



Dissecting tumor heterogeneity with single cell and spatial analyses

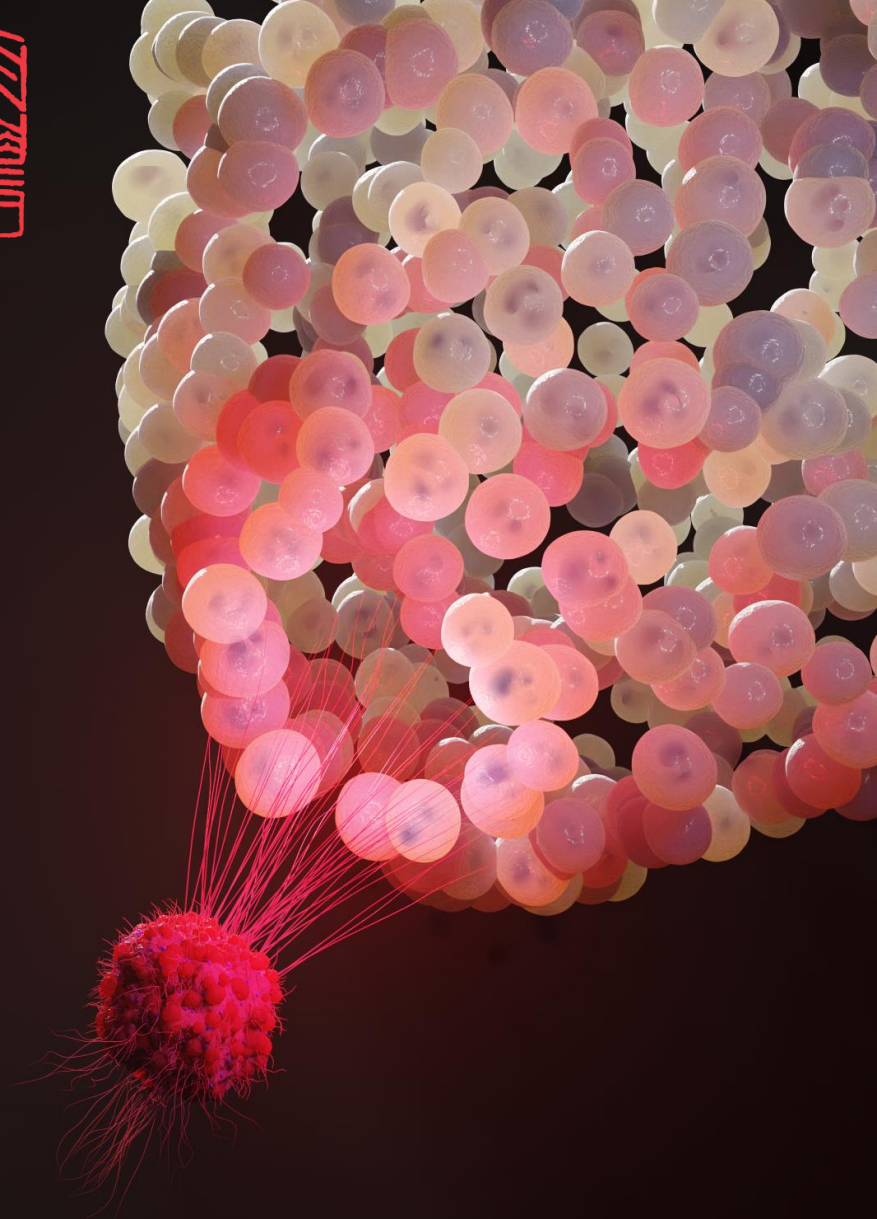
@ HKU MED Croucher Summer Course

Translational Immunology

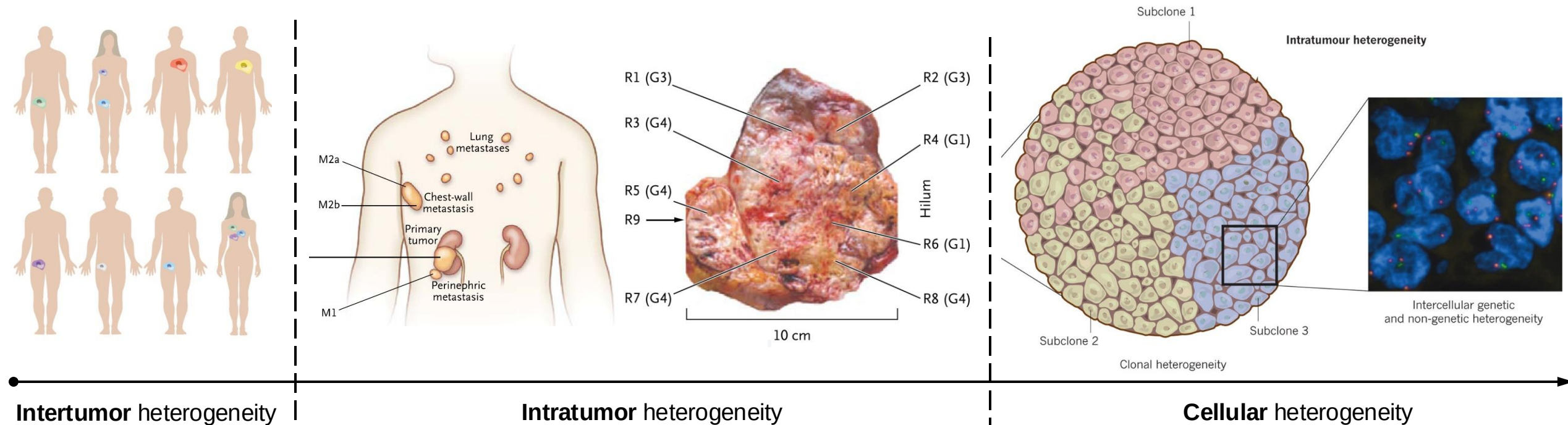
Jul.19 2024

Ruidong Xue 薛瑞栋 PhD

Peking University

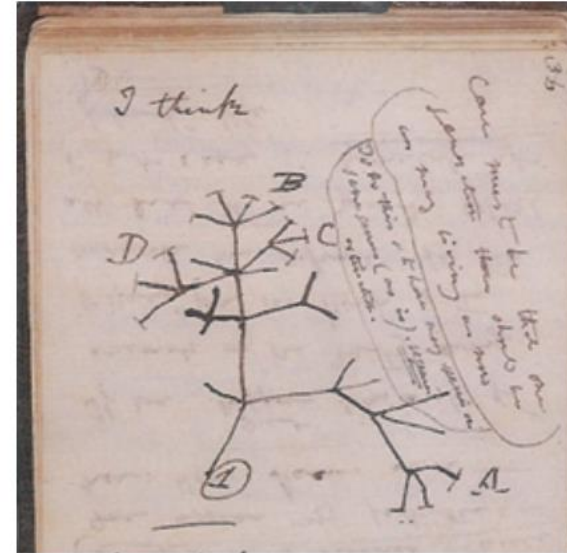
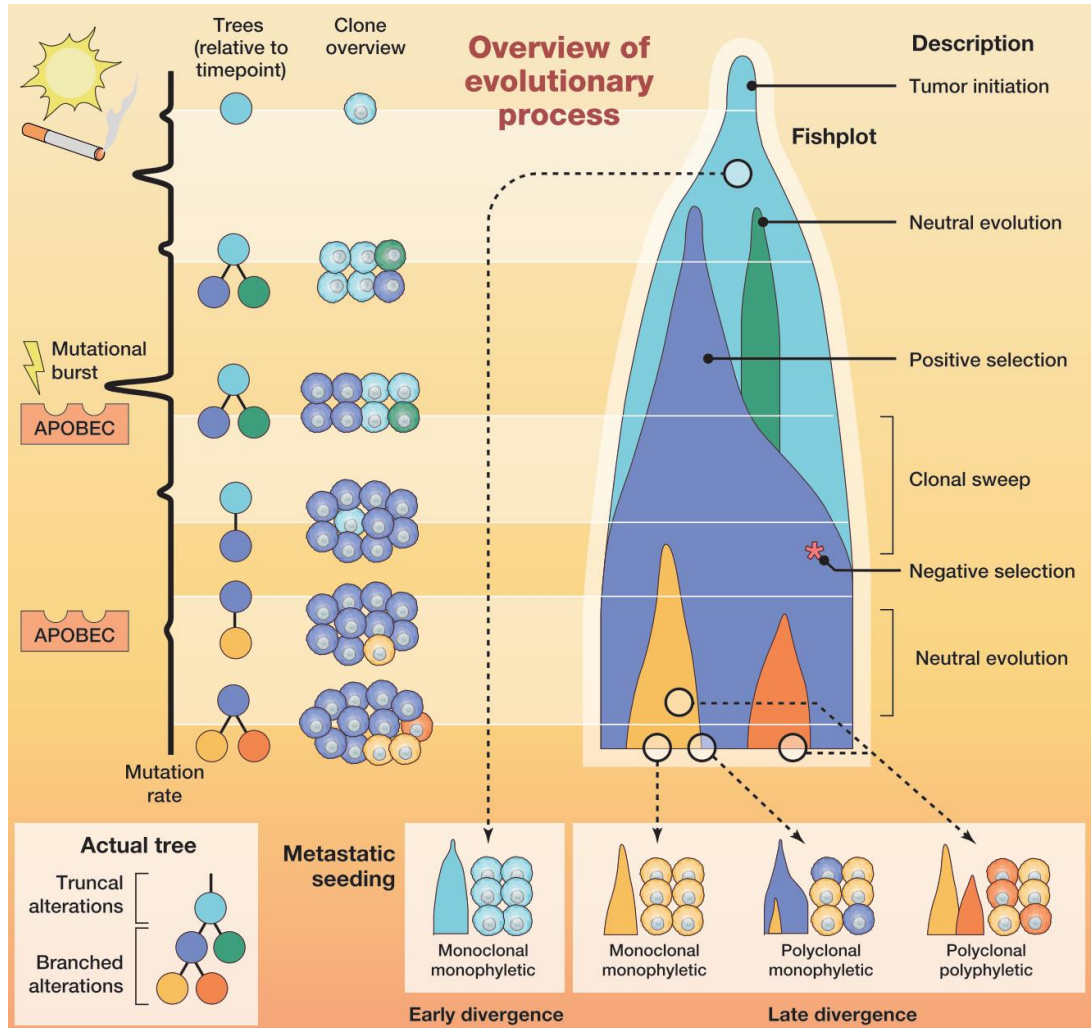


Tumor heterogeneity is a major hurdle for cancer precision medicine



associates with drug resistance, tumor metastasis, and relapse

Underneath the heterogeneity — tumor clonal evolution



Darwin's branching evolutionary tree of speciation, 1837

Tree structure

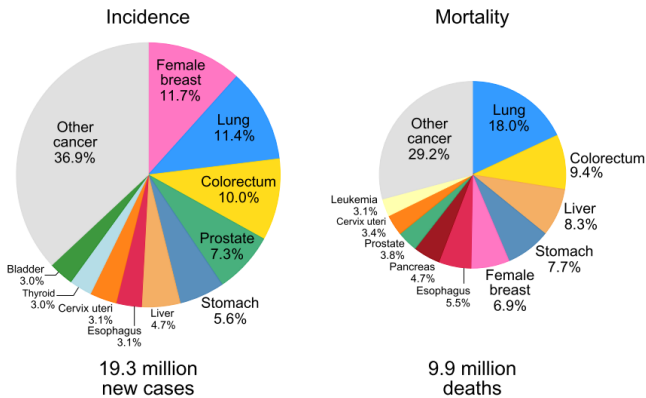
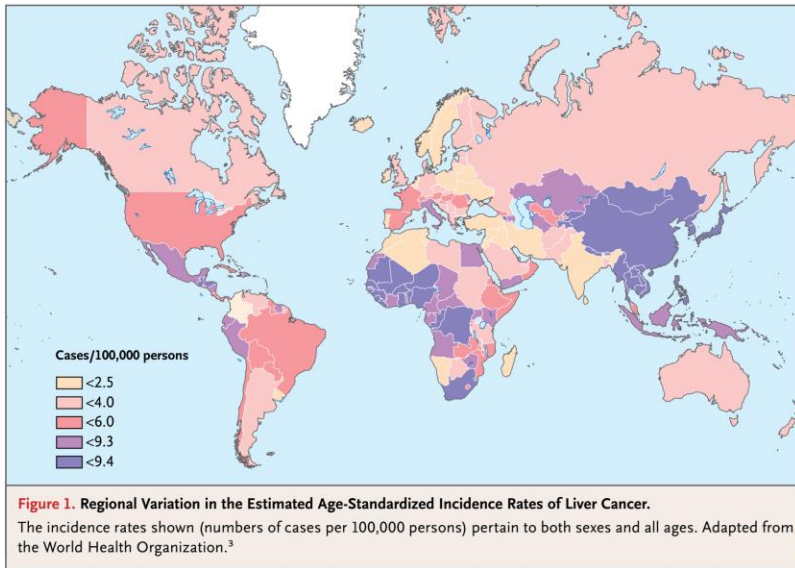


How to distinguish these tumors?
Which one develops faster?

McGranahan N. Cell, 2021

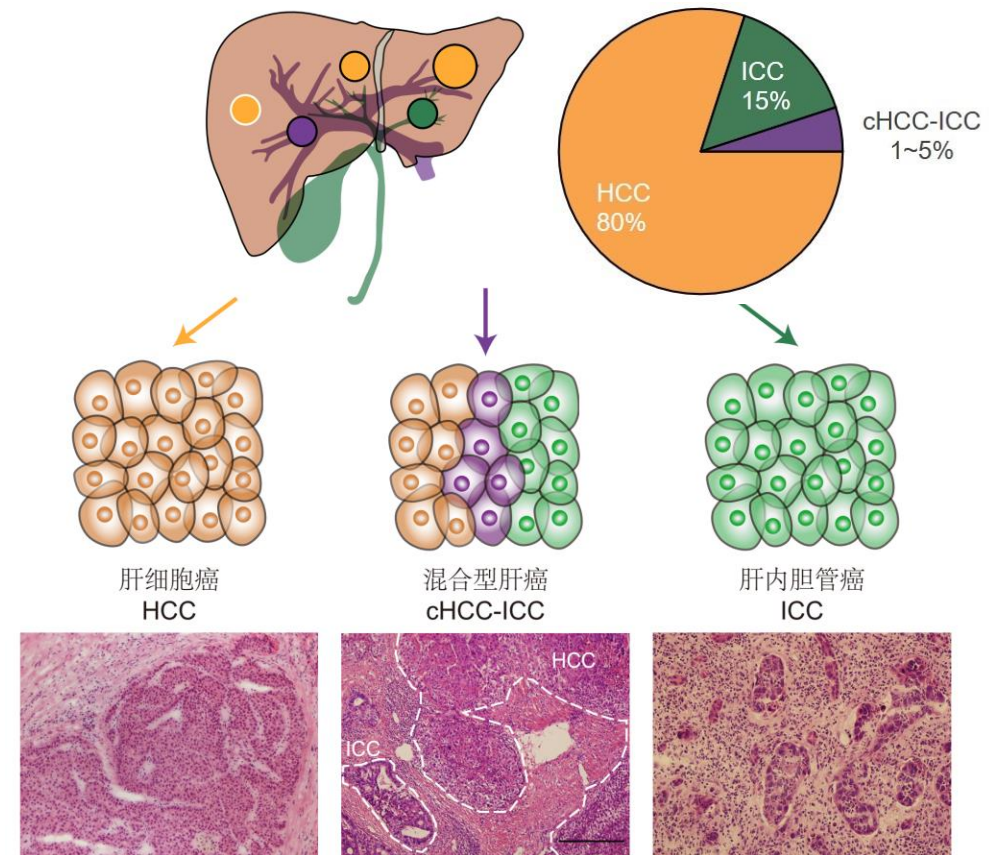
Mel Greaves & Carlo C. Maley. Nature, 2012

Liver cancer



- **HBV Aflatoxin**
- **HCV**
- **NAFLD NASH**
- **ASH**

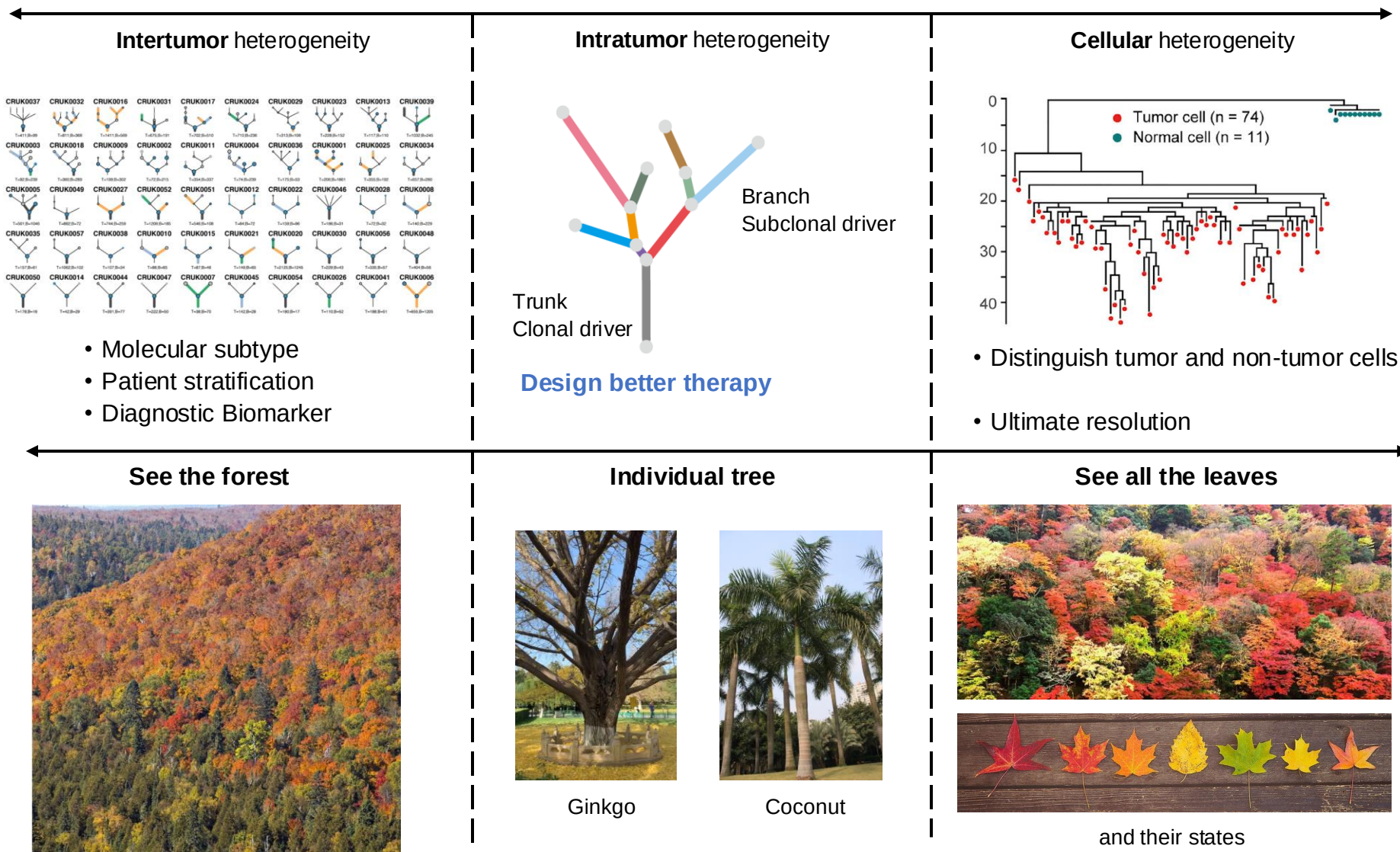
Primary liver cancer (PLC):
Hepatocellular carcinoma (**HCC**)
Intrahepatic cholangiocarcinoma (**ICC**)
Combined hepatocellular-cholangiocarcinoma (**cHCC-ICC**)



More than half of new incidences worldwide are from China

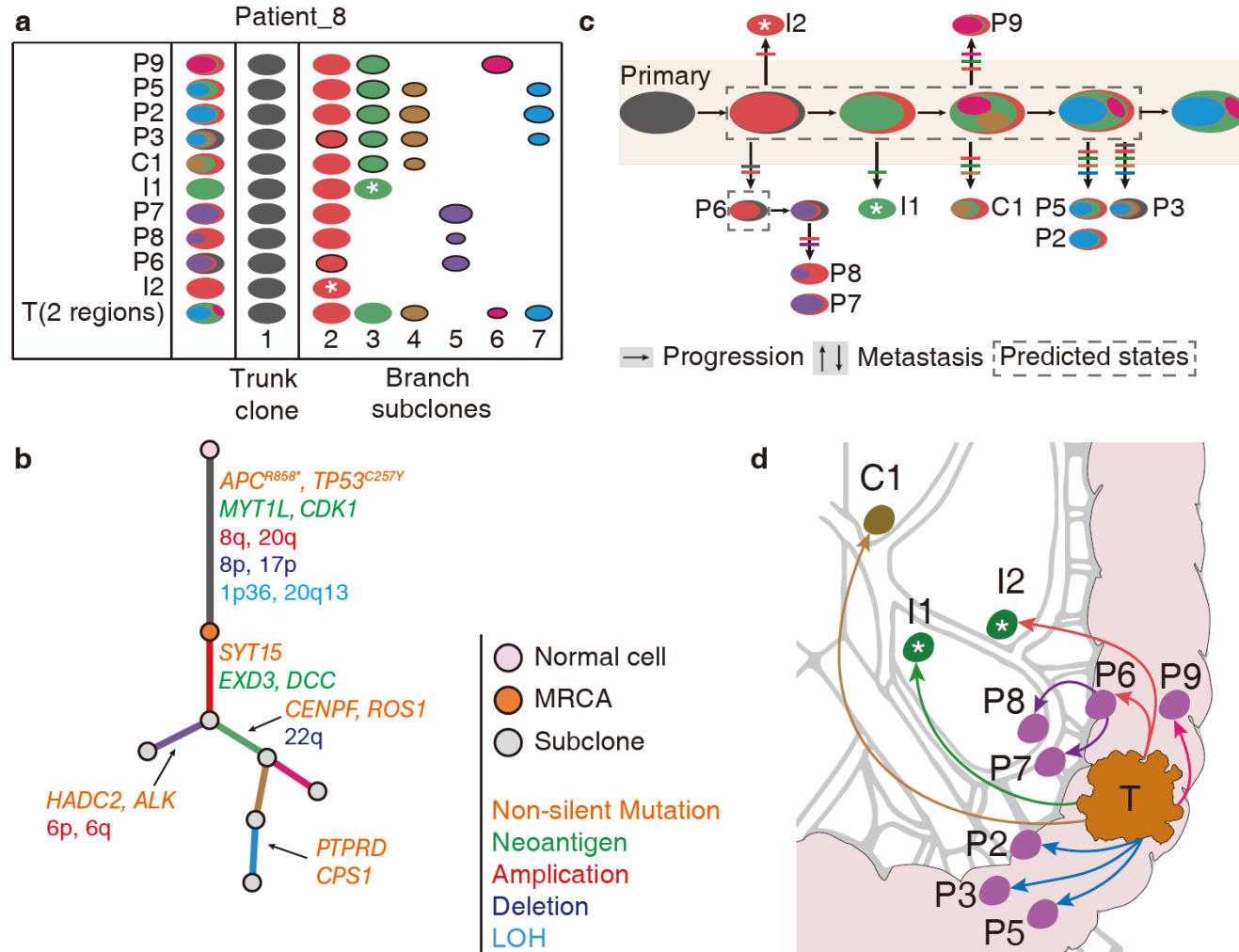
El-Serag, et al., NEJM, 2011
GLOBOCAN, CA, 2021
Cancer Statistics in China, CA, 2015
Xia, et al., CMJ, 2022

Investigation of tumor heterogeneity on three levels

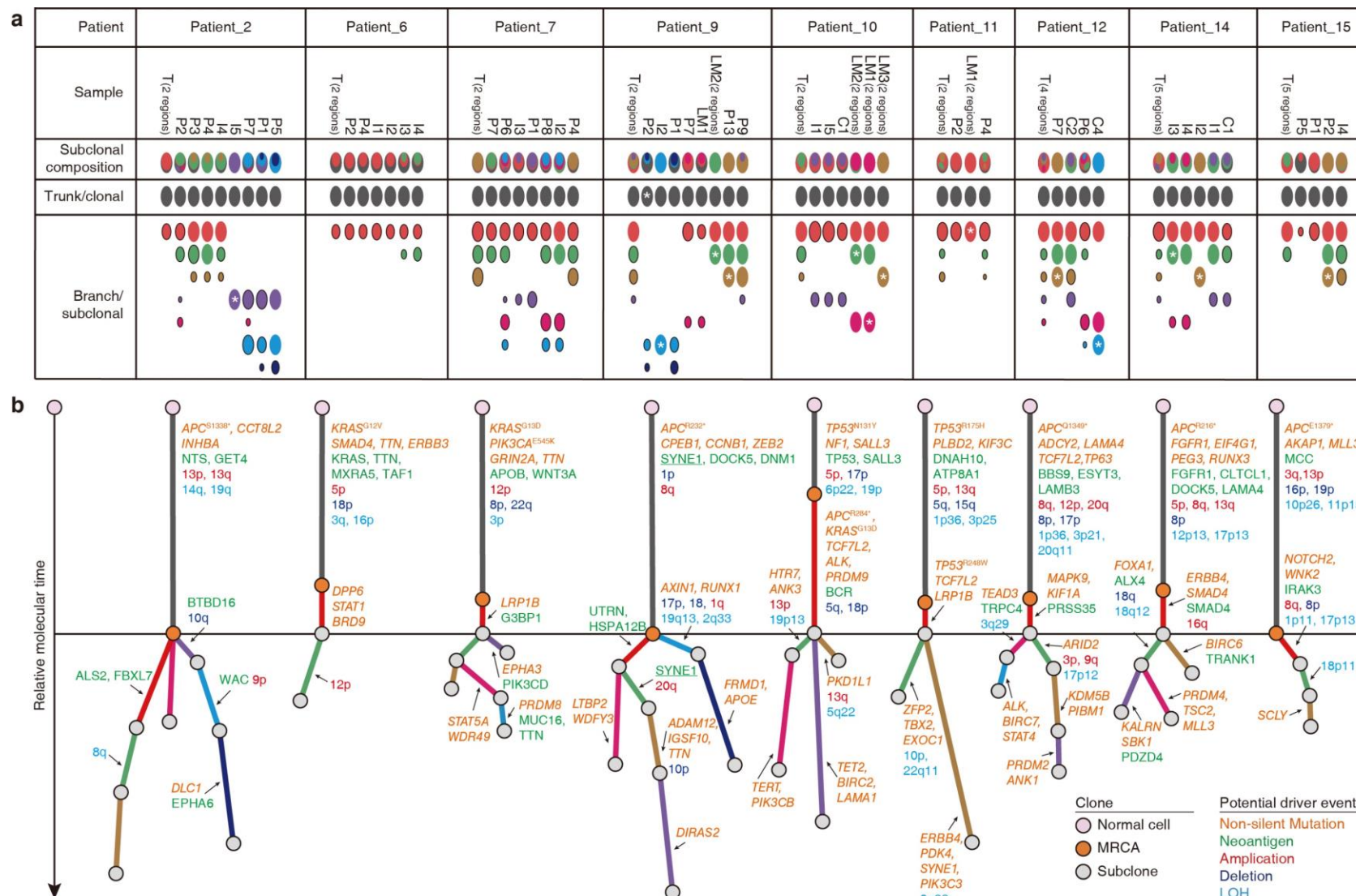


Multi-omics, single cell, spatial & Bioinformatics & Functional validation

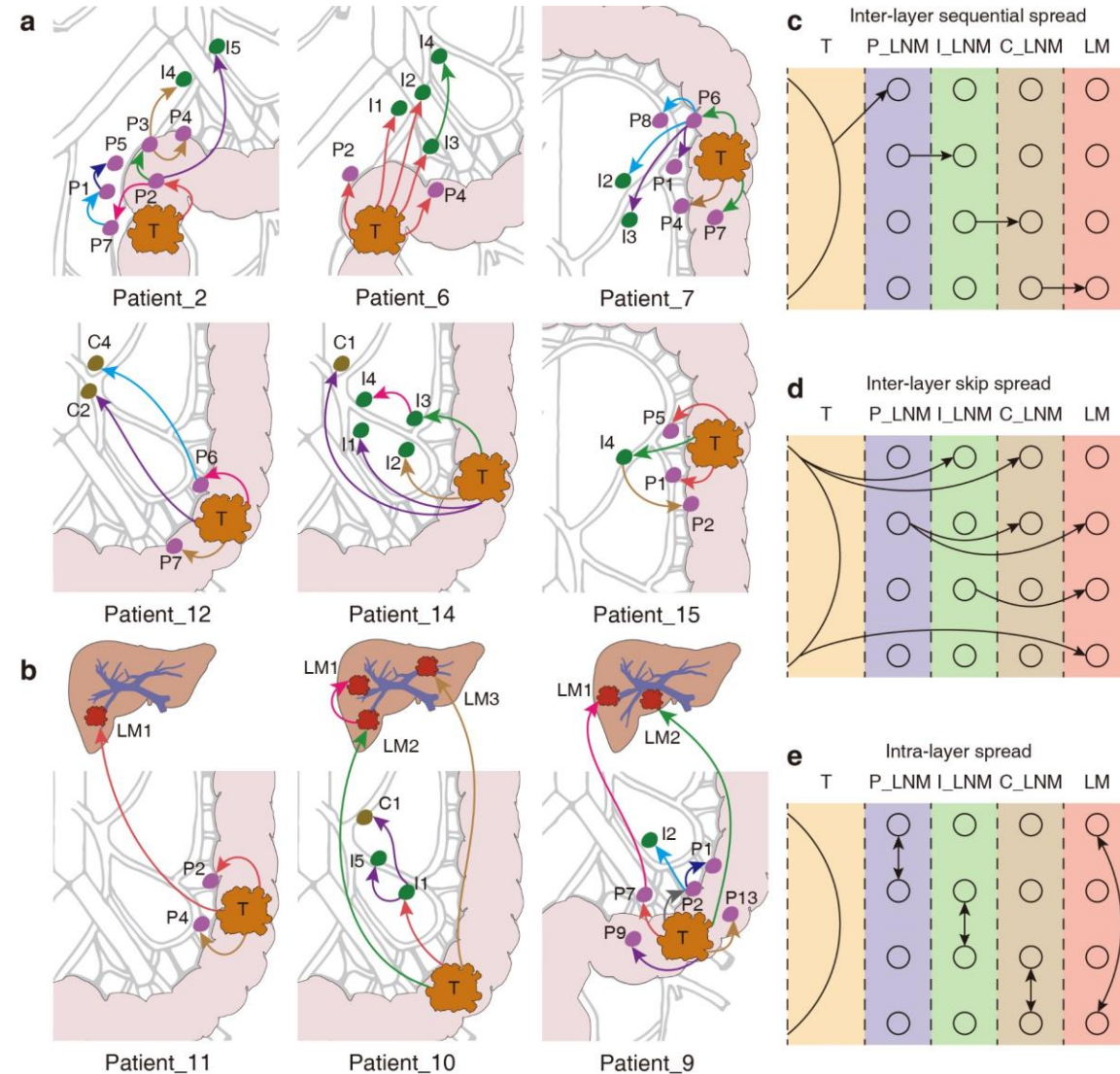
Reconstructing the evolutionary history



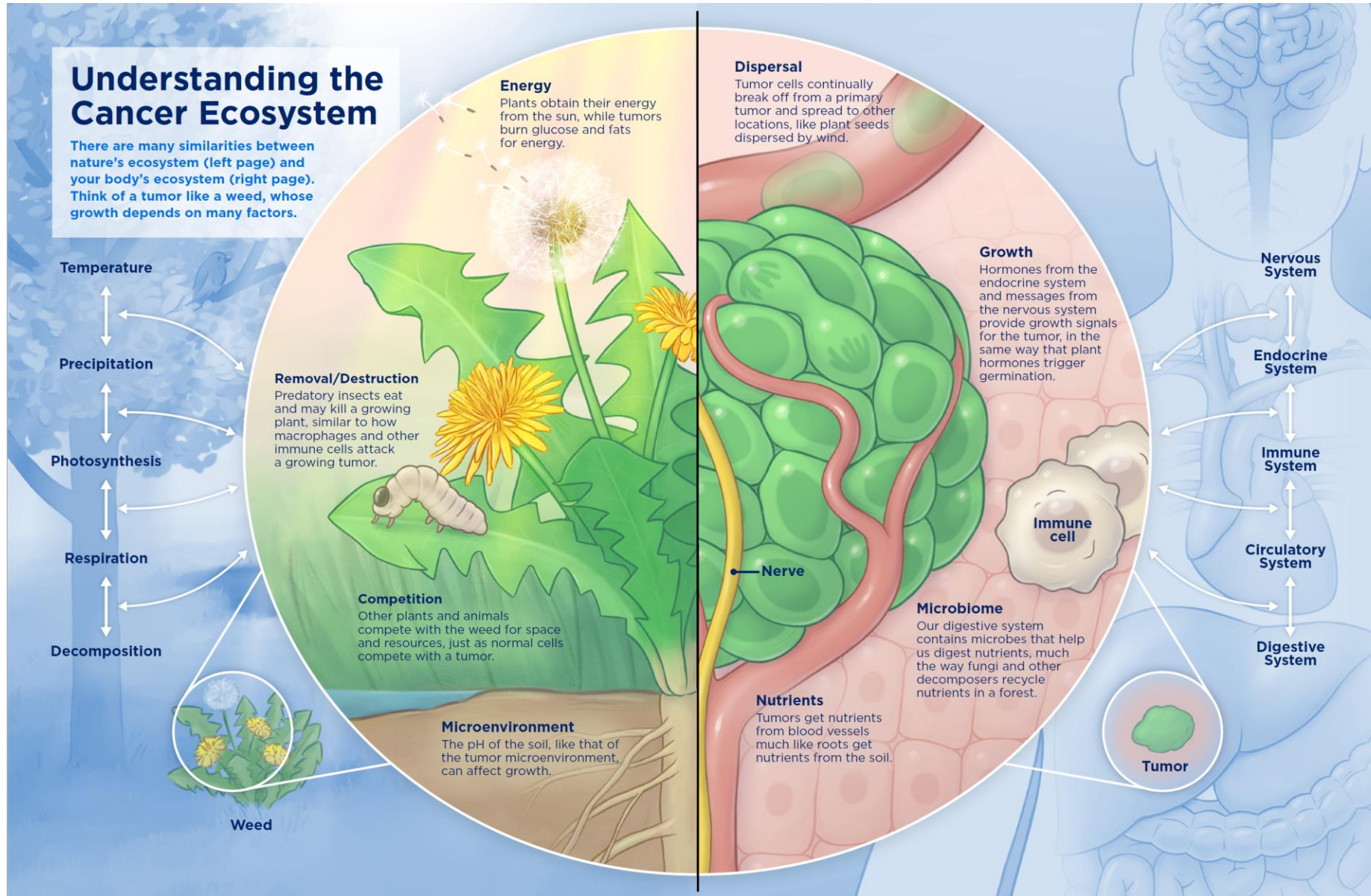
Reconstructing the evolutionary history



Spreading patterns of tumor cells

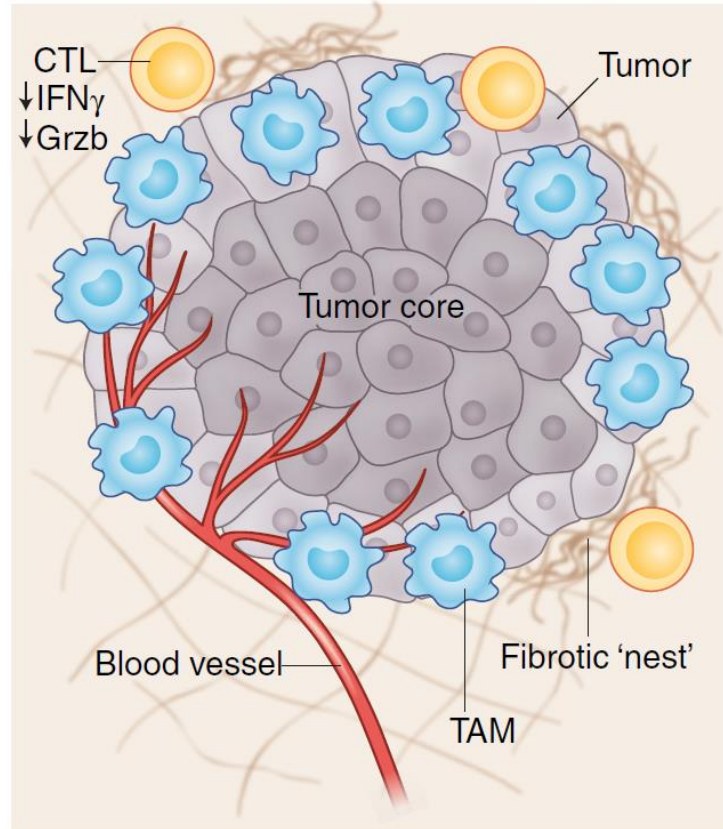


Nature versus Body

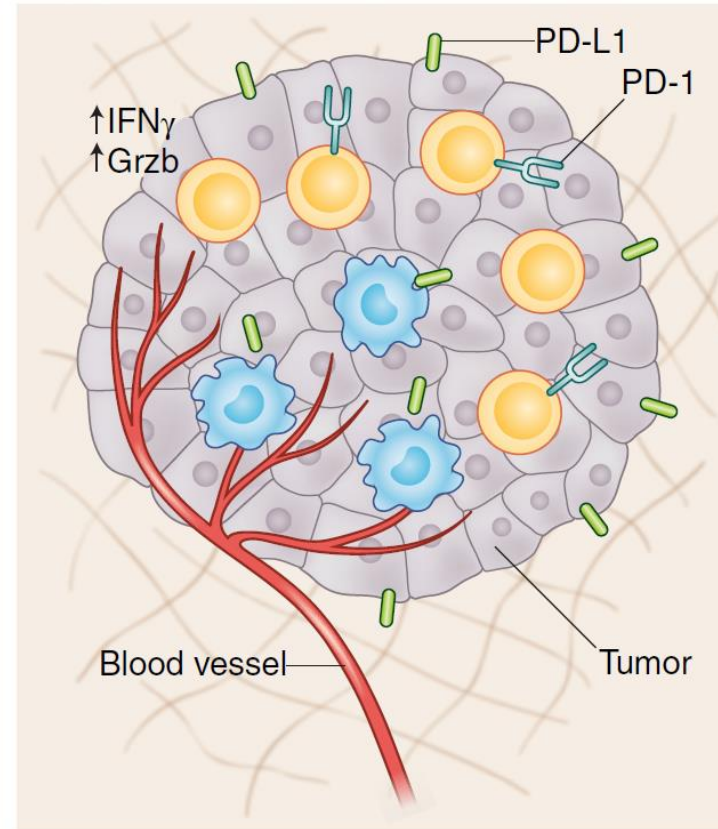


Tumor clonal evolution tree is shaped by its microenvironment

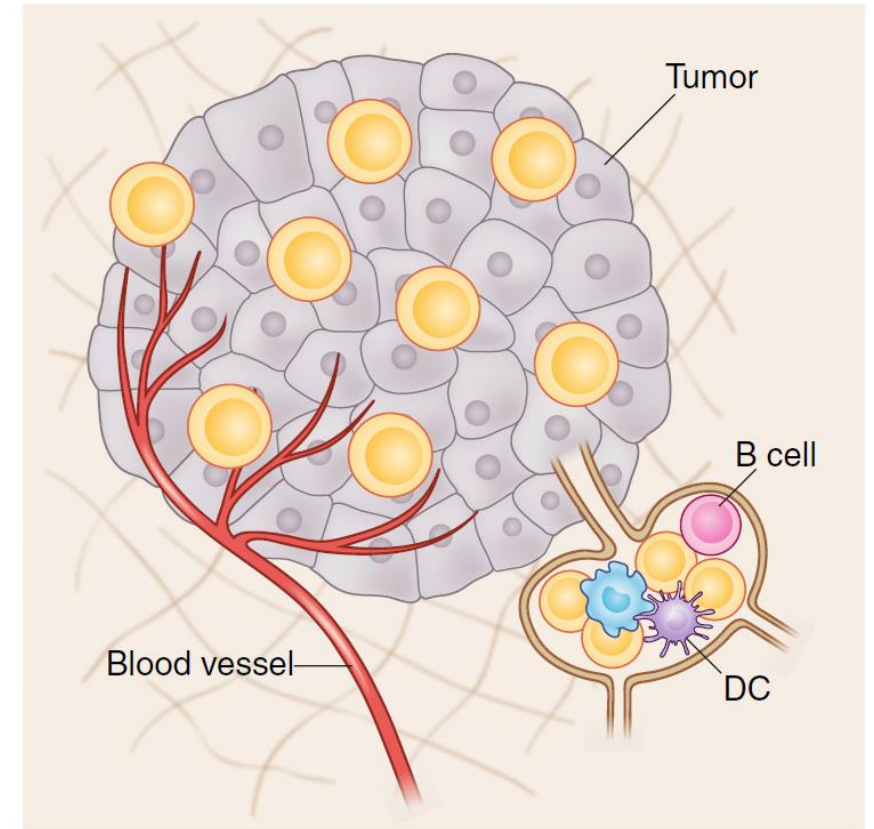
a Infiltrated-excluded



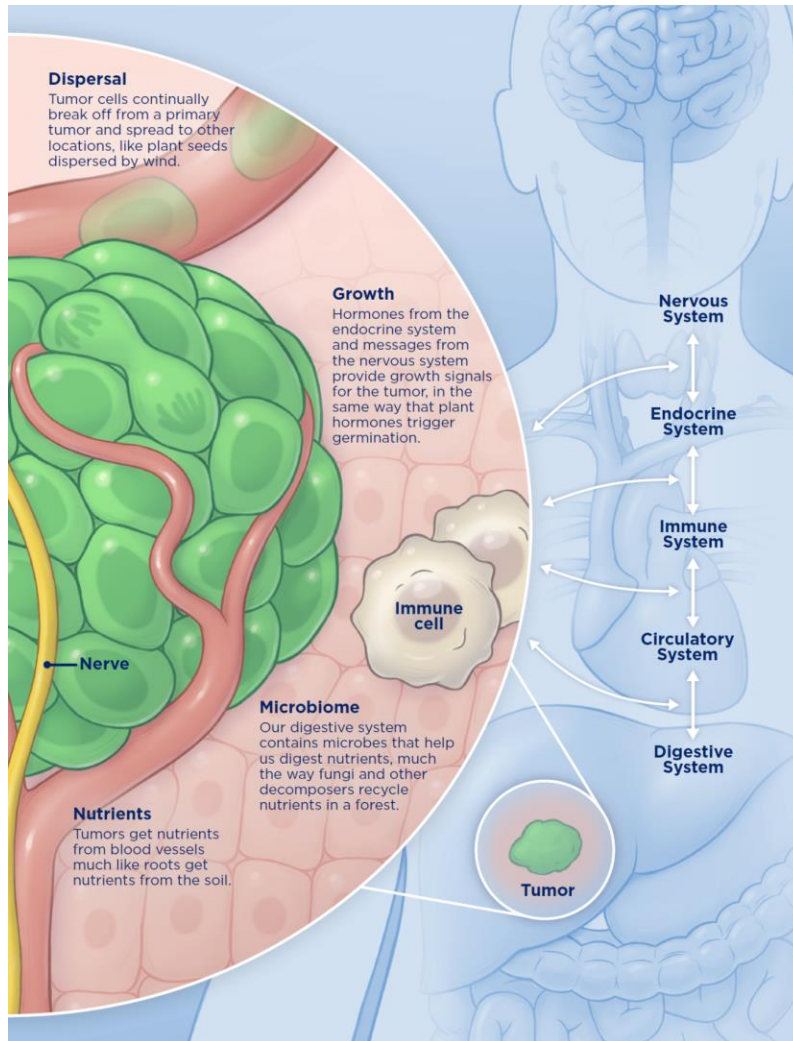
b Infiltrated-inflamed



c Infiltrated-TLS

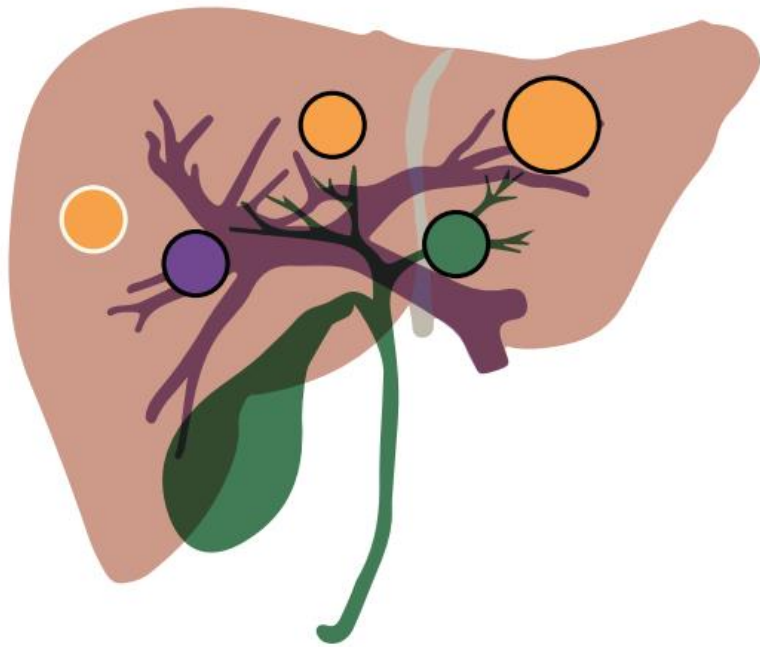


Outline



- Tumor clonal evolution
- Tumor immune microenvironment (TIME)
- Interplay between tumor and TIME

Liver cancer frequently develops intrahepatic metastasis, rather than distant metastasis



Multiple lesions:

Lesions in the liver tissue

- P: Primary
- IM: Intrahepatic Metastasis
- MO: Multiple Occurrence
or MCT: Multicentric Tumor
- SN: Satellite Nodule

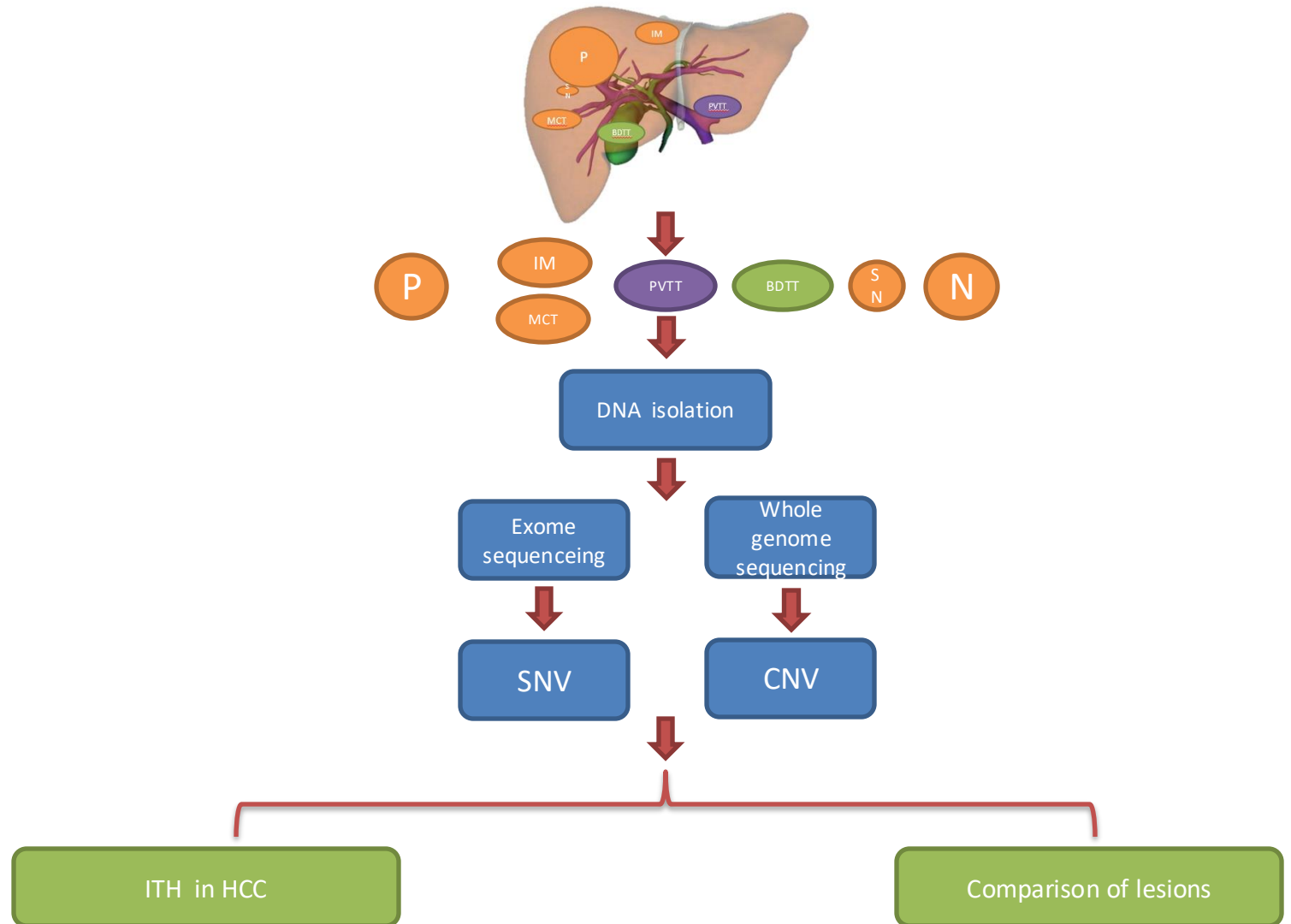
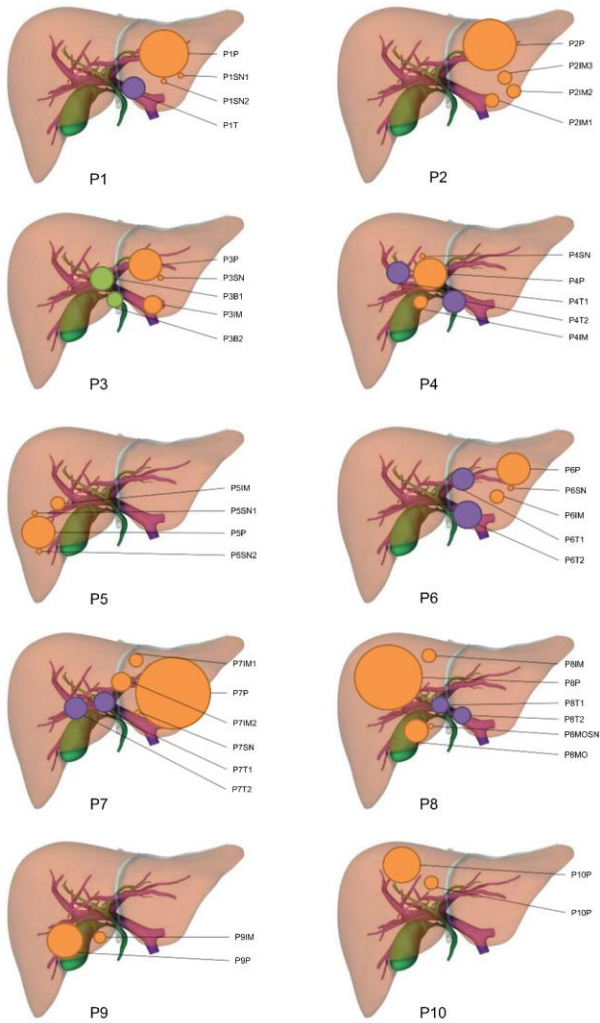
Lesions in veins and ducts

- PVTT: Portal Vein Tumor Thrombus
- BDTT: Bile Duct Tumor Thrombus

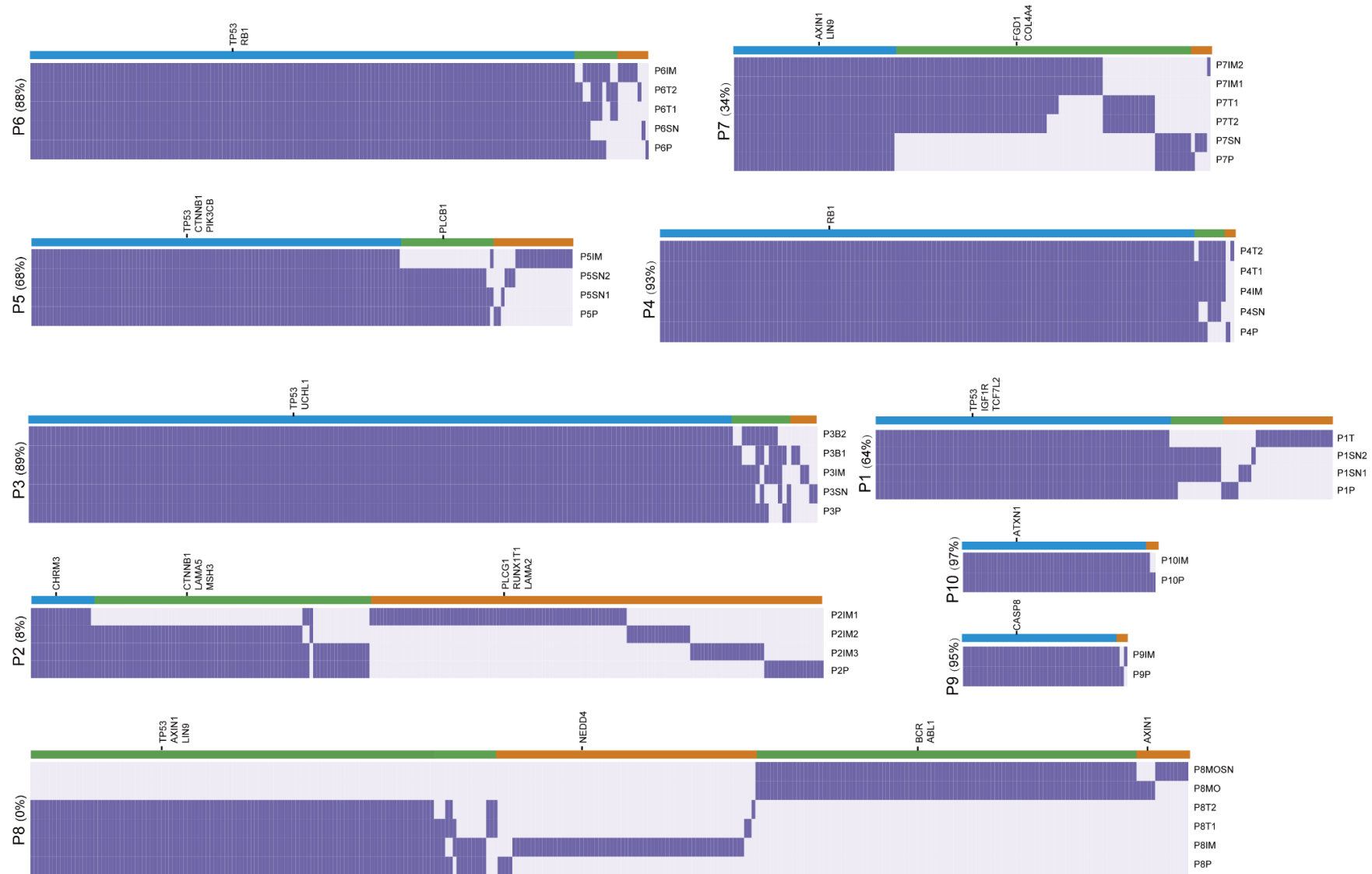
Genomic studies are very limited.

Q: The clonal relationship among multiple lesions in HCC?

Research strategy

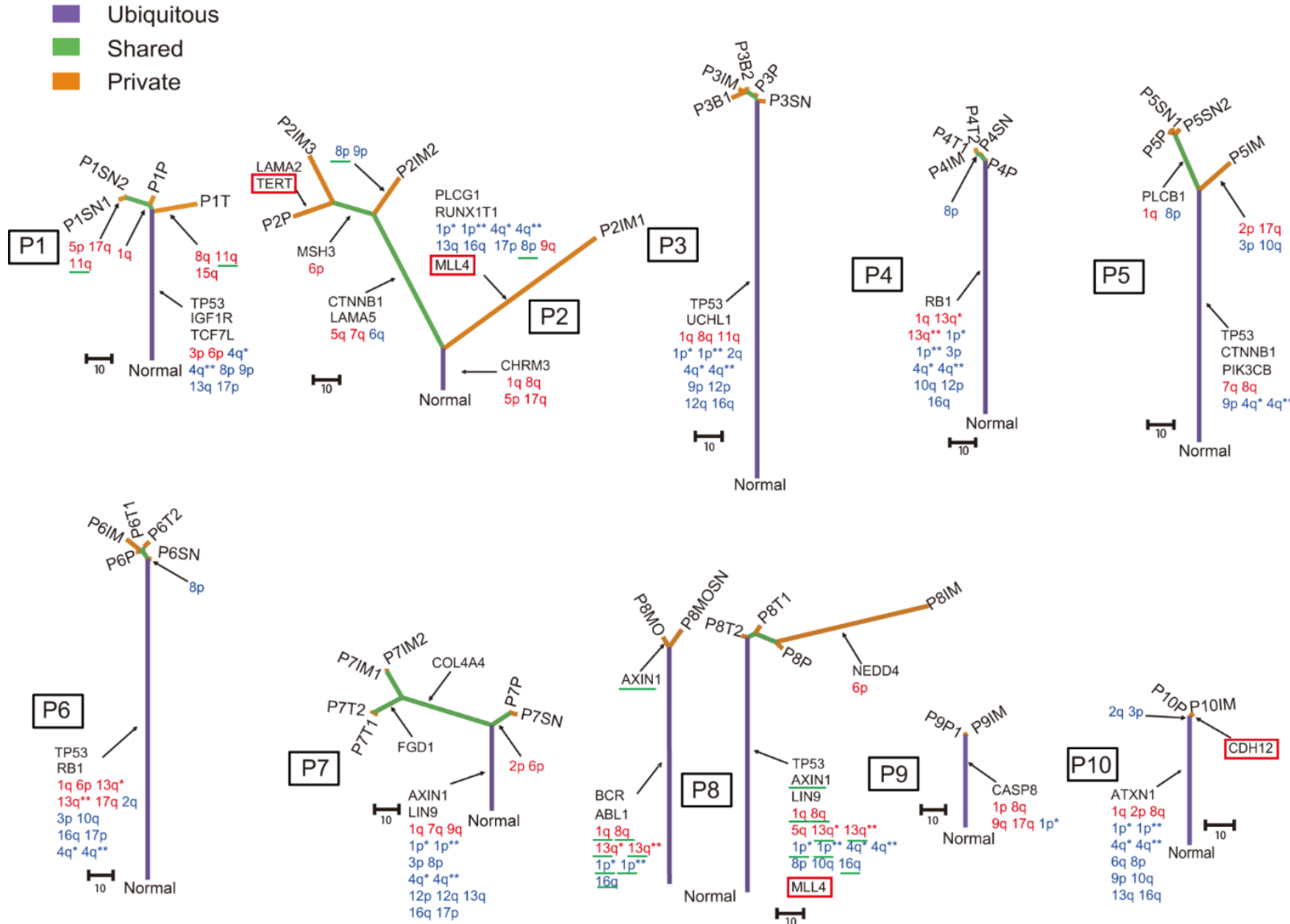


Variable extent of ITH as determined by SNV



ubiquitous mutations ranged from 0 to over 90% among the 10 patients

Branched evolution in HCC



Coconut



Baniam

Physically separate HCC and ICC lesions shared the same clonal origin

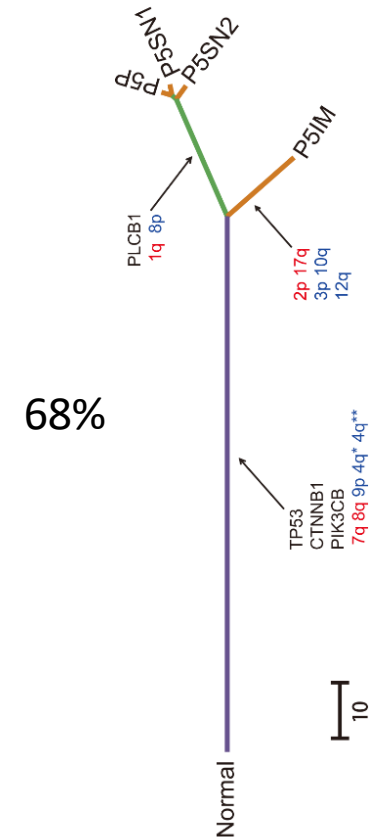
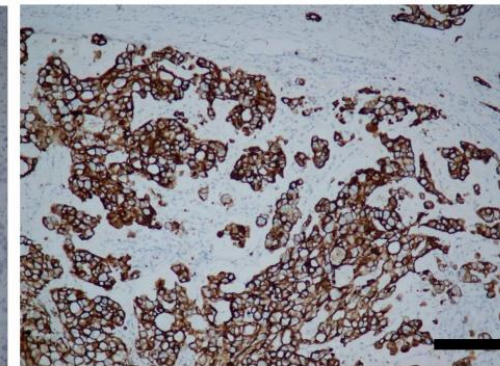
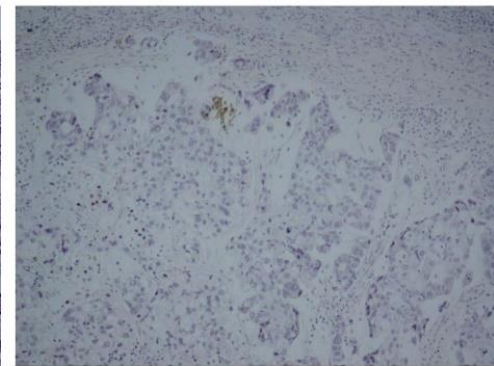
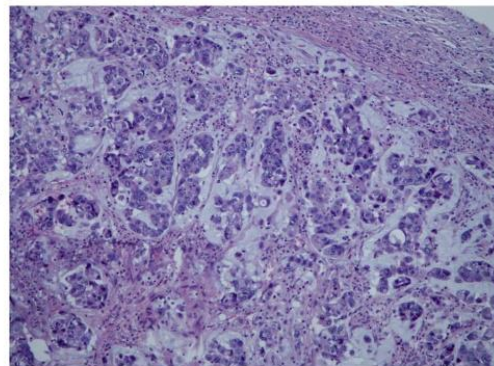
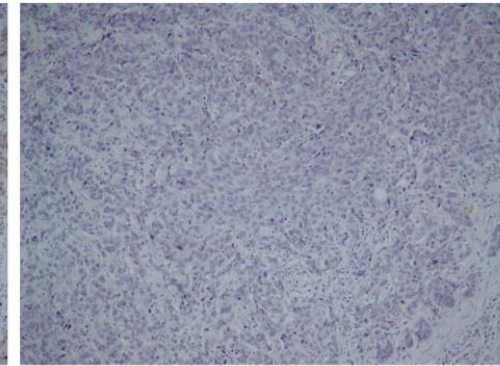
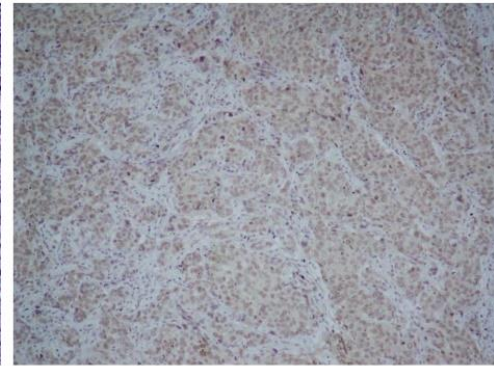
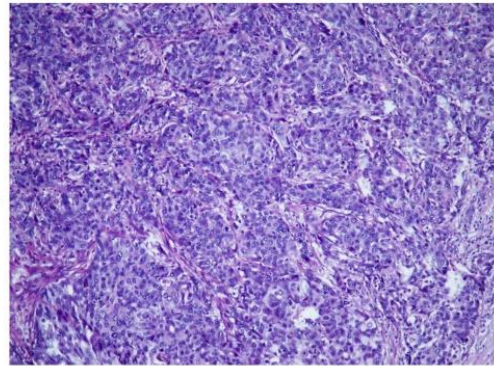
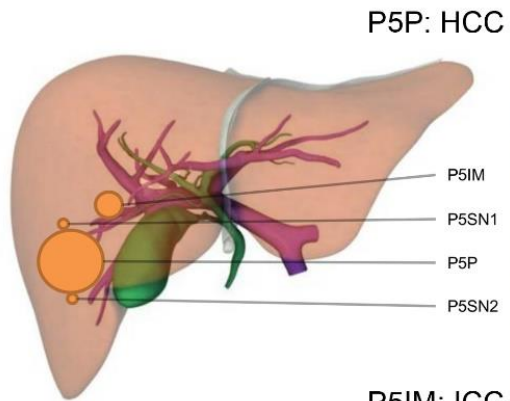
hepatocellular carcinoma

A

H&E

GPC3(HCC)

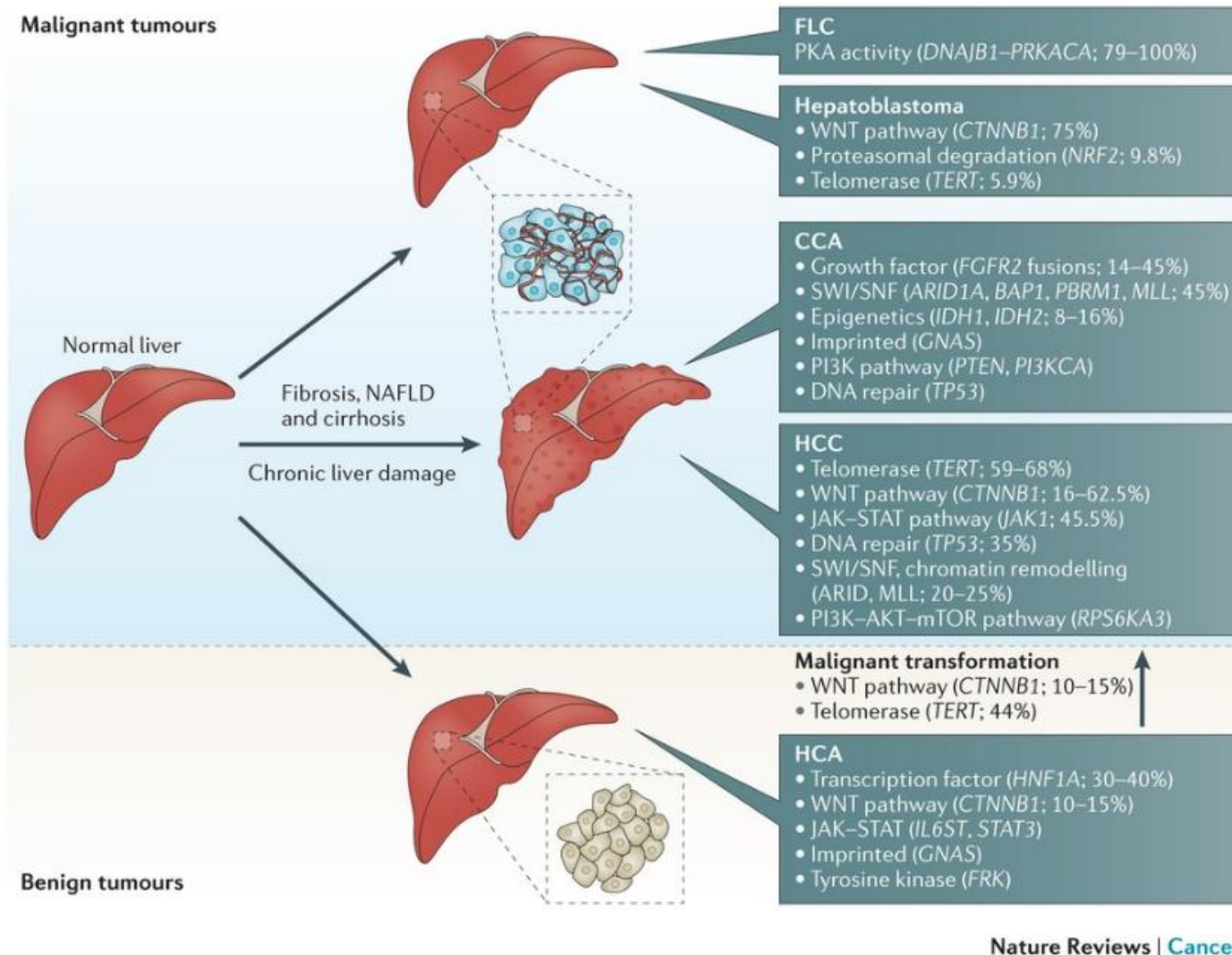
CK19(ICC)



intrahepatic cholangiocarcinoma

Phenotypic transition? Which one is the origin?

We know very little about cHCC-ICC



The very first pathological report about cHCC-ICC

COMBINED LIVER CELL AND BILE DUCT CARCINOMA*

RAYMOND A. ALLEN, M.D., and JAMES R. LISA, M.D.

(From the Laboratory of Pathology, City Hospital, Welfare Island, Department of Hospitals, New York 17, N.Y.)

Carcinomas of the liver displaying features indicating origin from both liver cells and bile ducts have been observed infrequently. They have been reported under various terms: mixed tumors,^{1,2} duplex type,³ intermediate type,^{3,4} carcinoma of dual origin,⁵ cholangiohepatoma.⁶ The first case so designated appears to be that of Wells.⁷ Others have been recorded by Goldzieher,⁸ 1 case; Tramontano and Fittipaldi,⁹ 2 cases; Koster and Kasman,⁸ 1 case; Rasario,^{10,11} 2 cases; Bonne,⁴ 1 case; and Cabot case no. 26441.¹² Gustafsen,⁵ reporting on 62 cases of primary carcinoma of the liver, mentioned 2 of intermediate type. In the reports of primary liver cell carcinoma by Prescott,¹³ Moon,¹⁴ Larson,¹⁵ and Neumann,¹⁶ the data were very suggestive that the bile ducts participated in the malignant process. This communication is a report of 5 additional cases, found among 35 cases of primary carcinomas of the liver in 8175 consecutive autopsies performed at City Hospital, Welfare Island, between May 1, 1911, and May 1, 1948.

Allen and Lisa **1949**

The American Journal of Pathology

Allen and Lisa

Type A

Double tumor (HCC and CC exist at different sites within the same liver)

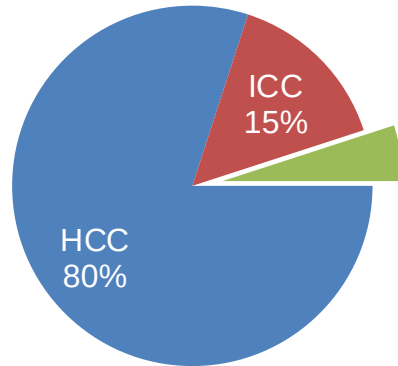
Type B

Combined type (HCC and CC exist at adjacent sites and mine with continued growth)

Type C

Mixed type (HCC and CC components are combined within the same mass)

cHCC-ICC: Unmet clinical need



Worst prognosis

cHCC-ICC
1~5%

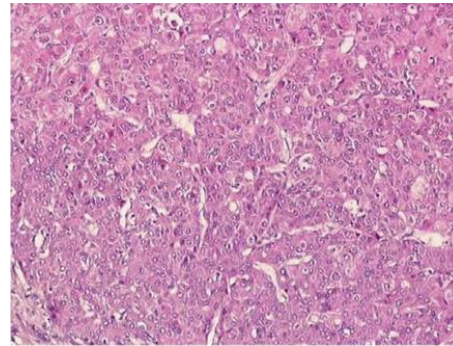
Primary liver cancer (PLC):

Hepatocellular carcinoma (**HCC**)

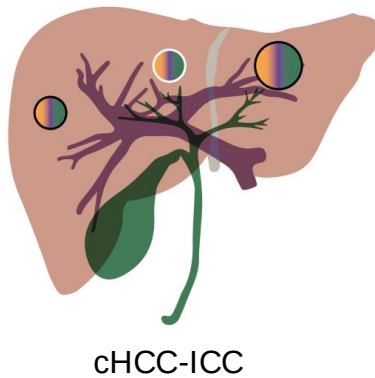
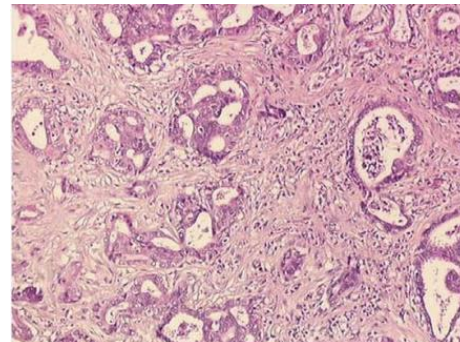
Intrahepatic cholangiocarcinoma (**ICC**)

Combined hepatocellular and intrahepatic cholangiocarcinoma (**cHCC-ICC**)

hepatocellular differentiation
(HCC component)



biliary epithelial differentiation
(ICC component)



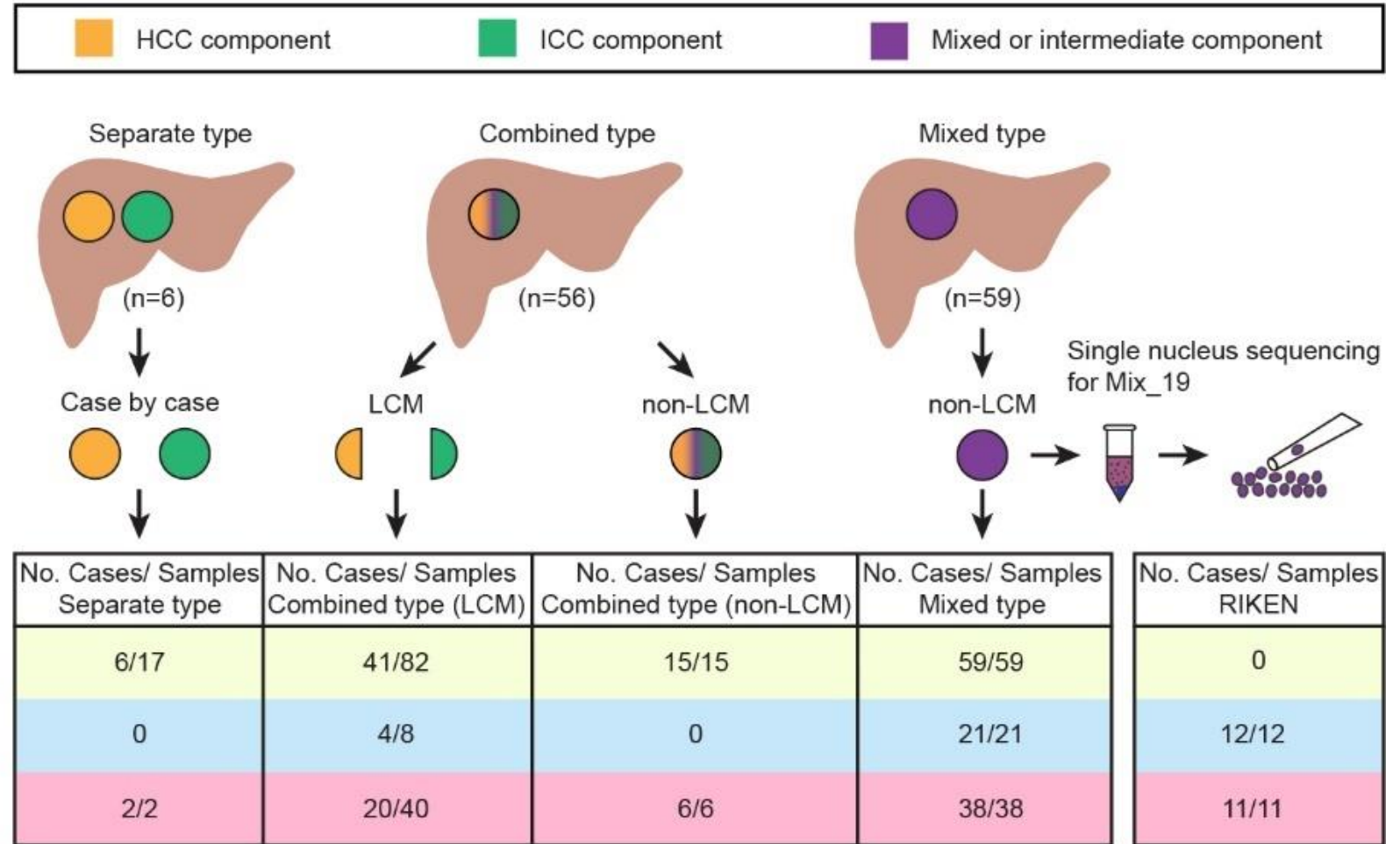
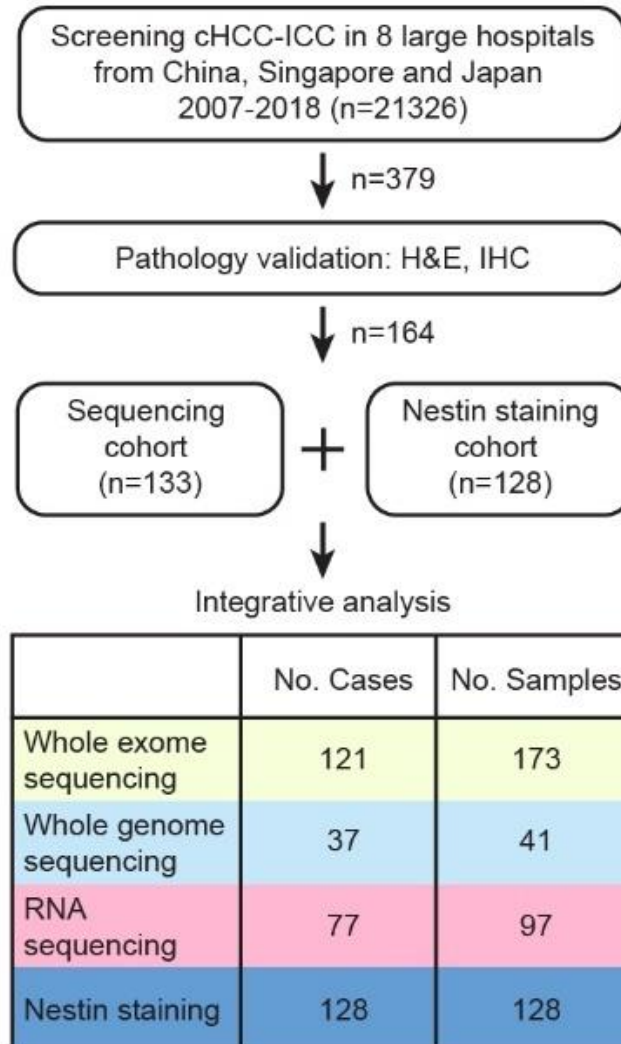
Clinical challenges:

- Often mis-diagnosed as HCC or ICC
- No specialized treatment

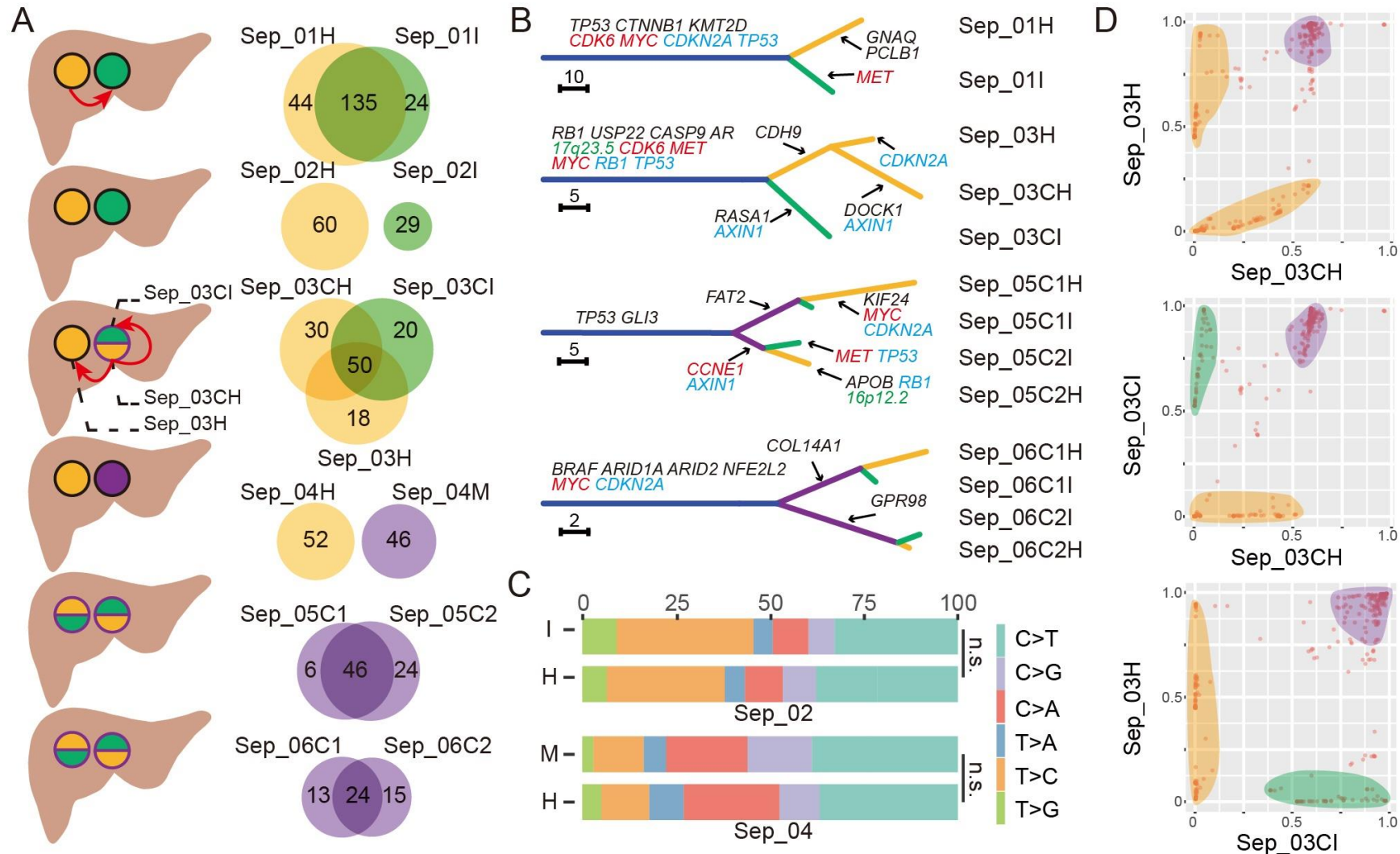
Scientific questions

- Clonal origin
- Phenotypic transition

Research design

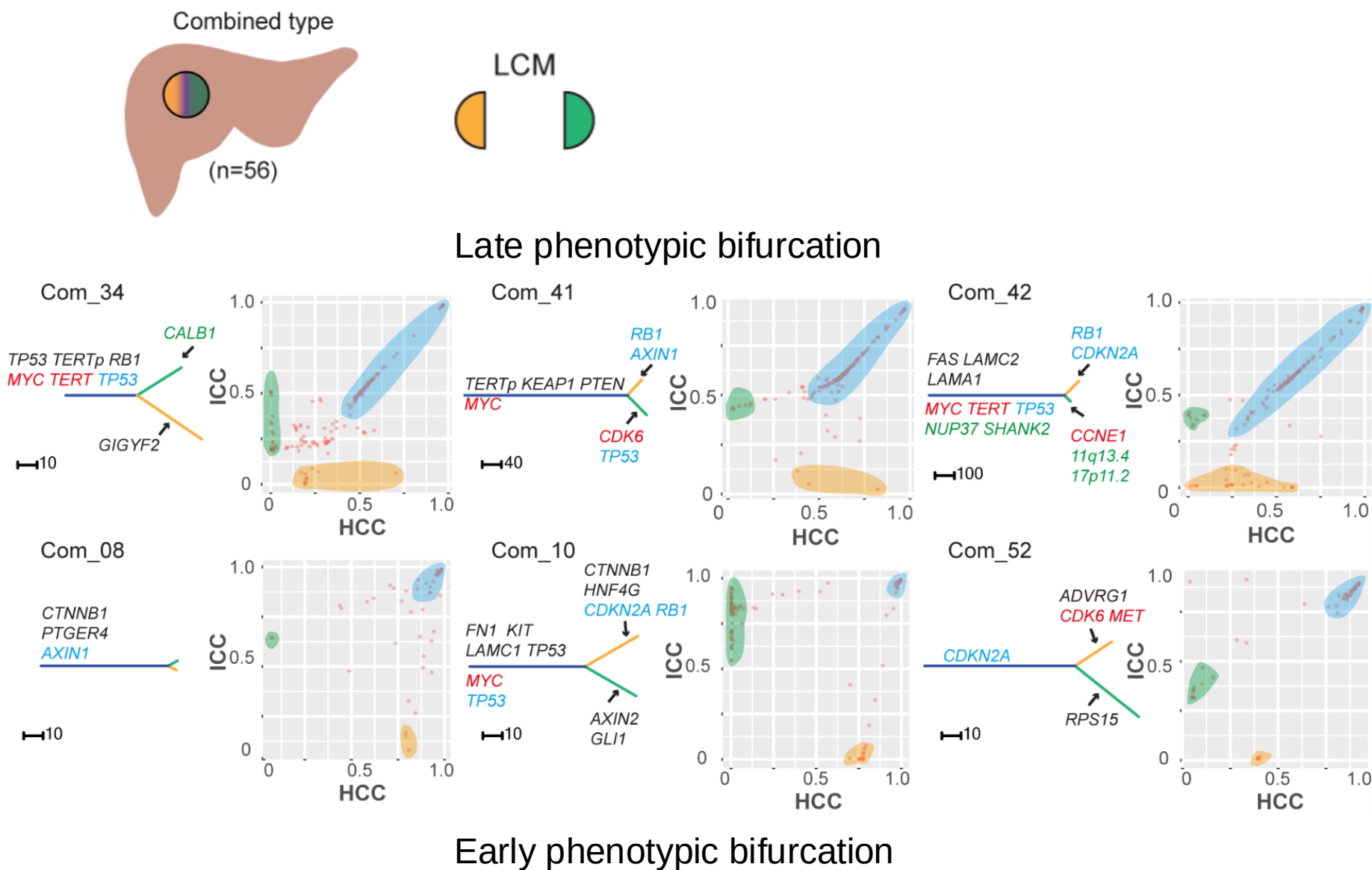


Separate type - Monoclonal origin and multiclonal origin

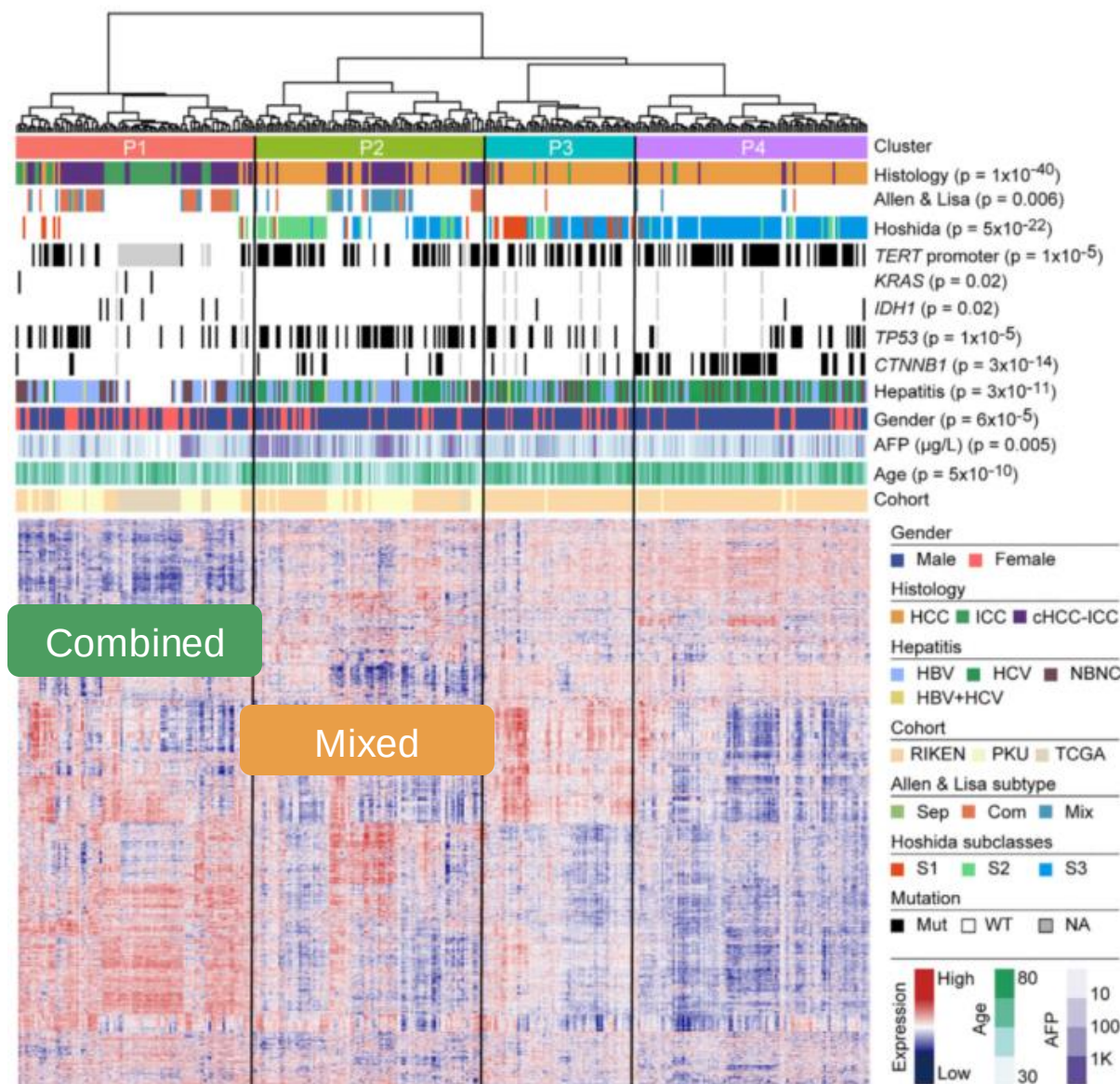


- Cancer cell fraction (CCF) analysis revealed the clonal structure
- Clonal analysis deduced the metastatic routes of two monoclonal cases

Combined type – Monoclonal origin with both early and late divergence



Integrative comparison of cHCC-ICC to HCC and ICC reveals distinct molecular subtypes



Published datasets

HCC

(Fujimoto et al., n=213)

ICC

(Fujimoto et al.,
(Farshidfar et al., n=49)

4 PLC molecular clusters

P1 :

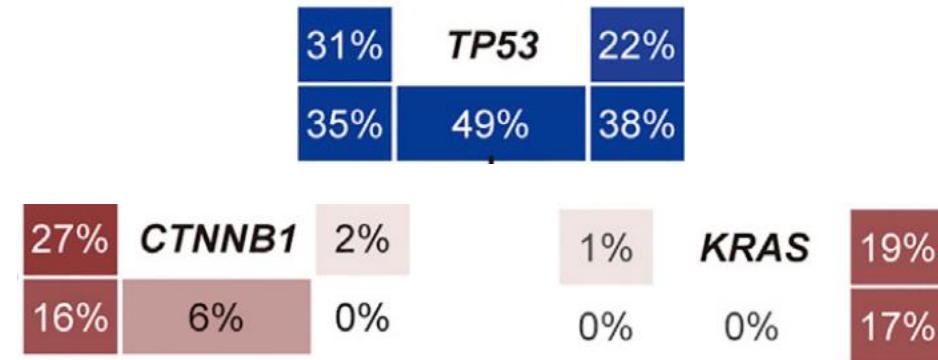
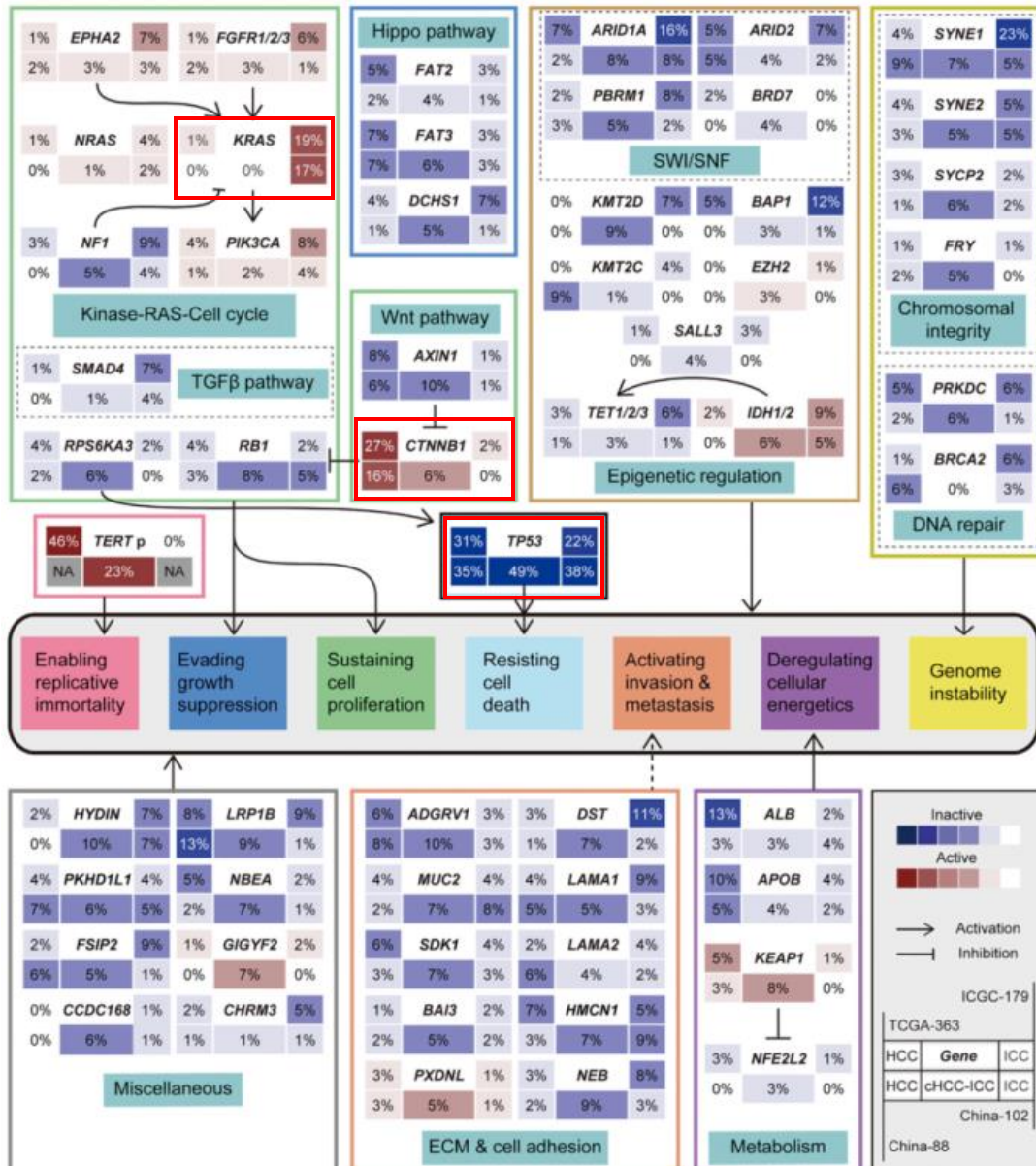
Combined type cHCC-ICC similar to **ICC**

P2

Mixed type cHCC-ICC
similar to **HCC**

(Hoshida-S2-poorly differentiated)

Genomic comparison of PLC



cHCC-ICC

Highest *TP53* mutation%

lower *CTNNB1* mutation% than **HCC**

lower *KRAS* mutation% than **ICC**

TP53-Nestin axis regulates cellular plasticity in liver cancer

Nestin (*NES*), a marker for the bipotent progenitor oval cell (Gleiberman et al., 2005).

GASTROENTEROLOGY 2012;142:1229–1239

Disruption of Trp53 in Livers of Mice Induces Formation of Carcinomas With Bilineal Differentiation

SARAH-FEE KATZ,* ANDRÉ LEHEL,* ANNA C. OBENAU,† YVONNE BEGUS-NAHRMANN,* JOHANN M. KRAUS,§
EVA M. HOFFMANN,‡ JOHANNA DUDA,|| PARISA ESHRAGHI,* DANIEL HARTMANN,**† BIRGIT LISS,||
PETER SCHIRMACHER,§ HANS A. KESTLER,§ MICHAEL R. SPEICHER,‡ and K. LENHARD RUDOLPH*

*Institute of Molecular Medicine and Max Planck Research Group on Stem Cell Aging, §Research Group Bioinformatics & Systems Biology, Institute of Neural Information Processing, and ||Research Group on Molecular Neurophysiology, University of Ulm, Ulm, Germany; ‡Institute of Human Genetics, Medical University of Graz, Graz, Austria; †Department of Surgery, Technical University Munich, Munich, Germany; and **Institute of Pathology, University of Heidelberg, Heidelberg, Germany

Cell

p53-Dependent Nestin Regulation Links Tumor Suppression to Cellular Plasticity in Liver Cancer

Darius F. Tschaharganeh,¹ Wen Xue,^{2,10} Diego F. Calvisi,³ Matthias Evert,³ Tatyana V. Michurina,² Lukas E. Dow,¹ Ana Banito,¹ Sarah F. Katz,⁴ Edward R. Kastenhuber,¹ Susann Weissmueller,^{1,2} Chun-Hao Huang,¹ Andre Lechel,⁴ Jesper B. Andersen,^{5,6} David Capper,⁷ Lars Zender,^{2,11} Thomas Longerich,⁸ Grigori Enikolopov,² and Scott W. Lowe^{1,2,9,*}

¹Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

²Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

³Institute of Pathology, University of Medicine, Greifswald 17487, Germany

⁴Department of Internal Medicine I, University of Ulm, Ulm 89070, Germany

⁵Laboratory of Experimental Carcinogenesis, NCI/CCR, NIH, Bethesda, MD 20892, USA

⁶Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen 2200, Denmark

⁷Institute of Pathology, University Hospital Heidelberg and Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg 69120, Germany

⁸Institute of Pathology, University Hospital Heidelberg, Heidelberg 69120, Germany

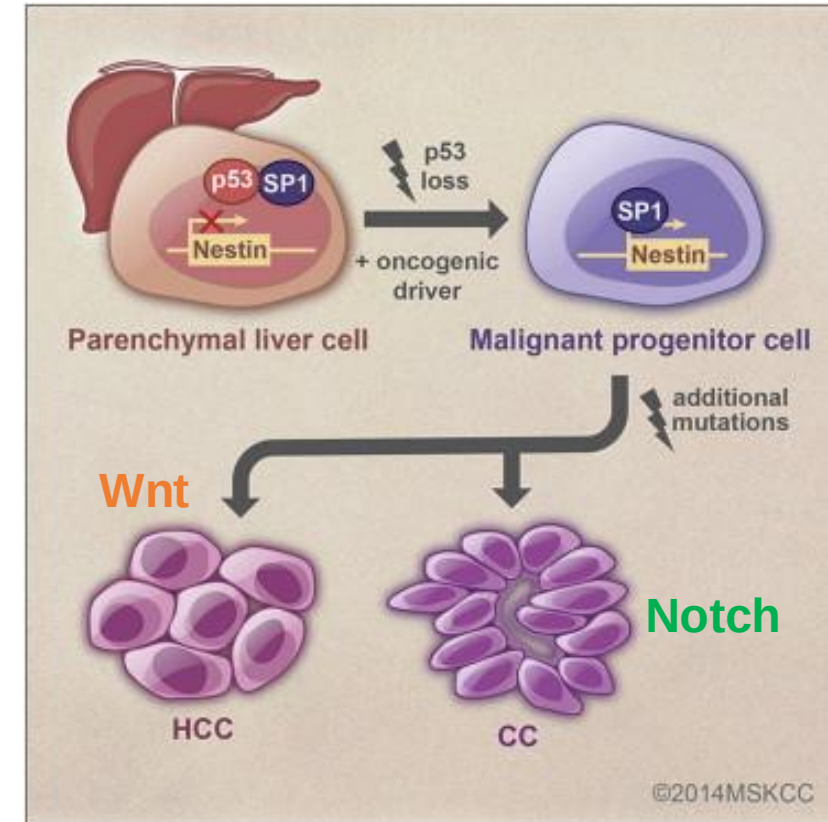
⁹Howard Hughes Medical Institute, New York, NY 10065, USA

¹⁰Present address: Koch Institute of Integrative Cancer Research, Department of Biology, MIT, Cambridge, MA 02139, USA

¹¹Present address: Division of Translational Gastrointestinal Oncology, Department of Internal Medicine I, Eberhard Karls University, Tuebingen 72076, Germany

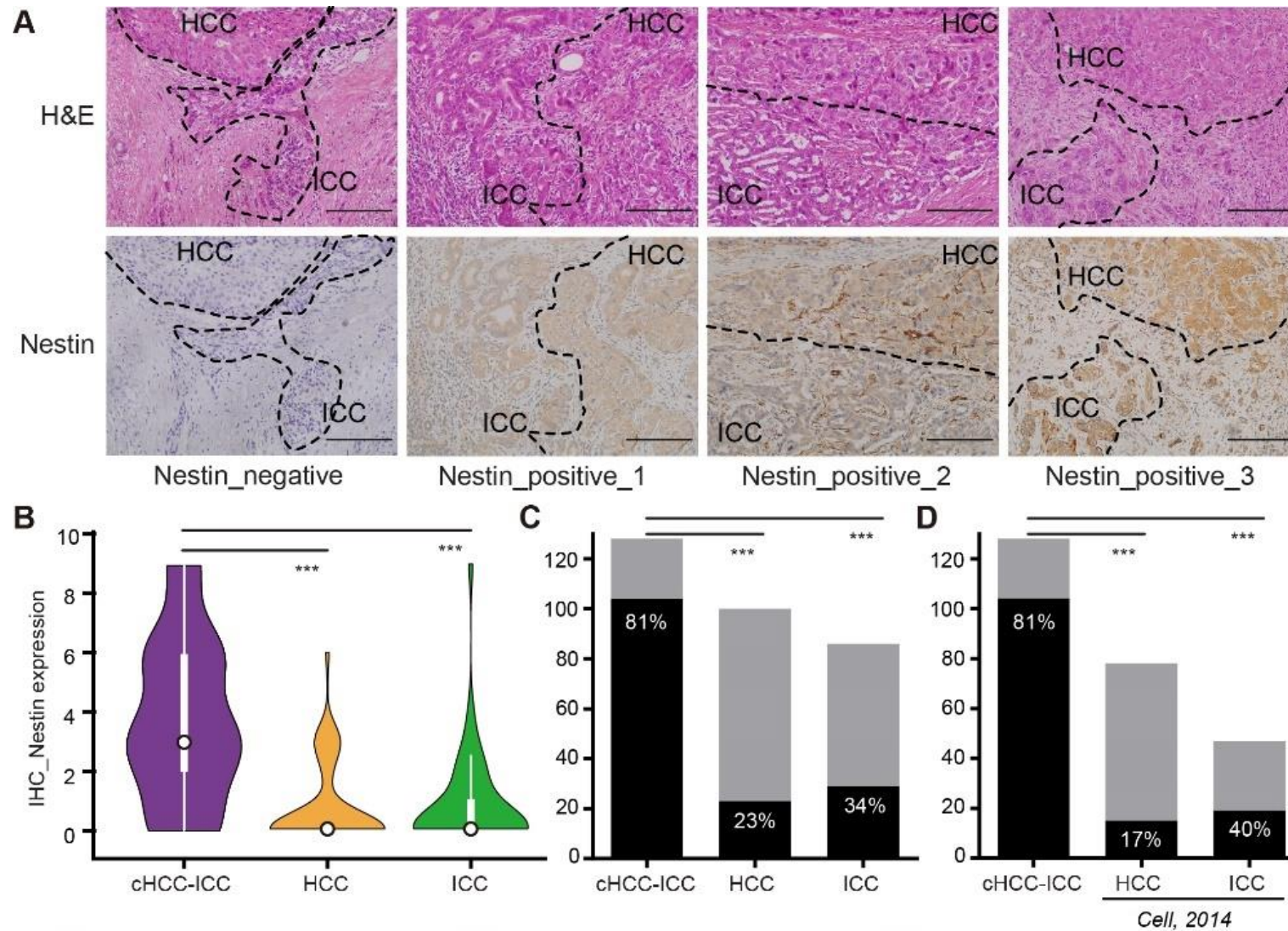
*Correspondence: lowes@mskcc.org

<http://dx.doi.org/10.1016/j.jcl.2014.05.051>



- High TP53 dysfunction in cHCC-ICC
- Low mutational frequency in lineage-related genes in cHCC-ICC
- Q: Nestin highly expressed in cHCC-ICC ?

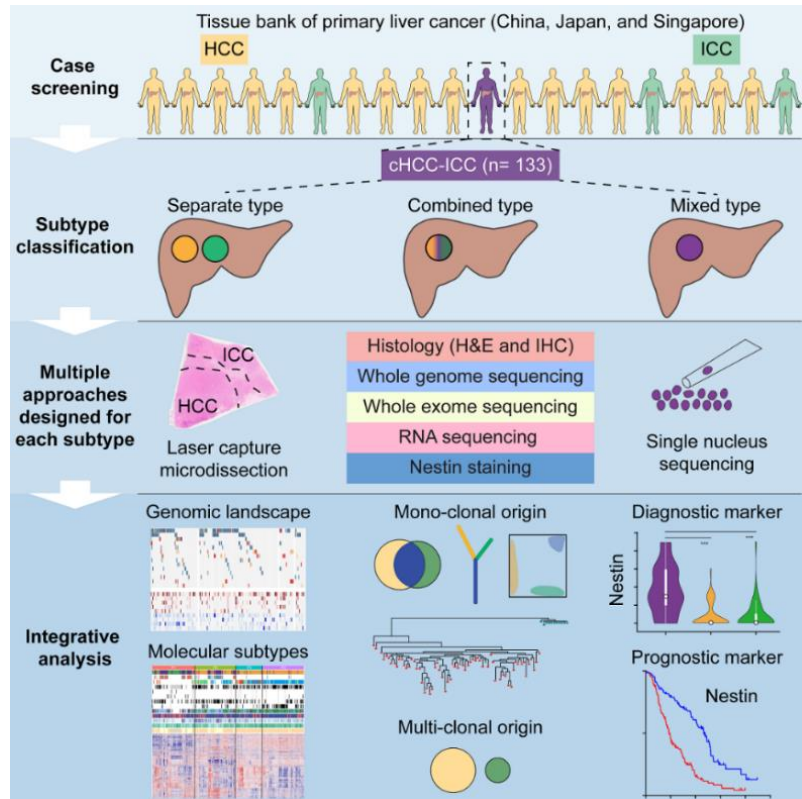
Nestin is a Biomarker for cHCC-ICC Diagnosis and Prognosis



Nestin might be a new therapeutic target.

Highlights

- Editorial by Cancer Cell
- News and Views by Nature Reviews G & H
- Liver cancer in 2019 by Nature Reviews G & H
- Cell Press 2019 Paper of the year China



Xue, R. et al., *Cancer Cell* 2019

Cancer Cell Previews

The Worst from Both Worlds: cHCC-ICC

Marco Seehawer,¹ Luana D'Artista,¹ and Lars Zender^{1,2,*}

LIVER CANCER

The jigsaw of dual hepatocellular–intrahepatic cholangiocarcinoma tumours

Patricia Munoz-Garrido and Pedro M. Rodrigues

A new study involving integrative genomic and transcriptomic analysis of dual hepatocellular–intrahepatic cholangiocarcinoma tumours has identified distinct subtypes that display different clinical and molecular features, and that could be used to guide therapeutic decisions.

Refers to Xue, R. et al. Genomic and transcriptomic profiling of combined hepatocellular and intrahepatic cholangiocarcinoma reveals distinct molecular subtypes. *Cancer Cell* 35, 932–947 (2019).

LIVER CANCER IN 2019

Joining the dots for better liver cancer treatment

Lo-Kong Chan and Irene Oi-Lin Ng

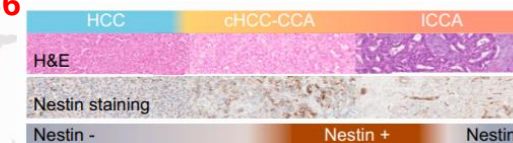
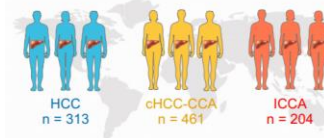
| *Cancer Cell* VOLUME 35, ISSUE 6, P932-947.E8, JUNE 10, 2019

基因组和转录组测序整合分析揭示混合型肝癌的不同分子亚型
Genomic and Transcriptomic Profiling of Combined Hepatocellular and Intrahepatic Cholangiocarcinoma Reveals Distinct Molecular Subtypes

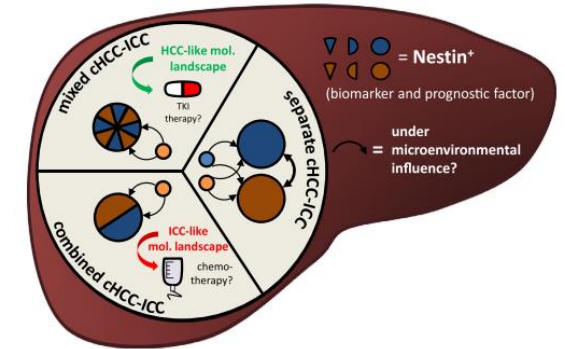
JOURNAL OF HEPATOLOGY

Nestin as a diagnostic and prognostic marker for combined hepatocellular-cholangiocarcinoma

AUC:0.86



Nestin in European cHCC-ICC 2022



nature reviews
gastroenterology & hepatology

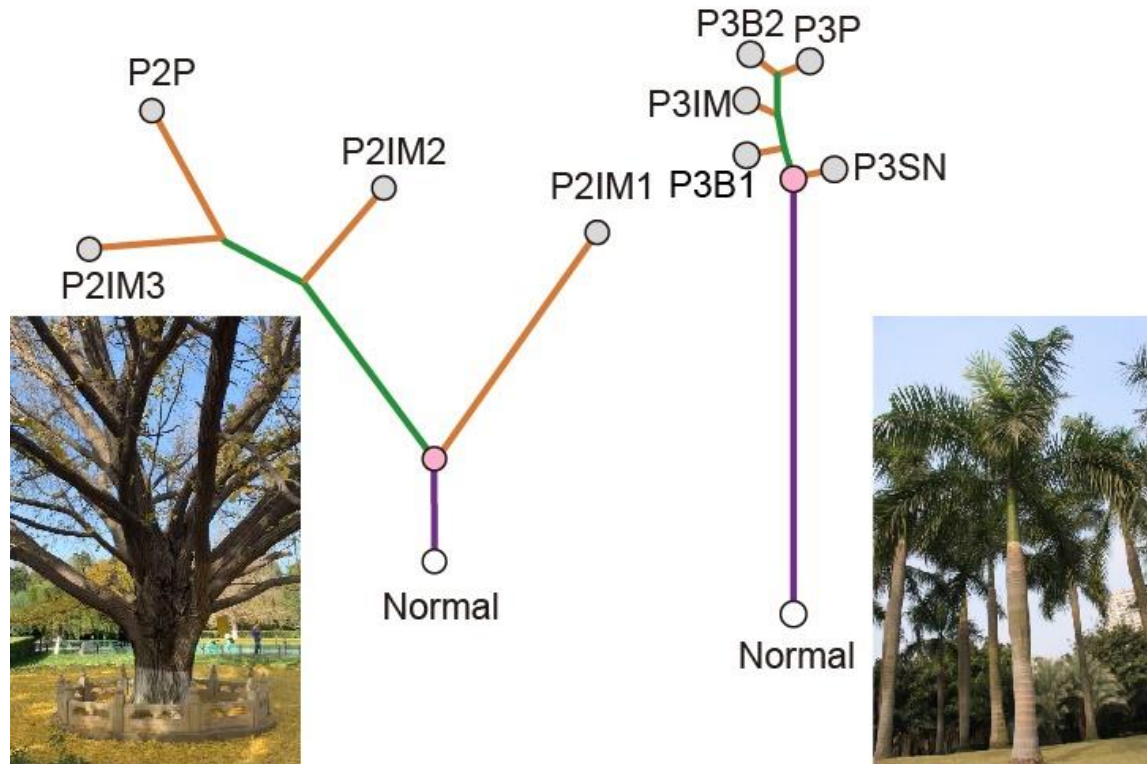
NEWS & VIEWS

nature reviews
gastroenterology & hepatology

YEAR IN REVIEW

细胞出版社2019中国年度论文
Cell Press Paper of the Year
2019·China

Investigating the underlying reason of ITH by scDNA-seq



Ginkgo 银杏树

Coconut 椰子树

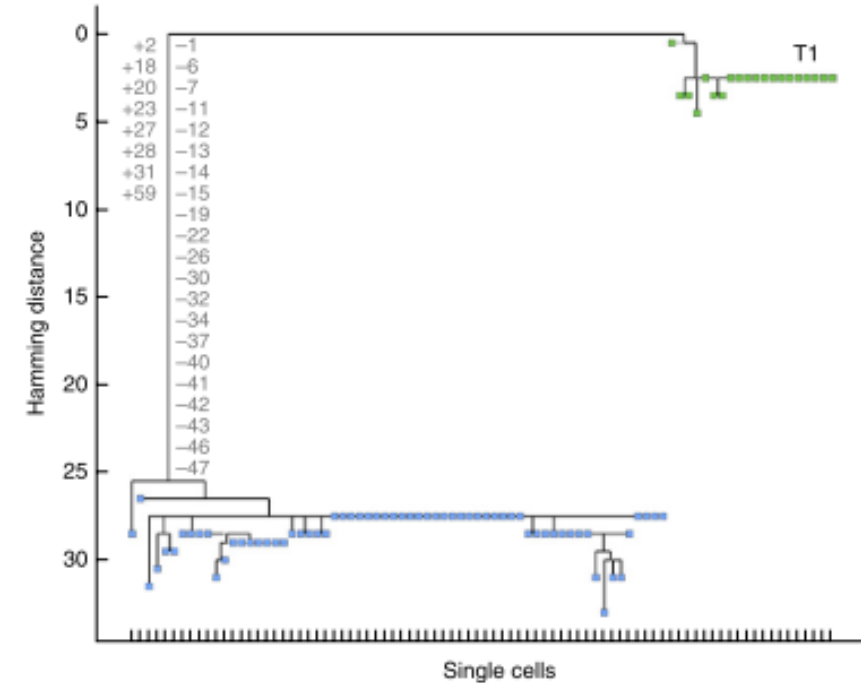
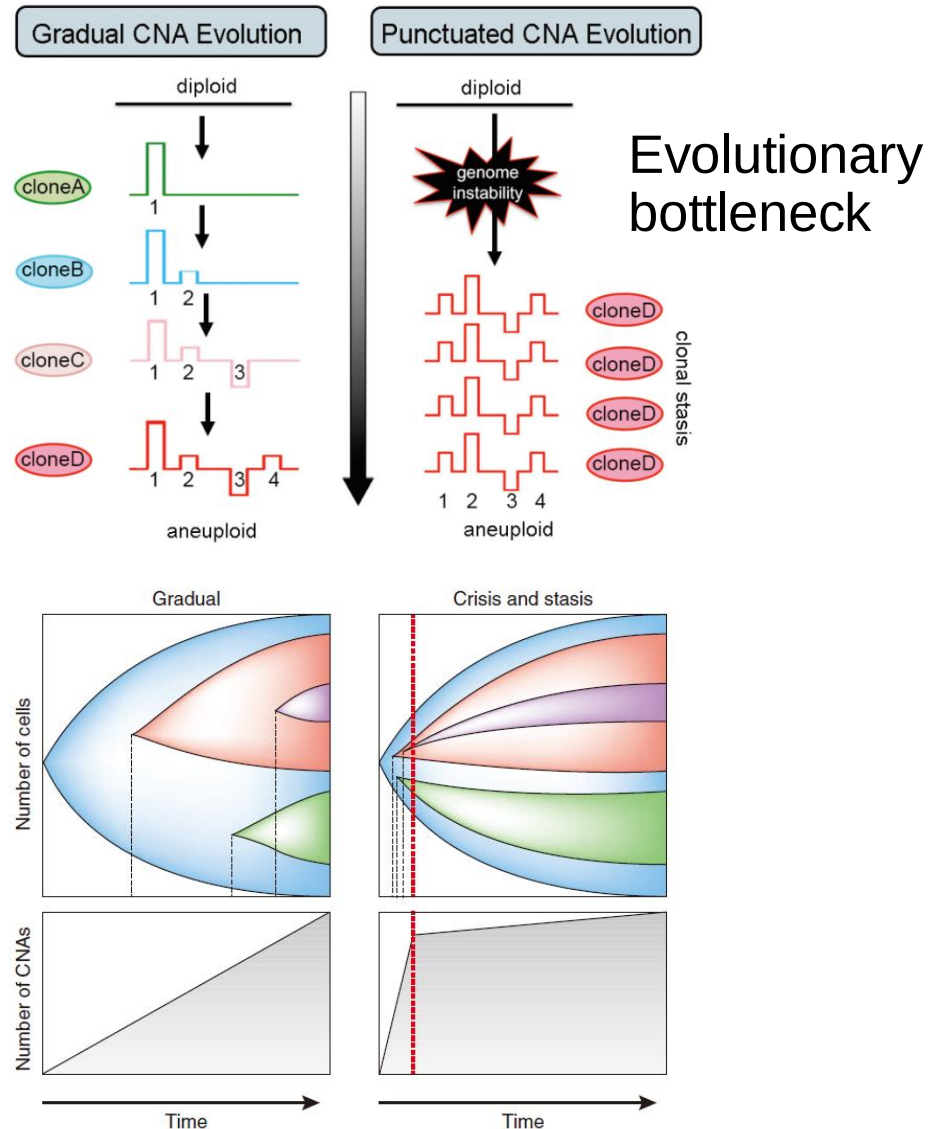
Early metastasis

Late metastasis

by scDNA-seq

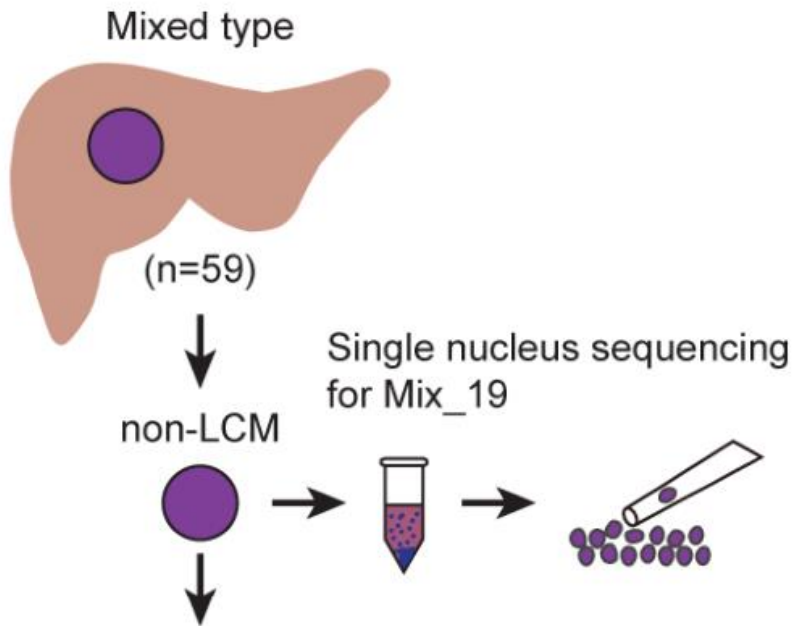
Copy number alterations (CNAs), elicited by genome instability, are a major source of ITH.

Investigating the underlying reason of ITH by scDNA-seq

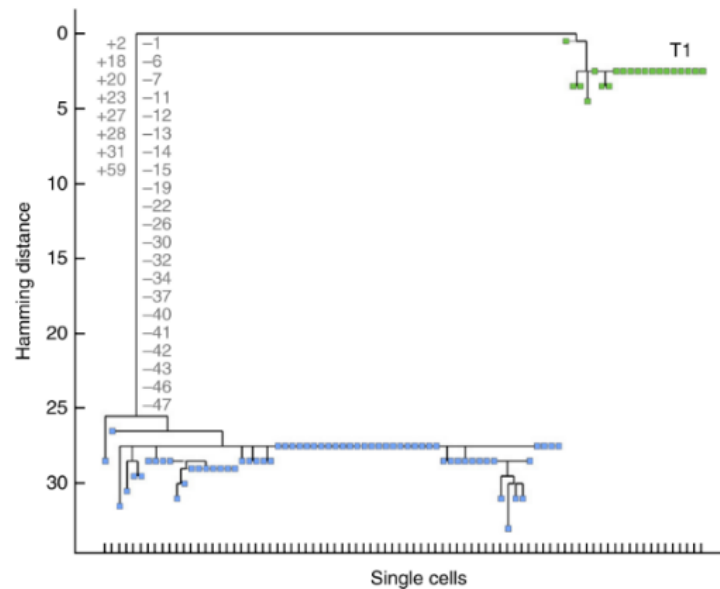


- Navin, et al, Nature, 2012
- Baca, et al, Cell, 2013
- Navin, et al, Nature, 2014
- Sottoriva, et al, Nature Genetics, 2014
- Gao & Navin, et al, Nature Genetics, 2016
- ...

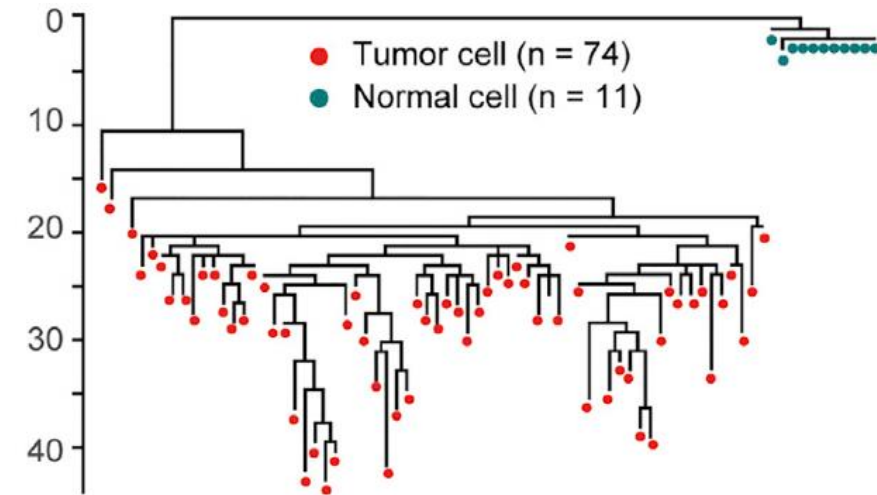
Investigating the underlying reason of ITH by scDNA-seq



Navin's data (PCNE)

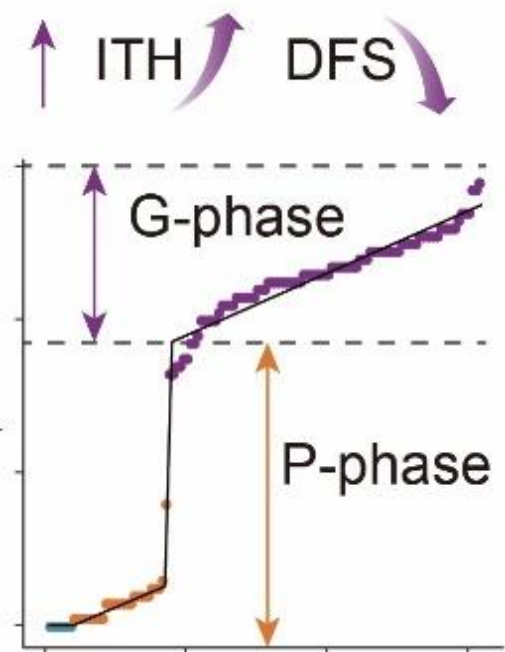


Our data



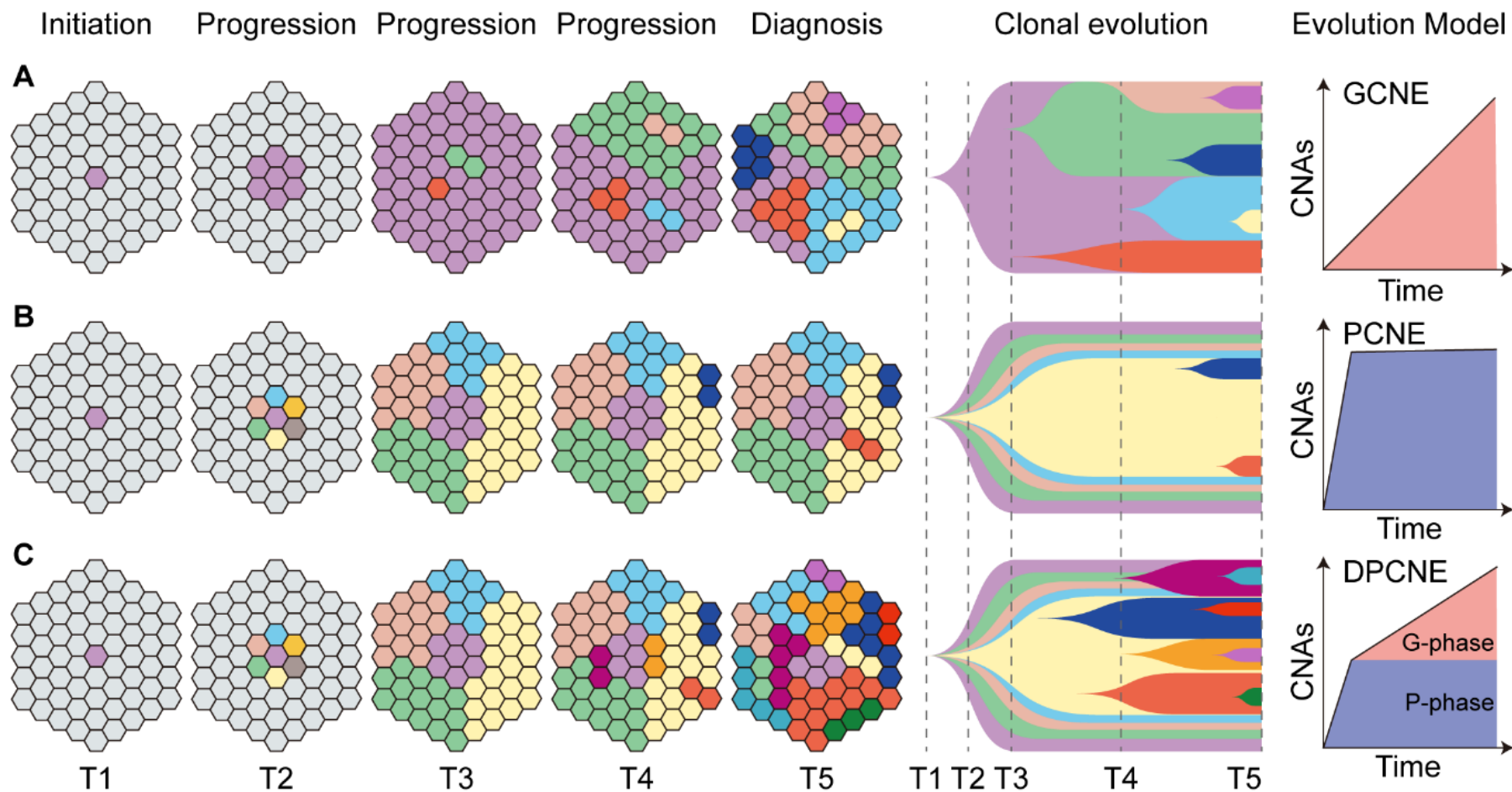
Gradual or punctuated? Our data do not fit with the punctuated evolution

Dual phase copy number evolution (DPCNE) model



DPCNE is better for

- Liver
- Breast
- Colorectal



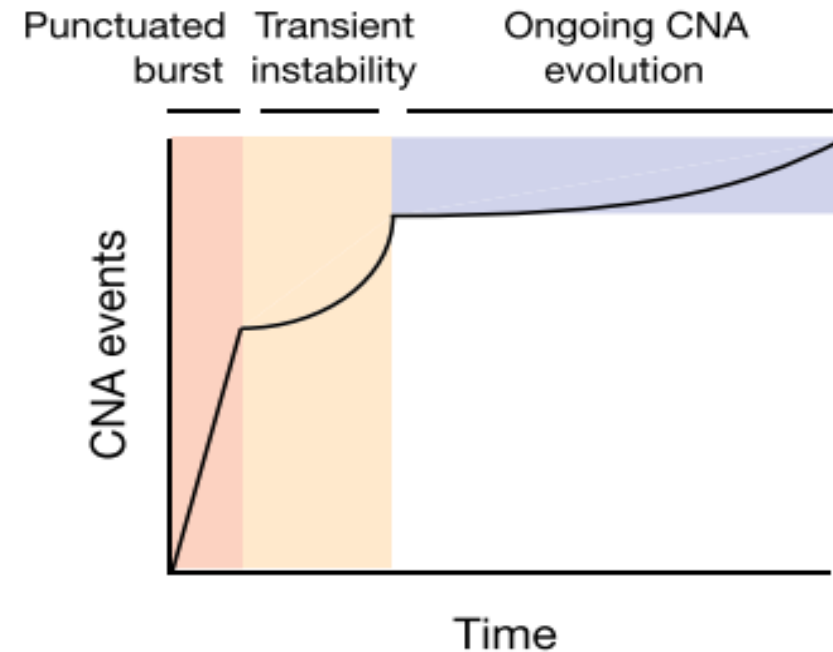
Dual phase copy number evolution (DPCNE) model

nature

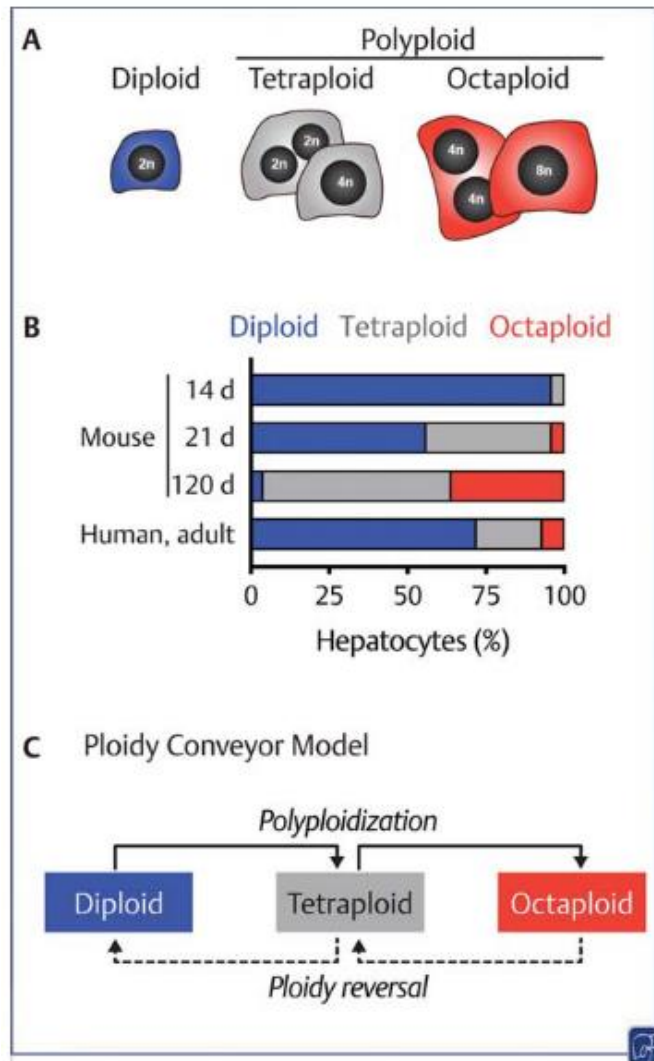
Article

Breast tumours maintain a reservoir of subclonal diversity during expansion

Nicholas E. Navin^{1,2,12} ✉



Liver is a mixture of diploid and polyploid cells



What is the origin of HCC? Diploid or Polyploid?

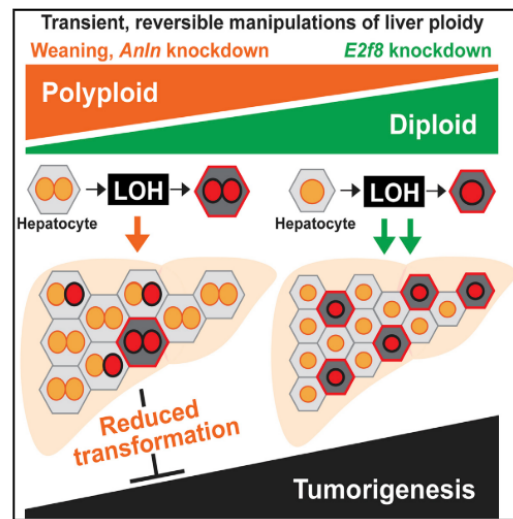
- A unique feature of liver tissue
- Accounts for 25-50% of hepatocytes
- Some HCC are diploid, some are polyploid

Previous investigation of polyploidy in murine models

Developmental Cell

The Polyploid State Plays a Tumor-Suppressive Role in the Liver

Graphical Abstract



Acute injury model

Suggests a protective role for polyploid cells, which can endure more mutagenic hits for TSG

Zhang et al., 2018, Developmental Cell

Article

Authors

Shuyuan Zhang, Kejin Zhou, Xin Luo, ...,
Branden D. Tarlow, Daniel J. Siegwart,
Hao Zhu

Correspondence

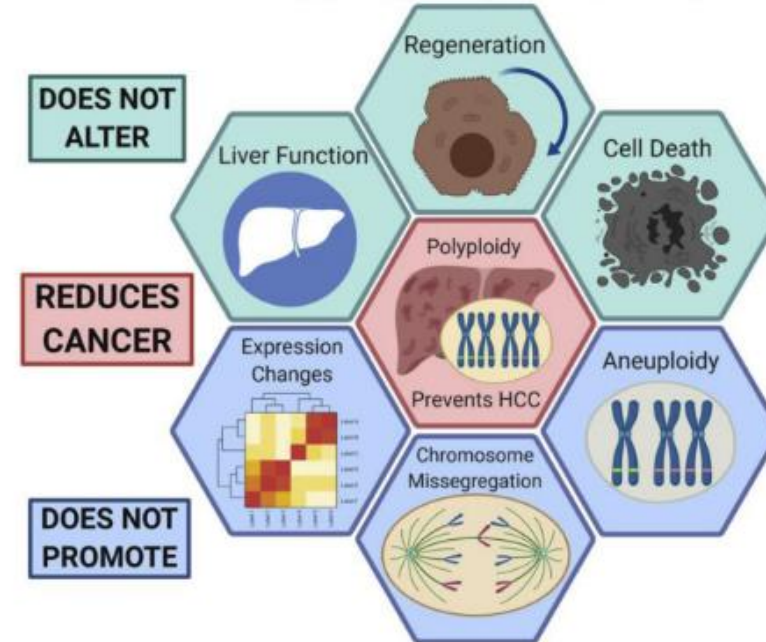
hao.zhu@utsouthwestern.edu

In Brief

Most liver cells are polyploid, but the functional role of wholesale genome duplications is unknown. To interrogate liver polyploidy function without irreversible manipulations of cell-cycle genes, Zhang et al. developed models to transiently alter ploidy, finding that polyploidy reduces tumor development by buffering against tumor-suppressor loss of heterozygosity.

Gastroenterology

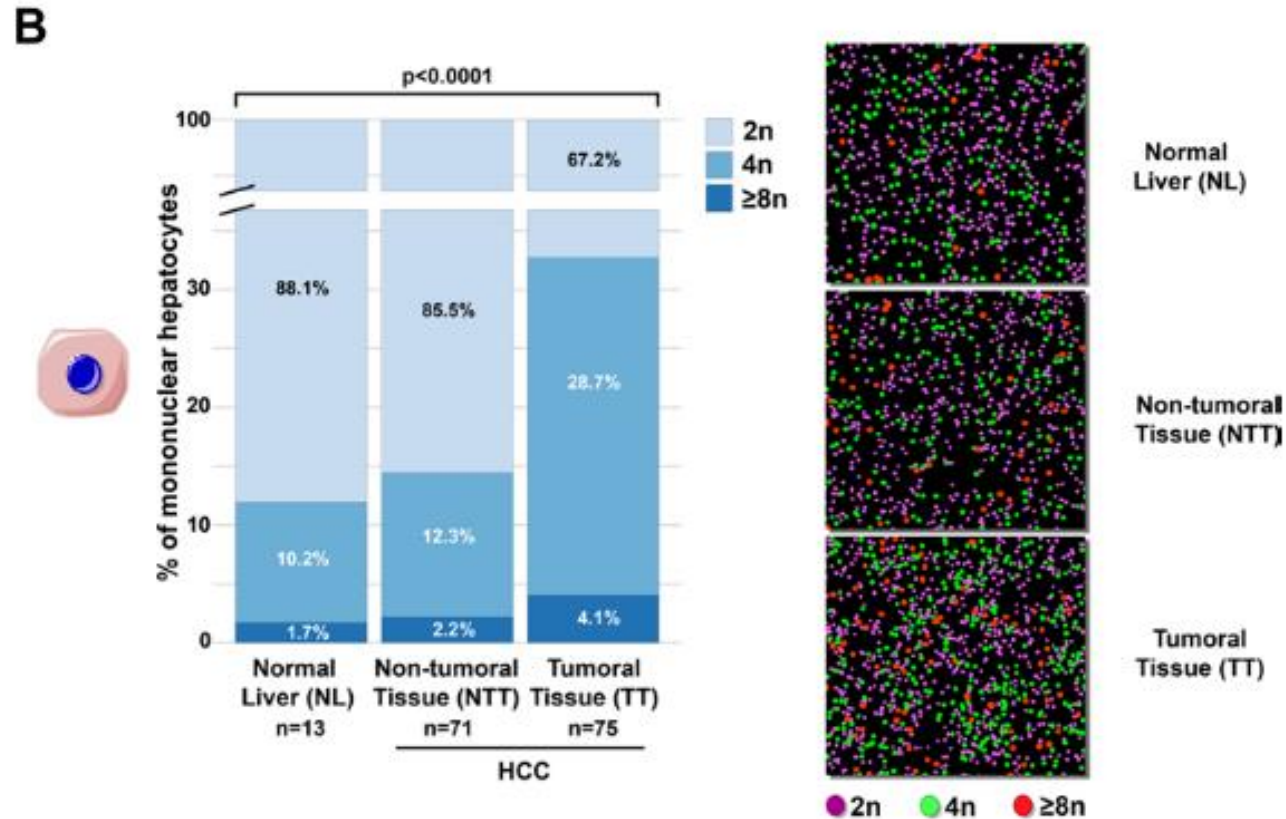
Increasing polyploidy in hepatocytes



Chronic injury model

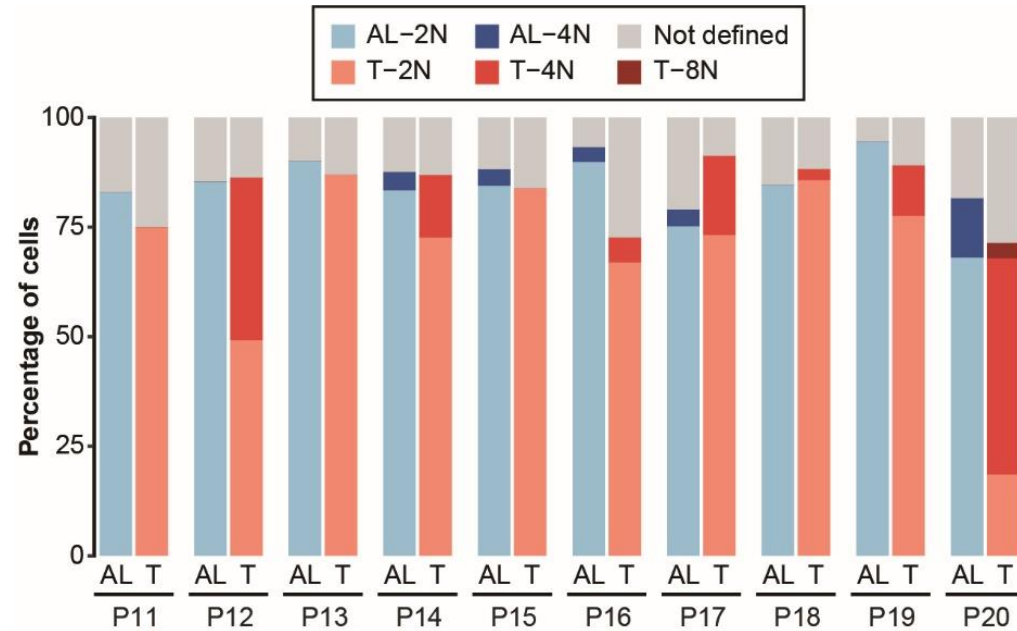
Lin et al., 2020, Gastroenterology

A big proportion of human HCC samples are polyploid

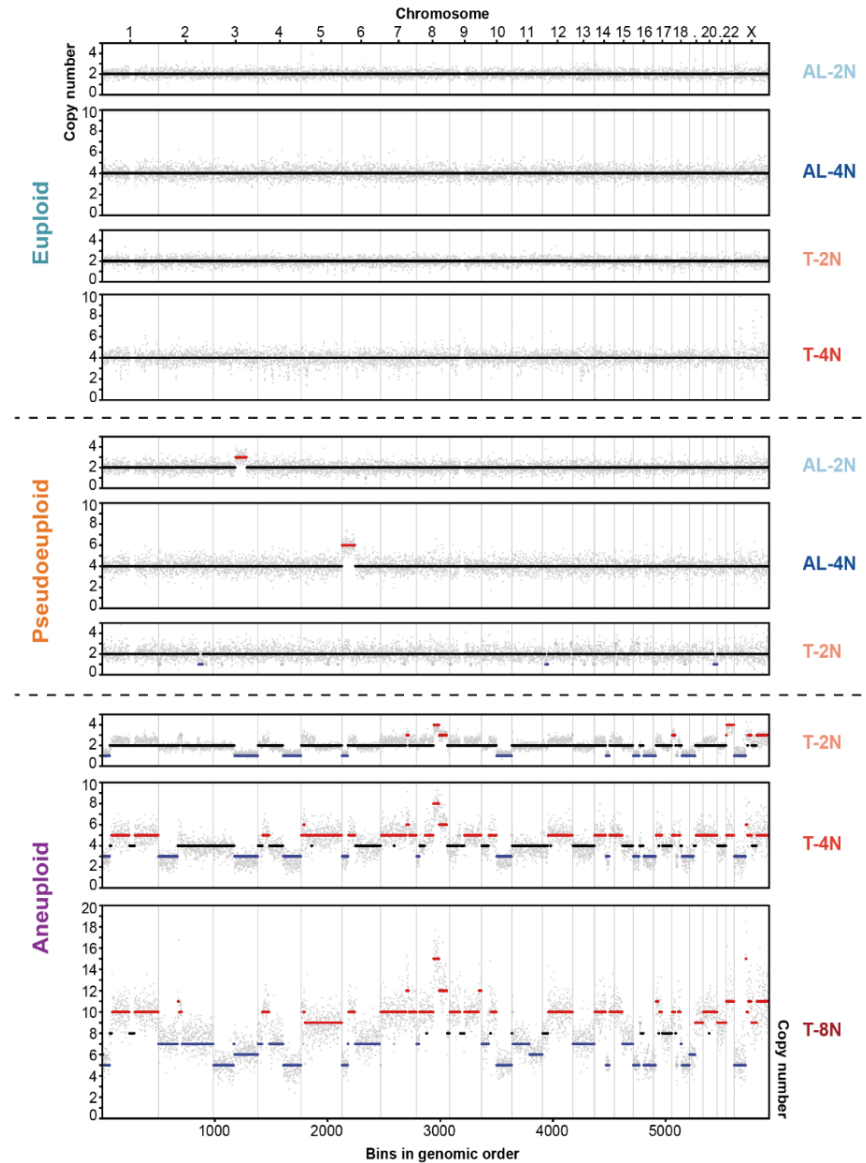


Suggests that human HCC are from polyploid cells?

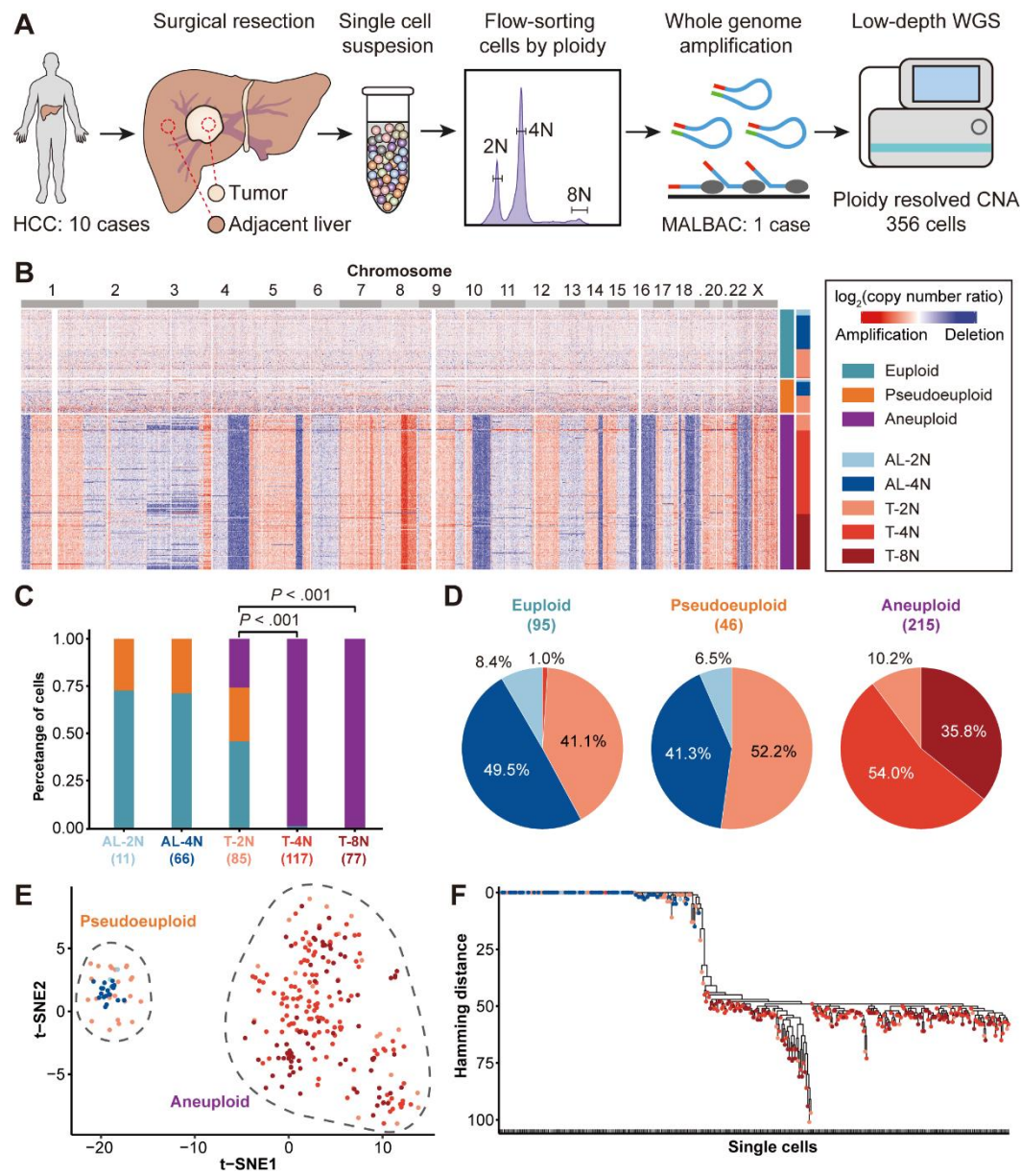
scDNA-seq to infer the origin



Ploidy-scDNA-seq



scDNA-seq to infer the origin



Two key observations in this case

- Anueploid 2N 与 4N 8N 共同起源
- Aneuploid 富集在多倍体 (4N 8N)

Diploid to Polyploid?

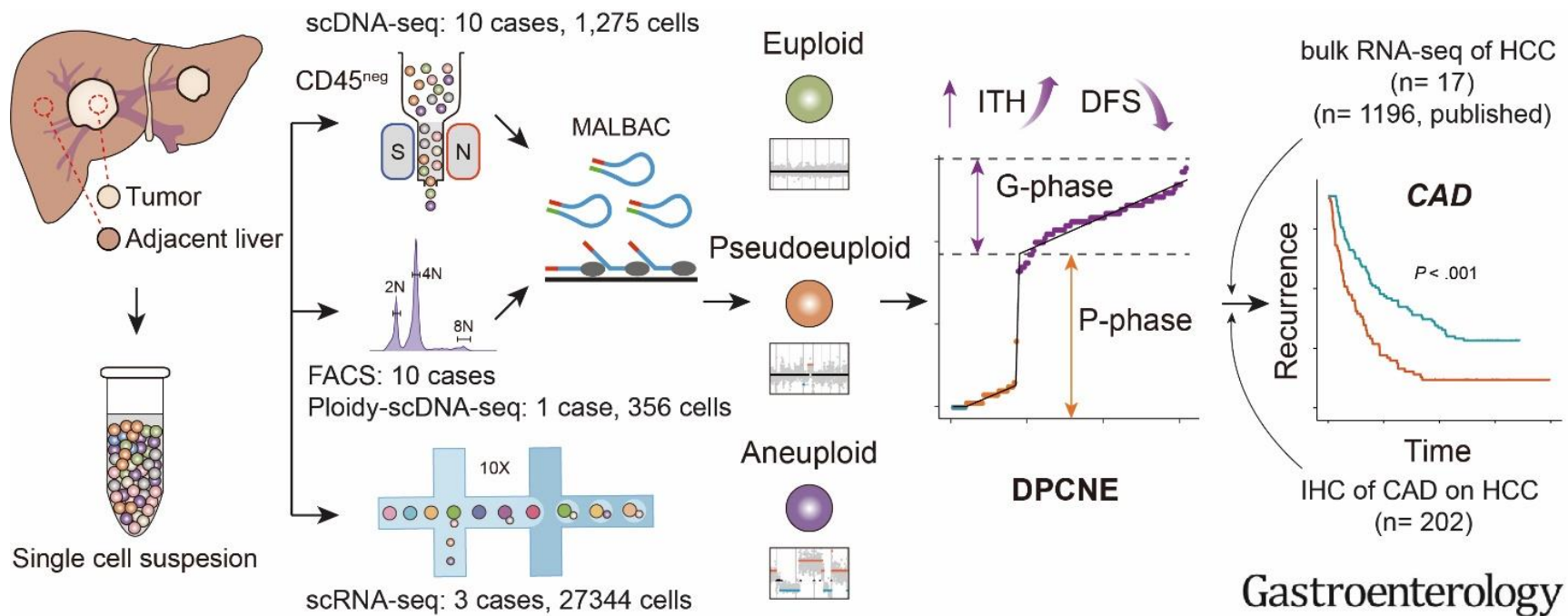
Polyploid to Diploid?

T_2N > WGD > T_4N, T_8N

Reconcile the conflict

Supported the diploid origin

Summary

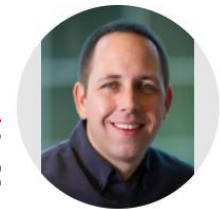


- ✓ A new clonal evolution model
- ✓ A diploid origin of HCC

EDITORIALS

Single-Cell DNA Sequencing Reveals Chromosomal Diversity in HCC: A Model of HCC Evolution

Sierra R. Wilson, Andrew W. Duncan, et al.



Andrew Duncan

are CNAs among patients with HCC and within individual tumors? How do these heterogeneous HCC cells evolve during tumor formation? In the current issue of *Gastroenterology*, Guo and colleagues⁶ tackle these questions by characterizing HCC heterogeneity at the single-cell level. They performed single-cell DNA sequencing (scDNA-seq) of HCC cells, and using a combination of modeling, validation, and patient tracking, they demonstrated extensive CNA-mediated tumor heterogeneity and identified a novel mechanism of HCC evolution.

A conflicting point remains: catastrophic event

- Kataegis

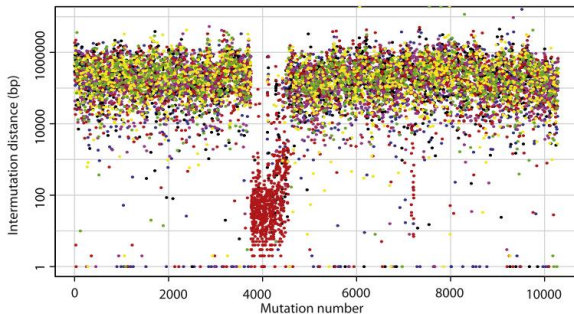
Cell

The Life History of 21 Breast Cancers

Serena Nik-Zainal,^{1,19} Peter Van Loo,^{1,2,3,19} David C. Wedge,^{1,19} Ludmil B. Alexandrov,¹ Christopher D. Greenman,^{1,4,5} King Wai Lau,¹ Keiran Raine,¹ David Jones,¹ John Marshall,¹ Manasa Ramakrishna,¹ Adam Shlien,¹ Susanna L. Cooke,¹ Jonathan Hinton,¹ Andrew Menzies,¹ Lucy A. Stebbings,¹ Catherine Leroy,¹ Mingming Jia,¹ Richard Rance,¹ Laura J. Mudie,¹ Stephen J. Gamble,¹ Philip J. Stephens,¹ Stuart McLaren,¹ Patrick S. Tarpey,¹ Elli Papaemmanuil,¹ Helen R. Davies,¹ Ignacio Varela,¹ David J. McBride,¹ Graham R. Bignell,¹ Kenric Leung,¹ Adam P. Butler,¹ Jon W. Teague,¹ Sancha Martin,¹ Goran Jönsson,¹ Odette Mariani,¹ Sandrine Boyault,¹ Penelope Miron,¹ Aquila Fatima,¹ Anita Langerød,¹⁰ Samuel A.J.R. Aparicio,^{1,11,12} Andrew Tutt,¹ Aniela M. Siowert,¹³ Åke Borg,¹⁴ Gilles Thomas,¹⁴ Anne Vincent Salomon,⁷ Andrea L. Richardson,^{8,15} Anne-Lise Borresen-Dale,^{10,16} P. Andrew Futreal,¹ Michael R. Stratton,¹ Peter J. Campbell,^{1,17,18} and Breast Cancer Working Group of the International Cancer Genome Consortium

Mutational Processes Molding the Genomes of 21 Breast Cancers

Serena Nik-Zainal,¹ Ludmil B. Alexandrov,¹ David C. Wedge,¹ Peter Van Loo,^{1,2,3} Christopher D. Greenman,^{1,4,5} Keiran Raine,¹ David Jones,¹ Jonathan Hinton,¹ John Marshall,¹ Lucy A. Stebbings,¹ Andrew Menzies,¹ Sancha Martin,¹ Kenric Leung,¹ Lina Chen,¹ Catherine Leroy,¹ Manasa Ramakrishna,¹ Richard Rance,¹ King Wai Lau,¹ Laura J. Mudie,¹ Ignacio Varela,¹ David J. McBride,¹ Graham R. Bignell,¹ Susanna L. Cooke,¹ Adam Shlien,¹ John Gamble,¹ Ian Whitmore,¹ Mark Maddison,¹ Patrick S. Tarpey,¹ Helen R. Davies,¹ Elli Papaemmanuil,¹ Philip J. Stephens,¹ Stuart McLaren,¹ Adam P. Butler,¹ Jon W. Teague,¹ Goran Jönsson,¹ Judy E. Garber,¹ Daniel Silver,¹ Penelope Miron,¹ Aquila Fatima,⁷ Sandrine Boyault,¹⁰ Anita Langerød,¹⁰ Andrew Tutt,¹⁰ John W.M. Martens,¹¹ Samuel A.J.R. Aparicio,^{1,12} Åke Borg,¹³ Anne Vincent Salomon,¹⁴ Gilles Thomas,¹⁴ Anne-Lise Borresen-Dale,^{10,16} Andrea L. Richardson,^{7,16} Michael S. Neuburger,¹⁷ P. Andrew Futreal,¹ Peter J. Campbell,^{1,18,19} Michael R. Stratton^{1,2} and the Breast Cancer Working Group of the International Cancer Genome Consortium

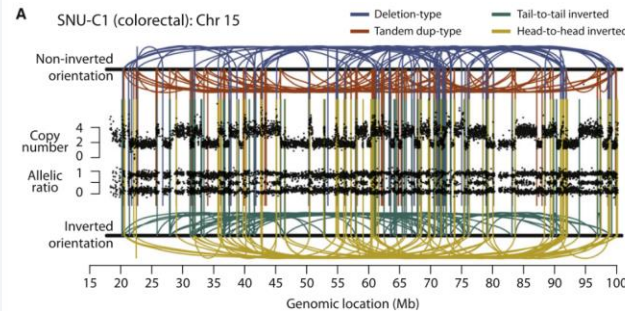


- Chromothripsis

Cell

Massive Genomic Rearrangement Acquired in a Single Catastrophic Event during Cancer Development

Philip J. Stephens,¹ Chris D. Greenman,¹ Beiyuan Fu,¹ Fengtang Yang,¹ Graham R. Bignell,¹ Laura J. Mudie,¹ Erin D. Plessano,¹ King Wai Lau,¹ David Beare,¹ Lucy A. Stebbings,¹ Stuart McLaren,¹ Meng-Lay Lin,¹ David J. McBride,¹ Ignacio Varela,¹ Serena Nik-Zainal,¹ Catherine Leroy,¹ Mingming Jia,¹ Andrew Menzies,¹ Adam P. Butler,¹ Jon W. Teague,¹ Michael A. Quail,¹ John Burton,¹ Harold Sverdlow,¹ Nigel P. Carter,¹ Laura A. Monsberger,² Christine Iacobuzio-Donaue,³ George A. Follows,⁴ Anthony R. Green,^{5,6} Adrienne M. Flanagan,^{6,8} Michael R. Stratton,^{1,2} P. Andrew Futreal,¹ and Peter J. Campbell^{1,4,9}

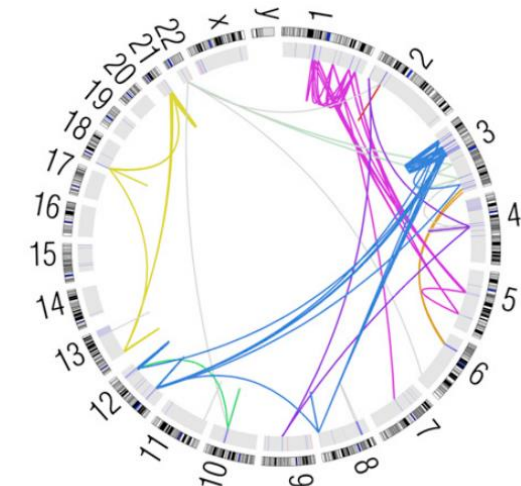


- Chromoplexy

Cell

Punctuated Evolution of Prostate Cancer Genomes

Sylvan C. Baca,^{1,2,3} Davide Prandi,⁴ Michael S. Lawrence,² Juan Miguel Mosquera,⁵ Alessandro Romanel,⁶ Yotam Drier,^{2,7} Kyung Park,⁸ Naoki Kitabayashi,⁹ Theresa Y. MacDonald,⁹ Mahmoud Ghandi,⁹ Eliezer Van Allen,^{2,9} Gregory V. Kryukov,^{1,2,10} Andrea Sboner,^{8,9} Jean-Philippe Theurillat,² T. David Soong,⁹ Elizabeth Nickerson,² Daniel Auclair,² Ashutosh Tewari,^{10,11} Himisha Beltran,¹² Robert C. Onofrio,² Gunther Boysen,⁶ Candace Guiducci,² Christopher E. Barbieri,^{8,11} Kristian Cibulskis,² Andrey Sivachenko,² Scott L. Carter,² Gordon Saksena,² Douglas Voet,² Alex H. Ramos,^{1,2} Wendy Winckler,² Michelle Cipicchio,² Kristin Ardlie,² Philip W. Kantoff,^{1,3} Michael F. Berger,^{1,4} Stacey B. Gabriel,² Todd R. Golub,^{2,4,6,15} Matthew Meyerson,^{1,2,3,4} Eric S. Lander,^{1,2,16,17} Olivier Elemento,⁹ Gad Getz,² Francesca Demicheli,^{8,9,18,2} Mark A. Rubin,^{8,11,18} and Levi A. Garraway^{1,3,3,4,18,2}



- ✓ Generate multiple alteration in a single event
- ✓ Regarded as evidence for Punctuated Evolution
- ✓ Need WGS data with high depth !

Few whole genome studies on Chinese HCC

- ICGC



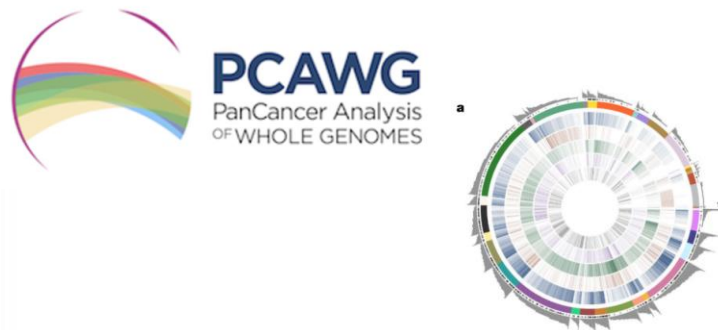
Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer

Akihiro Fujimoto^{1,2,4}, Mayuko Furuta^{1,2,4}, Yasushi Totoki^{2,24}, Tatsuhiko Tsunoda^{3-5,24}, Mamoru Kato^{5,6,24}, Yuichi Shiraishi⁷, Hiroko Tanaka⁷, Hiroaki Taniguchi¹, Yoshiiku Kawakami⁸, Masaki Ueno⁹, Kunihiro Gotoh¹⁰, Shun-ichi Arizumi¹¹, Christopher P Wardell¹, Shinya Hayami⁹, Toru Nakamura¹², Hiroshi Aikata⁸, Koji Arihiro¹³, Keith A Boroevich³, Tetsuo Abe³, Kaoru Nakano¹, Kazuhiro Maejima¹, Aya Sasaki-Oku¹, Ayako Ohsawa¹, Tetsuo Shibuya¹⁴, Hiromi Nakamura², Natsuko Hama², Fumie Hosoda², Yasuhito Arai², Shoko Ohashi², Tomoko Urushidate¹⁵, Genta Nagae¹⁶, Shogo Yamamoto¹⁶, Hiroki Ueda¹⁶, Kenji Tatsuno¹⁶, Hidenori Ojima¹⁷, Nobuyoshi Hiraoka¹⁸, Takuji Okusaka¹⁹, Michiaki Kubo²⁰, Shigeru Marubashi¹⁰, Terumasa Yamada¹⁰, Satoshi Hirano¹², Masakazu Yamamoto¹¹, Hideki Ohdan²¹, Kazuaki Shimada²², Osamu Ishikawa¹⁰, Hiroki Yamaue⁹, Kazuki Chayama²³, Satoru Miyano^{2,14}, Hiroyuki Aburatani¹⁶, Tatsuhiro Shibata^{2,15} & Hidewaki Nakagawa¹

Japanese HCC WGS cohort:
~300 cases
Depth: ~ 40x

- PCAWG

nature



PCAWG
336 Liver cancer cases
305 HCC cases

- TCGA



Resource



Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma

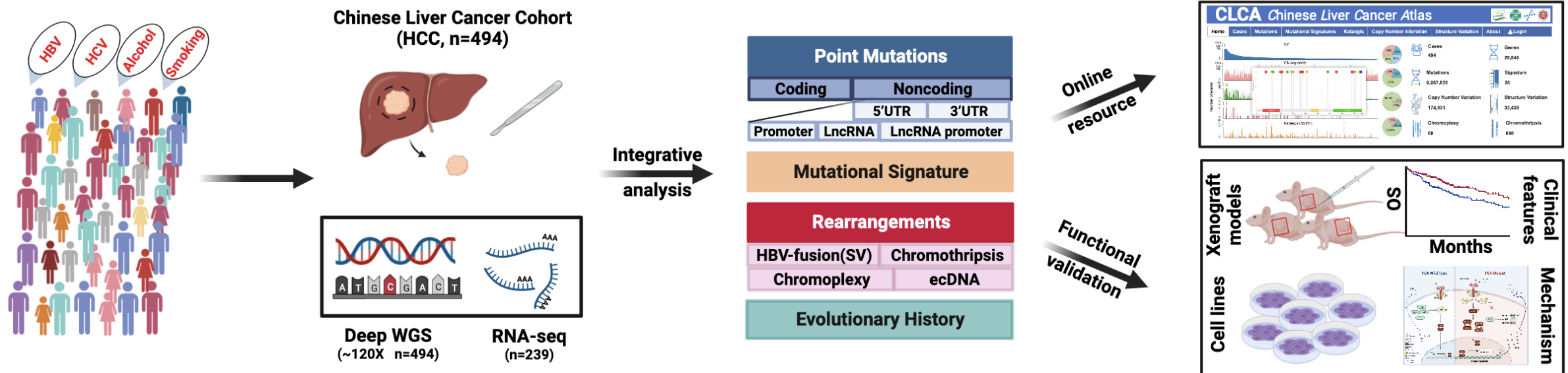
The Cancer Genome Atlas Research Network^{1,*}

¹Lead Contact (David A. Wheeler)

*Correspondence: wheeler@bcm.edu (David A. Wheeler), roberts.lewis@mayo.edu (Lewis R. Roberts)
<http://dx.doi.org/10.1016/j.cell.2017.05.046>

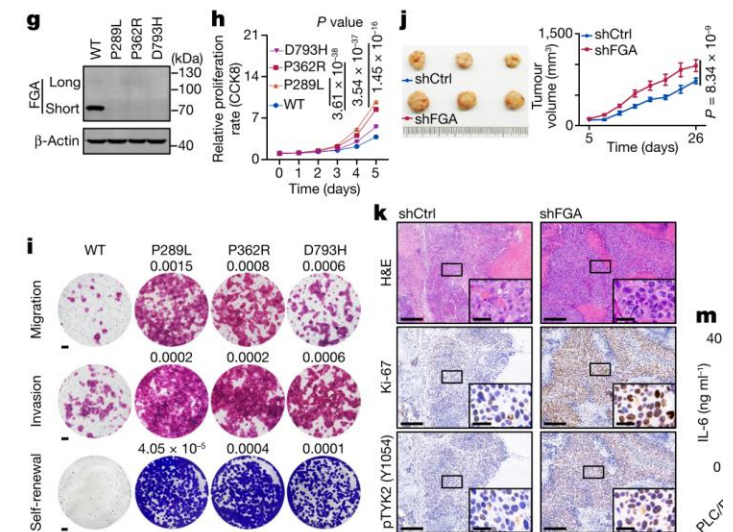
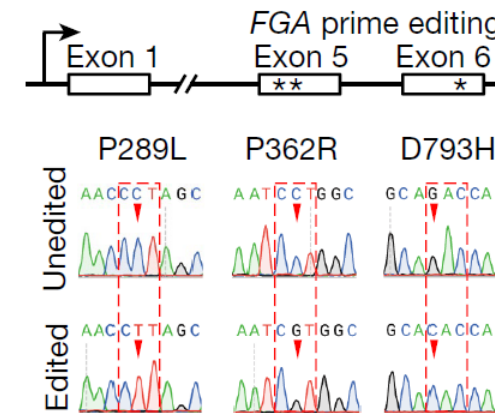
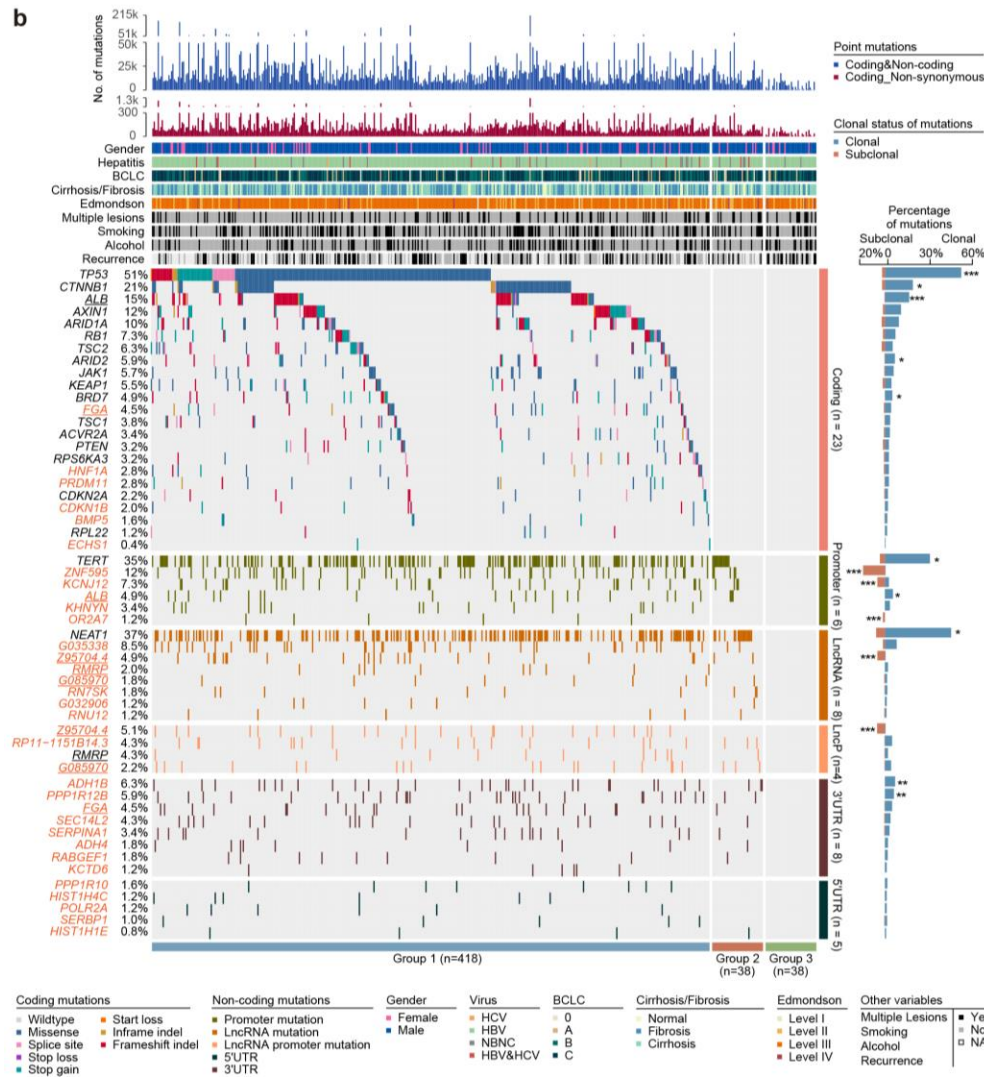
TCGA HCC WES cohort:
363 cases
Depth: ~ 100x

Deep Whole Genome Sequencing of Chinese HCC

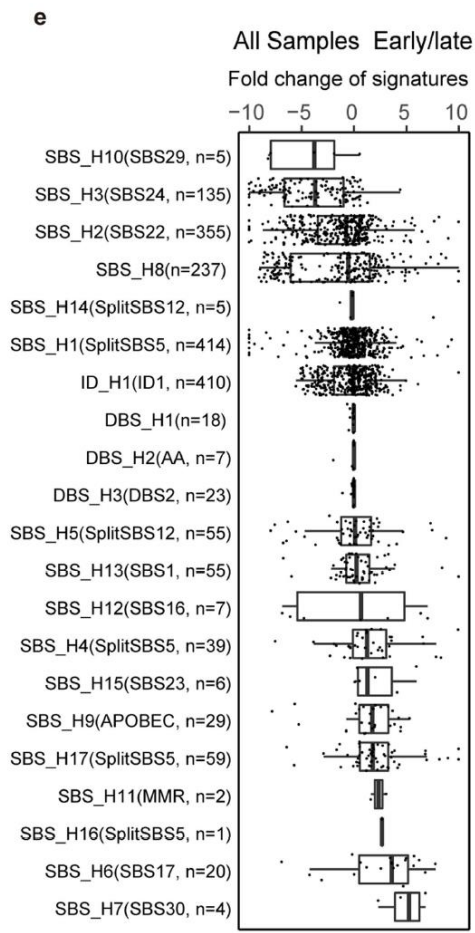
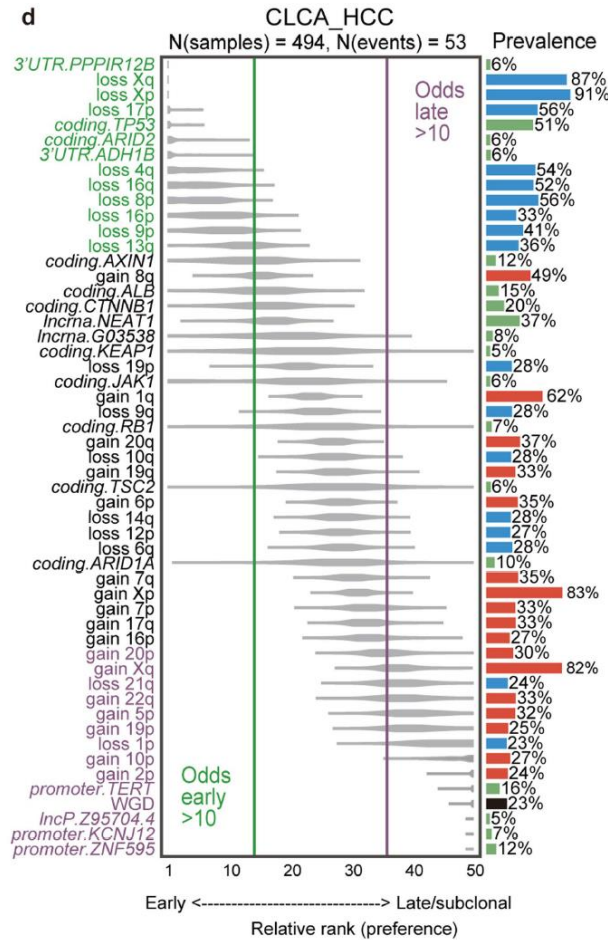
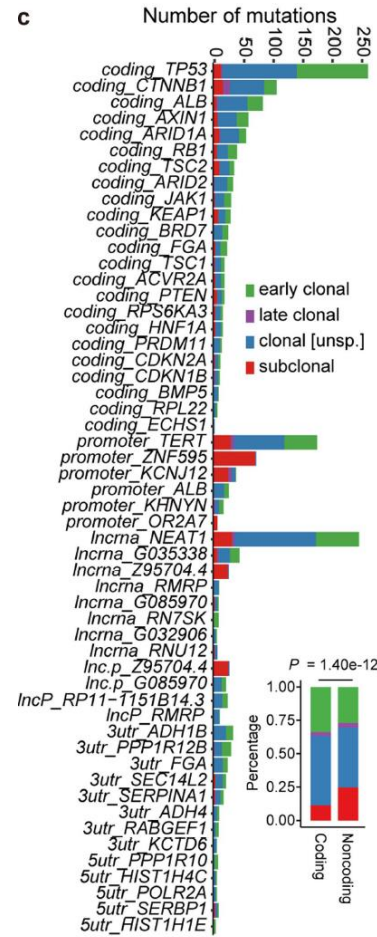
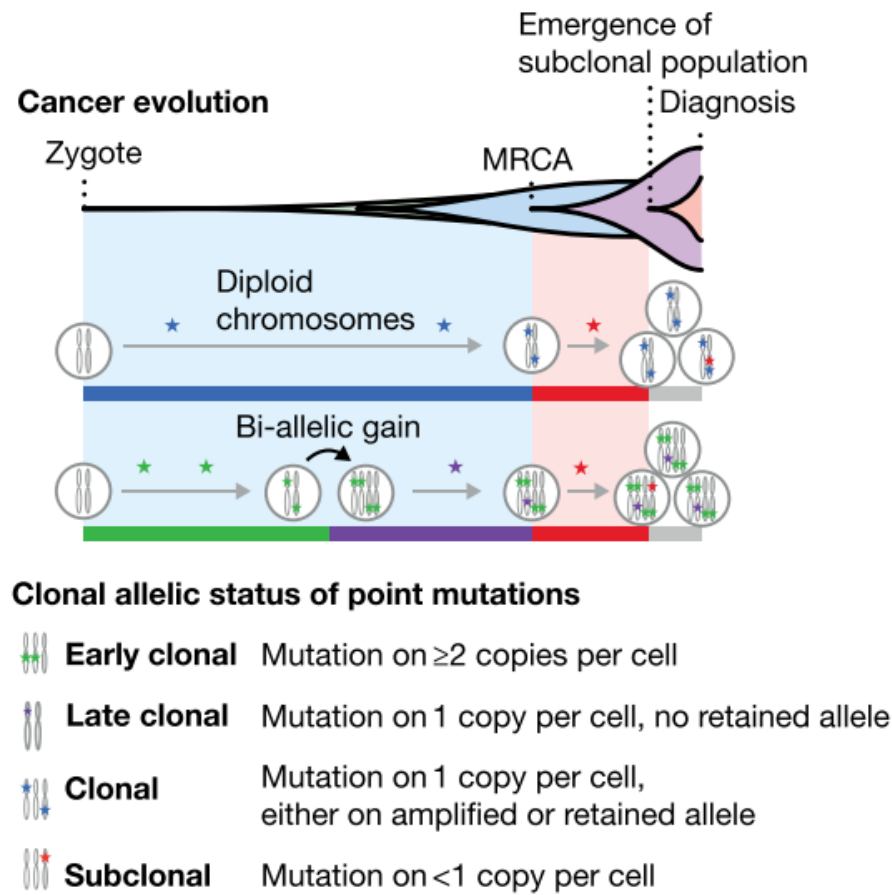


Mutational landscape— 6+28 novel drivers

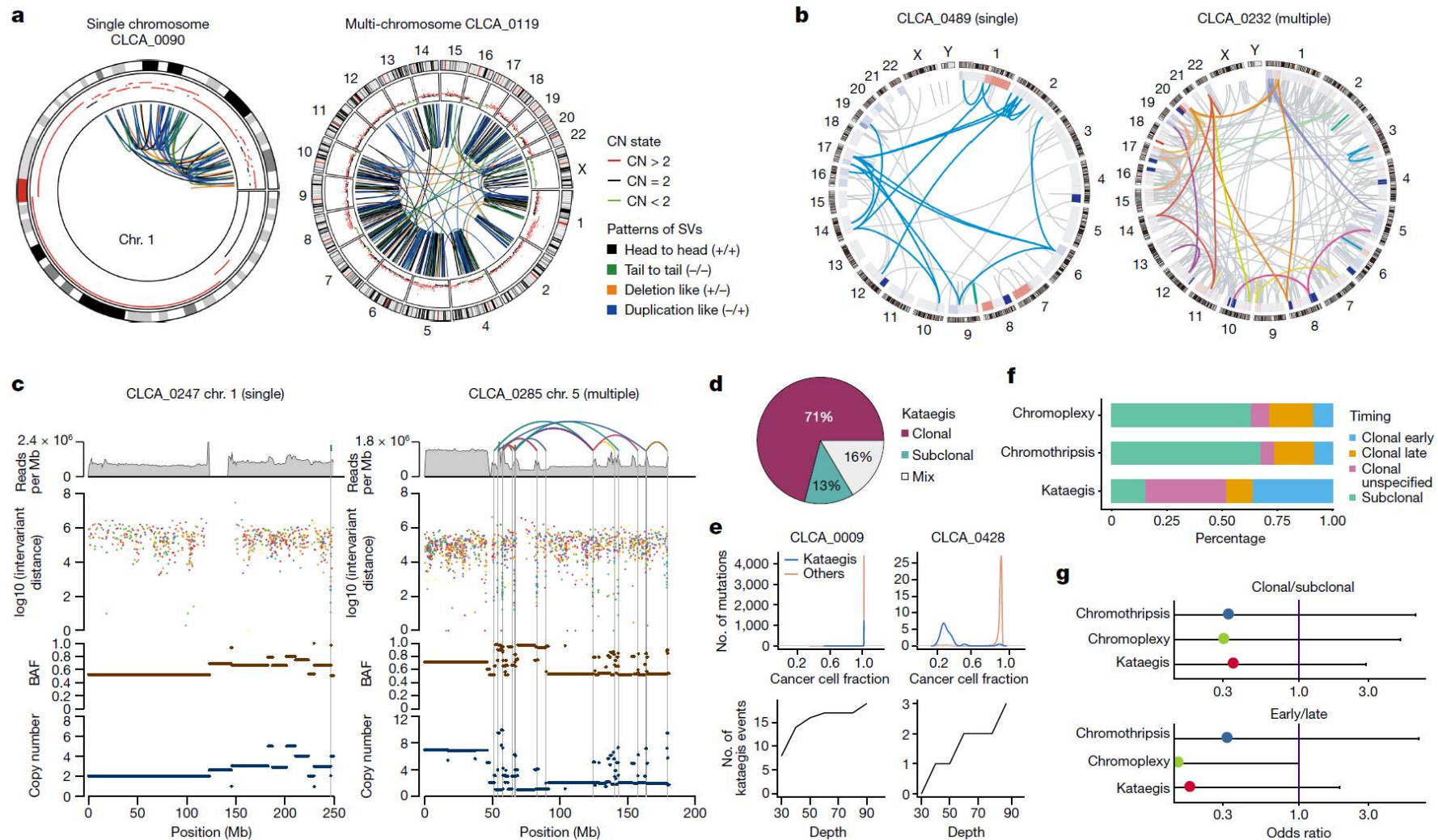
- 23 candidate coding drivers, **6** novel; 31 candidate noncoding drivers, **28** novel



Pervasive involvement of candidate noncoding drivers during HCC evolution

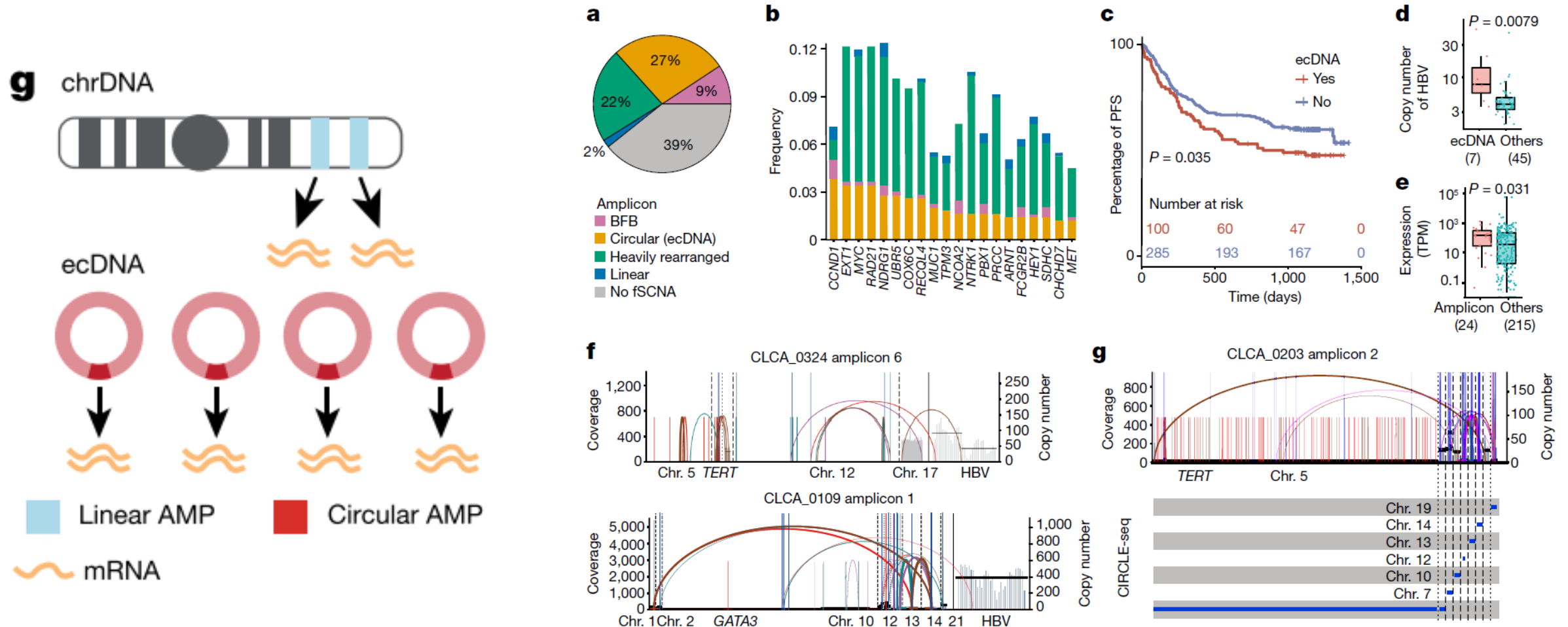


First evidence for subclonal catastrophic event



✓ Support our DPCNE model !

New **X** factor for clonal evolution and intratumor heterogeneity: ecDNA



All single tumor cells are different, at least true for these with ecDNA

Clonal evolution of HCC

nature

Article | Published: 14 February 2024

Deep whole-genome analysis of 494 hepatocellular carcinomas

nature reviews
gastroenterology & hepatology

HCC genomic landscape in Chinese individuals

Eleni Kotsiliti

Nature Reviews Gastroenterology & Hepatology

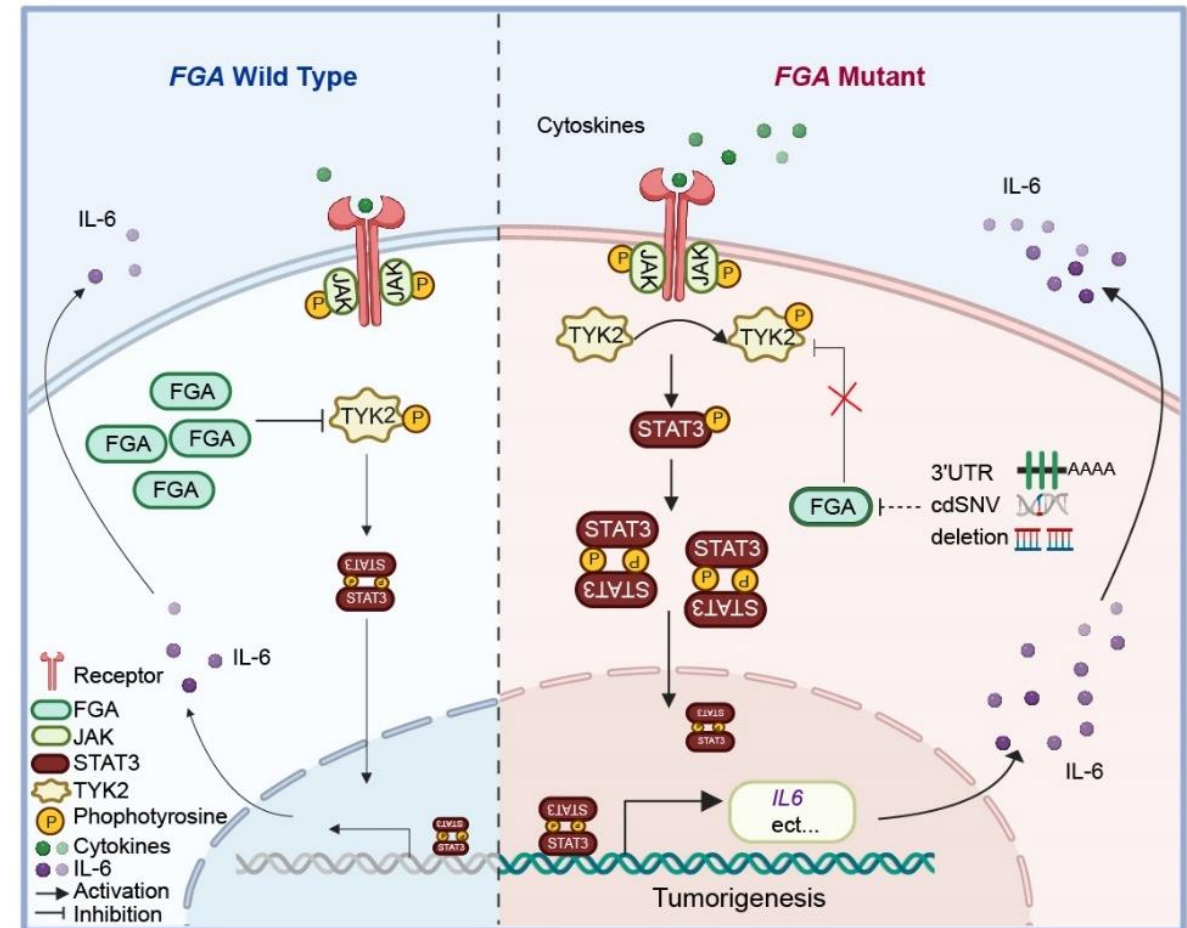
Research Highlight | 22 Mar 2024

Chen, L. et al., *Nature*, 2024

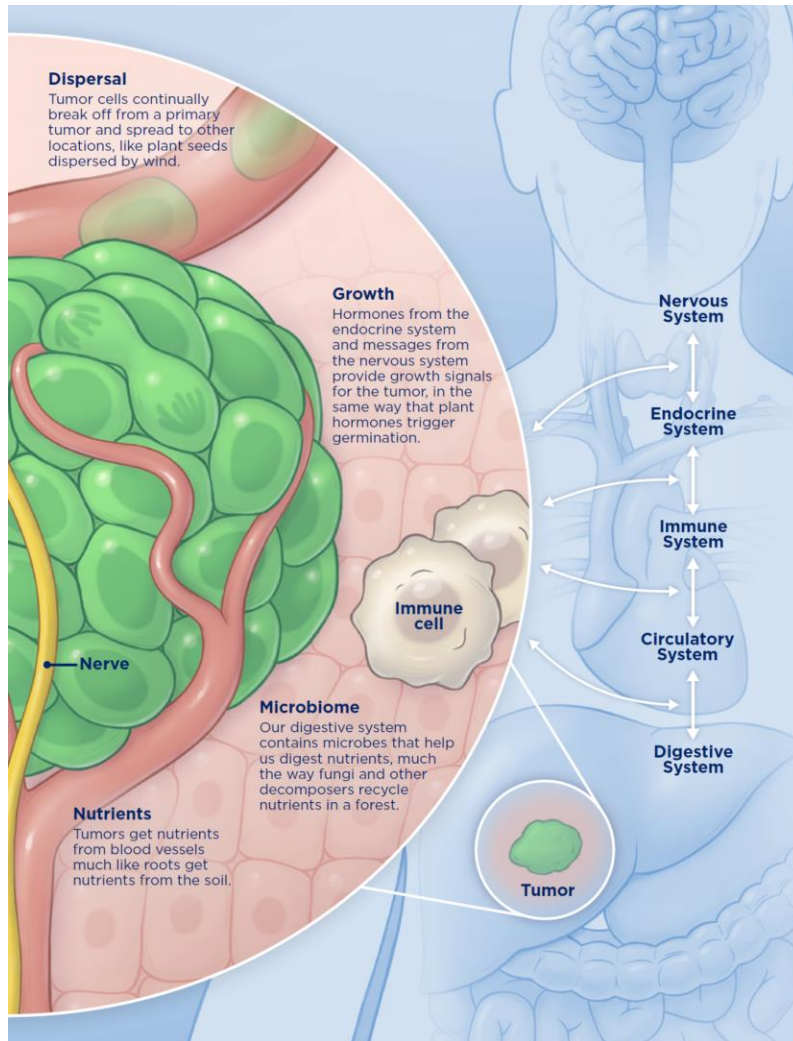
Guo, L. et al., *Gastroenterology*, 2022

Xue, R. et al., *Cancer Cell*, 2019

Xue, R. et al., *Gastroenterology*, 2016



Outline

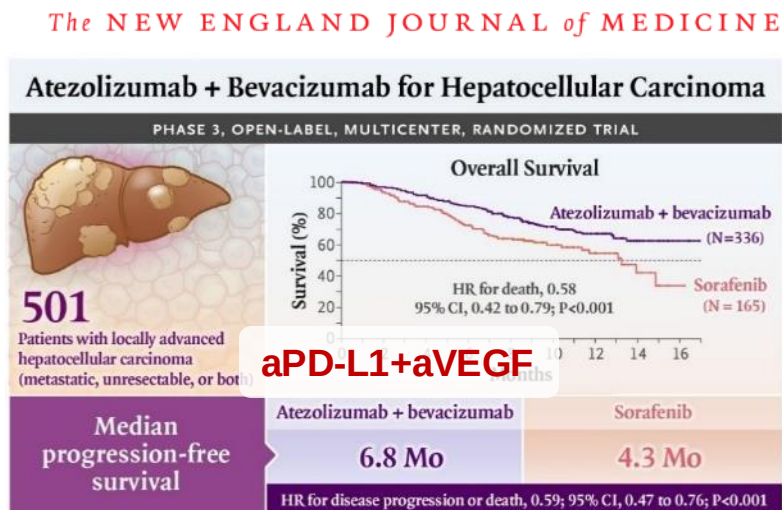


➤ Tumor clonal evolution

➤ Tumor immune microenvironment (TIME)

➤ Interplay between tumor and TIME

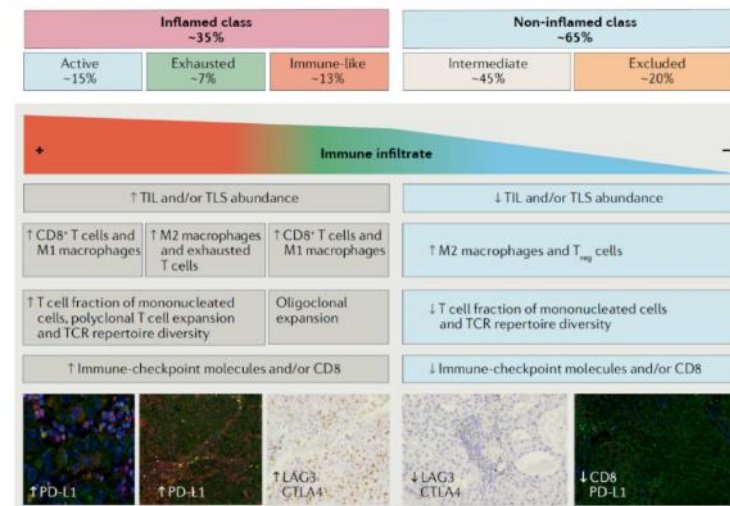
Low response rate of immunotherapy asks for **TIME** classification



Finn, et al., *NEJM*, 2020

Low response rate

- First-line immunotherapy
- ORR low as **27.3%**
- **Lacks suitable biomarker**



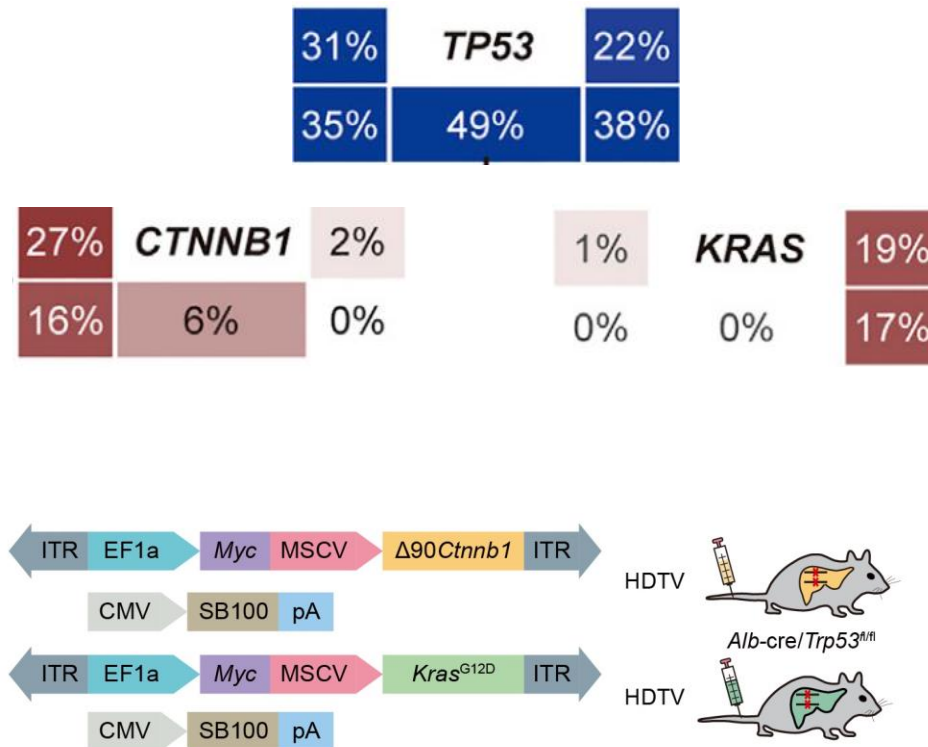
Johnson, et al., *Nature Reviews Clinical Oncology*, 2022

No suitable subtypes

- Pathology, not quantitative
- Bulk data, not accurate
- FACS sorting, cells missed

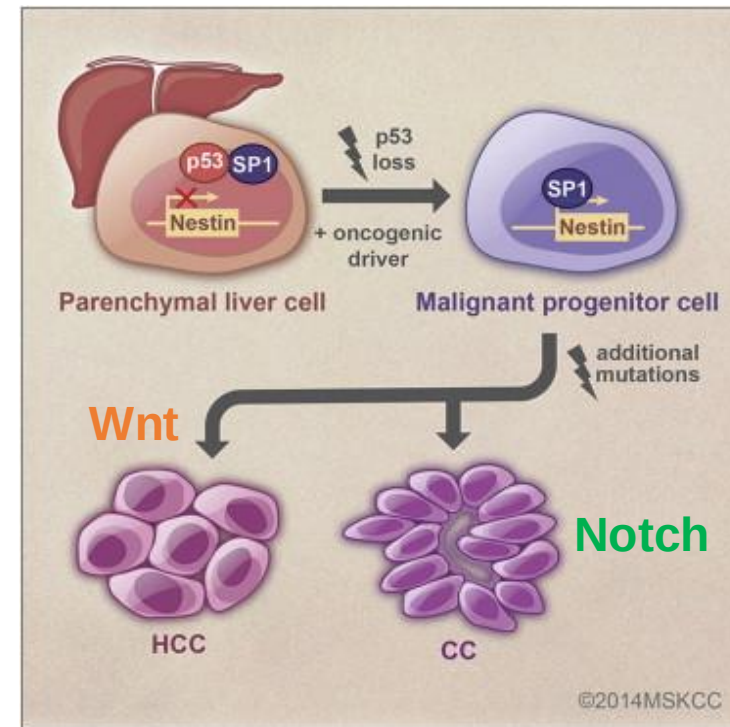
Our understanding of the baseline TIME landscape in PLC is limited, precluding biomarker identification for better patient stratification.

TP53-Nestin axis regulates cellular plasticity in liver cancer



p53-Dependent Nestin Regulation Links Tumor Suppression to Cellular Plasticity in Liver Cancer

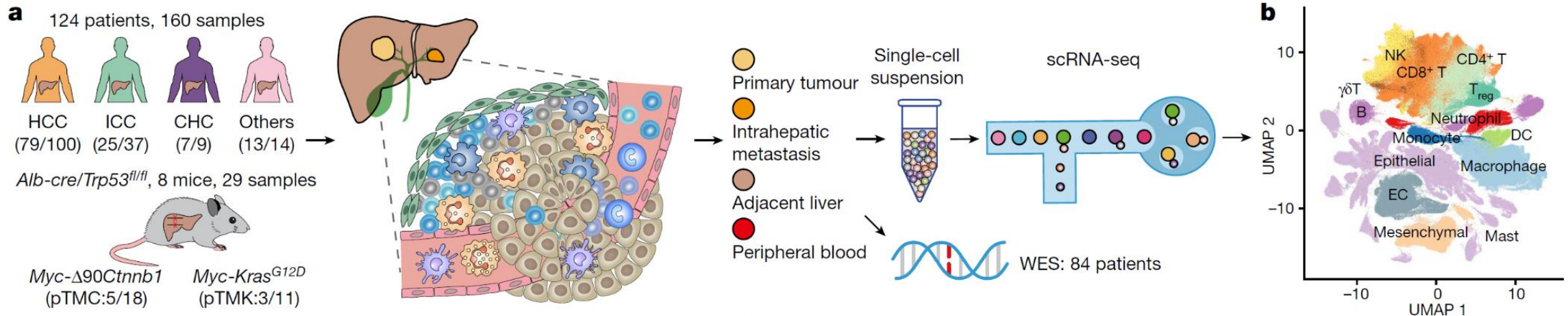
Cell



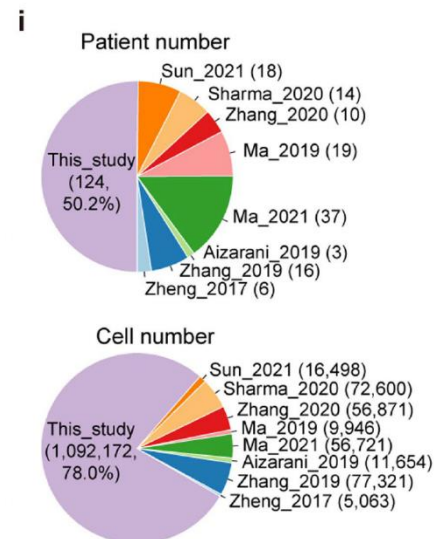
- High TP53 dysfunction in cHCC-ICC
- Low mutational frequency in lineage-related genes in cHCC-ICC
- Q: Nestin highly expressed in cHCC-ICC ?

A large-scale scRNA-seq analysis of 124 PLC patients

Experimental design



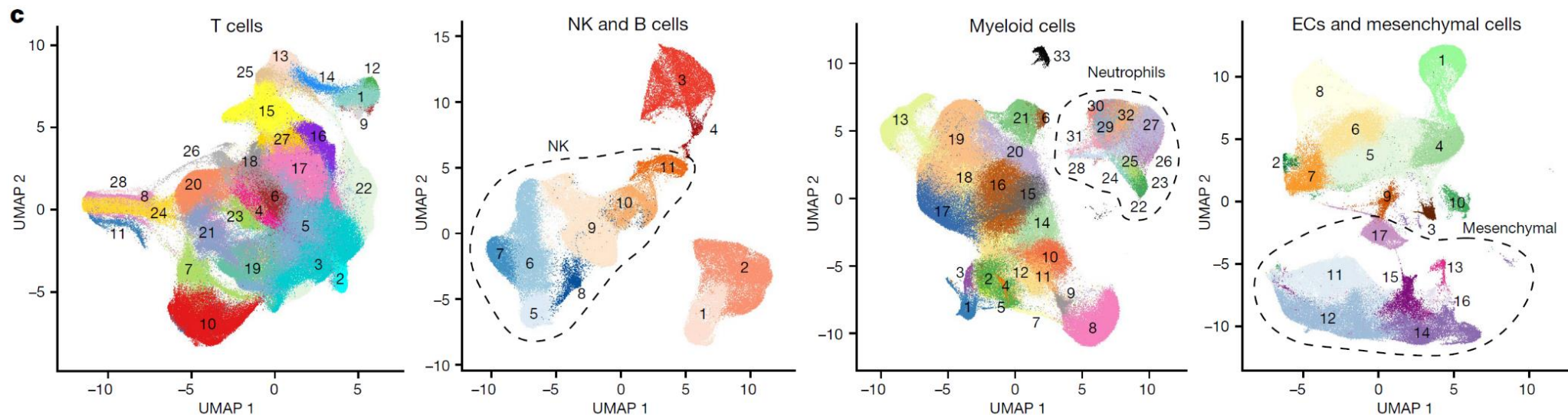
- 124 patients, 160 libraries
 - HCC (79 patients, 100 libraries)
 - ICC (25 patients, 37 libraries)
 - CHC (7 patients, 9 libraries)
 - SLC (9 patients, 10 libraries)
 - Others (4 patients, 5 libraries)
- 8 mice (*Alb-cre/Trp53^{fl/fl}*)
 - Myc-Δ90Ctnnb1*, pTMC
 - Myc-Kras^{G12D}*, pTMK



QC, ~1.1 M high quality cells

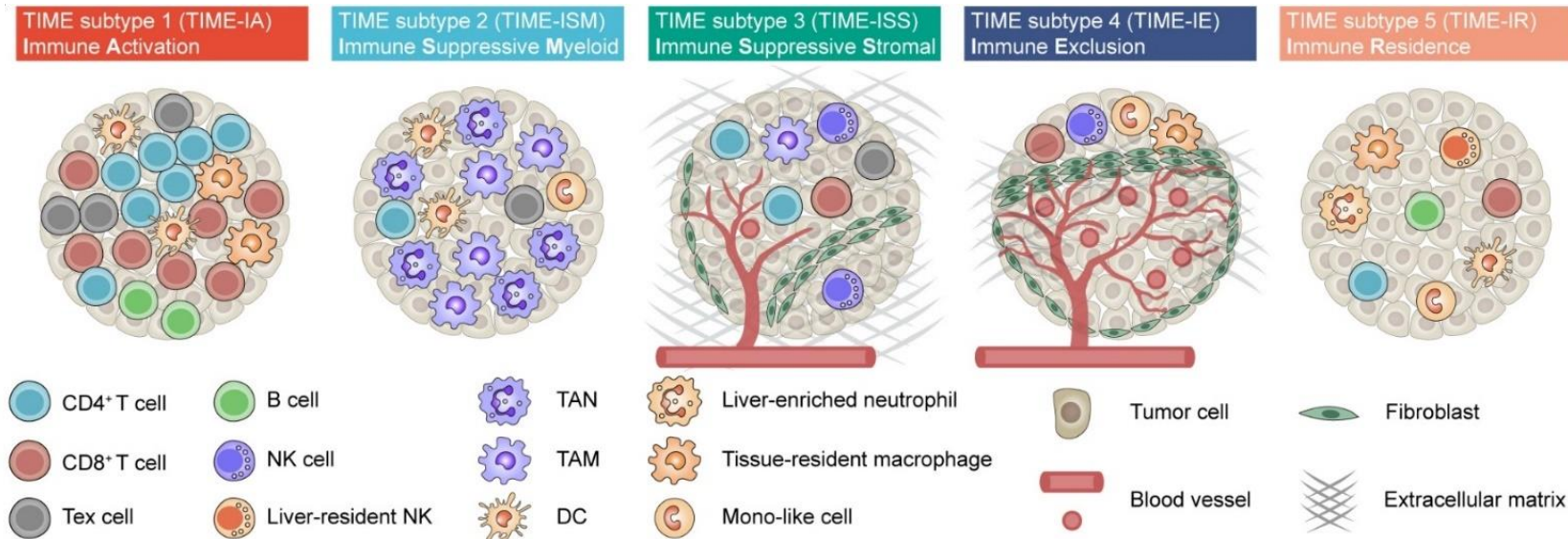
Can we quantitatively deduce the TIME subtypes across PLC and their molecular features?

Decoding 13 major populations further divided into 89 subpopulations of TIME

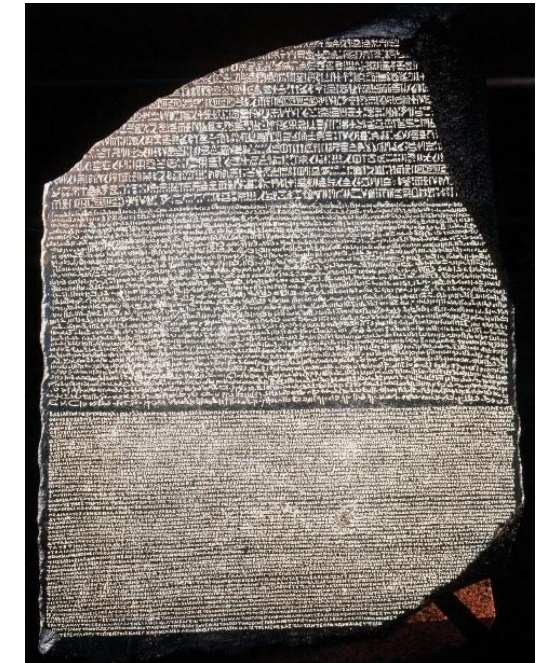


CD4⁺ T cells	15 CD8T_04_GNLY	2 B_02_MS4A1_CD83	6 DC_06_STMN1	21 Mph_09_STMN1	2 EC_02_CLEC4A_APOA2
1 CD4T_01_CCR7	16 CD8T_05_KLRD1	3 B_03_MZB1	7 MonoDC	Neutrophils	3 EC_03_TFF3
2 CD4T_02_SELL	17 CD8T_06_CD69	4 B_04_STMN1	Monocytes	22 Neu_01_MMP8	4 EC_04_ACKR1
3 CD4T_03_GPR183	18 CD8T_07_PLCG2	NK cells	8 Mo_01_CD14	23 Neu_02_S100A12	5 EC_05_KDR
4 CD4T_04_BAG3	19 CD8T_08_GZMK	5 NK_01_FCGR3A_CX3CR1	9 Mo_02_CD16	24 Neu_03_ISG15	6 EC_06_KDR_ESM1
5 CD4T_05_CD69	20 CD8T_09_PDCD1_IFNG	6 NK_02_FCGR3A_CXCR4	Macrophages	25 Neu_04_TXNIP	7 EC_07_KDR_APOA2
6 CD4T_06_PLCG2	21 CD8T_10_PDCD1	7 NK_03_FCGR3A_IFNG	10 Mono-like_01_CD14	26 Neu_05_ELL2	8 EC_08_IGFBP3
7 CD4T_07_CXCL13	22 CD8T_11_SLC4A10	8 NK_04_PLCG2	11 Mono-like_02_CD16	27 Neu_06_PTGS2	9 EC_09_PLCG2
8 CD4T_08_STMN1	23 CD8T_12_IFIT3	9 NK_05_CD160	12 Mono-like_03_CD14_CD16	28 Neu_07_APOA2	10 EC_10_STMN1
T_{reg} cells	24 CD8T_13_STMN1	10 NK_06_ITGA1	13 Mph_01_MARCO	29 Neu_08_CD74	Mesenchymal cells
9 CD4T_09_FOXP3	γδT cells	11 NK_07_STMN1	14 Mph_02_CCL20	30 Neu_09_IFIT1	11 Mu_01_MYH11
10 CD4T_10_FOXP3_CTLA4	25 γδT_01_GNLY_S1PR5	Dendritic cells	15 Mph_03_SPP1	31 Neu_10_SPP1	12 Mu_02_ABCC9
11 CD4T_11_FOXP3_STMN1	26 γδT_02_GNLY	1 DC_01_CLEC9A	16 Mph_04_TREM2	32 Neu_11_CCL4	13 Mu_03_STMN1
CD8⁺ T cells	27 γδT_03_KLRD1	2 DC_02_CD1C	17 Mph_05_IL1B	Mast cells	14 Fb_01_FAP
12 CD8T_01_CCR7	28 γδT_04_STMN1	3 DC_03_LAMP3	18 Mph_06_CXCL9	33 Mast	15 Fb_02_CXCL12
13 CD8T_02_CX3CR1	B cells	4 DC_04_CD207	19 Mph_07_SLC40A1	Endothelial cells	16 Fb_03_KLF4
14 CD8T_03_GZMK_S1PR1	1 B_01_MS4A1	5 DC_05_LILRA4	20 Mph_08_APOE	1 EC_01_CLEC4A	17 Fb_04_FABP4

Molecular features of TIMELASER



Rosetta stone



Features

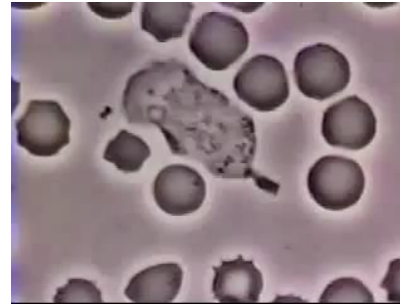
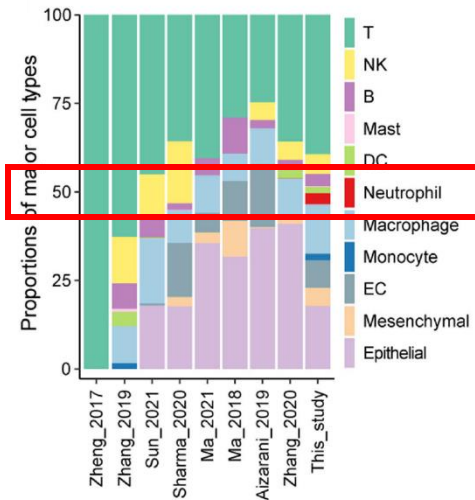
- Cancer type enrichment
- Chemokine network
- Driver mutations
- Patient prognosis
- Spatial organization

Therapeutic relevance:

- TIME-IE, aPD-L1+aVEGF
- TIME-ISS, anti-CAF
- TIME-ISM, anti-TAM, anti-TAN

- Bulk data
- scRNA-seq data
- Spatial data

First identification of neutrophils in scRNA-seq of liver cancer



- ~70% of all peripheral leukocytes in humans
- Granules, an **arsenal** of cytotoxic factors
- First responders to infection and inflammation, a critical component of innate immune defense

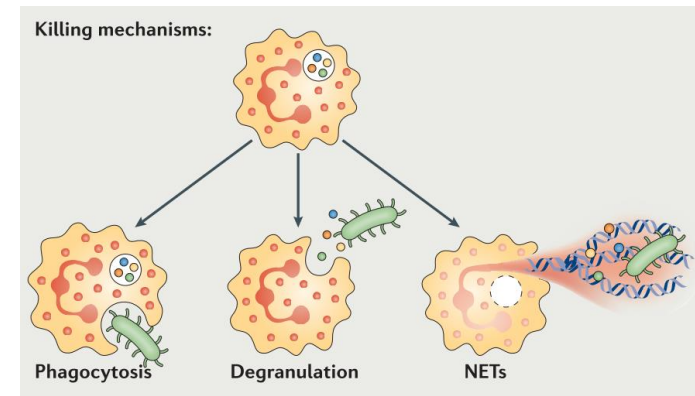
REVIEWS

Single-cell technologies in hepatology: new insights into liver biology and disease pathogenesis

Granulocytes

The term granulocytes refers to white blood cells that contain granules in their cytoplasm. Neutrophils^{82,83}, eosinophils⁸⁴, basophils⁵¹ and mast cells⁸⁵ have all been associated with liver injury and repair in different contexts. However, granulocytes often have low RNA content and contain large quantities of RNases, making scRNAseq of granulocytes challenging, especially when using high-throughput droplet-based approaches. Hence, granulocyte populations are often

Ramachandran, *Nature Reviews G&H*, 2020



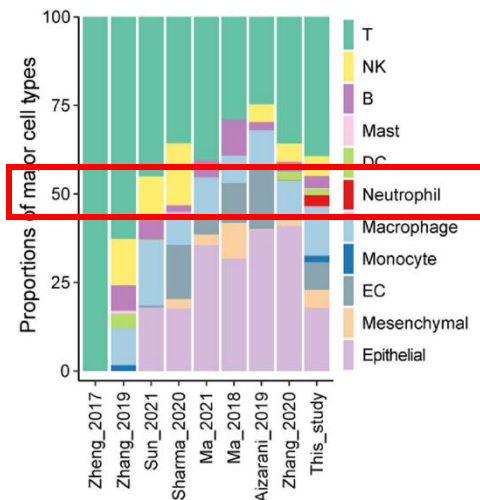
Paul Kubes. *Nature Reviews Immunology*, 2013

- **Fragile and short-lived**
- **Low RNA content**

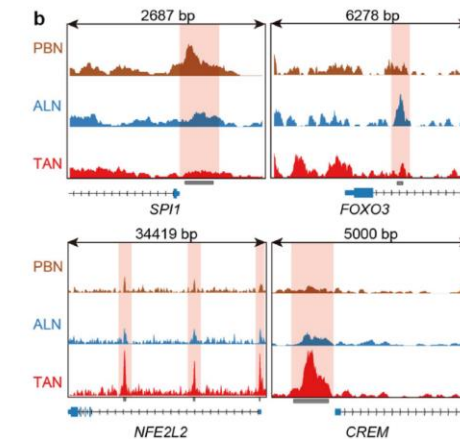
Improvement

- **Experiment:** quick and no Ab-enrichment
- **Analysis:** custom cutoff (UMI > 200)
- 34,307 neutrophils

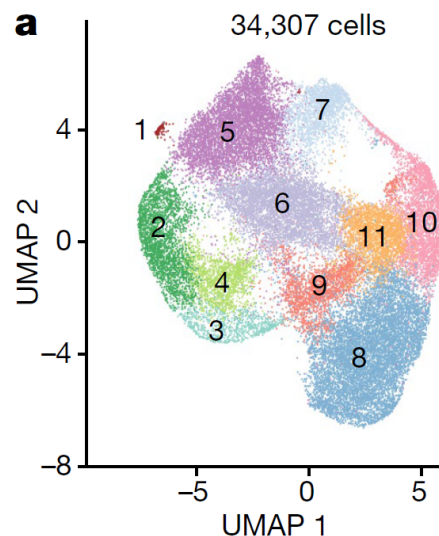
11 subsets of neutrophils



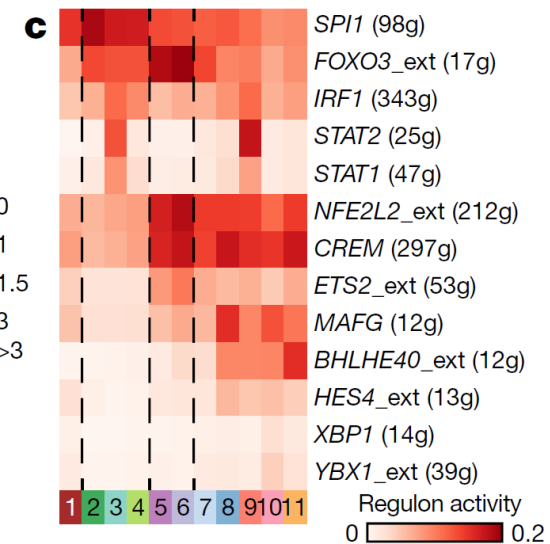
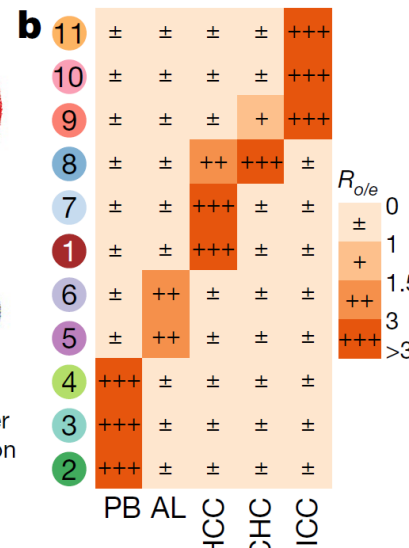
- 11 clusters enriched in PB, AL and tumor
- 6 clusters of TANs
- A clear tissue separation and differentiation order
- Key TF for each subset



ATAC-seq validation

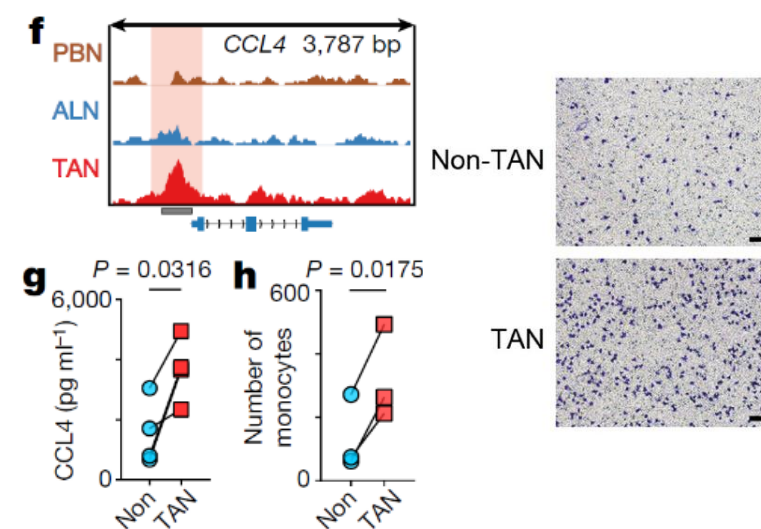
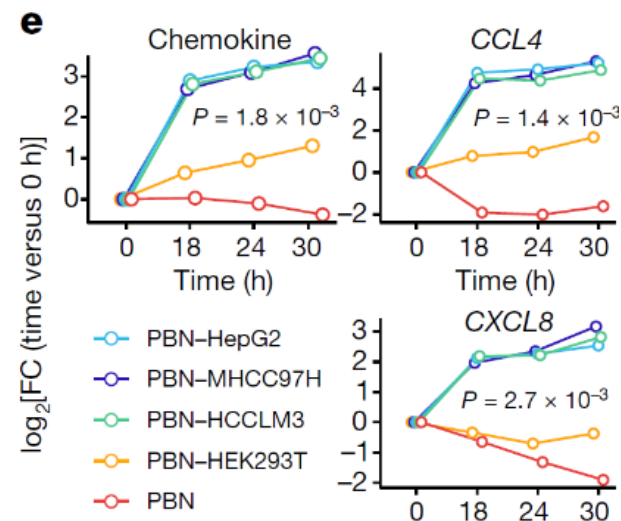
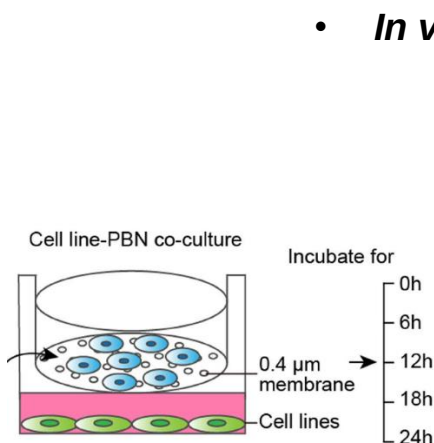
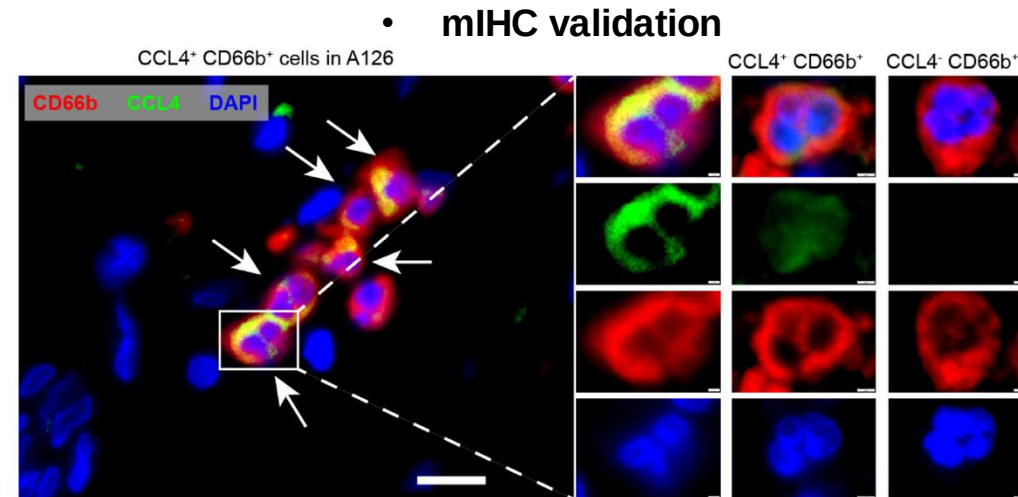
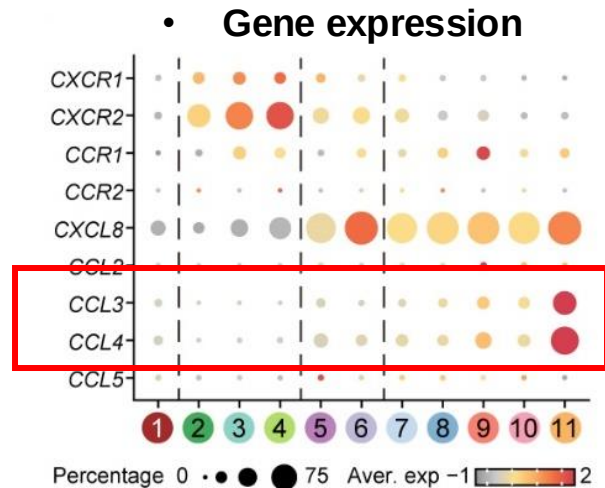


- 1 Neu_01_MMP8
- 2 Neu_02_S100A12
- 3 Neu_03_ISG15
- 4 Neu_04_TXNIP
- 5 Neu_05_ELL2
- 6 Neu_06_PTGS2
- 7 Neu_07_APOA2
- 8 Neu_08_CD74
- 9 Neu_09_IFIT1
- 10 Neu_10_SPP1
- 11 Neu_11_CCL4



CCL4⁺ TAN and PD-L1⁺ TAN for functional validation

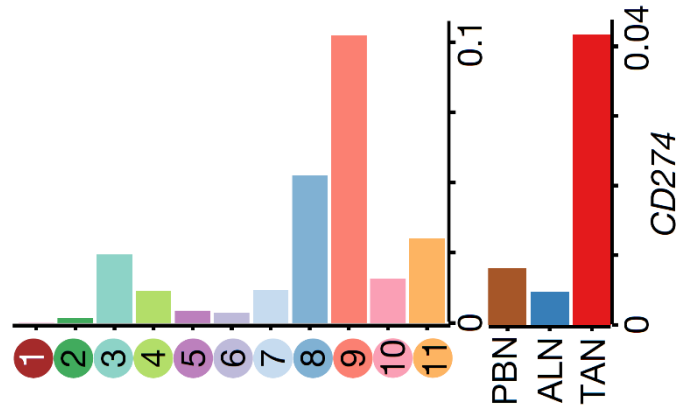
CCL4⁺ TAN secretes CCL4 to recruit macrophage



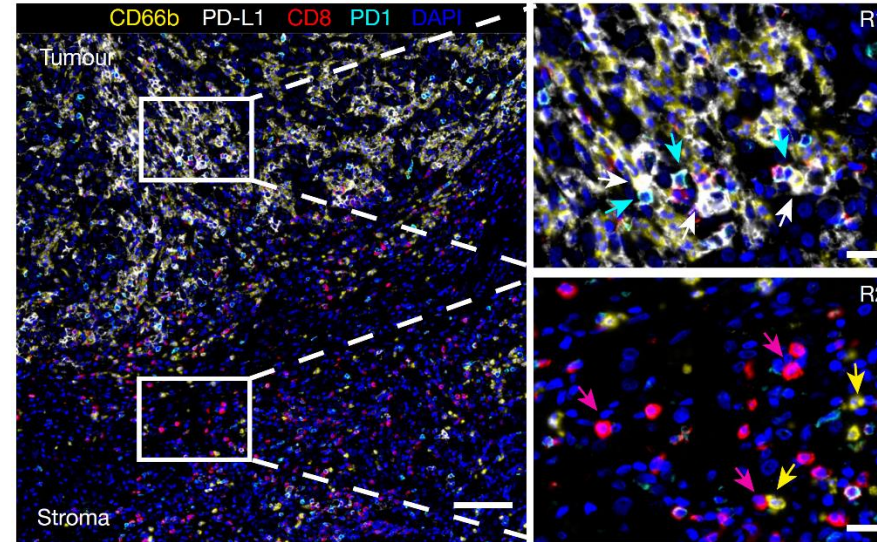
PD-L1⁺ TAN suppresses CD8⁺ T cell

- Gene expression

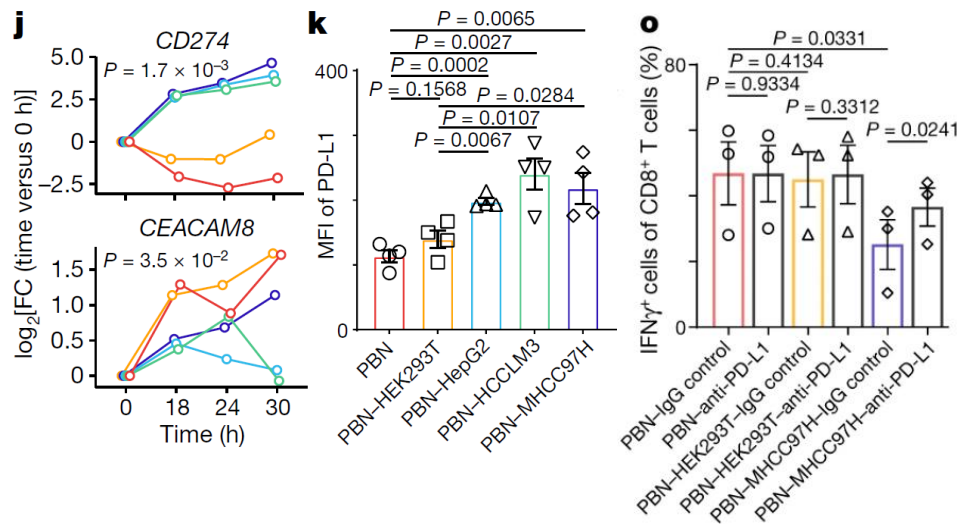
CD274 (encoding PD-L1)



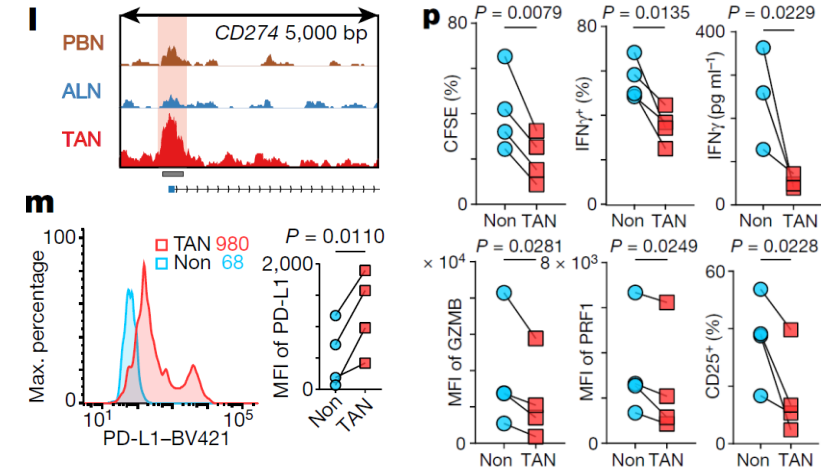
- mIHC validation



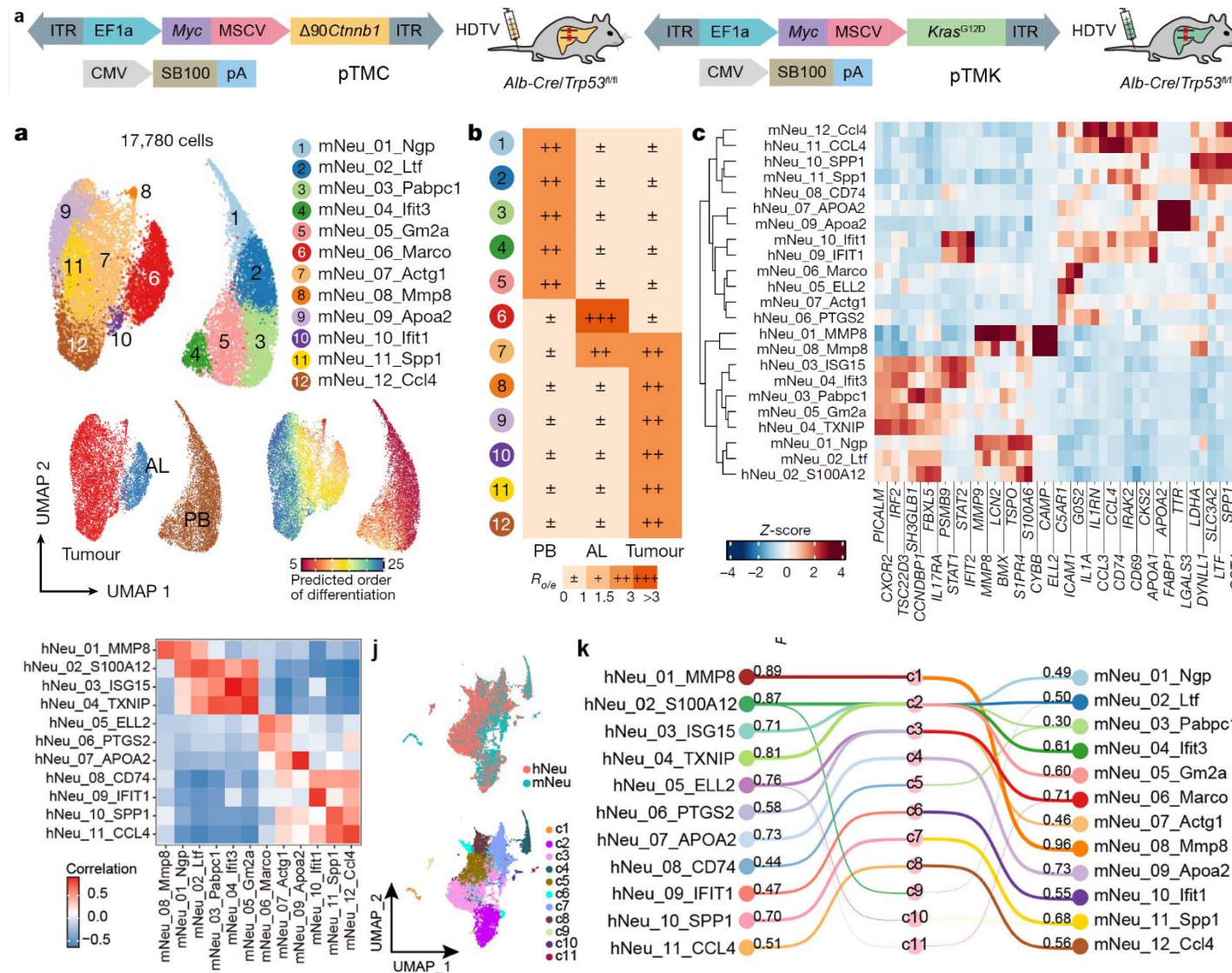
- In vitro* induced TANs



- Ex vivo* patient-derived TANs



Conserved neutrophil subsets in human and mouse liver cancer



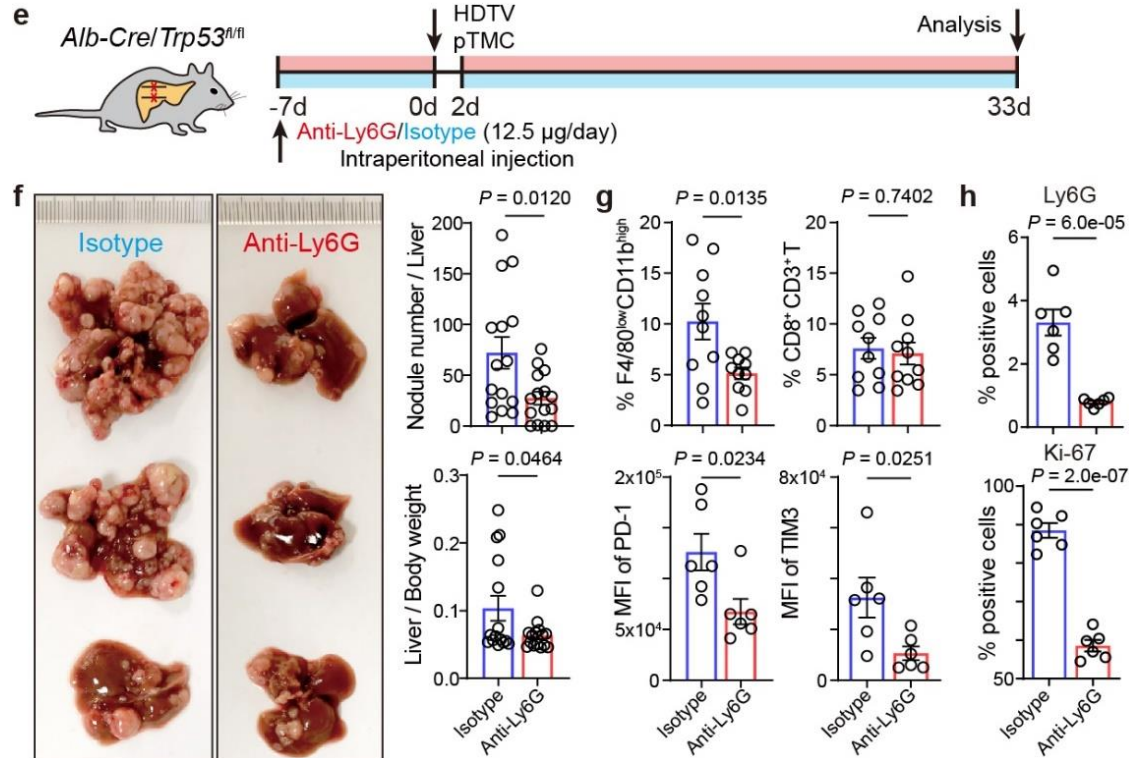
- two new spontaneous liver cancer mouse models

- Neutrophils in mice

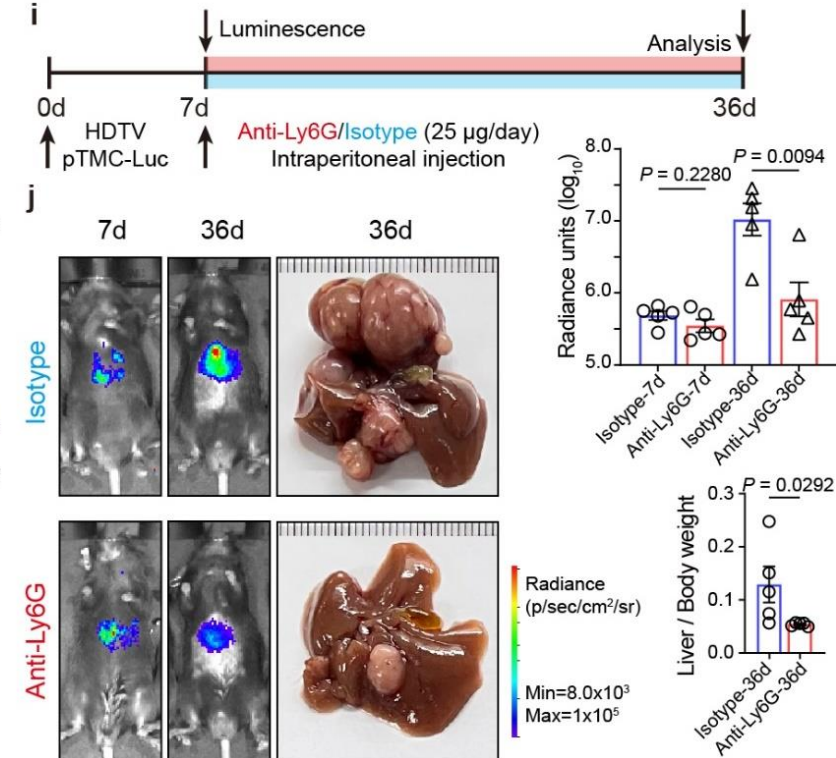
- Cross-species data integration
- Good in vivo model

Neutrophil depletion *in vivo* attenuates tumour progression

- Before HDTV



- After tumor growth



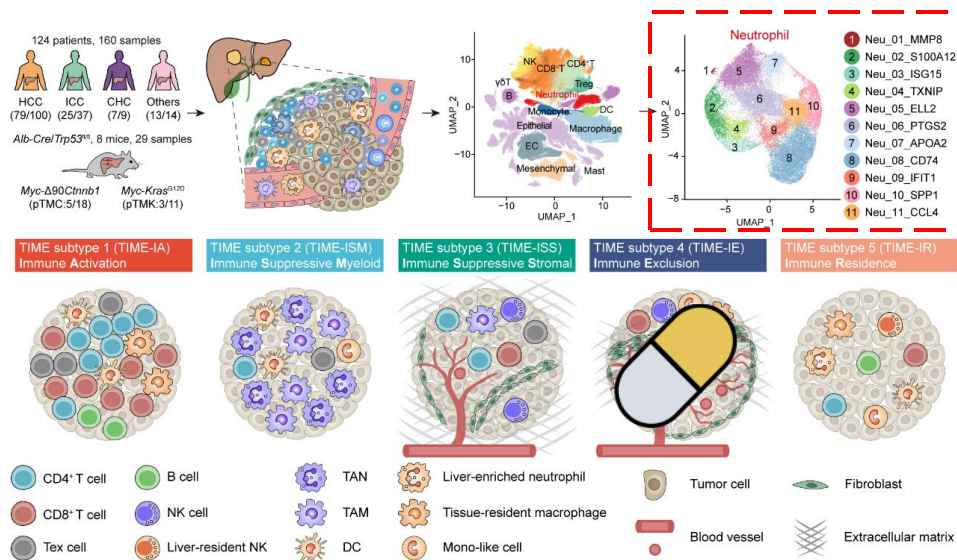
- attenuate macrophage recruitment and T cell suppression, resulting in tumour inhibition

Summary

Article

Liver tumour immune microenvironment subtypes and neutrophil heterogeneity

Ruidong Xue^{1,7}, Qiming Zhang^{2,7}, Qi Cao^{1,7}, Ruirui Kong^{1,7}, Xiao Xiang^{3,7}, Hengkang Liu¹, Mei Feng¹, Fangyanni Wang¹, Jinghui Cheng¹, Zhao Li³, Qimin Zhan⁴, Mi Deng⁴, Jiye Zhu^{3,8}, Zemin Zhang^{2,5,8} & Ning Zhang^{1,4,6,8}

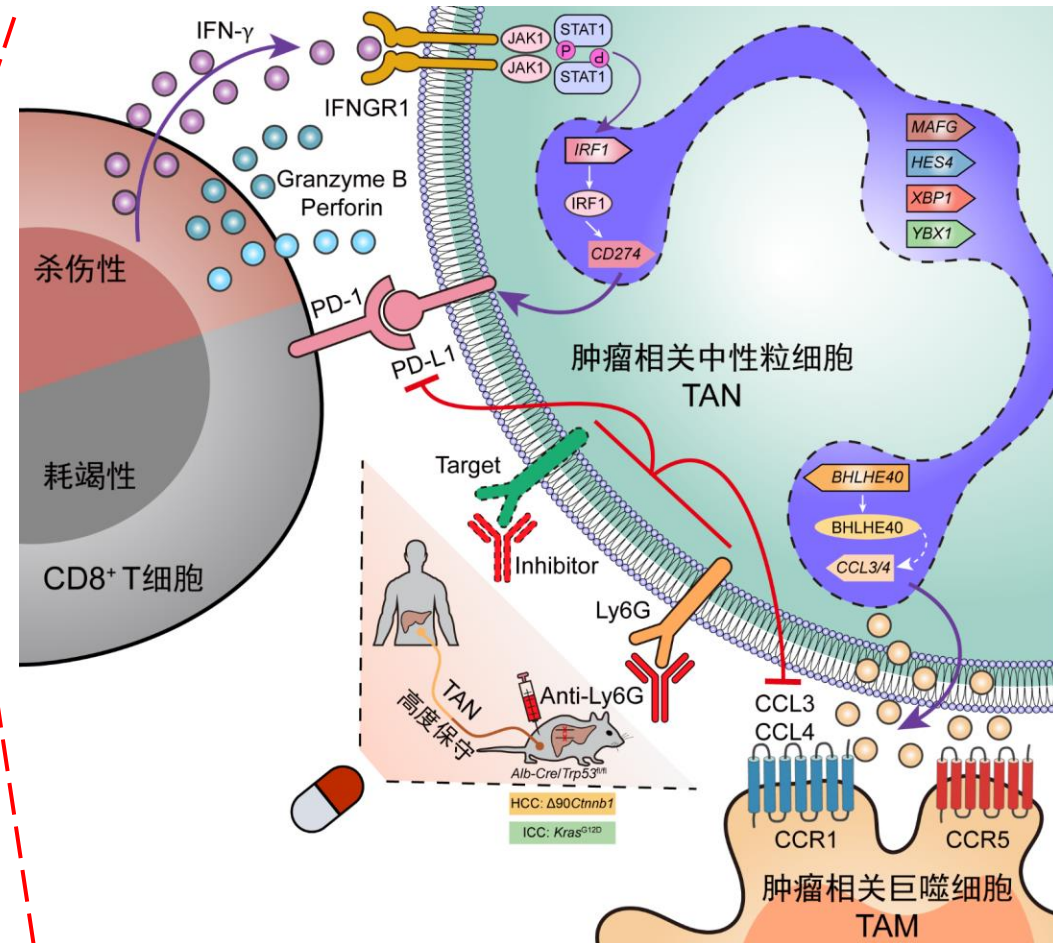


TIMELASER

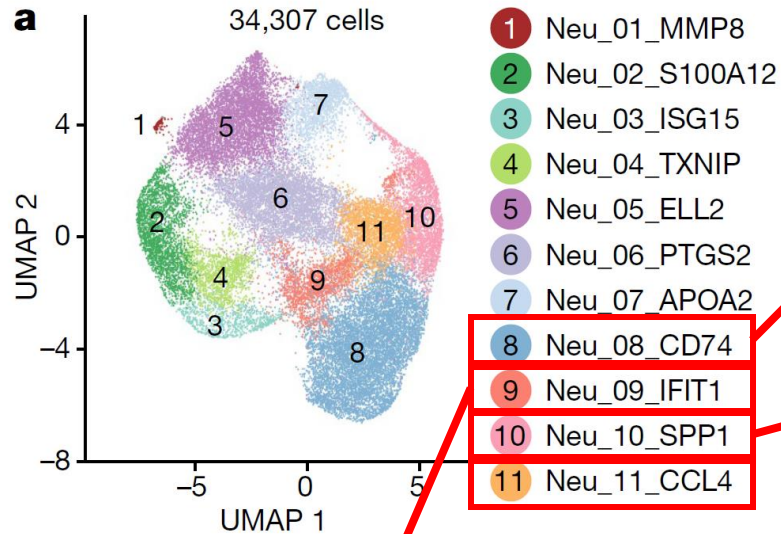
- Distinctive features
- Suit for different therapies

TAN

- Different pro-tumor functions
- Neutrophil based therapies



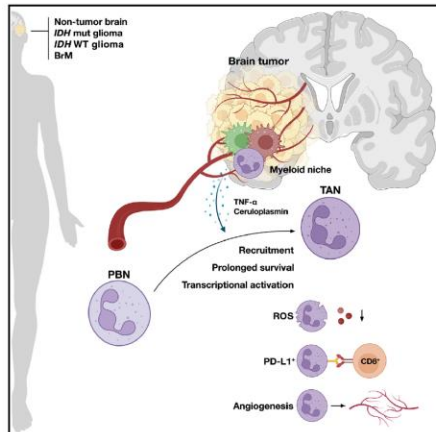
Our TAN subsets are validated in multiple cancer types



Cell

The local microenvironment drives activation of neutrophils in human brain tumors

Graphical abstract



Authors

Roeltje R. Maas, Klara Soukup,
Nadine Fournier, ..., Roy T. Daniel,
Monika E. Hegi, Johanna A. Joyce

Correspondence

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In brief

Comprehensive analyses of brain tumor-associated neutrophils in humans and mice reveal a multifaceted pro-tumoral phenotype, which is induced by soluble factors secreted by tumor-associated myeloid cells.

Article

Cell

Neutrophil profiling illuminates anti-tumor antigen-presenting potency

RESEARCH ARTICLE SUMMARY

CANCER IMMUNOLOGY

Deterministic reprogramming of neutrophils within tumors

Cell

T cell immunotherapies engage neutrophils to eliminate tumor antigen escape variants

Cell

A neutrophil response linked to tumor control in immunotherapy

Article

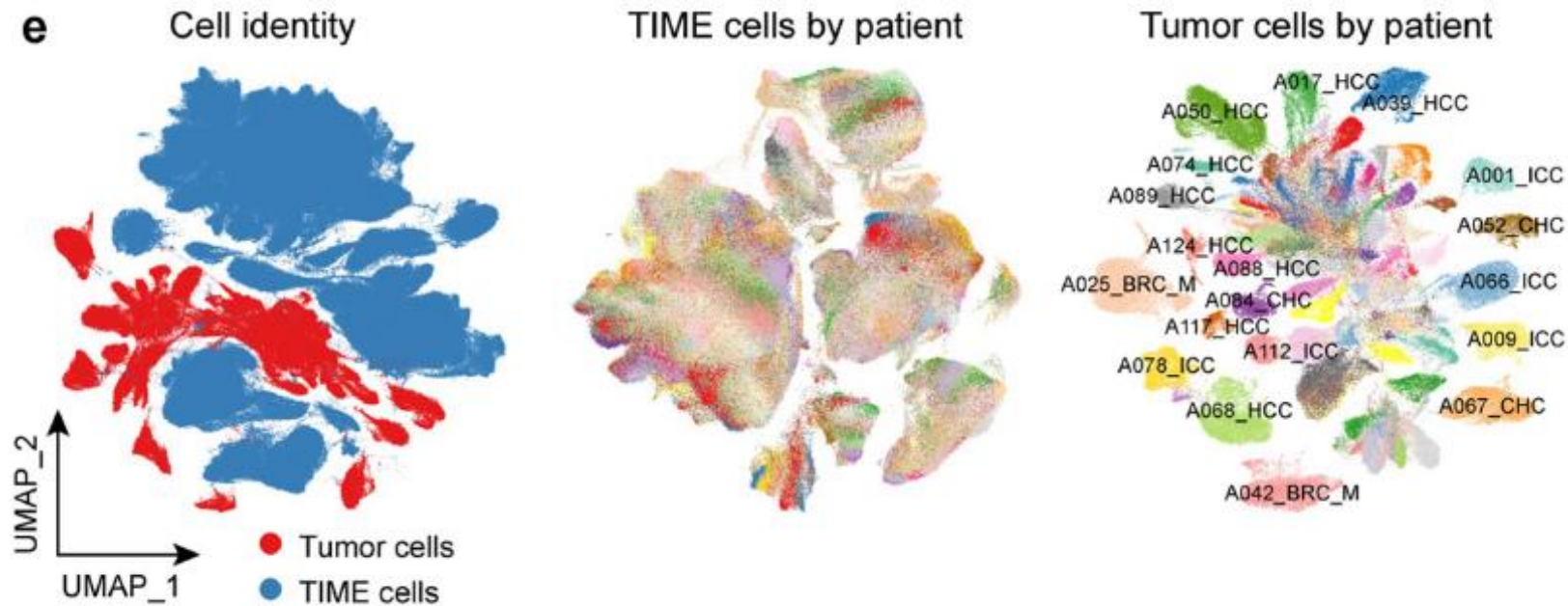
CD300ld on neutrophils is required for tumour-driven immune suppression

Article

Article

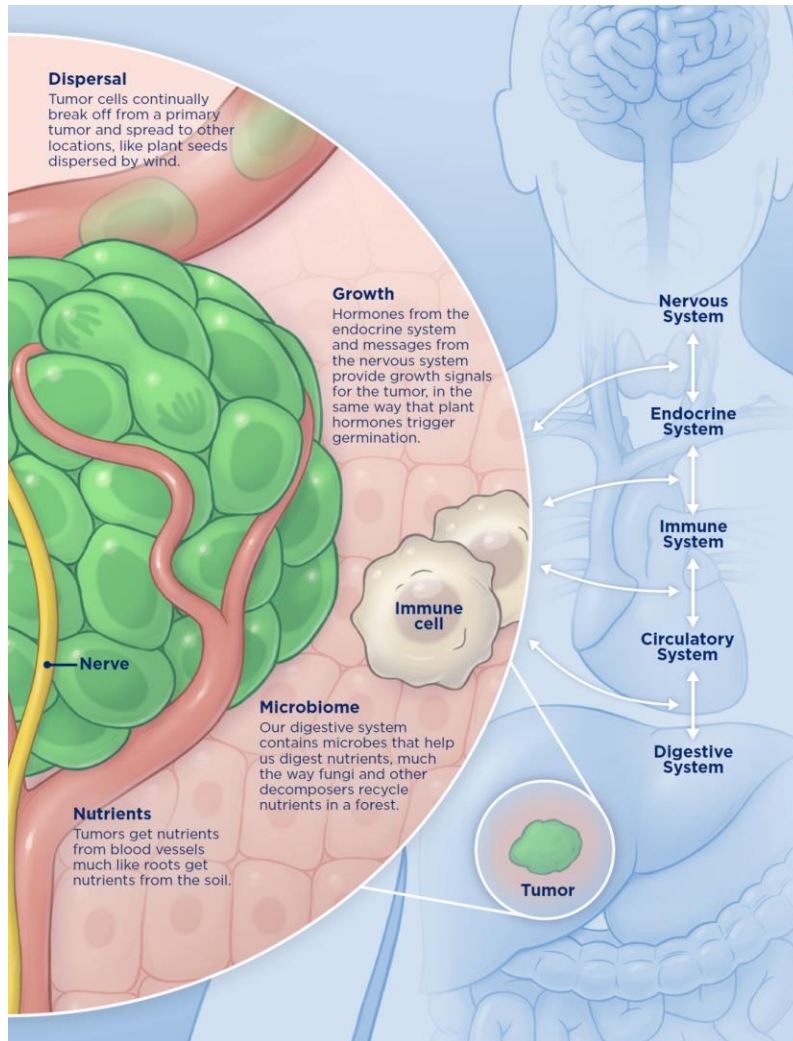
Article

The heterogeneity of tumor cells on the transcriptomic level



Driver mutations (KRAS and IDH1/2) and neutrophils

Outline

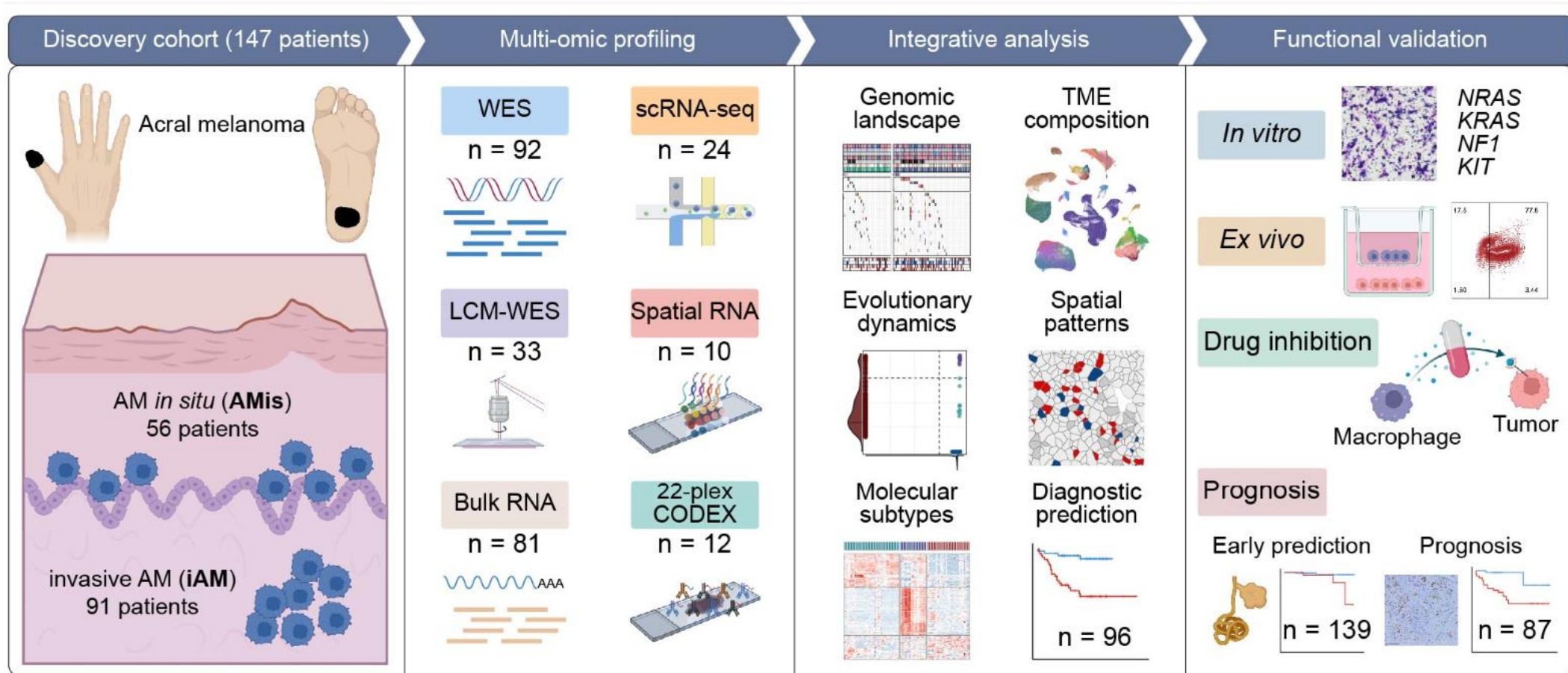


➤ Tumor clonal evolution

➤ Tumor immune microenvironment (TIME)

➤ Interplay between tumor and TIME

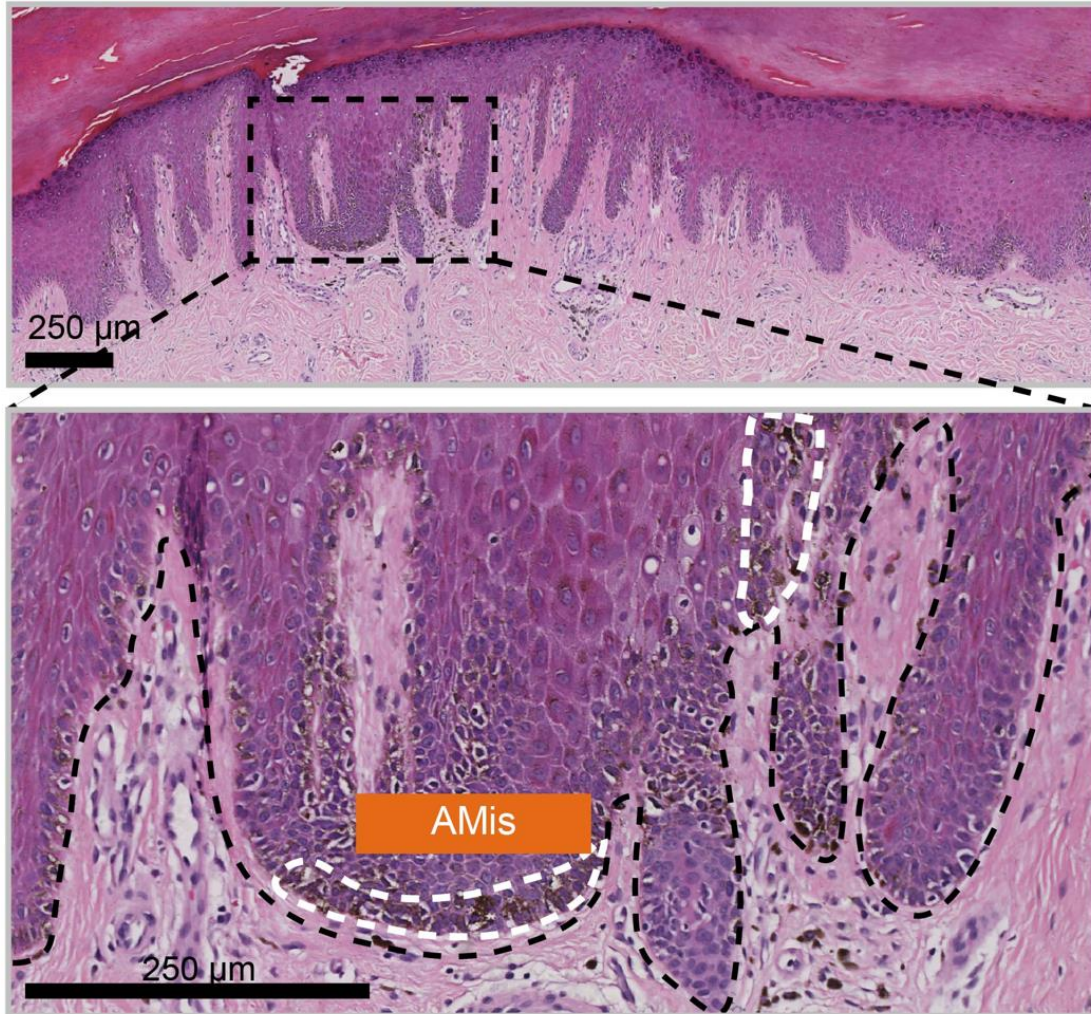
An early stage tumor model——Acral melanoma



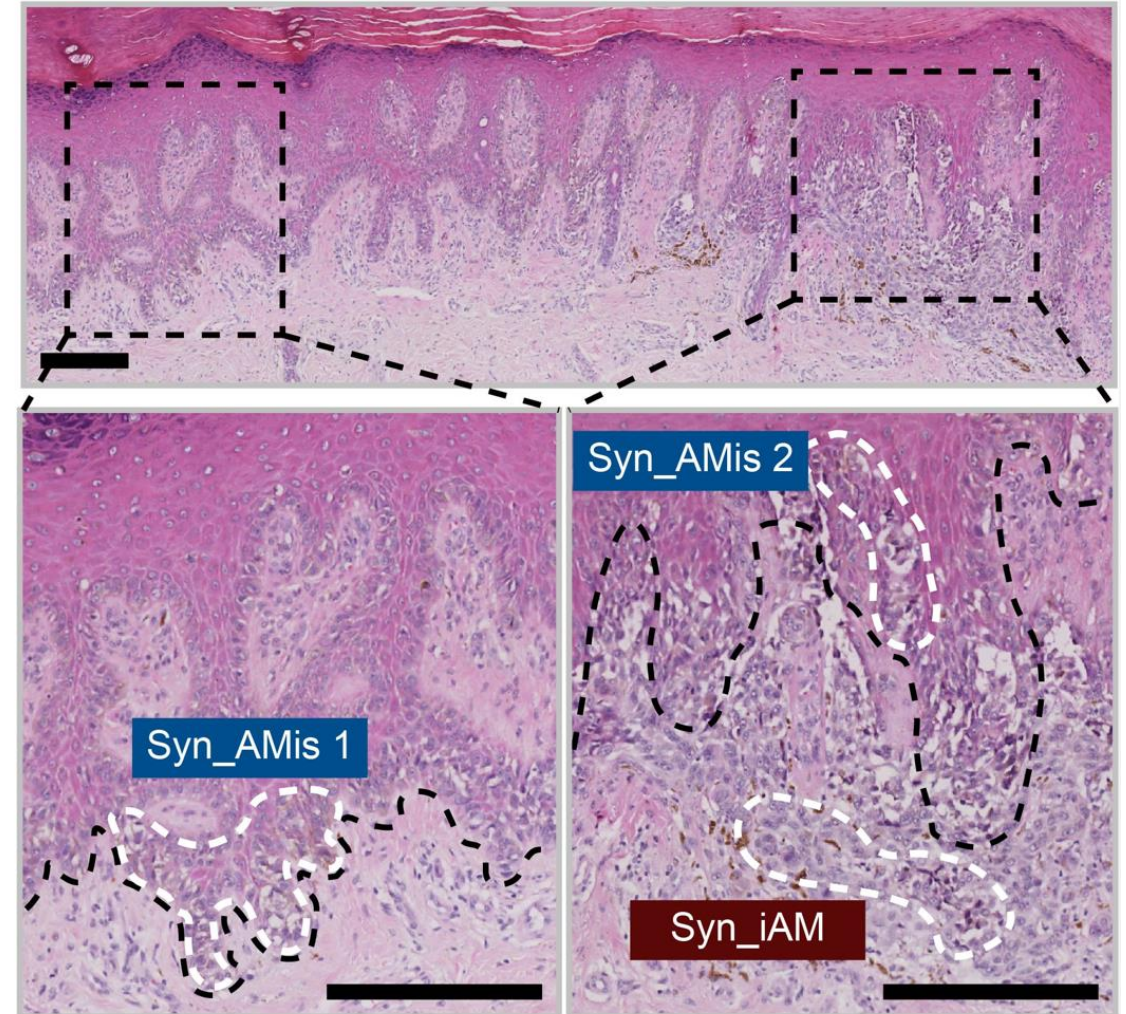
The most frequent type of melanoma in Chinese population
A good model to investigate the tumor & TME cooperation

An early stage tumor model——Acral melanoma

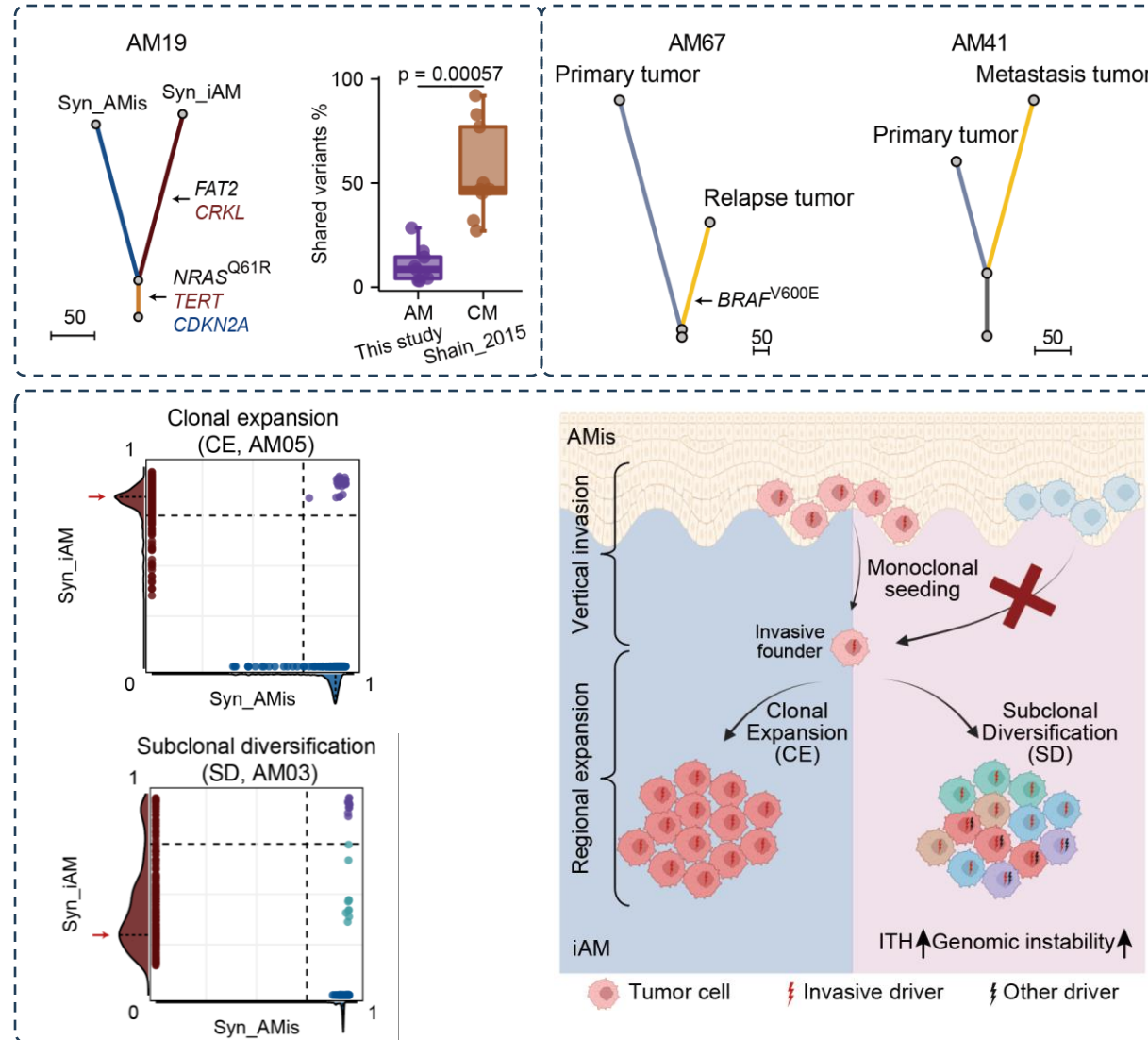
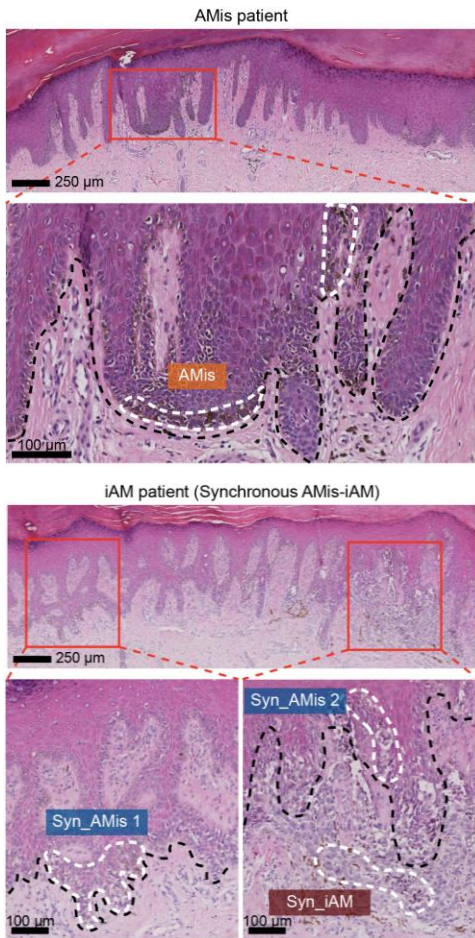
AMis patient



iAM patient (Synchronous AMis-iAM)



Two distinct evolutionary patterns during regional expansion

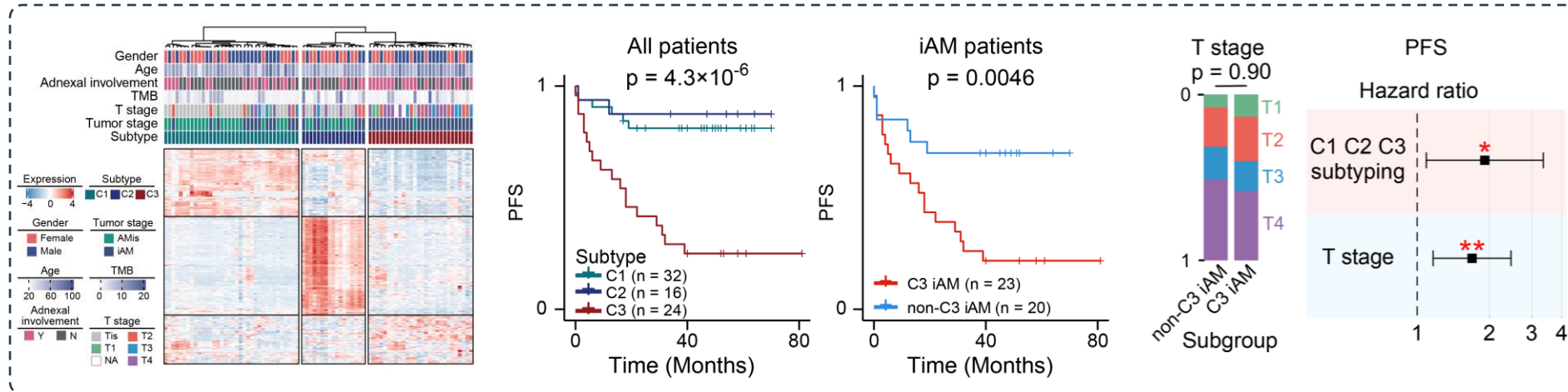


Early seeding

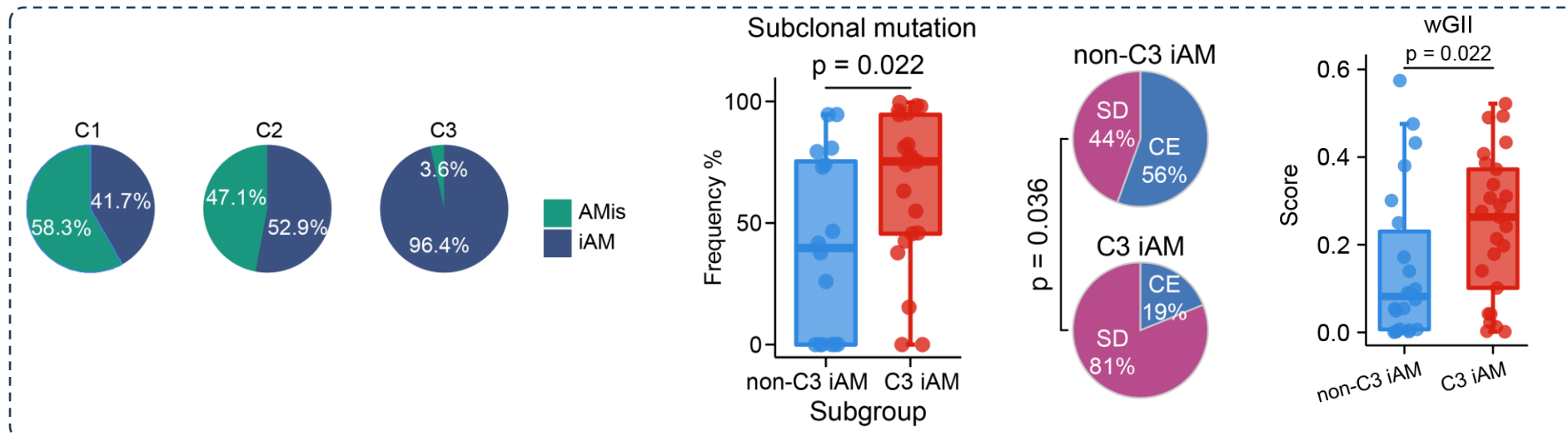
Two patterns

Three molecular subtypes of AM

C3 shows the worst prognosis.

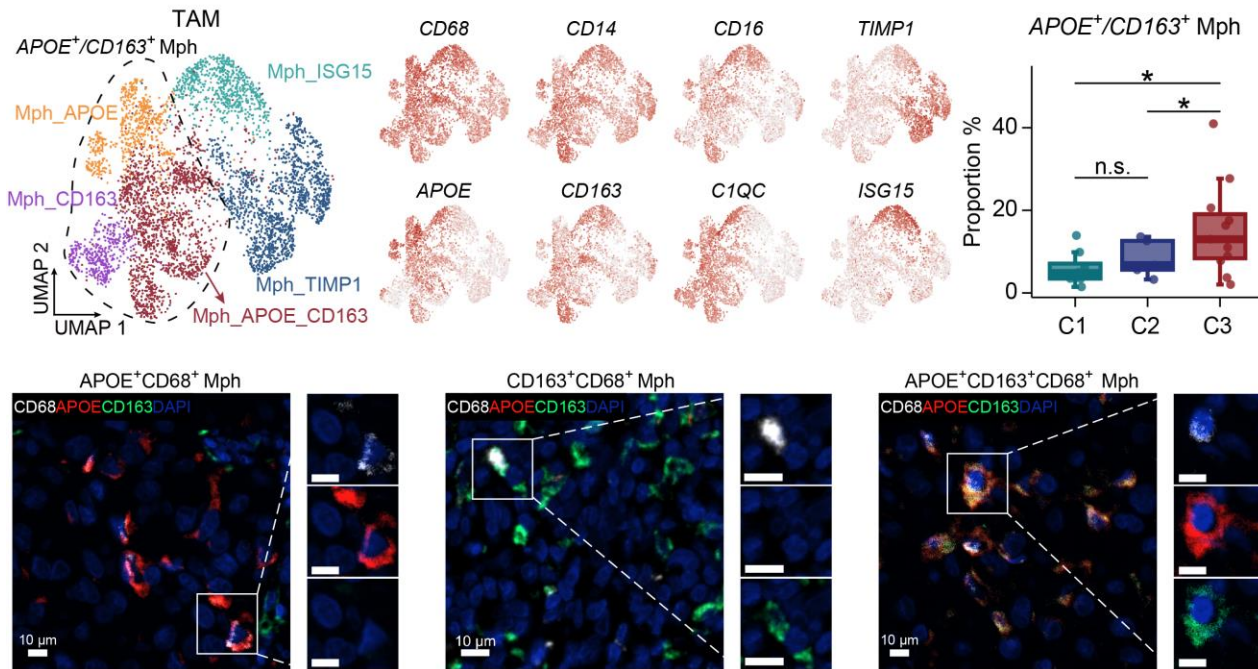


C3: subclonal diversification, high ITH, high genomic instability

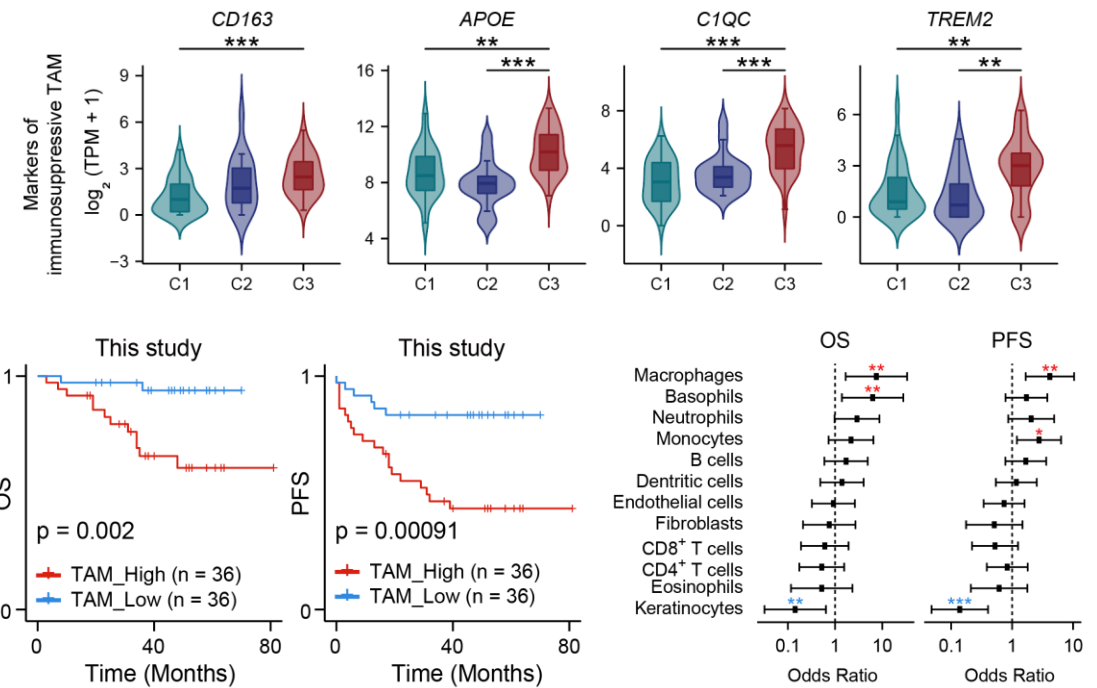


$APOE^+/CD163^+$ macrophages are enriched in the C3 subtype

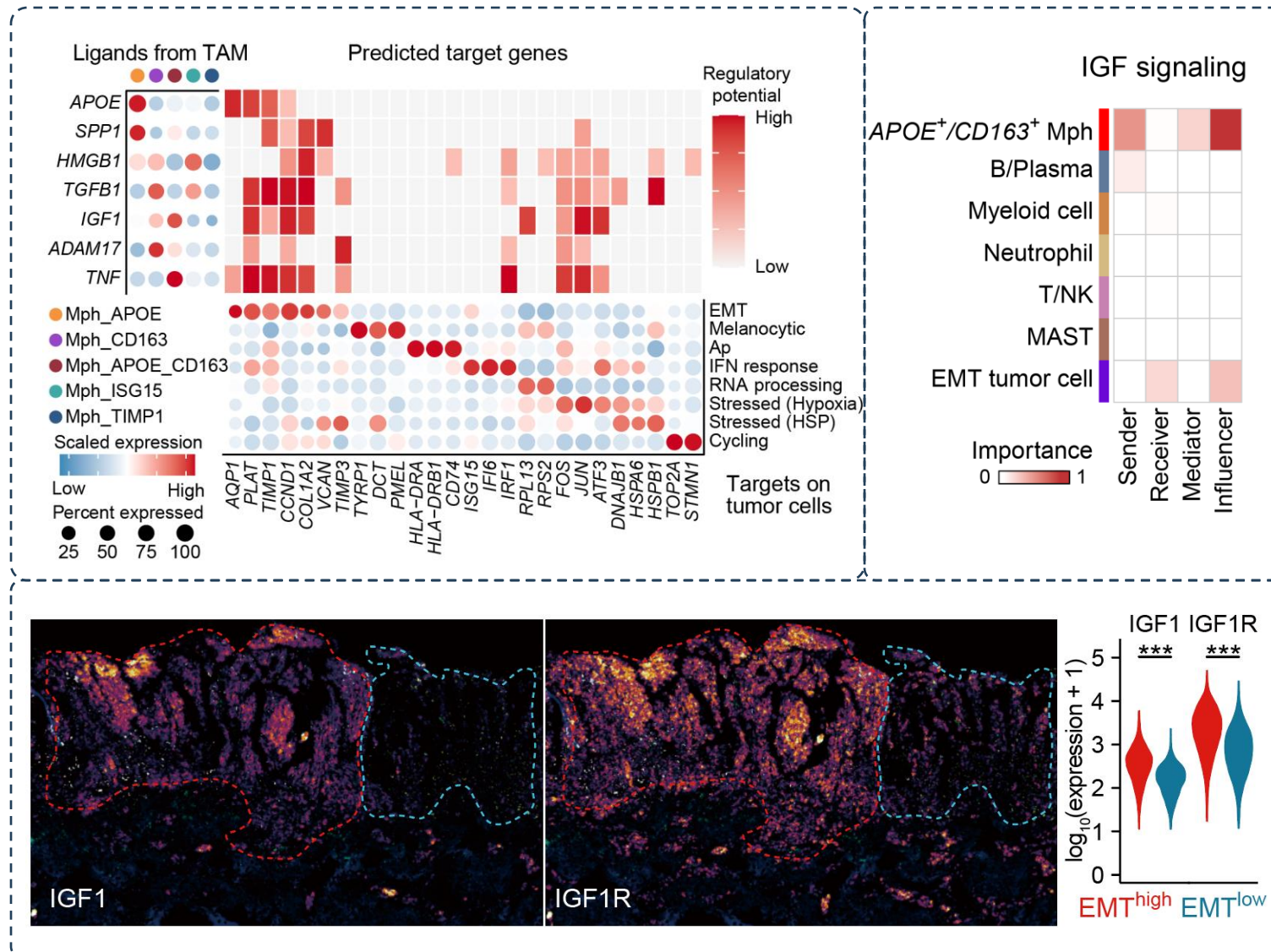
Single cell level



Bulk tissue level



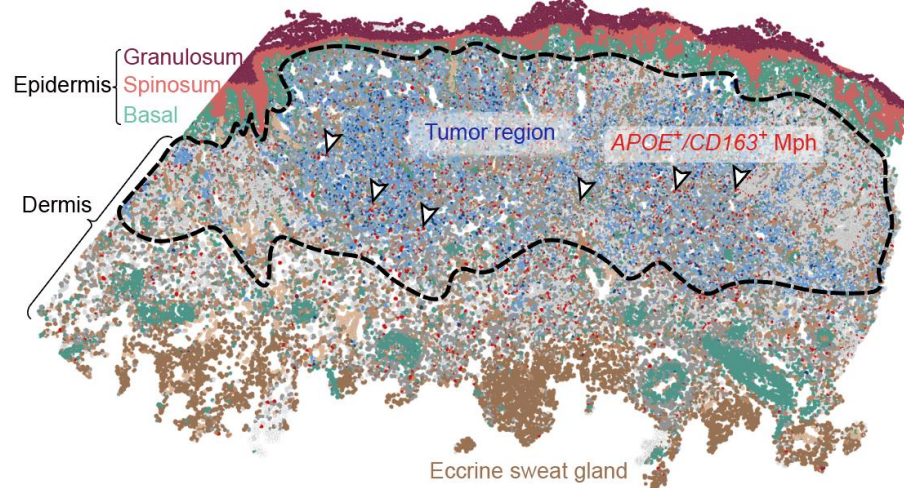
APOE⁺CD163⁺ TAMs promote tumor EMT via IGF1-IGF1R interaction



Spatial analyses confirms the direct and close interaction between mph and EMT

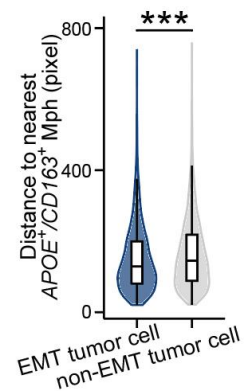
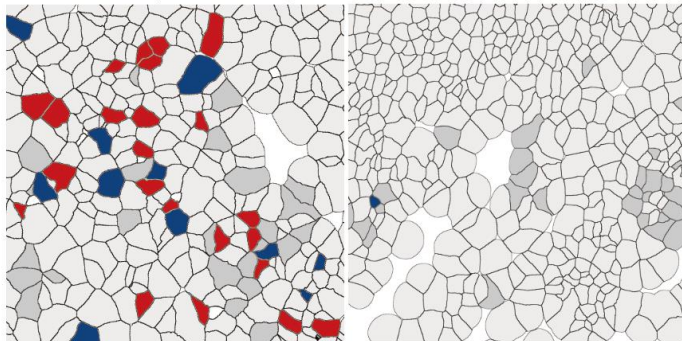
Spatial transcriptome

AM140 (high $APOE^+/CD163^+$ Mph infiltration)



EMT^{high} (AM140, C3)

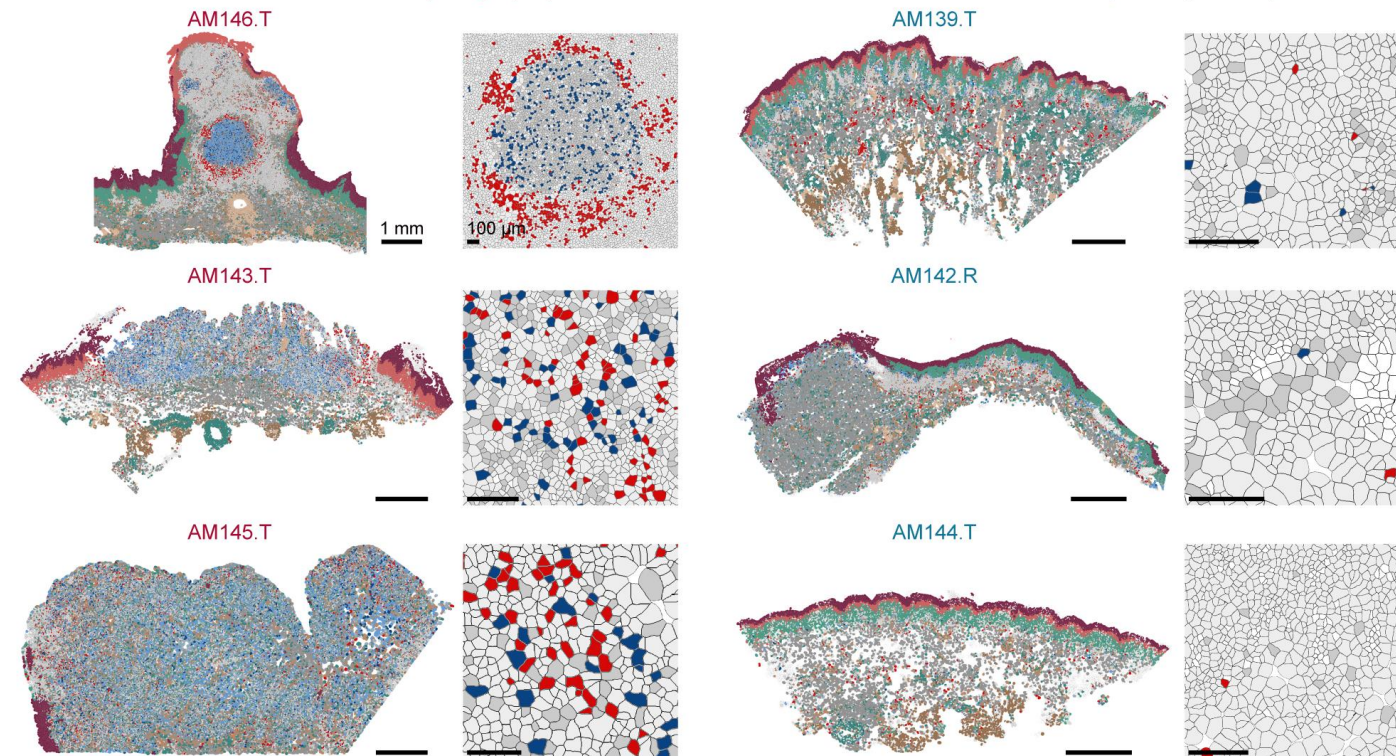
EMT^{low} (AM147, non-C3)



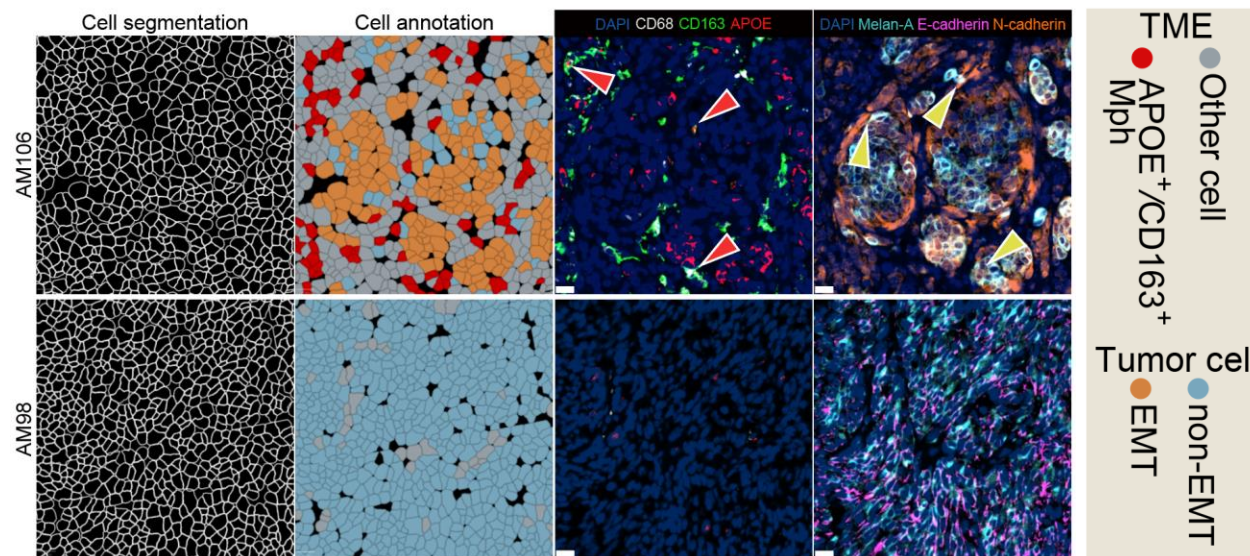
A

EMT^{high}; $APOE^+/CD163^+$ Mph high (C3)

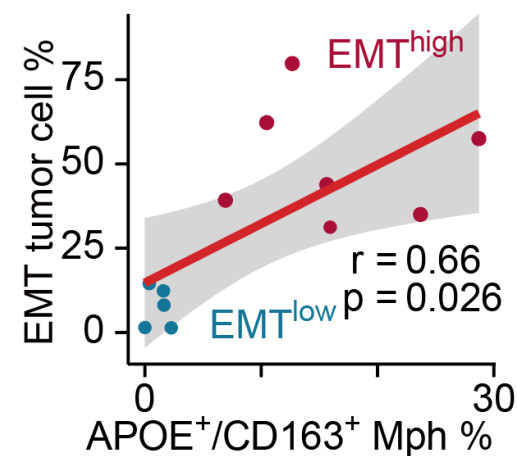
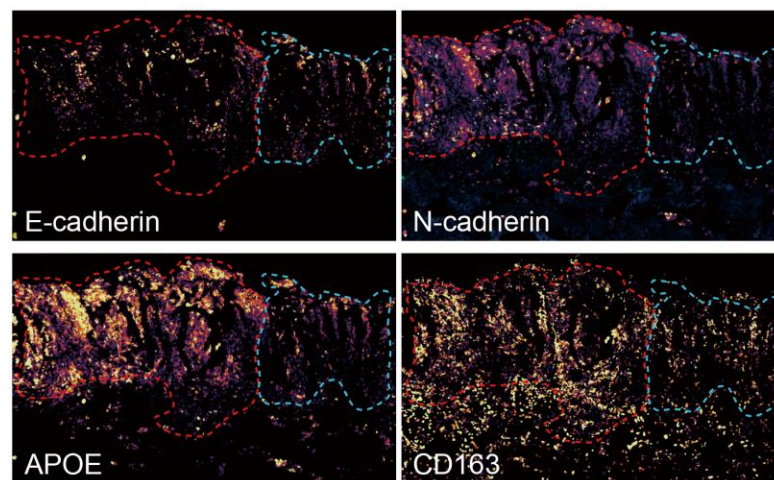
EMT^{low}; $APOE^+/CD163^+$ Mph low (non-C3)



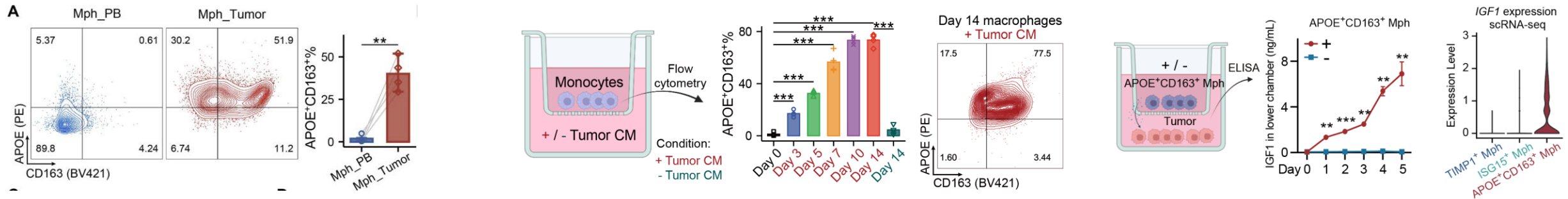
Spatial analyses confirm the direct and close interaction between mph and EMT



Spatial proteome



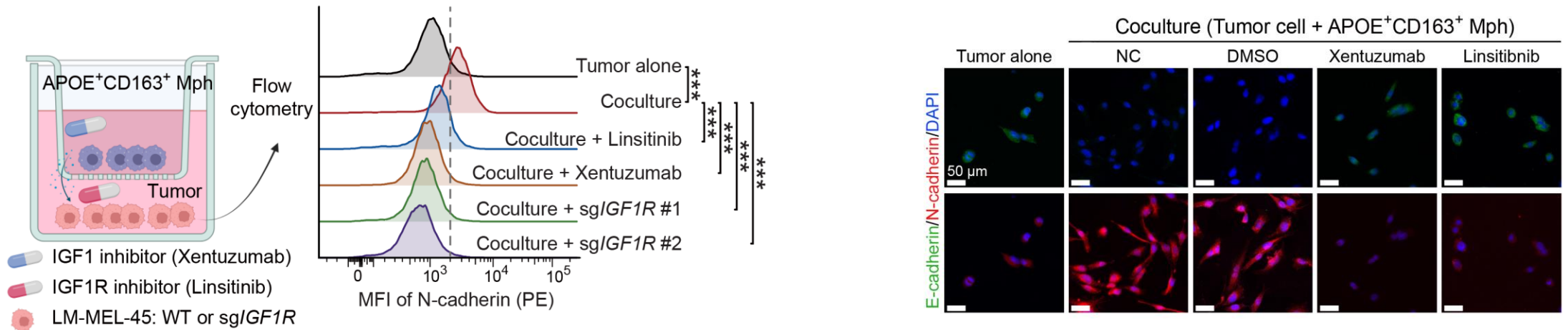
APOE⁺CD163⁺ TAMs promote tumor EMT via IGF1-IGF1R interaction



✓ Ex vivo

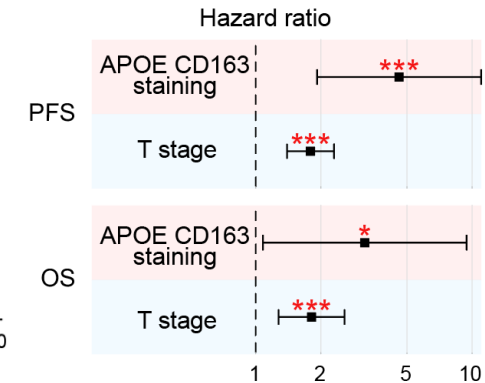
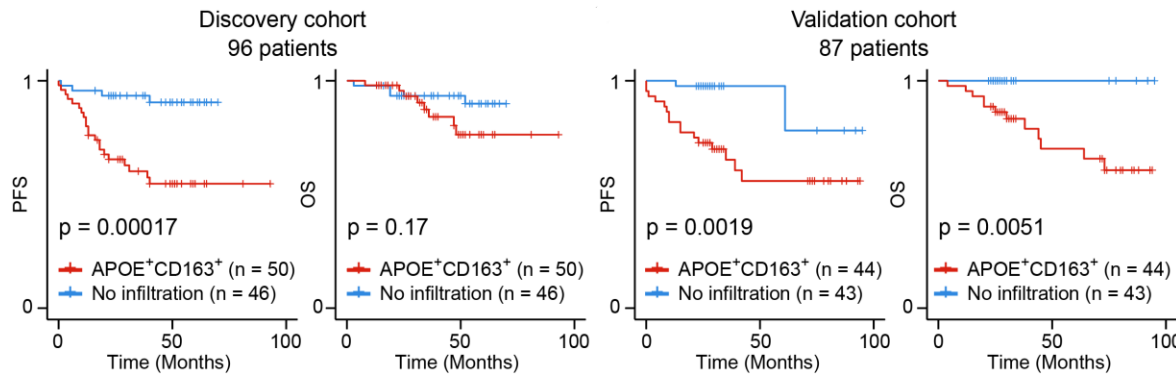
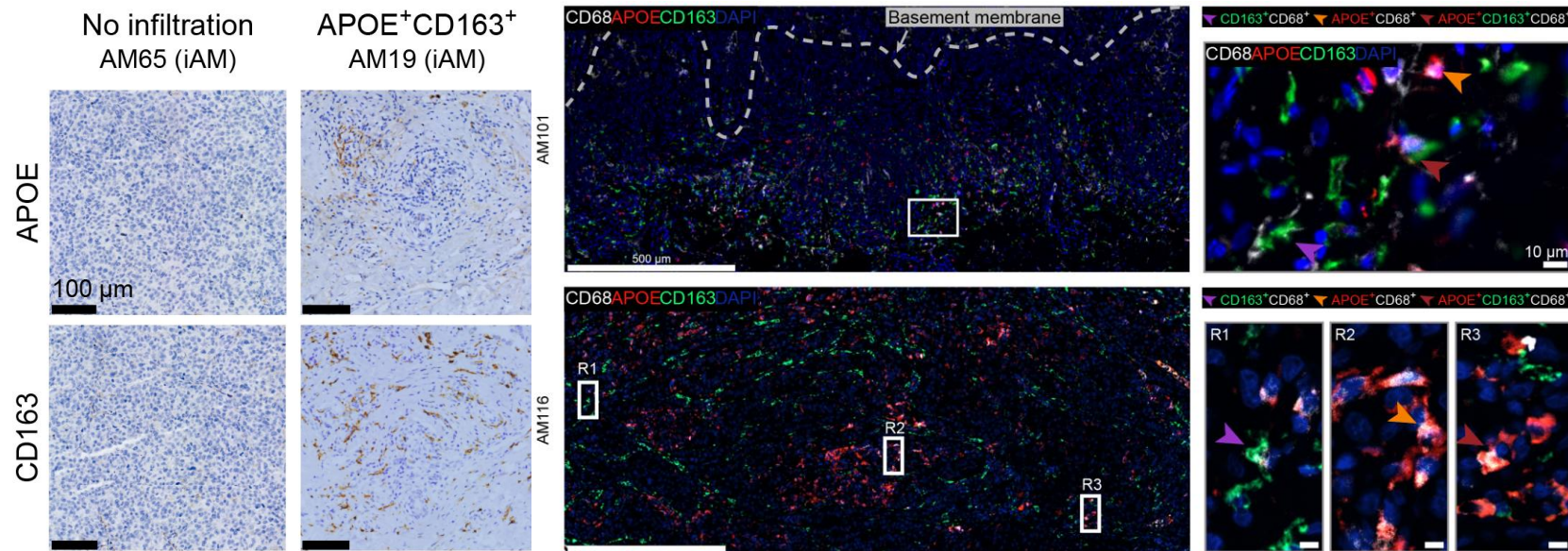
✓ Co-culture

✓ IGF1- IGF1R

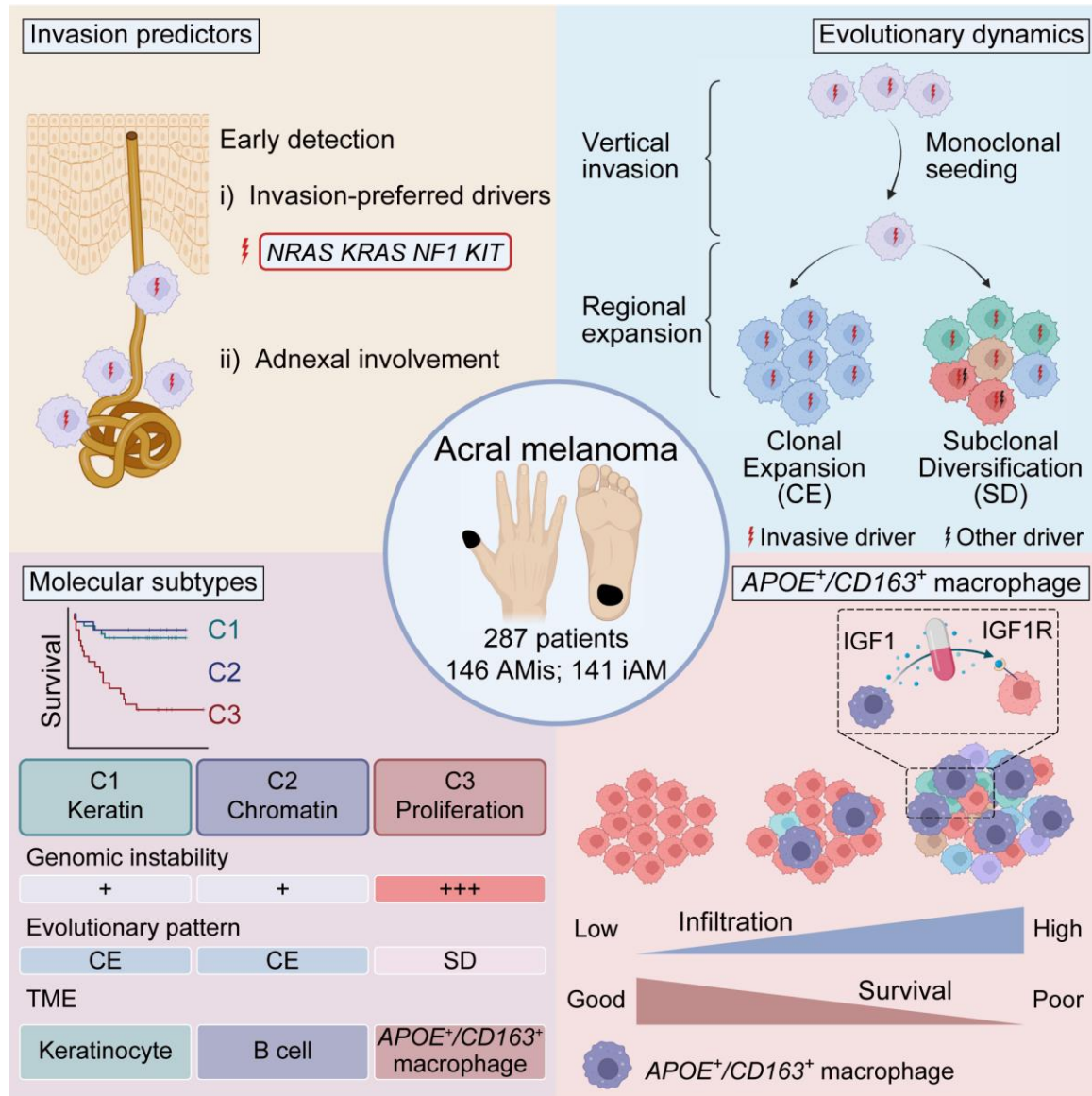


✓ Inhibition

APOE+CD163+ staining is a biomarker for inferior AM prognosis



Subclonal evolution cooperates with TAMs during AM invasion



Cancer Cell

CellPress

Article

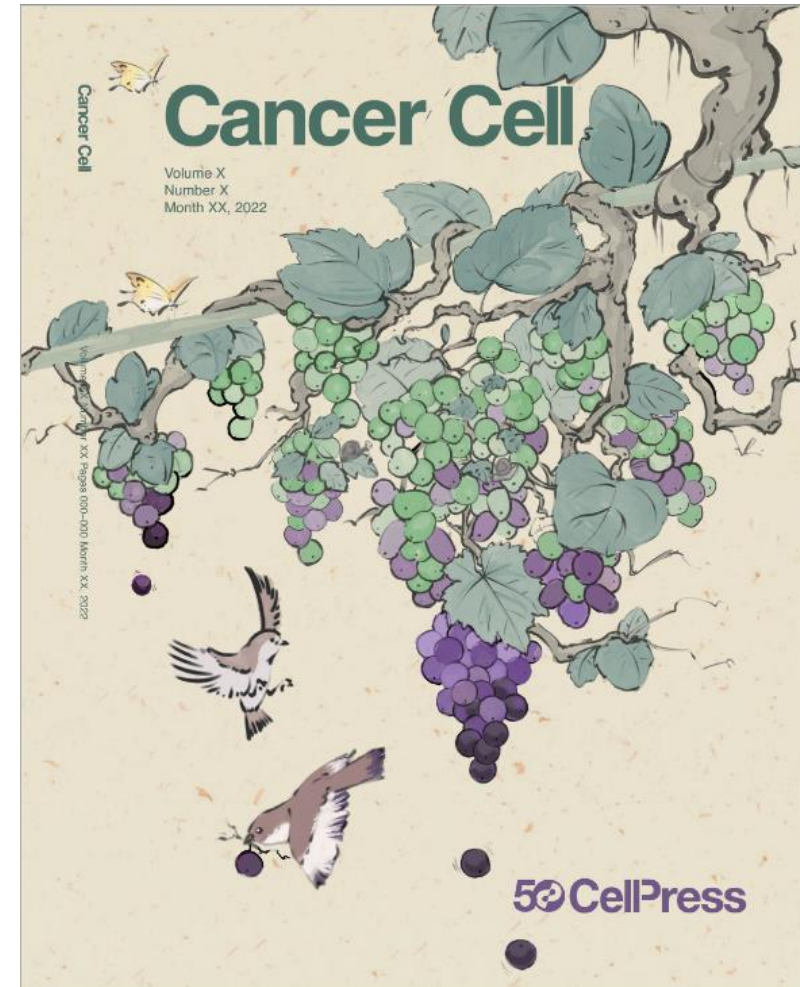
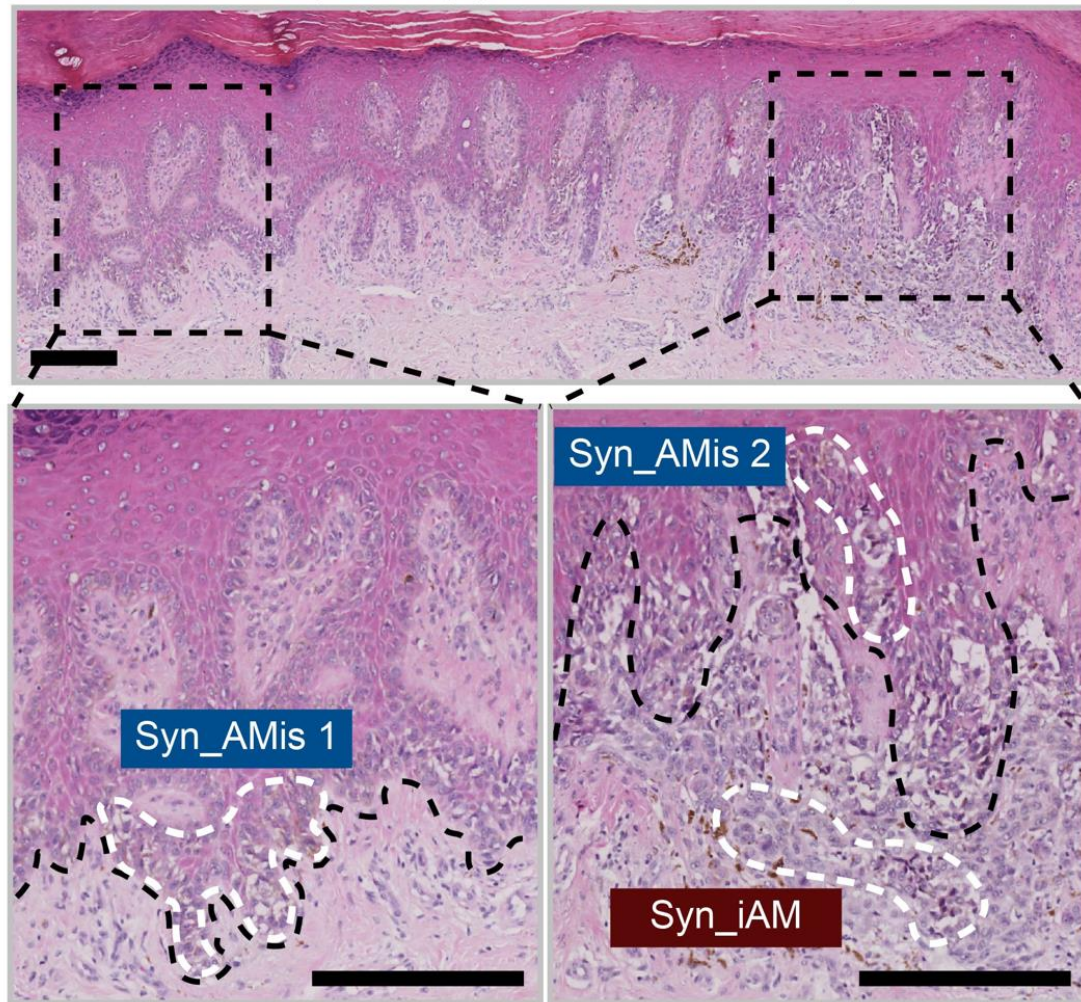
Integrative molecular and spatial analysis reveals evolutionary dynamics and tumor-immune interplay of *in situ* and invasive acral melanoma

Hengkang Liu,^{1,2,6} Jiawen Gao,^{3,4,6} Mei Feng,^{1,6} Jinghui Cheng,¹ Yuchen Tang,³ Qi Cao,¹ Ziji Zhao,¹ Ziqiao Meng,¹ Jiarui Zhang,³ Guohong Zhang,³ Chong Zhang,³ Mingming Zhao,³ Yicen Yan,³ Yang Wang,³ Ruidong Xue,^{1,2,*} Ning Zhang,^{1,2,5,7,*} and Hang Li^{1,3,5,*}

- Invasion-preferred drivers and adnexal involvement predict early invasion of AMis
- Subclonal diversification accelerates the regional expansion of iAM
- APOE⁺/CD163⁺ macrophages promote tumor EMT via IGF1-IGF1R interaction
- APOE and CD163 staining is a prognostic biomarker for AM

Liu, H. et al., *Cancer Cell* 2024

Selected as Cover Story of the June Issue

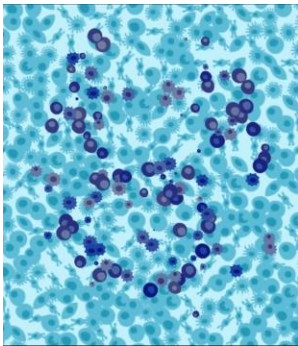




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- PKU-International Cancer Institute
北京大学国际癌症研究院
- Peking University First Hospital
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Prof. Zhao Li
Dr. Xiang Xiao
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Prof. Lei Chen



International collaboration



Prof. Hidewaki Nakagawa



Prof. Charles Swanton
Prof. Nicolas Mcgranhan



NATIONAL CANCER INSTITUTE
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