

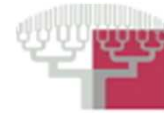


International
Cancer Genome
Consortium



Inserm

Institut national
de la santé et de la recherche médicale



UNIVERSITÉ
PARIS DESCARTES



Molecular profiling of hepatocellular carcinomas related to viral infections

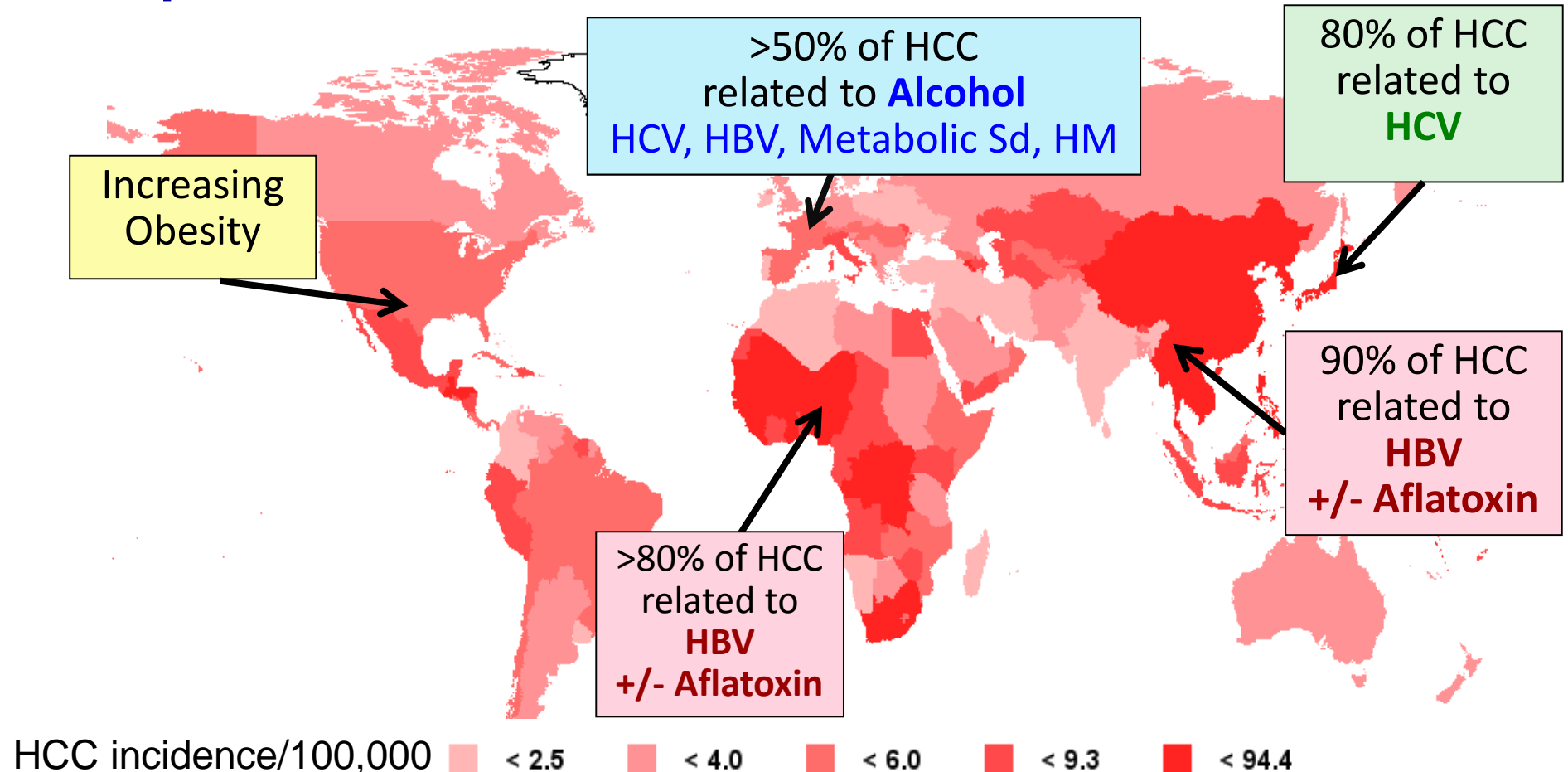
Jessica Zucman-Rossi

Inserm U1162

Paris

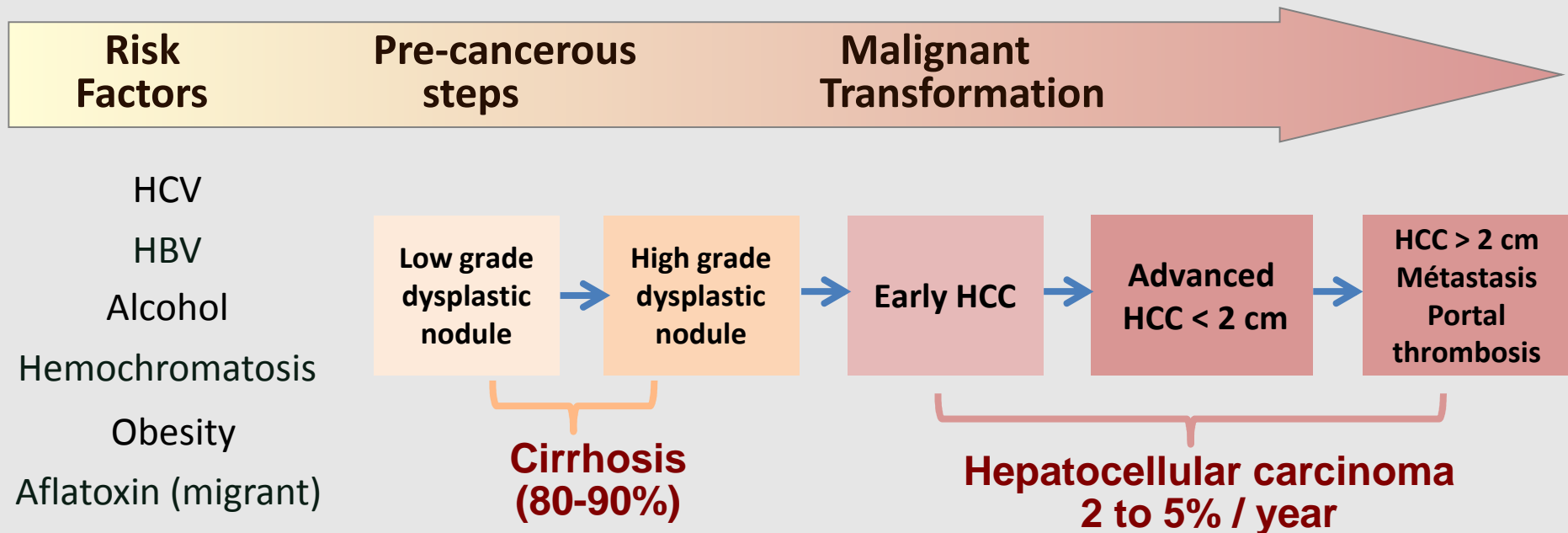


Geographical heterogeneity in hepatocellular carcinoma distribution

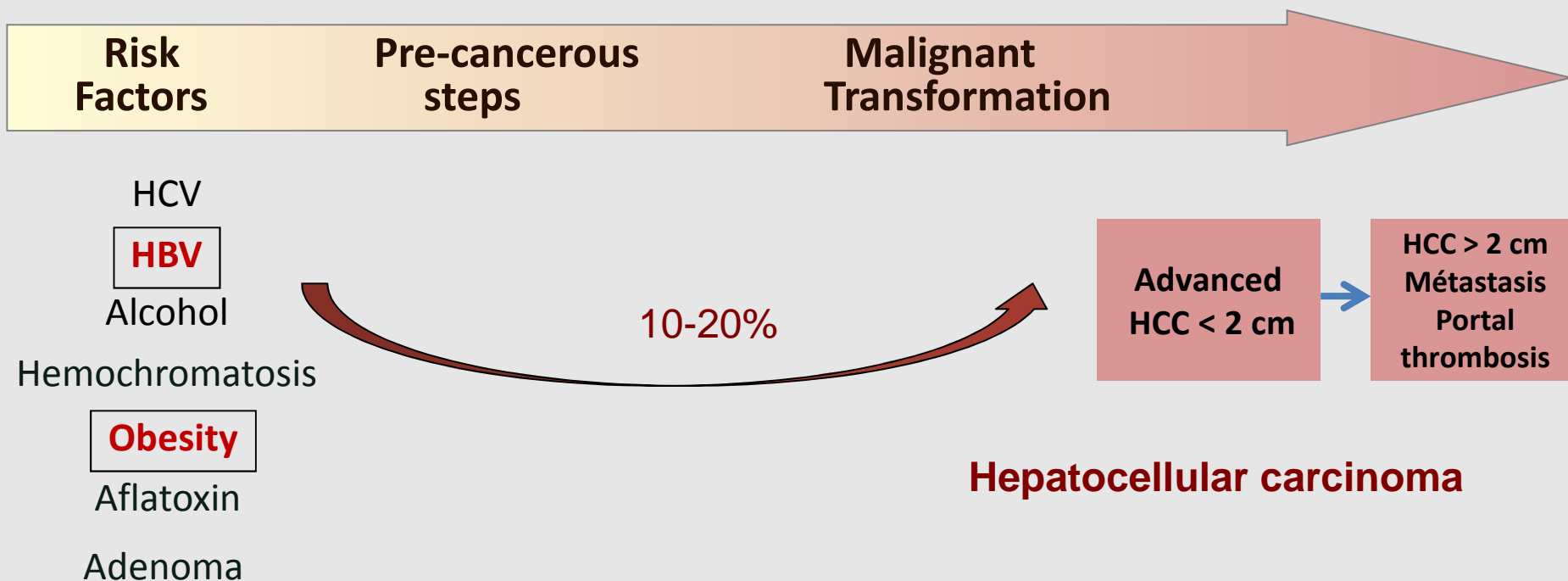


Hepatocellular carcinoma is the second leading cause of death among cancer patients worldwide

Hepatocarcinogenesis is a multistep disease



Hepatocarcinogenesis is a multistep disease



Aims of our research in genomics

Cancer is a disease of the Genome

We need to understand **HCC genomic diversity** to translate the molecular diversity in **biomarkers to improve clinical care** of patients with



Prediction of HCC
occurrence



Diagnosis



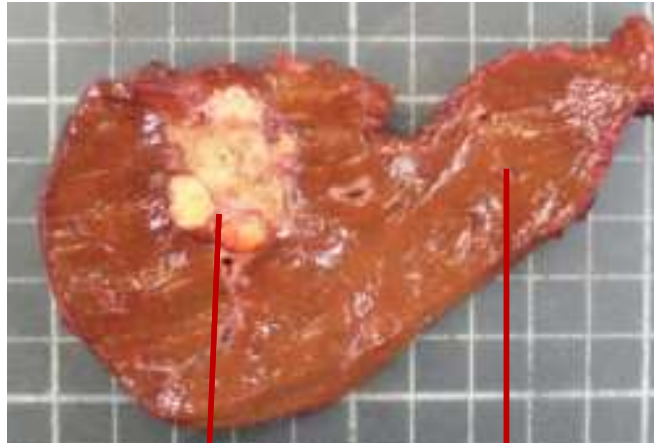
Prognosis



Prediction of
response
to treatment



Exome sequencing in HCC



Tumor

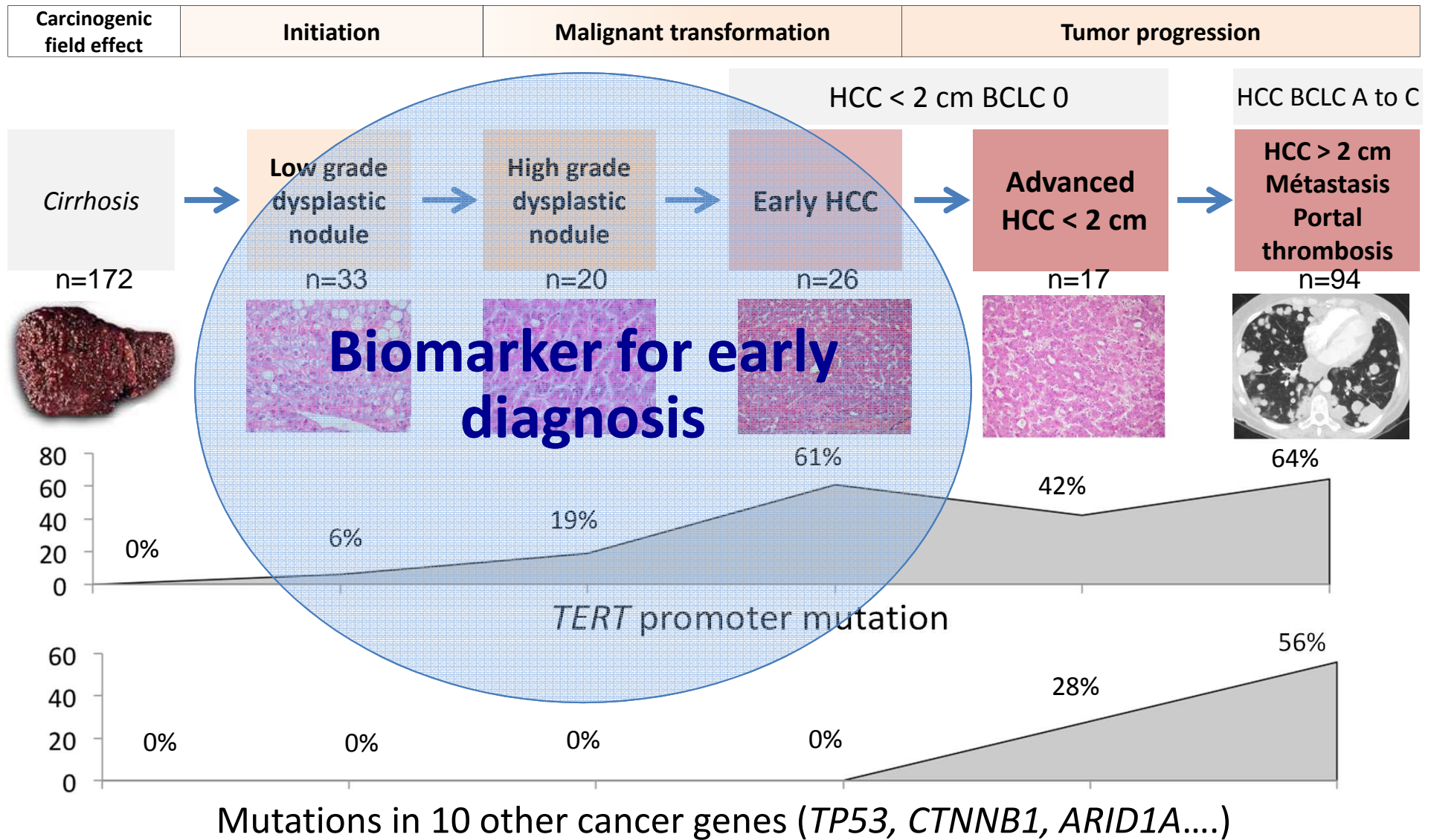
Non-tumor

Sequencing the whole coding
region of the genome (20,000
genes)

Mean of 40 damaging somatic mutations / tumor
Range: 5 to 121 /tumor

Each tumor result from a unique combination of mutations

Role of TERT promoter mutation in malignant progression



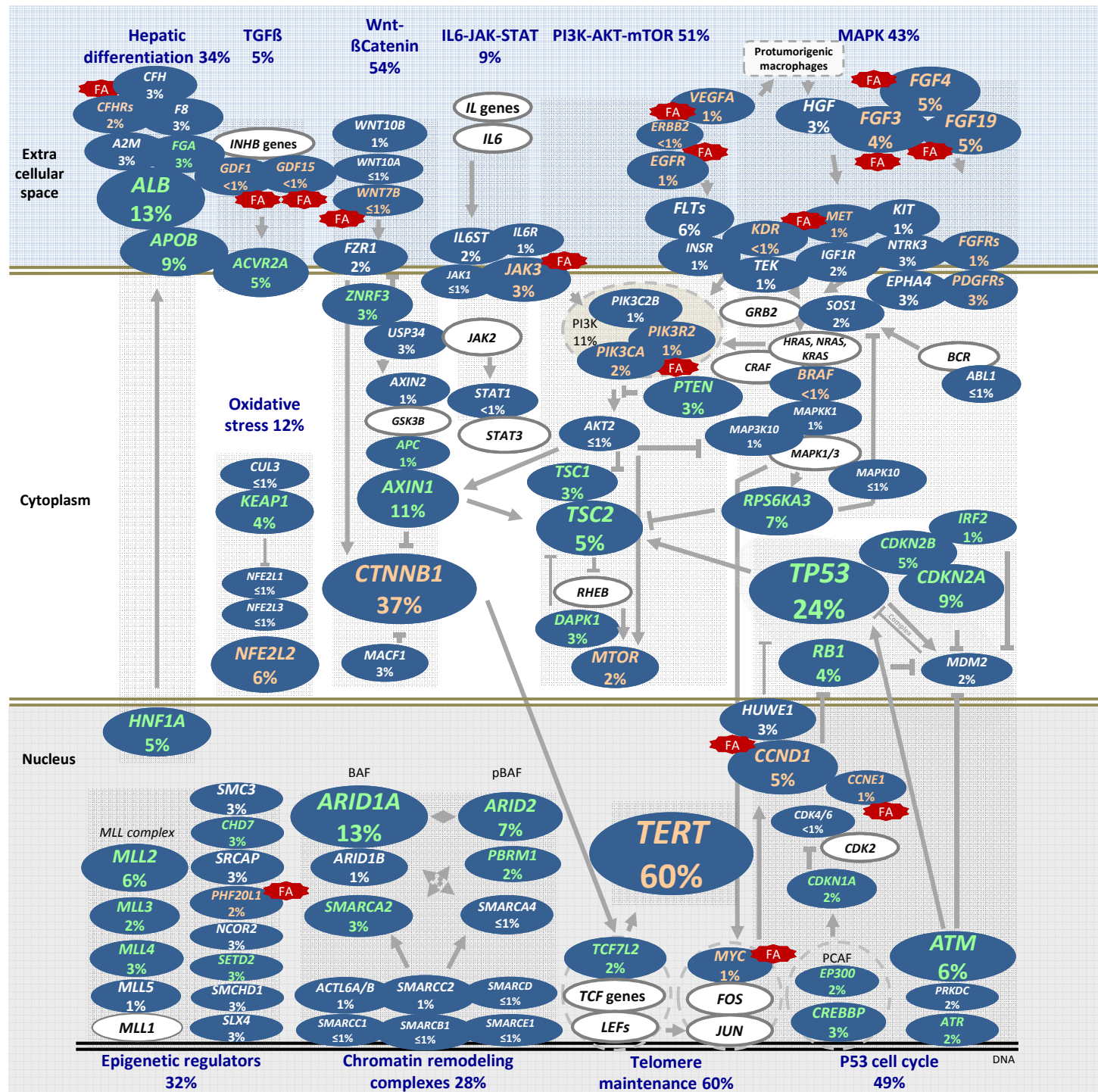
Nault et al. Nature Communications 2013
Nault JC, Calderaro J, et al. Hepatology 2014

Virtual slides at <http://mnd-tert2014.inserm-u1162.fr/>

Landscape of 161 driver genes belong in 11 pathways



Schulze, Imbeaud, Letouzé et al, Nature Genetics, March 30th, 2015

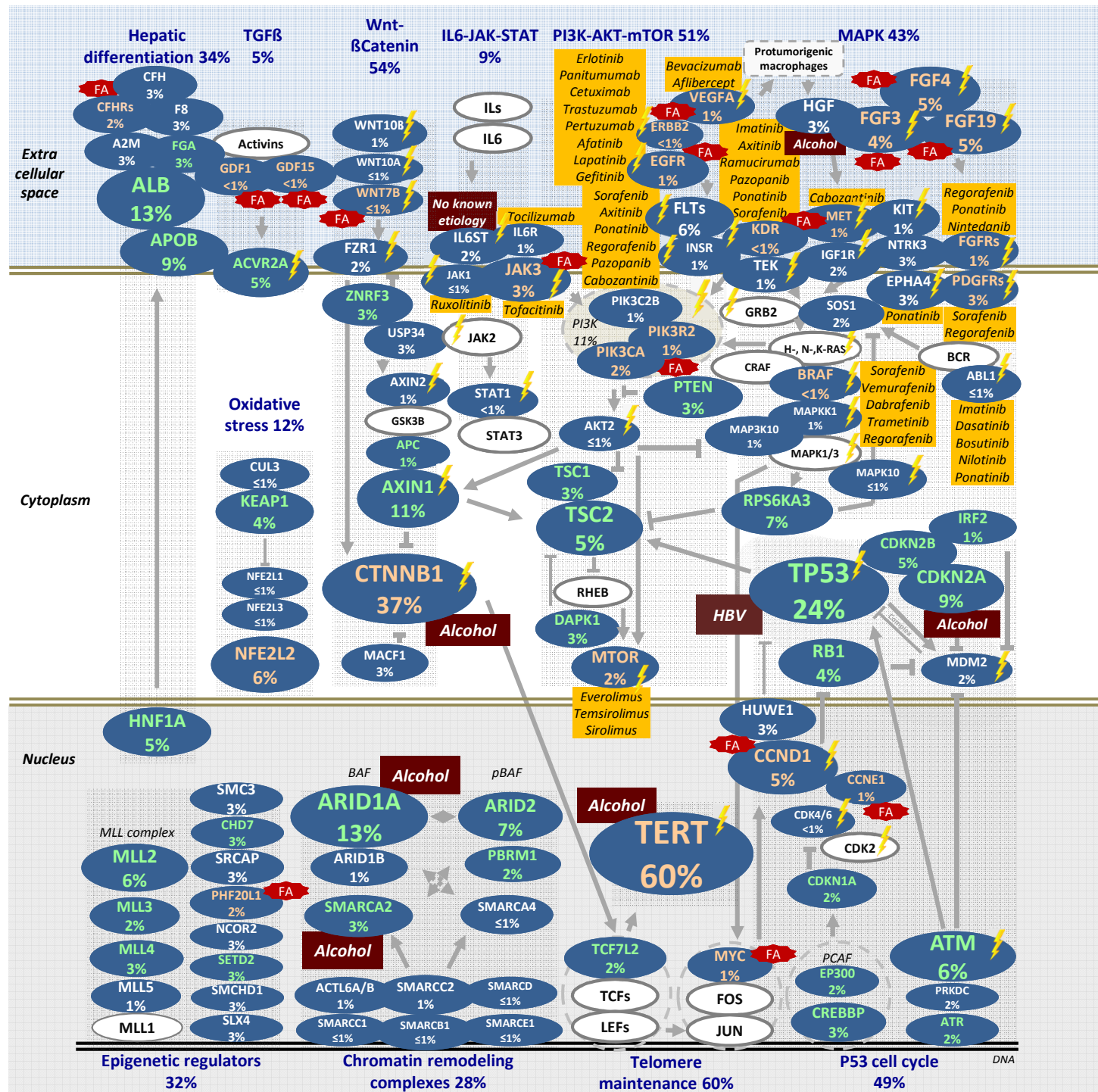


161 cancer driver genes: Targetable Genes

Drugs in
clinical
use


Drugs in
clinical trial

Schulze, Imbeaud,
Letouzé et al, Nature
Genetics, March 30th,
2015



Targetable

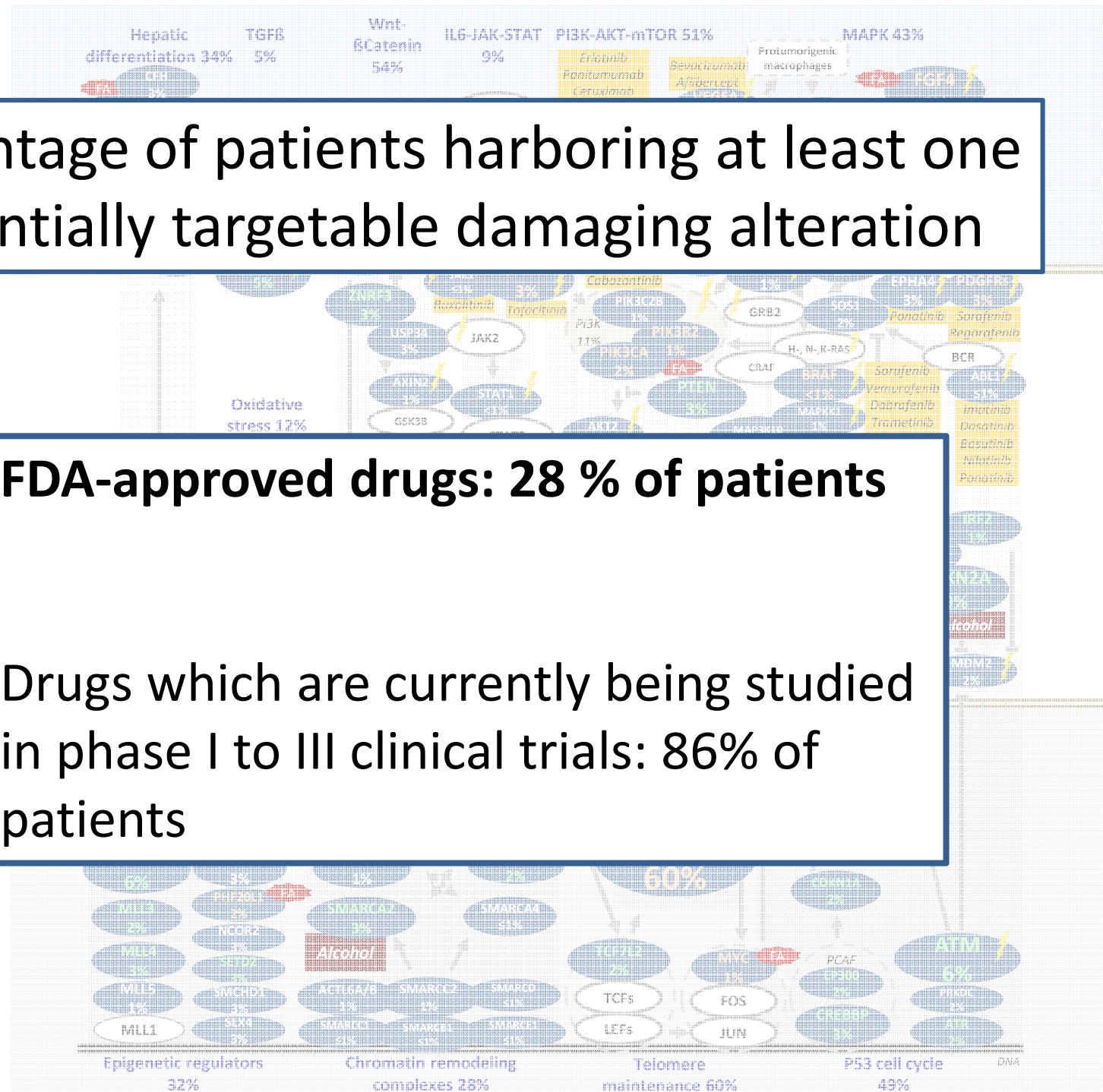
Genetic

Percentage of patients harboring at least one potentially targetable damaging alteration

- FDA-approved drugs: 28 % of patients
- Drugs which are currently being studied in phase I to III clinical trials: 86% of patients

Drugs in clinical use

Drugs in clinical trial



Next questions

Tumor sequencing

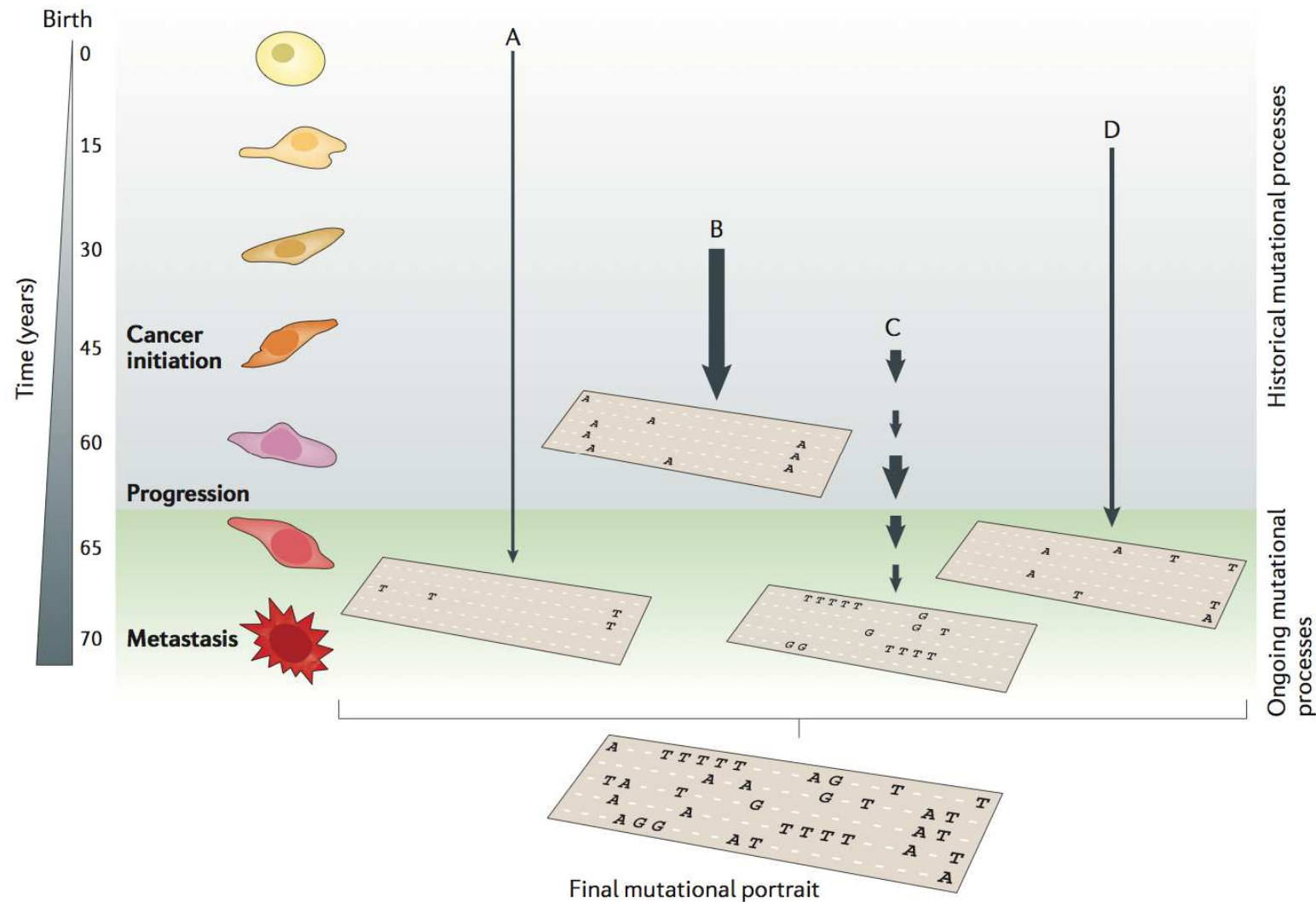
Epidemiological
studies

Spectrum of
somatic
nucleotidic
mutation



Identification of
new risk factors?

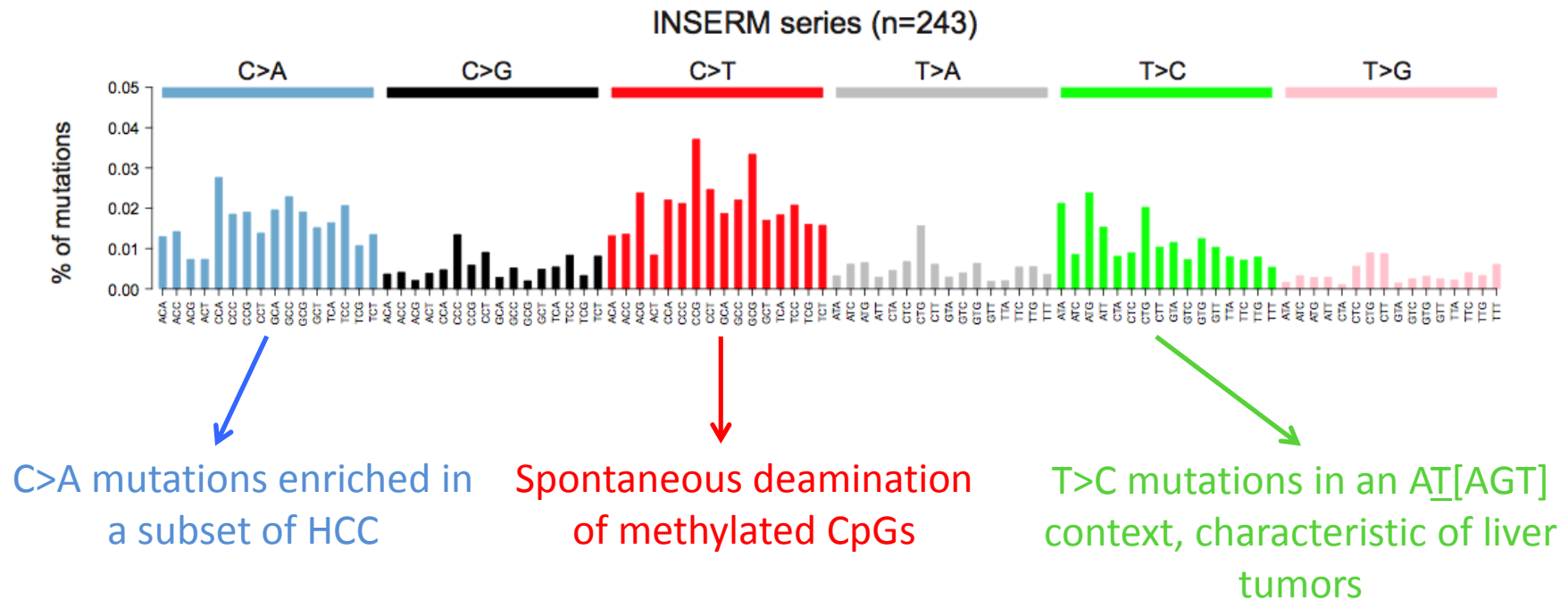
The mutational spectrum of a tumor reflects the accumulation of several mutational processes



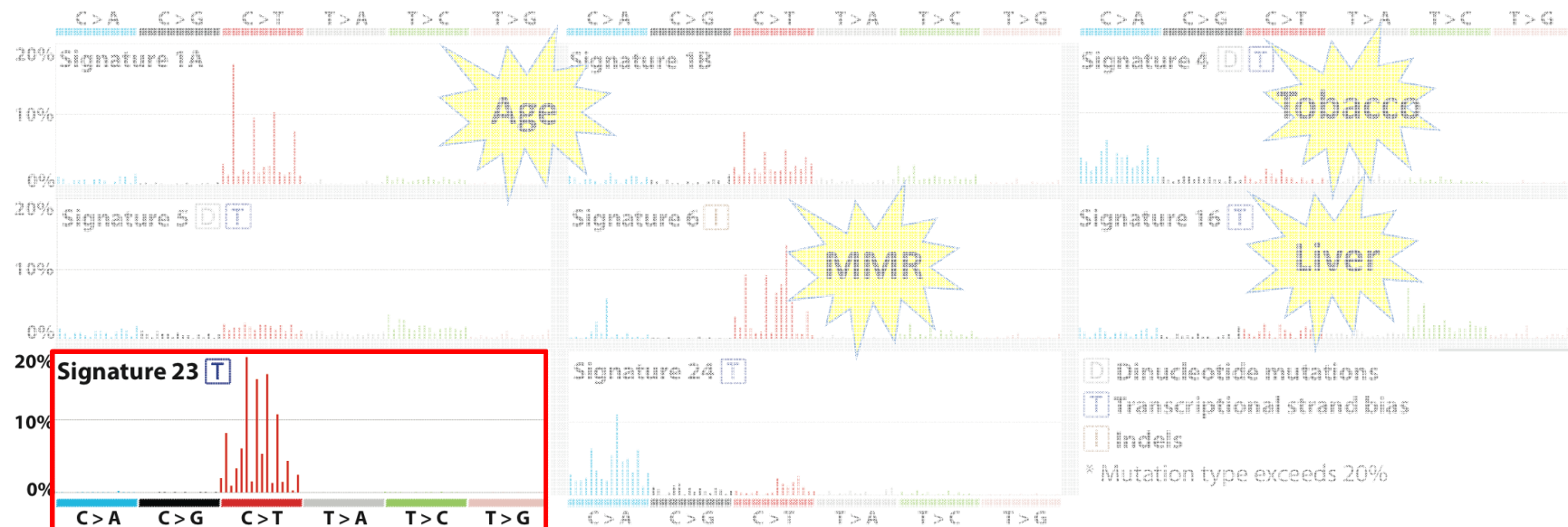
Helleday *et al.*, Nat Rev Genet 2014

The mutational spectrum of HCC

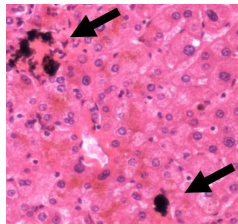
- Average mutational spectrum over 243 HCC analyzed by WES:



- Using the Wellcome Trust Sanger Institute method, we identified 8 signatures in our HCC series:

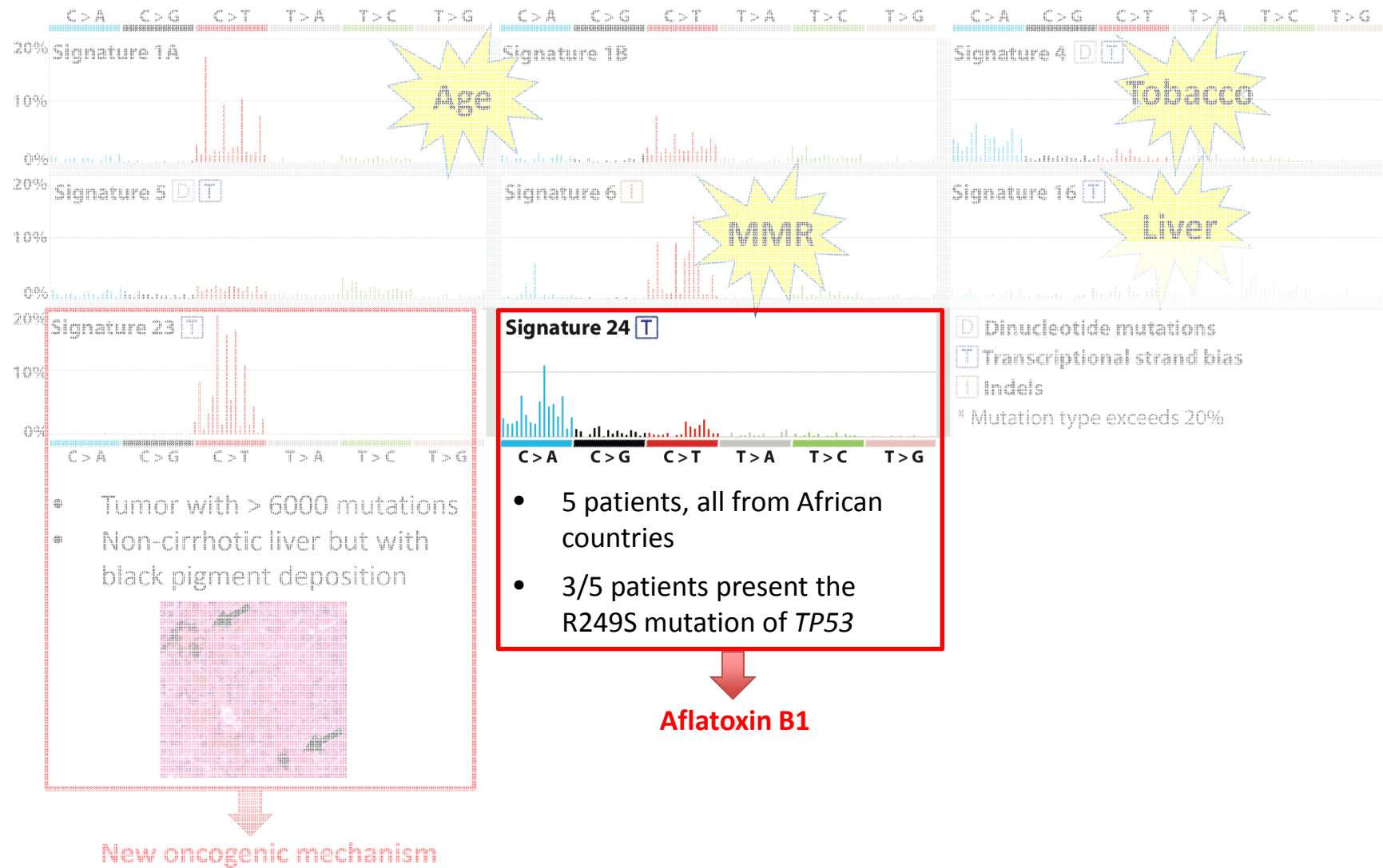


- Tumor with > 6000 mutations
- Non-cirrhotic liver but with black mineral deposits

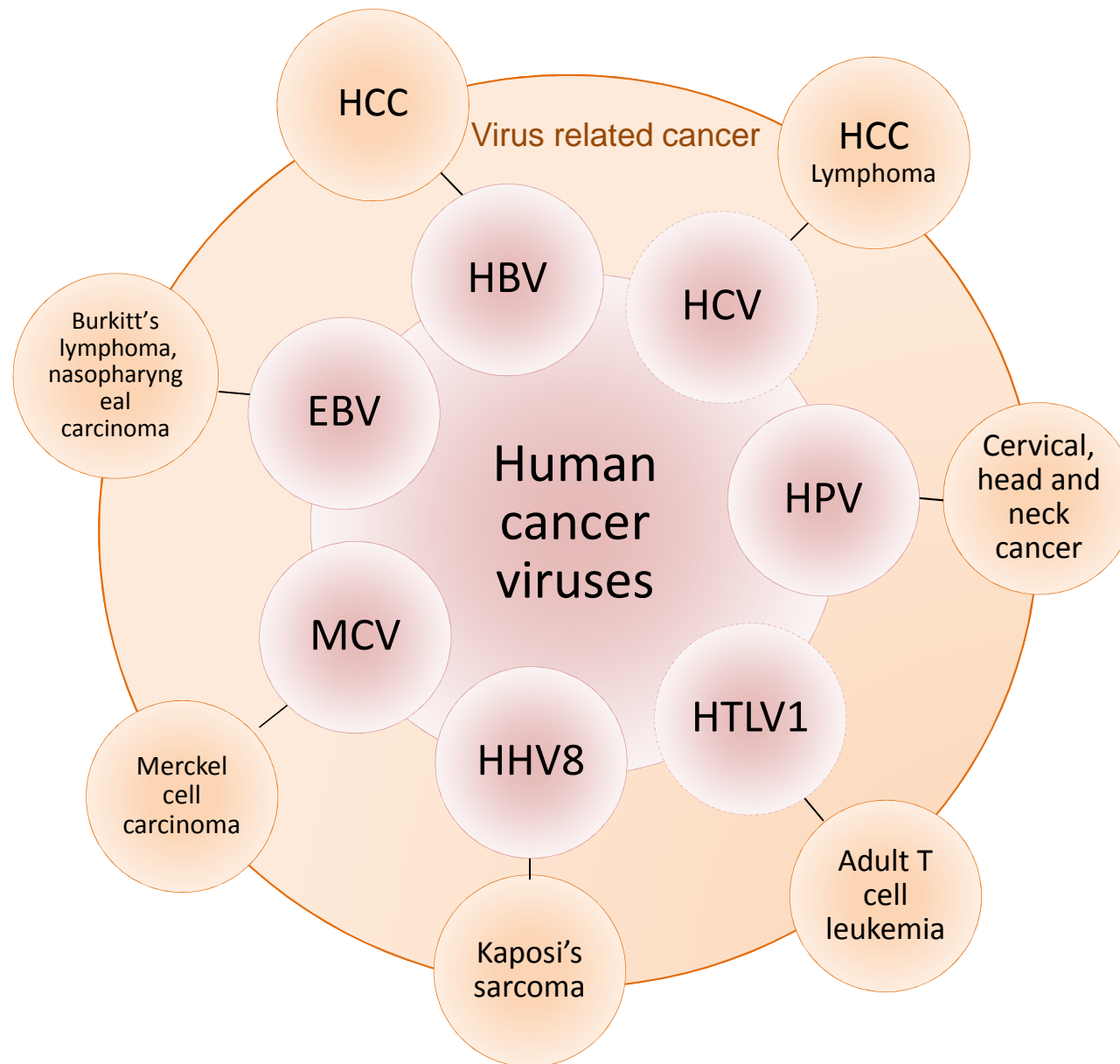


New oncogenic mechanism

- Overall 8 signatures were found operative in at least one HCC in our series:

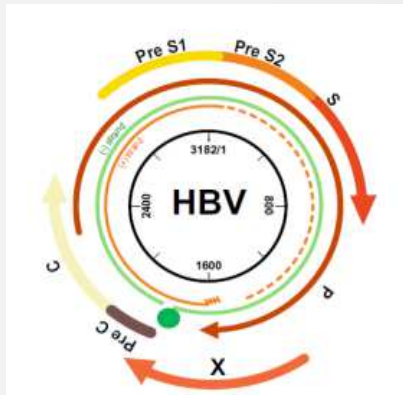


Virus induced oncogenesis in humans



Hepatitis B virus (HBV) related liver carcinogenesis

Chronic hepatitis B (HBV) infection



350 millions of chronic carriers



Normal liver



Chronic hepatitis

Inflammation
Necrosis and regeneration
Oxydative stress
Telomere shortening



Cirrhosis

Telomerase reactivation

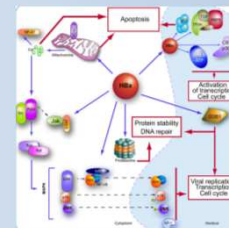


HCC

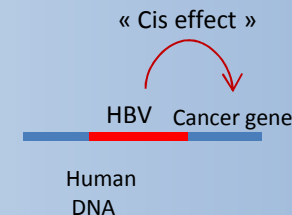
Indirect mechanism leading to HCC development

Direct mechanism leading to HCC development

Viral oncoprotein



Insertionnal mutagenesis



Insertional mutagenesis of HBV

Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma

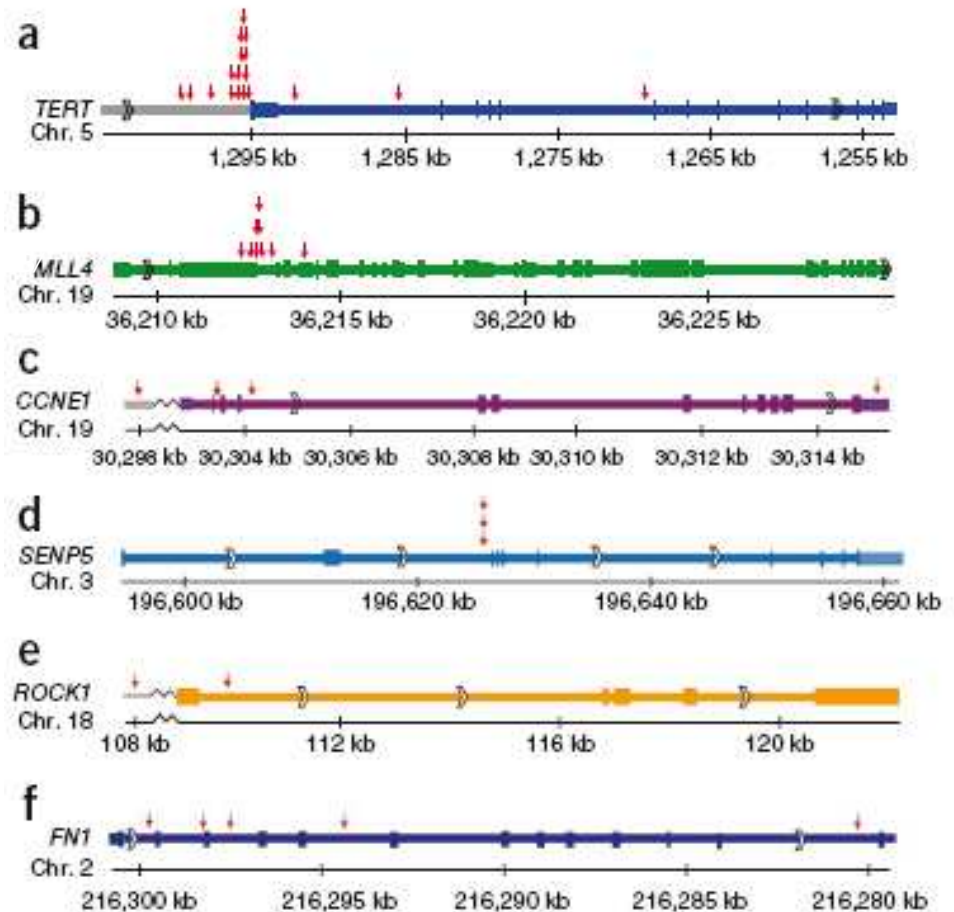
Jian Wang*, Xavier Chenivresse*, Berthold Henglein*
& Christian Bréchet*†

NATURE · VOL 343 · 8 FEBRUARY 1990

Oncogene (2003) 22, 3911–3916
© 2003 Nature Publishing Group All rights reserved 0950-9232/03 \$25.00
www.nature.com/onc

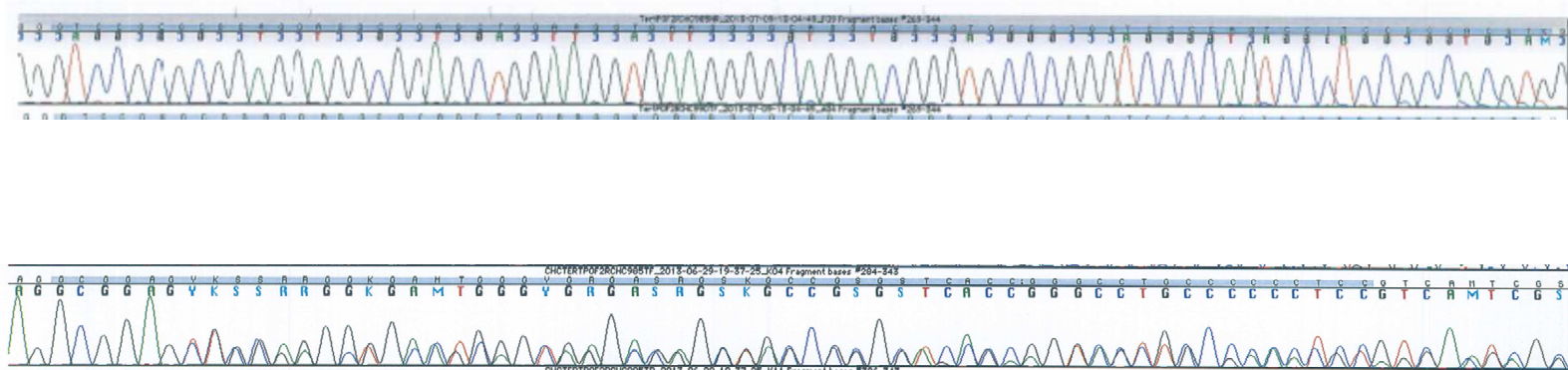
Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene

Patrizia Paterlini-Bréchet^{*1}, Kenichi Saigo², Yoshiki Murakami¹, Mounia Chami¹,
Devrim Gozuacik¹, Claude Mugnier³, David Lagorce, Christian Bréchet¹



Sung Wk, et al. Nat Gen 2012

A viral sequence in the *TERT* promoter of one hepatocellular carcinoma

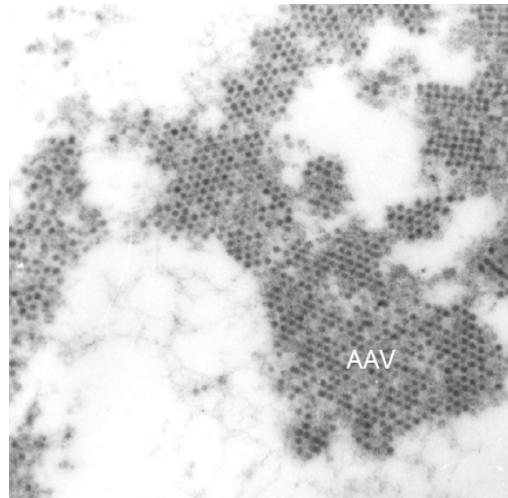


Adeno-associated virus type 2

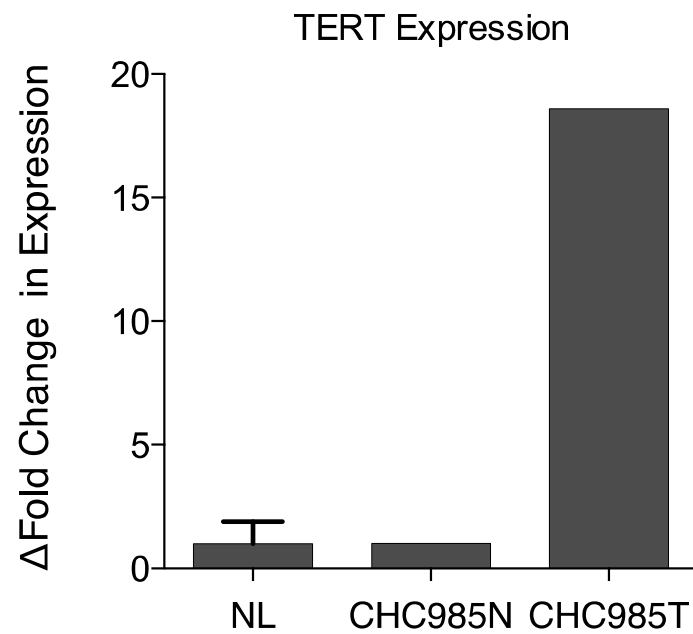
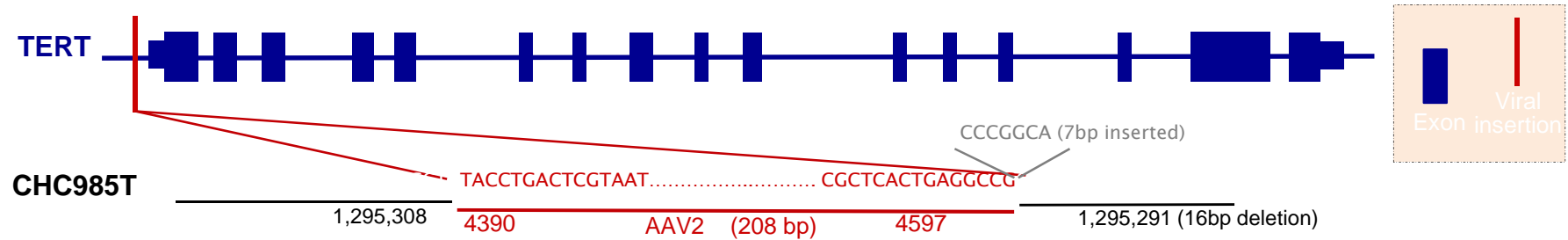
Nault JC, Datta S, Imbeaud S et al. Nature genetics, In press

Adeno-Associated virus type 2 (AAV2)

- Monostrand DNA virus
- Parvoviridae family
- Defective virus that requires another virus (adenovirus) to replicate and infect cells
- Described as a non-pathogenic infection (40-60% of the population)

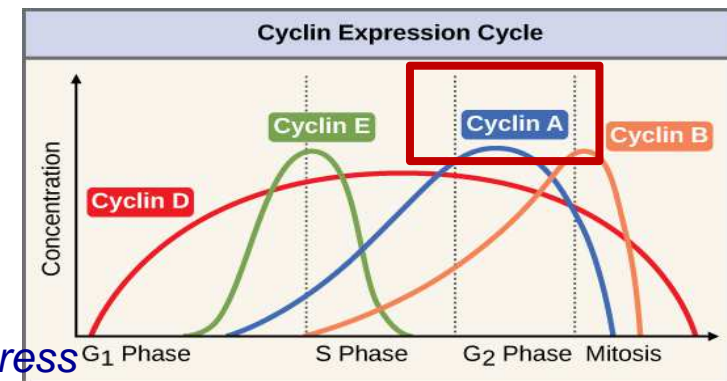
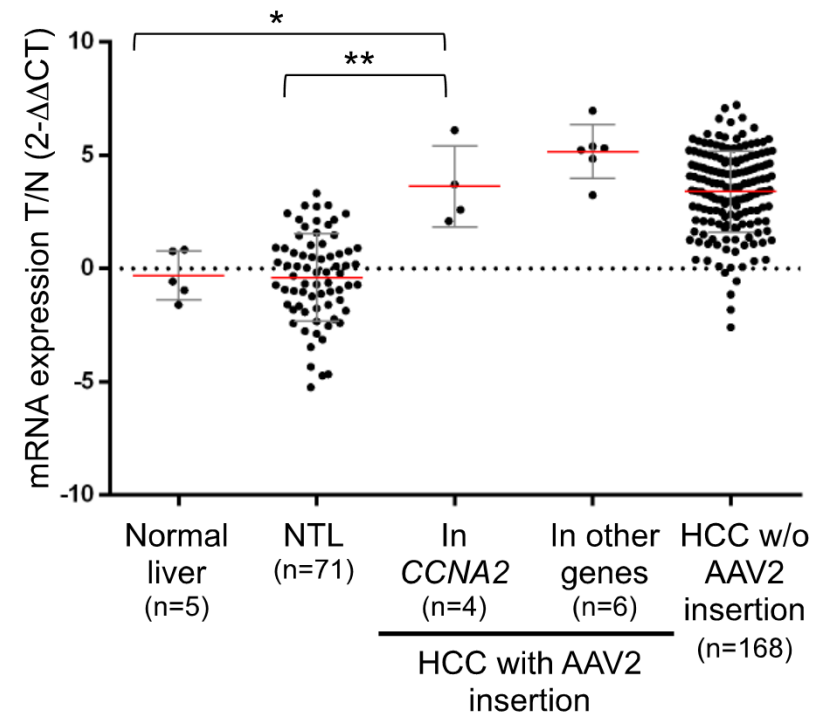
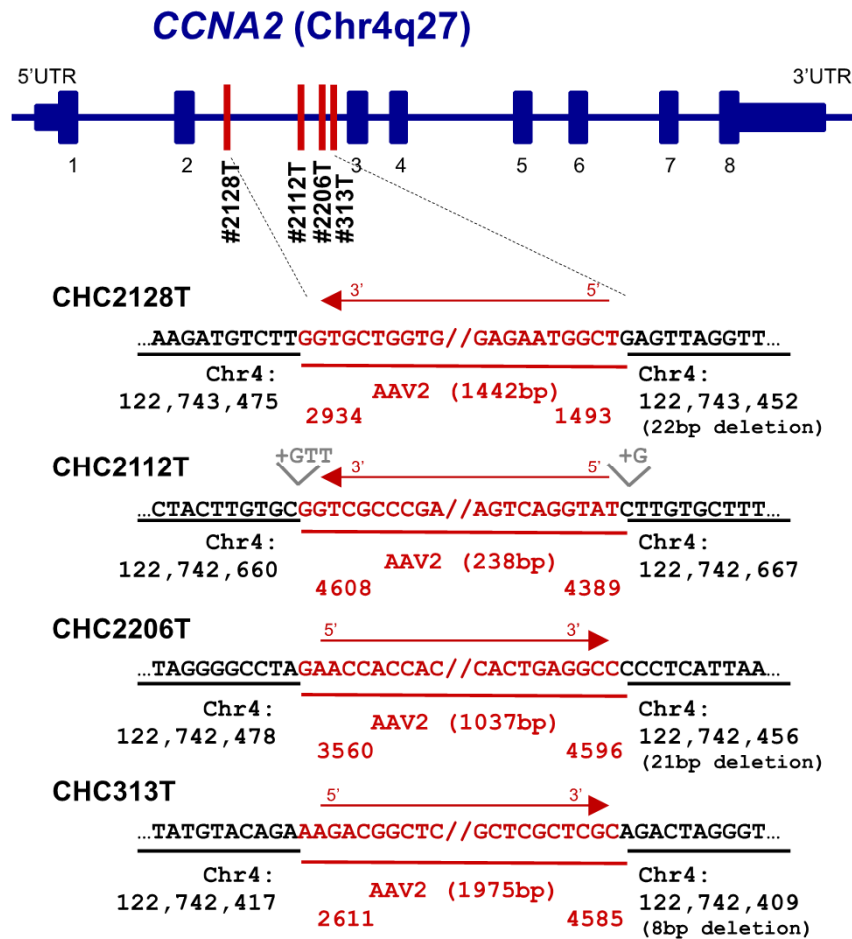


Insertion of AAV2 in the *TERT* promoter



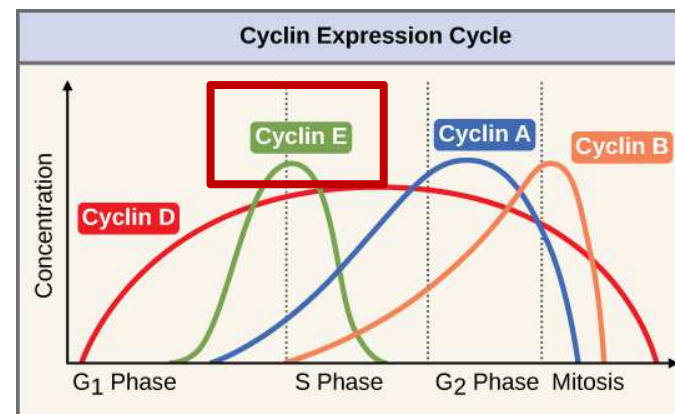
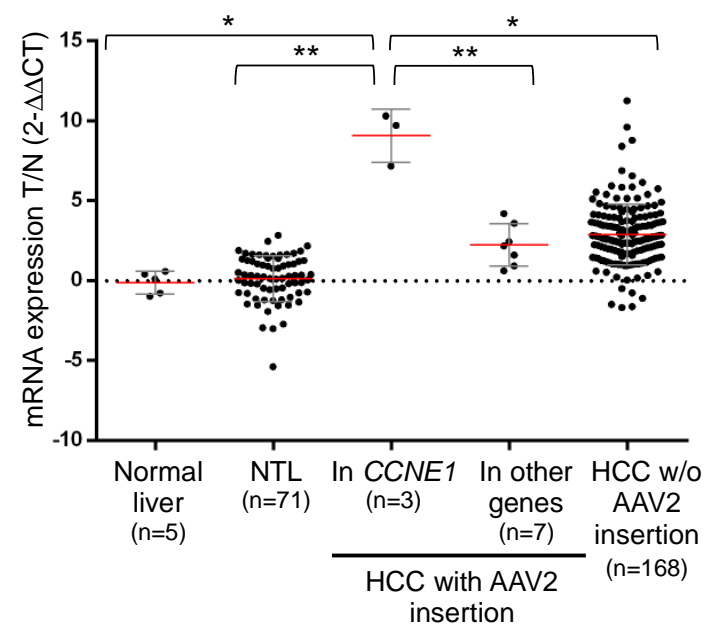
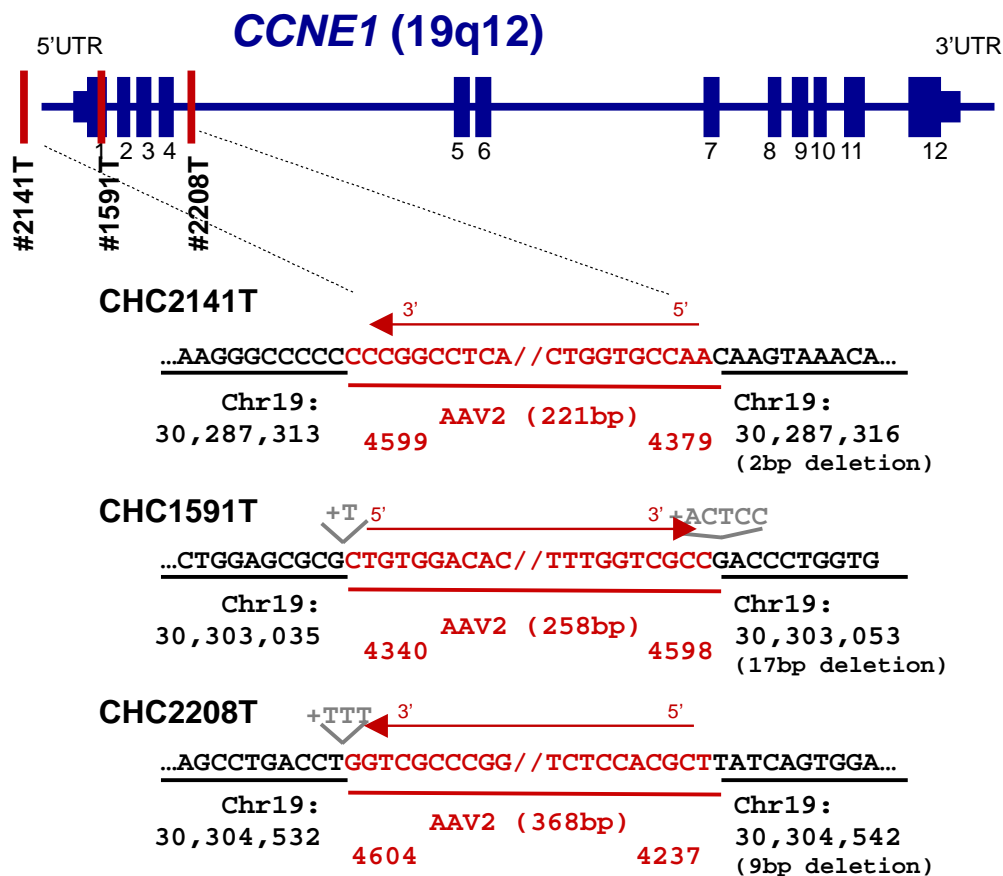
Nault JC, Datta S, Imbeaud S et al. *Nature genetics*, In press

Recurrent AAV2 insertion in *CCNA2* (Cyclin A)



Nault JC, Datta S, Imbeaud S et al. Nature genetics, In press

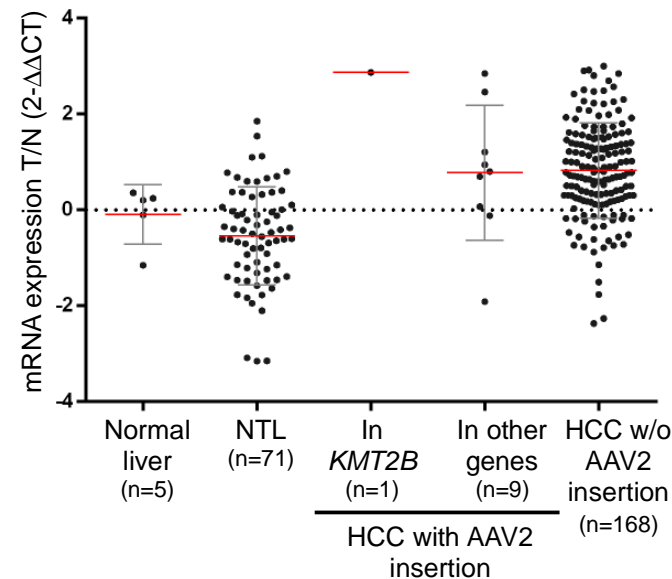
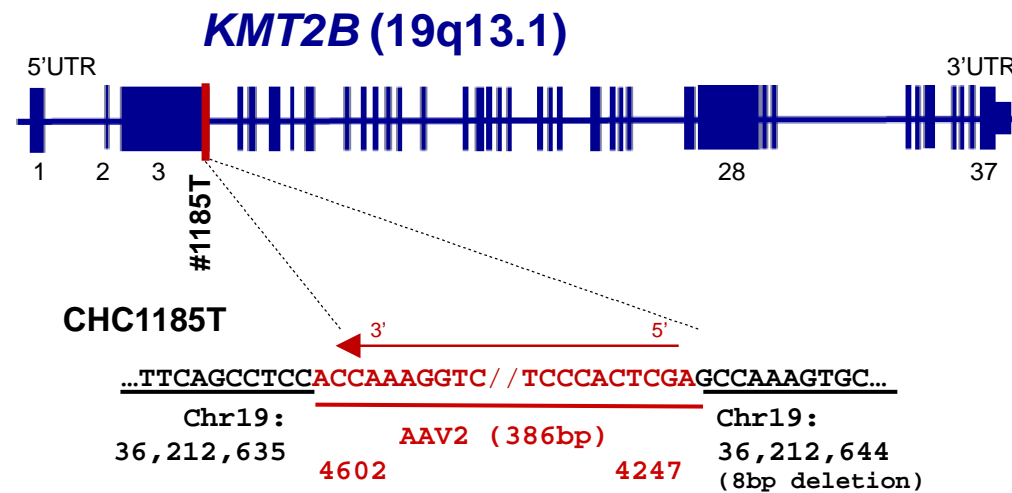
Recurrent AAV2 insertion in *CCNE1* (Cyclin E)



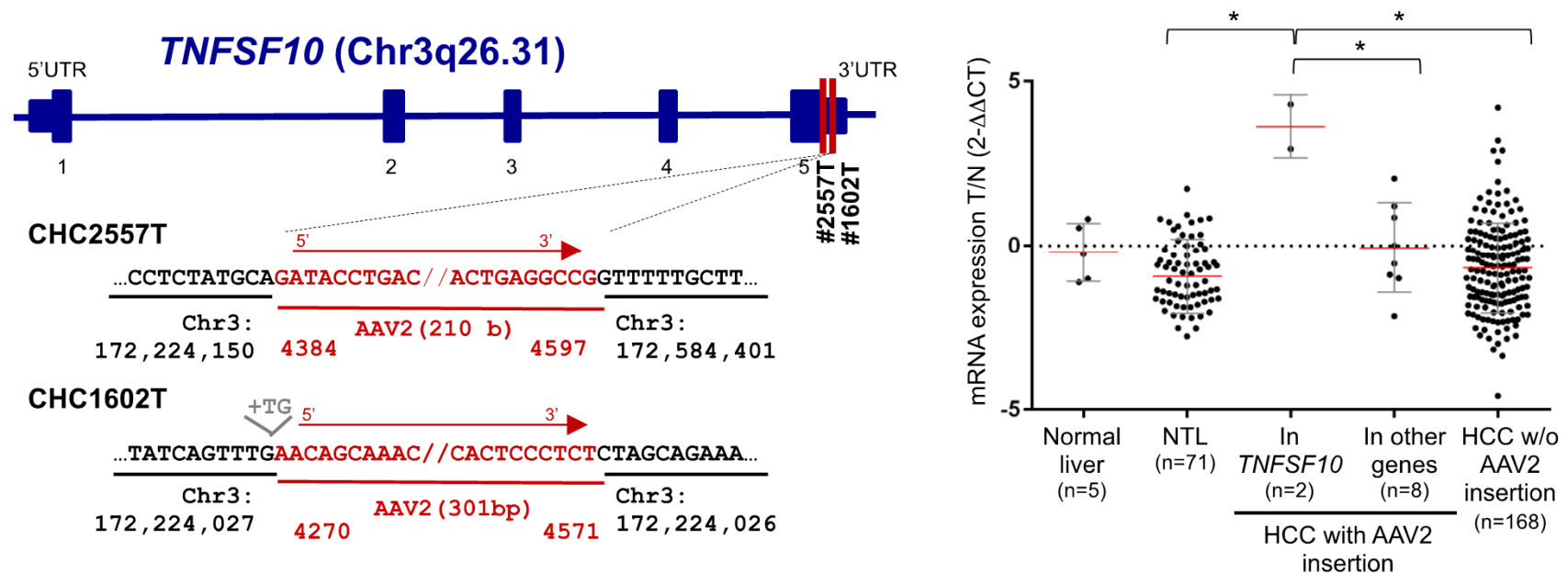
Nault JC, Datta S, Imbeaud S et al. Nature genetics, In press

AAV2 insertion in *KMT2B*

(*MLL4*, trithorax, Lysine (K)-Specific Methyltransferase 2B)



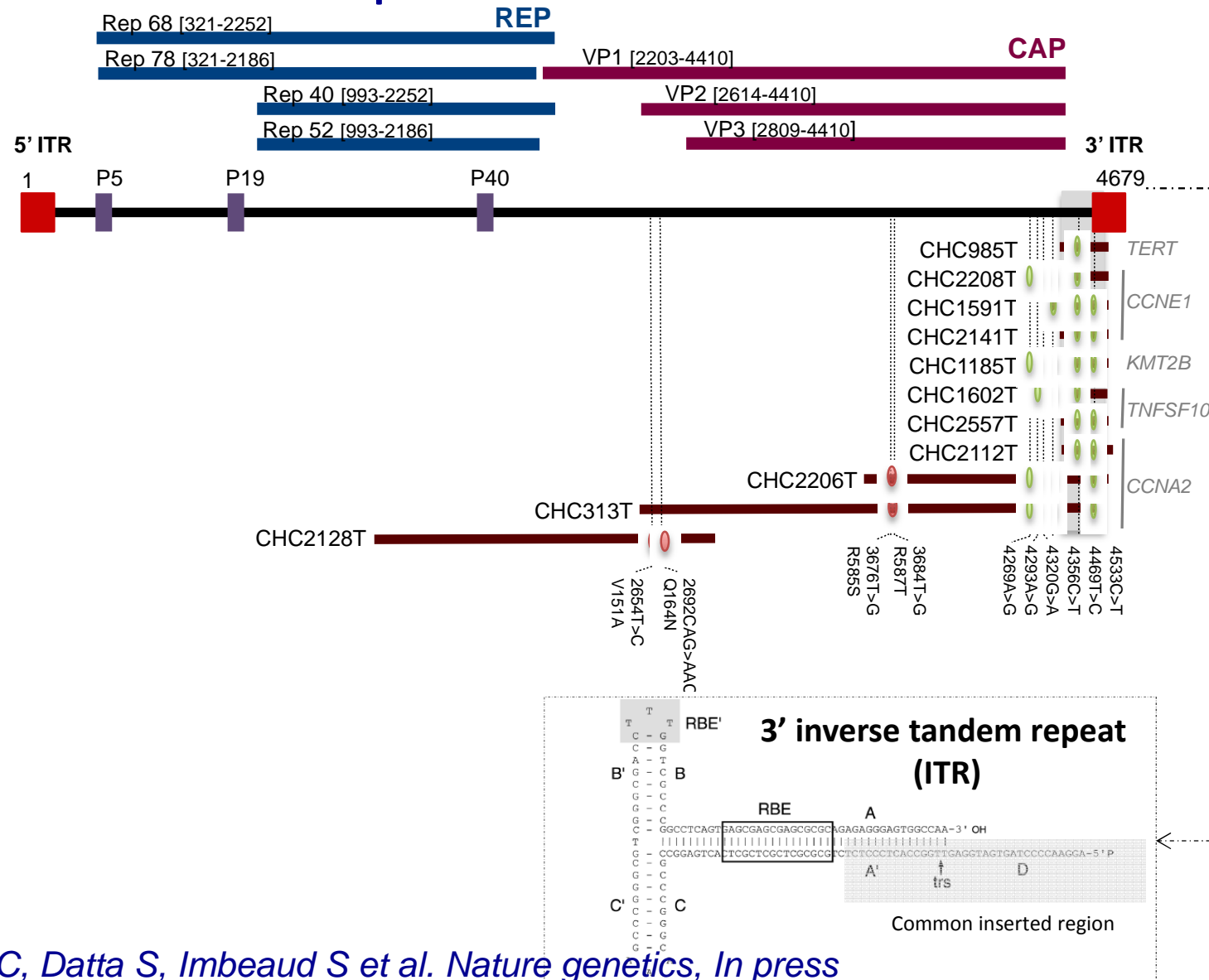
Recurrent AAV2 insertion in *TNFSF10* (*TRAIL*)



TNFSF10 codes for TRAIL

Dual role in cancer: apoptosis VS proliferative signalling

Recurrent integration of the 3' ITR region of AAV2 in 11 hepatocellular carcinomas



Nault JC, Datta S, Imbeaud S et al. *Nature genetics*, In press

AAV2 insertional mutagenesis in HCC on normal liver

Variable (number of available data)		AAV2 insertion (n=11)	No AAV2 insertion (n=182)
Age (n=193)	≤60 years	7 (64%, HR=5, q=0.06)*	63 (35%)
Risk Factor (n=193)	Alcohol	4 (36%)	91 (50%)
	HBV	1 (9%)	21 (12%)
	HCV	1 (9%)	35 (19%)
	Without etiology	6 (55%, HR=5, q=0.02)*	37 (20%)
Metavir Score in non-tumor liver (n=193)	F0-F1	8 (73%)	76 (42%)
	F2-F3	1 (9%)	45 (25%)
	F4	2 (18%)	61 (34%)
	F0-F1 (versus F2-F4)	8 (73%, HR=4, q=0.05)*	76 (42%)

*Significant enrichment using Monte-Carlo test with 2,000 permutations (p<0.05)

AAV2 insertions are more identified in HCC developed on normal liver without etiology

without *TERT* promoter mutation (0/11 HCC)

Nault JC, Datta S, Imbeaud S et al. Nature genetics, In press

AAV as a vector for gene therapy in humans

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

DECEMBER 22, 2011

VOL. 365 NO. 25

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S., Cecilia Rosales, Ph.D., Jenny McIntosh, Ph.D., David C. Linch, M.B., B.Chir., Pratima Chowdary, M.B., B.S., Anne Riddell, B.Sc., Arnulfo Jaquilmac Pie, B.S.N., Chris Harrington, B.S.N., James O'Beirne, M.B., B.S., M.D., Keith Smith, M.Sc., John Pasi, M.D., Bertil Glader, M.D., Ph.D., Pradip Rustagi, M.D., Catherine Y.C. Ng, M.S., Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D., John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D., Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D., Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial

Michael G Kaplitt, Andrew Feigin, Chengke Tang, Helen L Fitzsimons, Paul Mattis, Patricia A Lawlor, Ross J Bland, Deborah Young, Kristin Strybing, David Eidelberg, Matthew J During

Lancet 2007; 369: 2097–105

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis

Albert M. Maguire, M.D., Francesca Simonelli, M.D., Eric A. Pierce, M.D., Ph.D., Edward N. Pugh, Jr., Ph.D., Federico Mingozzi, Ph.D., Jeannette Benniselli, Ph.D., Sandro Banfi, M.D., Kathleen A. Marshall, C.O.T., Francesco Testa, M.D., Enrico M. Surace, D.V.M., Settimio Rossi, M.D., Arkady Lyubarsky, Ph.D., Valder R. Arruda, M.D., Barbara Konkle, M.D., Edwin Stone, M.D., Ph.D., Junwei Sun, M.S., Jonathan Jacobs, Ph.D., Lou Dell'Osso, Ph.D., Richard Hertle, M.D., Jian-xing Ma, M.D., Ph.D., T. Michael Redmond, Ph.D., Xiaosong Zhu, M.D., Bernd Hauck, Ph.D., Olga Zelenai, Ph.D., Kenneth S. Shindler, M.D., Ph.D., Maureen G. Maguire, Ph.D., J. Fraser Wright, Ph.D., Nicholas J. Volpe, M.D., Jennifer Wellman McDonnell, M.S., Alberto Auricchio, M.D., Katherine A. High, M.D., and Jean Bennett, M.D., Ph.D.

N ENGL J MED 358;21 WWW.NEJM.ORG MAY 22, 2008

Phase I Trial of Intramuscular Injection of a Recombinant Adeno-Associated Virus Serotype 2 α_1 -Antitrypsin (AAT) Vector in AAT-Deficient Adults

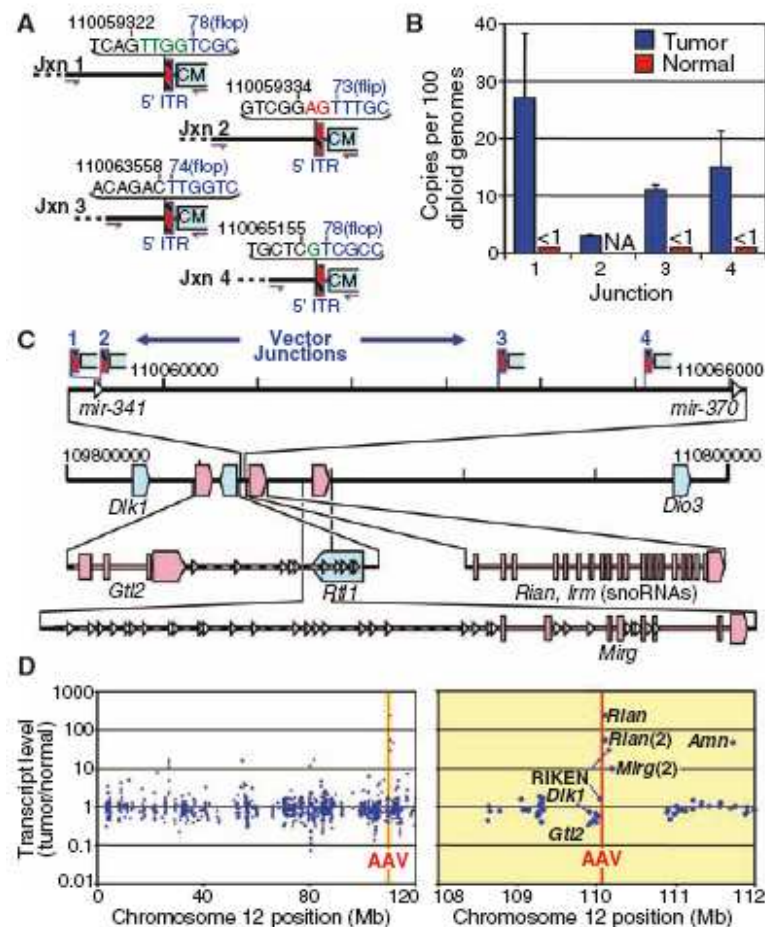
MARK L. BRANTLY,^{1,2} L. TERRY SPENCER,³ MARGARET HUMPHRIES,^{2,3} THOMAS J. CONLON,^{2,3} CAROLYN T. SPENCER,^{2,3} AMY POIRIER,^{2,3} WENDY GARLINGTON,^{2,3} DAWN BAKER,^{2,3} SIHONG SONG,^{2,4} KENNETH I. BERNIS,^{2,5} NICHOLAS MUZYCZKA,^{2,5} RICHARD O. SNYDER,^{2,5} BARRY J. BYRNE,^{2,3,5} and TERENCE R. FLOTTE^{2,3,5}

HUMAN GENE THERAPY 17:1177–1186 (December 2006)

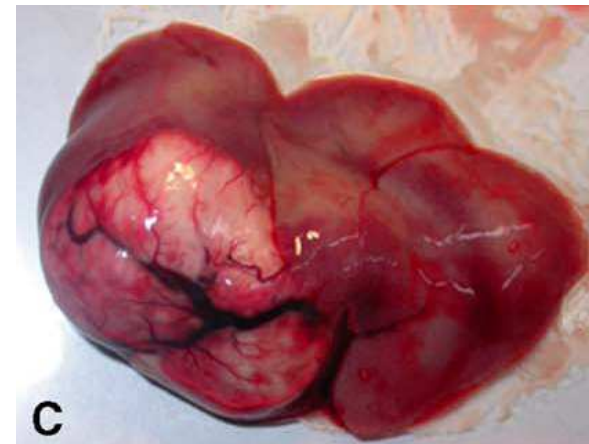
AAV Vector Integration Sites in Mouse Hepatocellular Carcinoma

Anthony Donsante,^{1*} Daniel G. Miller,^{2*} Yi Li,^{3,4} Carole Vogler,⁵ Elizabeth M. Brunt,⁵
David W. Russell,^{3,4†} Mark S. Sands^{1,6†}

SCIENCE VOL 317 27 JULY 2007



High incidence of HCC in mice treated with AAV mediated gene therapy



Vector design influences hepatic genotoxicity after adeno-associated virus gene therapy

Randy J. Chandler,¹ Matthew C. LaFave,² Gaurav K. Varshney,² Niraj S. Trivedi,³ Nuria Carrillo-Carrasco,⁴ Julien S. Senac,¹ Weiwei Wu,⁵ Victoria Hoffmann,⁶ Abdel G. Elkahoul,⁵ Shawn M. Burgess,² and Charles P. Venditti¹

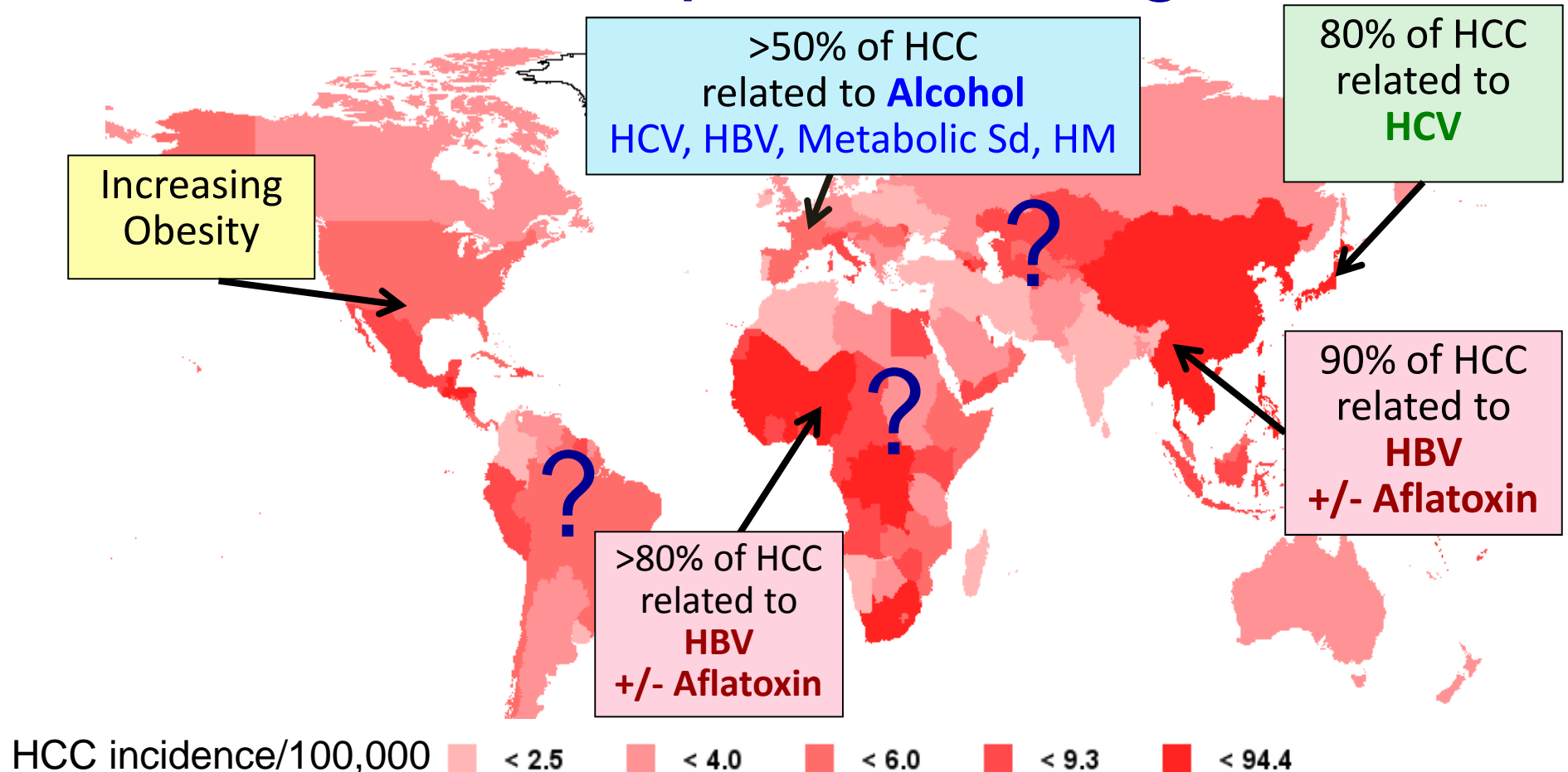
The Journal of Clinical Investigation

February 2015

Conclusion

- **TERT promoter mutation** is the earliest recurrent genomic alteration in hepatocarcinogenesis
- **160 cancer driver genes** in 11 pathways are recurrently mutated in HCC => new therapeutic opportunities
- **AAV2** is the fourth virus showing evidence of **insertional mutagenesis** in human tumors (after Human Papilloma virus, Merkel cell polyomavirus and Hepatitis B virus).
- **Molecular epidemiology** is possible thanks to deep sequencing

Genomics could help to better understand hepatocarcinogenesis



Hepatocellular carcinoma is the second leading cause of death among cancer patients worldwide

Inserm U1162, Paris

Direction :

Jessica Zucman-Rossi

Shalini Datta

Sandrine Imbeaud

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Gabrielle Couchy

Eric Letouze

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Guillaume Morcrette

Josselin Bouchaud

Emilie Gelabale

Jayendra Shinde

Fabien Calvo

Eric Letouzé

Gabrielle Couchy

Karine Poussin

Julien Calderaro

Giuliana Amaddeo

Maxime Mallet

Qian Cao

Cécile Guichard

Clement Meiller

Jean Verdier

Nathalie Ganne-

Carrie

Pierre Nahon

Marianne Ziol

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Jean Saric

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Créteil

Alexis Laurent

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