

# Cancer ecology and evolution

*N. Alcala*

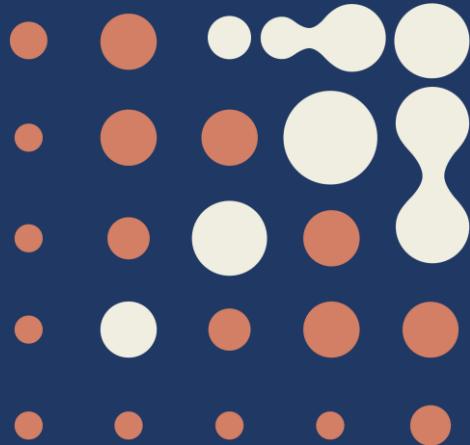
Rare Cancers Genomics Team

February 2022

International Agency  
for Research on Cancer



RARE  
CANCERS  
GENOMICS



# Plan

## IARC and the Rare Cancers Genomics team

1. Organization
2. Rationale
3. Research approach

## Cancer ecology and evolution

1. Concepts
2. A tentative genotype-phenotype map (**MESOMICS** project)
3. Measuring intra-tumor genetic diversity (**lungNENomics** project)
4. Diversity and FST-like statistics for cancer genomics (pan-cancer re-analysis)

## Mission: Cancer research for cancer prevention

The International Agency for Research on Cancer (IARC) is the specialized cancer agency of the World Health Organization.



Current location in Lyon



Nouveau centre (relocation end of 2022)

## Mission: Cancer research for cancer prevention

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q&a

Check for updates

### Cancer research that matters

Elisabete Weiderpass is an expert in cancer epidemiology and cancer prevention. She has been the Director of the International Agency for Research on Cancer, the specialized cancer agency of the World Health Organization, since January 2019. She spoke with *Nature Cancer* about 2021 and the years ahead.

■ 2021 has been another difficult year for the world, with the COVID-19 pandemic still not under control. What was the continuing impact of the pandemic on cancer research and patients with cancer?

EW: In response to the pandemic, the International Agency for Research on Cancer (IARC) assessed the impact of COVID-19 on health systems at the national level and looked at the outcomes of current and future patients with cancer. IARC also participated in the COVID-19 and Cancer Global Taskforce and became a founding partner of the COVID-19 and Cancer Global Modelling Consortium, with a remit to co-develop tools and provide evidence to aid decision-making during and after the pandemic.

In this manner, IARC was able to clearly assess the negative impact that the pandemic has had on cancer health systems and cancer outcomes. COVID-19 has interrupted registry operations, disrupted screening programs, and delayed patient diagnosis and initiation of treatment. The long-term, large-scale cancer aftershock will be strongly



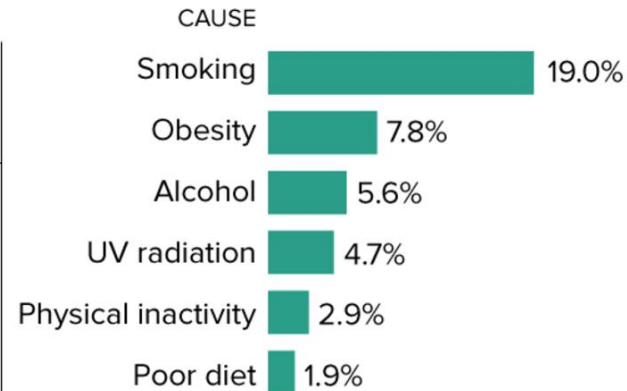
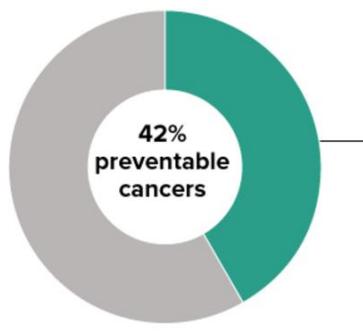
Credit: CC-BY-SA 3.0 IGO. IARC/M Stenmark

and achieving more rapid increases in treatment levels during the post-outbreak period can reduce the number of accumulated patients needing treatment and decrease the additional risk of dying due to longer time to treatment initiation.

Finally, IARC and partner institutions comprehensively assessed the impact of the COVID-19 pandemic on pediatric oncology diagnoses and provision of healthcare, for the first time covering an entire country. The researchers compared the incidence of childhood cancer in Germany, which has 13.5 million people younger than 18 years, using nationwide high-quality cancer registry data, and found that the estimated age-standardized incidence rates were markedly higher, overall and across diagnostic groups, in 2020 than in 2015–2019. The results from a qualitative survey indicate that diagnostic processes, timeliness of diagnosis, and delivery of treatment were hardly affected during the COVID-19 pandemic, so the underlying reasons for the increase in incidence rates seen in this study remain speculative. However, continued

### Preventable cancers

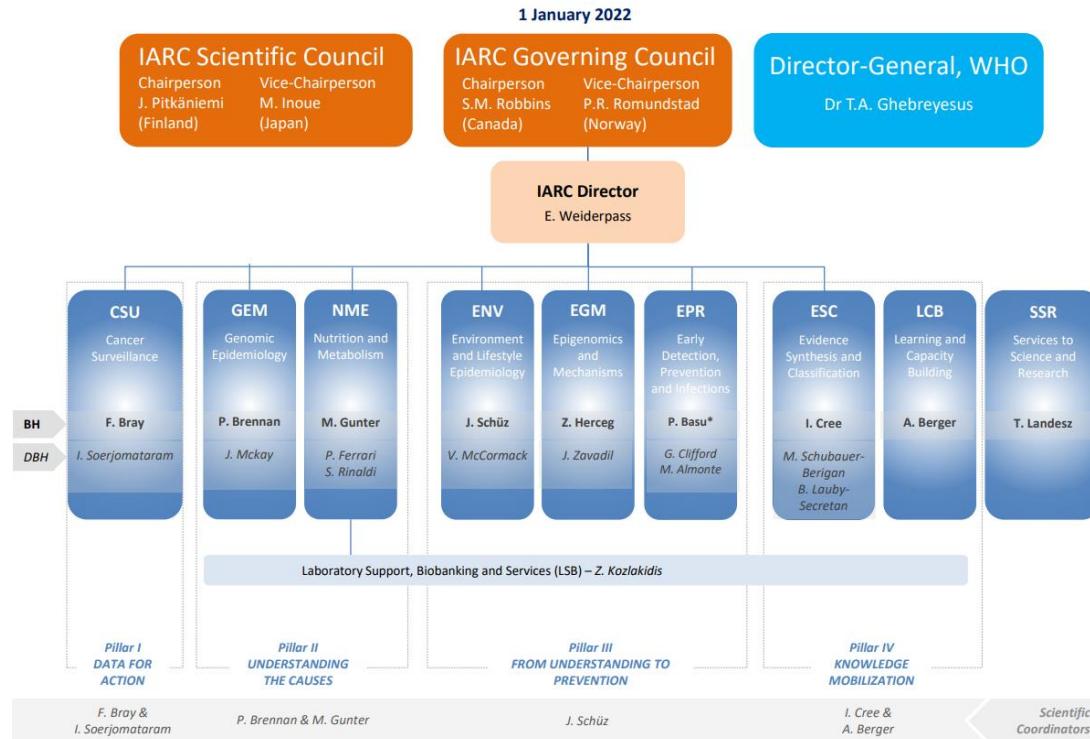
More than 40 percent of cancer cases can be prevented, the American Cancer Society finds in a new report. Here is a list of things people can change and their share of cancer cases:



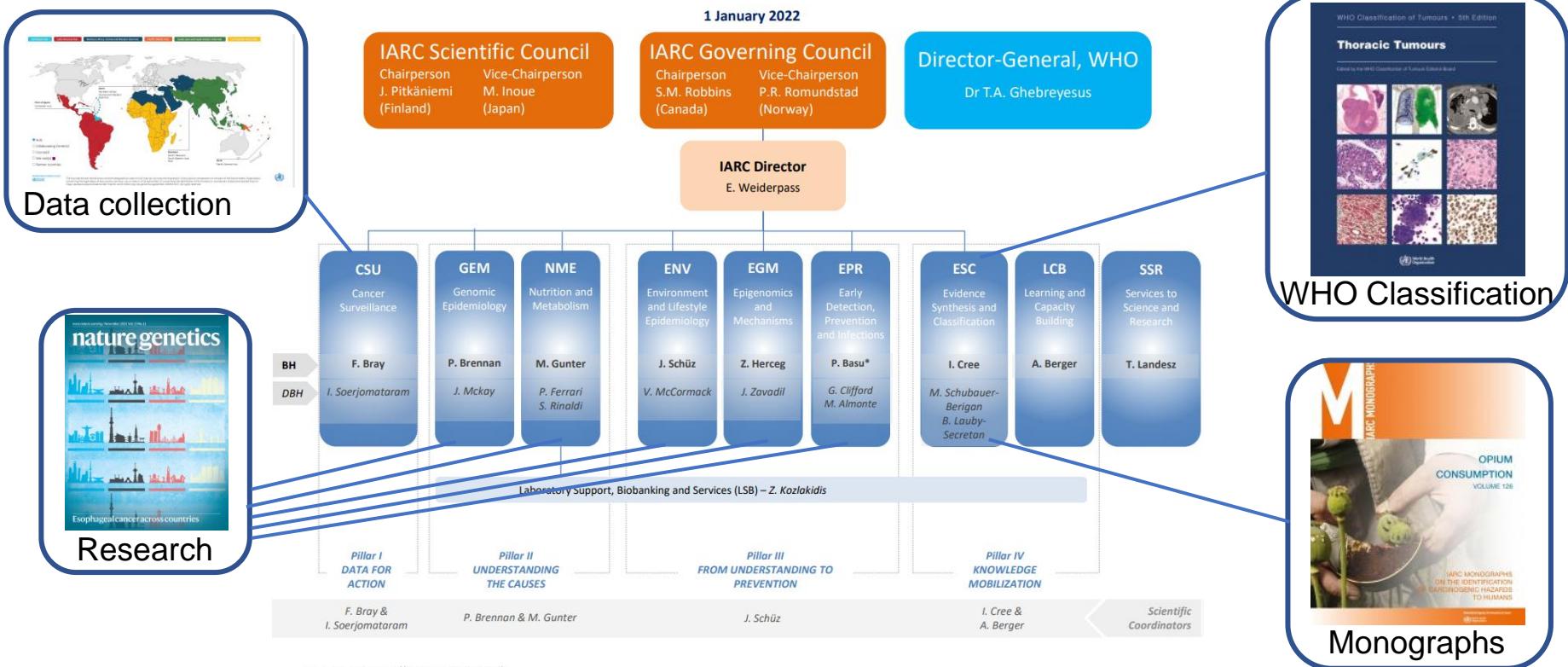
Source: American Cancer Society



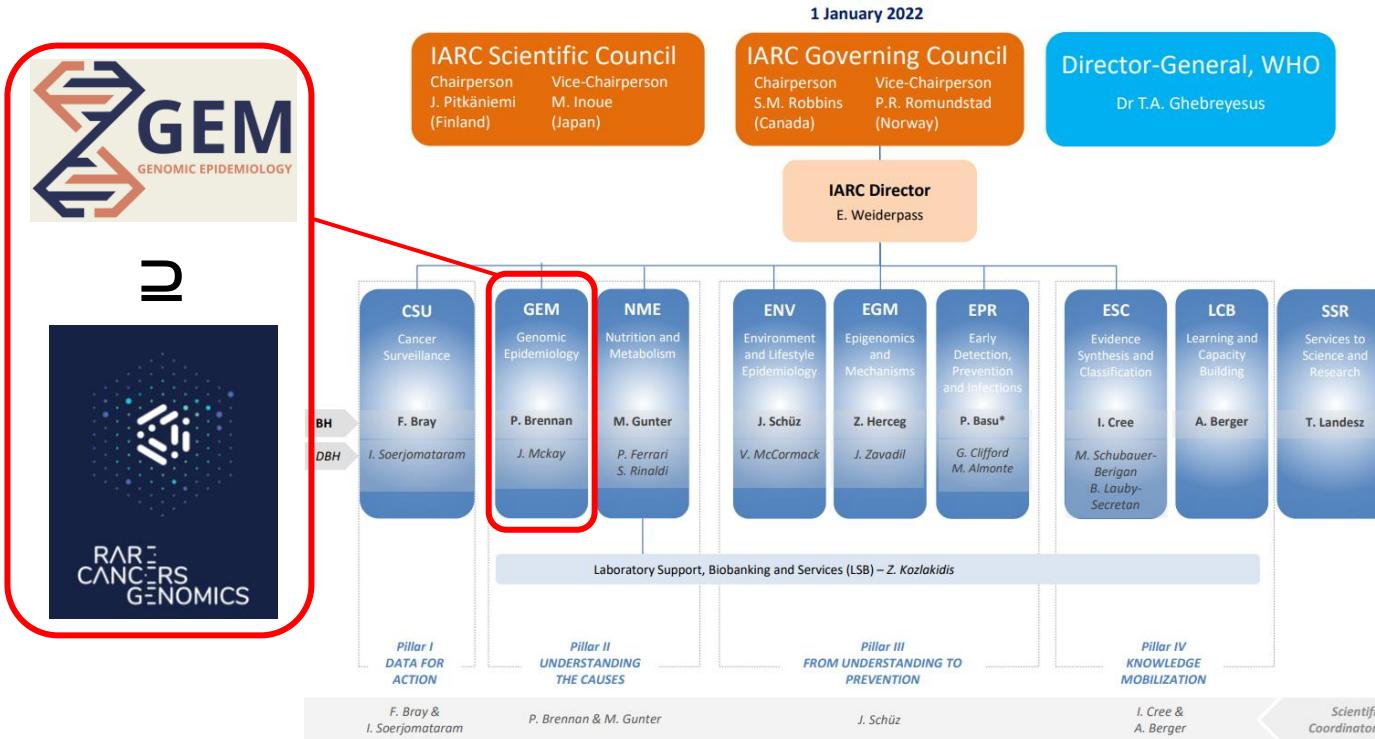
# IARC | Organization



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# IARC | Organization



## The RCG team at IARC | *Rationale*

- Rare cancers : 25–30% cancer diagnoses and 25% cancer deaths > **substantial burden of disease**
- Basic biological and clinical knowledge lacking à **suboptimal clinical outcome** > 47% 5-year survival rate vs 65% for patients with common cancers
- Limitations inherent to their rarity à **common cancers reclassified into rare subtypes** > limitations associated with the study of rare cancers will be extended to all cancer types

# The RCG team at IARC | *Rationale*

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- Limitations inherent to their rarity à **common cancers reclassified into rare subtypes** > limitations associated with the study of rare cancers will be extended to all cancer types
- **Rare Cancers Genomics initiative:** international multidisciplinary open-science effort to shed light on the molecular characteristics of rare cancers, to understand the etiology and carcinogenesis processes, to ultimately improve their clinical management and consequently, their prognosis

# The RCG team at IARC | *Organization and focus*



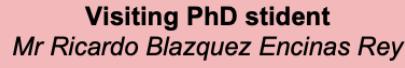
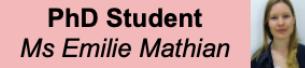
Statistics and cancer evolution -



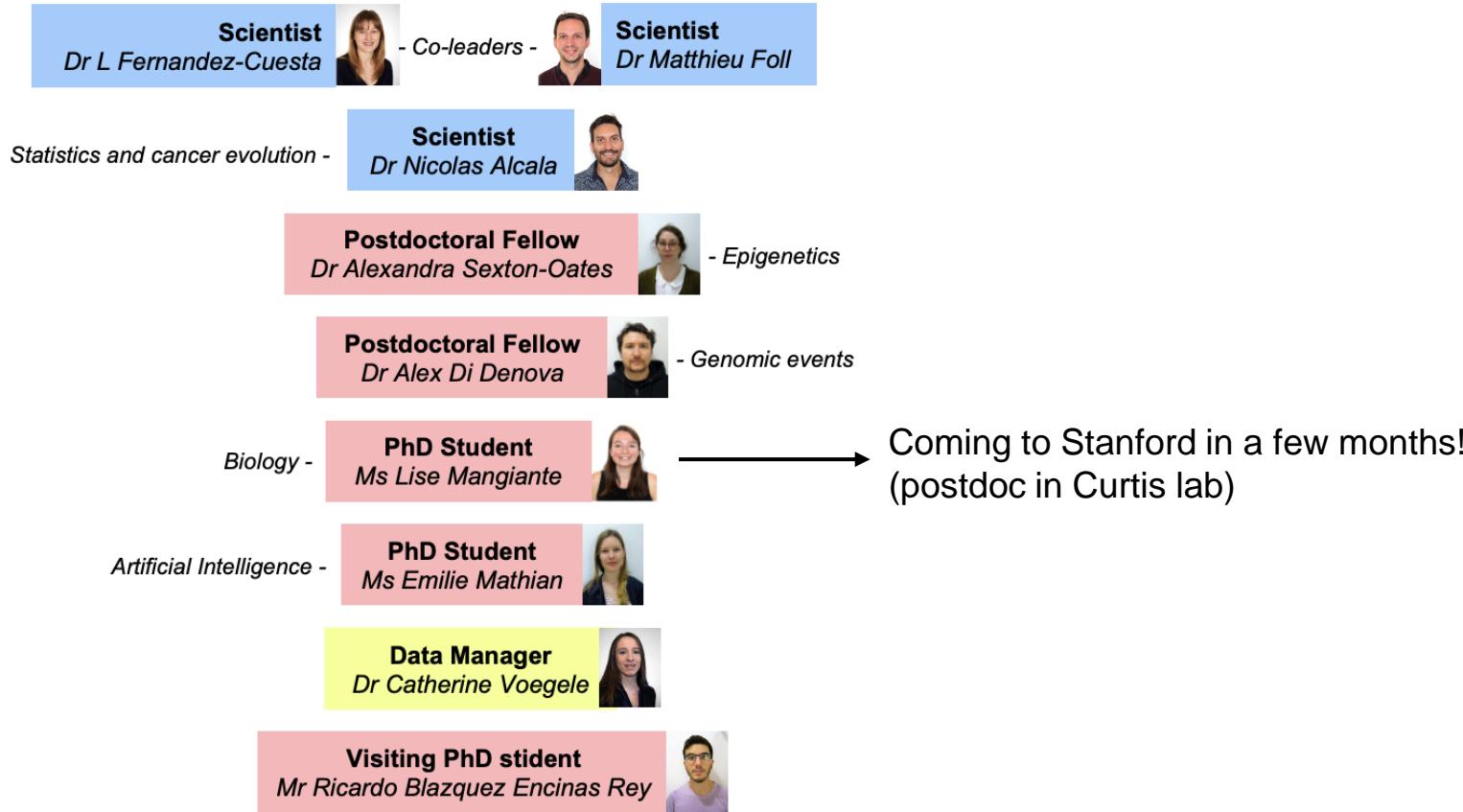
Biology -



Artificial Intelligence -



# The RCG team at IARC | Organization and focus



# The RCG team at IARC | Organization and focus

<p><b>Scientist</b> Dr L Fernandez-Cuesta</p>	<p>- Co-leaders -</p>	<p><b>Scientist</b> Dr Matthieu Foll</p>
<p>Statistics and cancer evolution -</p>		<p><b>Scientist</b> Dr Nicolas Alcala</p>
		<p><b>Postdoctoral Fellow</b> Dr Alexandra Sexton-Oates</p>
		<p><b>Postdoctoral Fellow</b> Dr Alex Di Denova</p>
<p>Biology -</p>		<p><b>PhD Student</b> Ms Lise Mangiante</p>
<p>Artificial Intelligence -</p>		<p><b>PhD Student</b> Ms Emilie Mathian</p>
		<p><b>Data Manager</b> Dr Catherine Voegele</p>
		<p><b>Visiting PhD student</b> Mr Ricardo Blazquez Encinas Rey</p>

## Cancers of interest:

- (lung) neuroendocrine neoplasms



lungNENomics



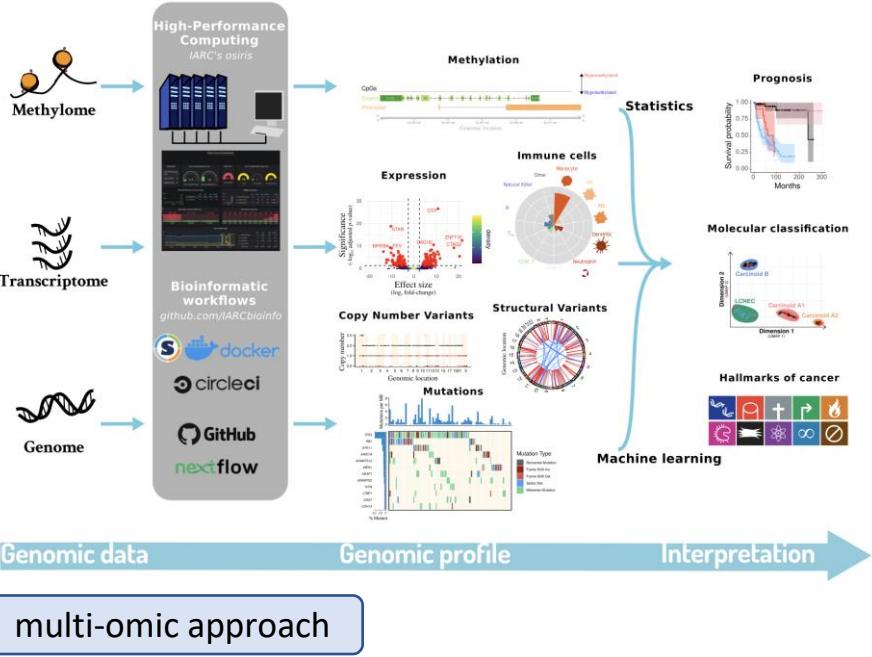
panNENomics

- malignant pleural mesothelioma

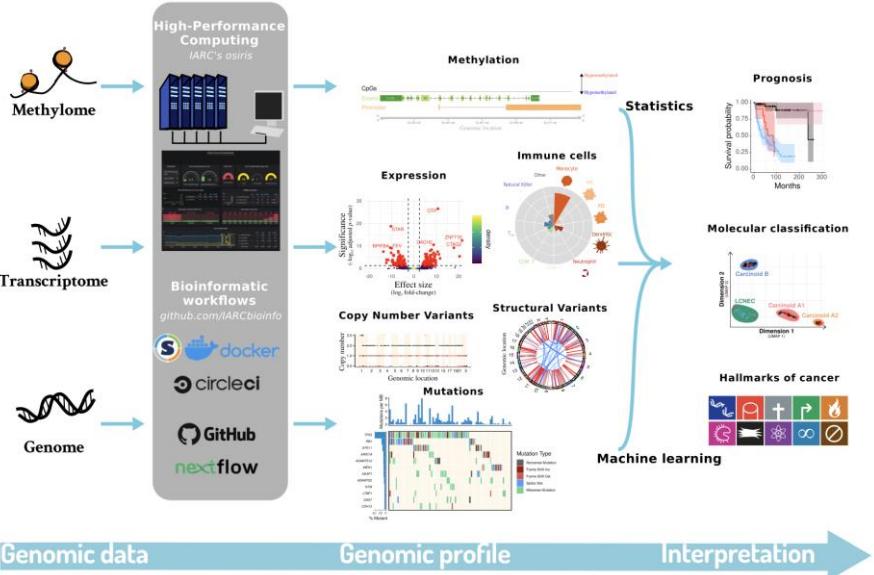


- more recently, high aggressive soft-tissue sarcomas (**SARCOMICS**)

# The RCG team | Study design

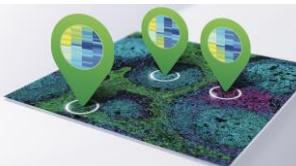


# The RCG team | Study design

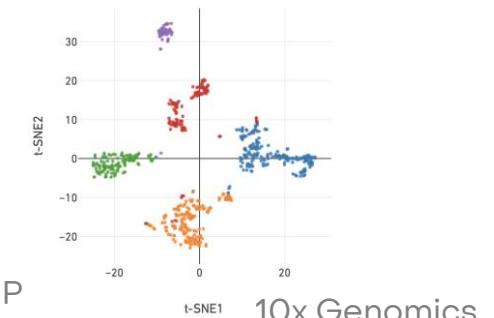


multi-omic approach

spatial and single-cell



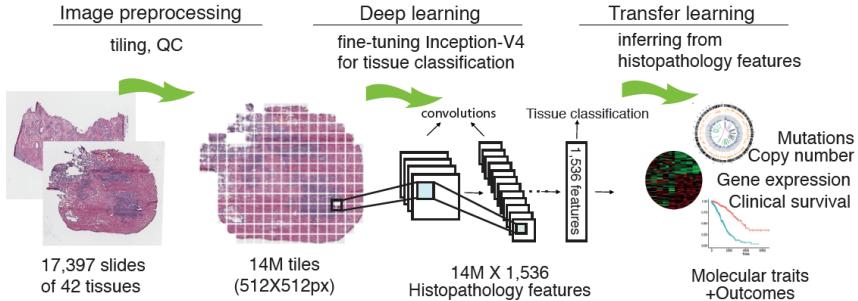
Nanostring GeoMx DSP



10x Genomics

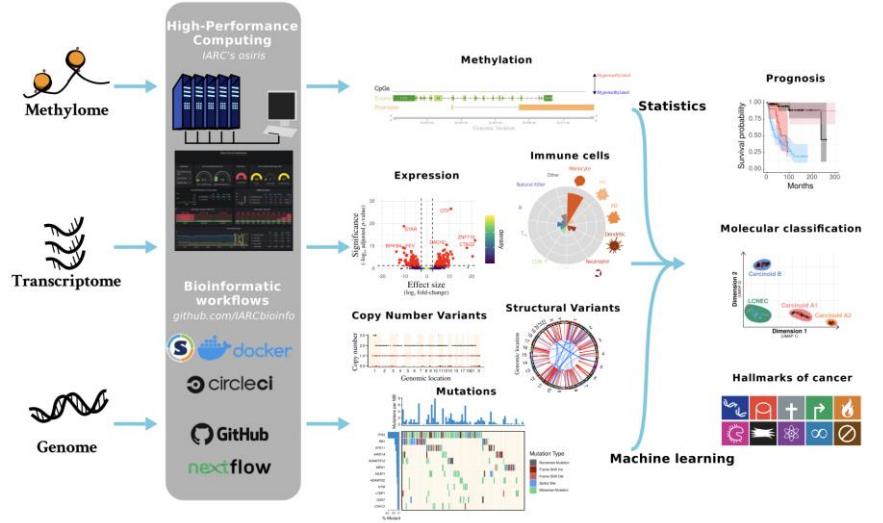
# The RCG team | Study design

Pan-Cancer Computational HistoPathology (PC-ChIP) analysis workflow



Fu et al. Nature Cancer 2020

AI and deep learning



Genomic data

Genomic profile

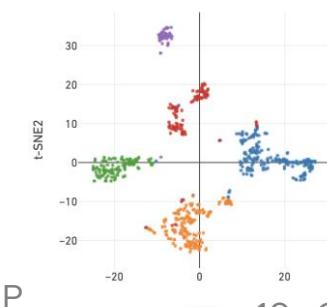
Interpretation

multi-omic approach

spatial and single-cell

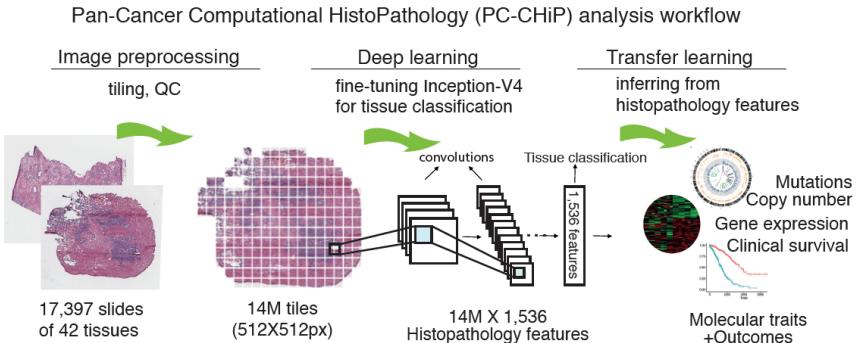


Nanostring GeoMx DSP

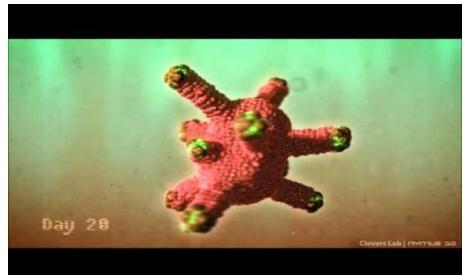


10x Genomics

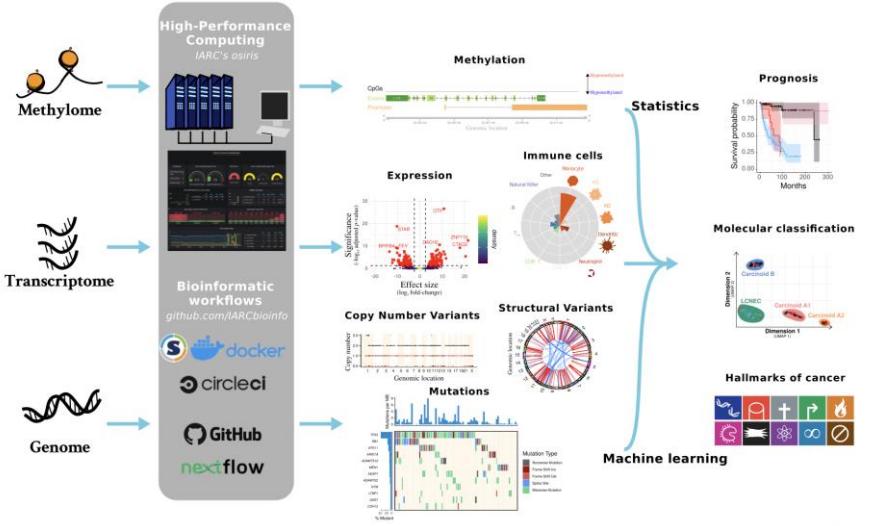
# The RCG team | Study design



Fu et al. Nature Cancer 2020



**Mini-gut organoids**  
Clevers group

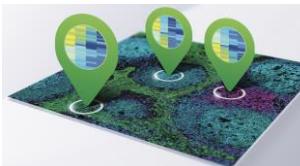


AI and deep learning

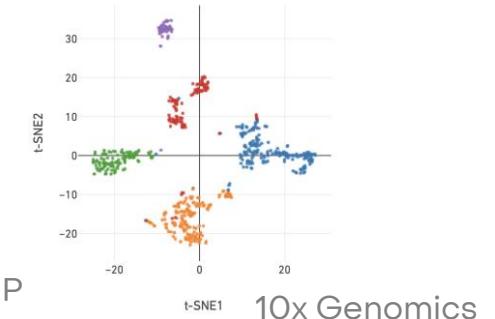
organoids

multi-omic approach

spatial and single-cell



Nanostring GeoMx DSP



# Tumor ecology & evolution

*Useful concepts (or an evolutionary biologist's simple view of cancer)*

# Tumor ecology & evolution | *Concepts*

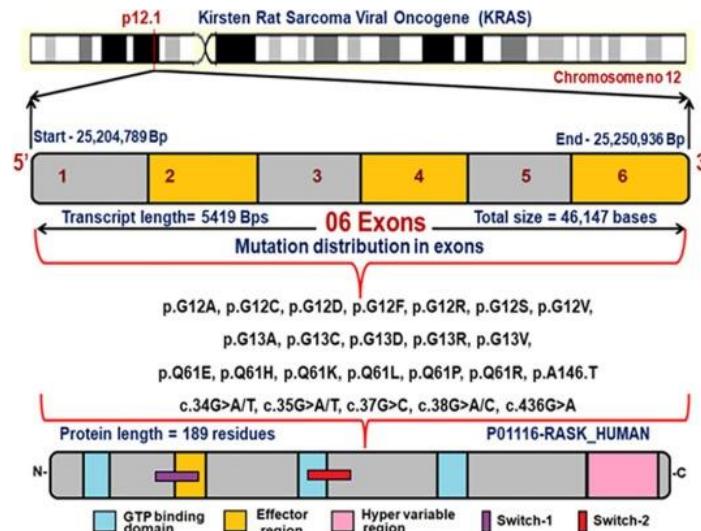
Cancer is a disease of the genome

# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

The current view is that the genome comprises "cancer genes" of 2 types:

- **Oncogenes**, proto-genes that are not normally expressed but can be expressed after a mutation at a specific site or epigenetic modification



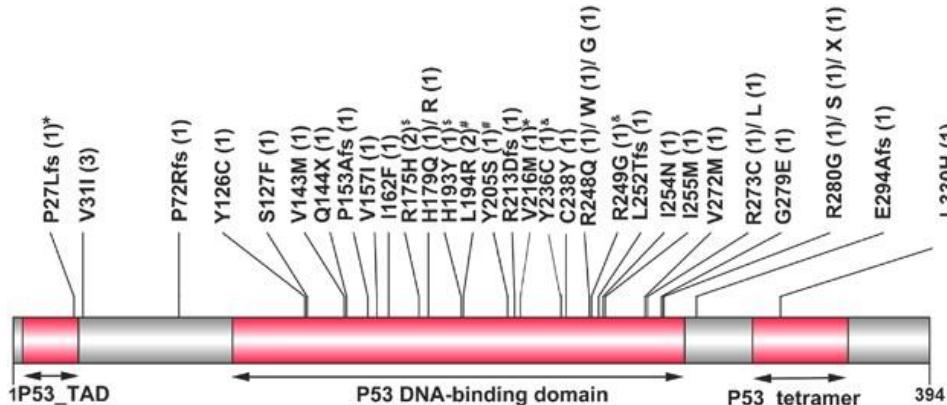
KRAS activating mutations (Upreti and Adjei 2020).

# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

The current view is that the genome comprises "cancer genes" of 2 types:

- **Oncogenes**, proto-genes that are not normally expressed but can be expressed after a mutation at a specific site or epigenetic modification
- **Tumor suppressor genes**, "house keeping" genes necessary to maintain the integrity of the cell function and where deleterious mutations cause uncontrolled growth

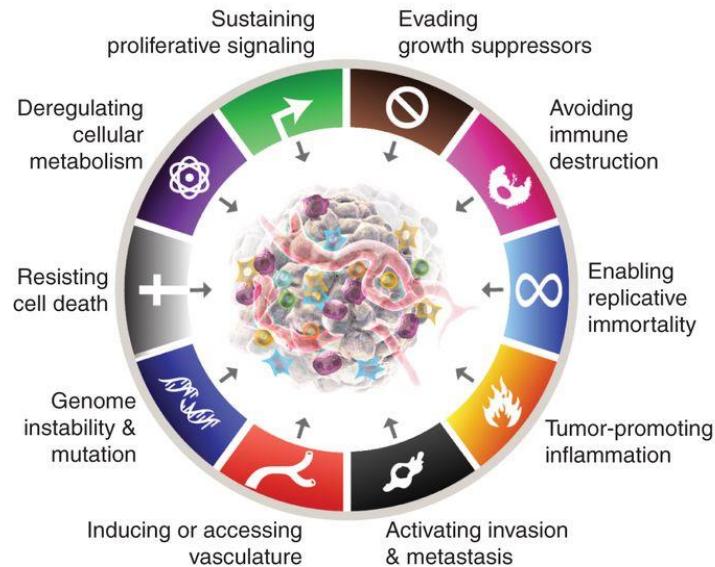


TP53 inactivating mutations (Hou et al. 2015).

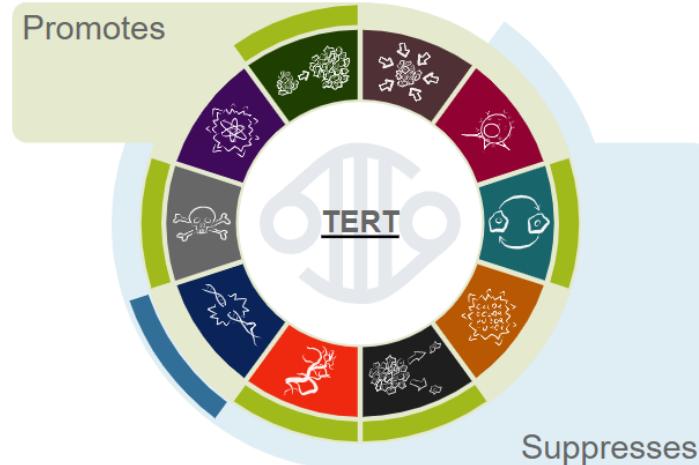
# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

Cancer genes contribute to the **cancer phenotype**, schematically represented by the **10 hallmarks of cancer**, 10 biological capabilities found at various degrees in cancer types



Hallmarks of cancer (Weinberg and Hanahan 2015).

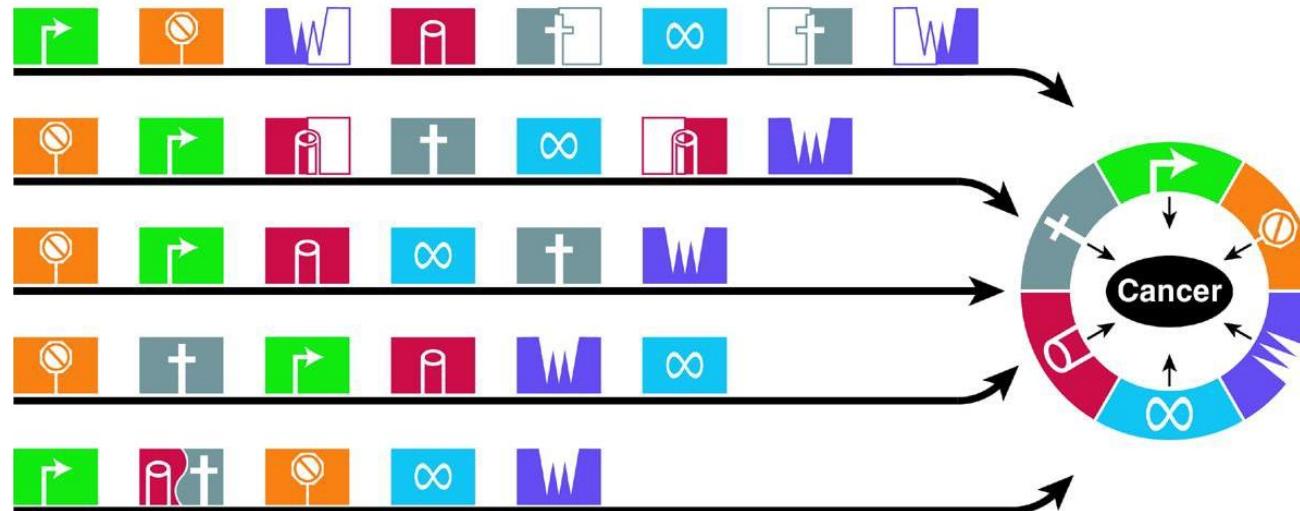


TERT Hallmarks profile (COSMIC database).

# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

**Multiple paths** to cancer, difference across and within tumor types

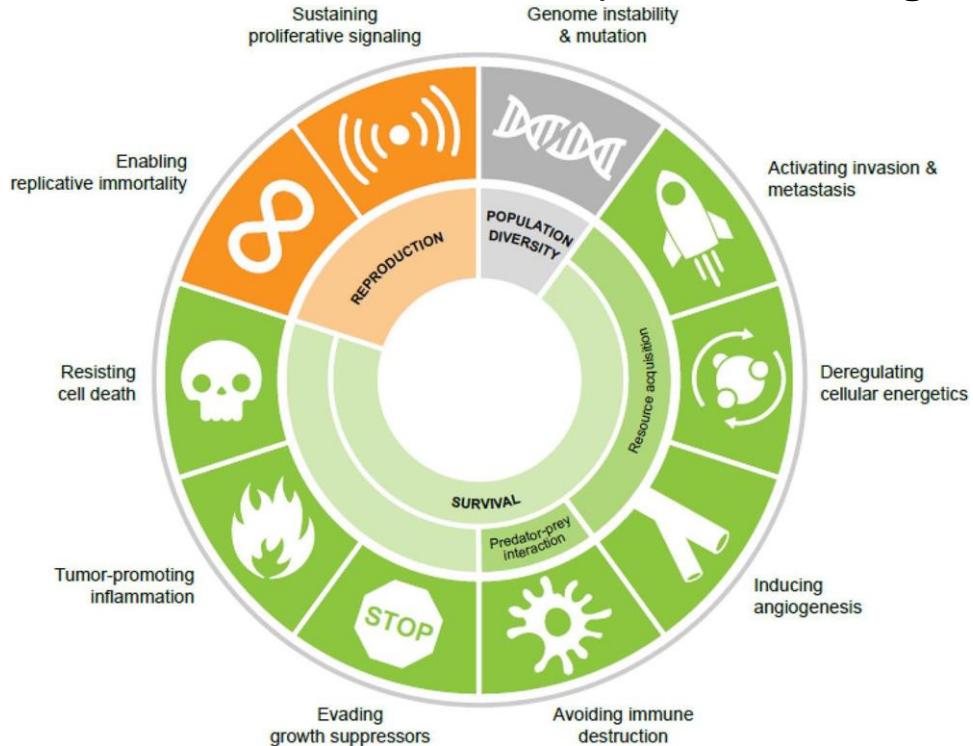


Hallmarks of cancer (Weinberg and Hanahan 2000).

# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

Hallmarks of cancer can be interpreted as **ecological strategies**

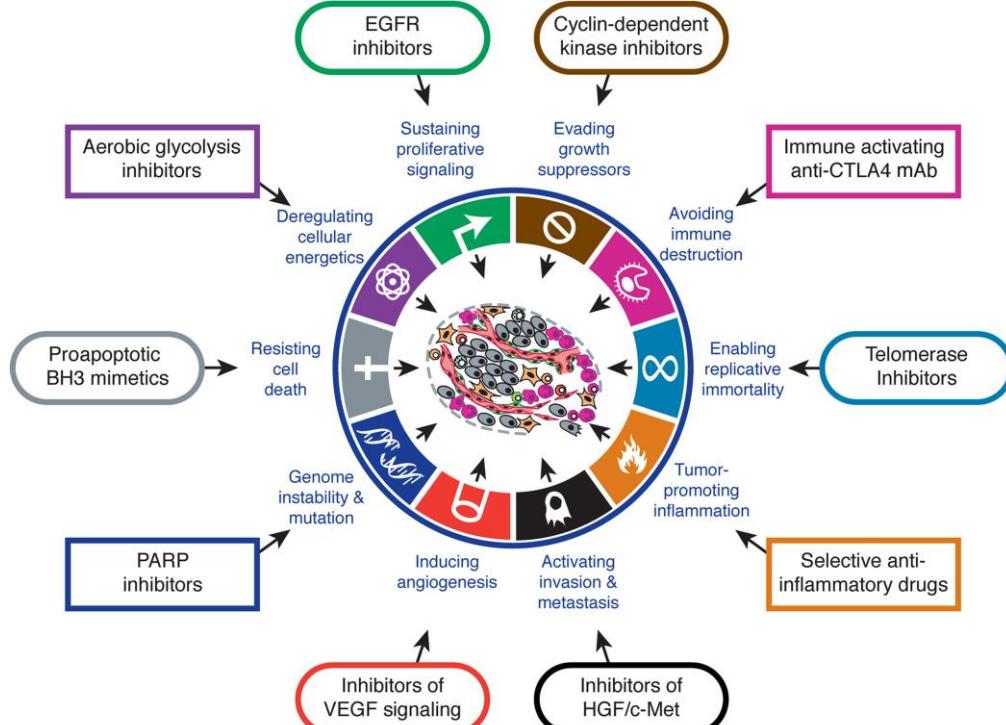


Hallmarks of cancer as ecological fitness parameters (Somarelli Front. Ecol. Evol. 2021).

# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

Each hallmark can be targeted, in an analogy with invasive species control

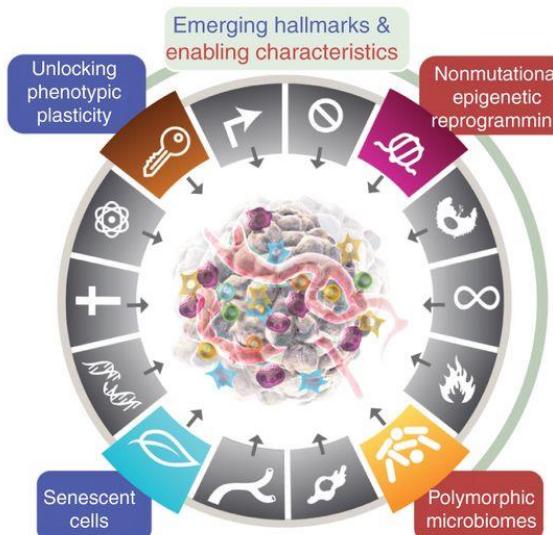
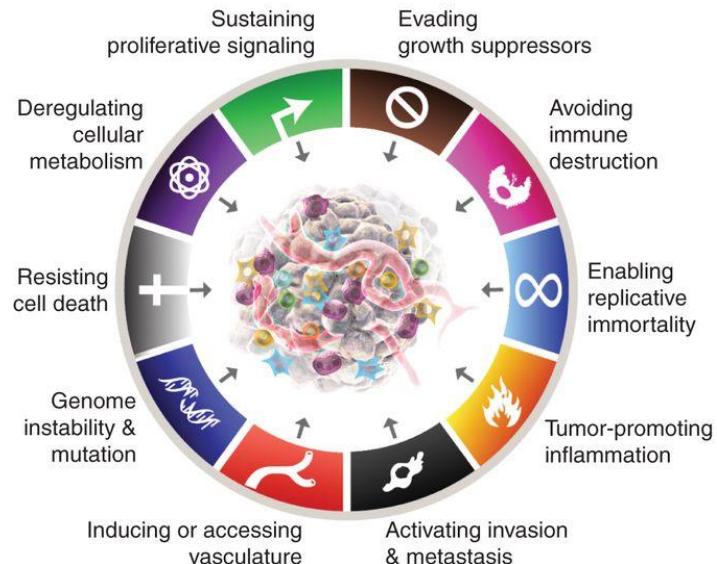


Therapeutic targeting of the hallmarks of cancer  
(Weinberg and Hanahan Cell 2014).

# Tumor ecology & evolution | Concepts

Cancer is a disease of the genome

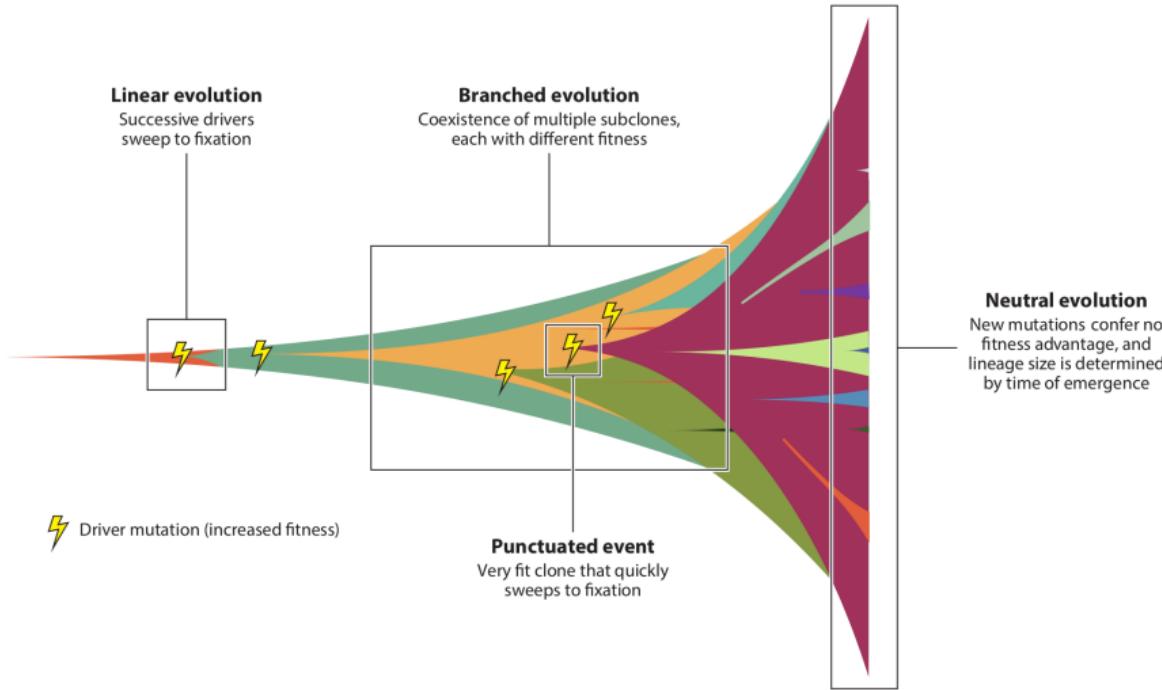
This is a conceptual model of cancer phenotypes, still under refinement



Hallmarks of cancer (Hanahan 2022).

# Tumor ecology & evolution | Concepts

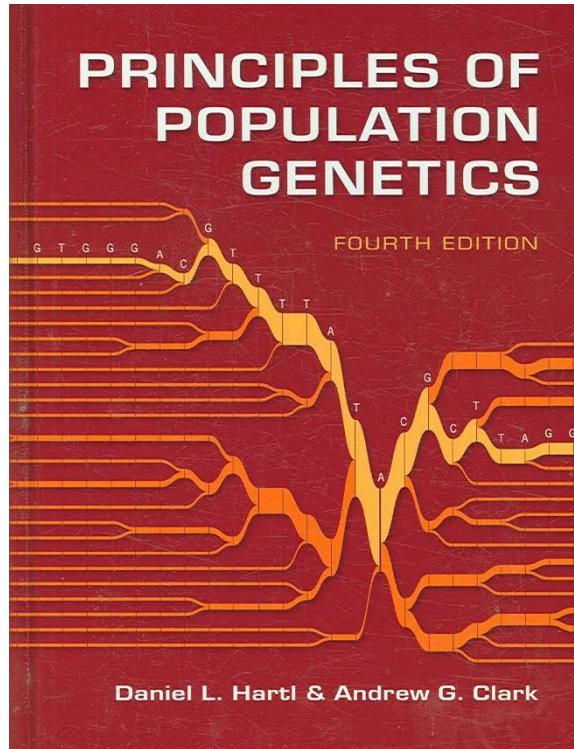
Cancer is a disease of the genome, and tumor initiation and progression are evolutionary processes



Schematic view of cancer evolution (Williams et al. 2019).

# Tumor ecology & evolution | Concepts

All classical evolutionary forces are at work



- Mutation
- Genetic drift
- Selection
- Migration

# Tumor ecology & evolution | *Concepts*

All **classical evolutionary forces** are at work

- **Mutation** → somatic alterations accumulate in the genome due to various mechanisms

# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

- **Mutation** → somatic alterations accumulate in the genome due to various mechanisms

Main framework: **mutational signatures**. Considering pyrimidines, 6 possible substitutions (C>A, C>G, C>T, T>A, T>C, T>G).

# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

- **Mutation** → somatic alterations accumulate in the genome due to various mechanisms

Main framework: **mutational signatures**. Considering pyrimidines, 6 possible substitutions (C>A, C>G, C>T, T>A, T>C, T>G). Generally, we consider the 3 nucleotide context (16 per single-nucleotide substitution), leading to 96 contexts (ACA>AAA, ACC>AAC, ACG>AAG, ACT>AAT, ...)

# Tumor ecology & evolution | Concepts

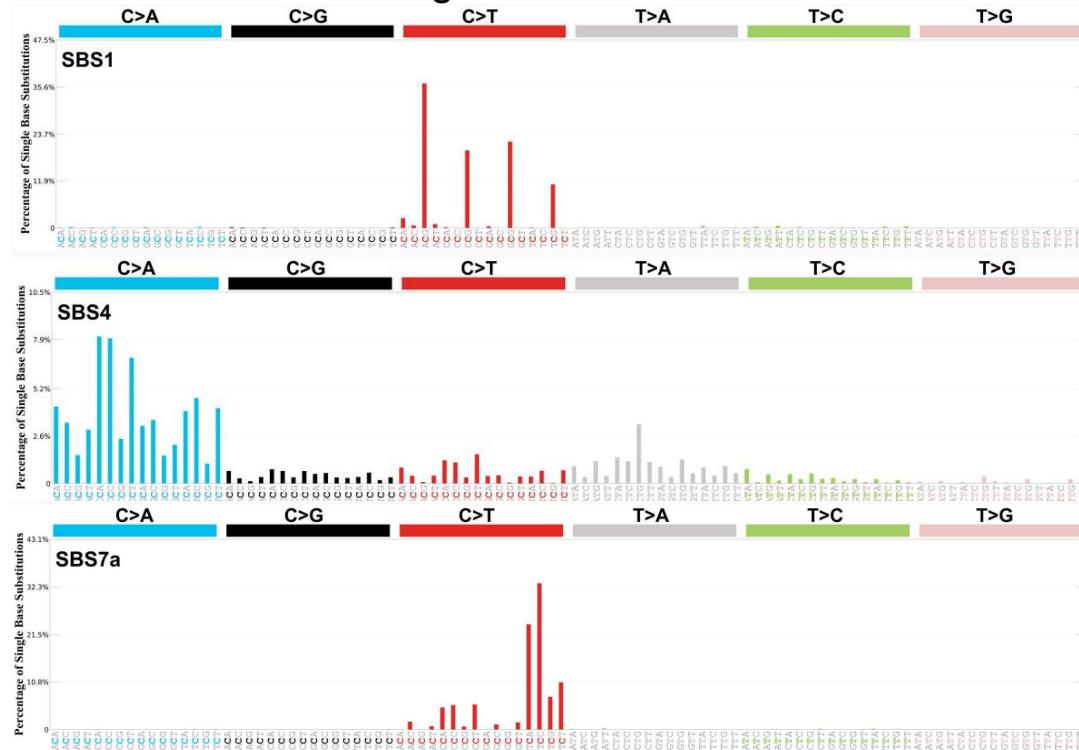
All classical evolutionary forces are at work

- Mutation → somatic alterations accumulate in the genome due to various mechanisms

Age (spontaneous deamination)

UV light

Tobacco

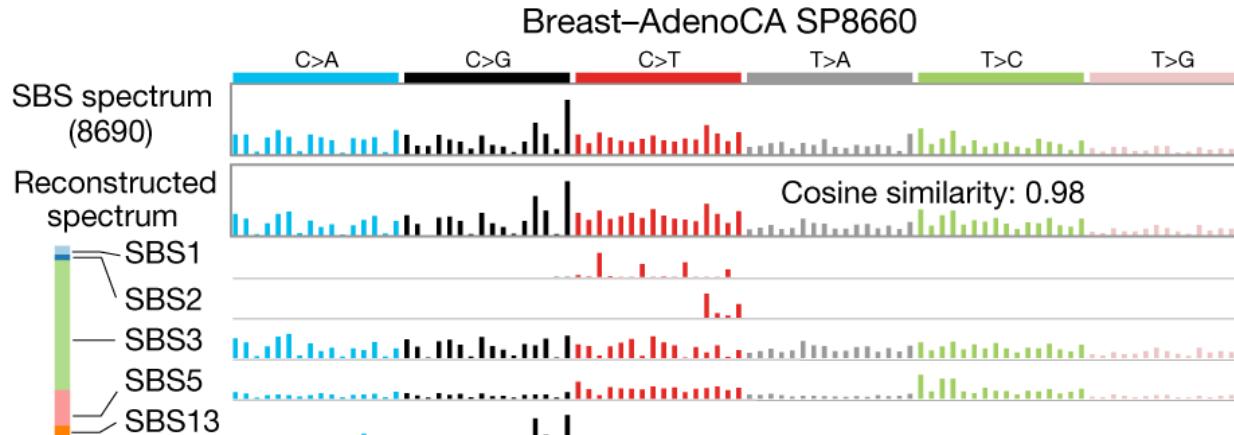


# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

- **Mutation** → somatic alterations accumulate in the genome due to various mechanisms

Individual mutational profiles can be decomposed into signatures (NNMF for discovery, NNLS for assignment of known signatures)



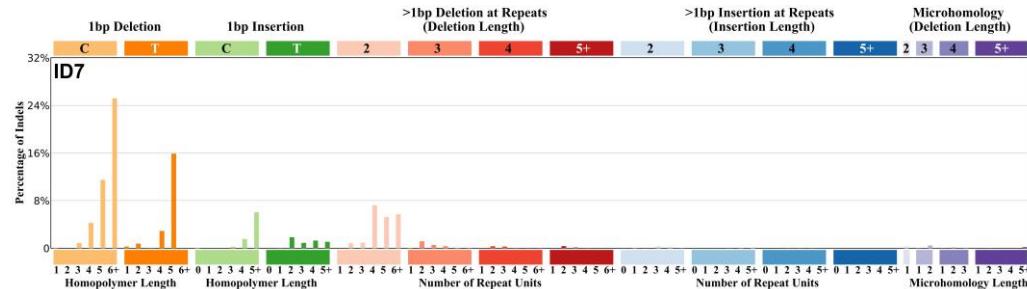
Example signature assignments (Alexandrov et al. Nature 2020)

# Tumor ecology & evolution | Concepts

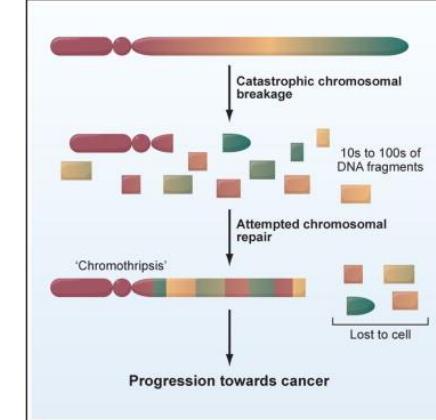
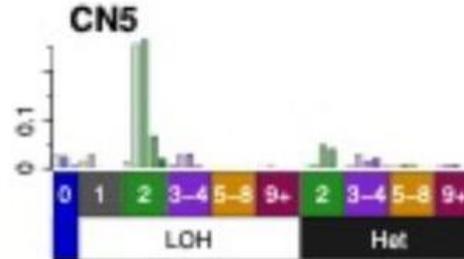
All classical evolutionary forces are at work

- Mutation → somatic alterations accumulate in the genome due to various mechanisms

Defective DNA mismatch repair



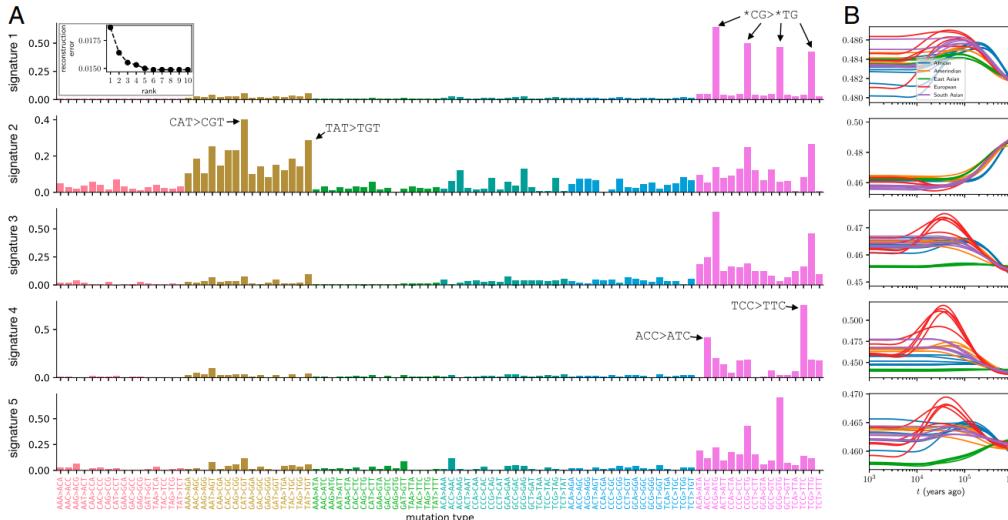
Chromothripsis



# Tumor ecology & evolution | Concepts

All classical evolutionary forces are at work

- **Mutation** → somatic alterations accumulate in the genome due to various mechanisms  
*Note: mutational signatures are also visible in the germline, but less diverse because genomic instability, and thus hypermutator phenotypes, is a hallmark of cancer*



Temporal dynamics of germline mutational signatures in populations from the 1KG.  
DeWitt et al. PNAS 2021

# Tumor ecology & evolution | *Concepts*

All **classical evolutionary forces** are at work

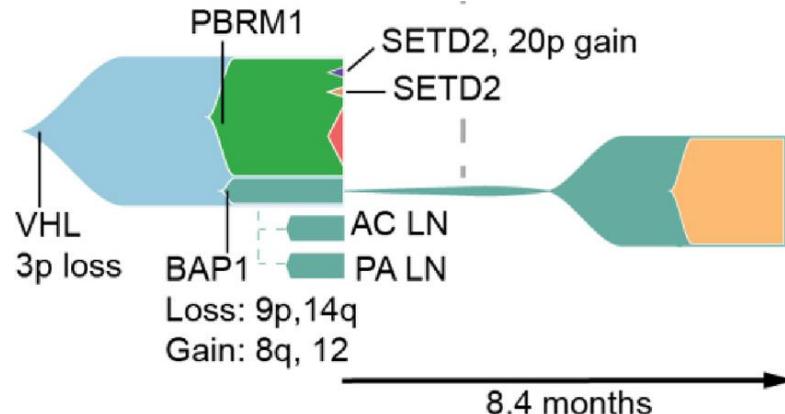
- **Genetic drift** → mutation frequency change due to random sampling of cells

# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

- **Genetic drift** → mutation frequency change due to random sampling of cells

Main demographic models: initial exponential growth, then plateau when reaching carrying capacity (e.g., Gompertz function).



Example Renal Cell Carcinoma evolution in primary and metastasis. Turajilic et al. *Cell* 2018

# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

- **Genetic drift** → mutation frequency change due to random sampling of cells

Main demographic models: initial exponential growth, then plateau when reaching carrying capacity (e.g., Gompertz function).

Appropriate genealogical model is unclear (generalized Moran models and other birth-death models proposed, no consensus). Nonetheless, because  $N \gg 1$ , drift is small and in a neutrally evolving tumor the allele frequency  $f$  of a mutation mostly depends on the tumor size at the time of appearance of allele ( $f=1/N(t_0)$ )

# Tumor ecology & evolution | *Concepts*

All **classical evolutionary forces** are at work

- **Selection** → certain "driver" mutations have better survival or better reproductive success

# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

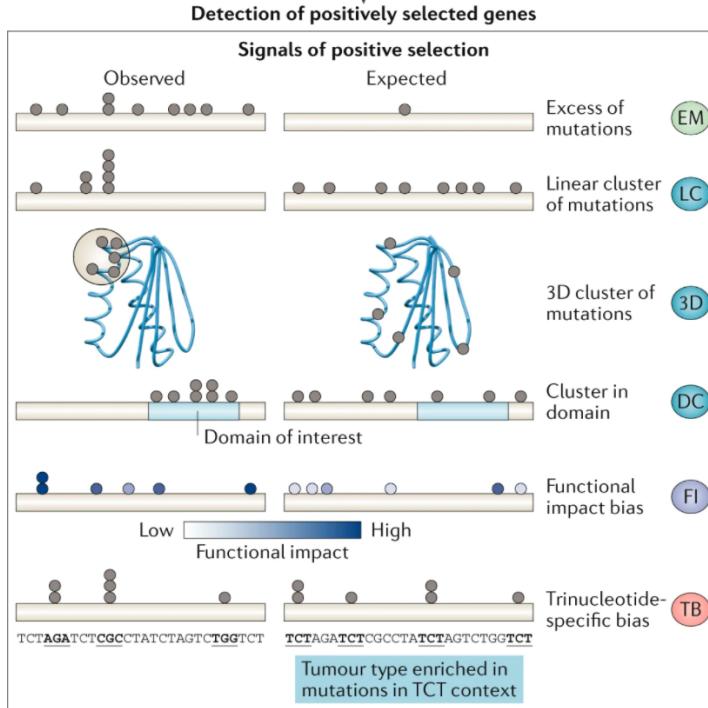
- **Selection** → certain "driver" mutations have better survival or better reproductive success

Main method: find genes undergoing convergent evolution toward a cancer phenotype, by finding **genes harboring recurrent mutations** in a cohort of independent patients with the same tumor type, after controlling for mutational hotspots and gene size

# Tumor ecology & evolution | Concepts

All classical evolutionary forces are at work

- **Selection** → certain "driver" mutations have better survival or better reproductive success



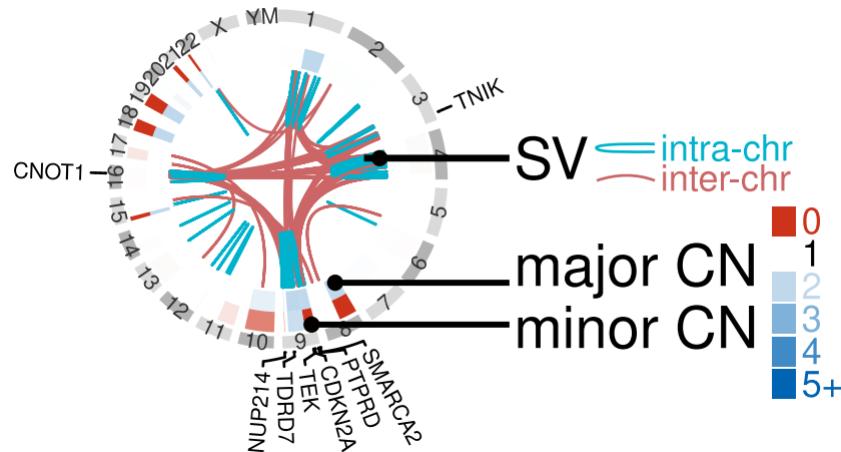
Favored software combine multiple methods ( $dN/dS$ , conservation scores, etc) to reduce false positives. Martinez-Jimenez et al., Nat Rev Cancer 2020

# Tumor ecology & evolution | Concepts

All **classical evolutionary forces** are at work

- **Selection** → certain "driver" mutations have better survival or better reproductive success

Note: catastrophic chromosomal events can simultaneously impact many cancer genes (punctuated evolution)



Structural variants in a lung neuroendocrine tumor with chromothripsis (Dayton\*, Alcala\* et al. In prep)

# Tumor ecology & evolution | *Concepts*

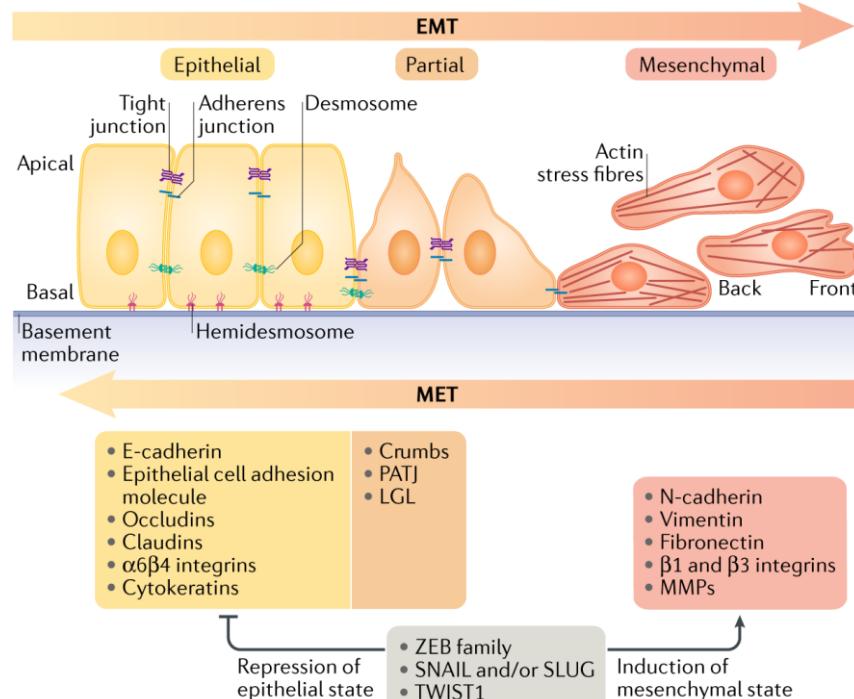
All **classical evolutionary forces** are at work

- **Migration** → cells move to other spatial locations

# Tumor ecology & evolution | Concepts

All classical evolutionary forces are at work

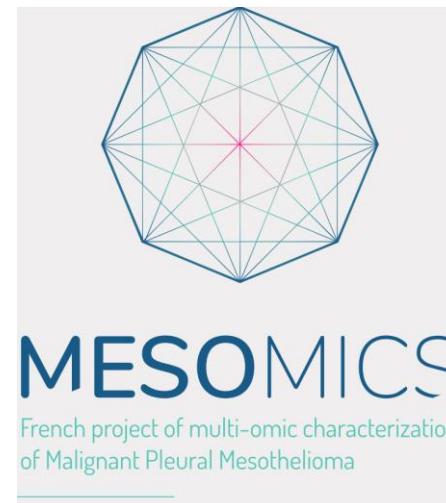
- **Migration** → cells move to other spatial locations



Epithelial-Mesenchymal Transition (EMT) and reverse program (MET). Dongre and Weinberg, Nat Rev Mol Cell Biol 2018.

# Tumor ecology & evolution

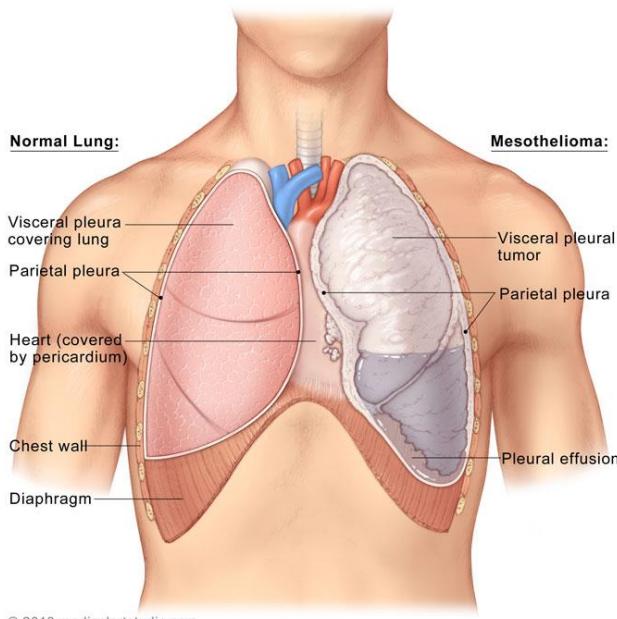
*Application I: a tentative genotype-phenotype map of malignant pleural mesothelioma*



# Tumor ecology & evolution | A genotype-phenotype map

## Malignant pleural mesothelioma

- Rare and deadly cancer arising in the linings of the lung (pleura)
- Mostly associated with **asbestos exposure**
- Asbestos is banned in many countries but **lag between exposure and disease ~30-40 years** ⇒ incidence still rising

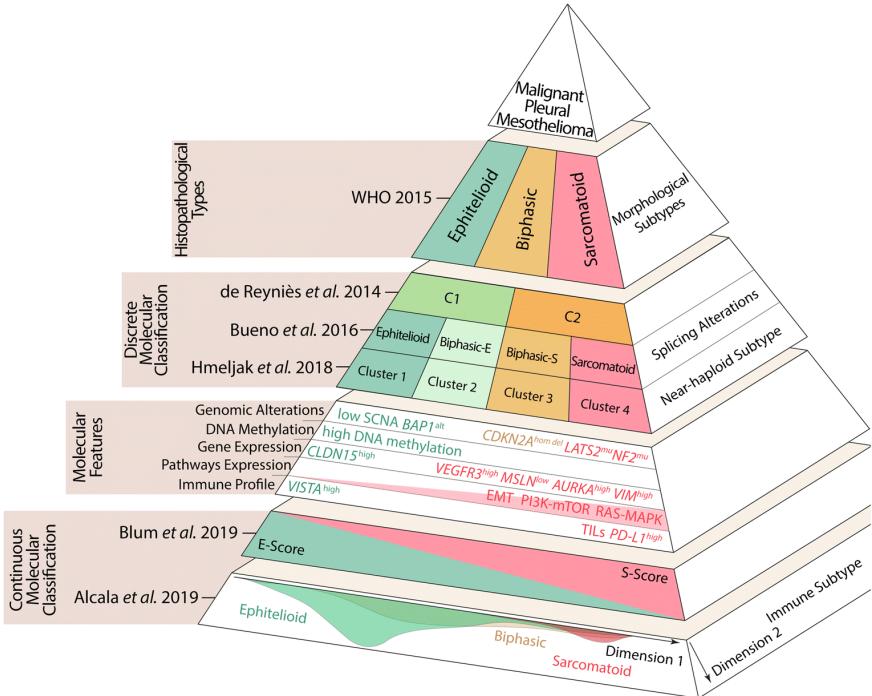


Stanford Medicine Dept of Surgery

# Tumor ecology & evolution | A genotype-phenotype map

## Malignant pleural mesothelioma (MPM)

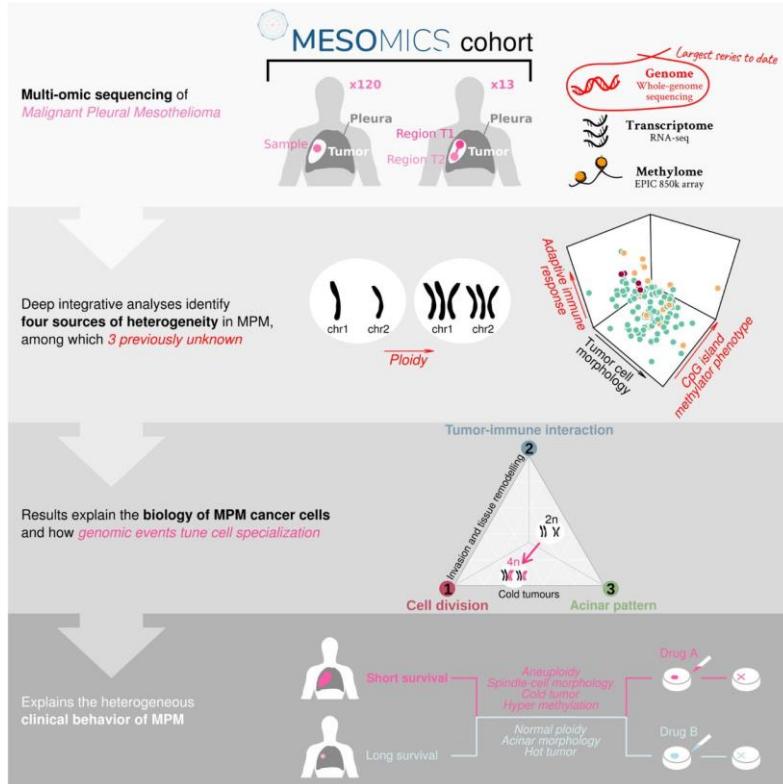
- Current WHO classification considers 3 types; molecular classifications further subdivide them
- Nevertheless, a number of observations not captured by this classification



Schematic representation of current MPM classifications (Fernandez-Cesta et al. Virchows Archive 2021)

# Tumor ecology & evolution | A genotype-phenotype map

MESOMICS study: further define inter-patient molecular variation

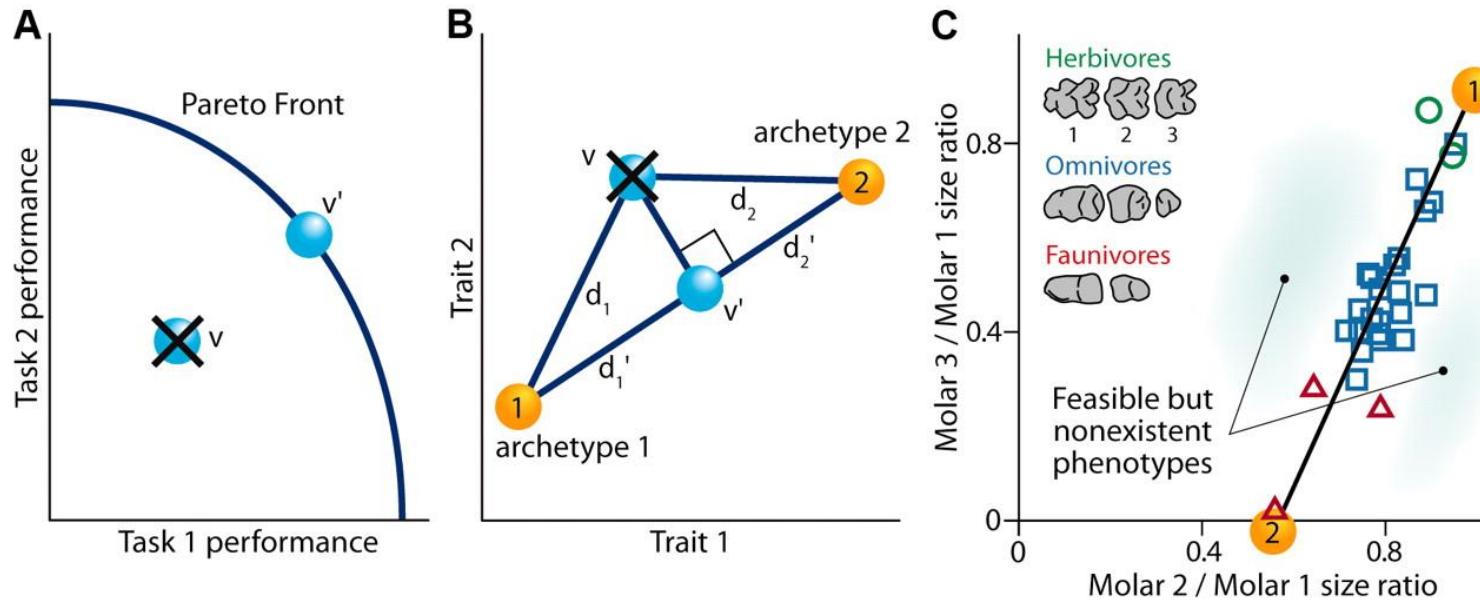


Mangiante\*, Alcala\*, Di Genova\*, Sexton-Oates\*, et al. (Under revision for Nat Genet)

# Tumor ecology & evolution | A genotype-phenotype map

## Step 1: build a phenotypic map of MPM

Method: Archetypal analysis

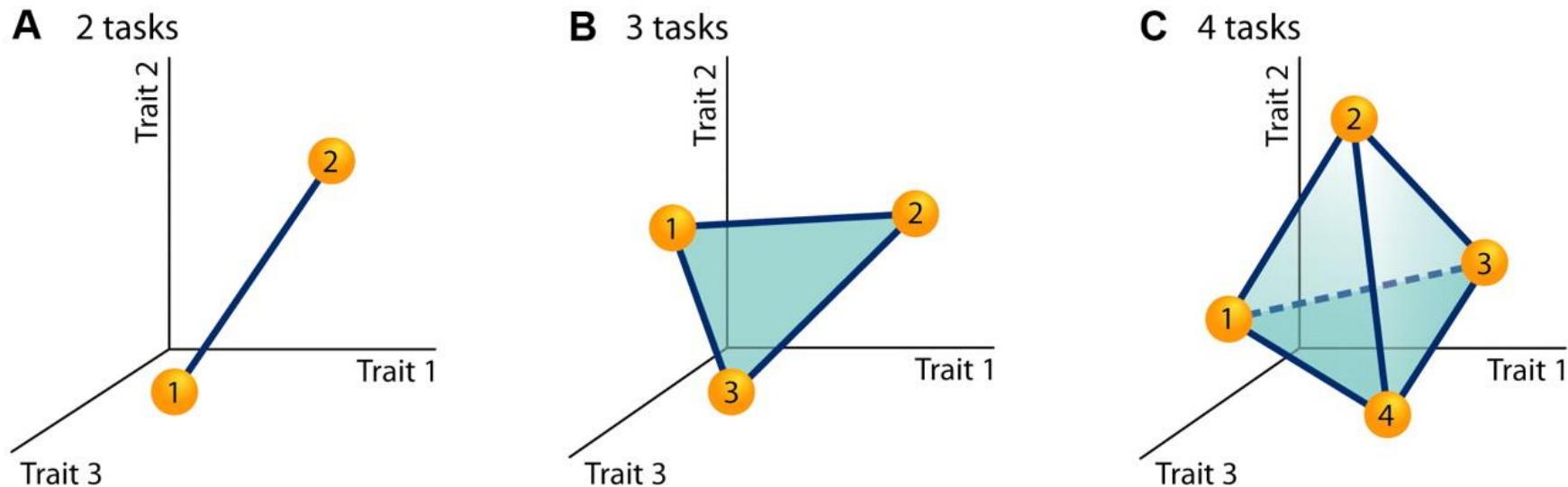


Evolutionary trade-offs and the geometry of phenotypic space (Shoval et al. Science 2012)

# Tumor ecology & evolution | A genotype-phenotype map

## Step 1: build a phenotypic map of MPM

Method: Archetypal analysis

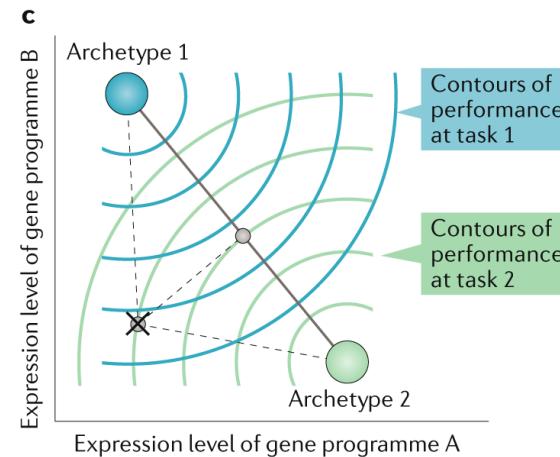
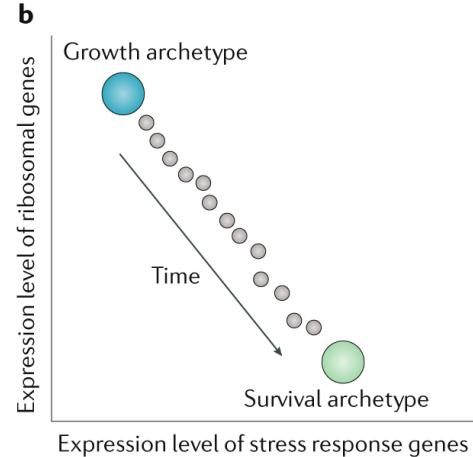
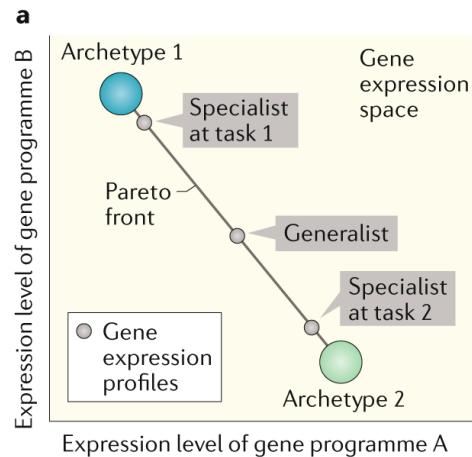


Evolutionary trade-offs and the geometry of phenotypic space (Shoval et al. Science 2012)

# Tumor ecology & evolution | A genotype-phenotype map

## Step 1: build a phenotypic map of MPM

Method: Archetypal analysis

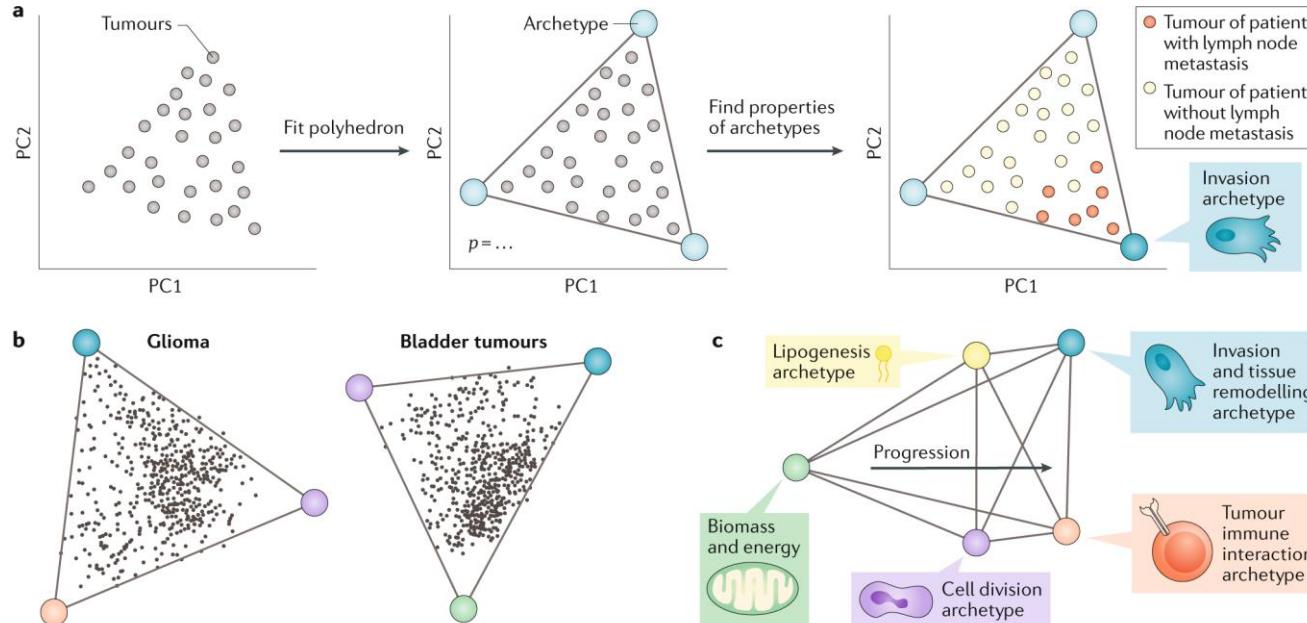


Evolutionary trade-offs in cancer. Expression is considered informative about cell function and thus a proxy for cancer phenotype (Hausser and Alon Nat Rev Cancer 2020)

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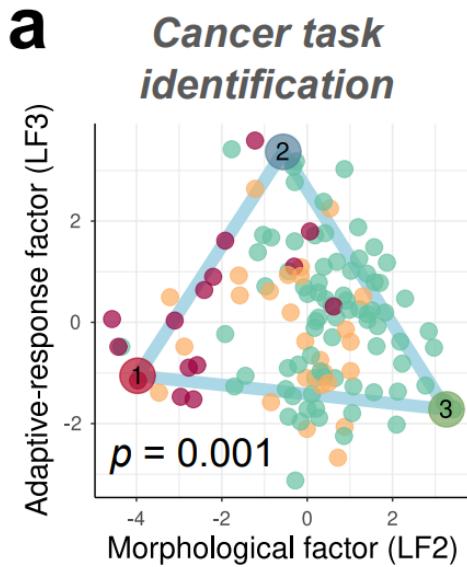


PCA is used as an unsupervised identification of latent expression variables that act as a proxy for cancer phenotypes (Hausser and Alon Nat Rev Cancer 2020)

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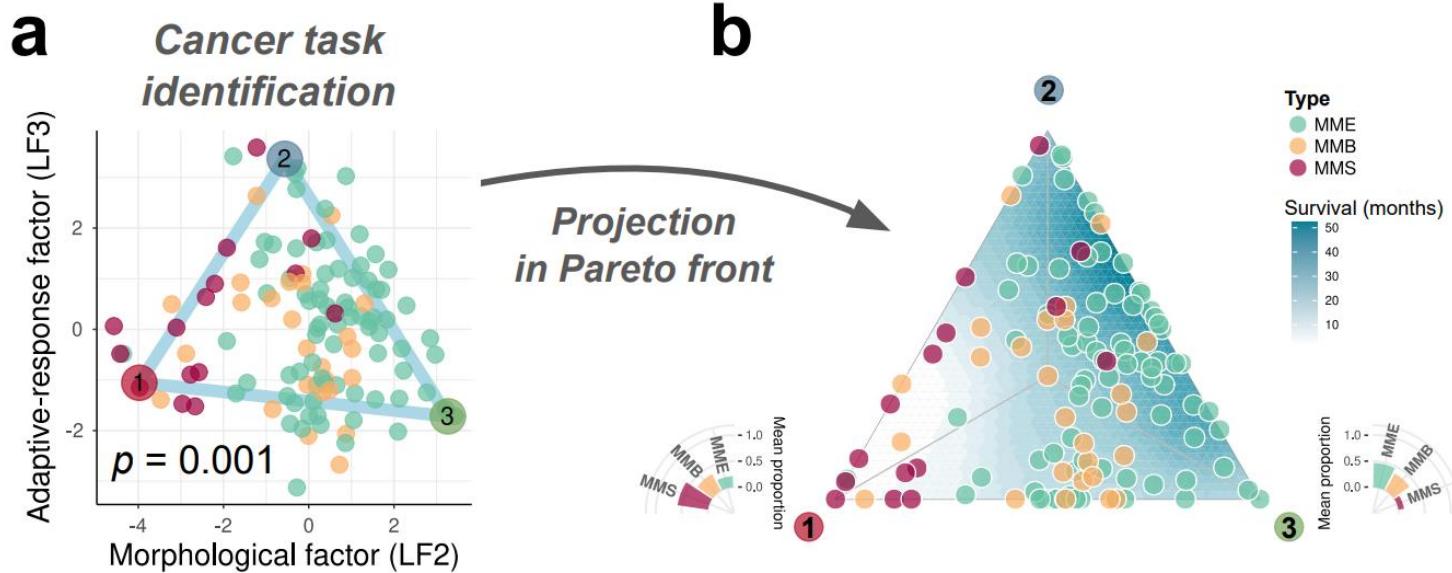


Evolutionary trade-offs in the MESOMICS cohort

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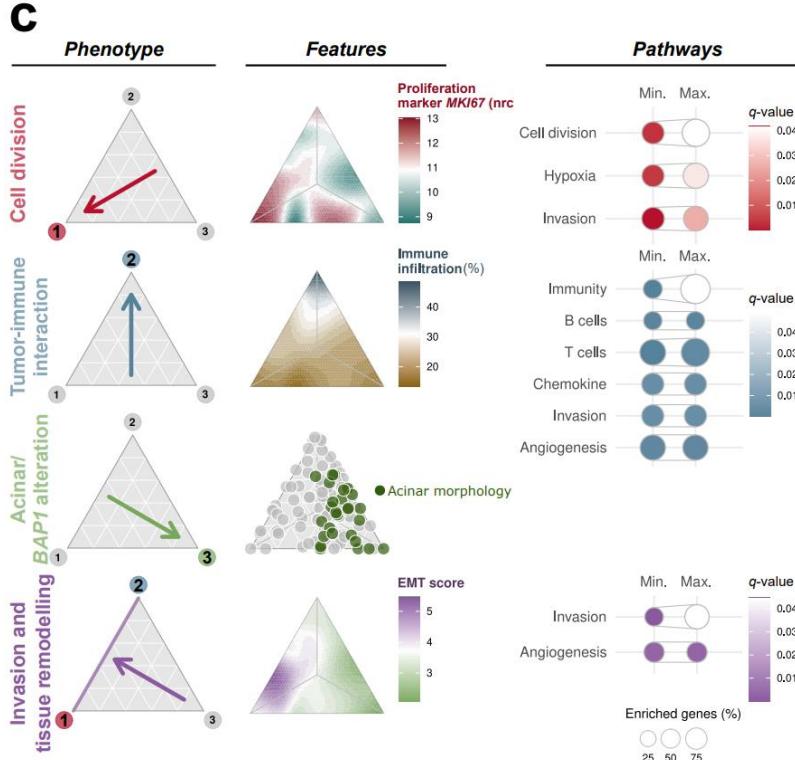


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# Tumor ecology & evolution | A genotype-phenotype map

## Step 1: build a phenotypic map of MPM

Method: Gene set enrichment analysis of archetypes, association with cell morphology



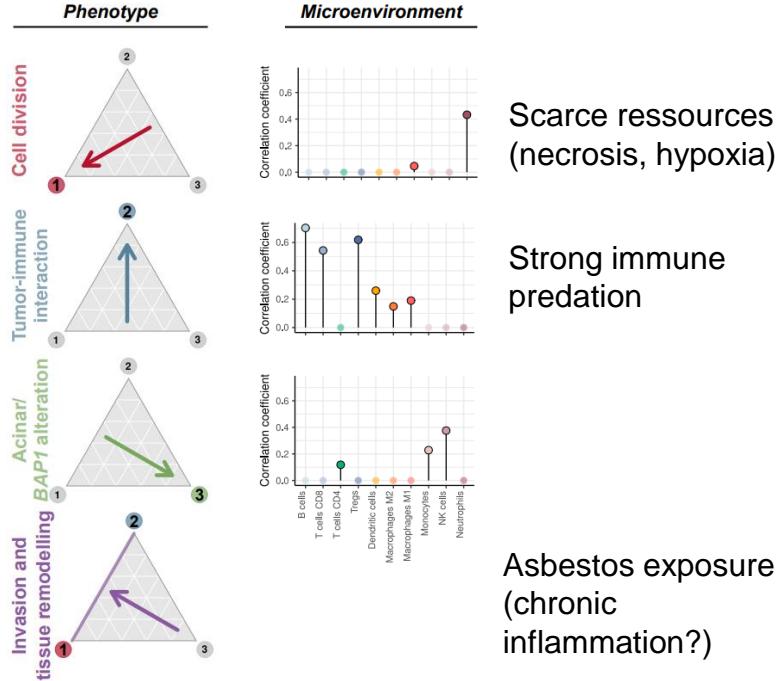
Evolutionary trade-offs in the MESOMICS cohort

# Tumor ecology & evolution | A genotype-phenotype map

## Step 2: infer microenvironmental conditions of each phenotype

Method: Gene set enrichment analysis of archetypes, association with clinical variables

C

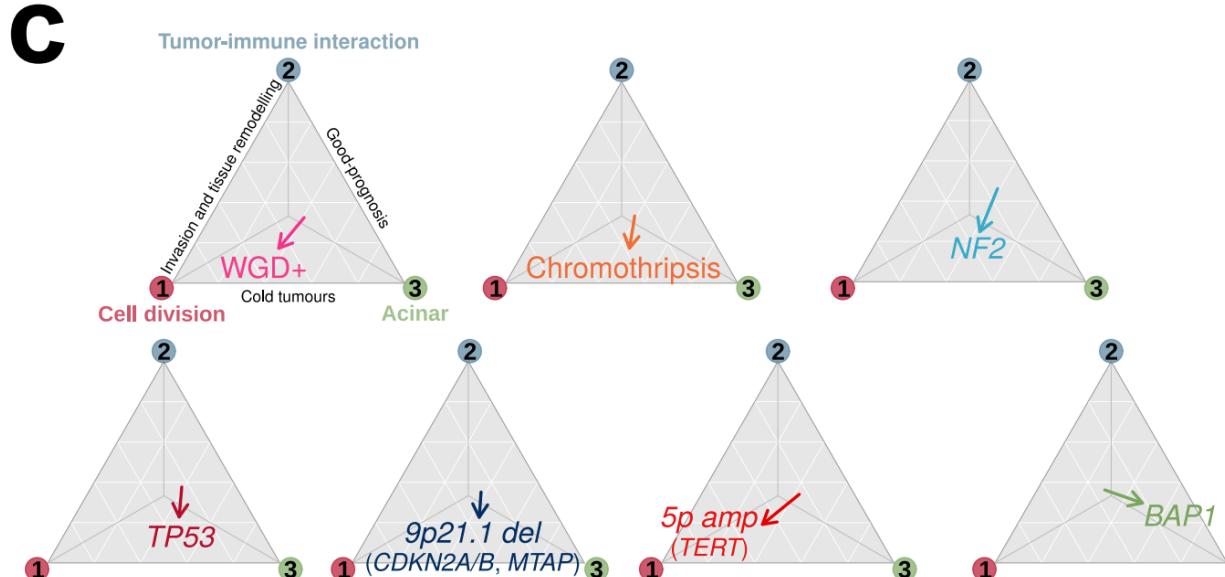


Microenvironments associated with MPM archetypes

# Tumor ecology & evolution | A genotype-phenotype map

## Step 3: find genomic alterations associated with each phenotype

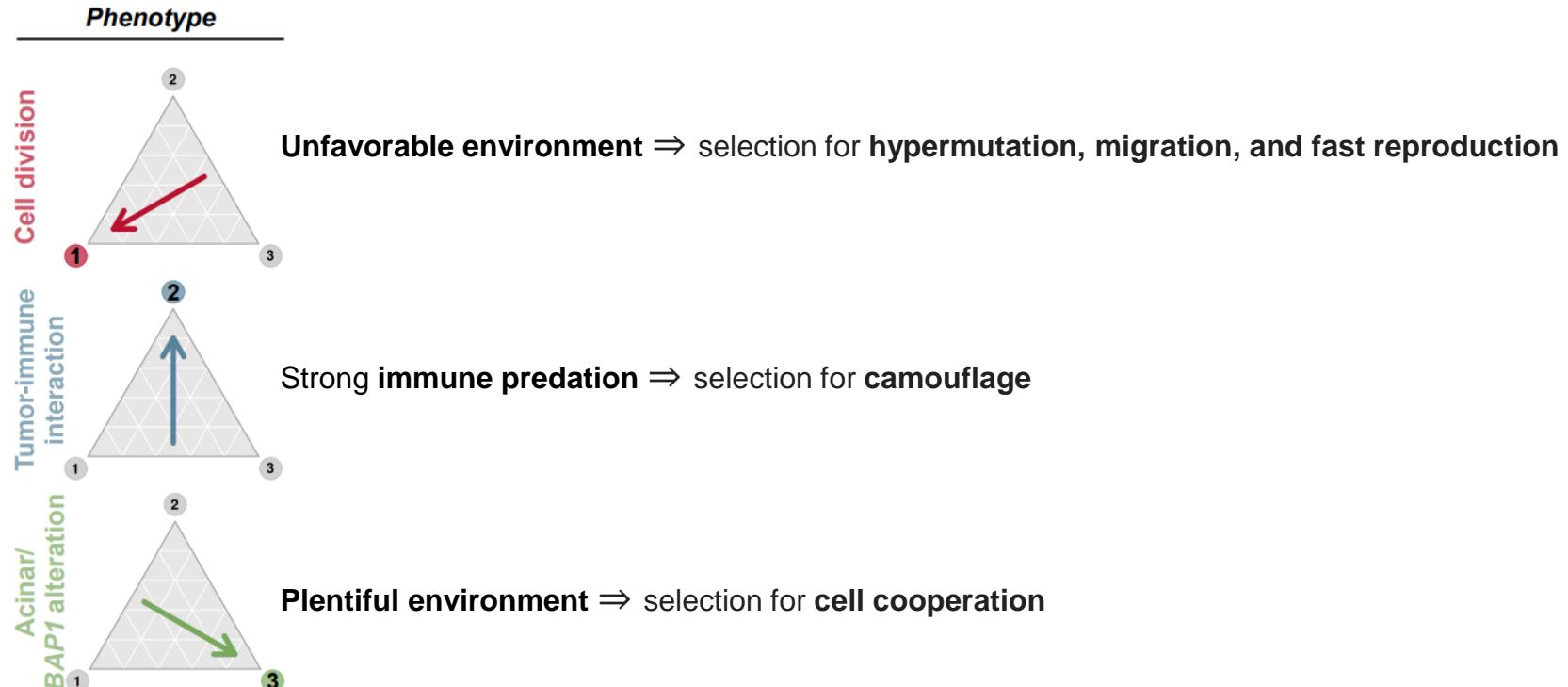
Method: Compute “effect vectors” linking WT and altered samples in phenotypic space



Genomic events tune phenotypic specialization in MPM

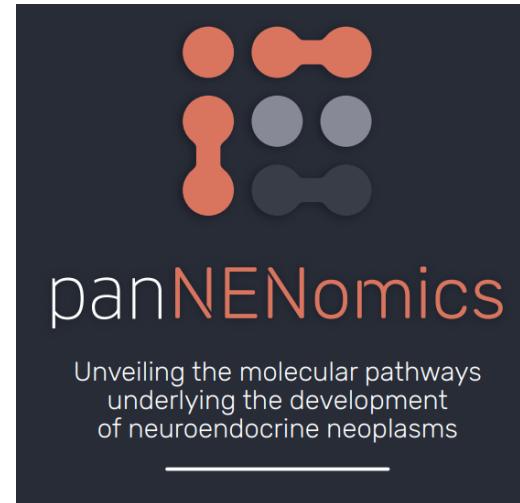
# Tumor ecology & evolution | A genotype-phenotype map

## Step 4: evolutionary hypotheses



# Tumor ecology & evolution

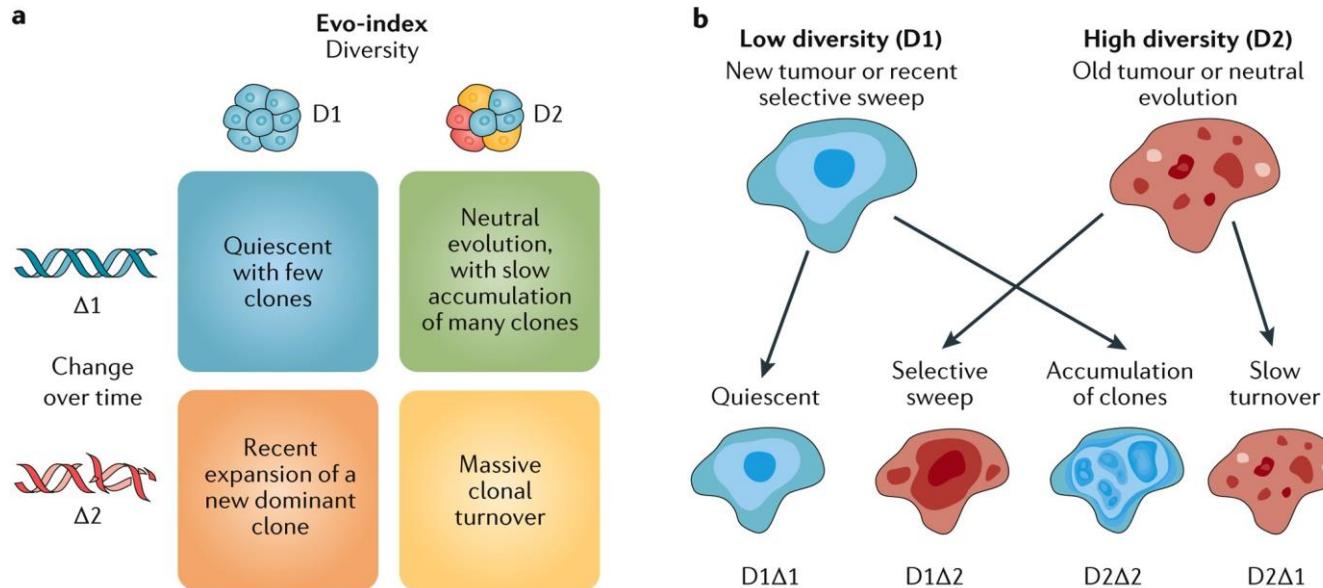
*Application II: developing cancer-cell-population-genetic statistics*



Unveiling the molecular pathways  
underlying the development  
of neuroendocrine neoplasms

# Tumor ecology & evolution | Statistics for cancer genomics

## On the importance of Intra-Tumor Heterogeneity (ITH)

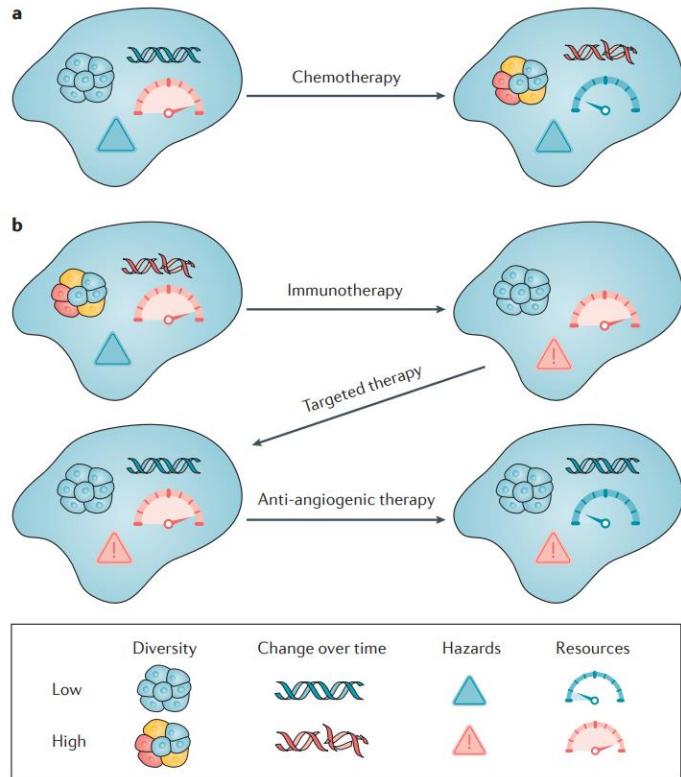


Nature Reviews | Cancer

Proposition of classification of tumors based on an evolutionary index (Malley et al. Nat Rev Cancer 2017)

# Tumor ecology & evolution | Statistics for cancer genomics

## On the importance of Intra-Tumor Heterogeneity (ITH)



Monitoring ITH is informative on “evolvability” and response to treatment (Malley et al. Nat Rev Cancer 2017)

# Tumor ecology & evolution | Statistics for cancer genomics

## On the importance of Intra-Tumor Heterogeneity (ITH)

Table 1 | Measures and assays for the factors that go into the Evo- and Eco-indices

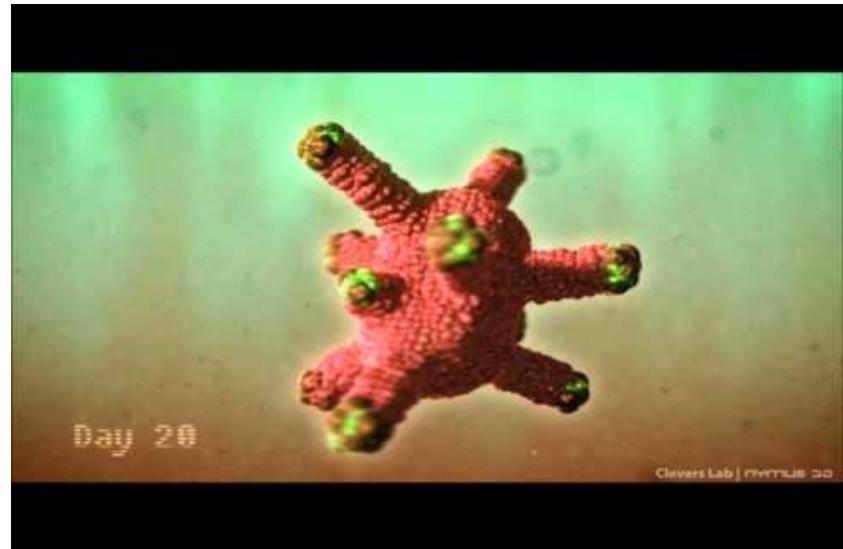
Icon	Factor	Statistics	Assays
High 	Diversity (D)	<ul style="list-style-type: none"><li>• Divergence<sup>12–14,46</sup></li><li>• Number of clones (richness)<sup>6,12–14</sup></li><li>• Shannon index<sup>12–14</sup></li><li>• Simpson's index<sup>12,13</sup></li><li>• Functional diversity<sup>115,169,170</sup></li><li>• Phylogenetic trees<sup>20,61–63</sup></li></ul>	<ul style="list-style-type: none"><li>• Whole-exome and whole-genome sequencing</li><li>• Multi-region sequencing</li><li>• SNP arrays</li><li>• Methylation arrays</li><li>• FISH</li><li>• Single-cell DNA and RNA sequencing</li><li>• Cell-free DNA sequencing<sup>19</sup></li><li>• RNA-Seq</li><li>• Proteomics</li><li>• Radiology</li></ul>
Low 			
High 	Change over time ( $\Delta$ )	<ul style="list-style-type: none"><li>• Mutation rates<sup>17,178</sup></li><li>• Estimates of selection<sup>17,179</sup></li><li>• Clonal expansion rates<sup>14</sup></li><li>• <math>F_{ST}</math> (REF. 60)</li><li>• Nei's standard genetic distance<sup>57,58</sup></li><li>• Change in above diversity statistics</li></ul>	<ul style="list-style-type: none"><li>• Longitudinal sampling</li><li>• Whole-exome and whole-genome sequencing</li><li>• Cell-free DNA analysis<sup>19</sup></li></ul>
Low 			

Some statistics have been proposed to monitor ITH but no real consensus (Malley et al. Nat Rev Cancer 2017)

# Tumor ecology & evolution | Statistics for cancer genomics

## Application: monitoring Intra-Tumor Heterogeneity (ITH) in tumor organoids

- > **Organoids** are "mini-organs" that summarize *in vitro* the structure of a tissue
- > **Groundbreaking medical applications**, such as regenerative medicine, personalized treatment<sup>1</sup>
- > Allow to create **mini-tumors** (Patient-Derived Tumor Organoids—PDTOs) to **model disease** with unprecedented detail

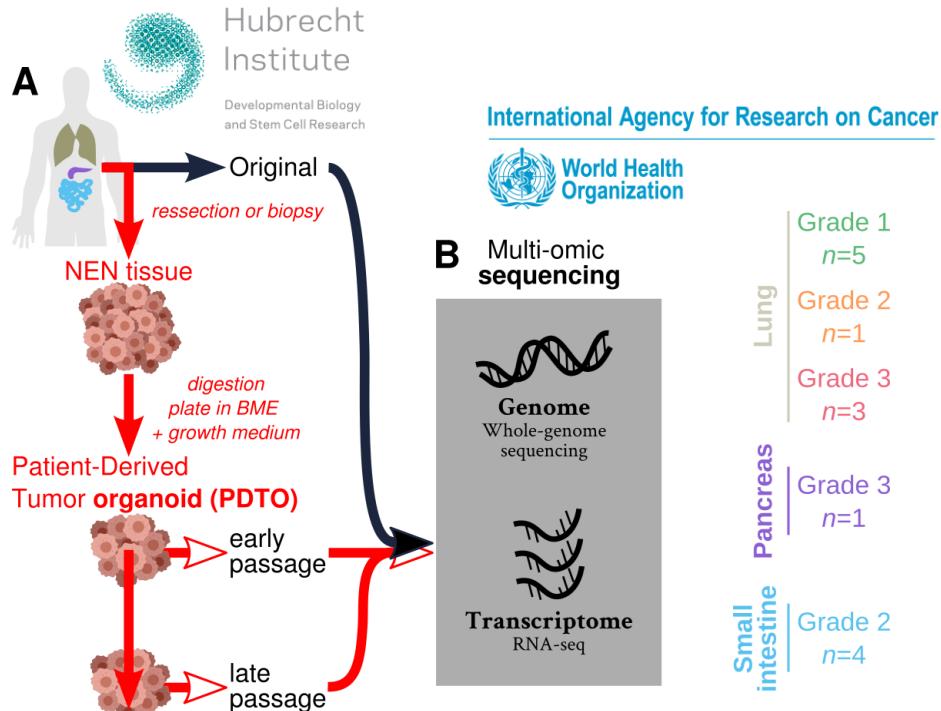


**Mini-gut organoids.** Clevers group, Hubrecht Institute.

<sup>1</sup>Tuveson & Clevers *Science* 2019

# Tumor ecology & evolution | Statistics for cancer genomics

> In collaboration with the Clevers group, we are **validating a biobank of neuroendocrine neoplasms (NEN) PDTOs<sup>2</sup>**



<sup>2</sup>Dayton, Alcala, et al. In prep

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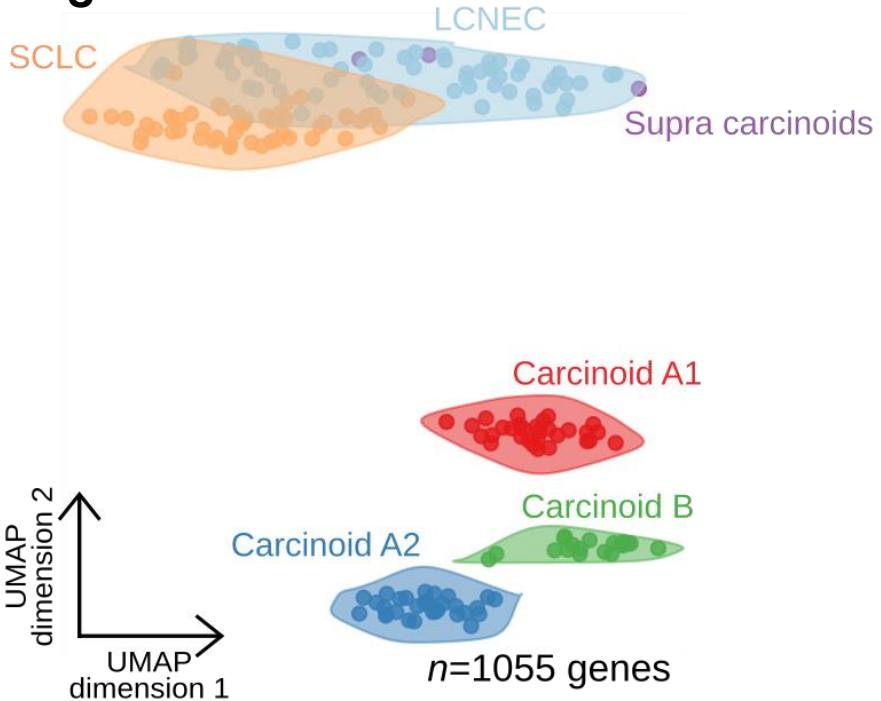
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## Lung



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<sup>3</sup>Alcala, Leblay, Gabriel et al. *Nat Commun* 2019

<sup>4</sup>Laddha et al. *Cancer Res* 2019

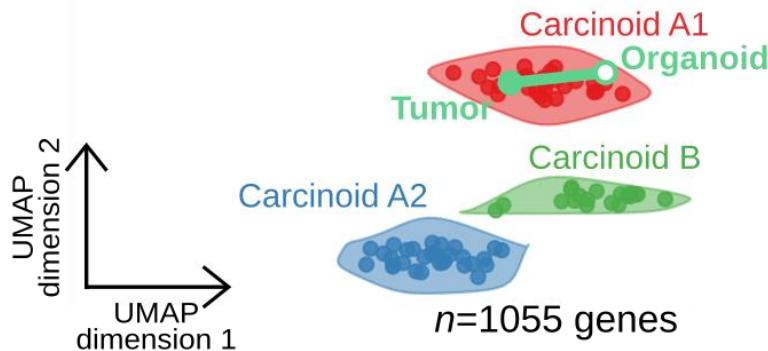
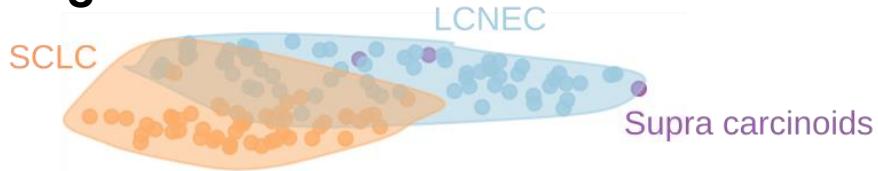
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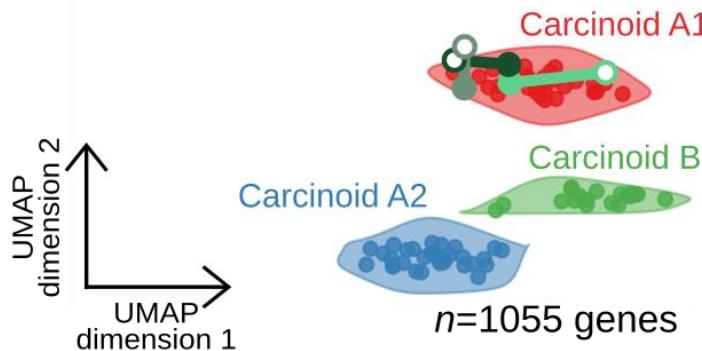
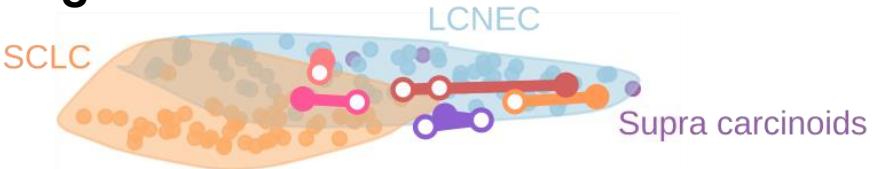
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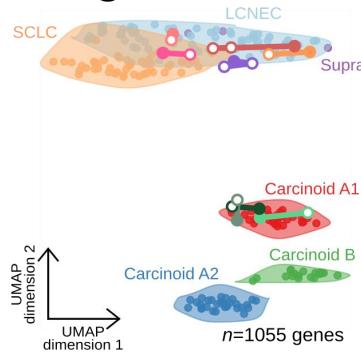
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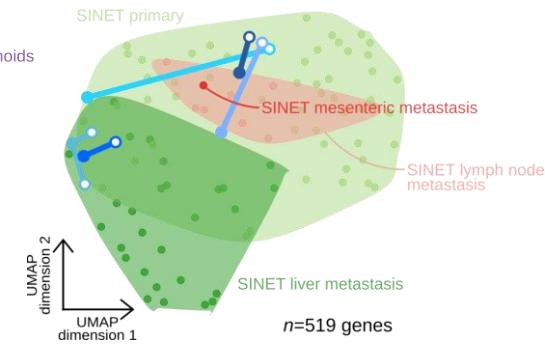
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## Lung



## Small Intestine



<sup>2</sup>Dayton, Alcala, et al. In prep

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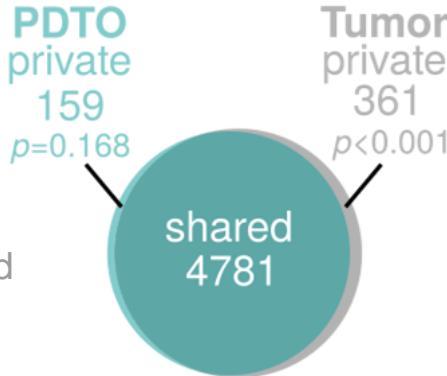
Compare the genetic composition of Tumor and PDTO

Usual strategy: the Tumor Mutational Burden (TMB), the proportion of shared SNVs

**Pros:** easy to compute and interpret

**Cons:** very sensitive to low frequency variants, so might not allow accurate comparisons

## Experiment LNET6



<sup>2</sup>Dayton, Alcala, et al. In prep

# Tumor ecology & evolution | Statistics for cancer genomics

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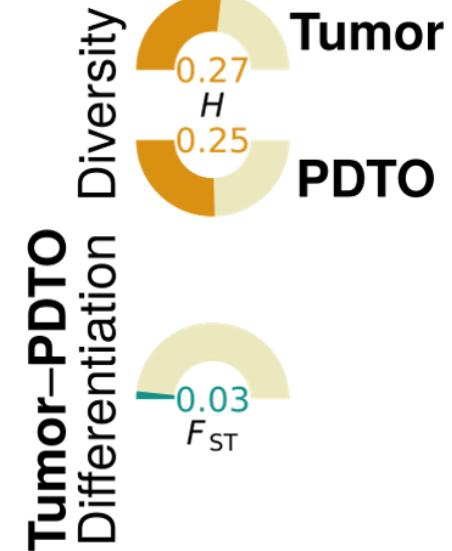
Alternative: use classical population-genetic statistics on within-tumor allelic frequencies (proportion of tumor cells carrying an allele)

Experiment LNET6

PDTO  
private  
159  
 $p=0.168$

Tumor  
private  
361  
 $p<0.001$

shared  
4781



<sup>2</sup>Dayton, Alcala, et al. In prep

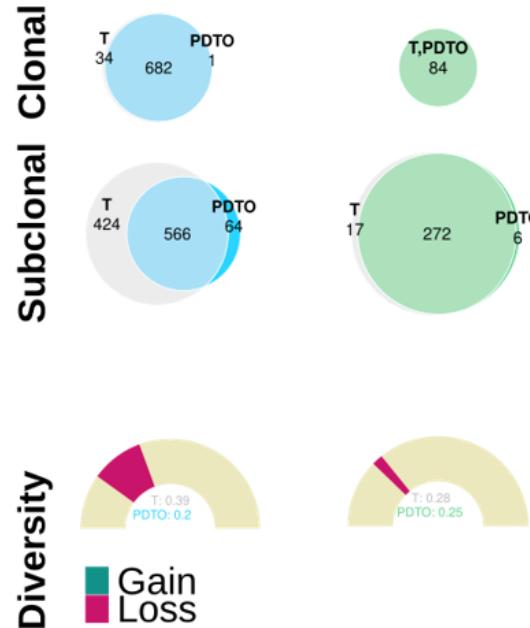
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Compare the genetic composition of Tumor and PDTO

Issue: while *FST* and *H* allow within-tumor comparisons, because they rely on average levels of diversity across loci, they are difficult to compare between tumors



<sup>2</sup>Dayton, Alcala, et al. In prep

# Tumor ecology & evolution | Statistics for cancer genomics

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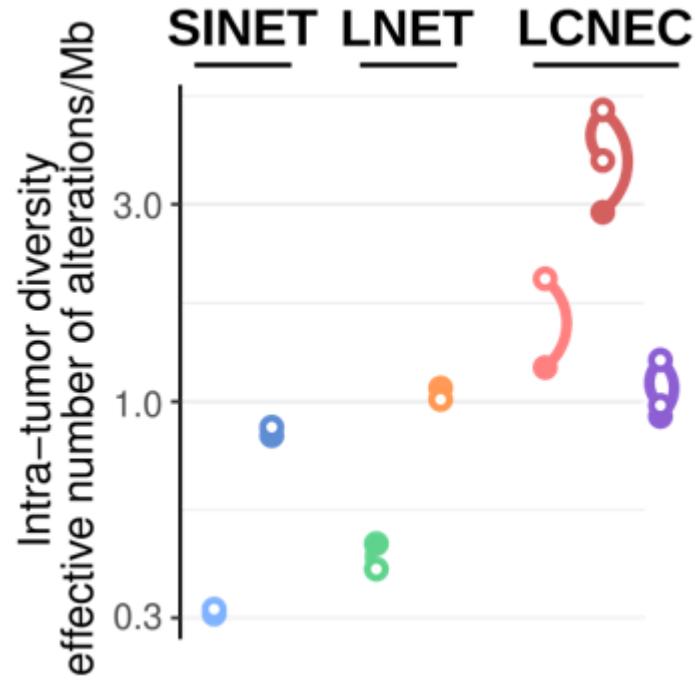
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Compare the genetic composition of Tumor and PDTO

Tentative solution: compute an effective tumor mutational burden, as the effective number of alleles -1 ( $\Delta$ -1) at each polymorphic locus, and sum across all variable positions.

**Pros:** value in [0,TMB].

**Cons:** for now still sensitive to tumor purity and does not follow some desirable properties of  $\Delta$



<sup>2</sup>Dayton, Alcala, et al. In prep

# Tumor ecology & evolution | Statistics for cancer genomics

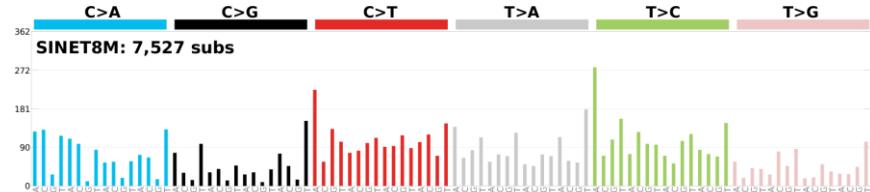
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- Q3** Do PDTOs **conserve the mutational processes** of the original tumor?

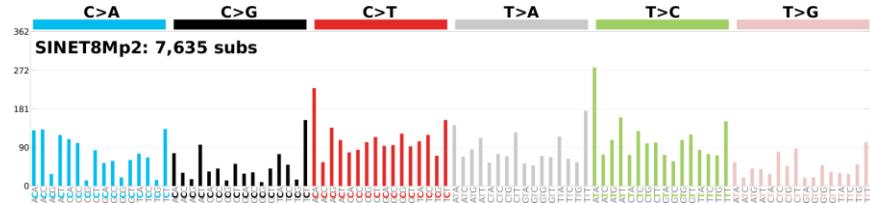
Compare the processes at work before and after PDTO formation

## Experiment SINET8

### Parental tumor



### Organoid



<sup>2</sup>Dayton, Alcala, et al. In prep

# Tumor ecology & evolution | Statistics for cancer genomics

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**Q1** Do PDTOs **conserve the global molecular profile** of the original tumor?



## Signatures

- SBS1
- SBS5
- SBS8
- SBS2&13
- SBS18

Age

Replication errors?

APOBEC

ROS

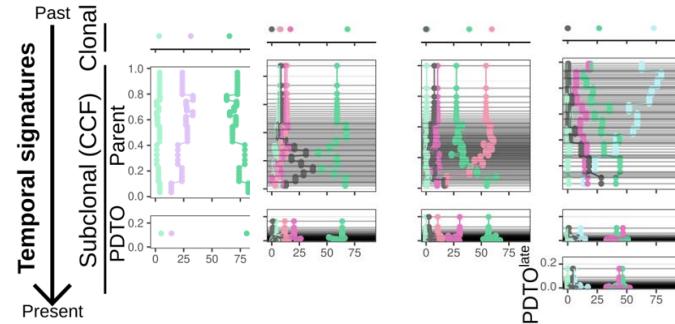
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**Q3** Do PDTOs **conserve the mutational processes** of the original tumor?



Compare the processes at work before and after PDTO formation

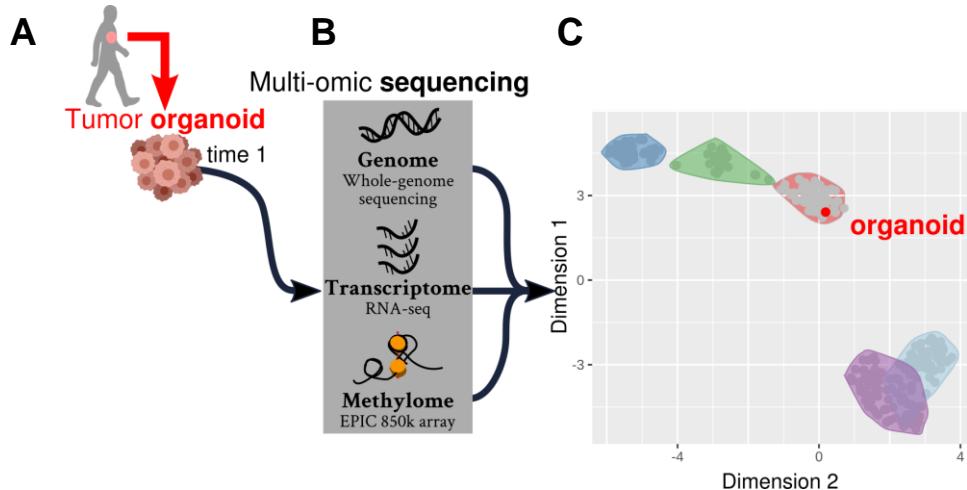


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# Tumor ecology & evolution | Statistics for cancer genomics

> Use the established **NEN biobank** to model disease initiation and progression

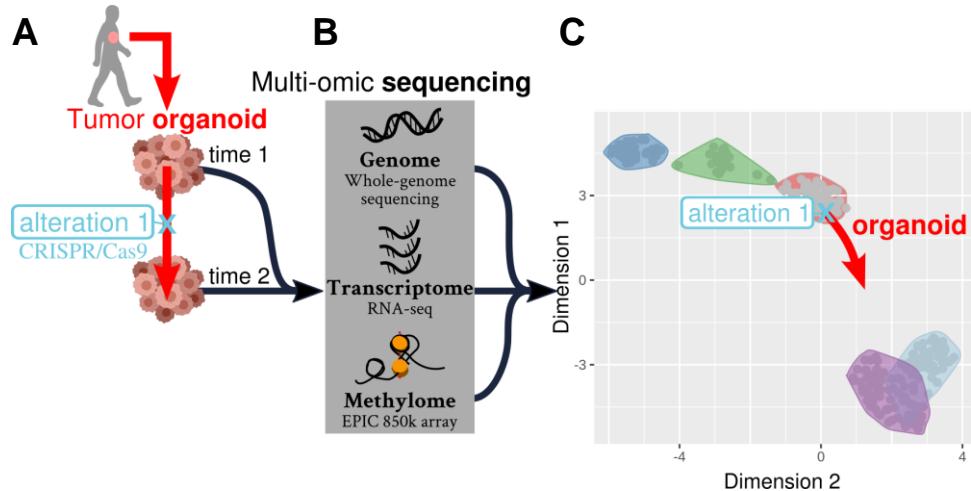
Introduce candidate alterations using the **CRISPR/Cas9 "genetic scissors"** and monitor impact with multi-omic analyses



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