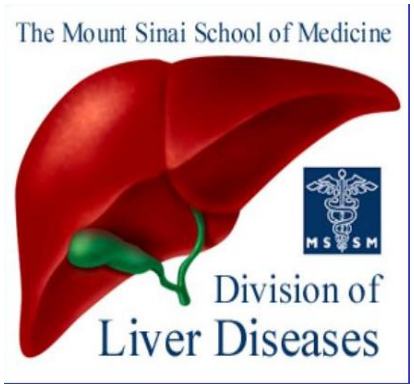

Pathological and molecular changes in hepatocellular carcinoma

Josep M Llovet, MD

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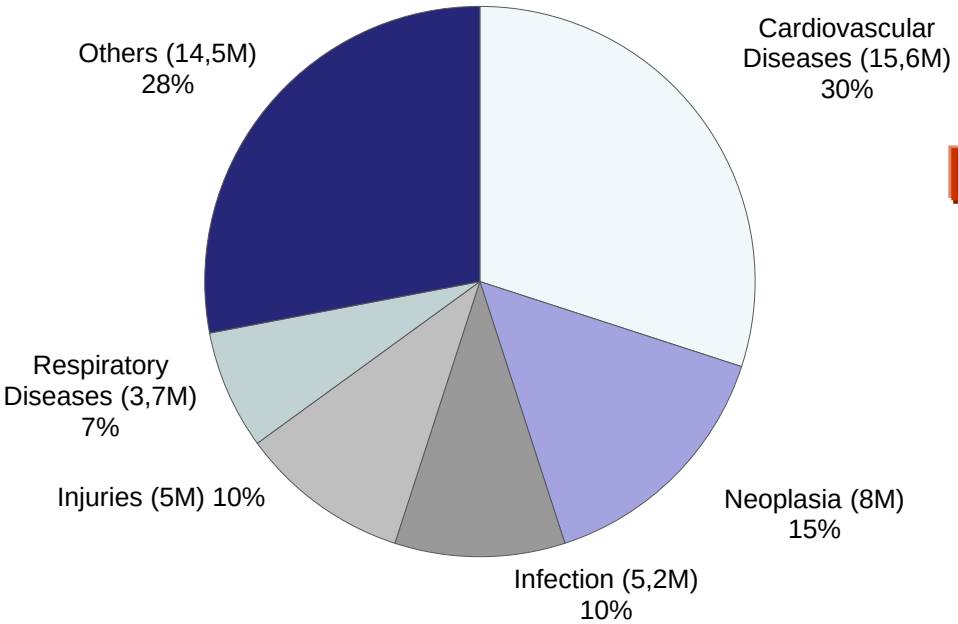
Professor of Medicine. Director of HCC Program. Mount Sinai School of Medicine, NY

Understanding liver cancer

1. Epidemiology and risk factors
2. Molecular traits and subclasses
   - Main genomic alterations
   - Molecular subclasses
3. Sorafenib as standard of care
4. Drugs in phase II-III
5. Personalized medicine: Improve outcomes with molecular therapies targeting specific subclasses

Global causes of death

- Global annual number of deaths: 52M



- Top 20 global causes of death

1 Ischaemic heart disease	1·0 (1 to 1)	35 (29 to 39)
2 Stroke	2·0 (2 to 2)	26 (14 to 32)
3 COPD	3·4 (3 to 4)	−7 (−12 to 0)
4 Lower respiratory infections	3·6 (3 to 4)	−18 (−24 to −11)
5 Lung cancer	5·8 (5 to 10)	48 (24 to 61)
6 HIV/AIDS	6·4 (5 to 8)	396 (323 to 465)
7 Diarrhoea	6·7 (5 to 9)	−42 (−49 to −35)
8 Road injury	8·4 (5 to 11)	47 (18 to 86)
9 Diabetes	9·0 (7 to 11)	93 (68 to 102)
10 Tuberculosis	10·1 (8 to 13)	−18 (−35 to −3)
11 Malaria	10·3 (6 to 13)	21 (−9 to 56)
12 Cirrhosis	11·8 (10 to 14)	33 (25 to 41)
13 Self-harm	14·1 (11 to 20)	32 (8 to 49)
14 Hypertensive heart disease	14·2 (12 to 18)	48 (39 to 56)
15 Preterm birth complications	14·4 (12 to 18)	−28 (-39 to -17)
16 Liver cancer	16·9 (14 to 20)	63 (49 to 78)
17 Stomach cancer	17·0 (13 to 22)	−2 (−10 to 5)
18 Chronic kidney disease	17·4 (15 to 21)	82 (65 to 95)
19 Colorectal cancer	18·5 (15 to 21)	46 (36 to 63)
20 Other cardiovascular and circulatory	19·7 (18 to 21)	46 (40 to 55)

Lozano et al, Lancet 2012

Epidemiology of HCC

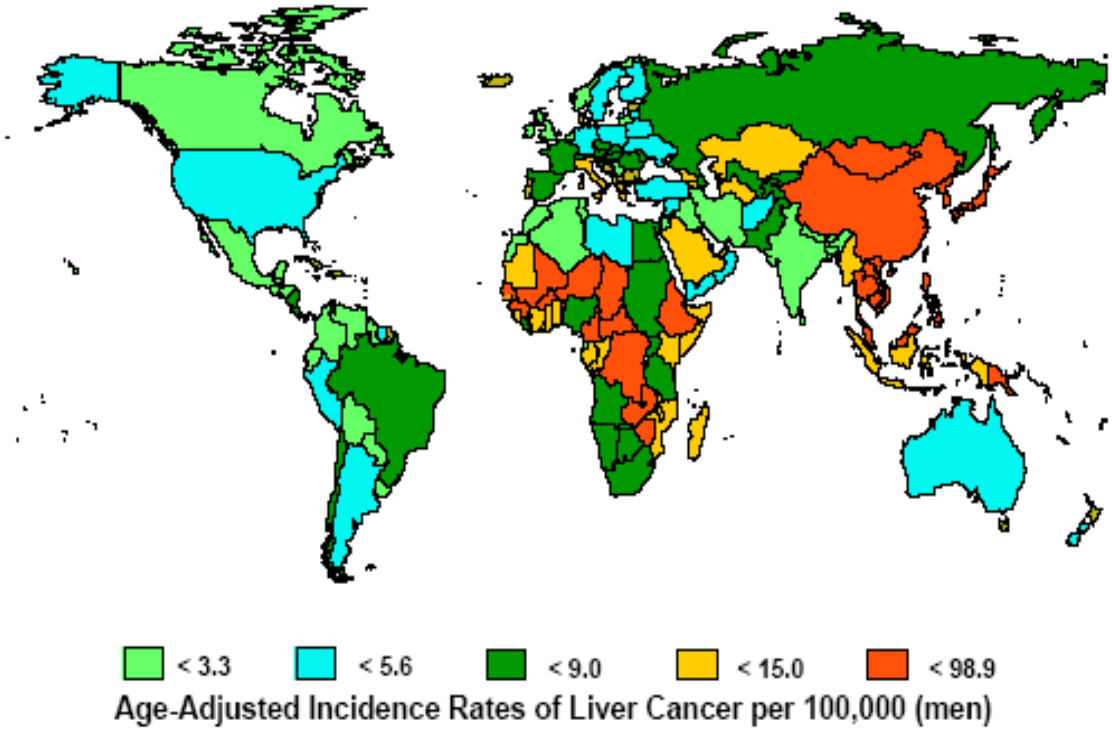
- Liver cancer is the sixth most common cancer globally[1]
- Over 750,000 new cases of liver cancer are diagnosed worldwide each year,[1] including
  - Eastern Asia: 470,000[1]
  - Japan: 39,000[1]
  - Europe: 58,000[1]
  - United States: 21,000[1]
- Liver cancer is the second leading cause of cancer-related mortality,[1] and is the leading cause of death in cirrhotic patients
- The incidence of HCC is increasing globally[2]

1. International Agency for Cancer Research. Globocan 2008. Available at: http://www-dep.iarc.fr. September 2, 2010.
2. Bosch XF, et al. Clin Liver Dis. 2005;9:191-21.

Incidence of HCC

Incidence rates of HCC according to geographical area.

Figure 2. Age-adjusted incidence rates of liver cancer among men. Estimates for the year 2000.[4]



Bosch X et al , Gastroenterology 2004

Risk factors and surveillance

- **Epidemiology and risk factors**

- The incidence of HCC is increasing in Europe and worldwide.
- Vaccination against hepatitis B is recommended to all newborns and high risk groups **(evidence: 2D; recommendation 1A)**
- Governmental health agencies should recommend policies for preventing HCV/HBV transmissions, encourage life styles preventing obesity and alcohol abuse **(evidence 3A; recommendation 1A)** and controlling metabolic conditions, such as diabetes **(evidence 3; recommendation 2B)**
- In patients with chronic hepatitis, antiviral therapies leading to maintained HBV suppression in chronic hepatitis B and sustained viral response in hepatitis C are recommended since they have been shown to prevent progression to cirrhosis, and hence HCC development **(evidence 1A; recommendation 1A)**. The application of antiviral therapies should follow the EASL guidelines for management of chronic hepatitis B and C infection
- Once cirrhosis is established, the benefits of anti-viral therapy in preventing HCC development are not robustly demonstrated **(evidence 1D; recommendation 2B)**

- **Risk factors**

Geographic area	AAIR	Risk factors		Alcohol	Others
	M/F	HCV (%)	HBV (%)	(%)	(%)
Europe	6.7/2.3	60-70	10-15	20	10
Southern	10.5/3.3				
Northern	4.1/1.8				
North America	6.8/2.3	50-60	20	20	10 (NASH)
Asia and Africa		20	70	10	10 (Aflatoxin)
Asia	21.6/8.2				
China	23/9.6				
Japan	20.5/7.8	70	10-20	10	10
Africa	1.6/5.3				
WORLD	16/6	31	54	15	

- **Surveillance: Target population**

1. Cirrhotic patients, Child-Pugh stage A and B*
2. Cirrhotic patients, Child-Pugh stage C awaiting liver transplantation**
3. Non-cirrhotic HBV carriers with active hepatitis or family history of HCC***
4. Non-cirrhotic patients with chronic hepatitis C and advanced liver fibrosis F3****

Understanding liver cancer

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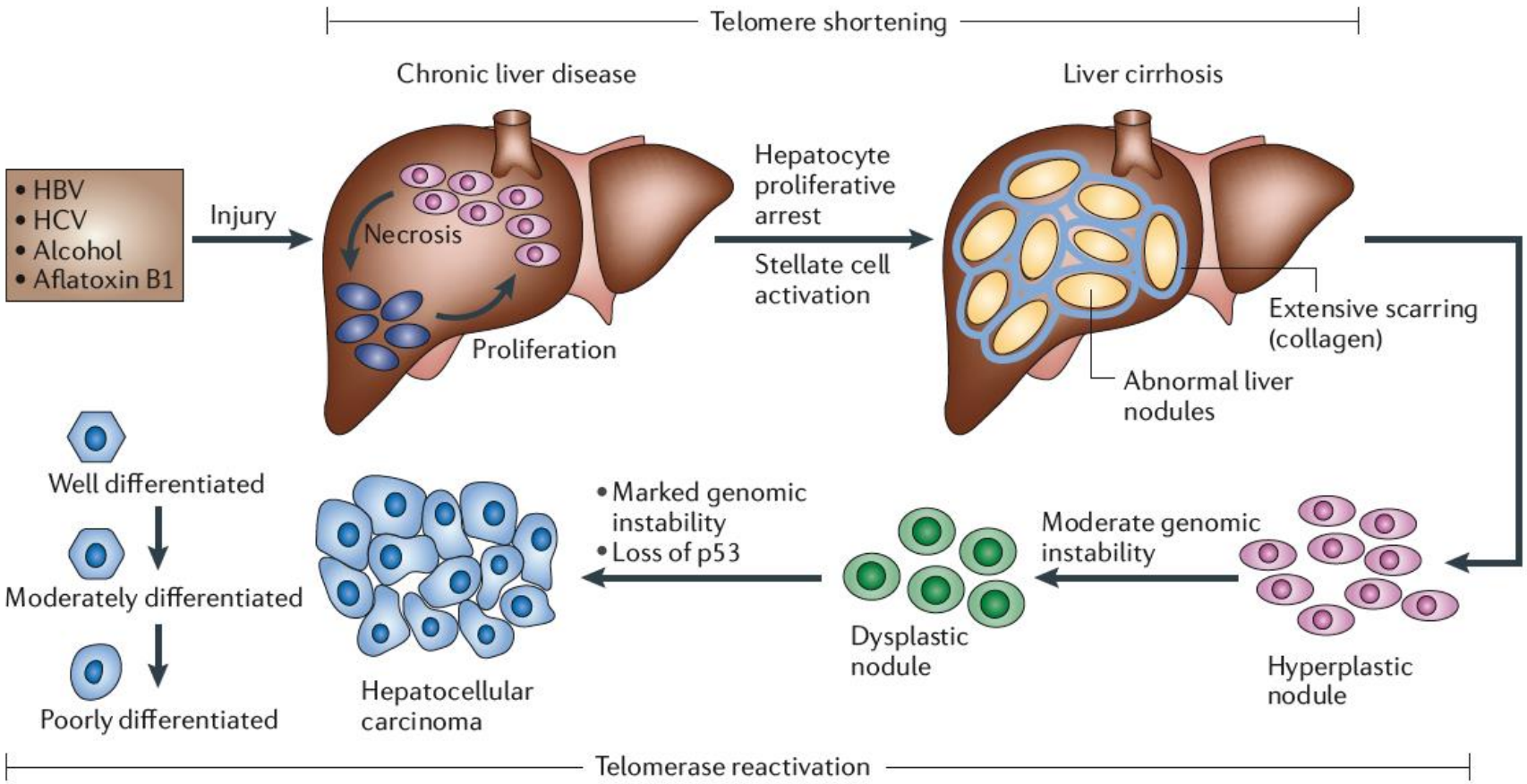
4. Drugs in phase II-III
5. Personalized medicine: Improve outcomes with molecular therapies targeting specific subclasses

The carcinogenic process

Hepatocellular carcinoma
Colorectal cancer



Vogelstein et al, Science 2013
Farazi PA, et al. Nat Rev Cancer. 2006

The hepatocarcinogenic process

HCV and HBV pathogenic mechanisms

HCV: (advanced fibrosis)

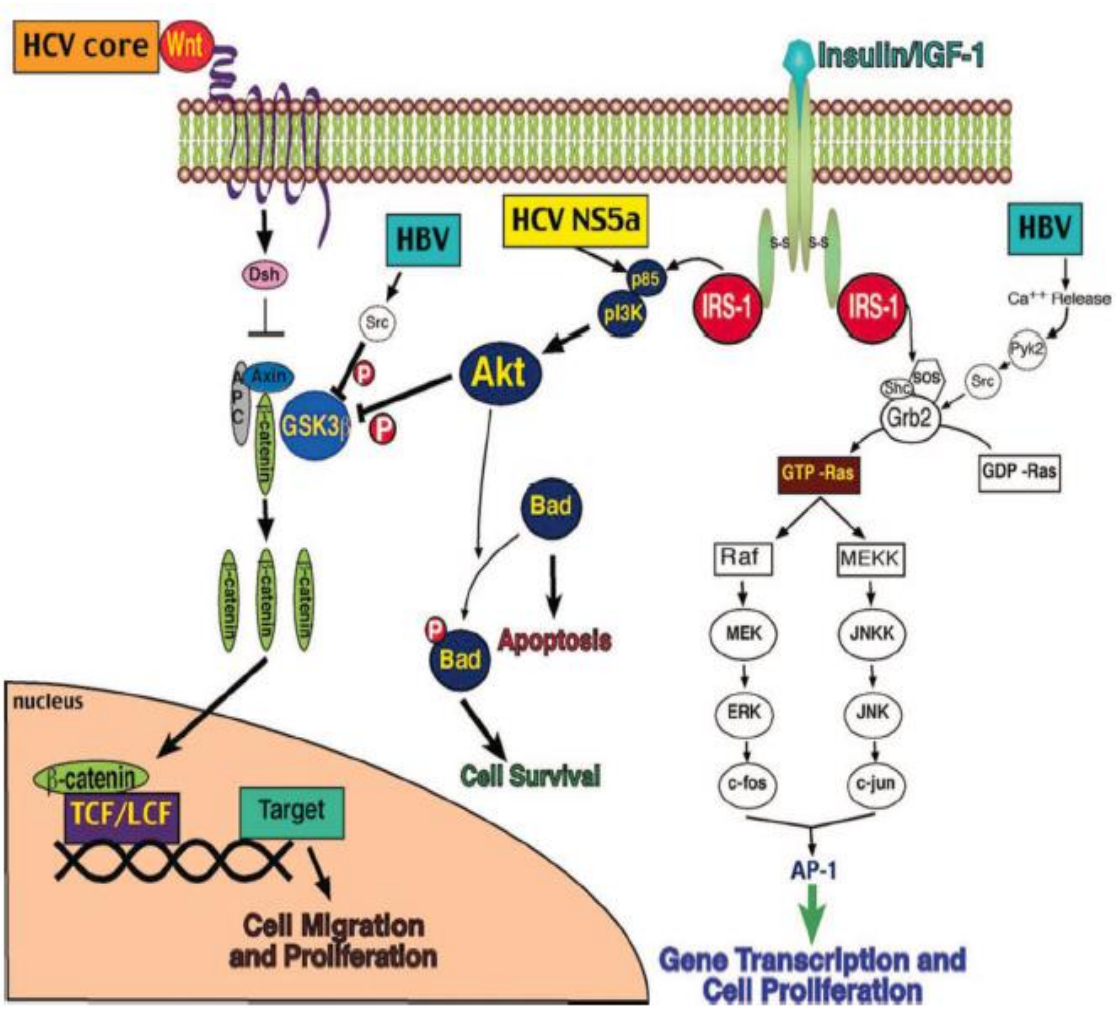
- Impairs immune fuctions
- **HCV core: Wnt activation**

  Mutation CTNNB1

  Raf,ERK activation
- HCV NS5: Activates akt
- Inactivation p53

HBV

- Integration genome
- Microdeletions TERT, PDGF
- **Inactivation /mutation of p53**
- Activation of Ras
- HBx protein: Transgenic mice

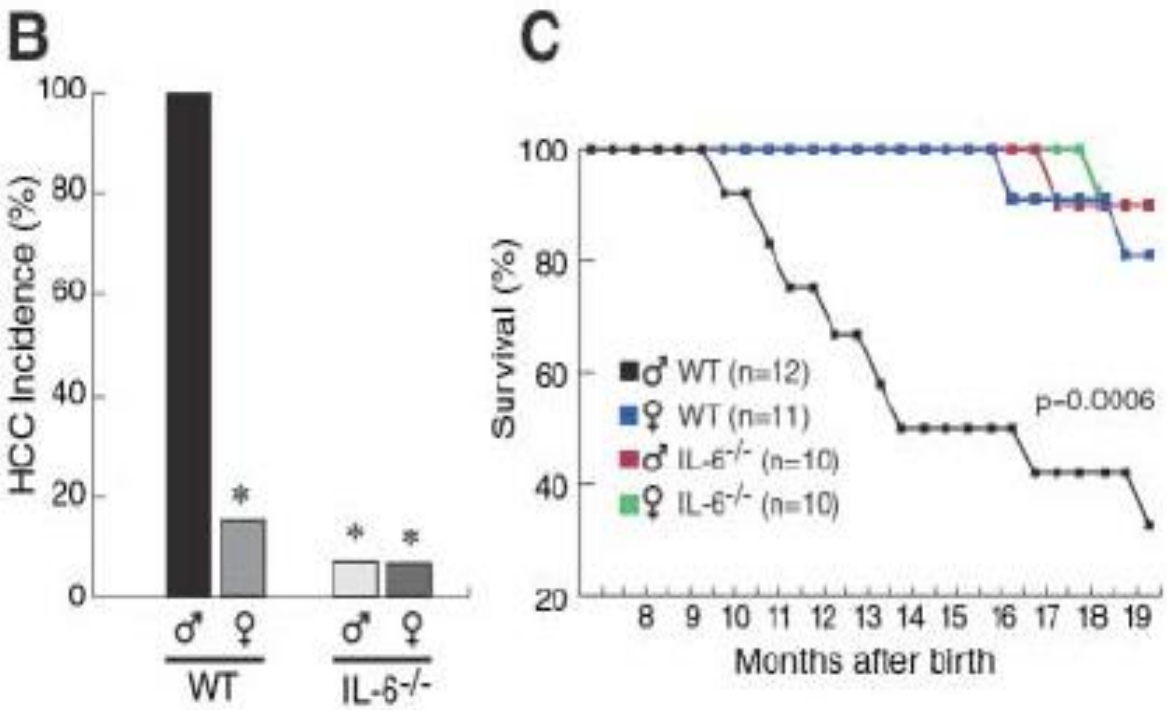


Branda M, Wands JR, Hepatology. 2006;43:891-902.
Levrero M, Oncogene. 2006;25:3834-47.

Gender as a risk factor for HCC

- Gender distribution of HCC : Male/Female ratio, 3:1
- Experimental model (DEN): <u>IL-6 mediated HCC</u>

  1. Greater concentrations of IL-6 in male>female produced by Kupffer cells.
  2. Estrogens inhibited secretion of IL-6, and decreased incidence of HCC.



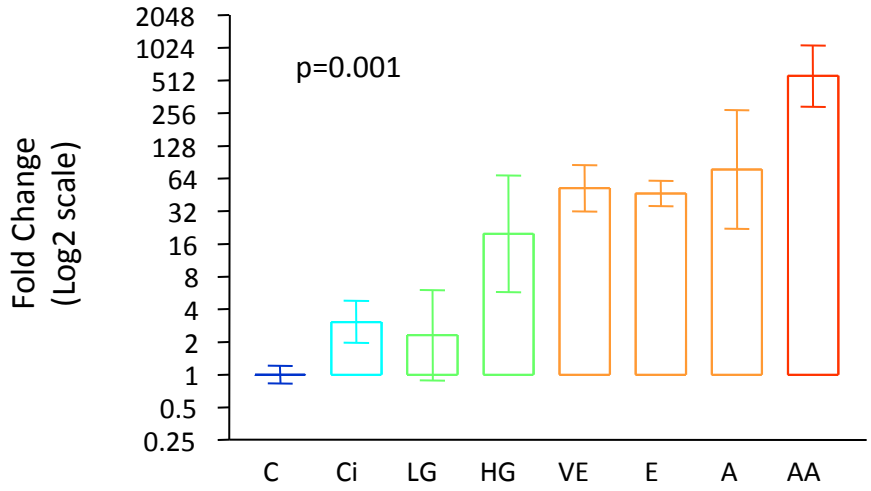
Naugler WE, et al. Science. 2007;317:121-4

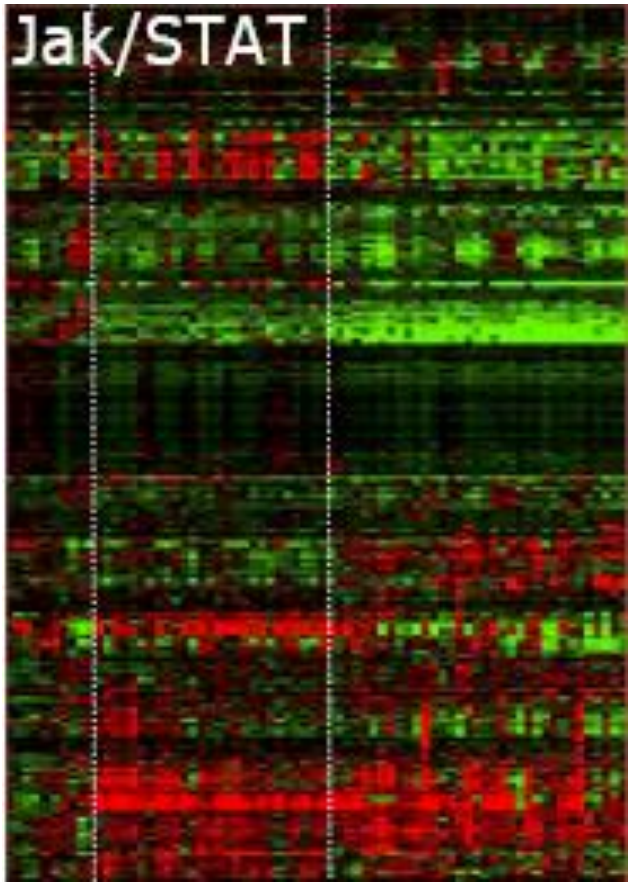
Pathogenesis of HCC

Dysplastic nodules

- Genomic alterations*:
  - Activation of Jak/Stat
  - Activation of TERT

Telomerase reverse transcriptase





C Dysplastic HCC

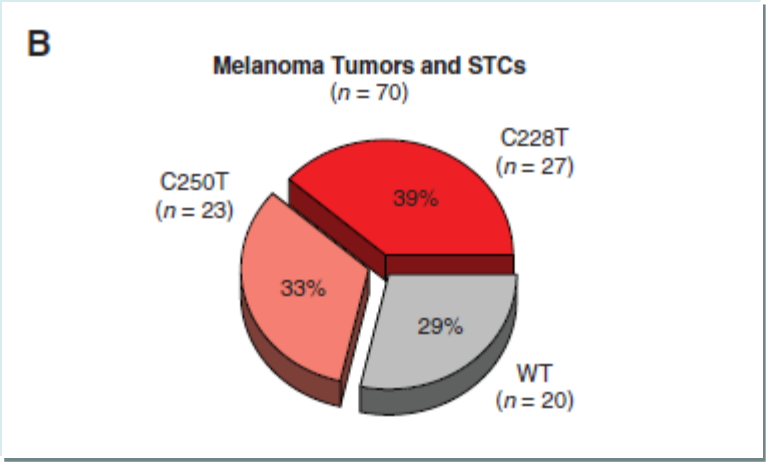
* Chromosome aberrations (<30%): Gain: 1q, 17q; loss: 16q,4q,17p

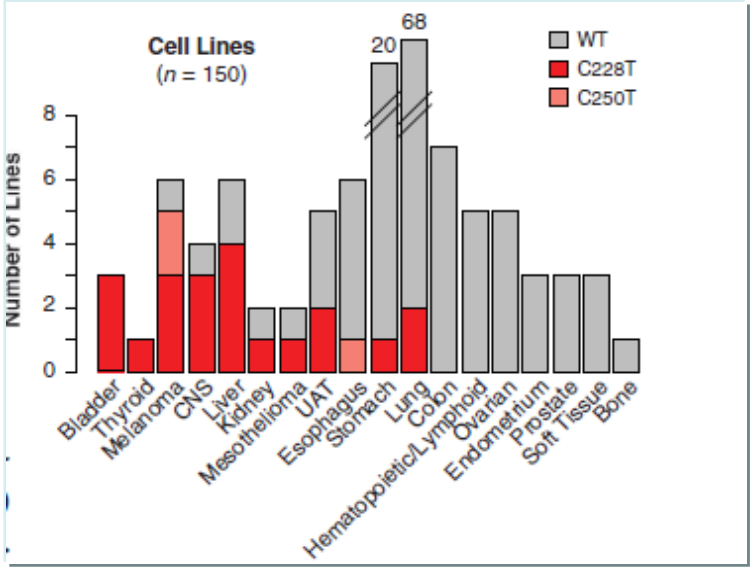
Wurmbach E, et al. Hepatology. 2007;45:938-47.

Pathogenesis of HCC

TERT promoter mutations

TERT promoter mutations- Melanoma (71%)

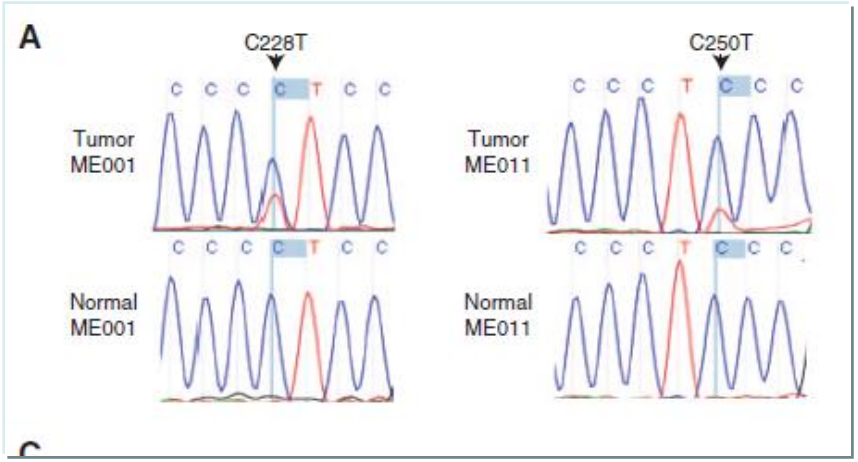




Huang et al, Science 2013

TERT promoter mutations-
HCC (60%)
Dysplastic nodules (25%)

- HCC (60% +): Significantly associated to :
  - TERT over expression
  - non-HBV infection
  - CTNNB1 ($p<0.0001$)
- Dysplastic nodules (25%+) associated to:
  - TERT overexpression
  - **First gatekeeper!!**



Nault et al, Nature comunications 2013

Major achievements in cancer genomics

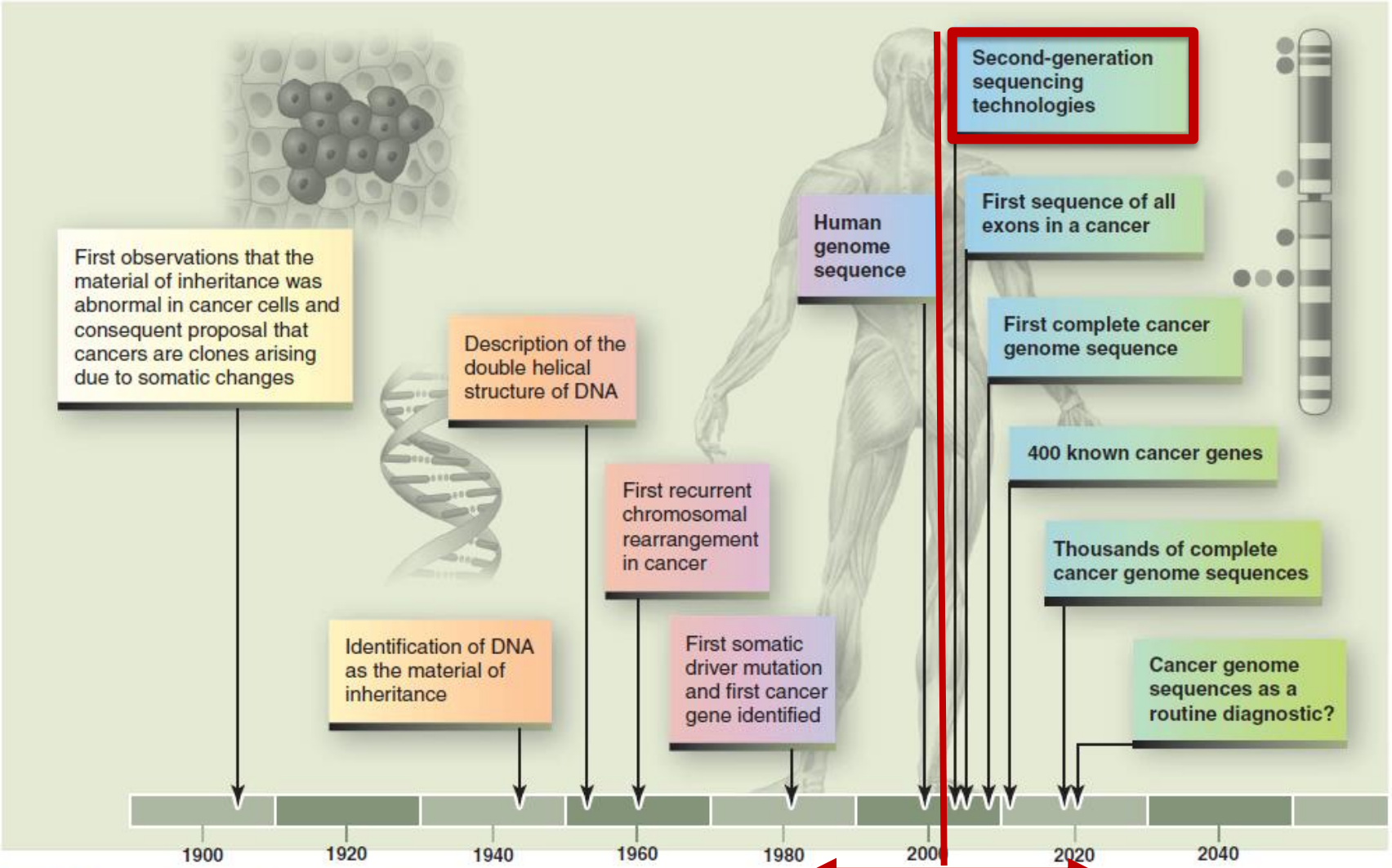
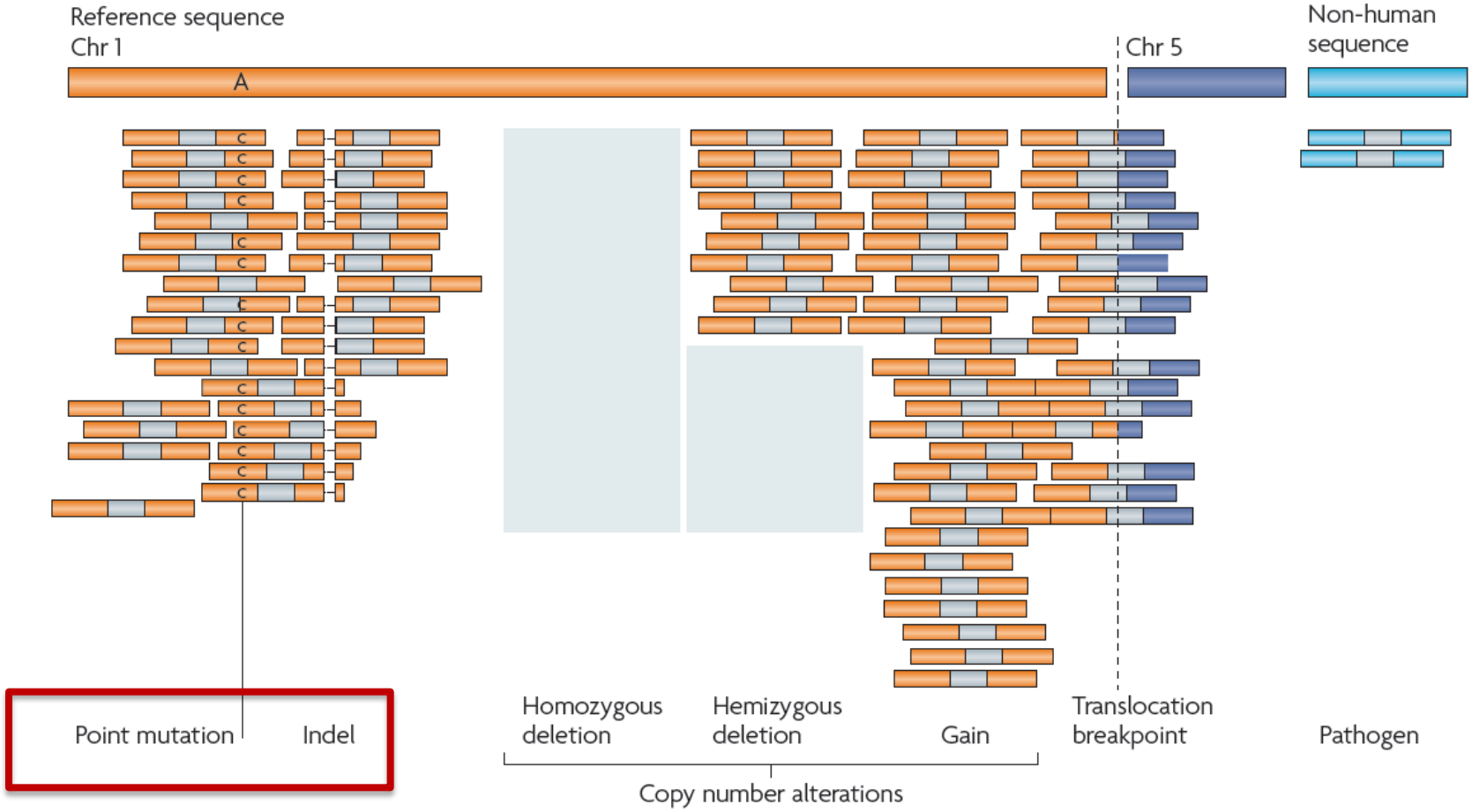


Fig. 1. Time line showing key events in the investigation of the cancer genome.

Next generation seq (RNA-Seq,Exome seq*)*
Meyerson, Nature Reviews 2010

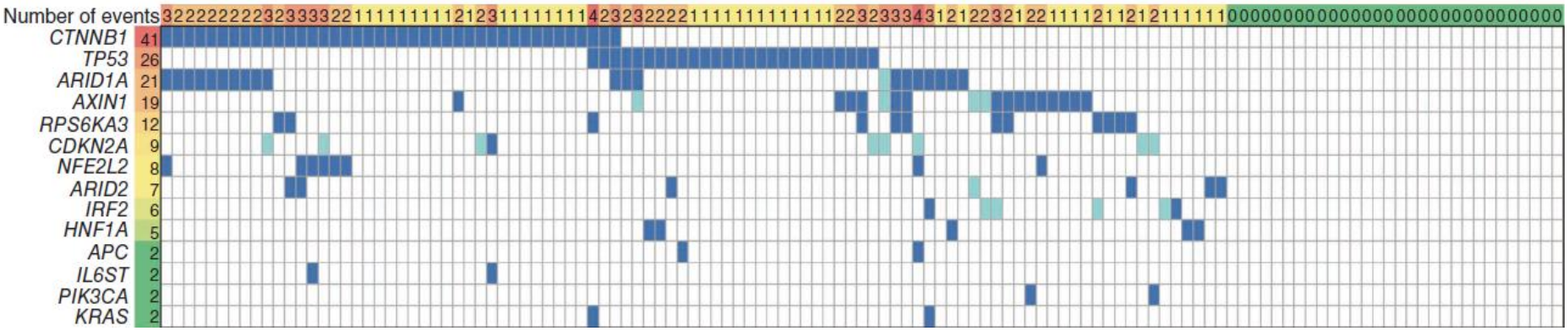
Stratton et al, Science 2011

Types of genomic alterations-2nd generation sequencing



Beroukim, Nature 2010

Genome sequencing in HCC



2nd generation sequencing (4 studies)

Known mutations: CTNNB1: 72/301: 24% (non-HBV),
p53: 109/ 401: 27% (HBV)
Axin:15/172 : 8%
RAS (~5%)
PI3k (3%)

Novel: ARID 1a: 49/392 :12%
ARID 1b: 19/341: 5.6%
RPS6KA3 9% (non-cirrhotics)

Fujimoto et al, Lin et al, Huang et al ;Guichard et al, Nat Gen 2012

Driver mutations in cancer

- Mutation-driver genes in cancer: 140
- Mutation-driver genes in HCC

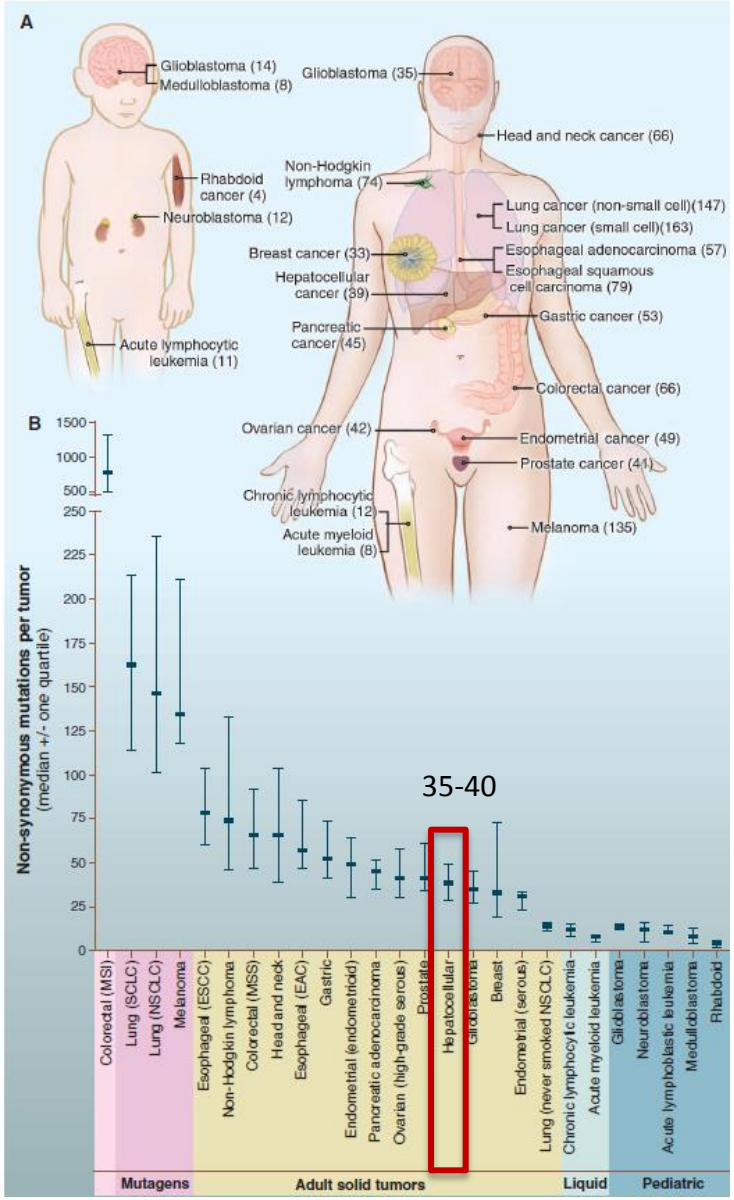
Table 2. Discoveries from Cancer Genome Characterization

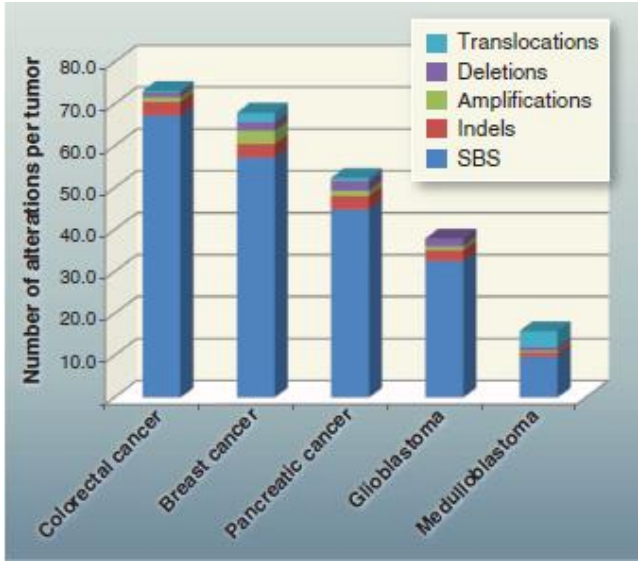
Cellular Process Altered by Genomic Alterations	Examples of Cancer Genes Discovered (or Extended to New Cancers*) by Genomics	
RTK signaling	*EGFR*,[a] *ERBB2*,*[,a] *MET*,*[,a] *ALK*,*[,a] *JAK2*,[a] *RET*,*[,a] *ROS*,*[,a] *FGFR1*,*[,a] *FGFR2*,[a] *PDGFRA*,*[,a] and *CRKL*[a]	<5%
MAPK signaling (oncogenes)	*KRAS*,*[,a] *NRAS*,*[,a] *BRAF*,[a] and *MAP2K1*[a]	<5%
MAPK signaling (TSG)	*NF1**[,b]	-
PI3K signaling (oncogenes)	*PIK3CA*,[a] *AKT1*,[a] and *AKT3*[a]	-
PI3K signaling (TSG)	*PTEN**[,b] and *PIK3R1*[b]	-
Notch signaling (oncogene or TSG)	*NOTCH1*,[c] *NOTCH2*,[c] and *NOTCH3*[b]	4%
TOR signaling (TSG)	*STK11*,*[,b] *TSC1*,*[,b] and *TSC2**[,b]	-
Wnt/β-catenin signaling (TSG)	*APC**[,b] and *CTNNB1**[,a]	24%
TGF-β signaling (TSG)	*SMAD2*,*[,b] *SMAD4*,*[,b] and *TGFBR2*[b]	-
NF-κB signaling (oncogene)	*MYD88*[a]	-
Other signaling	*RAC1*,[a] *RAC2*,[a] *CDC42*,[a] *KEAP1*,[b] *MAP3K1*,[b] *MAP2K4*,[b] *ROBO1*,[b] *ROBO2*,[b] *SLIT2*,[b] *SEMA3A*,[b] *SEMA3E*,[b] *ELMO1*,[d] and *DOCK2*[d]	-
Epigenetics DNA methylation	*DNMT3A*[b]	-
Epigenetics DNA hydroxymethylation	*TET2*[b]	-
Chromatin histone methyltransferases	*MLL*,*[,b] *MLL2*,[b] *MLL3*,[b] *EZH2*,[c] *NSD1*,[b] and *NSD3*[b]	4%
Chromatin histone demethylases	*JARID1A*,[b] *UTX*,[b] *KDM5A*,[b] and *KDM5C*[b]	-
Chromatin histone acetyltransferases	*CREBP*[b] and *EP300*[b]	-
Chromatin SWI/SNF complex	*SMARCA1*,*[,b] *SMARCA4*,[b] *ARID1A*,[b] *ARID2*,[b] *ARID1B*,[b] and *PBRM1*[b]	19%
Chromatin other	*CHD1*,[b] *CHD2*,[b] and *CHD4*[b]	-
Transcription factor lineage dependency or oncogene	*MITF*,[a] *NKX2-1*,[a] *SOX-2*,[a] *ERG*,[a] *ETV1*,[a] and *CDX2*[a]	-
Transcription factor other	*MYC*,*[,a] *RUNX1*,[b] *GATA3*,[b] *FOXA1*,[b] *NKX3.1*,[b] *SOX9*,[a] *NFE2L2*,[a] and *MED12*[d]	6%
Splicing	*SF3B1*,[d] *U2AF1*,[d] *SFRS1*,[d] *SFRS7*,[d] *SF3A1*,[d] *ZRSR2*,[b] *SRSF2*,[d] *U2AF2*,[d] and *PRPF40B*[d]	-
RNA abundance	*DIS3*[d]	-
Translation/protein homeostasis/ubiquitination	*SPOP*,[d] *FBXW7*,*[,b] *WWP1*,*[,b] *FAM46C*,[d] and *XBP1*[d]	-
Metabolism	*IDH1*[a] and *IDH2*[a]	34%
Genome integrity	*TP53*,*[,b] *MDM2*,[a] *MSH*,*[,b] *MLH*,*[,b] and *ATM**[,b]	60%
Telomere stability	*TERT* promoter mutations[a]	7%
Cell cycle (oncogene)	*CCND1**[,a] and *CCNE1**[,a]	7%
Cell cycle (TSG)	*CDKN2A*,*[,b] *CDKN2B*,*[,b] and *CDKN1B*[b]	-
Apoptosis regulation	*MCL1*,[a] *BCL2A1*,[a] and *BCL2L1*[a]	

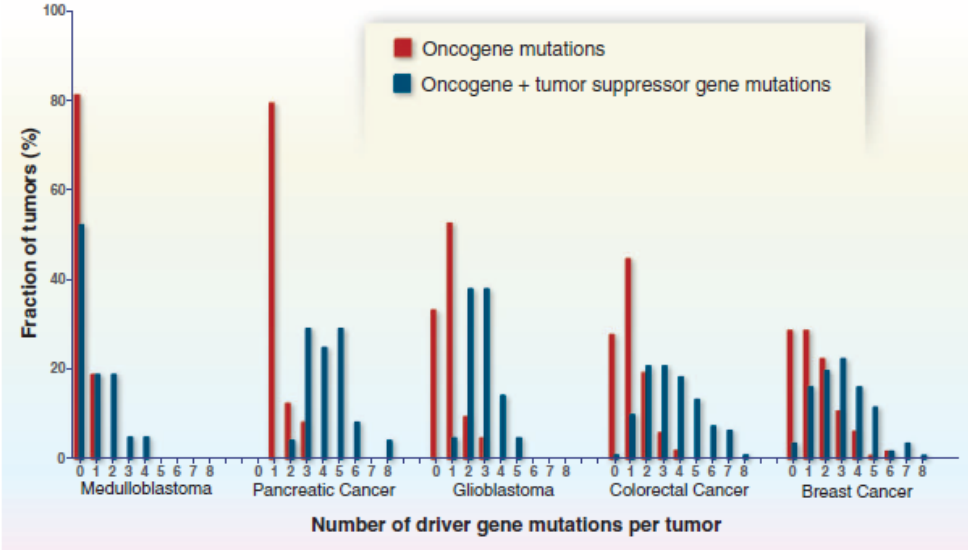
[a]Activating mutation or amplification.
[b]Inactivating mutation or deletion.
[c]Both activating and inactivating genomic events observed.
[d]Effect of mutations on protein function unknown.

Garraway et al, Cell 2013

Landscape of mutations in cancer

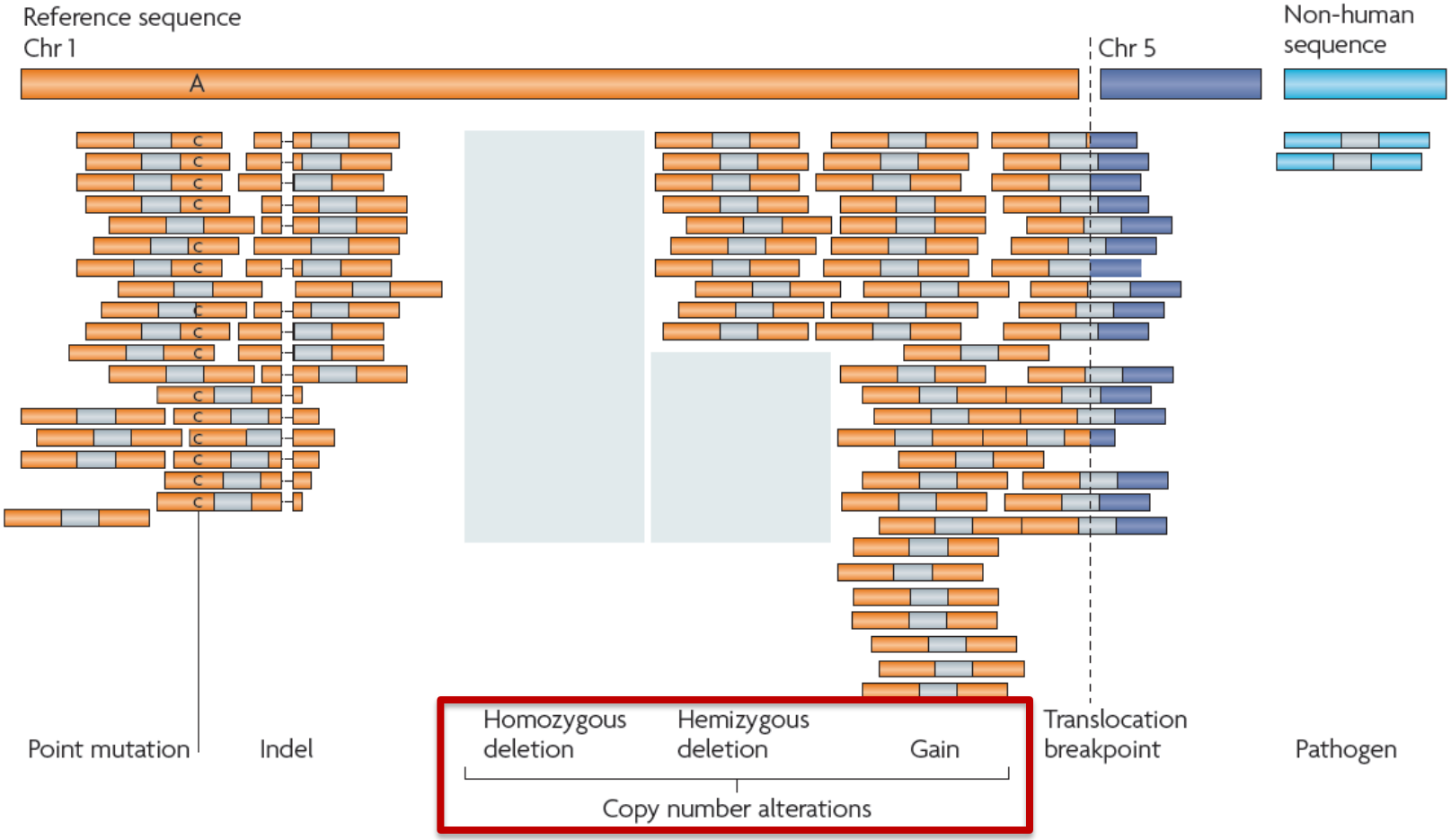






Vogelstein et al, Science 2013

Types of genomic alterations-2nd generation sequencing



Beroukim, Nature 2010

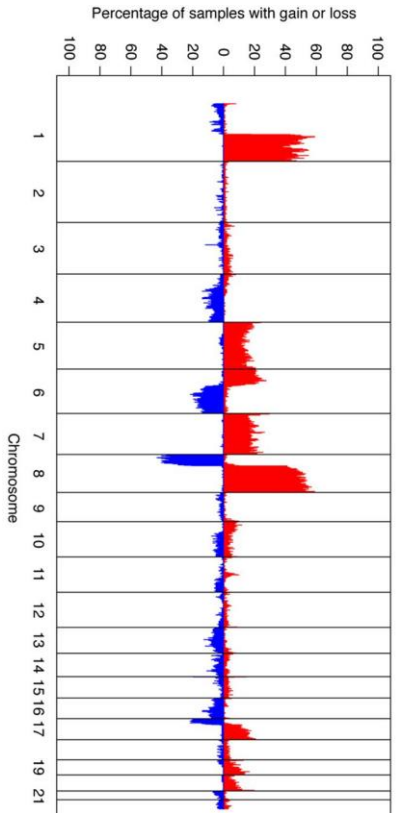
The hepatocarcinogenic process

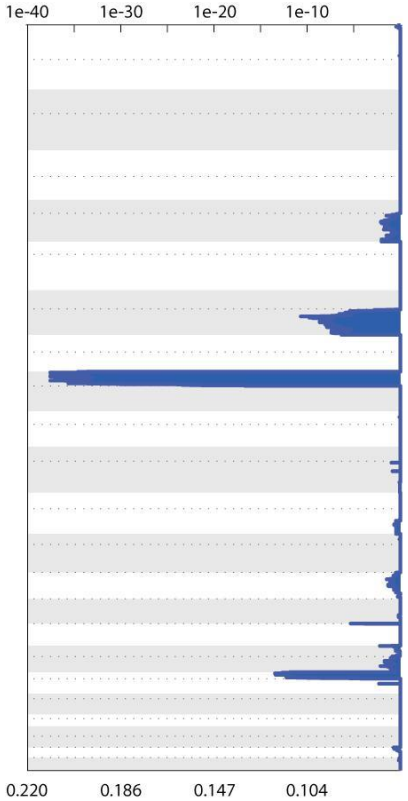
Structural alterations-summary

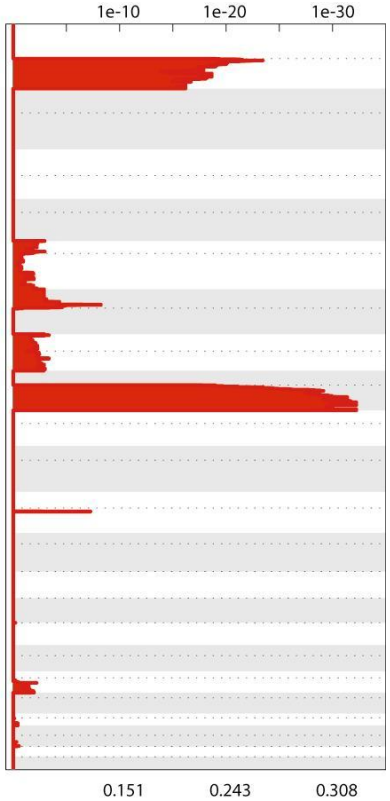
1. Structural alterations are present in almost all advanced HCC (HBV > HCV)

2. Gains in 1q21-23 and 8q 22-24 are common at early stages

3. High-level amplifications: 6p21 (VEGF) and 11q13 (Cyclin D1 and FGF19): 5-10%





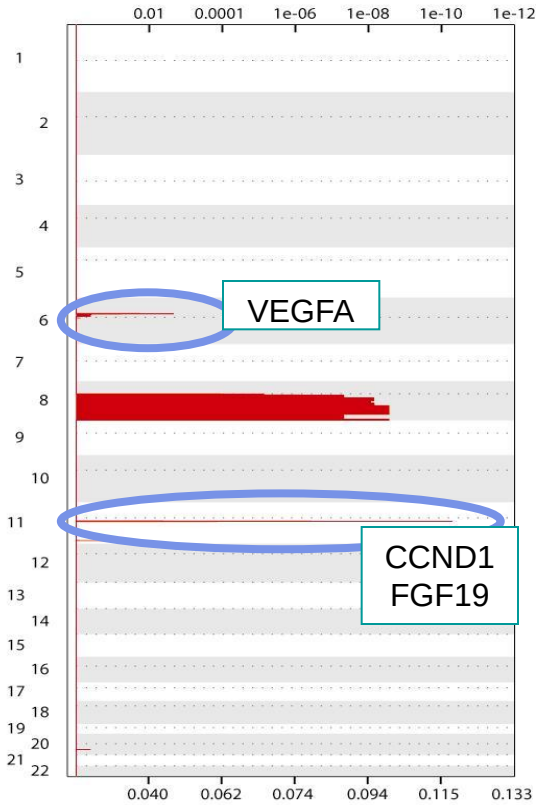


Chiang DY, et al. Cancer Res. 2008;68:6779-88

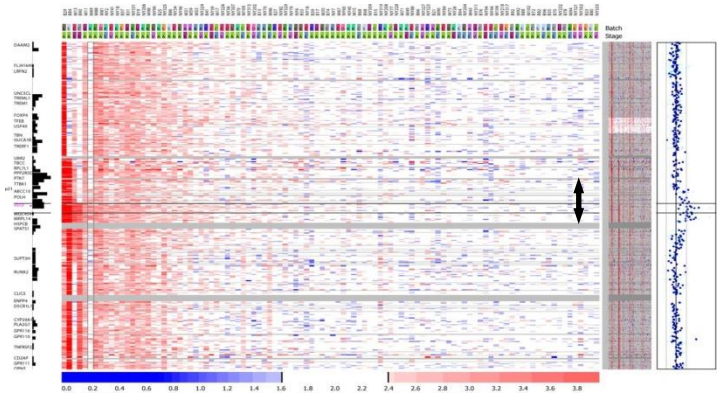
Molecular targets for HCC

Signaling pathways: molecular targets for new therapies.

High-level amplifications of 6p21 and 11q13 (HCC=103)

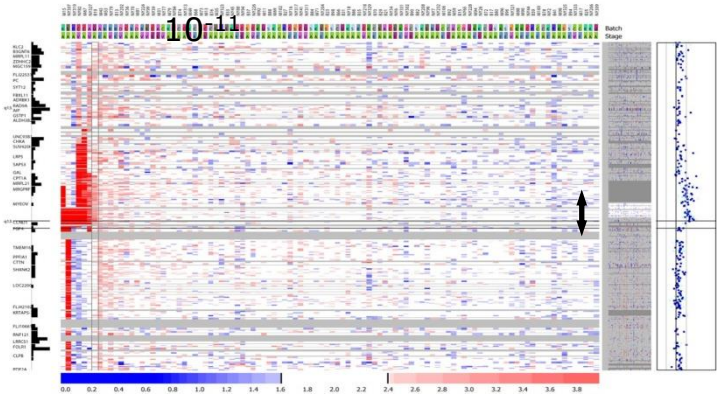


6p21.1 **4 samples**, *q*-val 2 x 10^{-3}



631 kb

11q13 **7 samples**, *q*-val 5 x 10^{-11}



470 kb

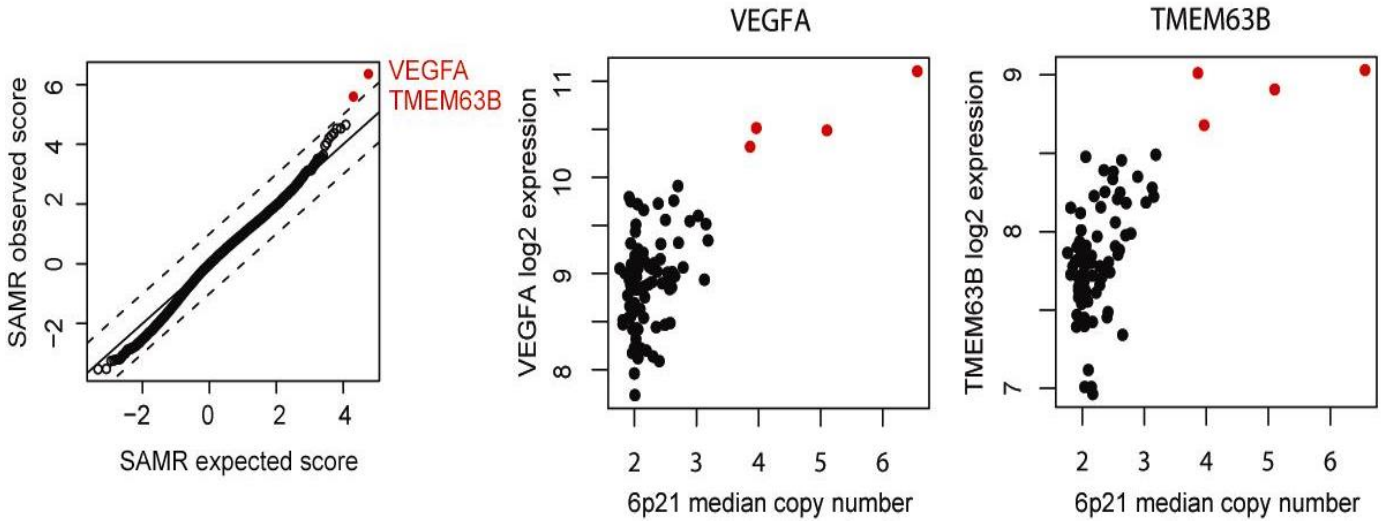
Chiang D et al, Cancer Research, 2008

Molecular targets for HCC

Integrative genomic analysis in 6p21 high-level amplifications

Focal VEGFA amplification

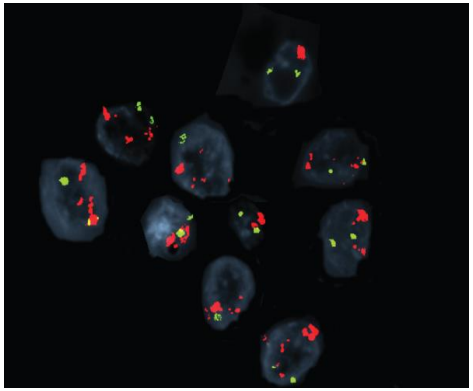
Training set : 4/103 (3.9%) HCV-HCC ***(SNP array; inferred copy number > 3.73)***



Validation set (samples all etiologies-HCC) ***(FISH)***

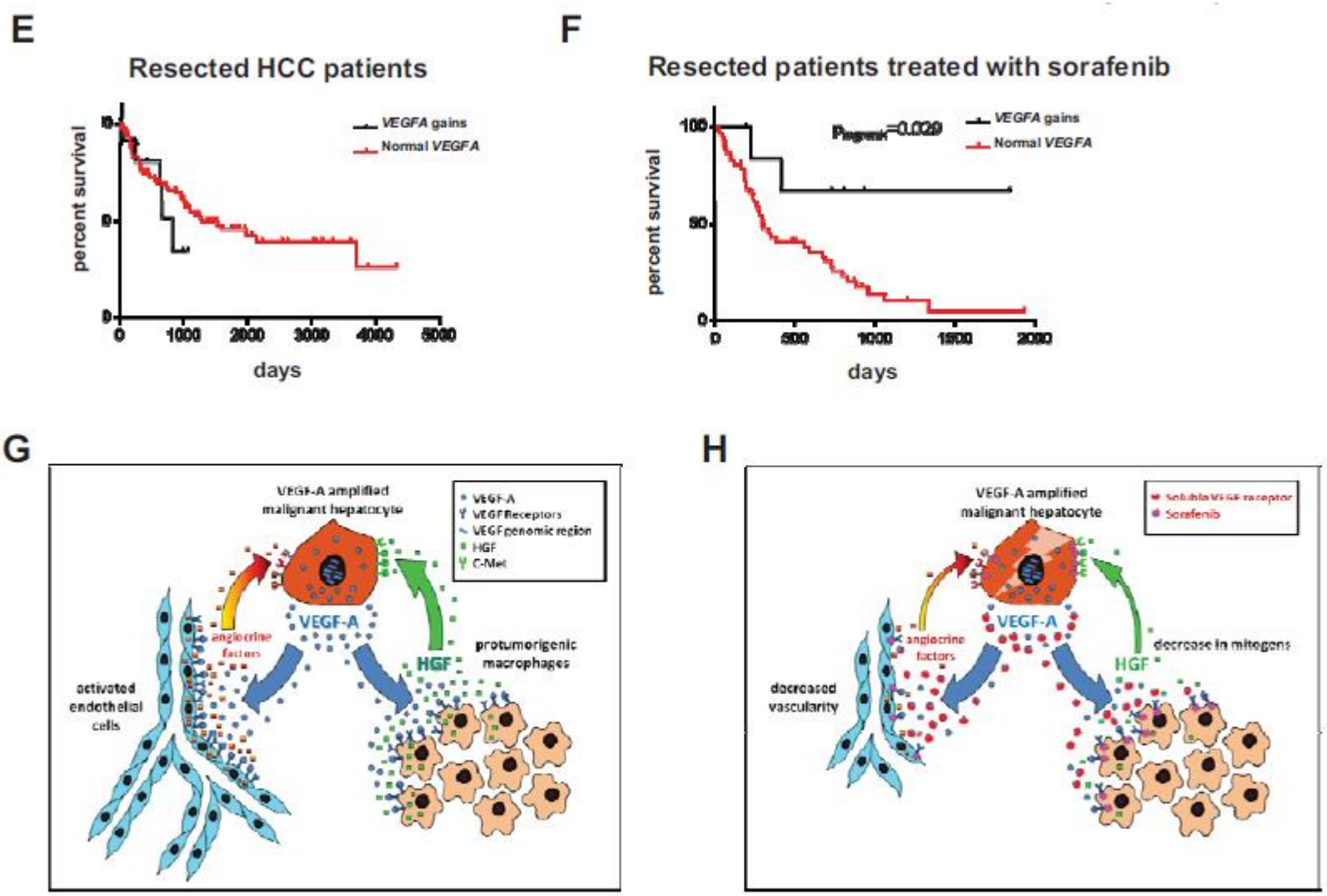
2/210 (1%) VEGF amplification

16/210 (7.7%) high-level gains



Molecular targets for HCC

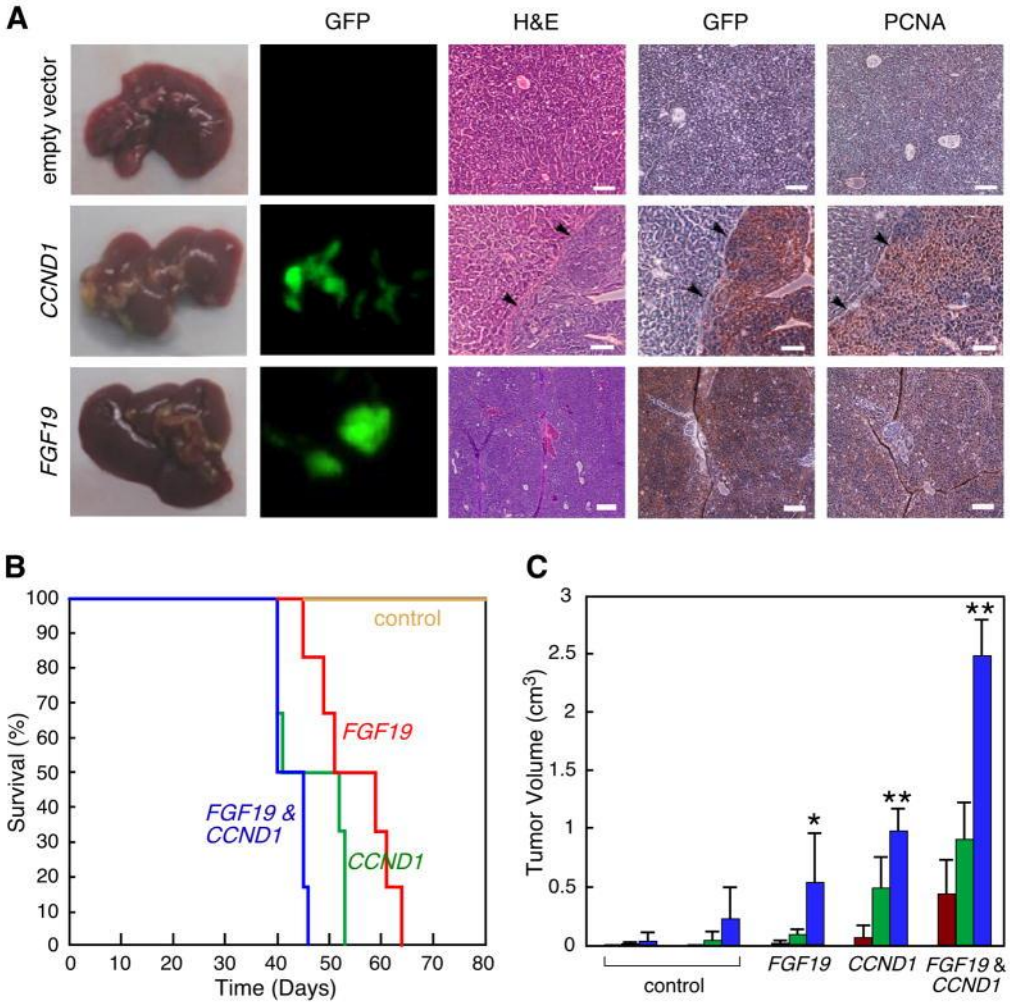
6p.21 amplifications: VEGF-A responding to sorafenib



Horwitz, Cancer Discovery 2014

Molecular targets for HCC

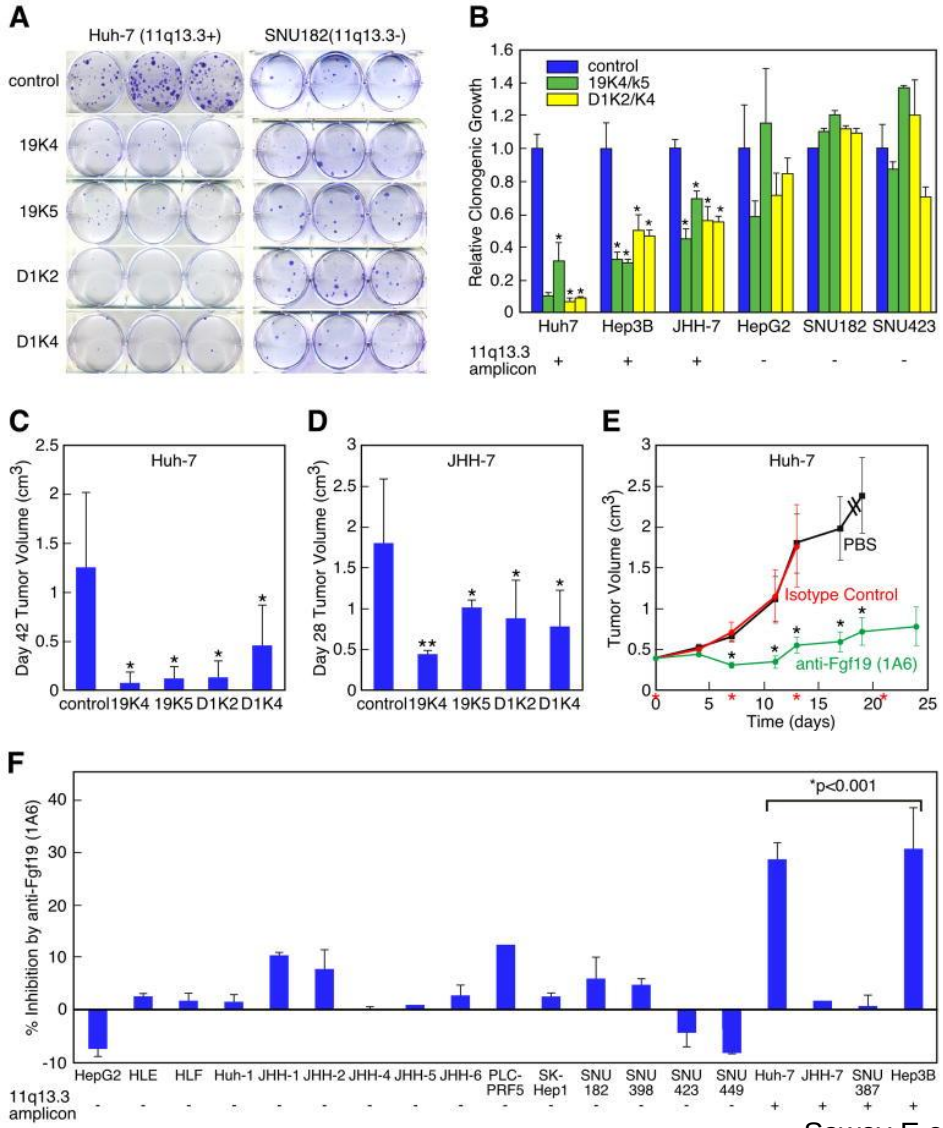
High-level amplification 11q13: FGF19 & CCND1



Sawey E et al, Cancer Cell 2011

Molecular targets for HCC

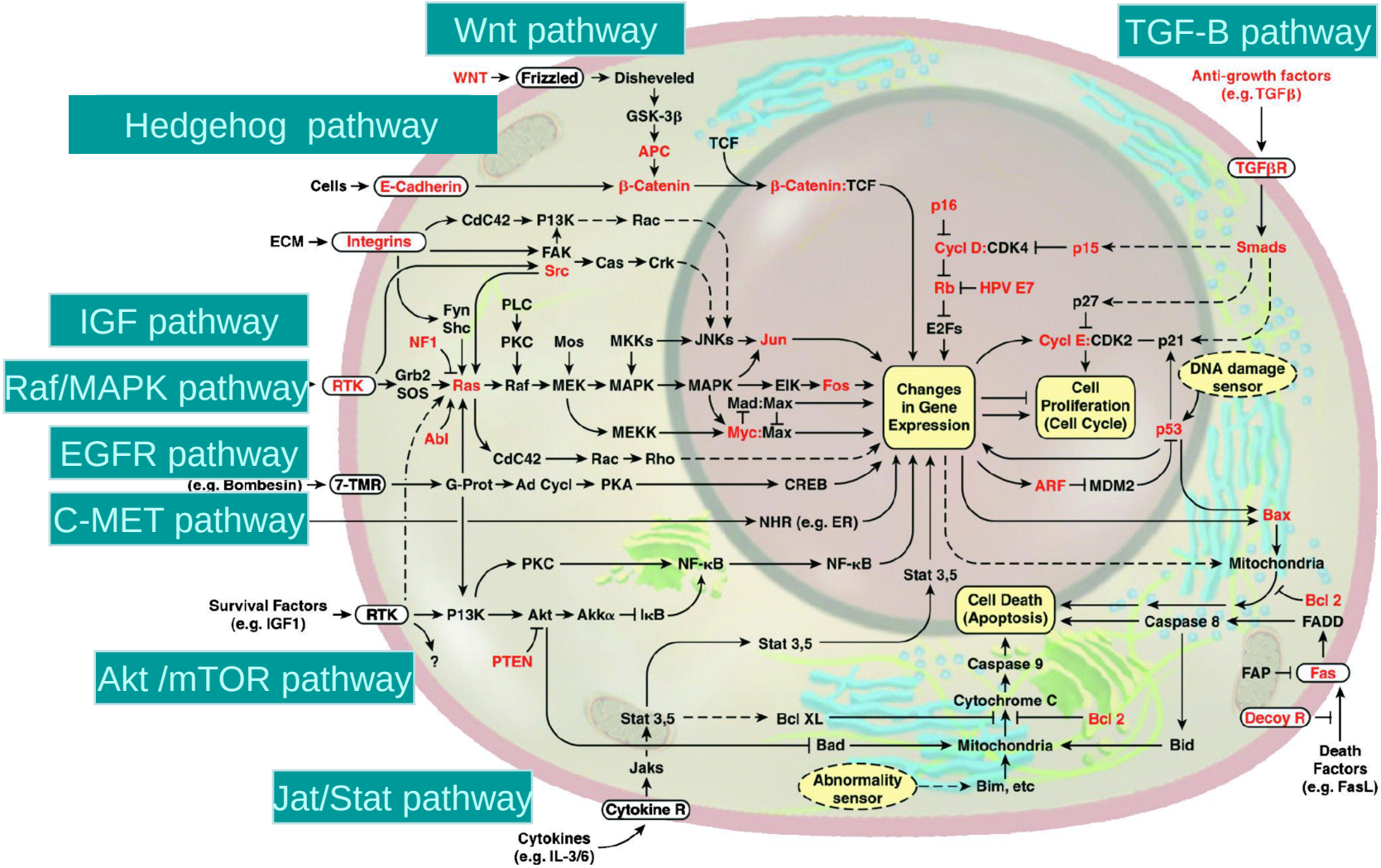
High-level amplification 11q13: FGF19 & CCND1



Sawey E et al, Cancer Cell 2011

Molecular targets for cancer

Signaling pathways: molecular targets for new therapies.

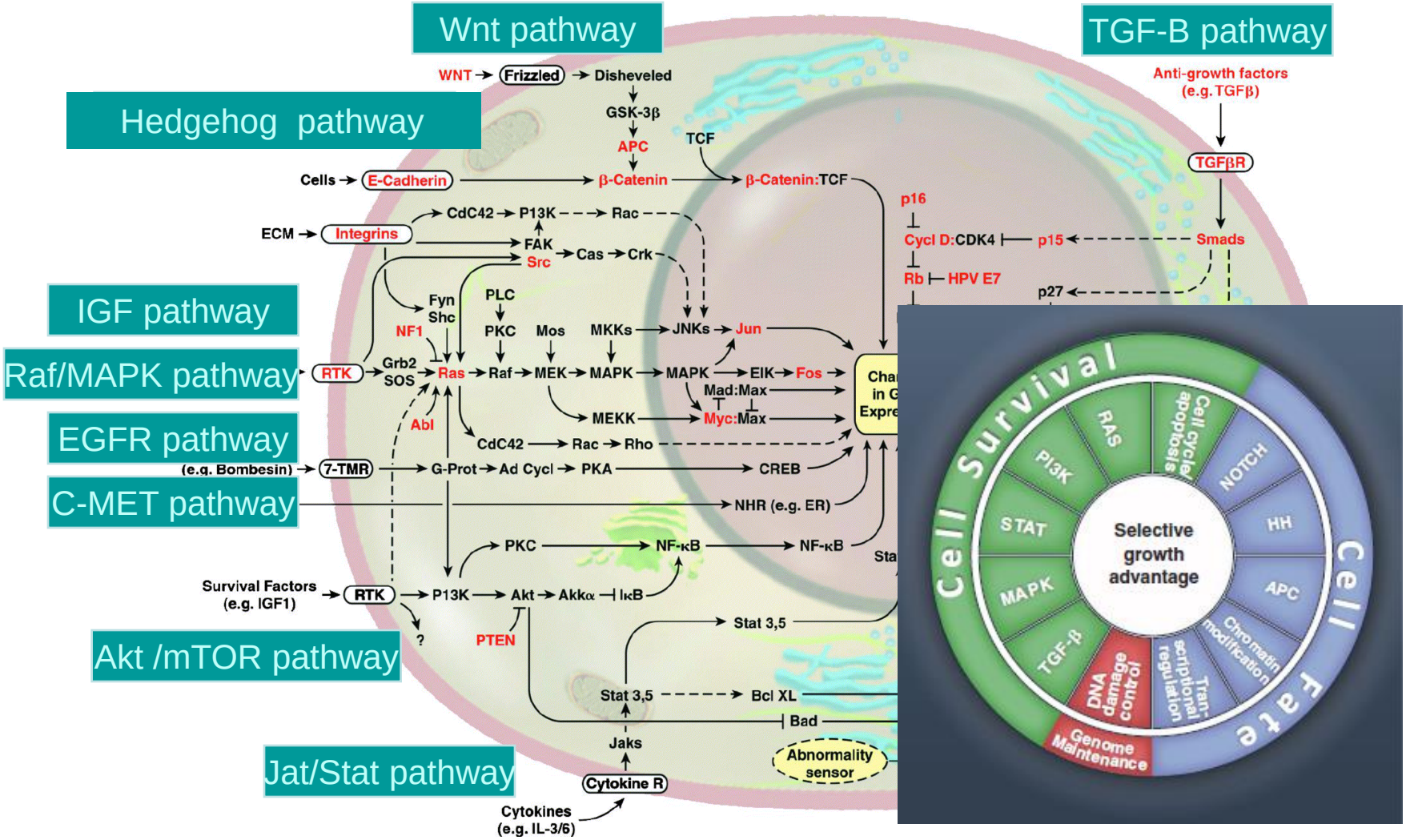


•***New pathways: FGF, Hippo, Notch,.***

Hanahan D, Weinberg RA. Cell. 2000;100:57-70.

Molecular targets for cancer

Signaling pathways: molecular targets for new therapies.

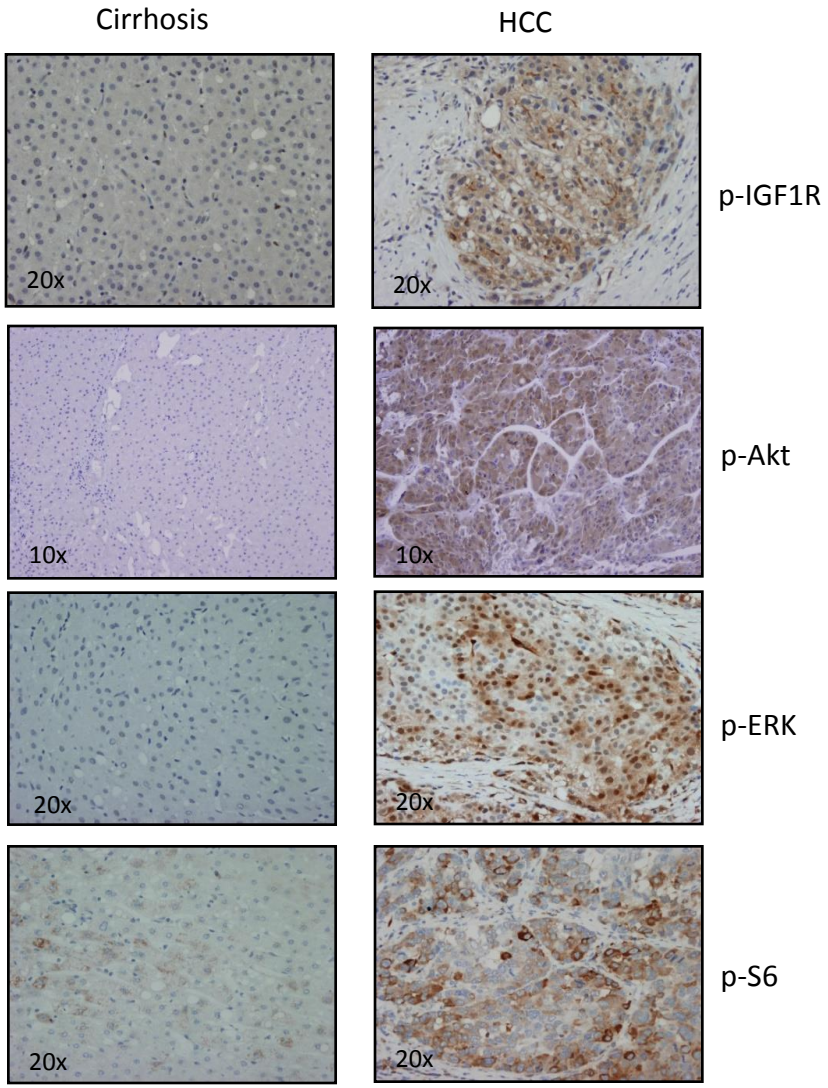


•***New pathways: FGF, Hippo, Notch,.***

Hanahan D, Weinberg RA. Cell. 2000;100:57-70.

Growth factor signaling pathway in HCC

Proliferation pathways



HCC positive samples (n=93)

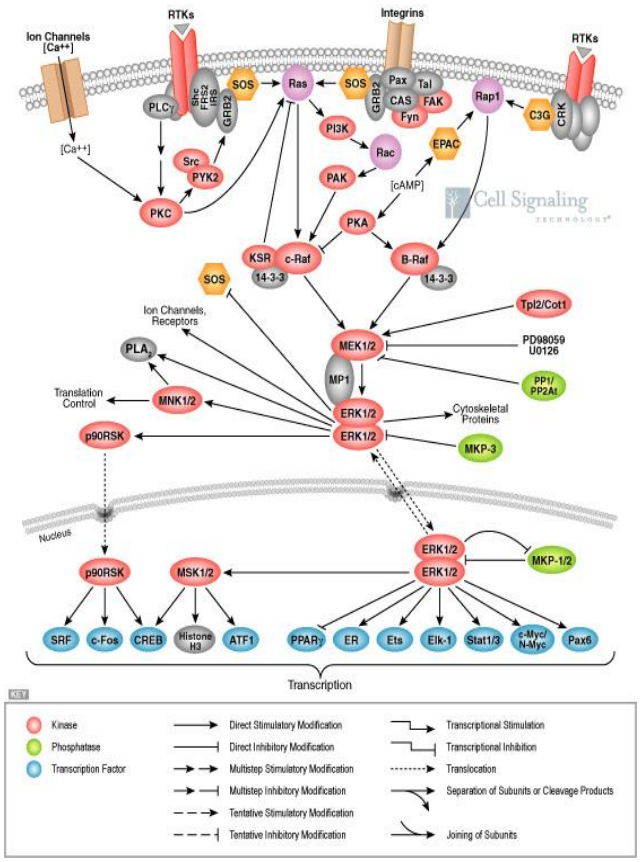
p-IGF1R	16/85	(19%)
p-Akt	31/93	(33%)
p-ERK	50/85*	(59%)
p-S6	45/85**	(53%)

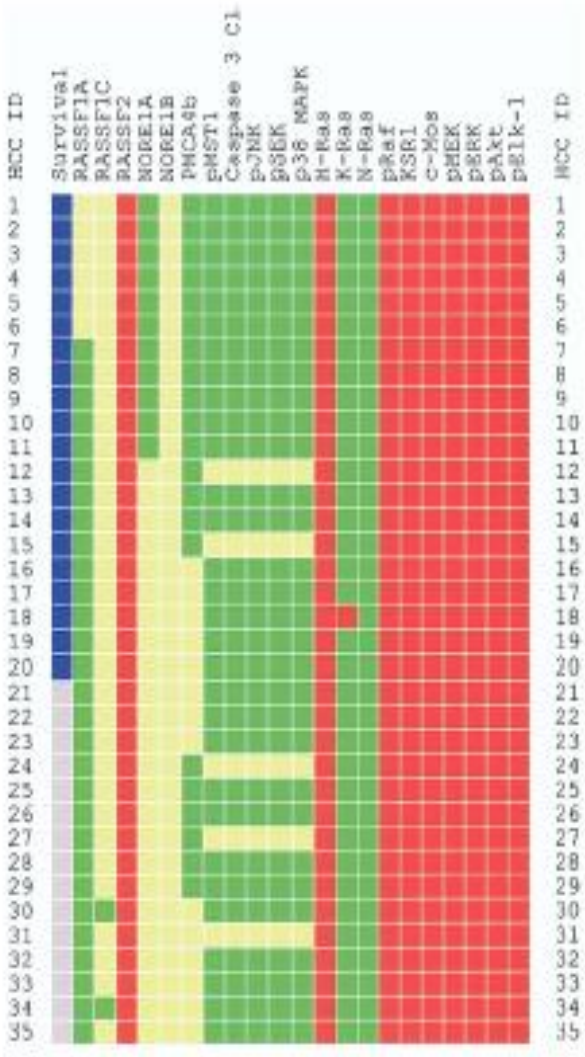
** p-ERK: Positive staining account for 10 cases of tumoral cells and 40 of endotelial cells*

*** p-S6: Postive staining account for 36 cases of tumoral cells and 9 cases of endothelial cells.*

Villanueva , Gastroenterology 2008

Alterations in RAF/MEK/ERK signaling in HCC





- **Activation of oncoproteins in human HCC:**

  H-Ras, pRaf, pERK

- **Promoter hypermethylation of TSG**

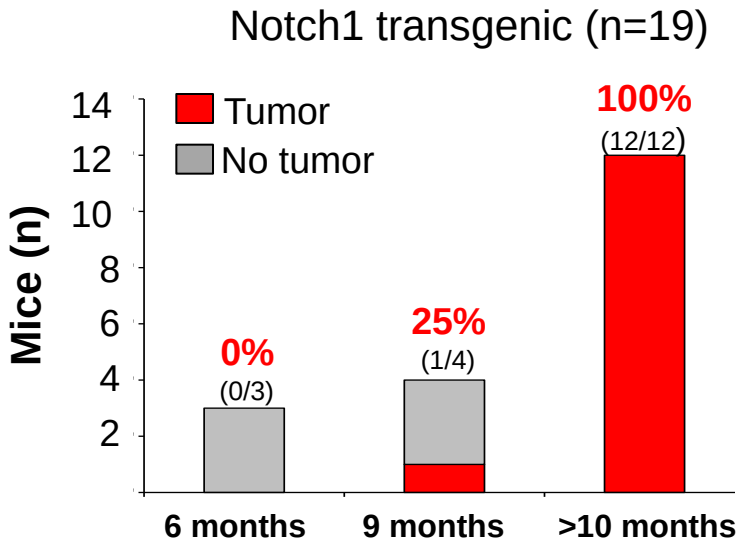
  RASSF1A, NORE1A

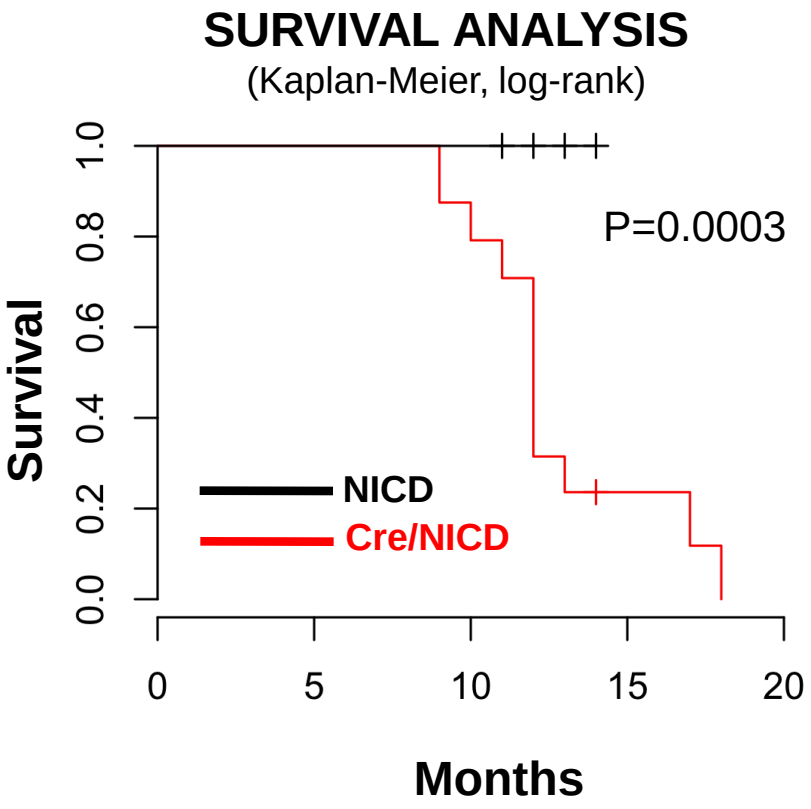
Calvisi DF, et al. Gastroenterology. 2006;130:1117-28.

Molecular targets for HCC

NOTCH Signaling pathway.

NOTCH1 as potential oncogene and target



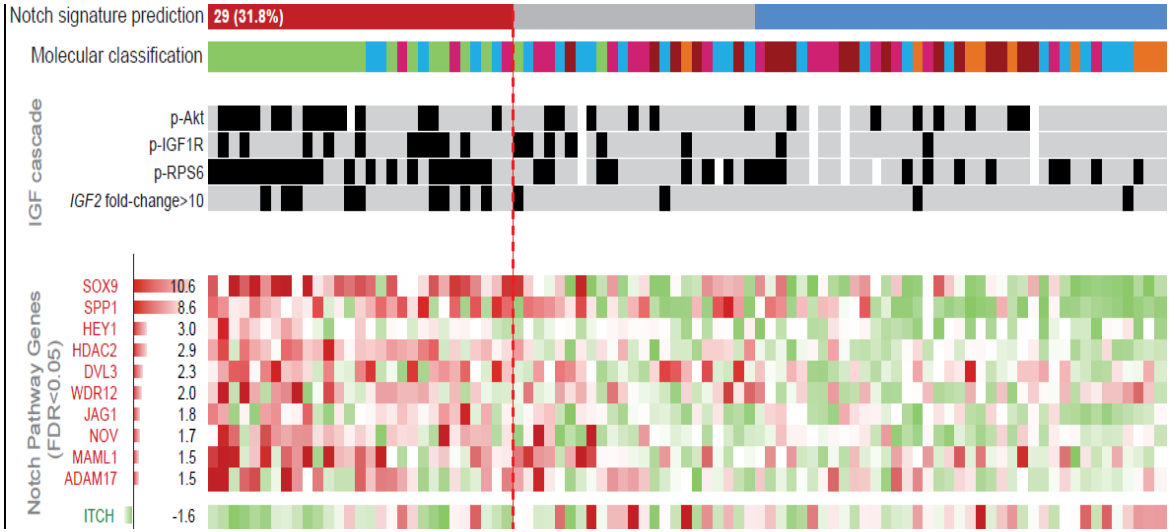


Villanueva , Gastroenterology 2012

Molecular targets for HCC

NOTCH Signaling pathway.

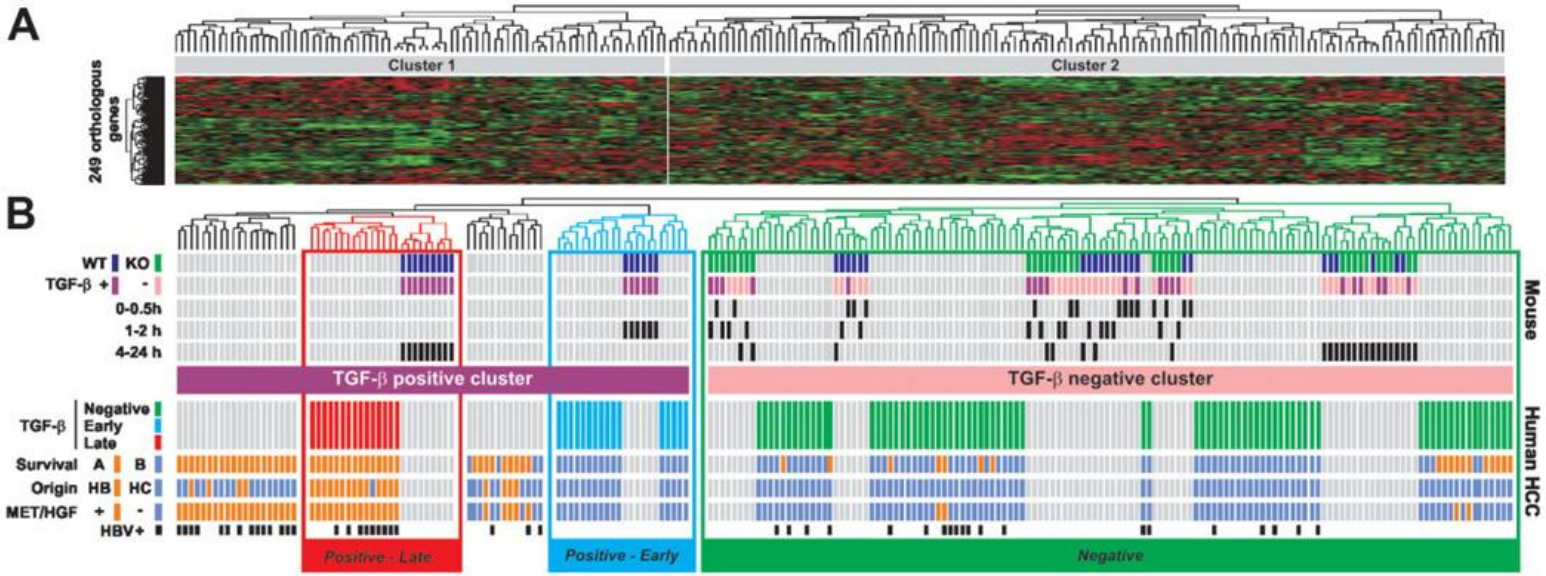
30% of human HCCs present activation of NOTCH1 signaling



Villanueva, Gastroenterology 2012

Molecular targets for HCC

TGF-beta signaling pathway.



Late TGF-β signature captured 17% (24/139) of HCC patients

Coulouarn et al, Hepatology 2008

Understanding liver cancer

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   - Main genomic alterations
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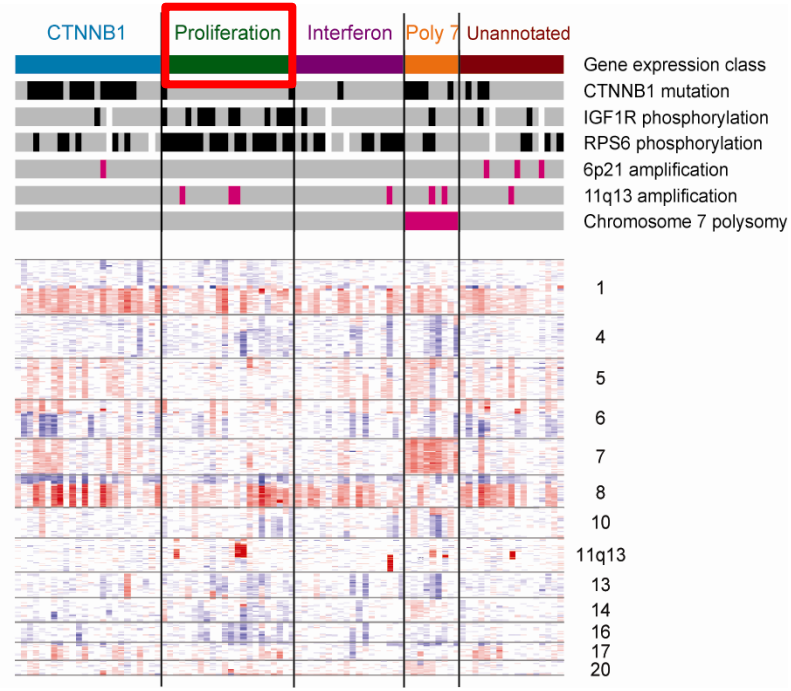
5. Personalized medicine: Improve outcomes with molecular therapies targeting specific subclasses

Molecular classification of HCC

Importance of tumor assessment

- Integrative genomic analysis (n=92)
- Transcriptome meta-analysis (~ 600 patients)

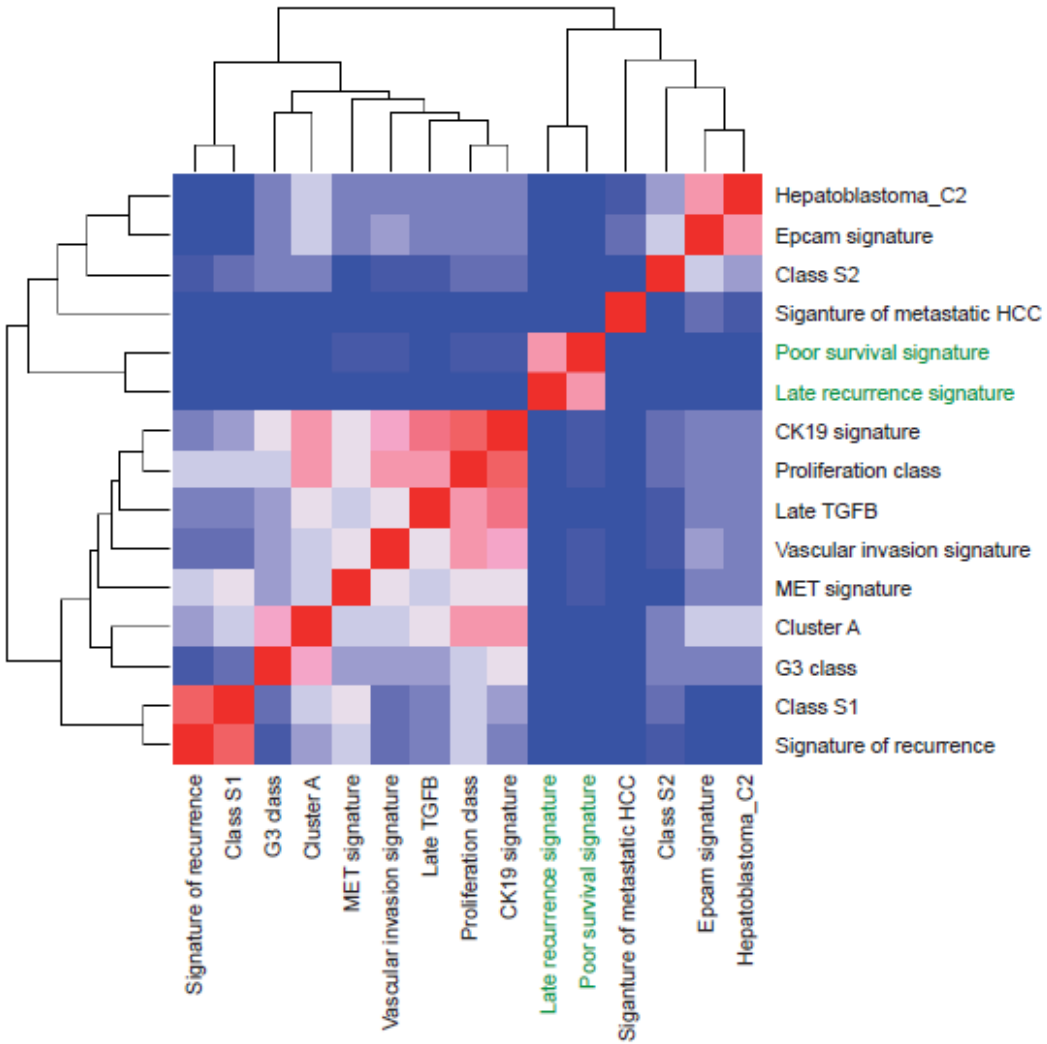


Chiang, et al., Can Res 2008

Hoshida et al., Can Res 2009

Molecular classification of HCC

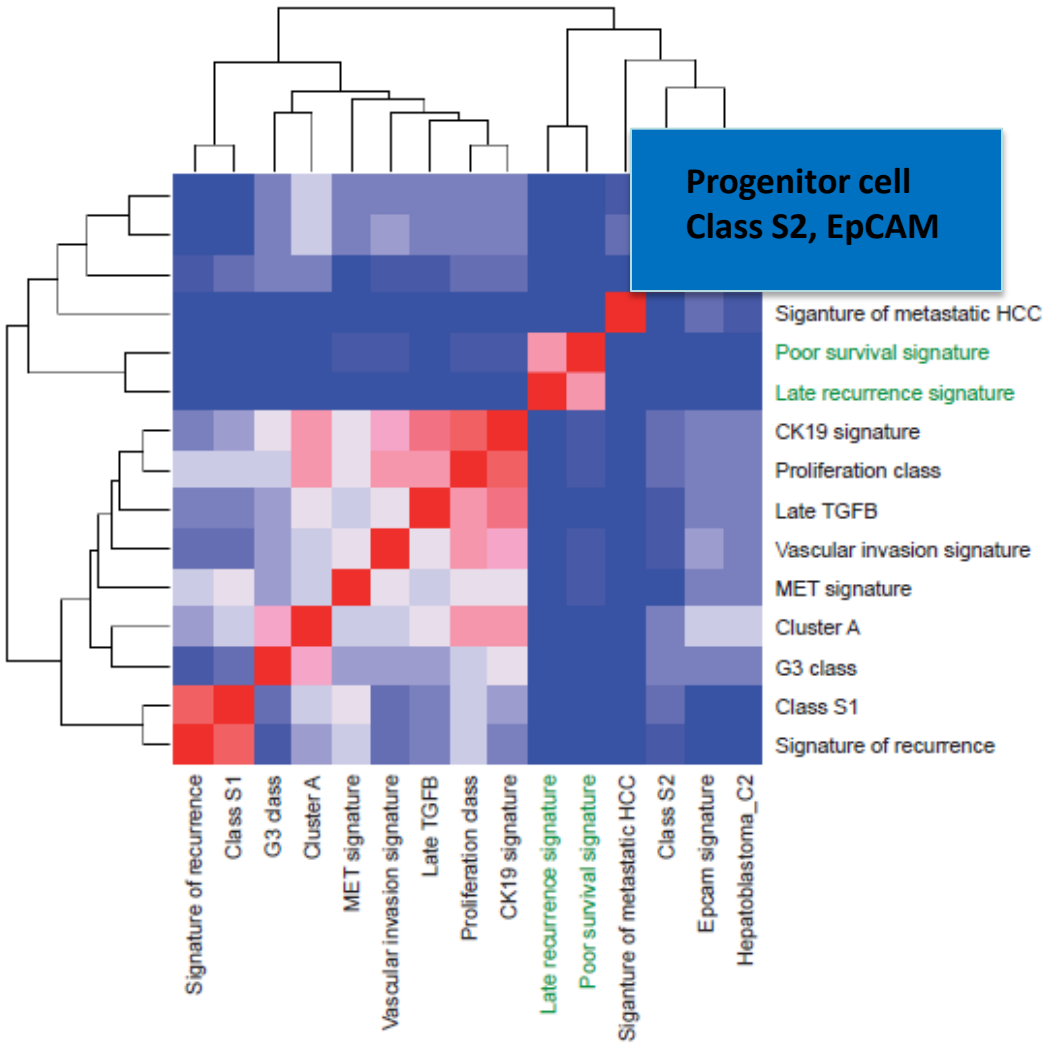
Integrative outcome approach



Villanueva A, Gastroenterology 2011

Molecular classification of HCC

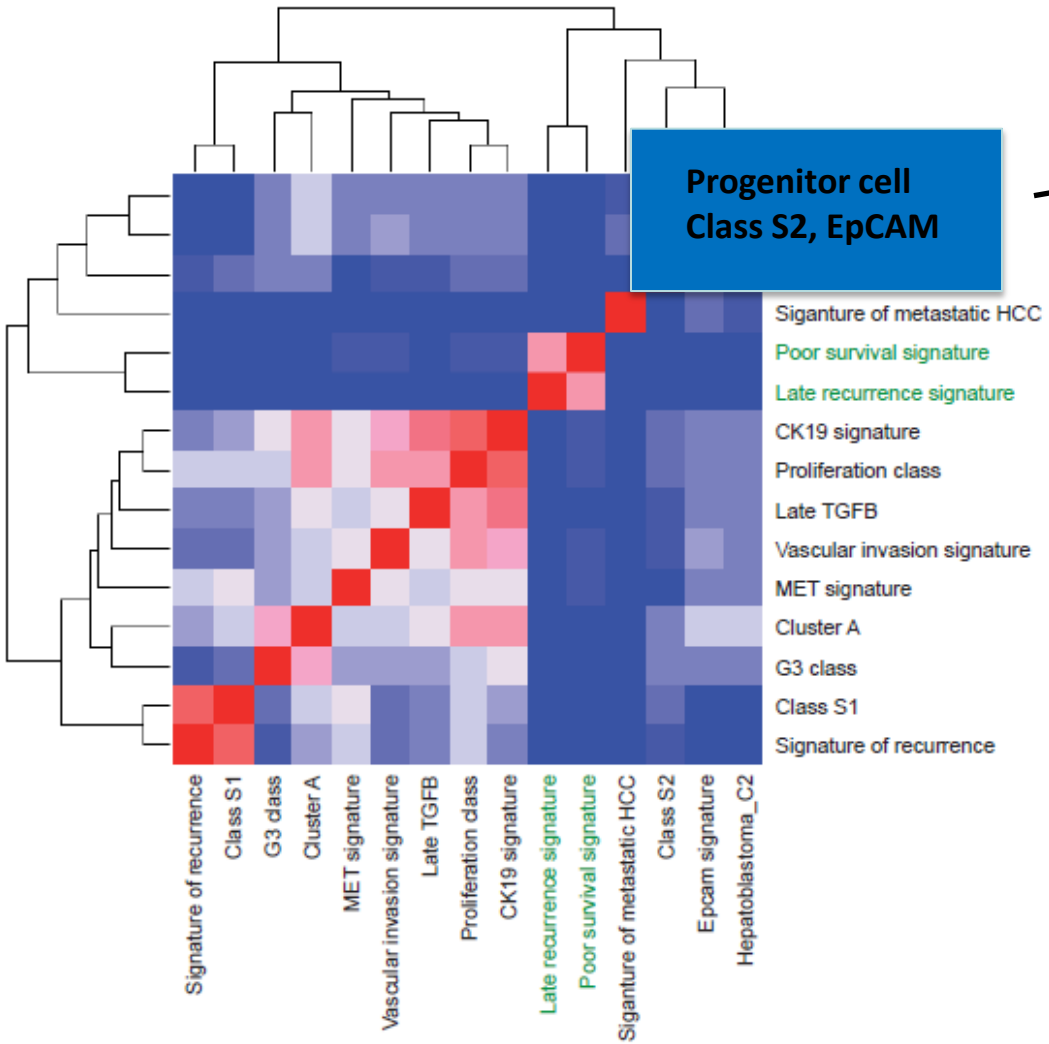
Integrative outcome approach



Villanueva A, Gastroenterology 2011

Molecular classification of HCC

Integrative outcome approach

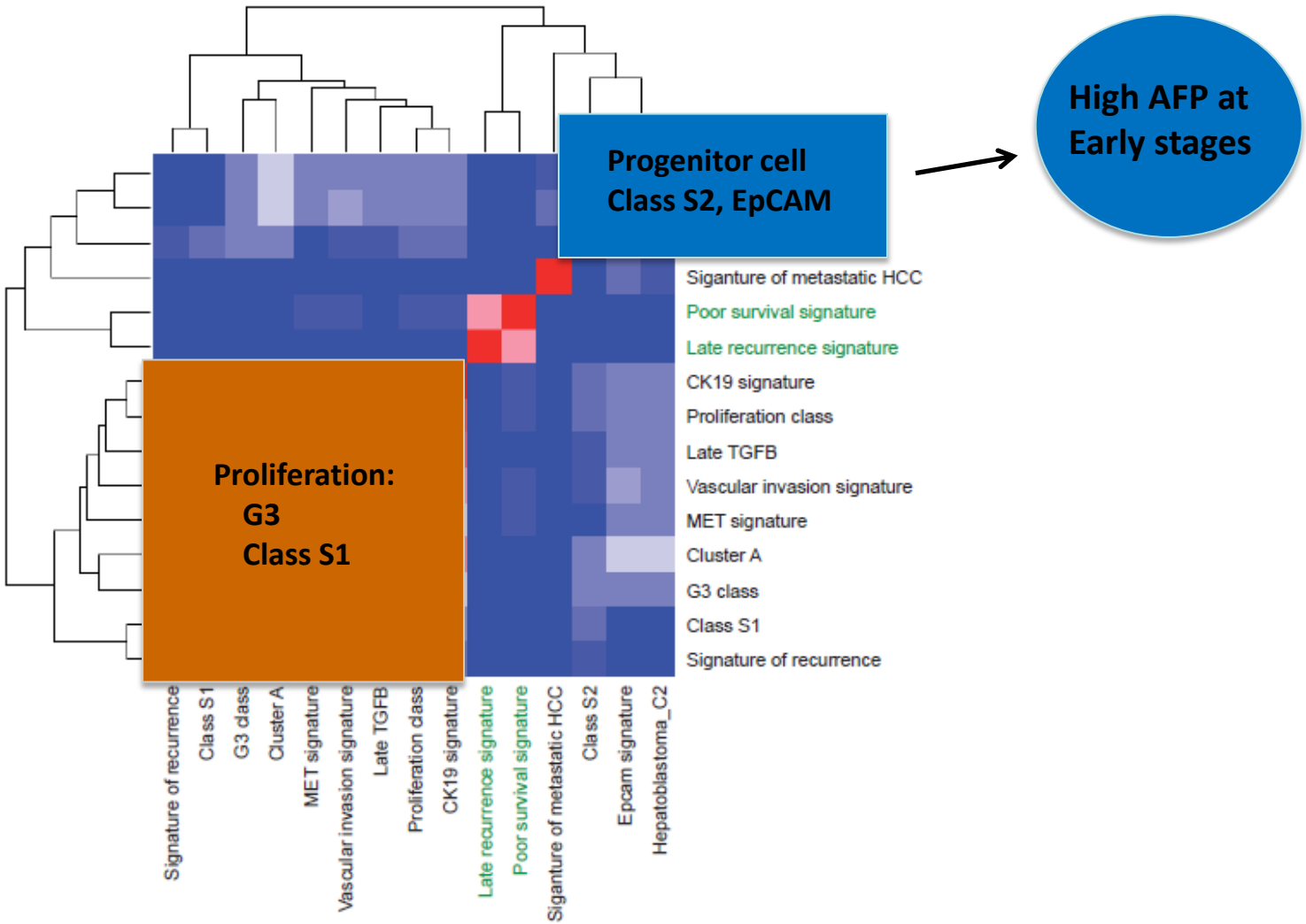


High AFP at Early stages

Villanueva A, Gastroenterology 2011

Molecular classification of HCC

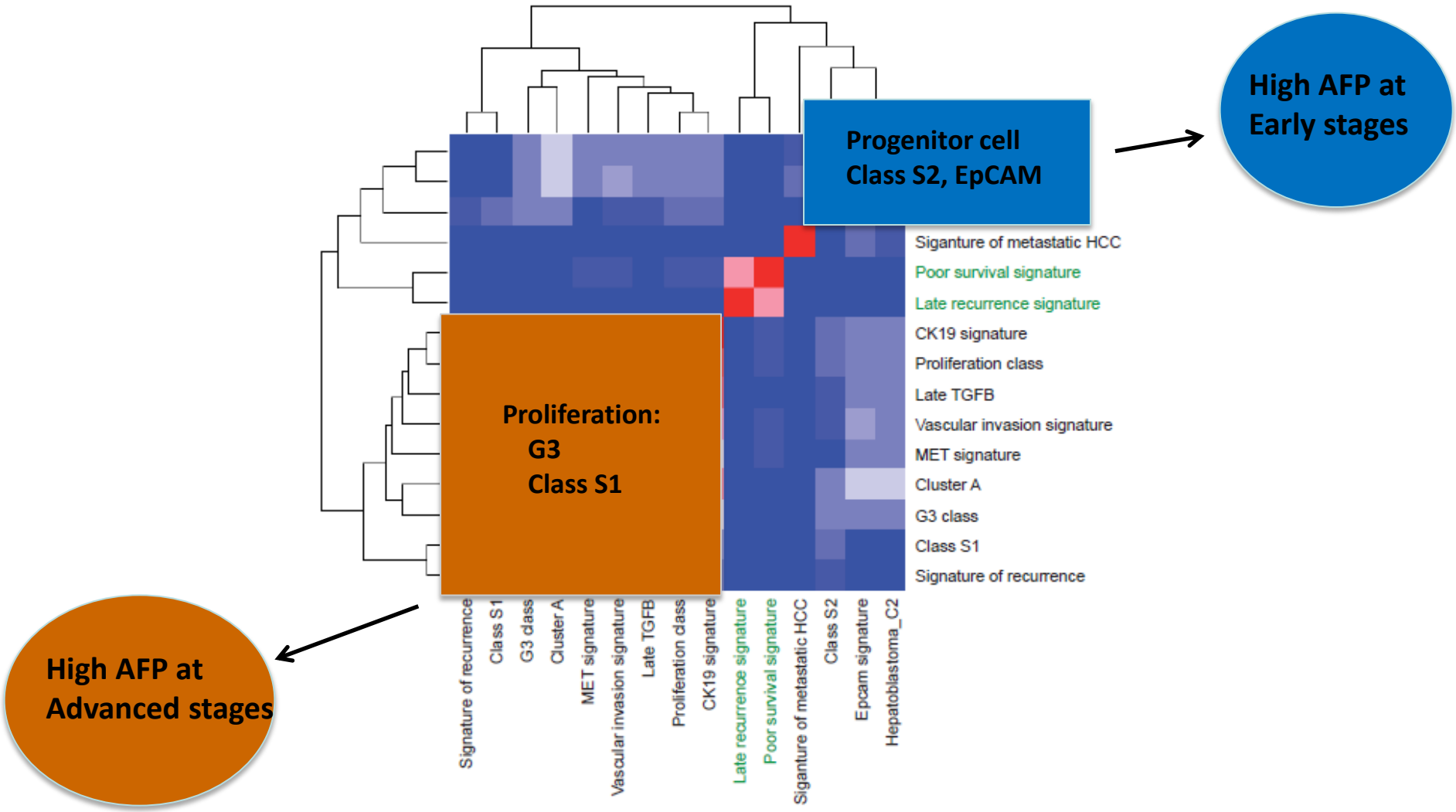
Integrative outcome approach



Villanueva A, Gastroenterology 2011

Molecular classification of HCC

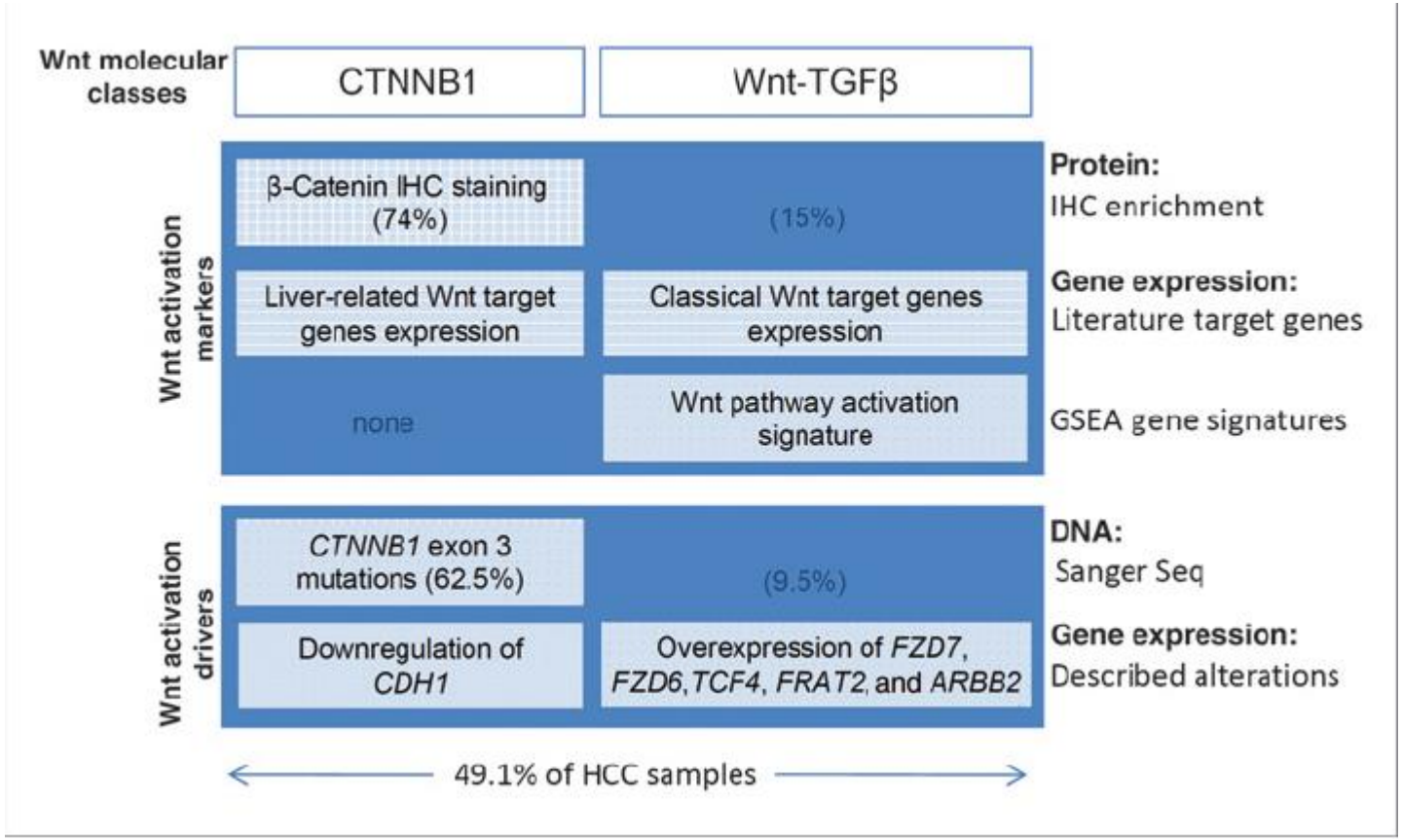
Integrative outcome approach



Villanueva A, Gastroenterology 2011

Molecular classification of HCC

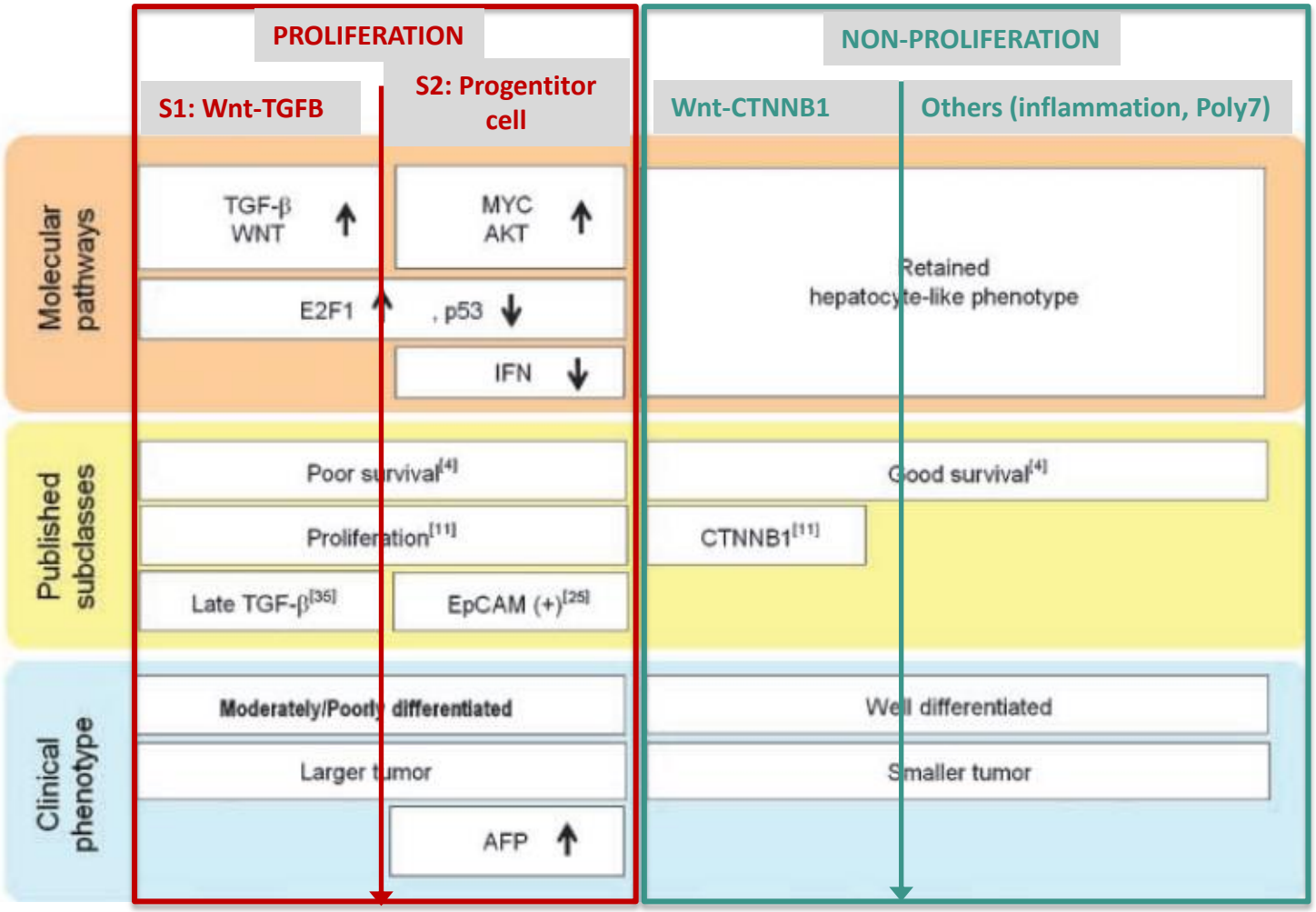
Molecular subclasses of Wnt



Lachenmeyer, Clin Can Res, 2012

Molecular classification of HCC

Importance of tumor assessment

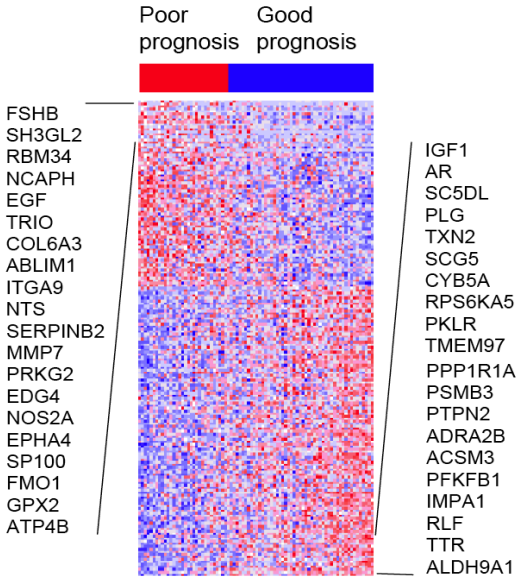


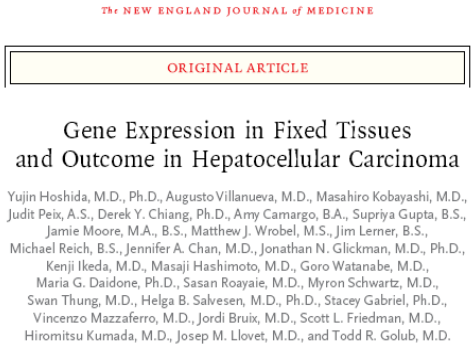
Modified from Hoshida et al., Can Res 2009

Molecular classification of HCC

Importance of microenvironment

Training set (n=82)

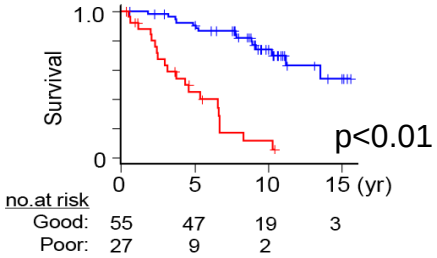



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

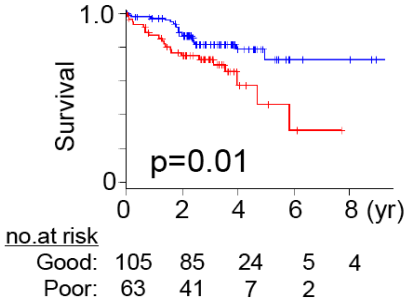
Gene Expression in Fixed Tissues and Outcome in Hepatocellular Carcinoma

Yujin Hoshida, M.D., Ph.D., Augusto Villanueva, M.D., Masahiro Kobayashi, M.D., Judit Peix, A.S., Derek Y. Chiang, Ph.D., Amy Camargo, B.A., Supriya Gupta, B.S., Jamie Moore, M.A., B.S., Matthew J. Wrobel, M.S., Jim Lerner, B.S., Michael Reich, B.S., Jennifer A. Chan, M.D., Jonathan N. Glickman, M.D., Ph.D., Kenji Ikeda, M.D., Masaji Hashimoto, M.D., Goro Watanabe, M.D., Maria G. Daidone, Ph.D., Sasan Roayaie, M.D., Myron Schwartz, M.D., Swan Thung, M.D., Helga B. Salvesen, M.D., Ph.D., Stacey Gabriel, Ph.D., Vincenzo Mazzaferro, M.D., Jordi Bruix, M.D., Scott L. Friedman, M.D., Hiromitsu Kumada, M.D., Josep M. Llovet, M.D., and Todd R. Golub, M.D.



Gene signature-poor prognosis: # 186 genes

Gene Set Enrichment : 1. Inflammation 2. NF-KB signaling 3. interferon-related immune response 4. Oxidative stress and 5. Proliferative signals (IL6, EGF)



Hoshida et al, NEJM 2008

Understanding liver cancer

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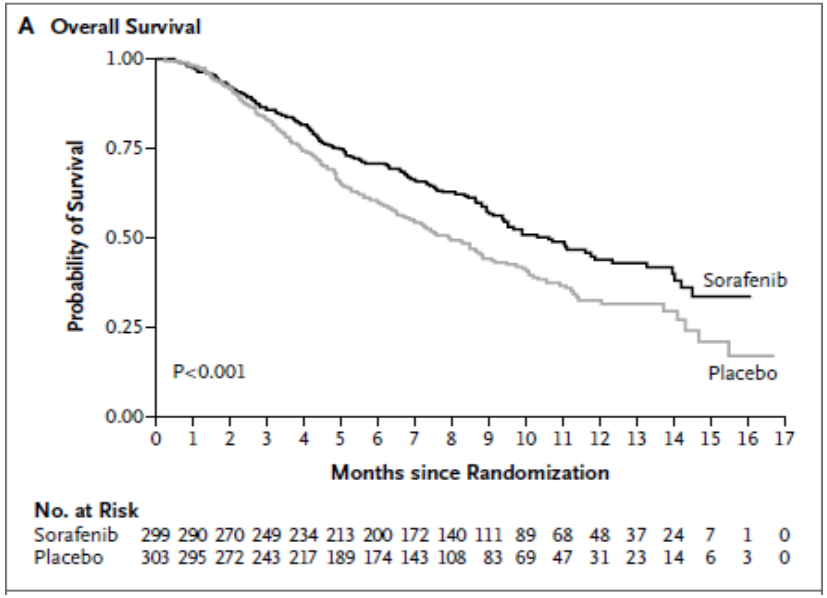
Phase III SHARP Trial

Sorafenib vs placebo in advanced HCC

ORIGINAL ARTICLE

Sorafenib in Advanced Hepatocellular Carcinoma

Josep M. Llovet, M.D., Sergio Ricci, M.D., Vincenzo Mazzaferro, M.D., Philip Hilgard, M.D., Edward Gane, M.D., Jean-Frédéric Blanc, M.D., Andre Cosme de Oliveira, M.D., Armando Santoro, M.D., Jean-Luc Raoul, M.D., Alejandro Forner, M.D., Myron Schwartz, M.D., Camillo Porta, M.D., Stefan Zeuzem, M.D., Luigi Bolondi, M.D., Tim F. Greten, M.D., Peter R. Galle, M.D., Jean-François Seitz, M.D., Ivan Borbath, M.D., Dieter Häussinger, M.D., Tom Giannaris, B.Sc., Minghua Shan, Ph.D., Marius Moscovici, M.D., Dimitris Voliotis, M.D., and Jordi Bruix, M.D., for the SHARP Investigators Study Group*



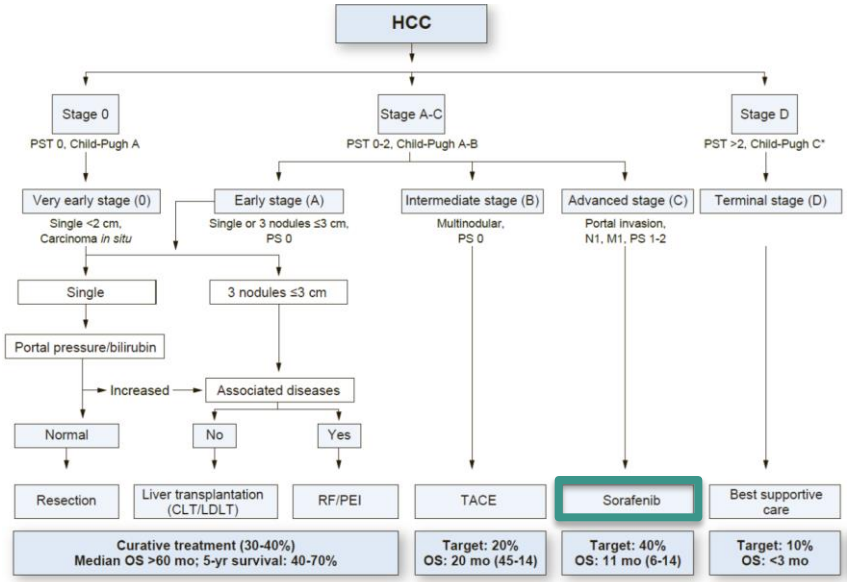
Stratification:

* Macroscopic vascular invasion (portal vein) and/or extrahepatic spread
* ECOG PS
* Geographical region

Randomization N=602

Sorafenib (n=299)
400 mg po bid
continuous dosing

Placebo (n=303)
2 tablets po bid
continuous
dosing



Llovet JM et al, NEJM 2008;359:378-90

Molecular-profiling approaches to the development of personalized therapies in cancer

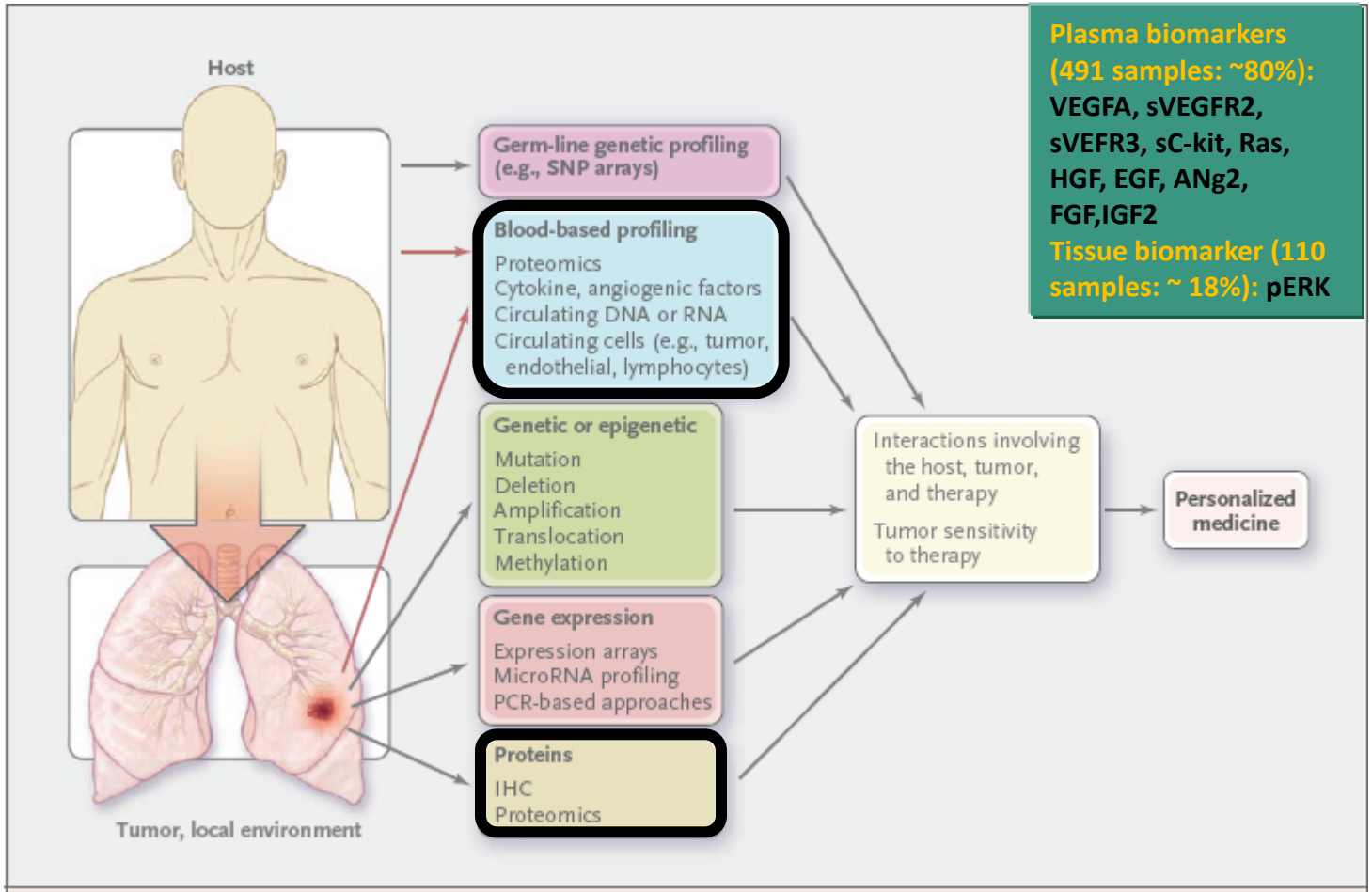


Figure 4. Molecular-Profiling Approaches to the Development of Personalized Therapy.

Herbst RS, et al. N Engl J Med 2008; 359:1367-80.

Predictors of Survival

All patients (multivariate analysis)

Sorafenib Cohort

	Multivariate Analysis	
Variable	*P*	HR
Macrovascular invasion	**0.005**	**1.74**
Extrahepatic spread	**0.016**	**1.60**
Baseline AFP	**0.015**	**1.57**
Baseline alkaline phosphatase	**0.002**	**1.82**
Baseline c-KIT	**0.004**	**0.56**
Baseline HGF	**0.017**	**1.69**

Placebo Cohort

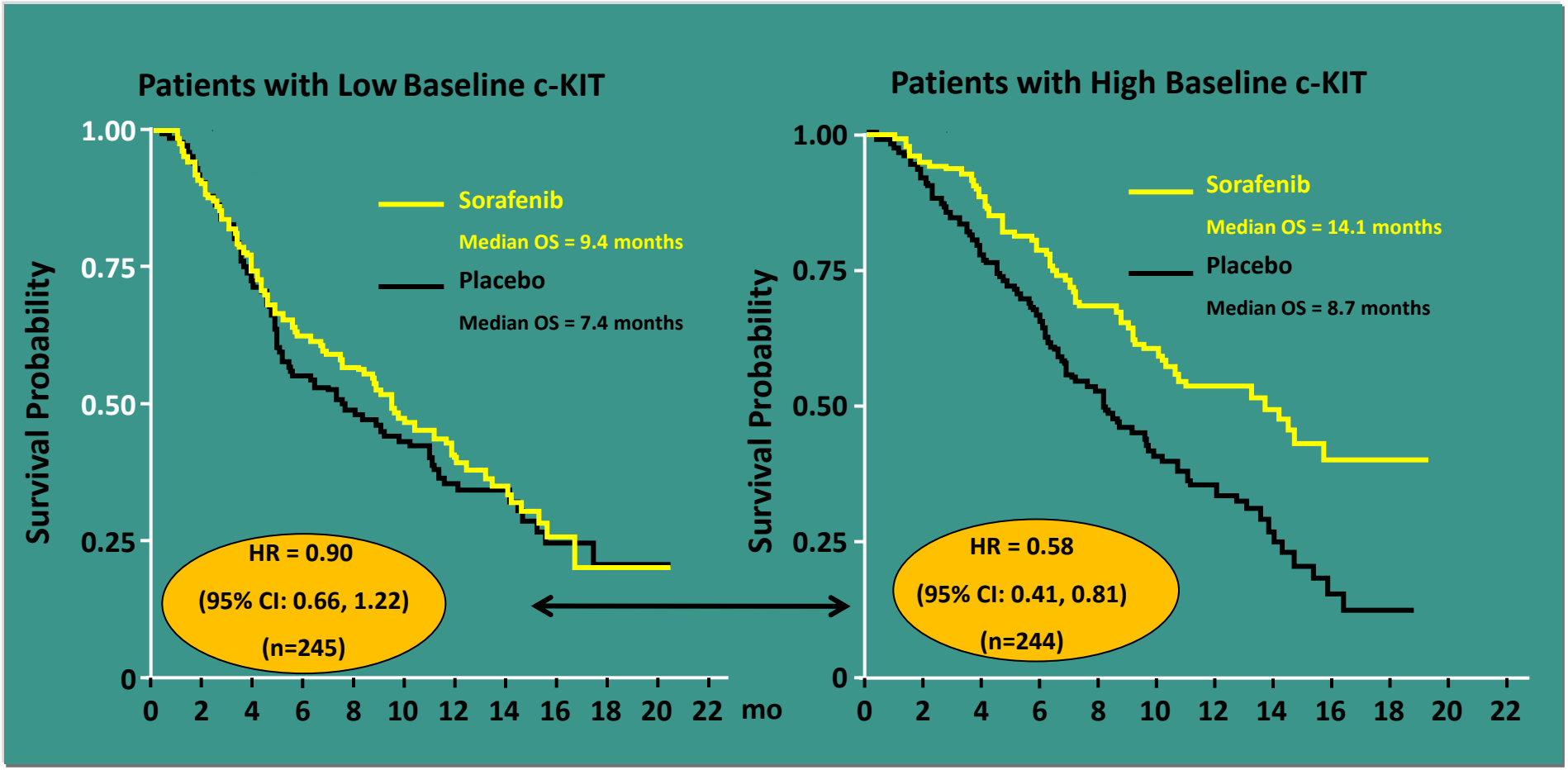
Baseline AFP	**0.007**	**1.58**
Macroscopic vascular invasion	**0.0003**	**1.88**
Baseline alkaline phosphatase	**0.013**	**1.56**
Baseline Ang2	**0.002**	**1.84**
Baseline VEGF	**0.002**	**1.97**

Llovet et al. Clin Cancer Res 2012;18:2290.

Level II-B

Baseline plasma c-KIT and Sorafenib

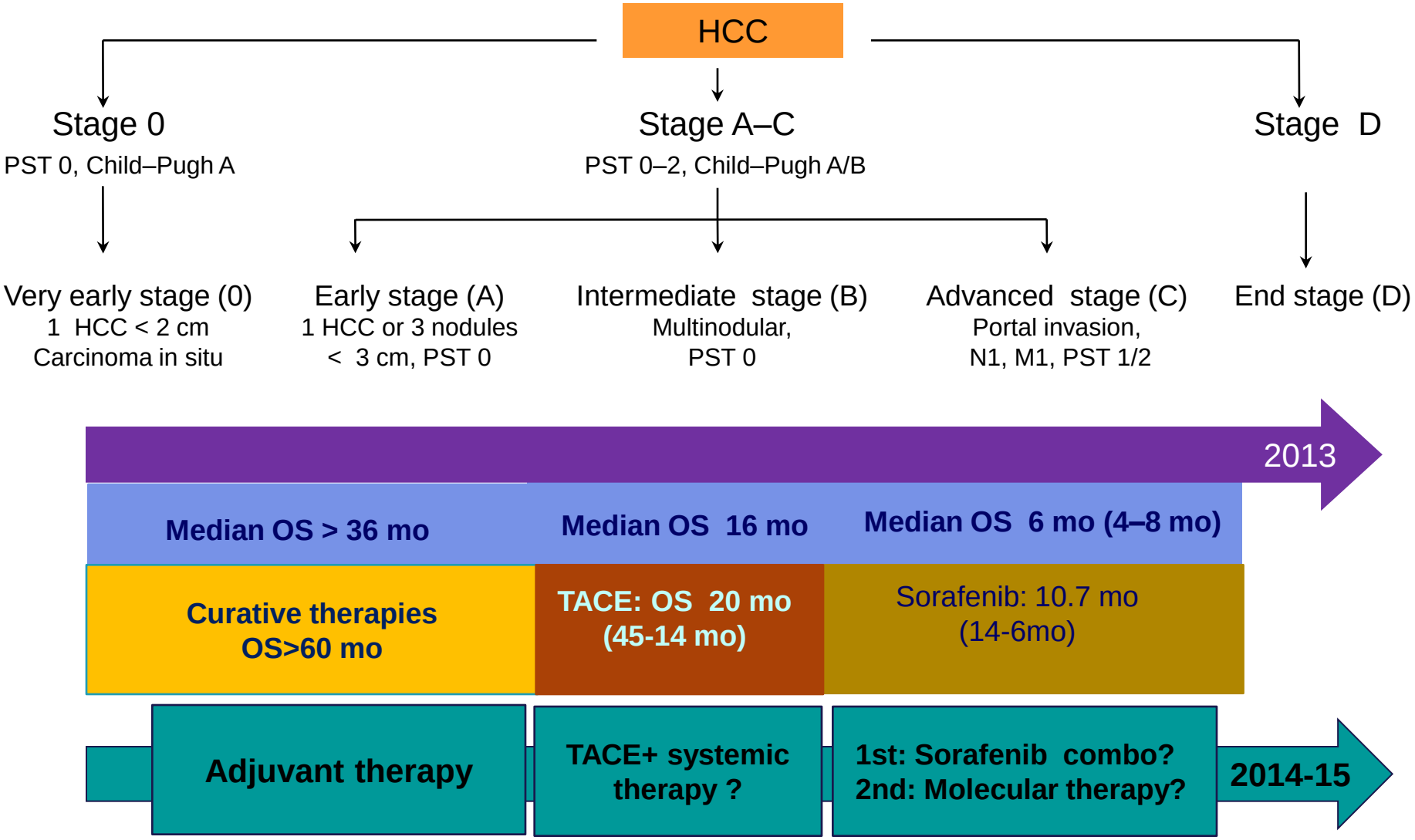
Prediction of survival



Patients with high c-KIT showed a trend of better survival benefit from sorafenib (interaction *P*-value=0.081).

Llovet et al. Clin Cancer Res 2012;18:2290.

Understanding survival outcomes in HCC patients



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Molecular targeted therapies tested in HCC

Table 1. Molecular Therapies (Including TKIs, mAbs, and Oligonucleotide Antisense) Currently Under Evaluation in HCC

	Drugs	Phases	Trials, n	Targets
1	Sorafenib	1, 1–2, 2, 3, 4	65	BRAF, VEGFR, PDGFR
2	Erlotinib	1, 1–2, 2, 3	13	EGFR
3	Everolimus	1, 1–2, 2, 3	7	MTORC1
4	Brivanib	1, 2, 3	6	FGFR, VEGFR, PDGFR
5	Sunitinib	2, 3	6	VEGFR, PDGFR, CKIT
6	Rapamycin	1, 2–3, 3	5	MTORC1
7	Linifanib	2, 3	2	VEGF, PDGFR
8	PI-88	2, 3	2	Endo-β-D-glucuronidase heparinase
9	Ramucirumab	3	1	VEGFR2
10	Bevacizumab	1, 1–2, 2	20	VEGF
11	AZD6244	1–2, 2	4	MEK
12	Bortezomib	1, 2	4	Proteasome
13	TAC-101	1–2, 2	4	RAR-a
14	Cediranib	1, 2	3	VEGFR
15	Cetuximab	1, 2	3	EGFR
16	Cixutumumab	1, 2	3	IGF-1R
17	Temsirolimus	1, 2	3	MTORC1
18	ARQ197	1, 2	2	MET
19	BIBF1120	2	2	VEGFR, PDGFR, FGFR
20	Dasatinib	2	2	BCR-ABL
21	GC33	1	2	GPC3
22	Gefitinib	2	2	EGFR
23	Lapatinib	2	2	EGFR, HER2/neu
24	Licartin	2, 4	2	HAb18G/CD147
25	Pazopanib	2	2	VEGFR, PDGFR, CKIT
26	Alvocidib	1, 2	2	Cyclin-dependent kinase
27	AEG35156	1–2	1	XIAP
28	AMG386	2	1	Angiopoietin
29	AVE1642	1, 2	1	IGF-1R
30	AZD8055	1–2	1	MTORC1, MTORC2
31	Regorafenib	2	1	VEGFR, TIE-2
32	BIIB022	1–2	1	IGF-1R
33	Belinostat	1–2	1	Histone deacetylase
34	CS-1008	2	1	TRAIL
35	CT-011	1–2	1	PD1
36	E7080	1–2	1	VEGFR, FGFR, SCFR
37	Foretinib	1	1	MET
38	IDN-6556	2	1	Caspase
39	IMC-1121B	2	1	VEGFR2
40	IMC-A12	2	1	IGF-1R
41	Ispinesib	2	1	Kinesin spindel protein
42	LBH589	1	1	Histone deacetylase
43	LY2181308	1–2	1	Survivin
44	Lonafarnib	2	1	Farnesyl-OH-transferase
45	MLN8237	2	1	Aurora kinase
46	Mapatumumab	1–2	1	TRAIL
47	OSI-906	2	1	IGF-1R, IR
48	Oblimersen	2	1	BCL2
49	Panobinostat	1	1	Histone deacetylase
50	Resminostat	2	1	Histone deacetylase
51	TSU-68	1–2	1	VEGFR, FGFR, PDGFR
52	Talabostat	1	1	Dipeptidyl peptidases
53	Tremelimumab	2	1	B7-CD28
54	Vandetanib	2	1	EGFR, VEGFR, RET
55	Vorinostat	1	1	Histone deacetylase
56	Z-208	1–2	1	RAR

NOTE. Data accessed on February 2011.
FGFR, fibroblast growth factor receptor; IGR, insulin-like growth factor.

UNCORRECTED PROOF

Main figures:

>300 phase II/III trials in clinical trials .gov

56 molecular targeted therapies currently tested

14 RCT for regulatory approval

Main novel targets are: mTOR, MEK, IGFR1, MET, FGF, TIE-2, HDAC.

Villanueva and Llovet, Gastroenterology 2011;140:1410-26

Molecular therapies tested for HCC in Phase III (2014)

Targeted population		Phase III comparison
Adjuvant	Prevent recurrences	**1. Sorafenib vs placebo (STORM)*** **2. Retinoids vs placebo***
Intermediate HCC	Improve TACE	**1. RF vs RF-Dox** **2.TACE+/- sorafenib*** **3. TACE +/- brivanib***
Advanced HCC	First line:	**1. Sorafenib + /- erlotinib*** **2. Sorafenib vs brivanib*** **3. Sorafenib vs sunitinib*** **4. Sorafenib vs linifanib*** 5. Sorafenib +/- Y90 6. Sorafenib +/- Doxorubicin 7. Sorafenib vs Lenvatinib
	Second line:	**1. Brivanib vs placebo*** 2. **Everolimus vs placebo*** **3. Ramucirumab vs placebo*** 4. Regorafenib vs placebo 5. Tivantinib vs placebo 6. Cabozantinib vs placebo

** RCT halted or with unconclusive or negative results*

Why these RCT are negative in HCC?

1. Limited understanding of the pathogenesis/subclasses/drivers
2. Two diseases: HCC and cirrhosis

   - Balance between efficacy and toxicity (sunitinib, linifanib)

   - Difficult trial design:

     Non-inferiority concept (Brivanib, linifanib)

     Surrogate end-points of survival? TTP (contradictory data), ORR
3. Moving to phase III without clear signals. (Sunitinib, erlotinib)
4. Drugs are not powerful enough (brivanib, linifanib, erlotinib, everolimus, ramucirumab).

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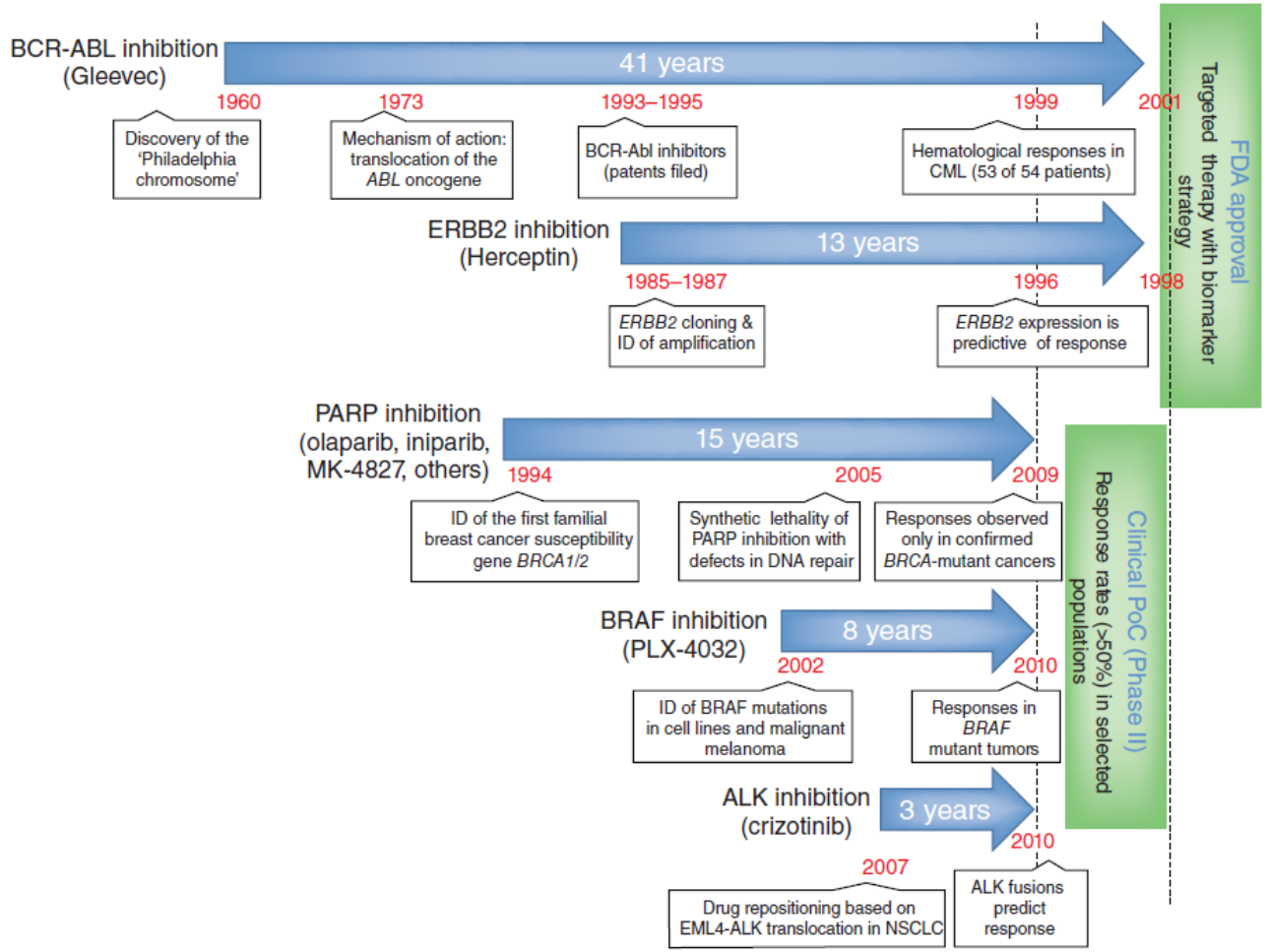
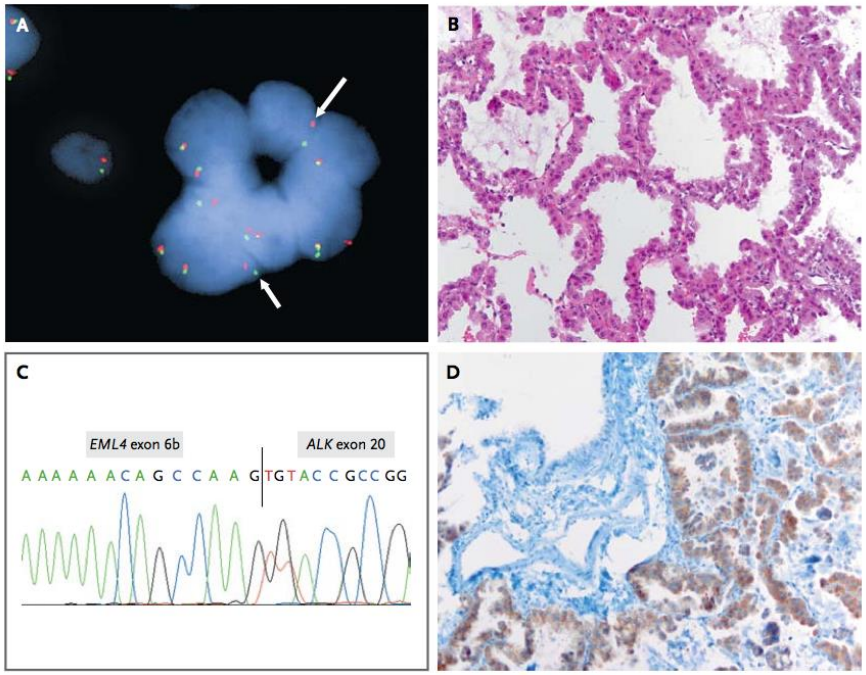
Personalized oncology:
Oncogenic addiction loops in cancer

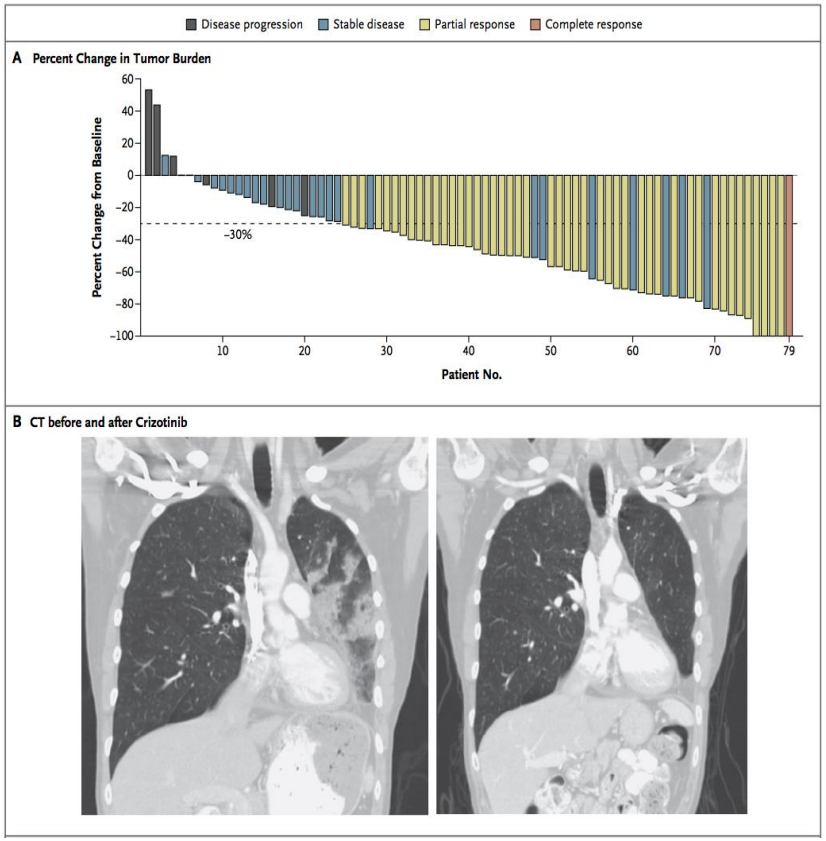


Oncogene addiction

Lung cancer (ALK rearrangements: 3%) : Crizotinib

~1,500 patients screened (18 months)
82 patients included **(3% ALK fusions)**





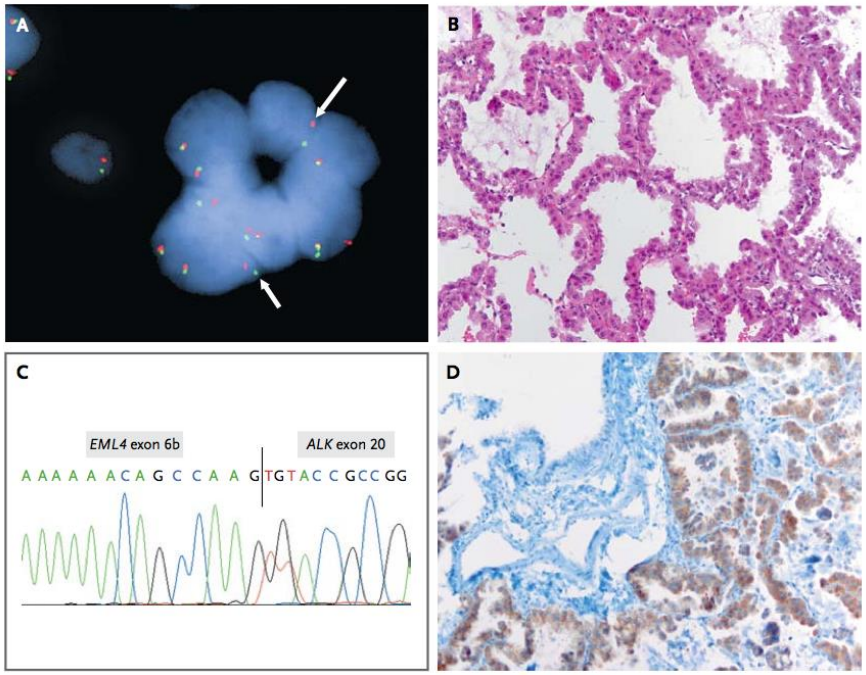
PFS at 6 months: 72% (vs 27% of historic cohorts - Mok TS NEJM 2009**)**

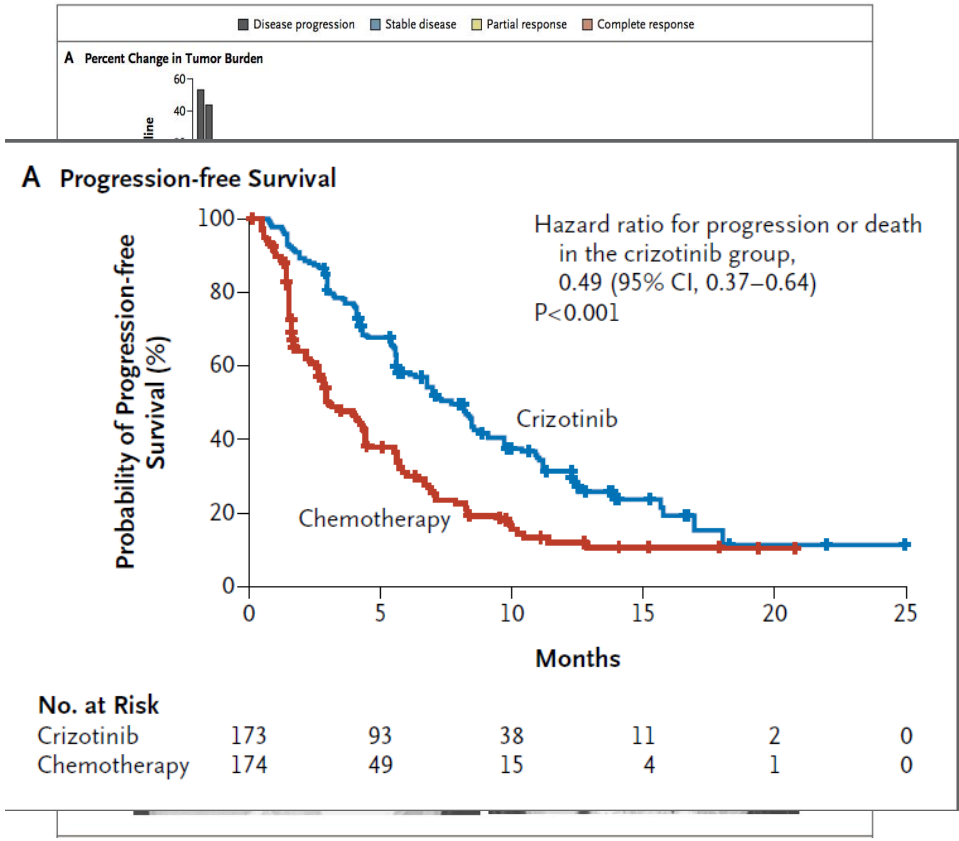
Kwak et al. New Engl J Med 2010

Oncogene addiction

Lung cancer (ALK rearrangements: 3%) : Crizotinib

~1,500 patients screened (18 months)
82 patients included **(3% ALK fusions)**





PFS at 6 months: 72% (vs 27% of historic cohorts - Mok TS NEJM 2009**)**

Shaw et al. New Engl J Med 2013

Kwak et al. New Engl J Med 2010

Oncogene addiction

Therapeutic applications

Table 1. Selected Genetic Markers and Their Application in Cancer Treatment

Genetic Marker	Application	Drug
BCR-ABL	Ph+ CML; Ph+ ALL	Imatinib, dasatinib, nilotinib
BCR-ABL/T315I	Resistance to anti-BCR-ABL agents	Imatinib, dasatinib, nilotinib
BRAF V600E	Metastatic melanoma	Vemurafenib
BRCA1/2	Metastatic ovarian cancer and breast cancer with BRCA 1/2 mutations	Olaparib, veliparib, iniparib
c-Kit	Kit (CD117)-positive malignant GIST	Imatinib
EGFR	Locally advanced, unresectable, or metastatic NSCLC	Erlotinib, gefitinib
EGFR T790M	Resistance to EGFR tyrosine kinase inhibitors in advanced NSCLC	Erlotinib, gefitinib
EML4-ALK	ALK kinase inhibitor for metastatic NSCLC with this fusion gene	Crizotinib
HER2 amplification	HER2-positive breast cancer or metastatic gastric or gastroesophageal junction adenocarcinoma	Trastuzumab
KRAS	Resistance to EGFR antibodies in metastatic colorectal cancer	Cetuximab, panitumumab
PML/RAR	Acute promyelocytic leukemia	ATRA, arsenic trioxide
TPMT	Deficiency is associated with increased risk of myelotoxicity	Mercaptopurine, azathioprine
UGT1A1	Homozygosity for UGT1A1*28 is associated with risk of toxicity	Irinotecan
DPD	Deficiency is associated with risk of severe toxicity	5-Fluorouracil

ATRA, all trans retinoic acid; Ph+, Philadelphia-positive chromosome; DPD, dihydropyrimine dehydrogenase; EGFR, epidermal growth factor receptor; EML4-ALK, echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase; HER2, human epidermal growth receptor 2; GIST, gastrointestinal stromal tumors; ALL, acute lymphocytic leukemia; NSCLC, non-small cell lung cancer; TPMT, thiopurine S-methyltransferase.

Dancey et al. Cell 2012

Oncogene addiction

Landscape of mutations and high-level amplifications in HCC

Table 2. Landscape of most prevalent mutations and high-level amplifications of HCC

Gene	Pathways/gene functions involved	Estimated frequency based on deep-sequencing studies (%)
Genes frequently mutated in HCC		
TERT promoter	Telomere stability	60
TP53	Genome integrity	20–30
CTNNB1	WNT signaling	15–25
ARID1A	Chromatin remodeling	10–16
TTN	Chromosome segregation	4–10
NFE2L2	Oxidative stress	6–10
JAK1	JAK/STAT signaling	0–9
Genes frequently mutated in other solid tumors, but rarely muted in HCC		
IDH1, IDH2	NAPDH metabolism	<5
EGFR	Growth factor signaling	<5
BRAF	RAS/MAPK signaling	<5
KRAS, NRAS	RAS/MAPK signaling	<5
PIK3CA	AKT signaling	<5
PTEN	AKT signaling	<5
Oncogenes contained in high-level amplifications in HCC		
FGF19	FGF signaling	5–10
Cyclin D1	Cell cycle	5–10

Adapted from Villanueva and Llovet (42).

Llovet JM et al, CCR 2014

Molecular targeted in HCC

Phase I-II studies ongoing

Agent	Targets	Phase	Patients (n)
LY2157299	TGFB	2	190
Refametininb	MEK	2	95
OPB-31121	JAK2	2	48
BIBF1120	PDGFR/FGFR	2	180
Apatinib	VEGFR, PDGFR, SRC	2	36
SGI-110	DNA hypomethylation	2	46
Nivolumab	PD-1	1	90
Ganetespib	HSP90	1	32
AZD9150	STAT3	1	80

www.clinicaltrials.gov

Oncogene addiction in HCC

Proof-of-concept trial: Refametinib in Ras + HCC (5%)

Screening
unresectable advanced or metastatic HCC
Stage 1 N=~350
Stage 2 N= ~2300

→

Refametinib (MEK inhibitor)
unresectable advanced or metastatic HCC carrying NRAS or KRAS mutation
Stage 1 N=~15
Stage 2 N=~ 80

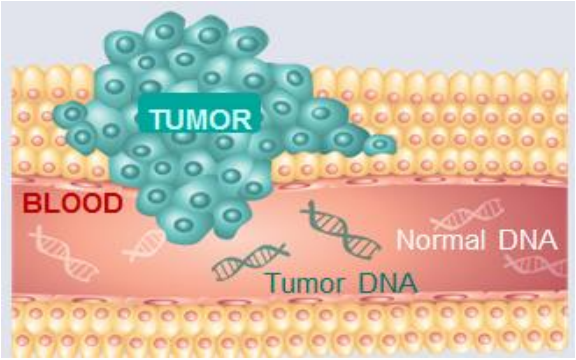
→

Refametinib 50 mg bid
(Sorafenib 400mg bid)

→

Primary endpoint:
ORR

Primary endpoint:	ORR (mRECIST)
Study treatment:	Refametinib 50mg twice daily (comb. with sorafenib 400mg twice daily)
Targeted ORR:	Stage 1: positive if ≥ 5/15 Responses Stage 2: trial positive if 47/80 Responses



BEAMing technique: Allows detection of mutations using DNA in plasma

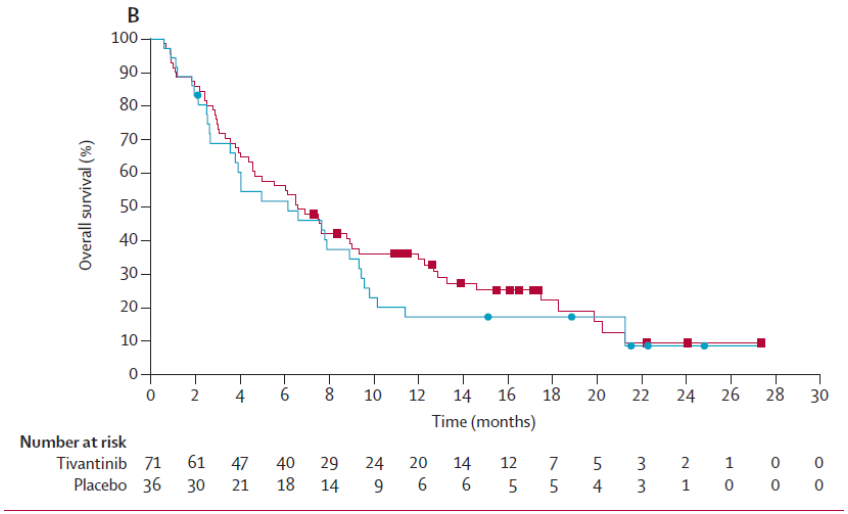
Li M *et al*. *Nat Methods* 2006; **3**: 95–97

Accuracy for PI3K mutations in breast cancer : 75-100%

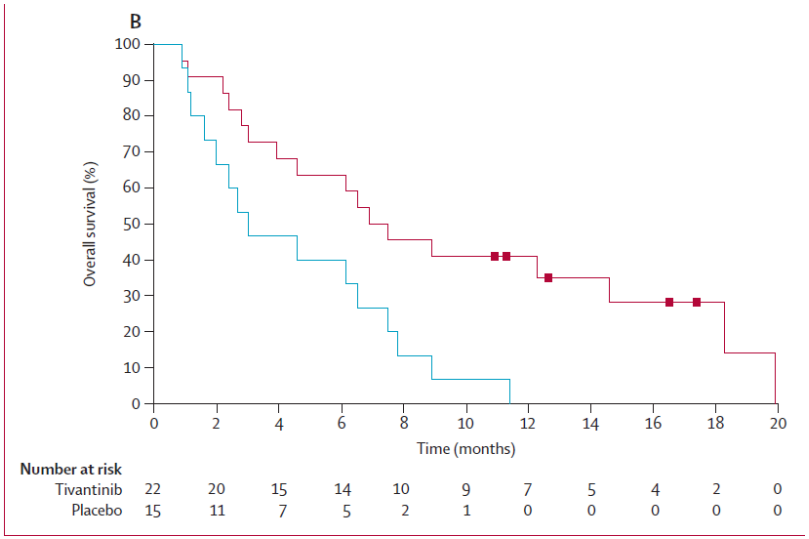
Higgins *et al*. Clin Can Res 2012

Tivantinib vs placebo in 2nd line: Phase II studies

- Tivantinib (n=71; OS= 6.6 mo) vs placebo (n=36; OS= 6.2 mo)



- Subgroup analysis-Enrichment for c-MET +: Tivantinib 7.2mo vs placebo 3.8 mo

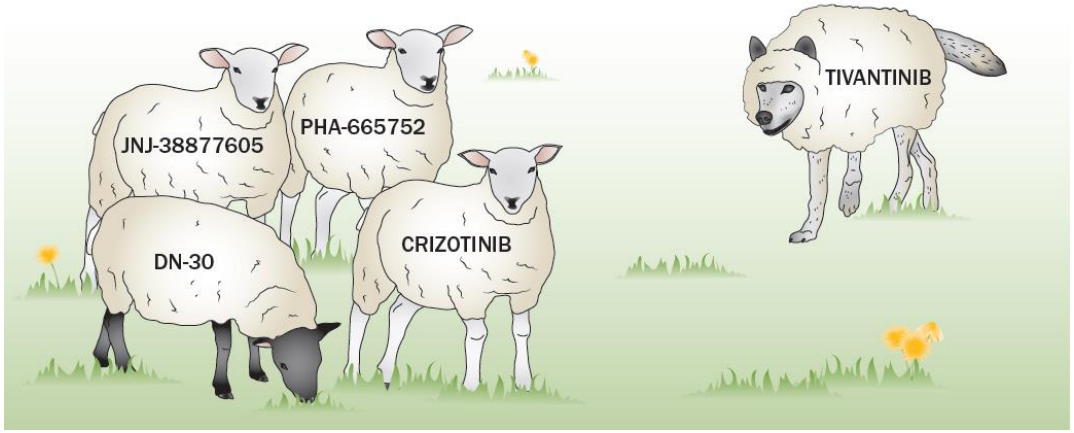


Santoro et al, Lancet Oncol 2013

Is tivantinib a c-MET inhibitor?

TARGETED THERAPIES

Tivantinib—a cytotoxic drug in MET inhibitor's clothes?



Michielli, Nat Rev Oncol 2013

- Tivantinib kills equally cells with MET amplification or not, as opposed to selective MET inhibitors.
- Tivantinib is a cytotoxic drug acting on microtubule dinamics independenly of MET.
- It might be very active against tumors with high proliferation (i.e cancers with KAS + and HGF-MET+ in liver!).

Phase III tivantinib vs placebo in 2nd line in MET+ patients

Conclusions

Molecular pathogenesis

- The key molecular aberrations in HCC:
    1. Mutations of TERT promoter, p53, CTNNB1, ARID1 , (<5%:KRAS and Pi3K)
    2. High-level amplifications of 6p21 (VEGF) and 11q13 (Cyclin D1 and FGF19)
    3. Angiogenesis: VEGF, PDGF,FGF
- Molecular classification of HCC defines subclasses depending on the tumor (CTNNB1, proliferation) and adjacent tissue

Molecular changes: implications for therapies

- Sorafenib is the sole molecular therapy approved for HCC
- Several phase III trials testing molecular therapies failed in improving survival
- New trials are designed targeting enriched populations/ potential oncogenic loops.

HCC GENOMIC CONSORTIUM

Mount Sinai Liver Cancer Program, NY.

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MOUNT SINAI SCHOOL OF MEDICINE

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Institut d'Investigacions Biomèdiques August Pi i Sunyer

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FONDAZIONE IRCCS ISTITUTO NAZIONALE DEI TUMORI

SEVENTH FRAMEWORK PROGRAMME

FUNDACIÓN aecc INVESTIGACIÓN CONTRA EL CÁNCER

AACR

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BCLC Group. Hospital Clínic Barcelona

Thank You