCC



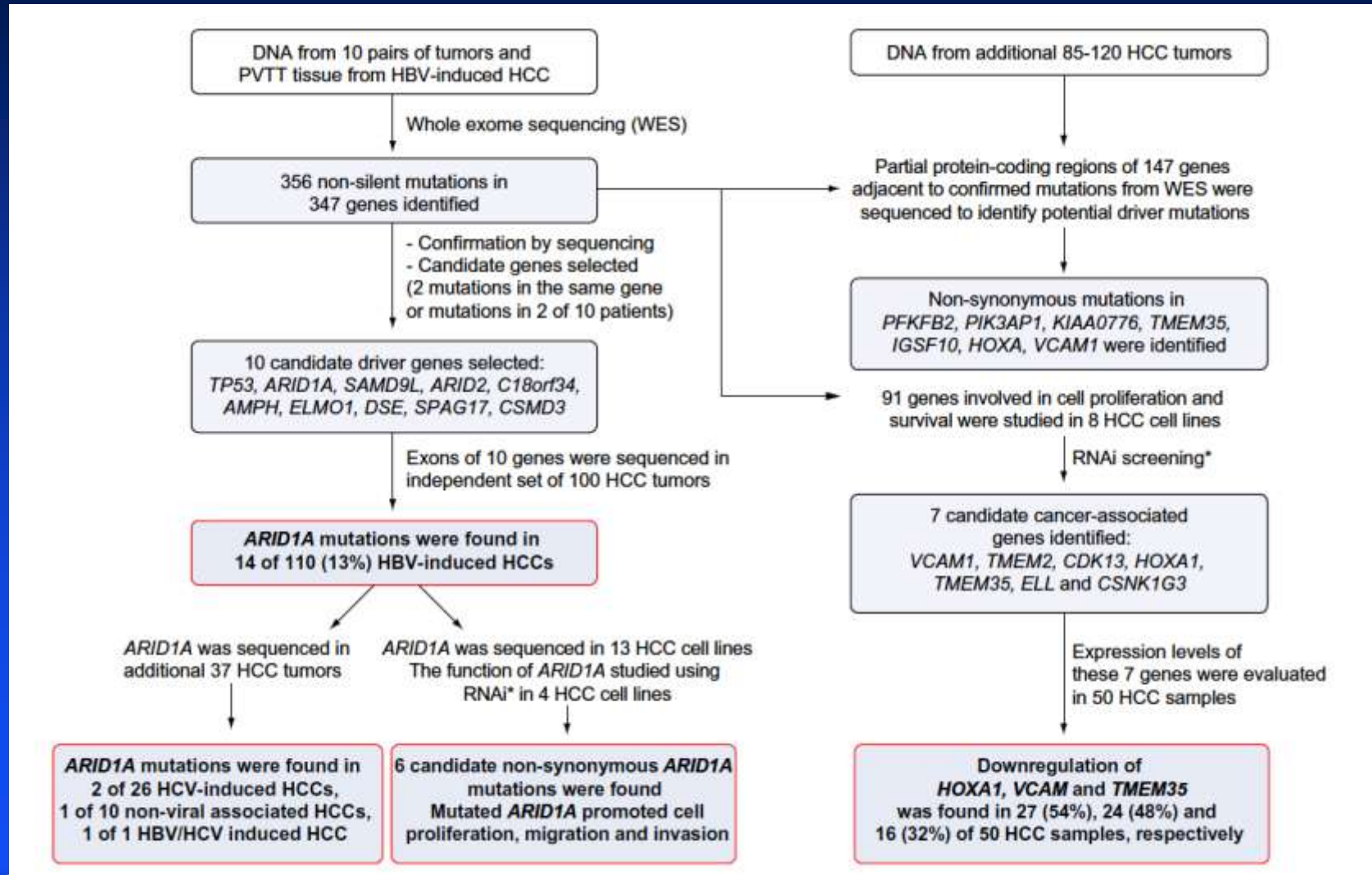
Genes with Recurrent HBV Integrations



Pathways Identified in HCC by Next Generation Sequencing



Chromatin Remodeling Gene Mutations in Advanced HCCs



Frequency of Chromatin Remodeling Gene Mutations in HCCs

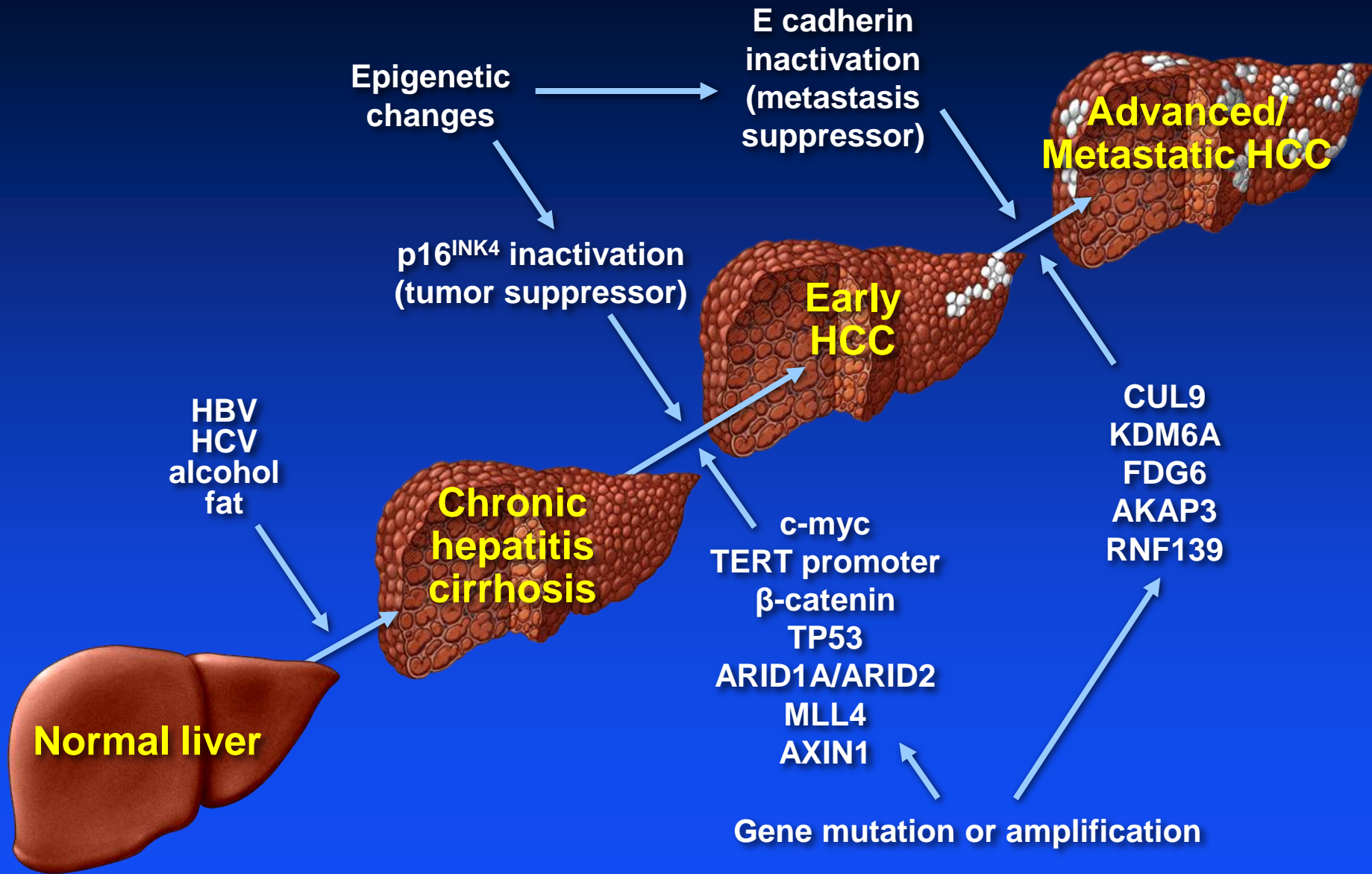
Table 1. Summary of mutations in chromatin remodeling gene family from recent NGS publications on HCC.

Author, [Ref.]	Country	ARID1A			ARID1B			ARID2		
		HBV	HCV	NBNC	HBV	HCV	NBNC	HBV	HCV	NBNC
Li <i>et al.</i> , [2]	US and Netherlands	-	-	-	-	-	-	0 of 15 (0%)	6 of 33 (18%)	2 of 30 (7%)
	China	-	-	-	-	-	-	1 of 35 (3%)	0 of 10 (0%)	0 of 14 (0%)
Fujimoto <i>et al.</i> , [4]	Japan	4 of 11 (36%)	3 of 14 (21%)	0 of 2 (0%)	2 of 11 (18%)	1 of 14 (7%)	0 of 2 (0%)	1 of 11 (9%)	1 of 14 (7%)	1 of 2 (50%)
Guichard <i>et al.</i> , [5]	France	4 of 35 (11%)	4 of 24 (17%)	12 of 66 (18%)	-	-	-	2 of 35 (6%)	1 of 24 (4%)	4 of 66 (6%)
Huang <i>et al.</i> , [6]	China	14 of 110 (13%)	-	-	-	-	-	2 of 50 (4%)	-	-

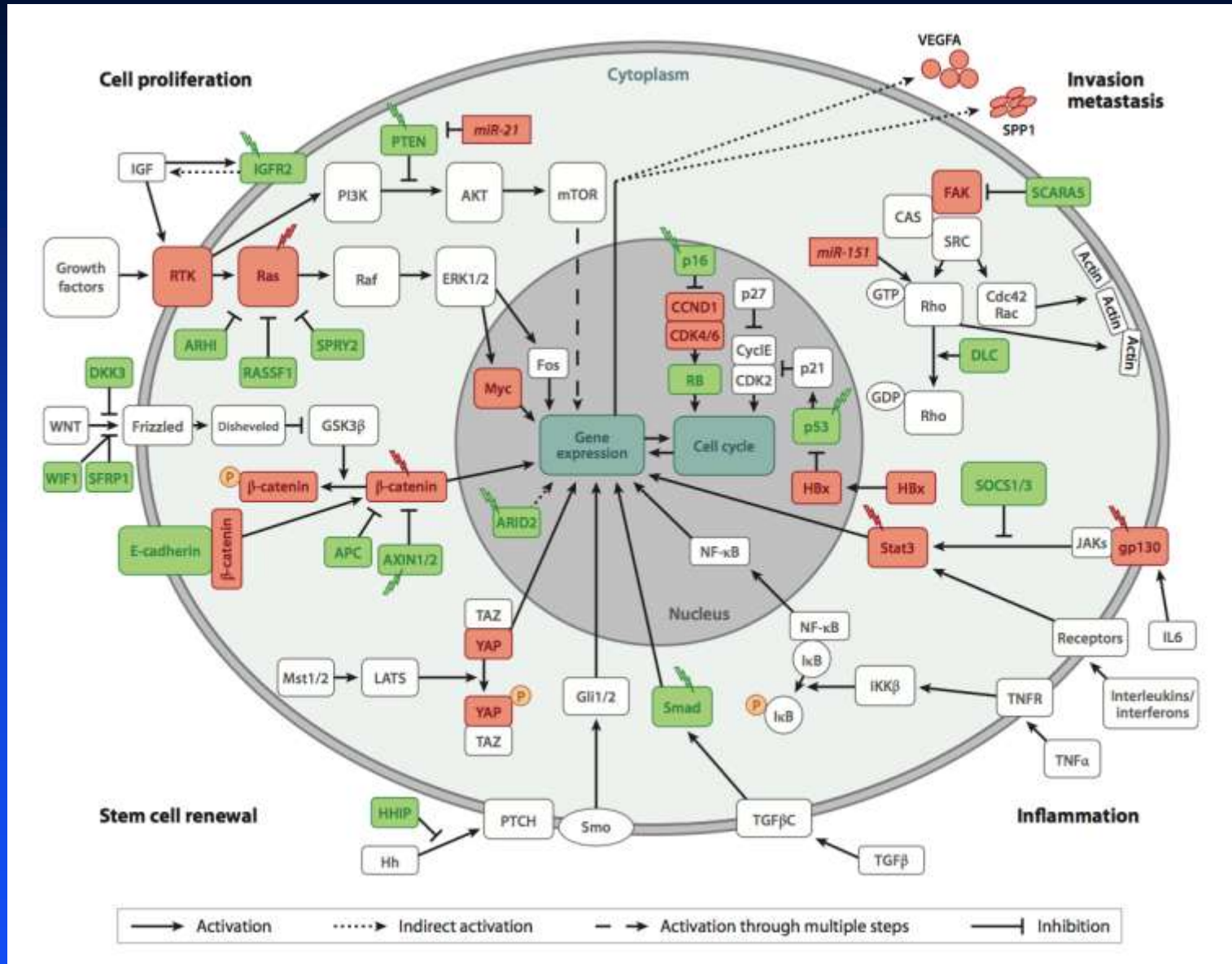
HBV, hepatitis B-related HCC; HCV, hepatitis C-related HCC; NBNC, non-viral hepatitis B/C-related HCC; HCC, hepatocellular carcinoma.

- Mutations occur in ARID1A, ARID1B and ARID2 genes
- Frequency ranges from 0-50% in different studies
- Frequency appears to be independent of etiology

Multistep Development of HCC

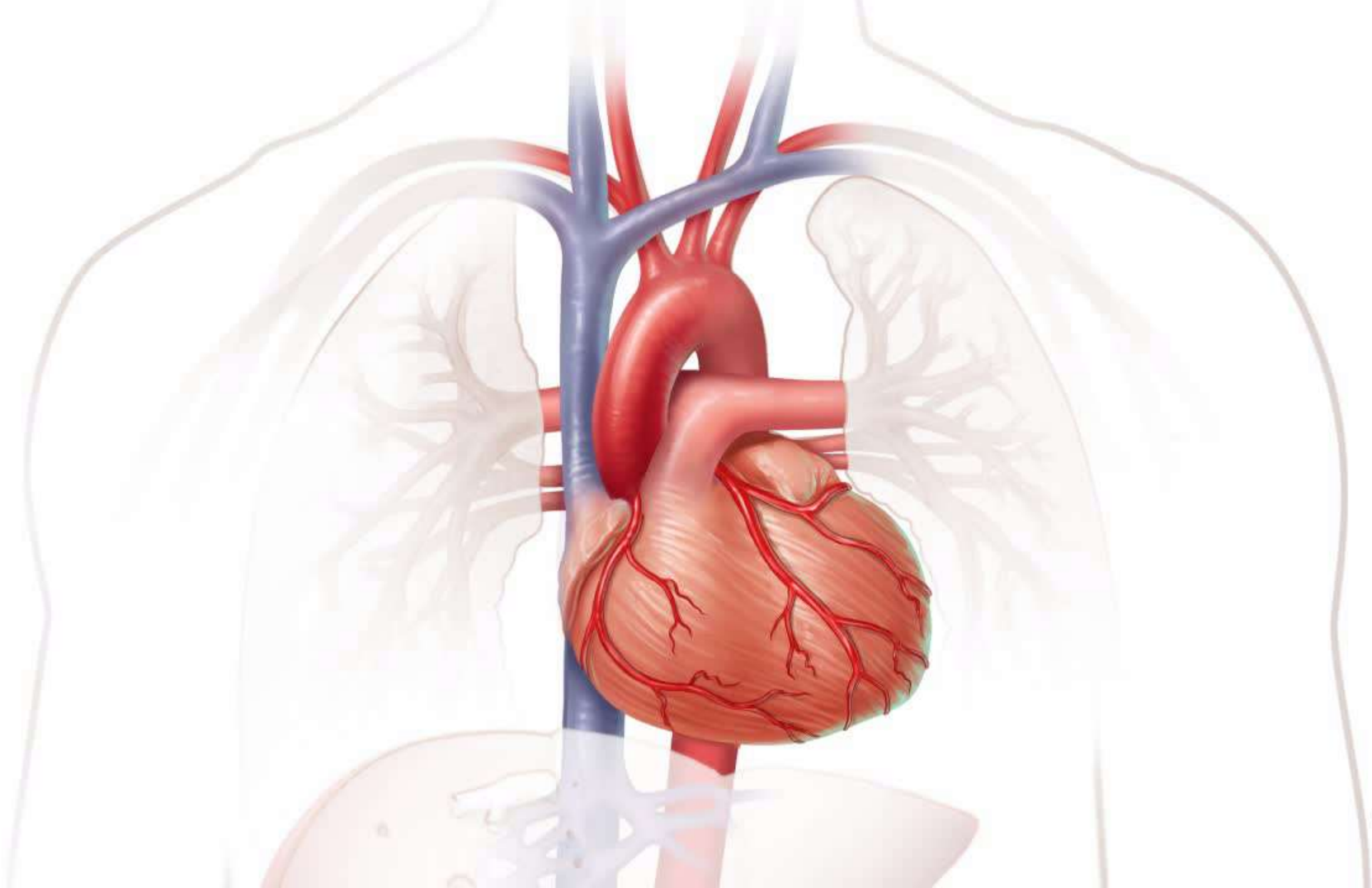


Summary of Pathway Alterations in HCC

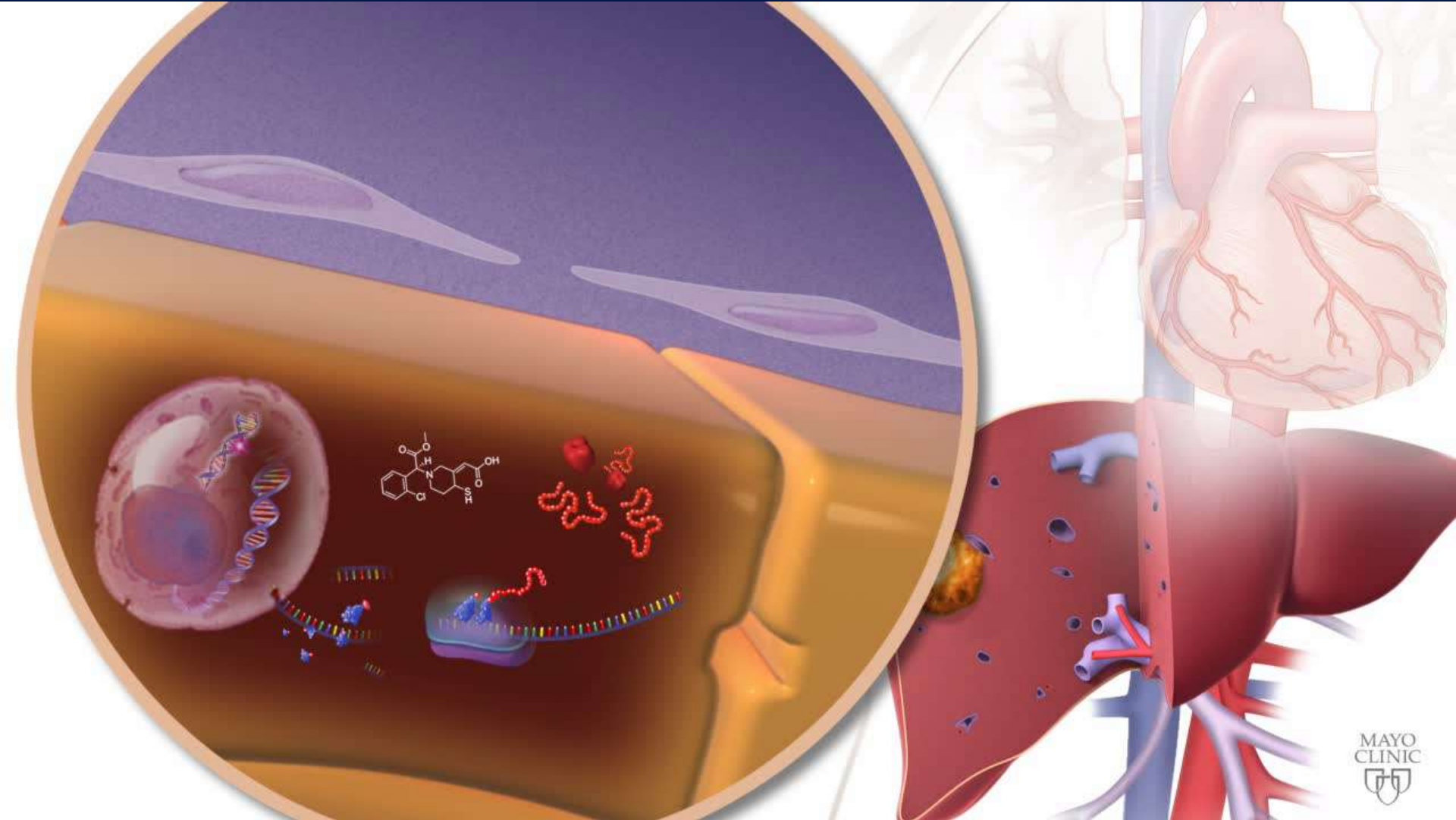


Evidence for Utility of Genomics for Diagnosis of HCC

Definition and Scope of HCC Biomarkers

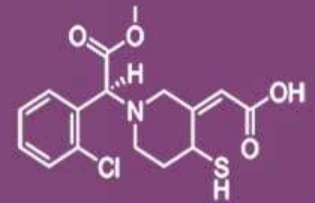
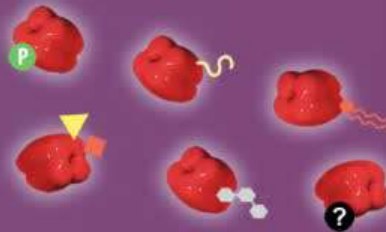


Definition and Scope of HCC Biomarkers

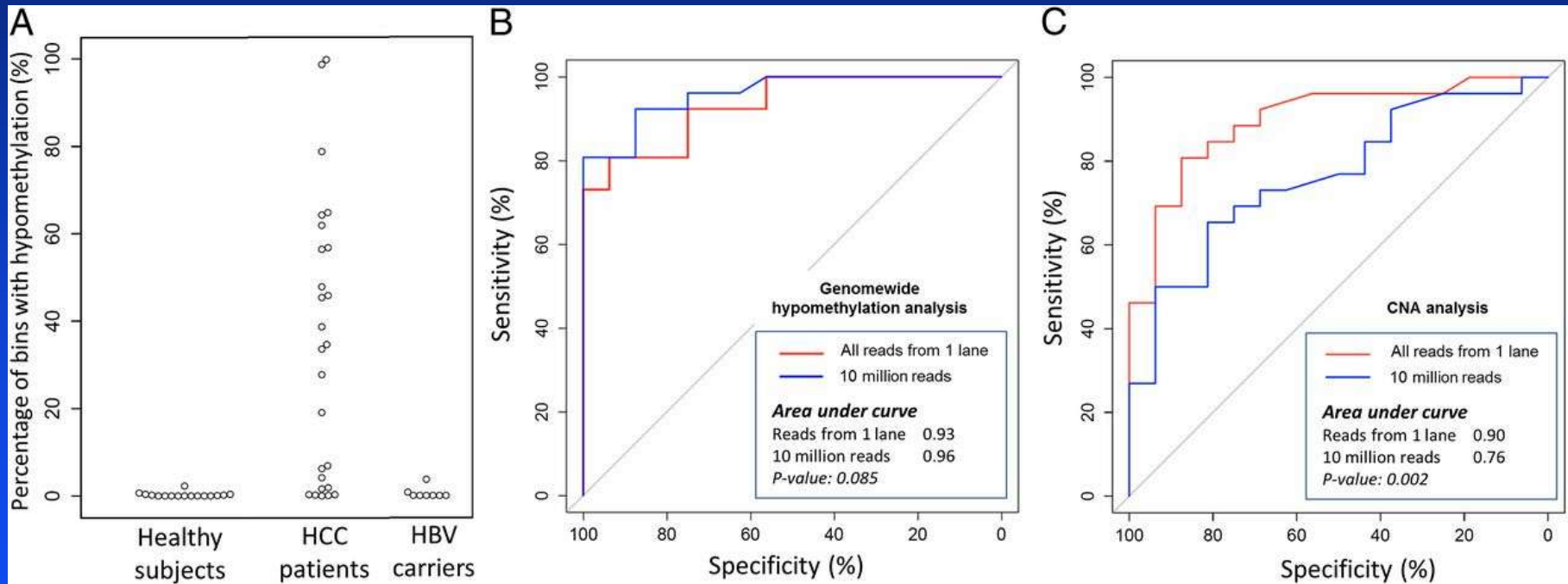


Definition and Scope of HCC Biomarkers

BLOODSTREAM



Evidence for Utility of Genomics for Diagnosis of HCC: Genomewide DNA Hypomethylation in Plasma

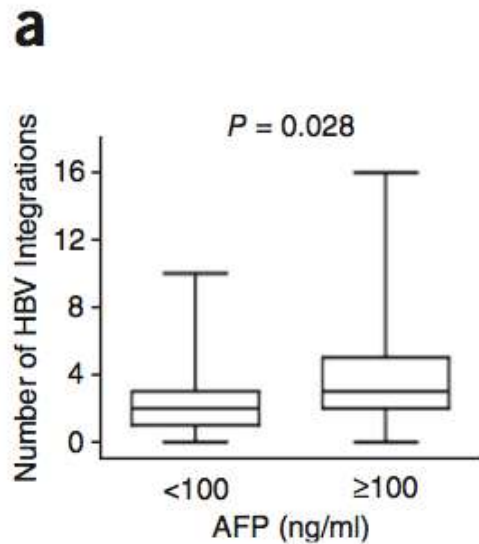


Overview

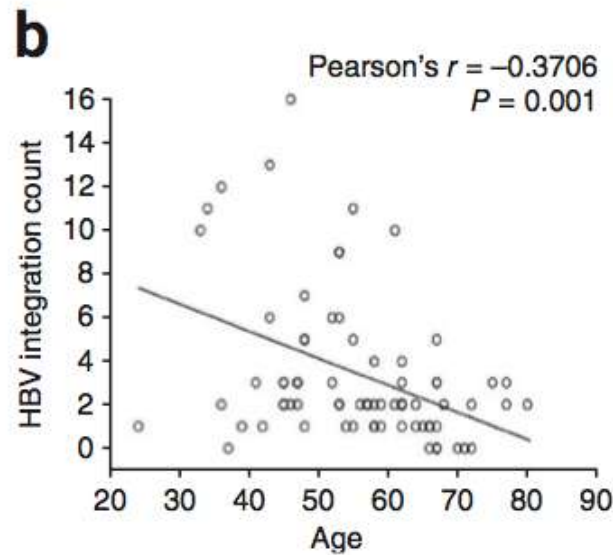
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Evidence for Ability of Genomics to Predict Clinical Outcomes in HCC

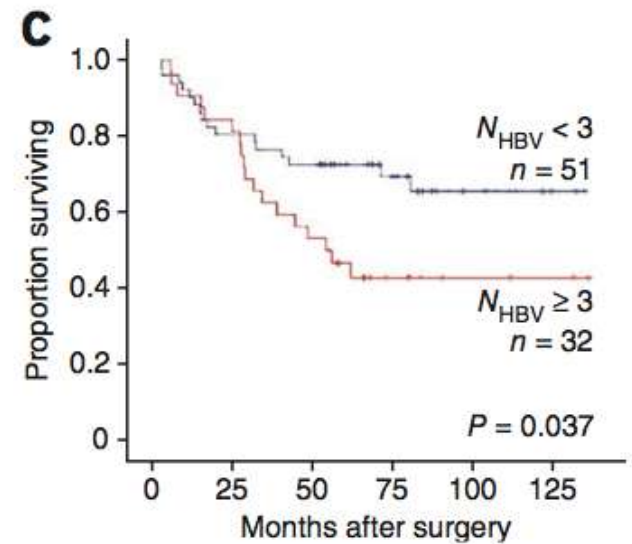
HBV Integrations are Associated with and Influence Clinical Outcomes



AFP

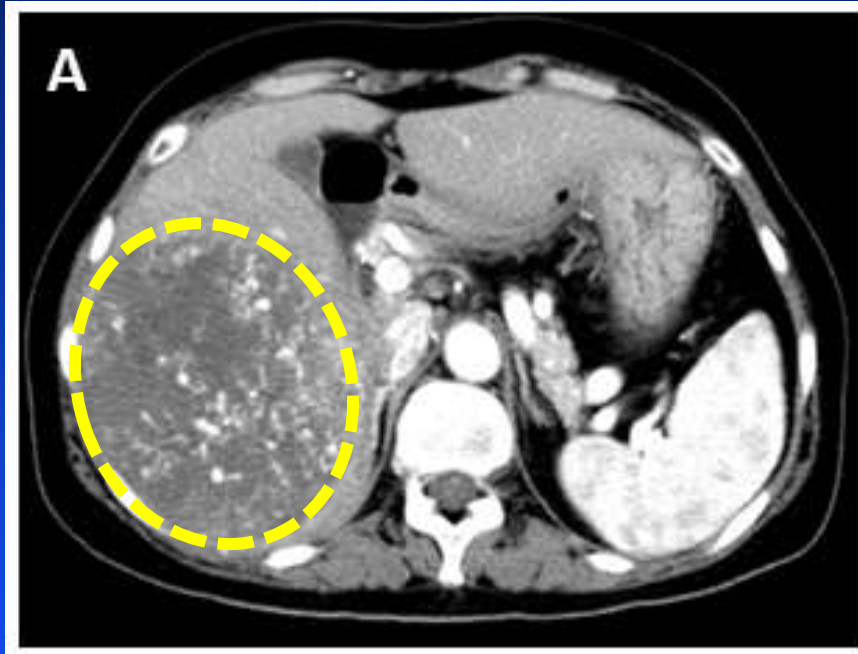


Age



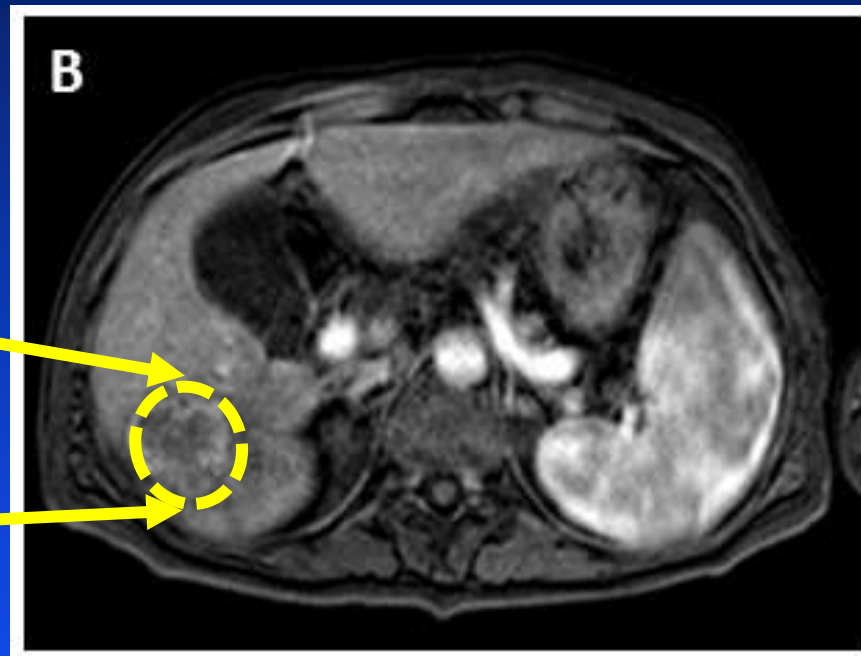
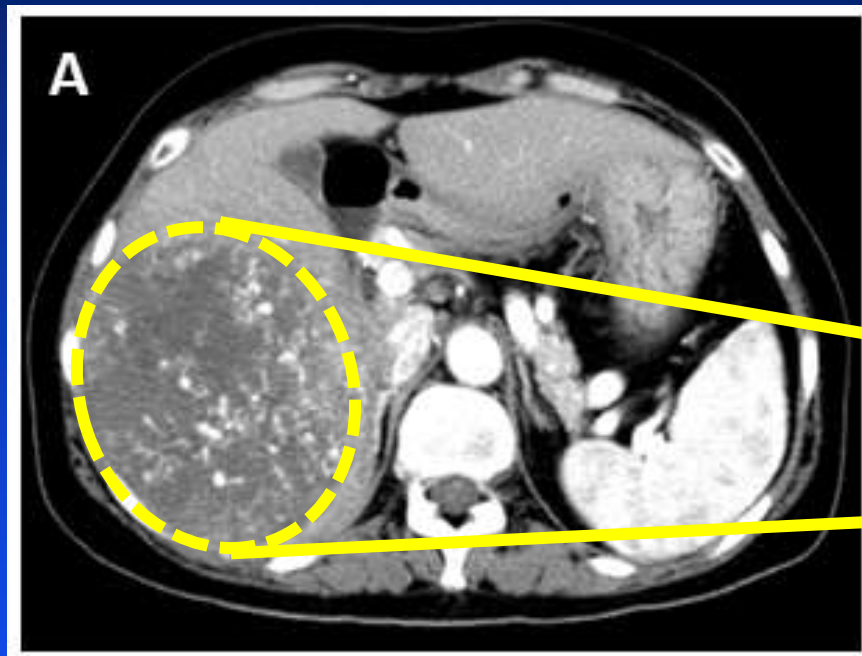
Survival

Biomarkers for Predicting Response to Sorafenib



Patient No. 9

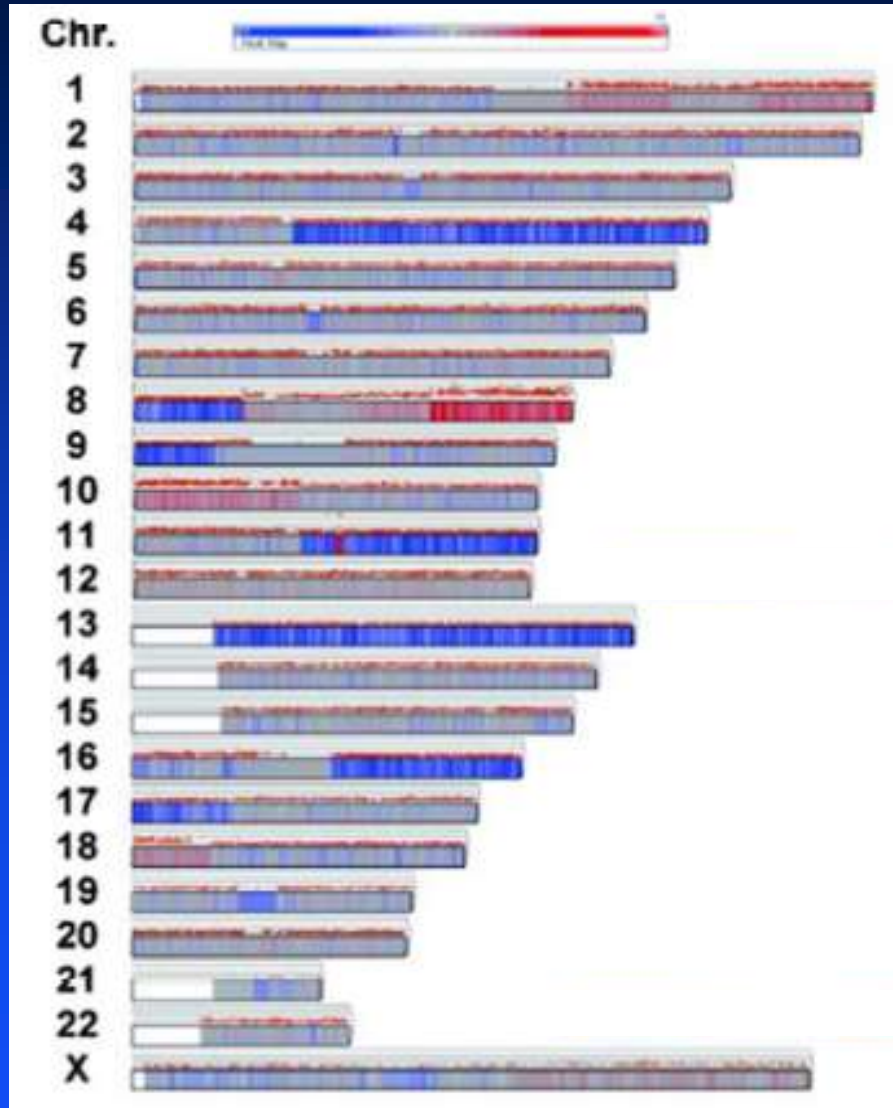
Biomarkers for Predicting Response to Sorafenib



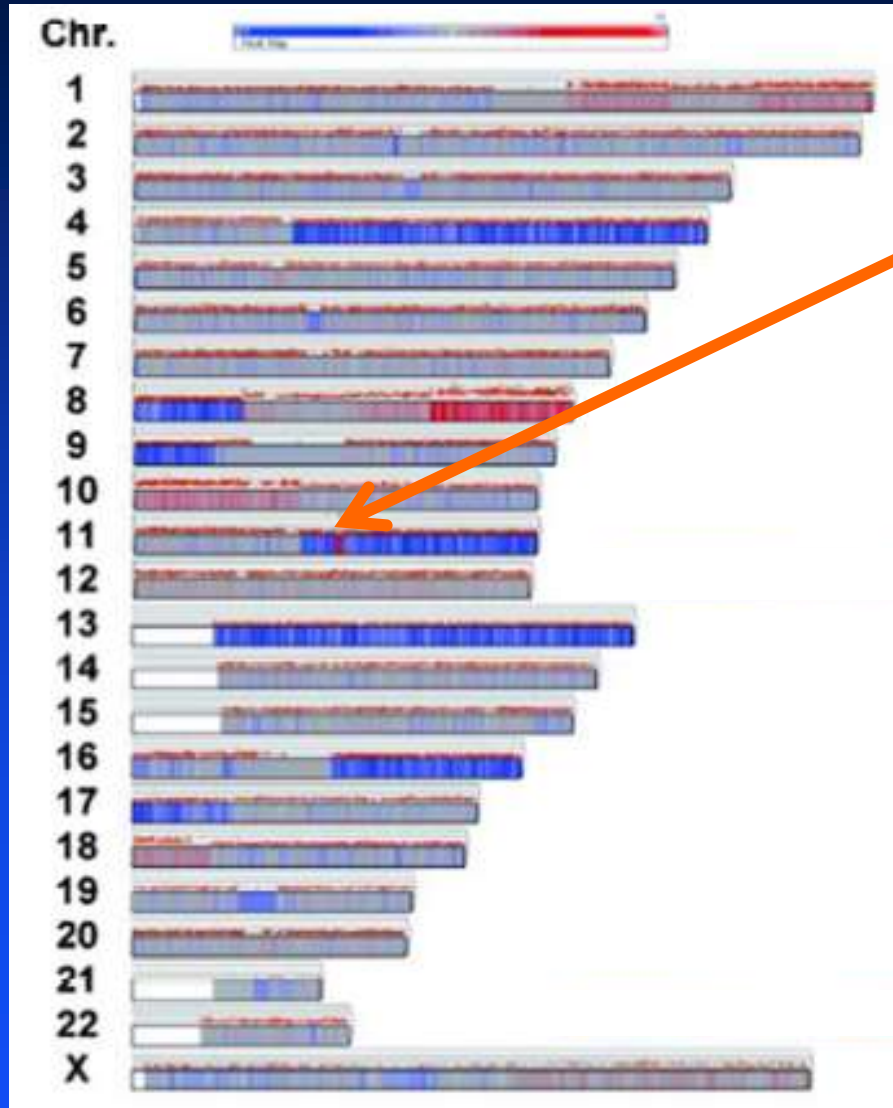
Patient No. 9

2 months sorafenib

Comparative Genomic Hybridization of HCC from Patient No. 9

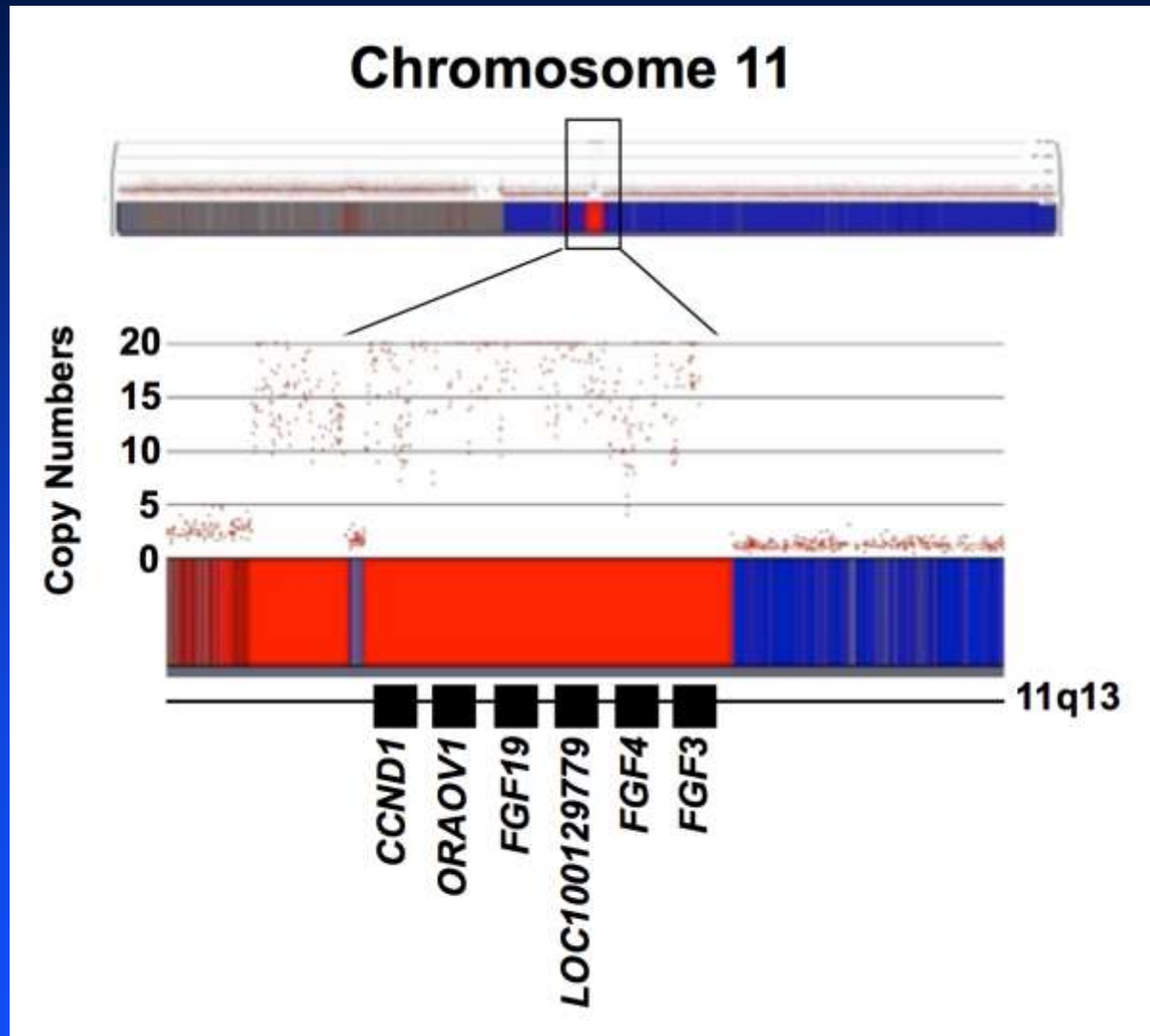


Comparative Genomic Hybridization of HCC from Patient No. 9

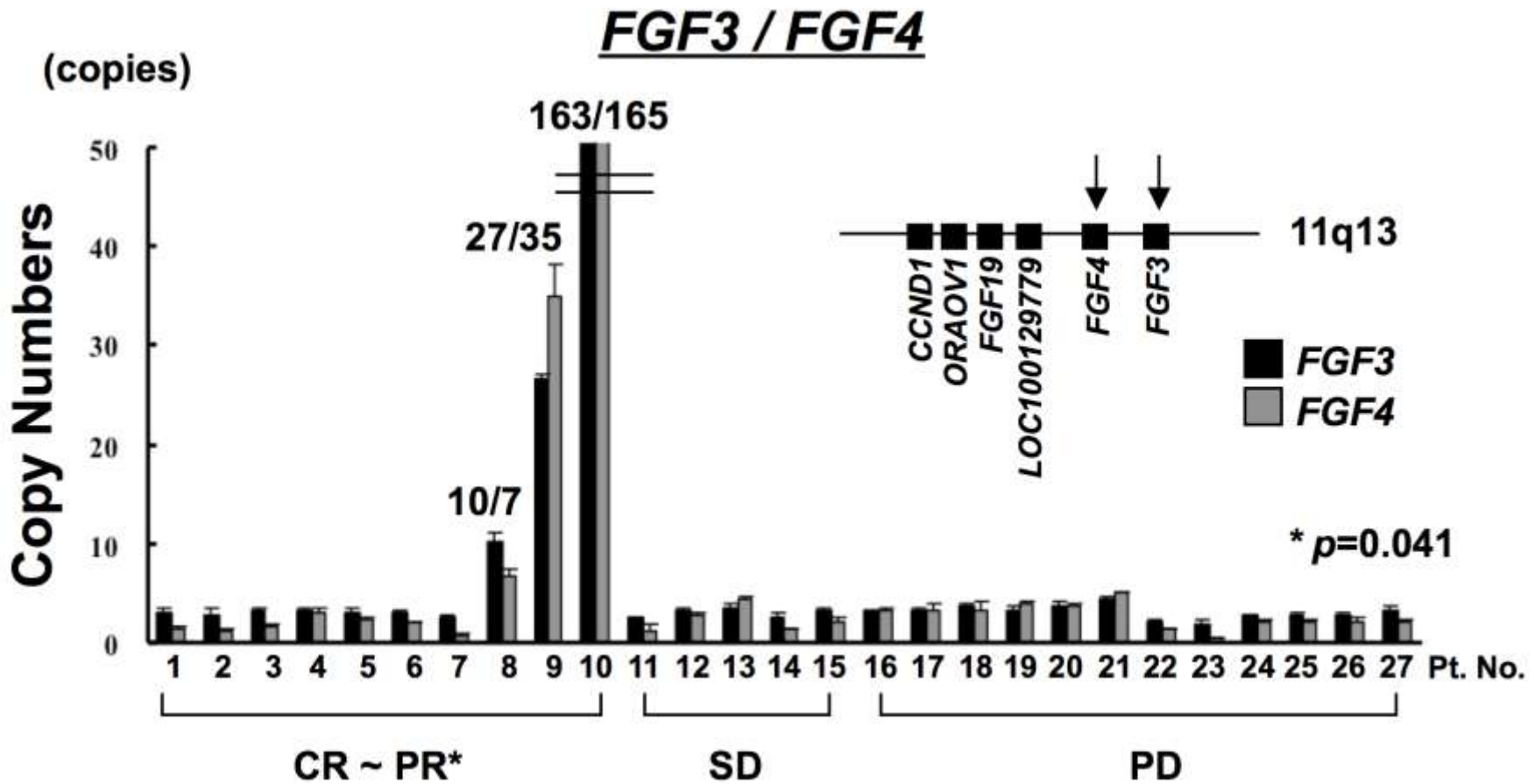


**Chromosome 11
Amplification**

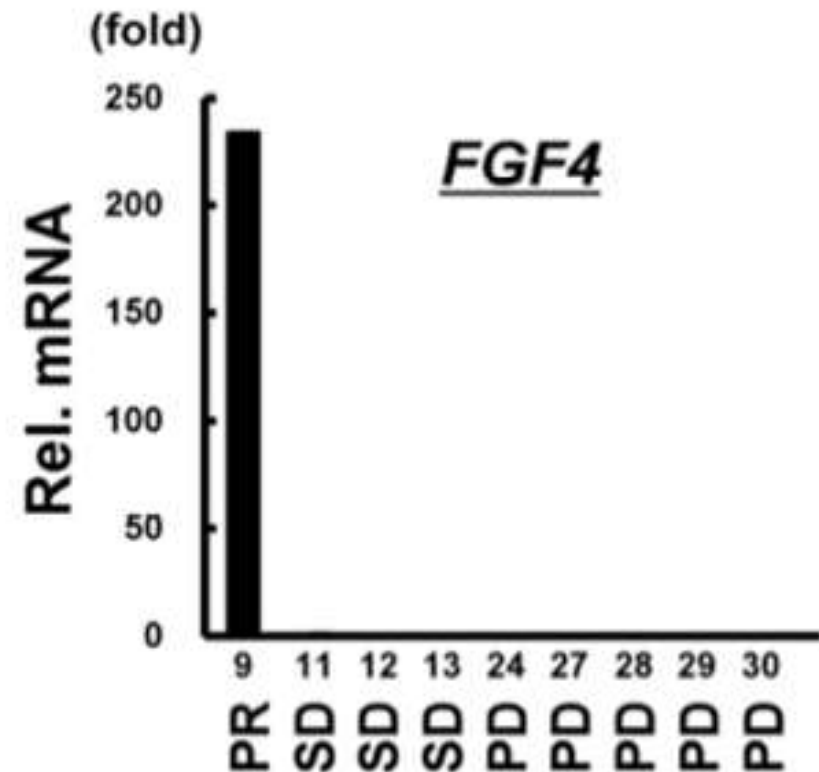
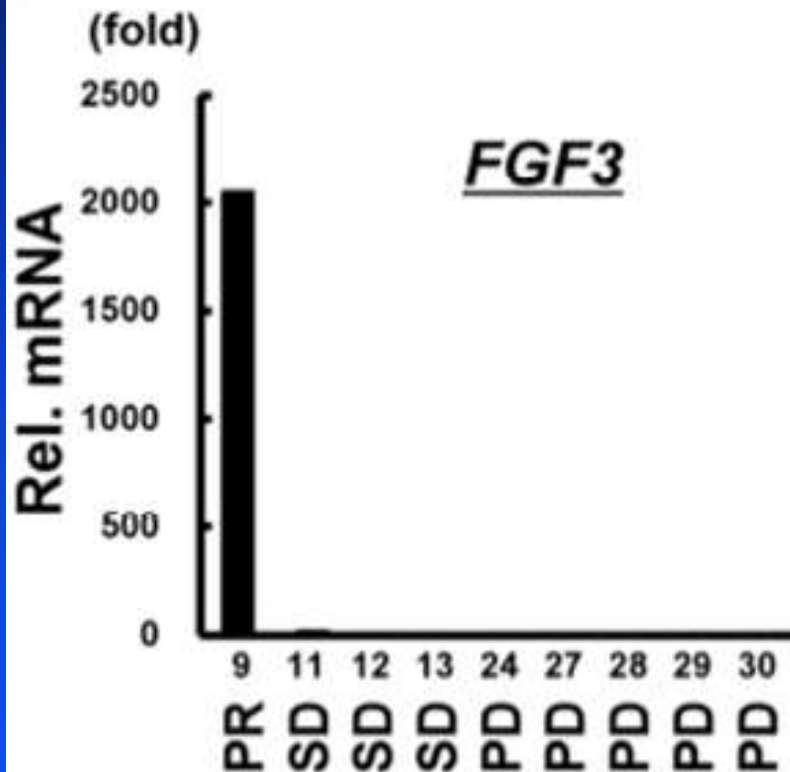
Expanded View of Chromosome 11 Amplification by CGH



FGF3/FGF4 Amplification in 3 of 10 HCC Patients With Complete or Partial Response to Sorafenib

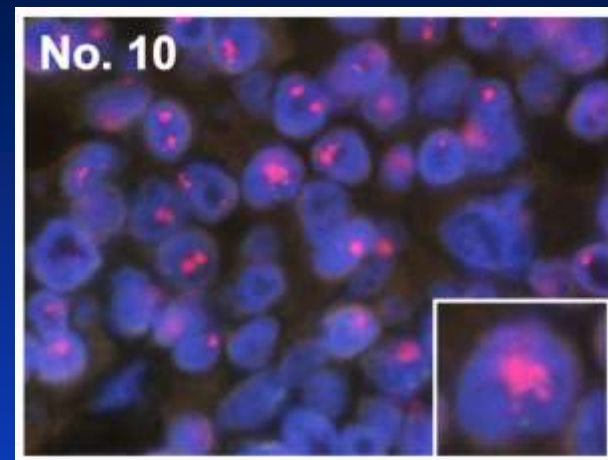
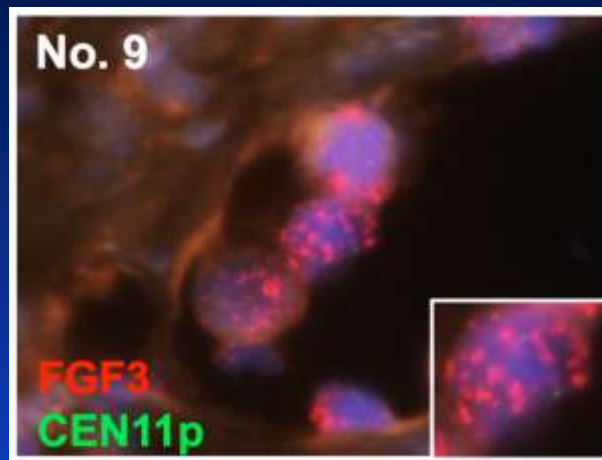
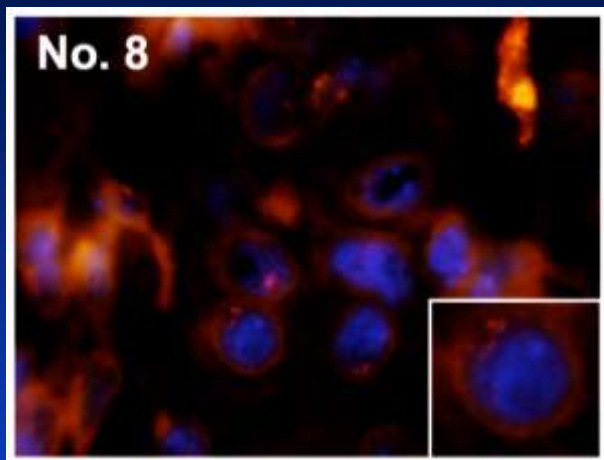


Increased FGF3 and FGF4 mRNA Expression in HCC from Patient No. 9

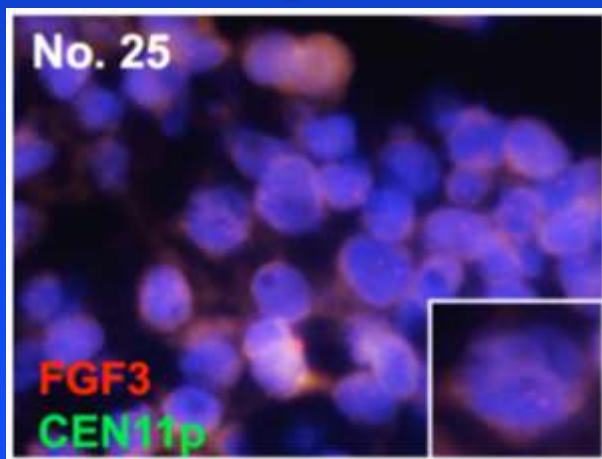


FISH Analysis for FGF3 Amplification

FGF3 Amplification +

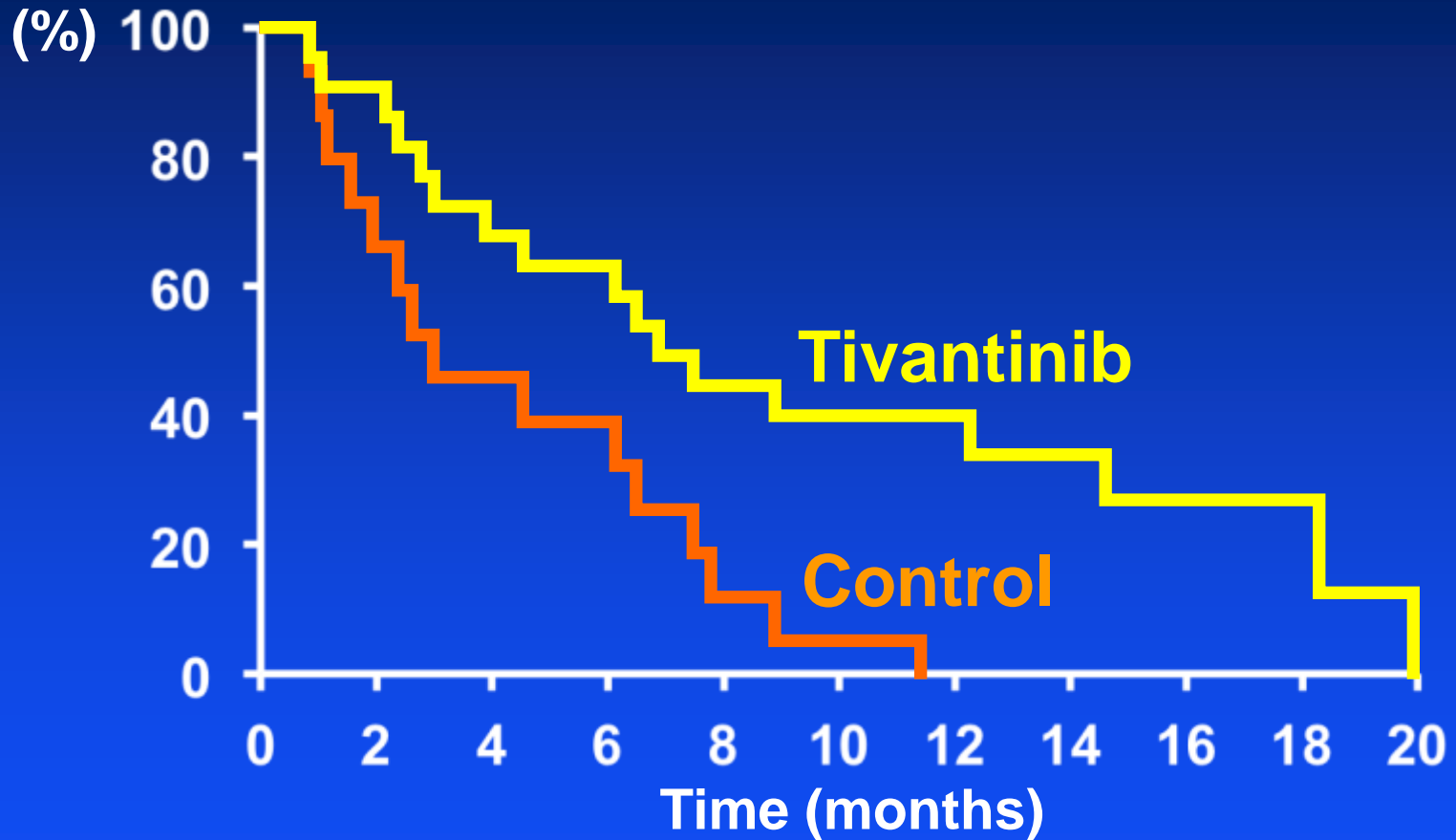


FGF3 Amplification –



MET Expression: Biomarker for Predicting Response to Tivantinib (MET Inhibitor)

Survival of High MET subgroup

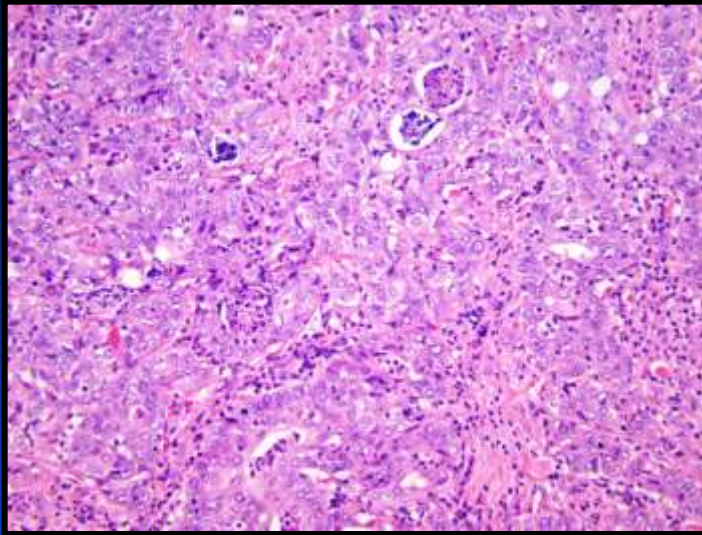


The Way Forward

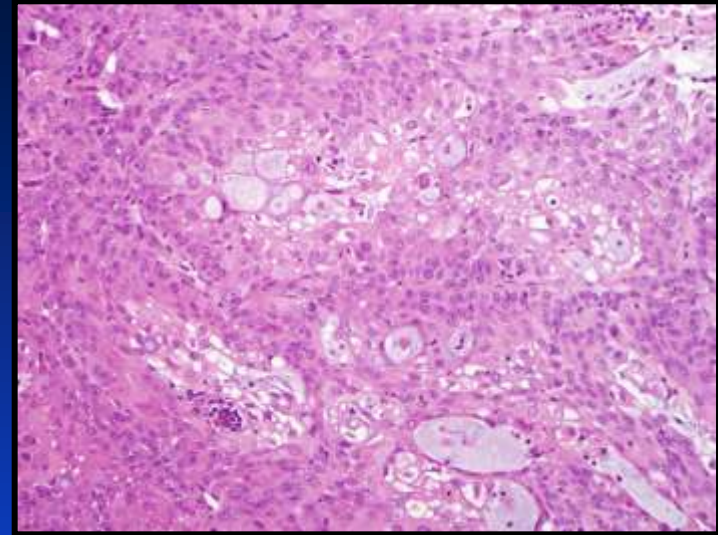
- **Still only a limited number of HCCs of any etiology/geographic origin analyzed, significantly limiting statistical analysis (eg. West Africans in France; Asians in US)**
- **Need enhanced biorepositories and collaborative studies to increase validity of findings (EU HEPTROMIC and MODHEP; US NCI TCGA; ICGC initiatives are underway)**
- **Molecular markers for HCC subclasses should be assayed to predict outcome and to ascertain treatment response in defined subsets of patients in clinical trials**

Patient Derived Xenografts as a Tool

Original Tumor



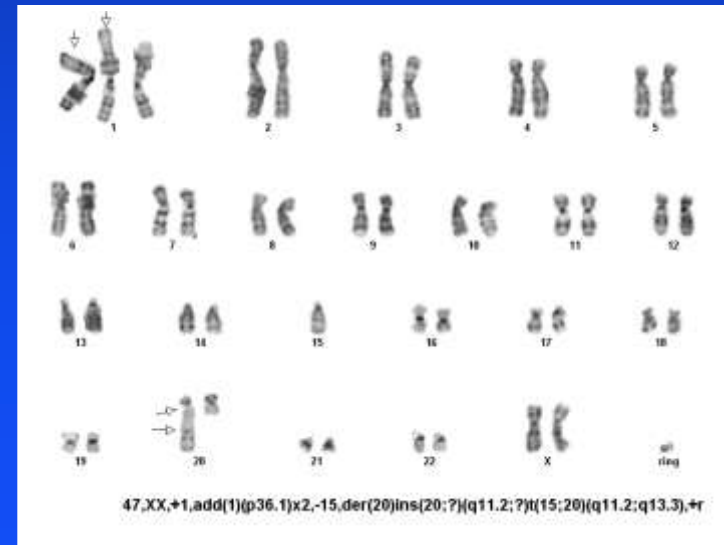
PDX



Tumor in Nude Mouse



Karyotype



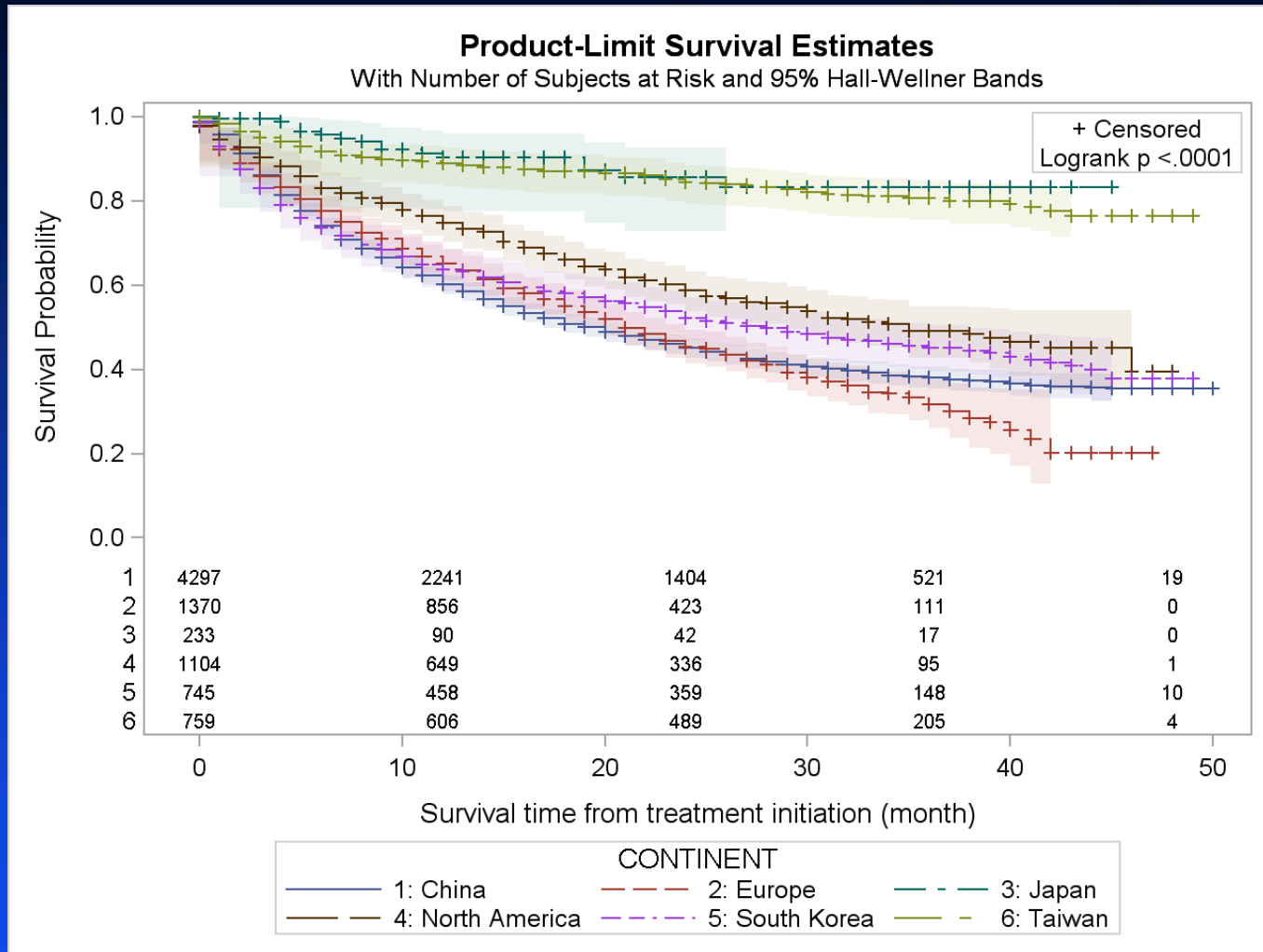
Hepatocellular Carcinoma Epidemiology Consortium Registries and Biorepositories

- Consent patients with hepatobiliary cancers or normal risk factors
- Family history, risk factor and performance status questionnaires
- Obtain serum, plasma and DNA from blood as well as frozen tissue from surgical resections
- Initiate projects to culture fresh tumors in vitro or in immunodeficient mice
- These become a rich resource for basic, translational and epidemiologic research on HCC

Overview

- **Global Epidemiology of HCC**
- **Cancer Hallmarks and Mechanisms – Exhausted Regeneration vs Oncogenic Etiology**
- **Cancer Heterogeneity – Observations and Pitfalls of Molecular Profiling**
- **New Insights from Genetic Testing – SNPs and Next Generation Sequencing**
- **Individualizing Therapy for HCC**
- **Keeping our Eyes on the Prize – Prevention, Early Diagnosis & Therapy**

What we can Learn by Comparing Notes



- Median follow-up time was approximately 24 months for this cohort
- Median OS was not reached for Taiwan and Japan
- Median OS was 35 mo for North America, 28 mo for South Korea, 21 mo for Europe, and 19 mo for China

Thank You

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