



**Journées du Cancéropôle Est 2023**

# Hétérogénéité tumorale

**Pr Celine Mascaux, MD, PhD**

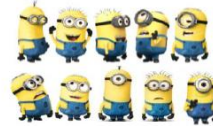
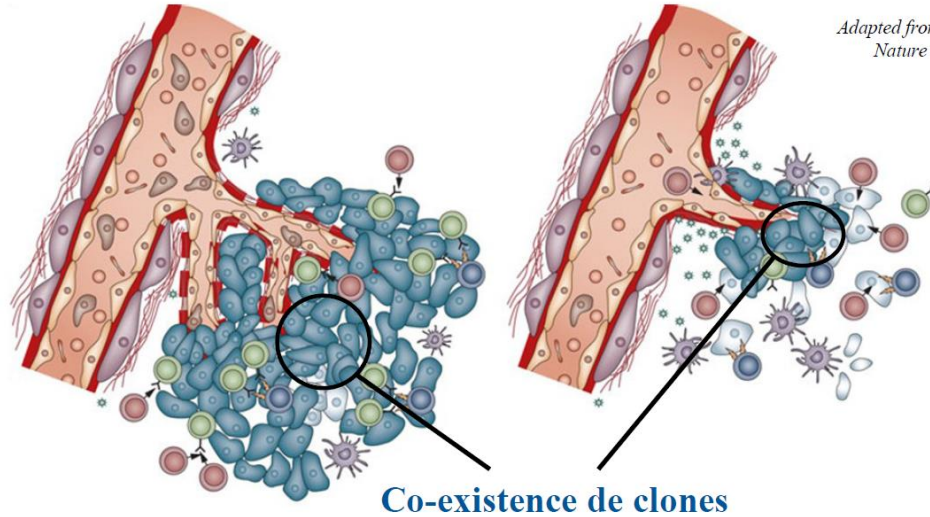
**Service de pneumologie, Hôpitaux Universitaires de Strasbourg**

**Inserm UMR 1260**

**[celine.mascaux@chru-strasbourg.fr](mailto:celine.mascaux@chru-strasbourg.fr)**

# Hétérogénéité intra-tumorale

*Adapted from André N, Carré M and Pasquier E,  
Nature Reviews Clinical Oncology 2014*



**De quoi s'agit-il exactement?**

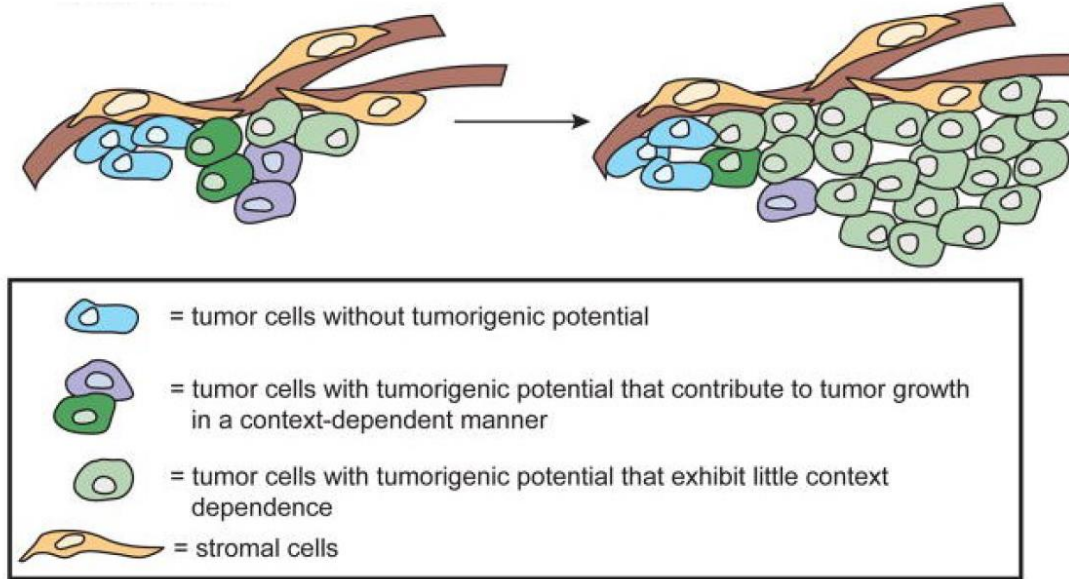
**Quelle(s) source(s) à l'origine de cette hétérogénéité ?**

**Défi à la médecine personnalisée ou obstacle clinique insurmontable ?**

**Comment prendre en compte l'hétérogénéité intra-tumorale en recherche pré-clinique?**

## Hétérogénéité intratumorale = diversité clonale au sein même d'une masse tumorale

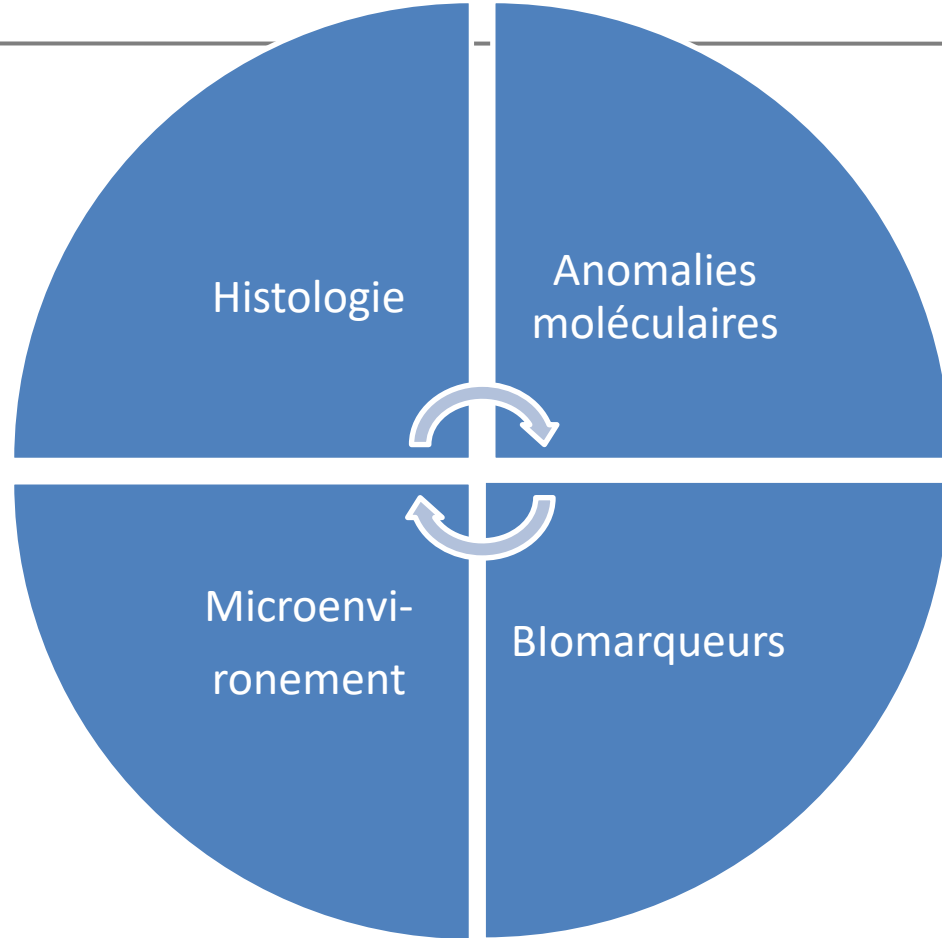
Une majorité de ces clones existe déjà dans les cancers nouvellement diagnostiqués



Meacham CE & Morrison SJ,  
*Nature* 2013

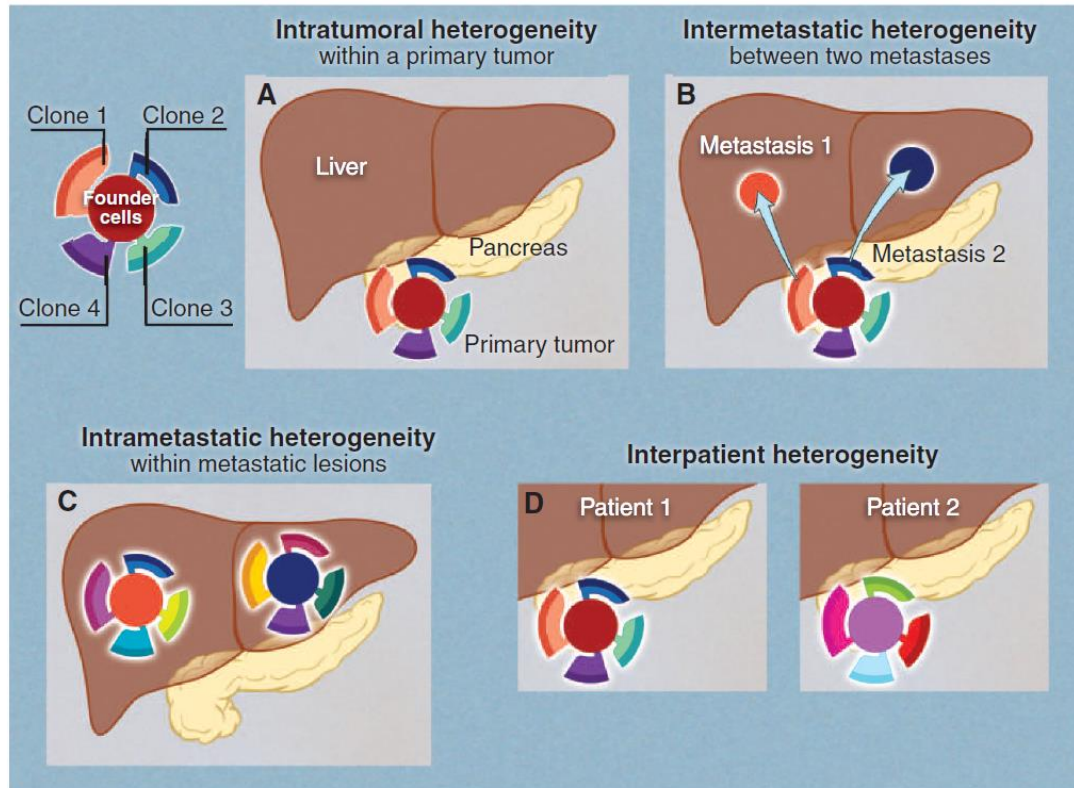
# Hétérogénéité tumorale

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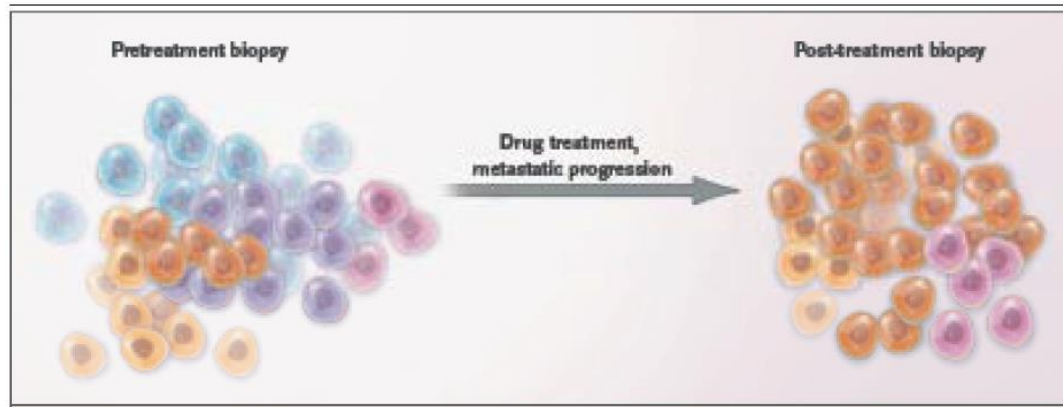




# Hétérogénéité tumorale



# Les biomarqueurs évoluent au cours du temps

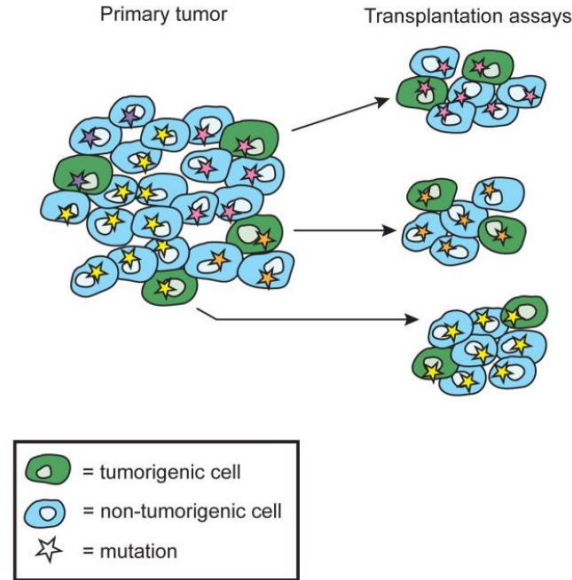


- Est-ce dû à l'hétérogénéité tumorale?
  - Développement de clones résistants sélectionnés par le traitement?
- Est-ce dû à l'échantillonnage?
  - Certains échantillons seraient sous le seuil de détection?
  - Ou variations réelles?

Mais finalement que se passe-t'il ?

## Cellules souches cancéreuses: modèle hiérarchique

**Exclusivité des CSC comme source de l'hétérogénéité intra-tumorale???**



**hhmi** | Howard Hughes  
Medical Institute

Published as: *Nature*, 2013 September 19; 501(7467): 328-337.

**Tumor heterogeneity and cancer cell plasticity**

Corbin E. Meacham and Sean J. Morrison  
Howard Hughes Medical Institute, Children's Research Institute and Department of Pediatrics,  
University of Texas Southwestern Medical Center, Dallas, Texas, 75390, USA

CSC tumorigéniques :

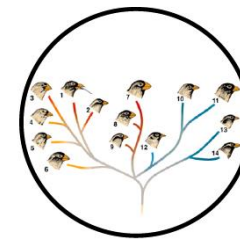
- **incapables de reproduire à elles seules l'hétérogénéité génétique complexe des tumeurs**
- donnent naissance à des **tumeurs organisées selon une hiérarchie mais génétiquement distinctes**



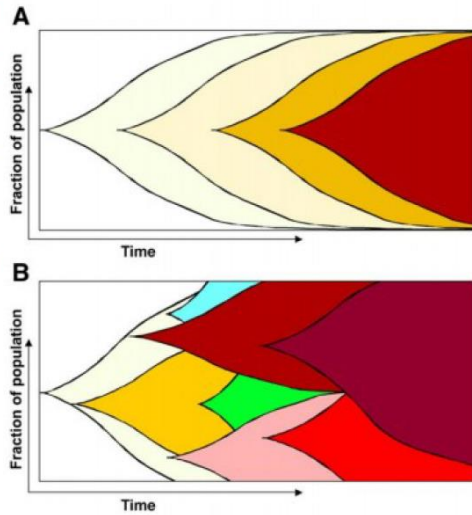
**mécanismes indépendants de la différenciation des CSC ?**



# Evolution clonale: modèle Darwinien



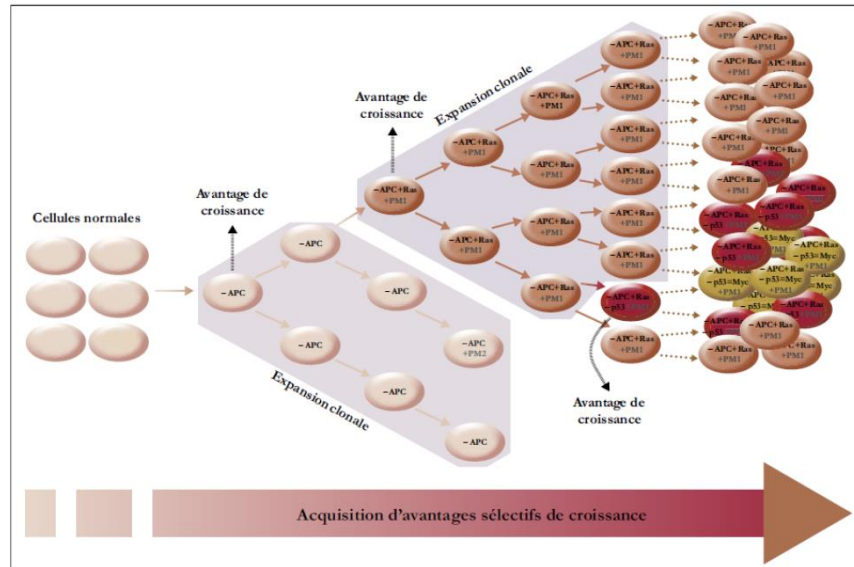
*Tumor heterogeneity: Causes and consequences.*  
 Marusyk A & Polyak K. BBA 2010



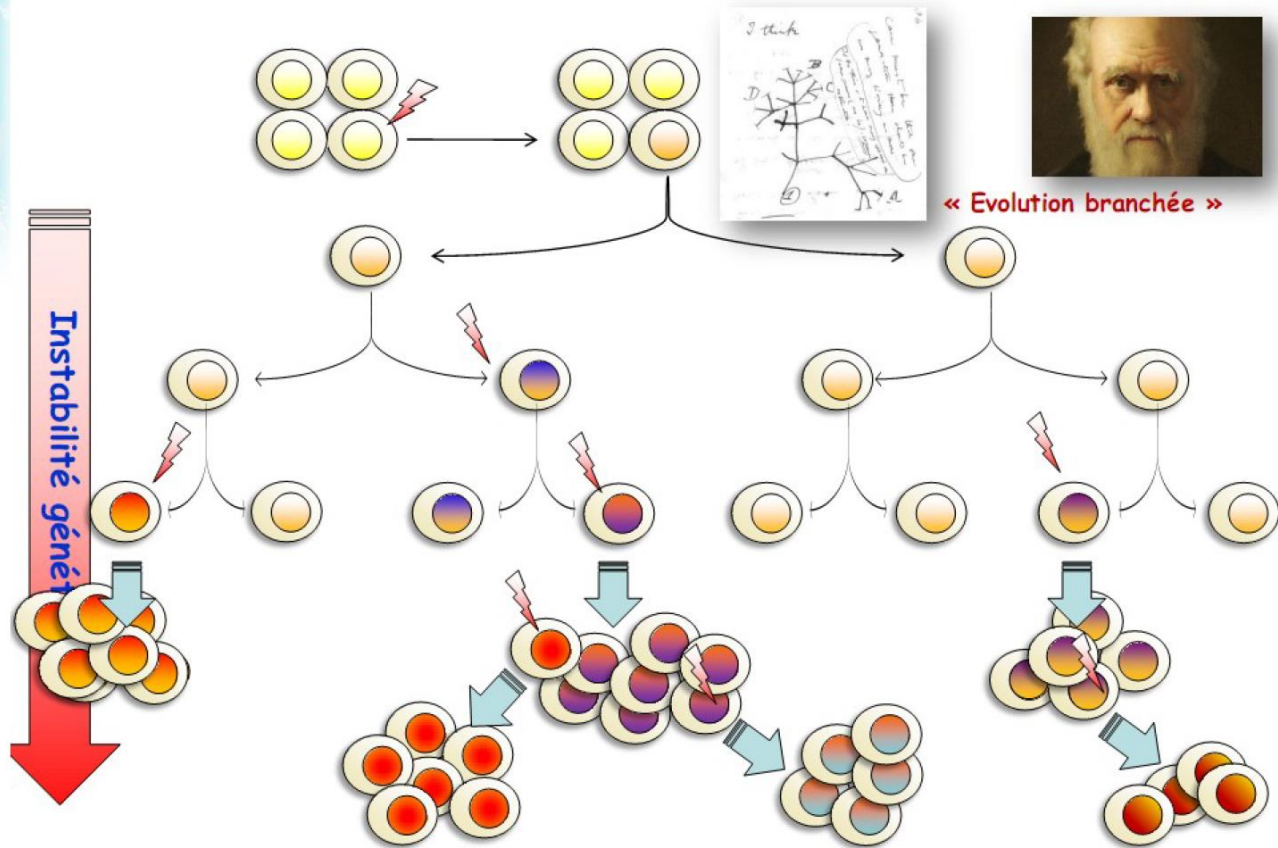
Sélection des clones tumoraux en fonction de leurs capacités prolifératrices (peu de hiérarchie): co-existence de différents clones

Instabilité génomique élevée mais seule une **minorité de mutations confère un avantage sélectif**

*Schéma d'évolution monoclonale (A) & multiclonale (B)*



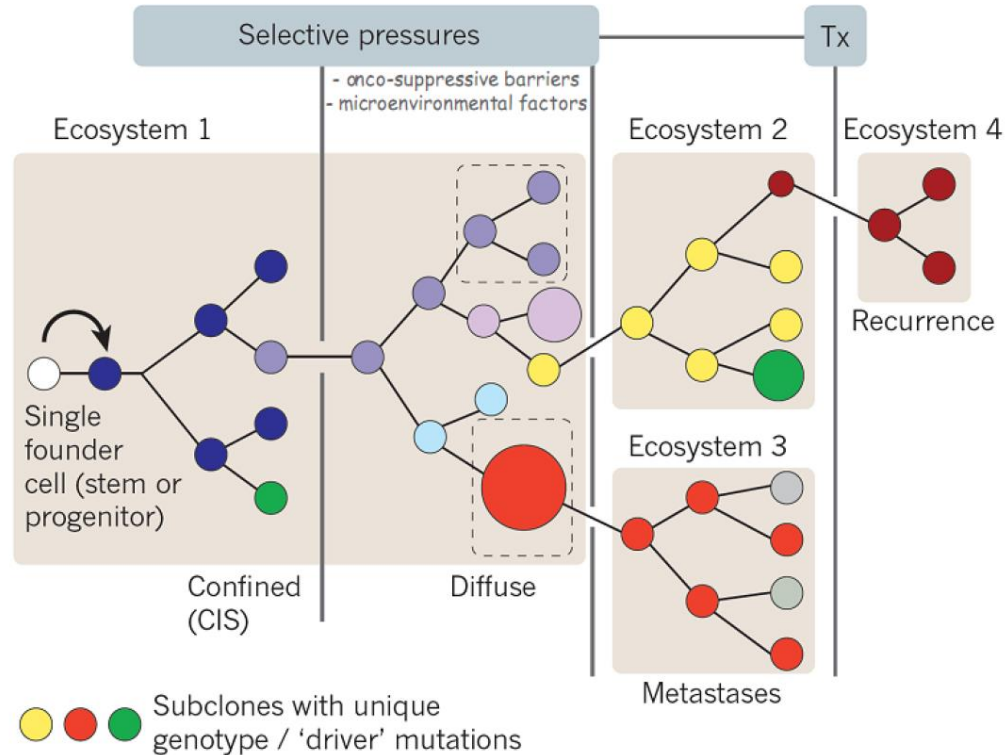
## Evolution clonale: modèle Darwinien

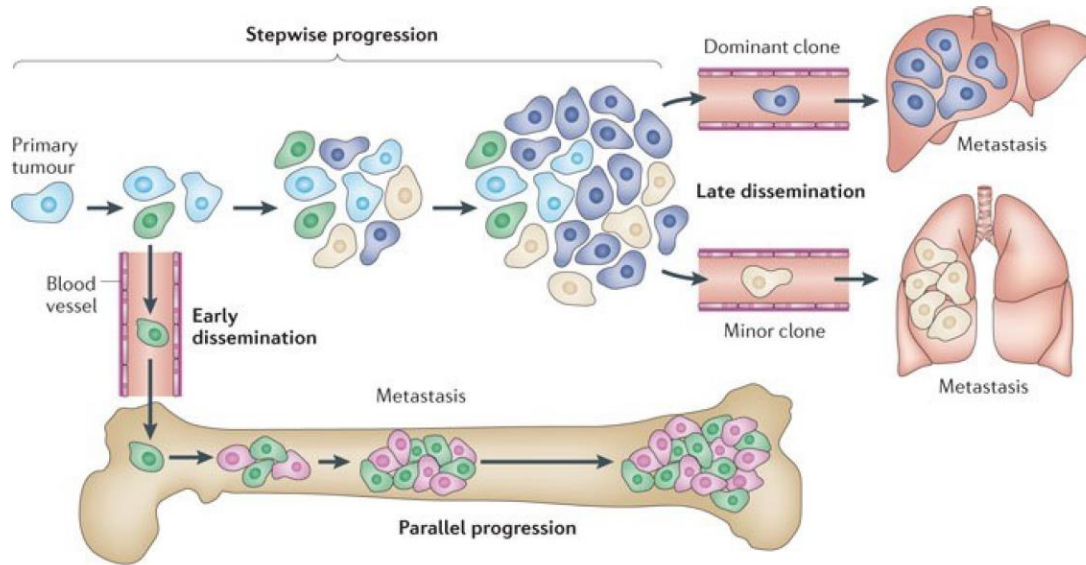




## Une conciliation possible?

CSC et sélection clonale pourraient contribuer à l'hétérogénéité intra-tumorale



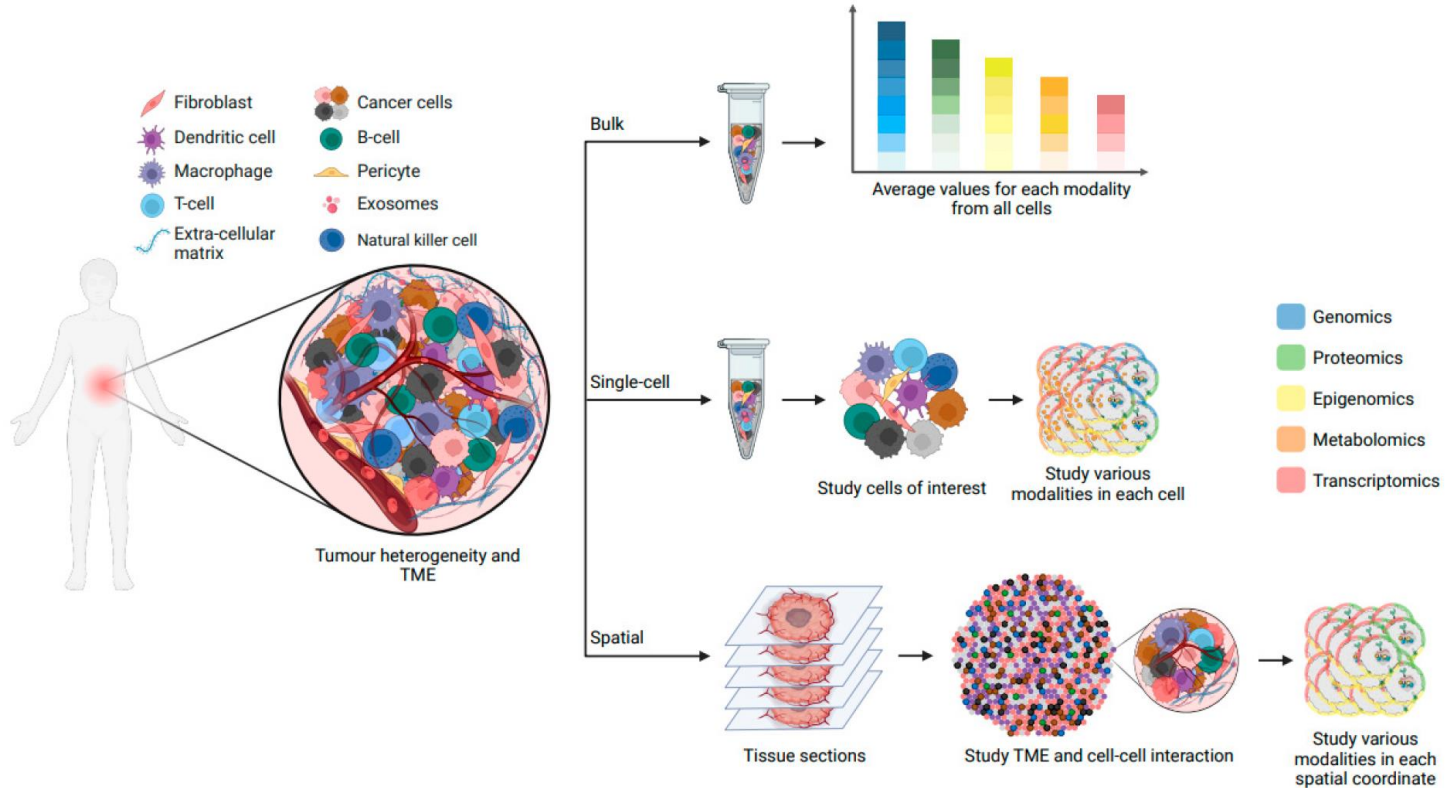


Marusyk A et al. Nat Rev Cancer 2012

**Relations clonales entre tumeur primaire et métastases:** dérivent-elles directement des clones présents ou divergent-elles à un stade précoce de l'évolution?

**Degré d'hétérogénéité des métastases:** sont-elles aussi hétérogènes que les tumeurs primaires?

# Etudes en single cell pour étudier l'hétérogénéité tumorale



# Le projet TRACERx

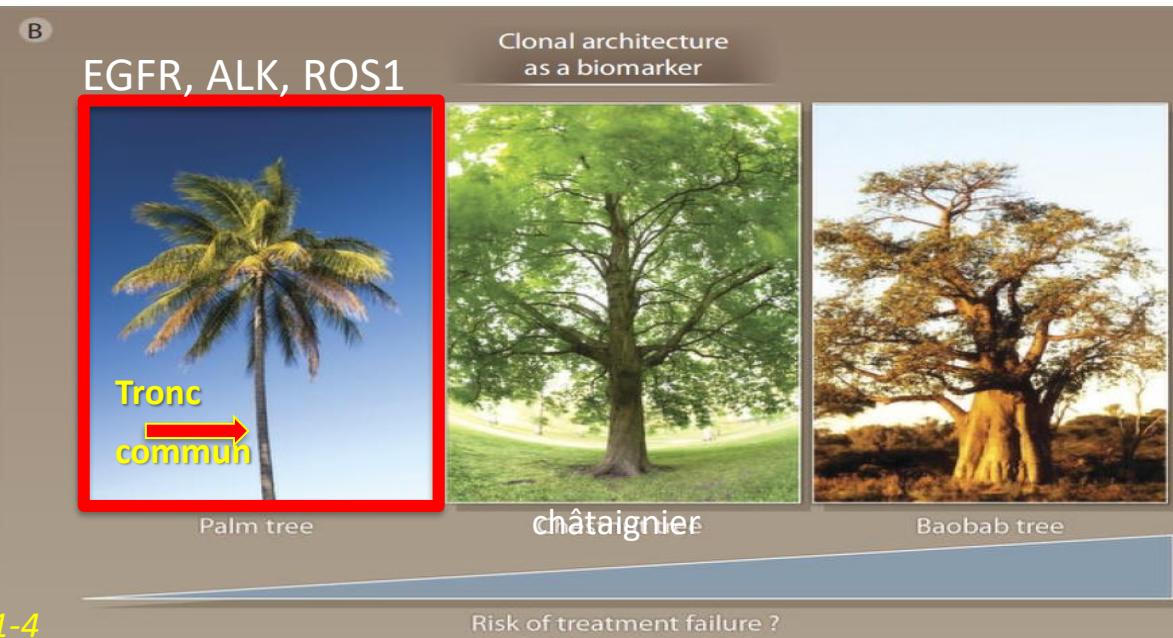
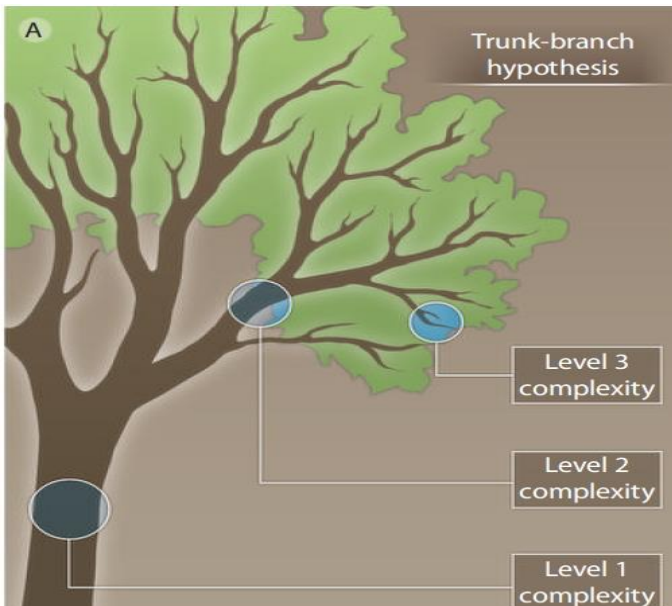
08:30 - 10:00 Mini oral session 2 - NSCLC, metastatic

CHAIRS : JESSICA LIN, TETSUYA MITSUDOMI, MARTIN RECK

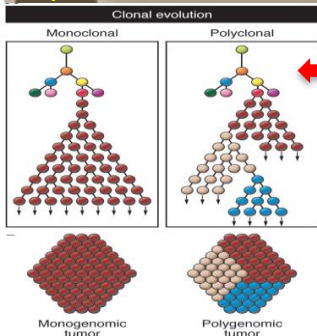


**Tetsuya Mitsudomi**  
Invited Discussant 1322MO, LBA72  
and 1323MO



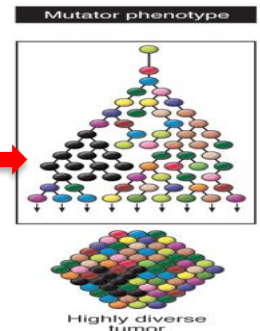


Yap TA et al. *Science Translat. Med.* 2012; 4:1-4



← **Evènements génétiques du tronc:** présents dans toutes les cellules cancéreuses: trunk drivers

**Evènements génétiques branchés:** présents dans certaines cellules cancéreuses => dynamique dans le temps



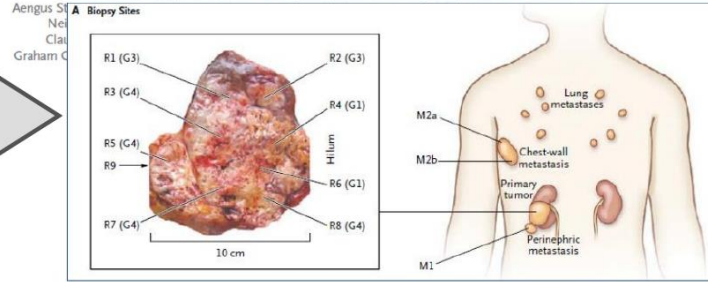
**C. Swanton**  
Francis Crick Institute  
University College London, UK

*The* **NEW ENGLAND**  
**JOURNAL** of MEDICINE

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

Intratumor Heterogeneity and Branched Evolution Revealed  
by Multiregion Sequencing

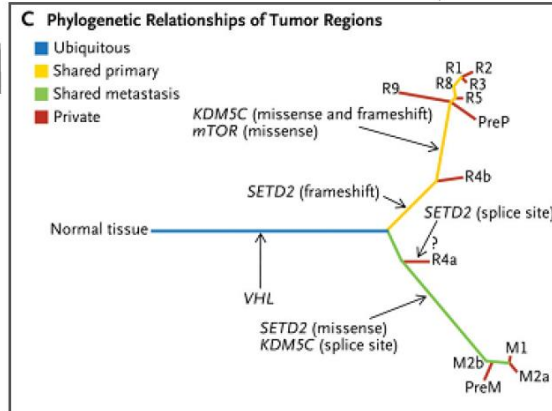
Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D.,  
David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc.,



Patients avec  
carcinomes  
rénaux ayant  
métastasé

Séquençage de  
l'exome complet  
& analyse  
transcriptomique

Pas de progression tumorale linéaire avec une  
même cellule accumulant des mutations  
génétiques successives



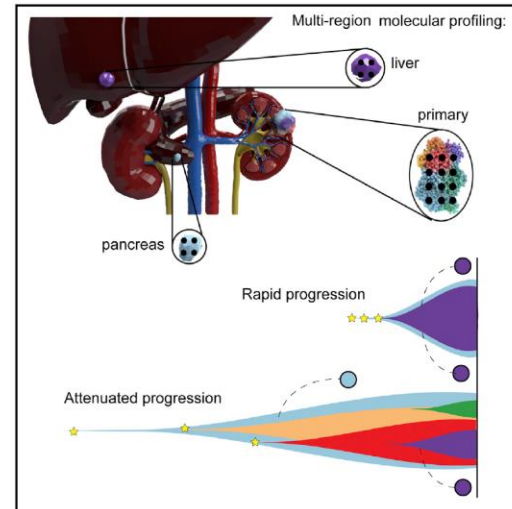
Architecture clonale évolutive générant :

- des sous-populations de **cellules malignes occupant des régions distinctes** de la même tumeur laire
- des **métastases** dont le profil mutationnel est plus proche d'une des sous-populations de la tumeur laire



## Tracking Cancer Evolution Reveals Constrained Routes to Metastases: TRACERx Renal

### Graphical Abstract



### Authors

Samra Turajlic, Hang Xu, Kevin Litchfield, ..., Charles Swanton, PEACE, the TRACERx Renal Consortium

### Correspondence

charles.swanton@crick.ac.uk

### In Brief

A multi-center prospective study and two validation cohorts of matched primary metastasis biopsies from 100 patients with clear-cell renal cell carcinoma provides a comprehensive picture of the genetic underpinnings and the evolutionary patterns of metastasis.

### Highlights

- Evolutionary study of matched primary metastasis biopsies from 100 ccRCC cases
- Metastasis competence is afforded by chromosome complexity, but not driver mutation load
- The hallmark genomic drivers of ccRCC metastasis are loss of 9p and 14q
- Punctuated and branched evolution result in distinct patterns of metastases

Metastasising clone: wGII: ↑↑ Ki67: ↑↑ Loss 9p, 14q: ↑↑

**“Punctuated Evolution”**  
Rapid Progression

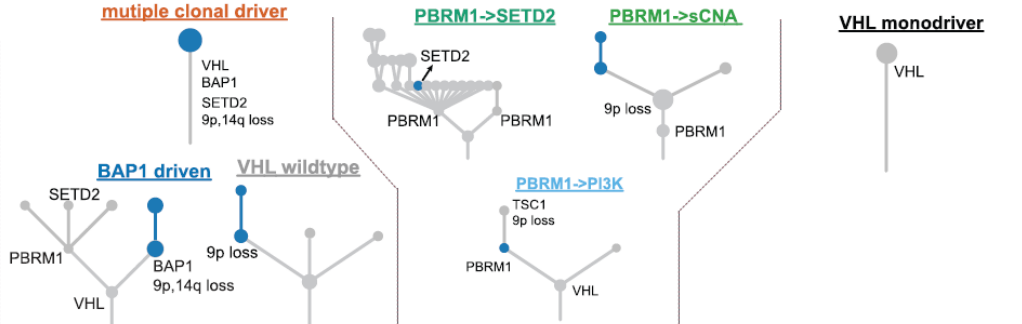
**“Branched Evolution”**  
Attenuated Progression

**“Linear Evolution”**

Metastasis



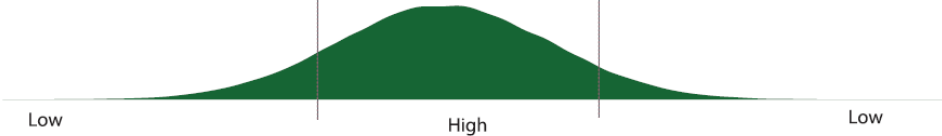
Primary tumour



Chromosomal complexity



Intratour heterogeneity



# Tracking Cancer Evolution through the Disease Course

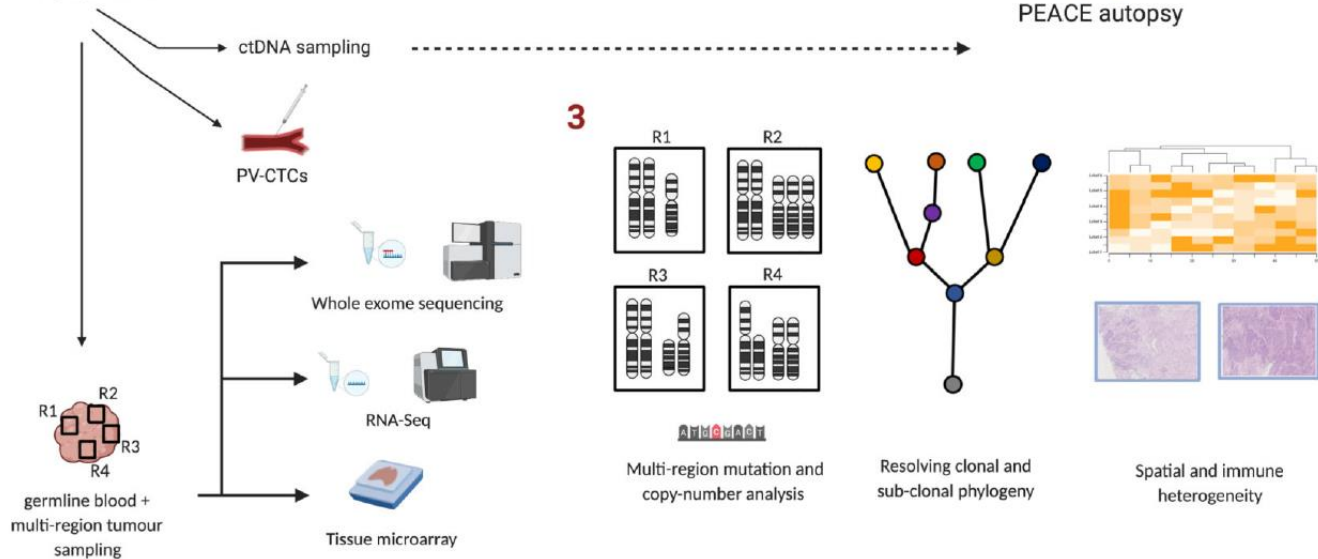
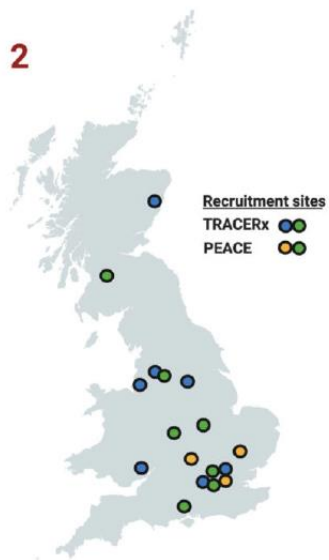
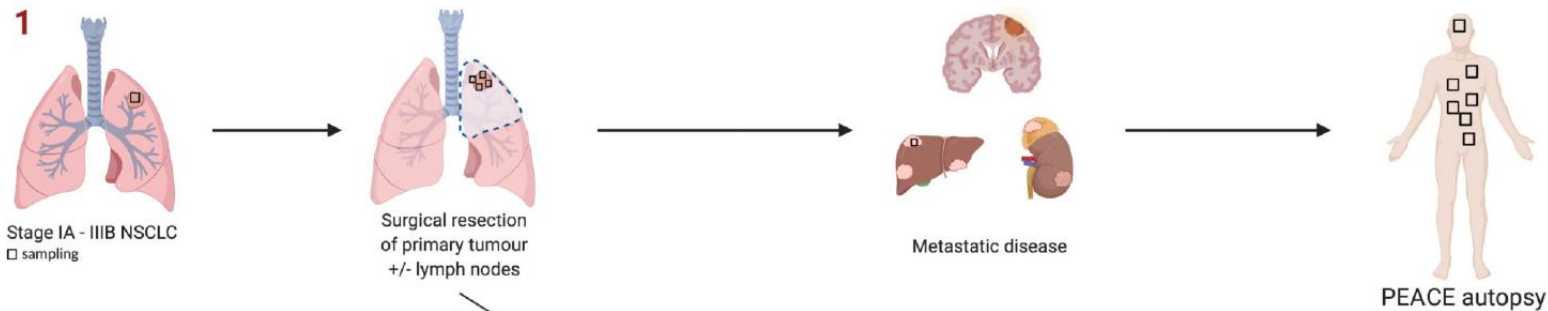
**Chris Bailey<sup>#1</sup>, James RM. Black<sup>#2,6</sup>, James L. Reading<sup>3</sup>, Kevin Litchfield<sup>4</sup>, Samra Turajlic<sup>5</sup>,  
Nicholas McGranahan<sup>2,6</sup>, Mariam Jamal-Hanjani<sup>6,7</sup>, Charles Swanton<sup>1,6,7</sup>**

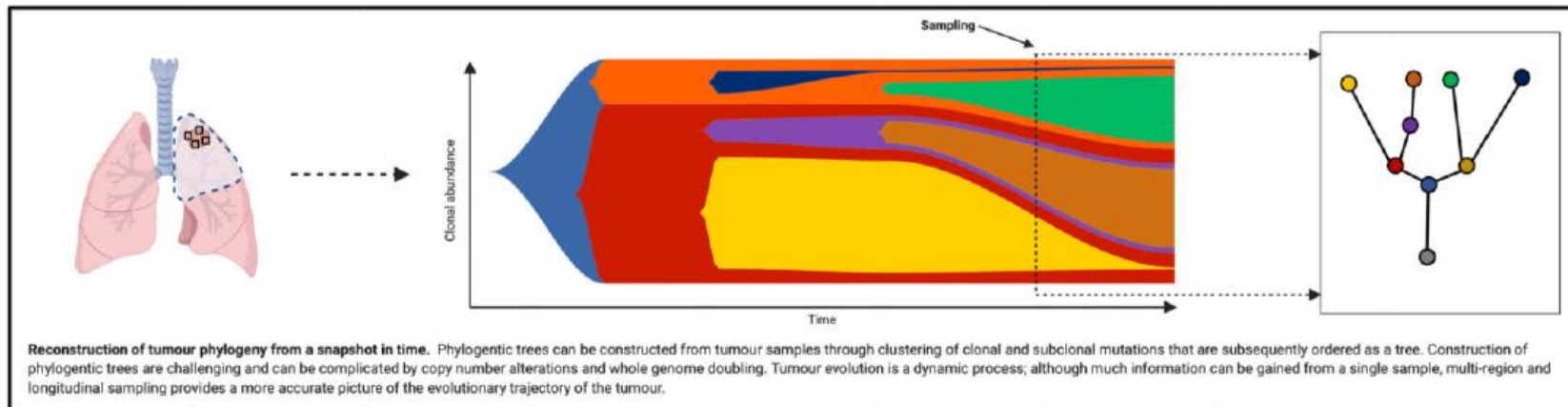
<sup>1</sup>Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute, London, United Kingdom

<sup>2</sup>Cancer Genome Evolution Research Group, University College London Cancer Institute, University College London, London, United Kingdom

<sup>3</sup>Research Department of Haematology, University College London Cancer Institute, University College London, London, United Kingdom

*Cancer Discov.* 2021 April 01; 11(4): 916–932. doi:10.1158/2159-8290.CD-20-1559.



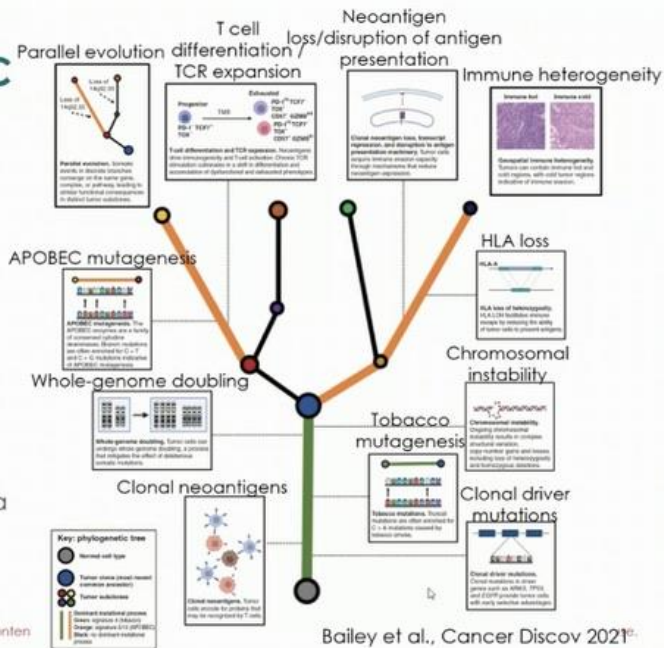


08:30 - 10:00 Mini oral session 2 - NSCLC, metastatic

CHAIRS : JESSICA LIN, TETSUYA MITSUDOMI, MARTIN RECK

# Tumor evolution in NSCLC

- intratumour heterogeneity (ITH) fuels lung cancer evolution which is mainly driven by tobacco exposure and later by APOBEC mutagenesis
- Cancers evolve and adapt to environmental challenges such as immune surveillance and treatment pressures, leading to immune evasion and resistance to therapy.
- TRACERx has revealed the potential therapeutic utility of targeting clonal neoantigens and ctDNA detection as a MRD detection



Bailey et al., Cancer Discov 2021



**Tetsuya Mitsudomi**  
 Invited Discussant 1322MO, LBA72 and 1323MO



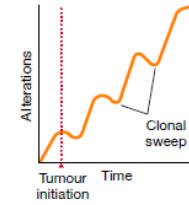
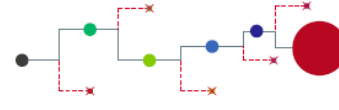
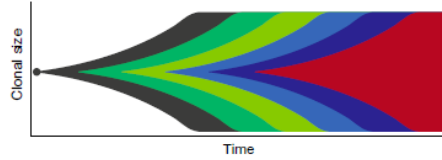
**Cancer Reviews 2021 series**

# Cancer evolution: Darwin and beyond

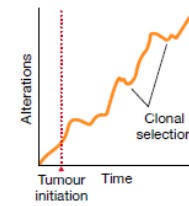
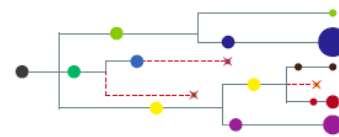
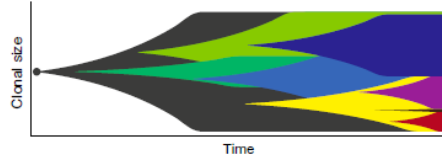
Roberto Vendramin<sup>1</sup> , Kevin Litchfield<sup>1,\*</sup> & Charles Swanton<sup>1,2,\*\*</sup>

# Modèles d'évolution tumorale

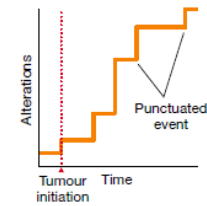
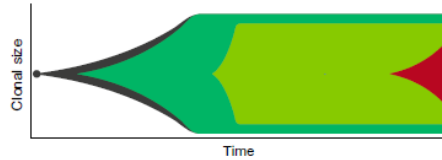
**A Linear evolution**



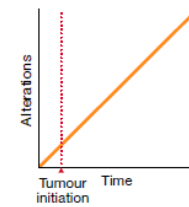
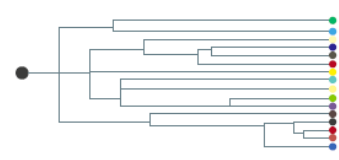
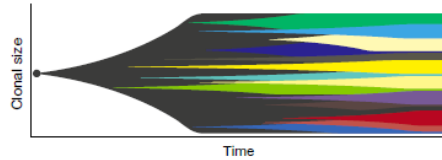
**B Branched evolution**



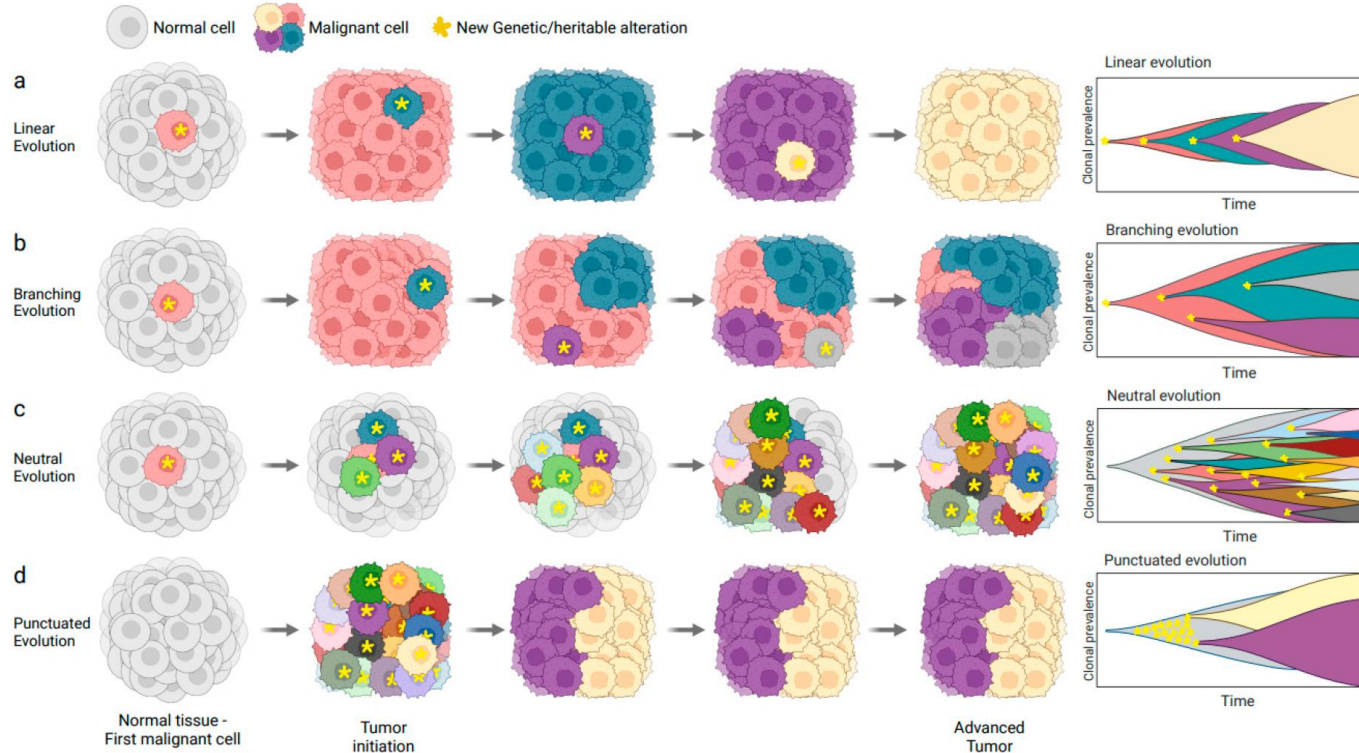
**C Macroevolution**

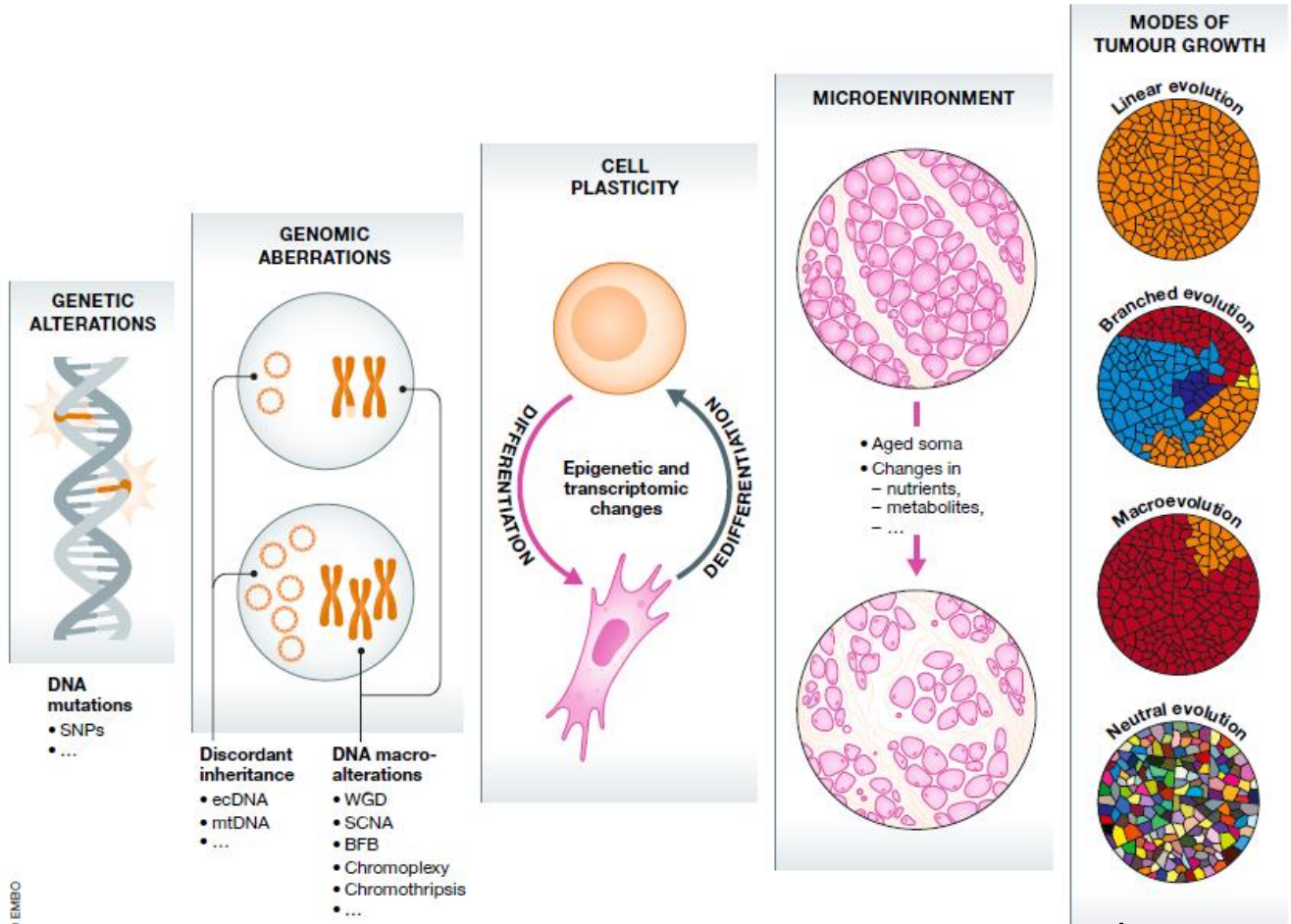


**D Neutral evolution**



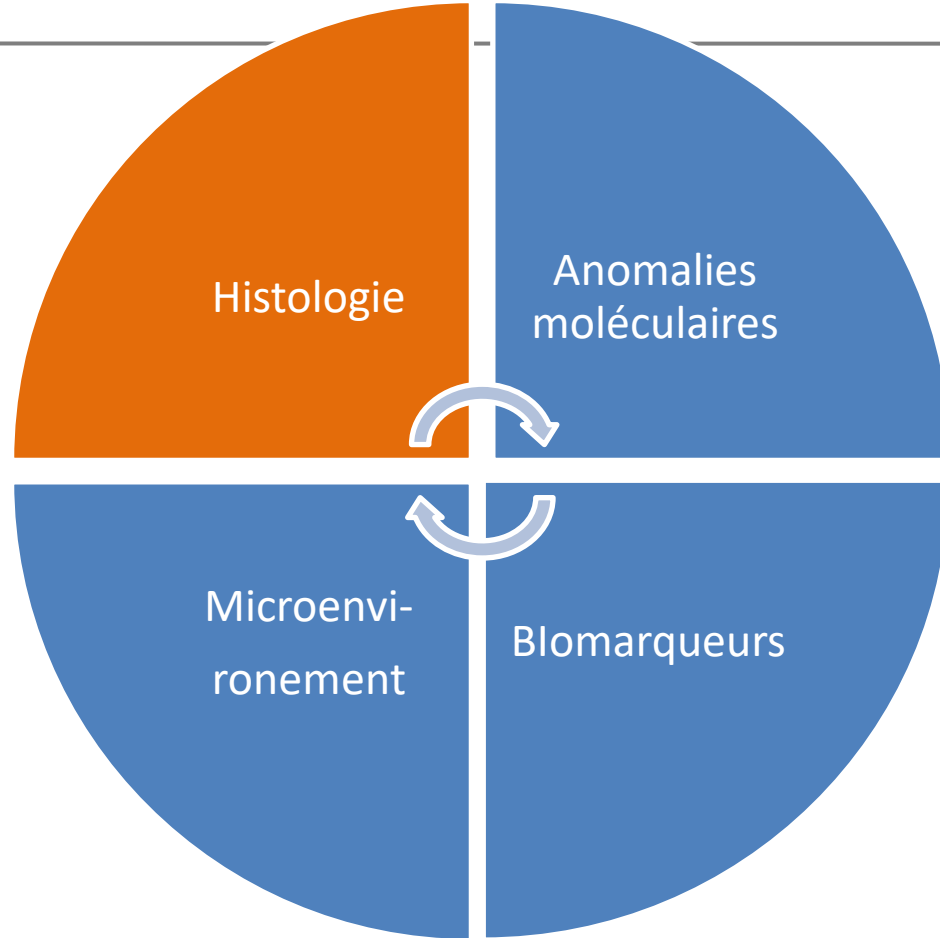
# Modèles d'évolution tumorale





# Hétérogénéité tumorale

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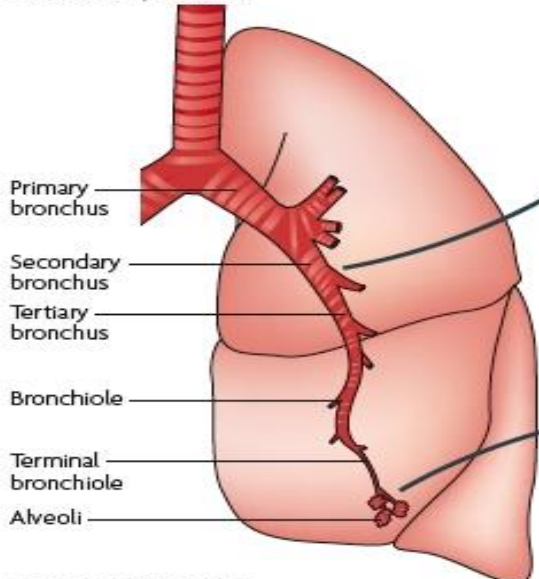


**CBNPC (80%)**

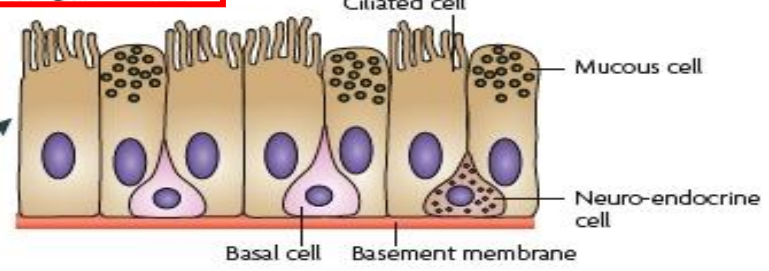
épidermoïde  
AdénoK  
bronchique  
Carcinome à  
grandes  
cellules  
+/-neuro-  
endocrine

Carcinome NE à  
petites cellules  
(17%)

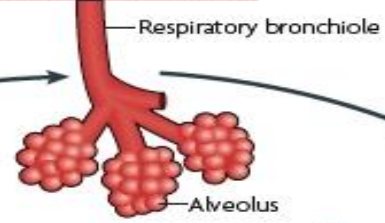
**Central compartment**



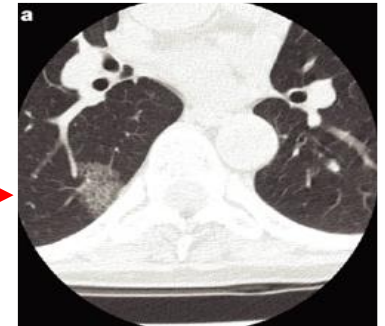
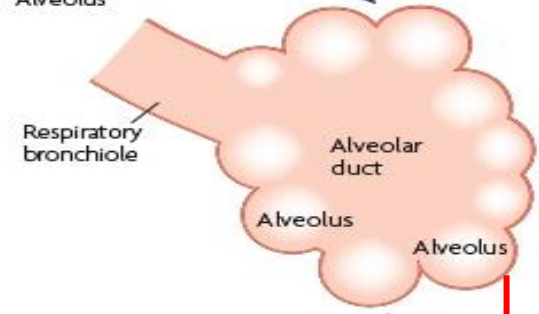
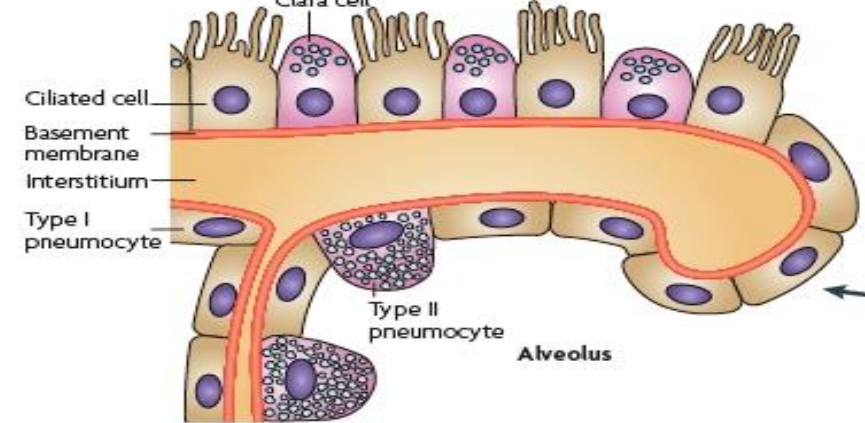
**Large bronchus**



**Peripheral compartment**

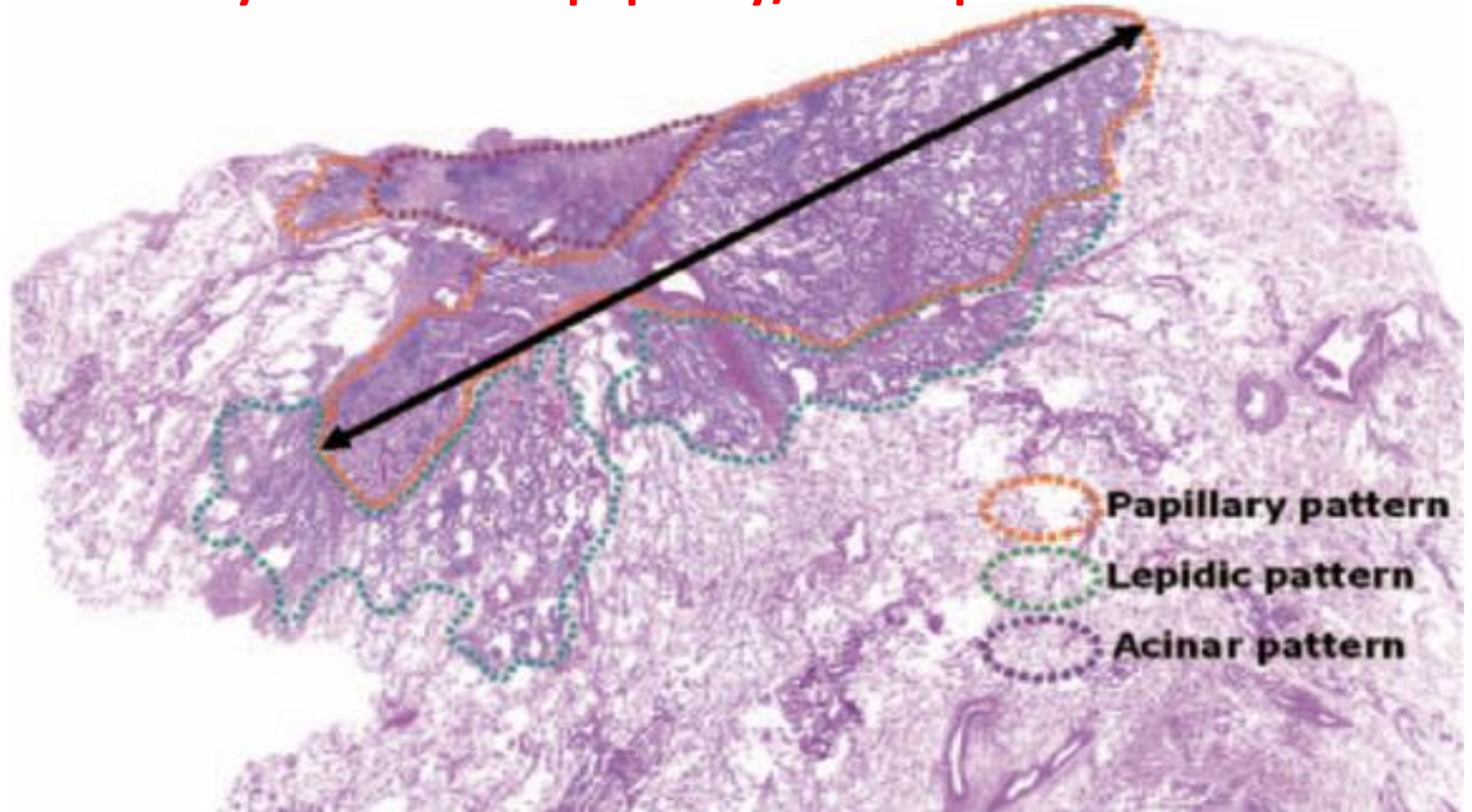


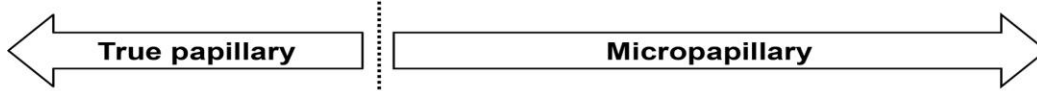
**Respiratory bronchiole**





# Histological heterogeneity of lung adenocarcinoma: beyond classical papillary/acinar patterns





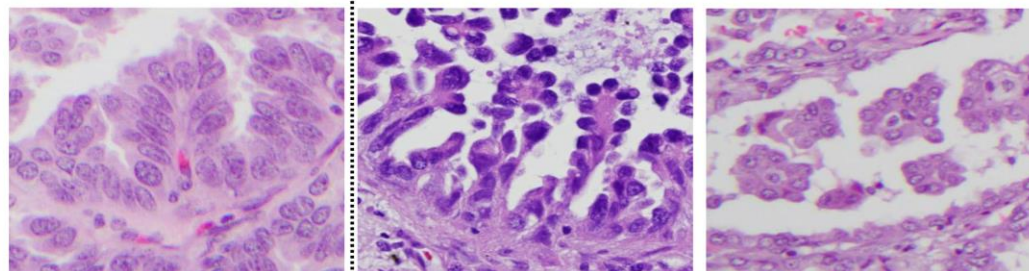
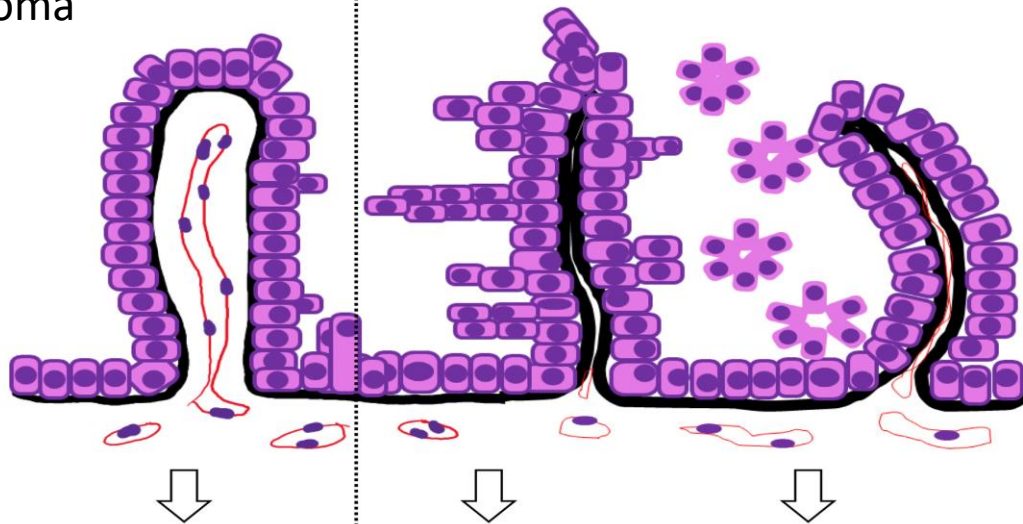
- A growth of glandular cells along central fibrovascular cores

**Filigree**

- Delicate lace-like narrow stacks
- At least 3 piled-up nuclei from the alveolar wall basal layer
- Up to three cells in width
- No definite florets
- No fibrovascular cores

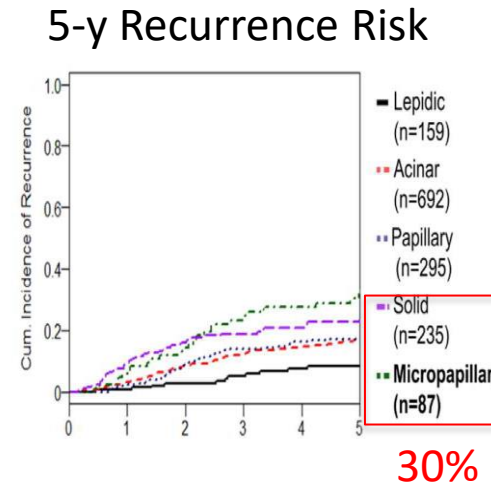
**Classical**

- Tumor cells growing in papillary tufts forming florets
- No fibrovascular cores



**n=1468 stage I lung adenocarcinoma MSKC**

- Lepidic= 11%
- Acinar= 47%
- Papillary= 20%
- Solid= 16%
- Micropapillary=6%



Only 5% is enough

# *The* NEW ENGLAND JOURNAL *of* MEDICINE

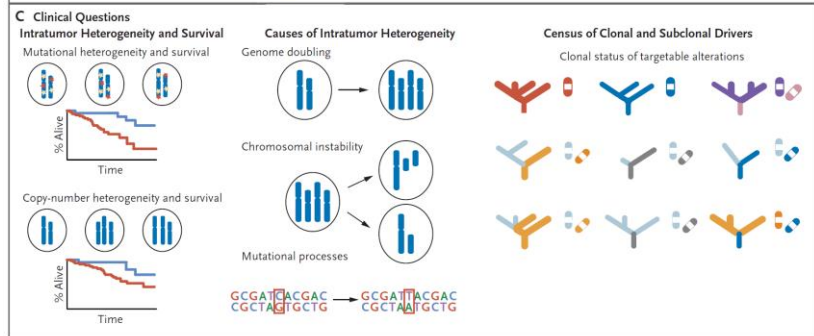
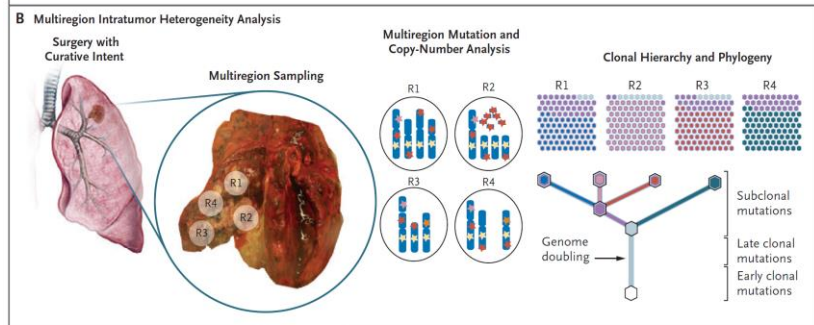
ESTABLISHED IN 1812

JUNE 1, 2017

VOL. 376 NO. 22

## Tracking the Evolution of Non–Small-Cell Lung Cancer

M. Jamal-Hanjani, G.A. Wilson, N. McGranahan, N.J. Birkbak, T.B.K. Watkins, S. Veeriah, S. Shafi, D.H. Johnson, R. Mitter, R. Rosenthal, M. Salm, S. Horswell, M. Escudero, N. Matthews, A. Rowan, T. Chambers, D.A. Moore, S. Turajlic, H. Xu, S.-M. Lee, M.D. Forster, T. Ahmad, C.T. Hiley, C. Abbosh, M. Falzon, E. Borg, T. Marafioti, D. Lawrence, M. Hayward, S. Kolvekar, N. Panagiotopoulos, S.M. Janes, R. Thakrar, A. Ahmed, F. Blackhall, Y. Summers, R. Shah, L. Joseph, A.M. Quinn, P.A. Crosbie, B. Naidu, G. Middleton, G. Langman, S. Trotter, M. Nicolson, H. Remmen, K. Kerr, M. Chetty, L. Gomersall, D.A. Fennell, A. Nakas, S. Rathinam, G. Anand, S. Khan, P. Russell, V. Ezhil, B. Ismail, M. Irvin-Sellers, V. Prakash, J.F. Lester, M. Kornaszewska, R. Attanoos, H. Adams, H. Davies, S. Dentro, P. Taniere, B. O’Sullivan, H.L. Lowe, J.A. Hartley, N. Iles, H. Bell, Y. Ngai, J.A. Shaw, J. Herrero, Z. Szallasi, R.F. Schwarz, A. Stewart, S.A. Quezada, J. Le Quesne, P. Van Loo, C. Dive, A. Hackshaw, and C. Swanton, for the TRACERx Consortium\*







**Abstr.# 135 – 70: Molecular drivers and therapeutic targets for neuroendocrine transformation in lung cancer**

Triparna Sen



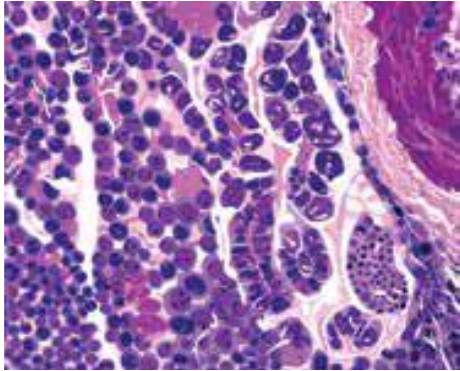
*Discussant: G. Zalcman*

*Proffered Paper session, 5 Oct. 2023*

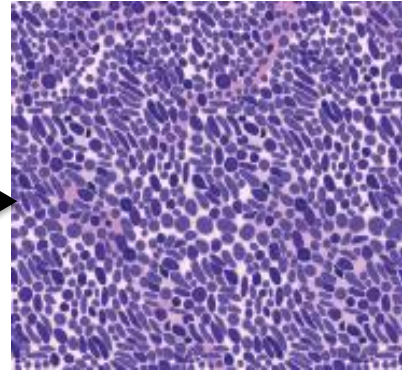


## Lineage plasticity—a mechanism of resistance to EGFR-targeted therapies

EGFR/ALK-mutant  
lung adenocarcinoma



Small cell lung cancer



TKI

— ? — ? —>  
**14-20%**

Although histologically typical,  
**not always clinically typical:**

- “small” lesions (# bulky mediastinum nodes)
- Peripheral (# central)
- “slow” **initial** growth (bias of observation ?)
- Qualitatively different response to SCLC chemo? (less durable, less volume shrinkage)

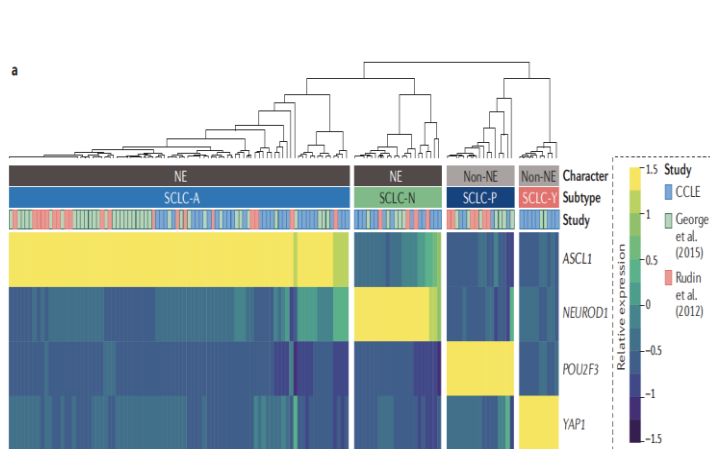
**Initial mixed histology tumors with therapeutic-induced clonal selection ?  
or cancer cell plasticity by Rb in a TP53 mutated background ?  
... both probably**

# TRANSCRIPTIONAL CLASSIFICATION OF SCLC

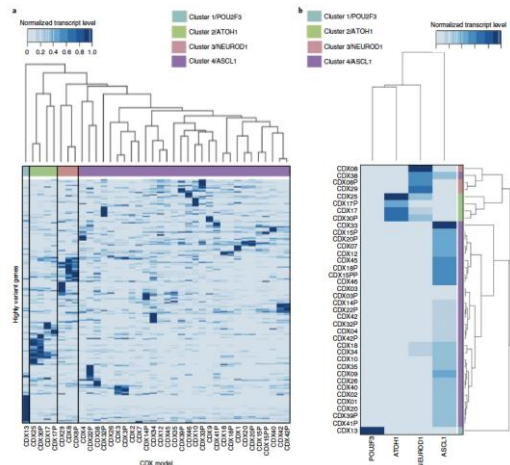
Rudin et al. Nature Rev Cancer 2019

Simpson et al. Nature Cancer 2020

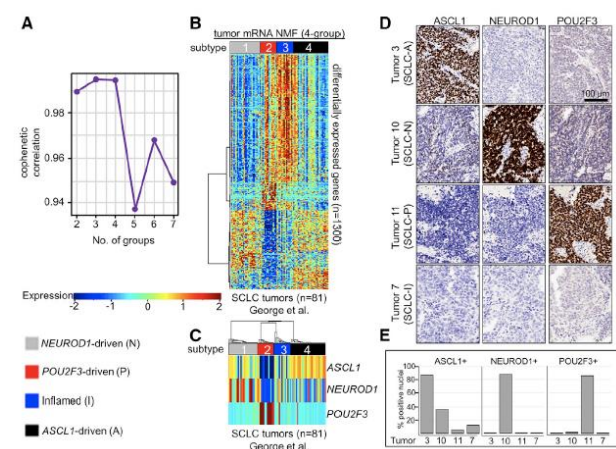
Gay et al. Cancer Cell 2021



ASCL1, NEUROD1, POU2F3, YAP1



ASCL1, NEUROD1, POU2F3, ATOH1



ASCL1, NEUROD1, POU2F3, Inflamed

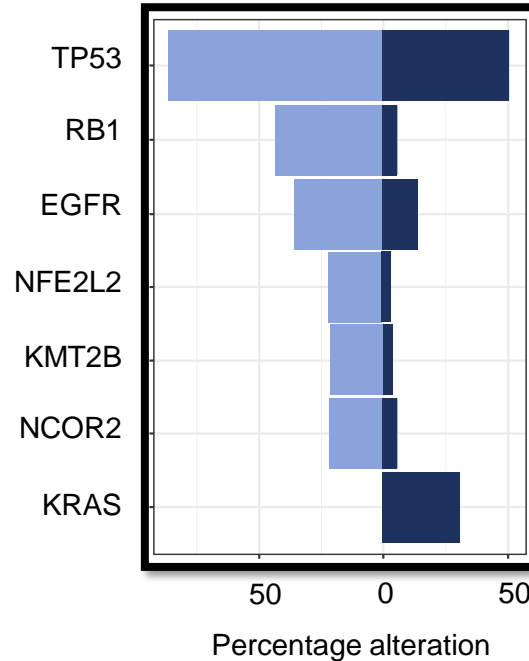
=> 3 dominant subtypes: ASCL1, NEUROD1, POU2F3

**YAP1 expressing cells are non-NE** and part of the intra-tumoral heterogeneity (YAP1 inhibits Notch driven NE differentiation)

Shue *et al.* Nat Comms 2022

# Abstr.# 135 – 70: Molecular drivers and therapeutic targets for neuroendocrine transformation in lung cancer

## TP53, RB1 loss are common alterations in SCLC transformation



No K-Ras mut in transformed SCLC

100% TP53

Only 45% of Rb loss...

Only 40% of EGFR mut ?

20% NFE2L2: TF response to oxidative stress

20% KMT2B: chromatin methyl-transferase

20% NCOR2: transcription repressor

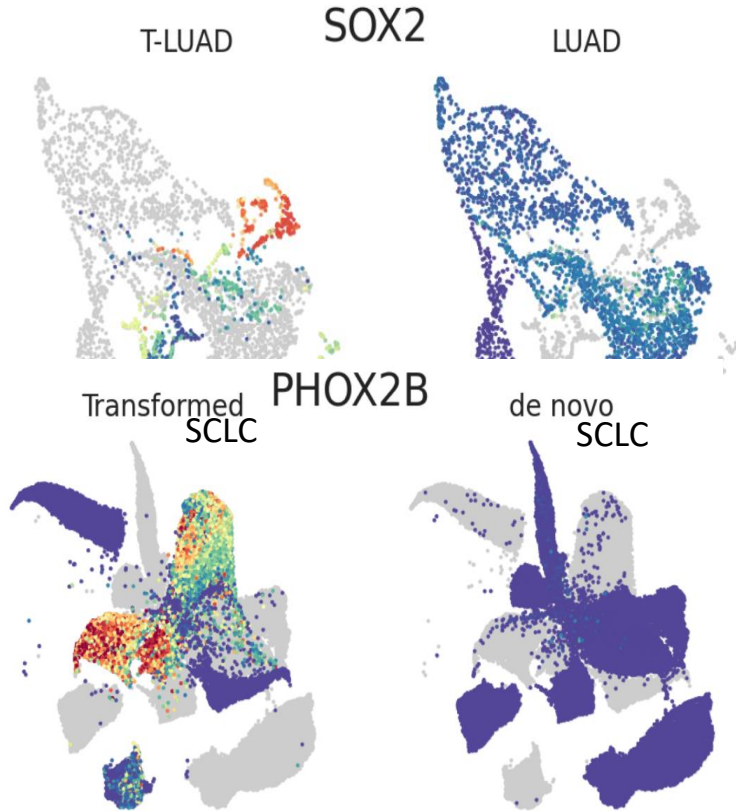
KMT2C loss in SCLC promotes metastasis through DNMT3A-mediated epigenetic reprogramming

(Chen *et al.* Nature Cancer 2022)

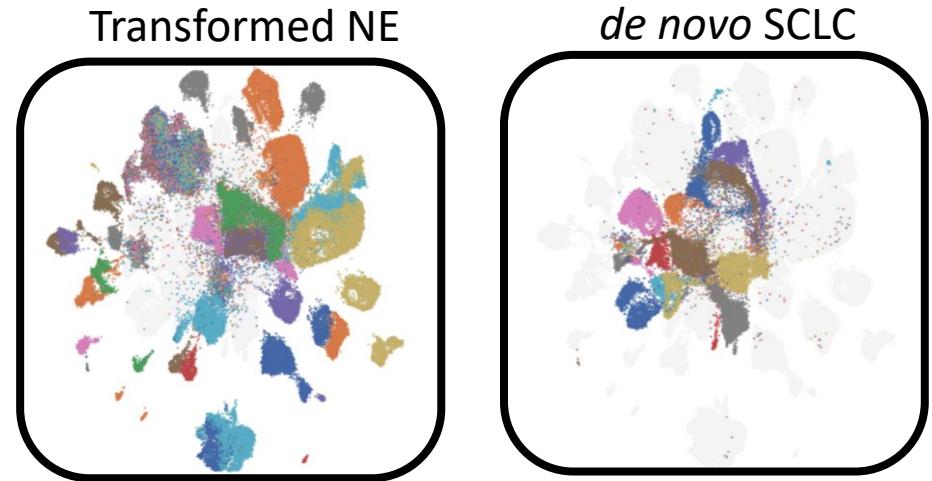
■ T-LUAD  
■ TCGA LUAD

**Abstr.# 135 – 70: T. Sen et al.**

Differentially expressed genes between transformed vs classical histology



Transformed SCLC displays greater inter-tumoral **heterogeneity**

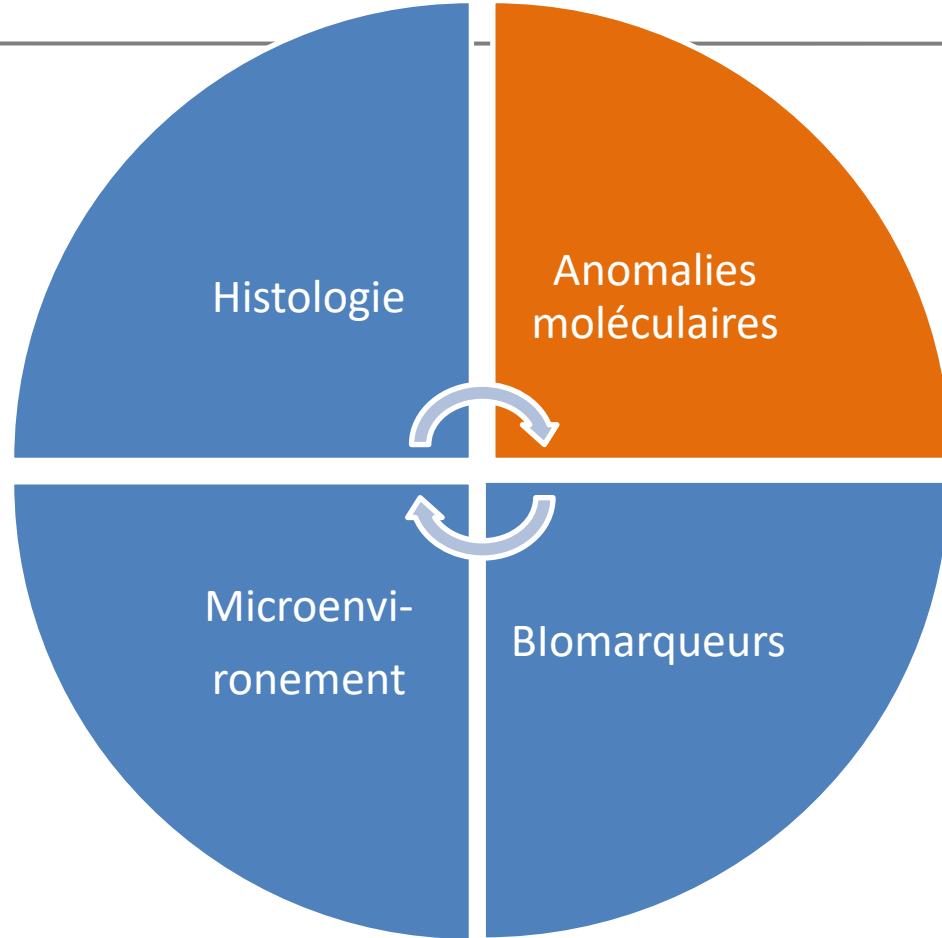


Annotated by patient

**Meaning ? : greater instability ?**

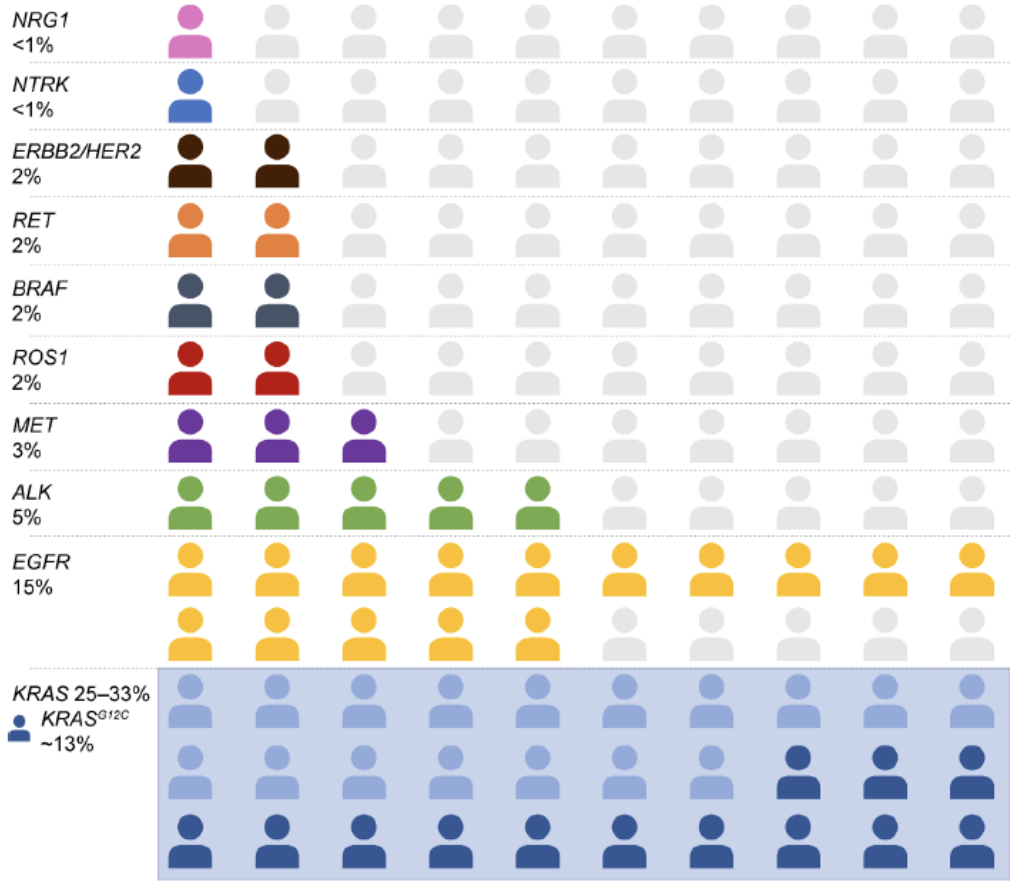
# Hétérogénéité tumorale

---



# Prevalence of common oncogenic driver alterations in NSCLC adenocarcinomas in Caucasian patients: molecular heterogeneity

“jamais fumeurs”  
 “petits fumeurs”  
 ex-fumeurs sevrés  
 > 10 ans



Lindsay CR et al.  
 Lung Cancer 2022

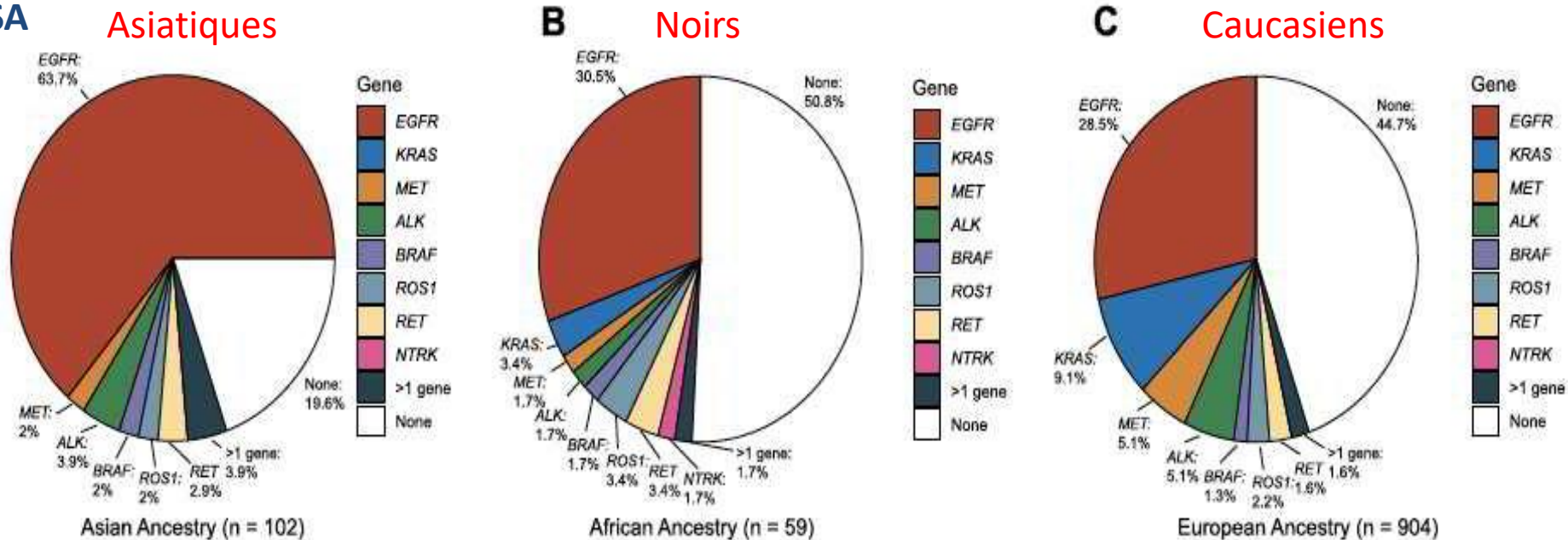


# Une maladie particulière: l'adénocarcinome des "petits" fumeurs, fumeurs sevrés ou jamais fumeurs

## Hétérogénéité du background moléculaire selon l'ethnicité

⇒ Hétérogénéité systèmes de réparation ADN/ métabolisme carcinogènes  
 ≥ susceptibilité génétique addiction tabagique ?

USA

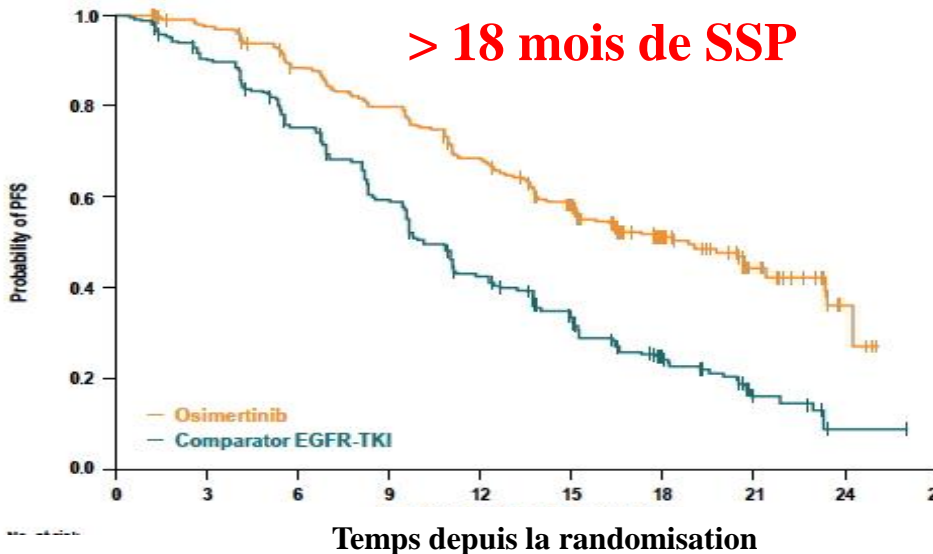


Distribution of targetable alterations by inferred ancestral groups among light smokers ( $\leq 15$  pack-year smoking history) with LUAD

# Etude FLAURA 1<sup>ère</sup> ligne osimertinib (ITK 3<sup>ème</sup> génération) vs. ITK de 1<sup>ère</sup> génération en première ligne thérapeutique chez les EGFR mutés

## Survie sans progression

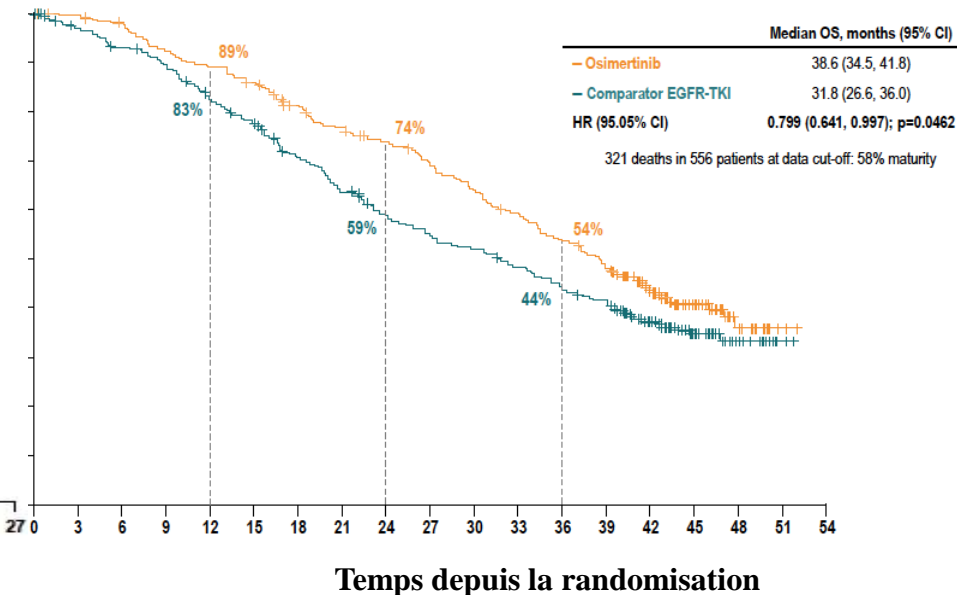
> 18 mois de SSP



		Temps depuis la randomisation									
		0	3	6	9	12	15	18	21	24	27
—	Osimertinib	279	262	233	210	178	139	71	26	4	0
—	Comparator EGFR-TKI	277	239	197	152	107	78	37	10	2	0

## Survie Globale

≥ 38 mois de SG



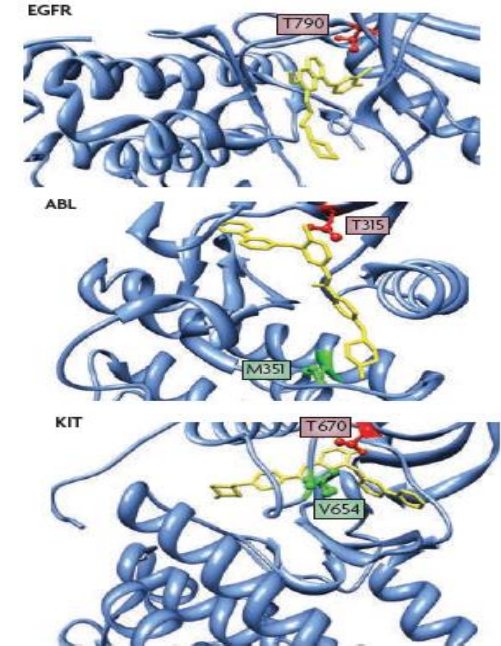
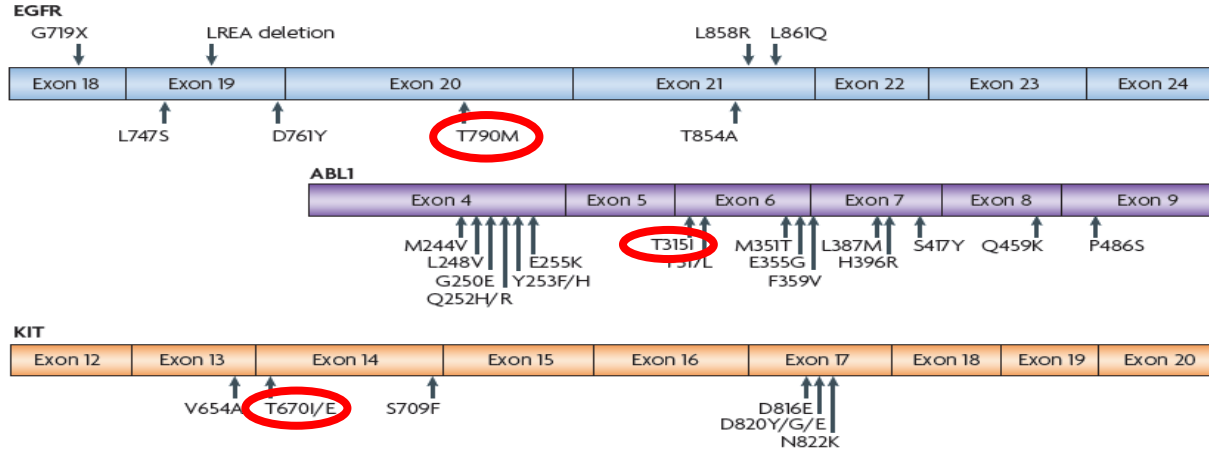
		Temps depuis la randomisation																		
		0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54
—	Osimertinib	279	276	269	253	243	232	154	87	29	4	0								
—	Comparator EGFR-TKI	277	263	252	237	218	200	126	64	24	1	0								

Median OS, months (95% CI)  
 — Osimertinib 38.6 (34.5, 41.8)  
 — Comparator EGFR-TKI 31.8 (26.6, 36.0)  
 HR (95.05% CI) 0.799 (0.641, 0.997); p=0.0462  
 321 deaths in 556 patients at data cut-off: 58% maturity

# Résistances secondaires (acquises) aux TKI

(1) avec persistance d'une dépendance à la voie de l'addiction oncogénique (oncogène-dépendante)

Mutations "gatekeeper" sur les RTK associées à une résistance aux inhibiteurs de kinase



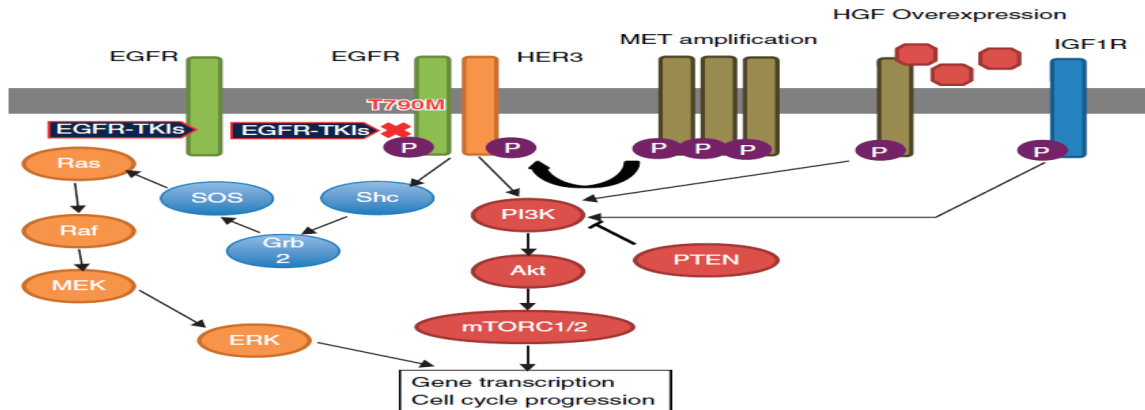
Disease	Protein	Mutation	Drug
CML	BCR-ABL	T315I	Imatinib
CML	BCR-ABL	T315I	Dasatinib
HES	PDGFRa	T674I	Imatinib
GIST	KIT	T670I	Imatinib
NSCLC	EGFR	T790M	Geftinib/Erlotinib
NSCLC	ALK	L1196M	Crizotinib

# Résistances secondaires (acquises) aux TKI

(2) avec indépendance (relative) de la voie oncogénique (oncogène-indépendante)

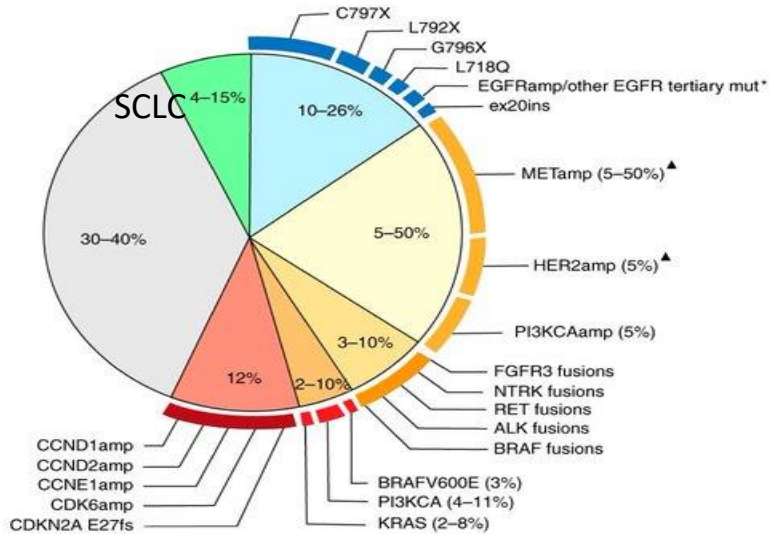
## Sélection d'un clone avec:

- Activation parallèle des voies de signalisation d'aval (bypass) : amplification de *c-MET*, de HER2, HER3, IGF1-R ...
- Acquisition d'altérations génétiques supplémentaires : mutations sur les voies de signalisation d'aval : *K-Ras*, *PI3K*, délétion *PTEN* ...
- « Transformation » phénotypique : cancer à petites cellules (Rb-TP53+), épidermoïde (SOX2) transition épithélio-mésenchymateuse (Wnt, Notch, YAP1)



# Mutations de résistance acquises sous Osimertinib

Resistance mechanisms to second-line osimertinib



\* Other EGFR tertiary mutations include G719X, G724S AND S768I

▲ Mutations have also been reported

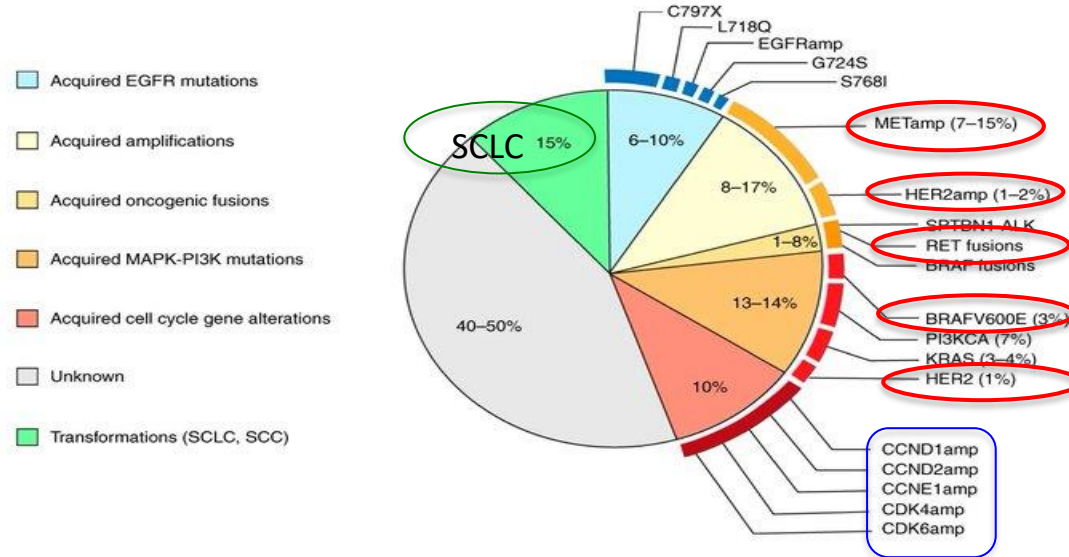
EGFR-T790M

Essai AURA 3 (plasma)

Papadimitrakopoulo, ESMO 2018

Piotrowska Cancer Discov. 2018, Oxnard JAMA Oncol 2018

Resistance mechanisms to first-line osimertinib

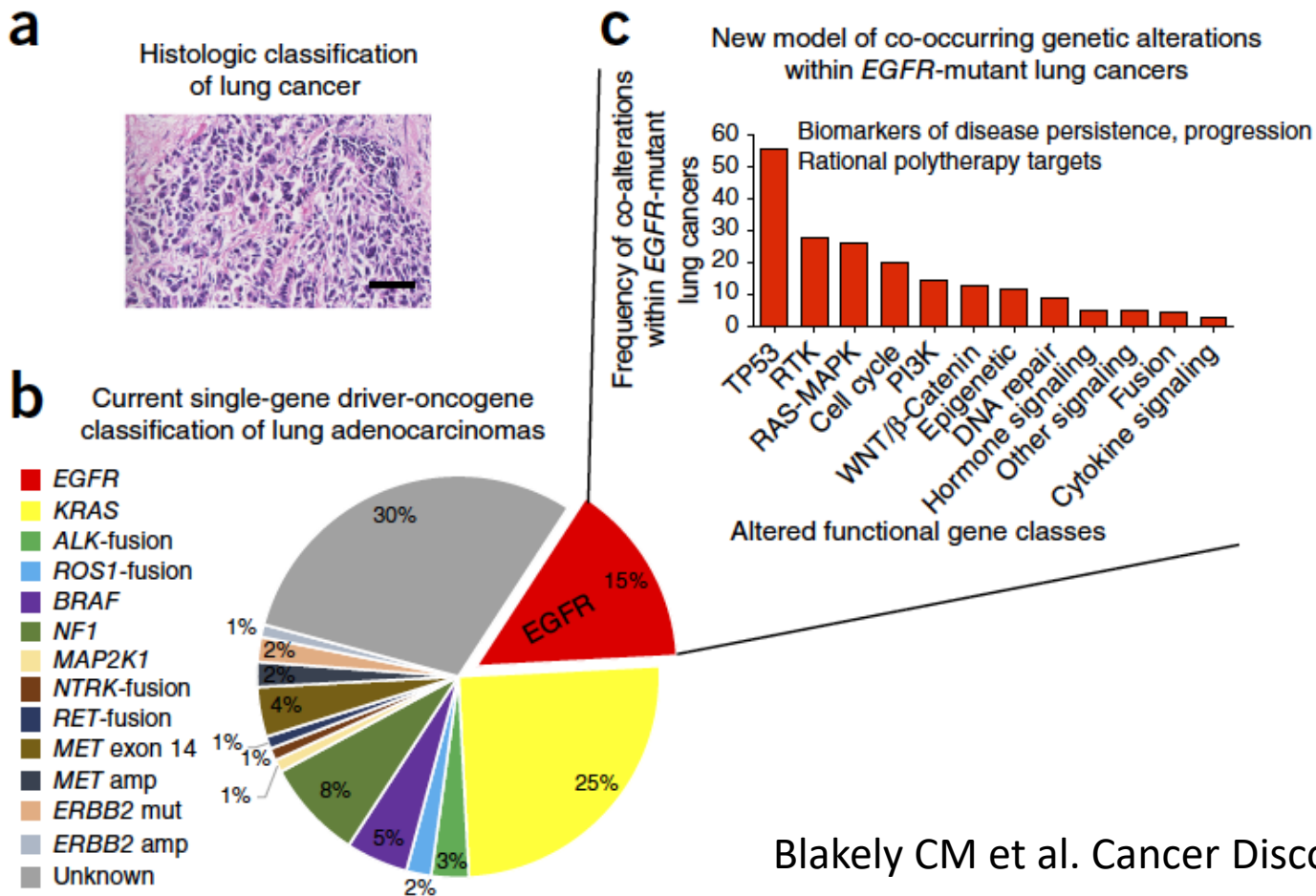


EGFR-del ex 19/ L858R

Essai FLAURA (plasma)

Ramalingham, ESMO 2018

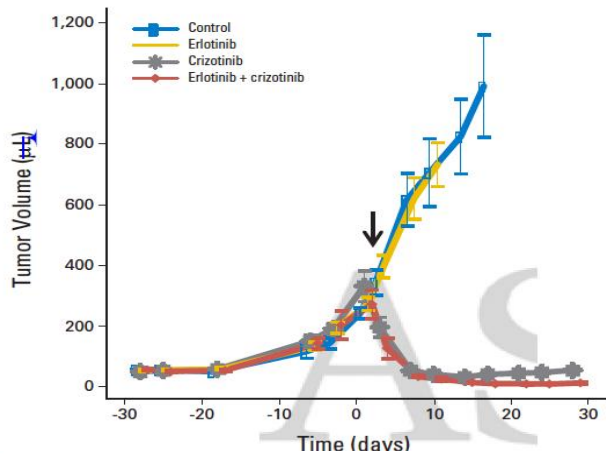
# Un niveau supplémentaire d'hétérogénéité tumorale: le rôle des co-mutations



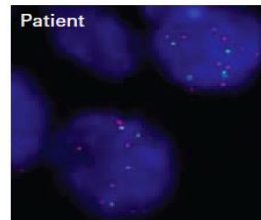


# EGFR Exon 19 del/ MET amplif: what is the driver oncogene?

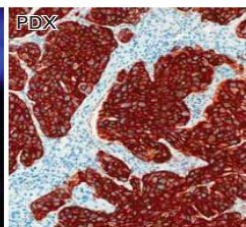
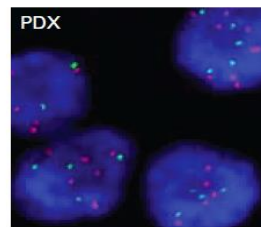
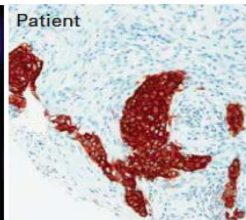
**A**



**B**

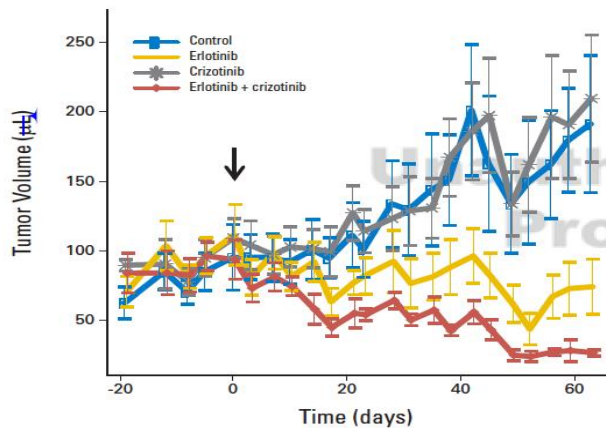


**C**

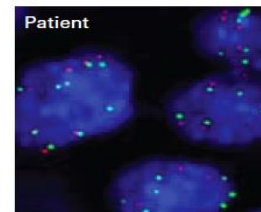


Model  
148

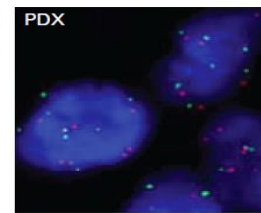
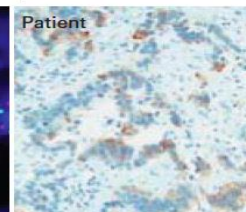
**D**



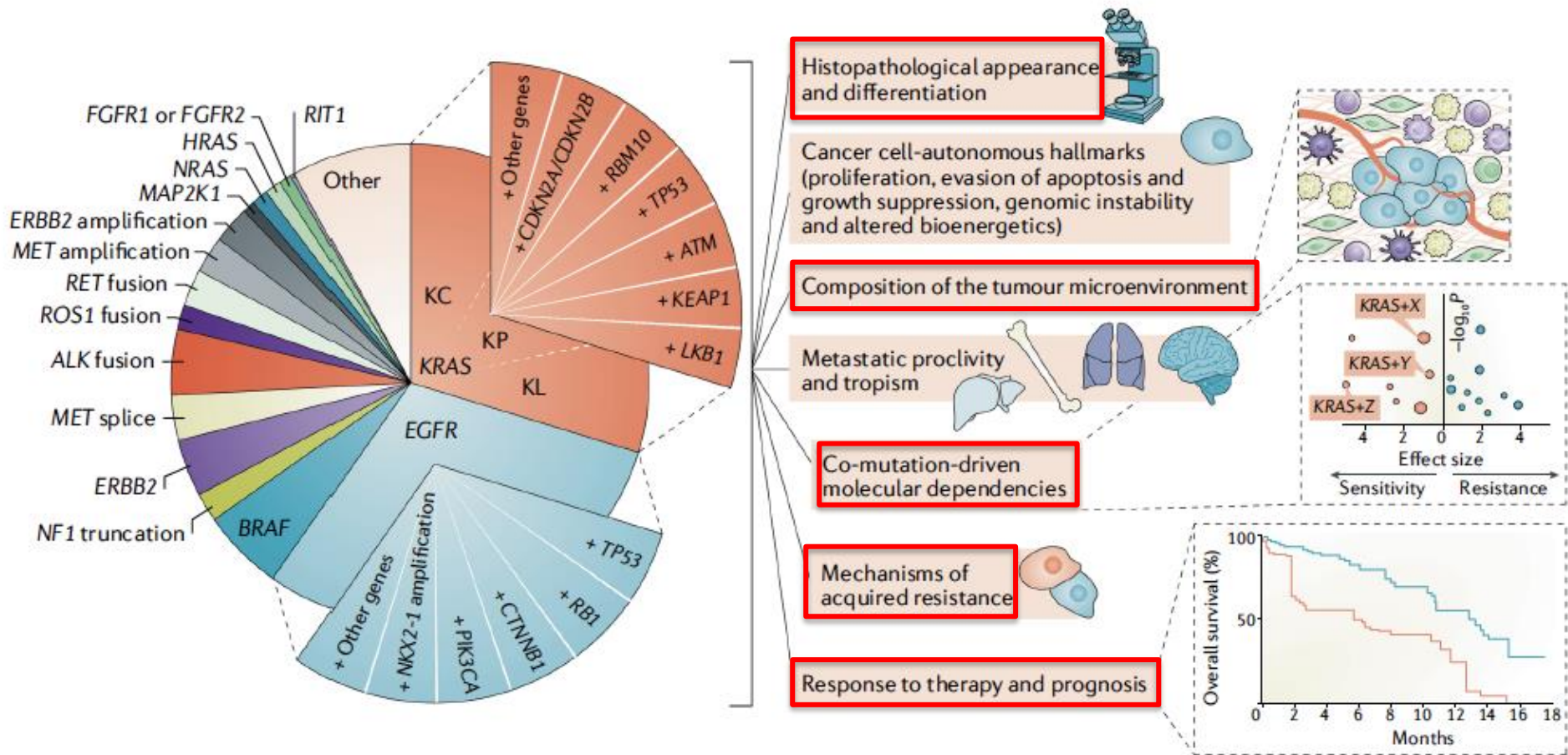
**E**



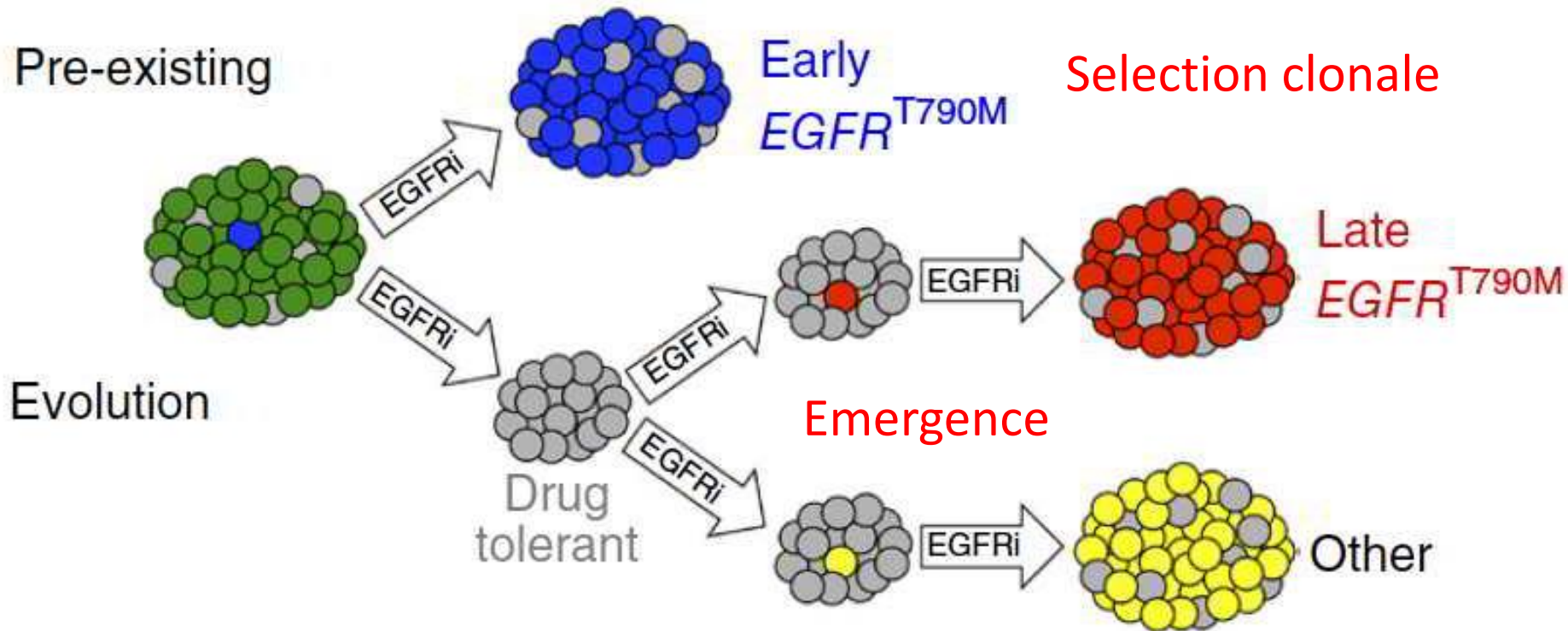
**F**



Model  
192



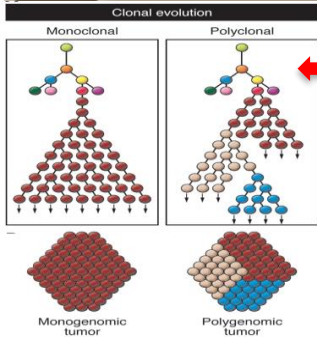
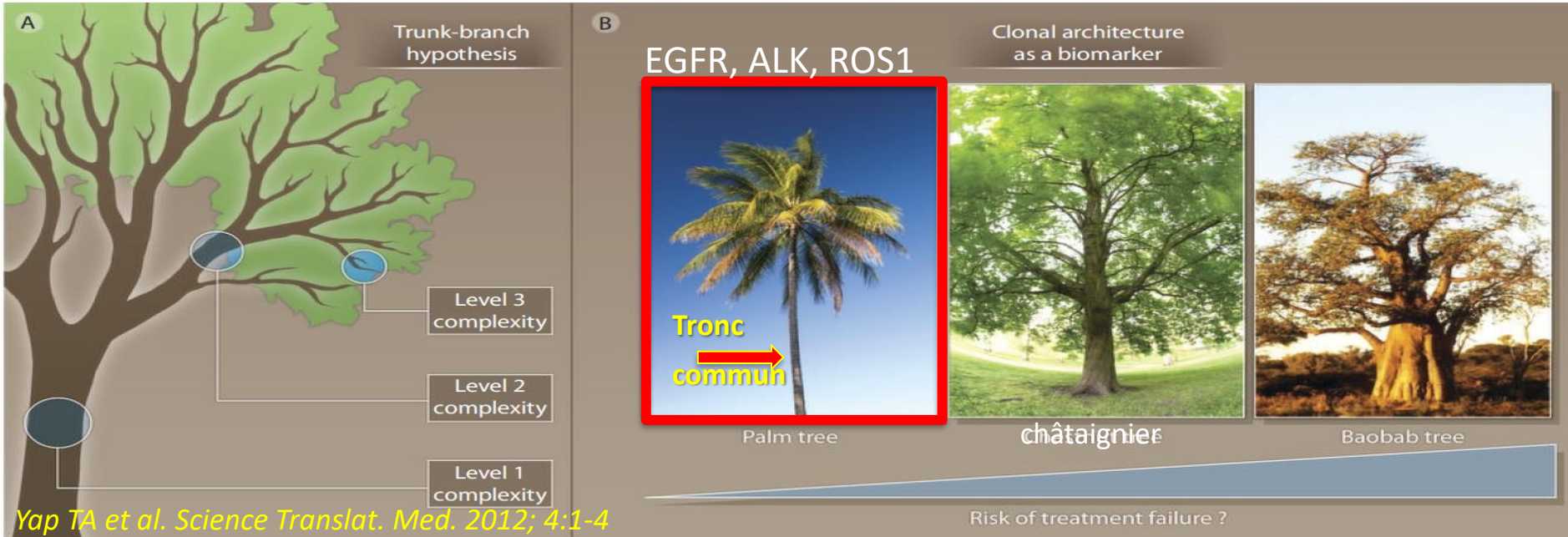
# Les résistances acquises aux ITK: la conséquence de l'hétérogénéité tumorale ?





# L'hétérogénéité tumorale n'est qu'un facteur tardif de résistance

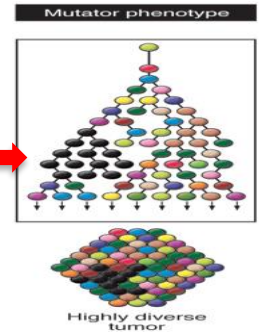
## En cas de mutation additive: modèle du palmier= dominance clonale



← **Evènements génétiques du tronc:** présents dans toutes les cellules cancéreuses: trunk drivers

**Evènements génétiques branchés:** présents dans certaines cellules cancéreuses

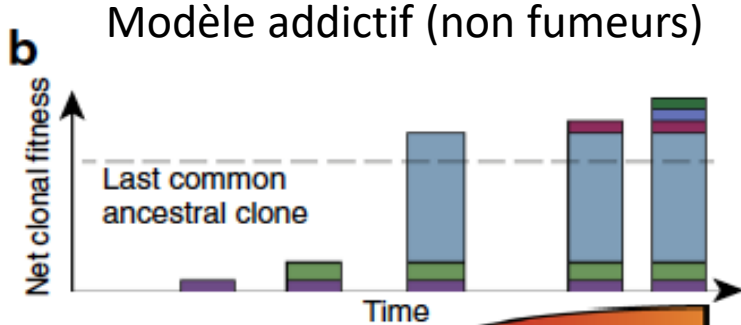
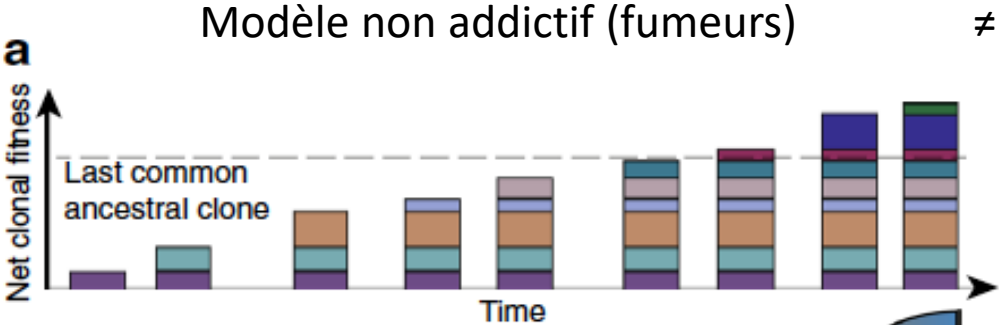
=> dynamique dans le temps



**C. Swanton**

Francis Crick Institute  
University College London, UK

# L'hétérogénéité moléculaire tumorale détermine le type d'évolutivité moléculaire

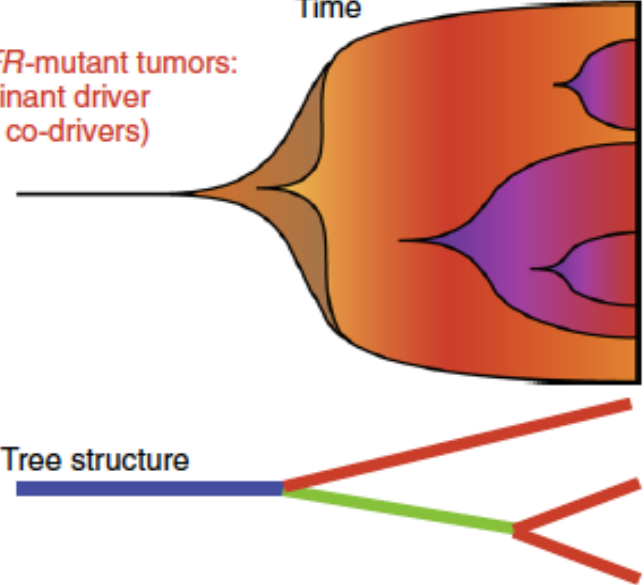
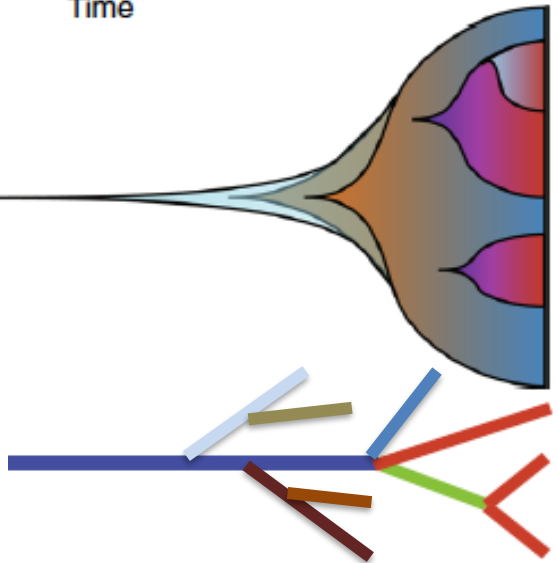


Smokers:  
many co-drivers

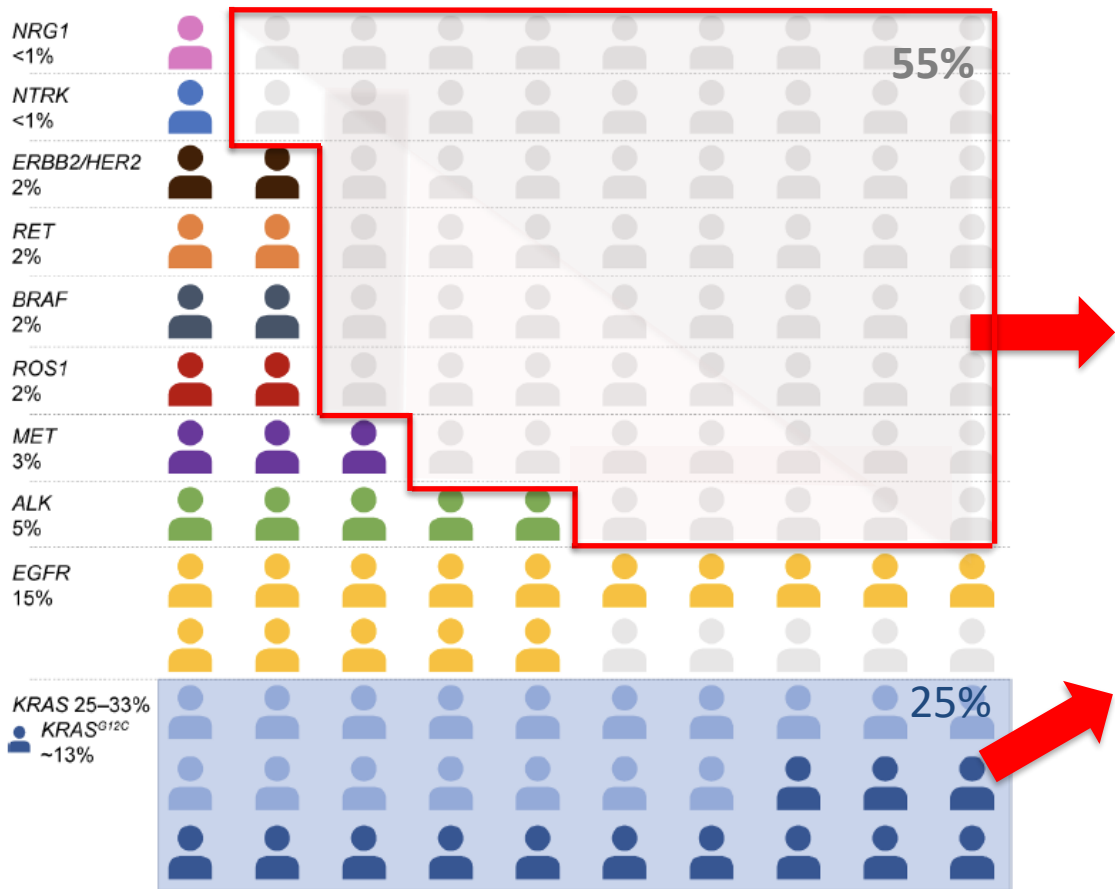
*EGFR*-mutant tumors:  
dominant driver  
(few co-drivers)

Tree structure

Tree structure



# Prevalence of common oncogenic driver alterations in NSCLC adenocarcinomas in Caucasian patients



En l'absence de mutation  
addictive (**fumeurs actifs # 80%**):

**La 2<sup>ème</sup> révolution  
thérapeutique du XXI<sup>ème</sup>  
siècle: l'immunothérapie**

= les Inhibiteurs de Checkpoints  
Immuns (ICI)

Lindsay CR et al.  
Lung Cancer 2022





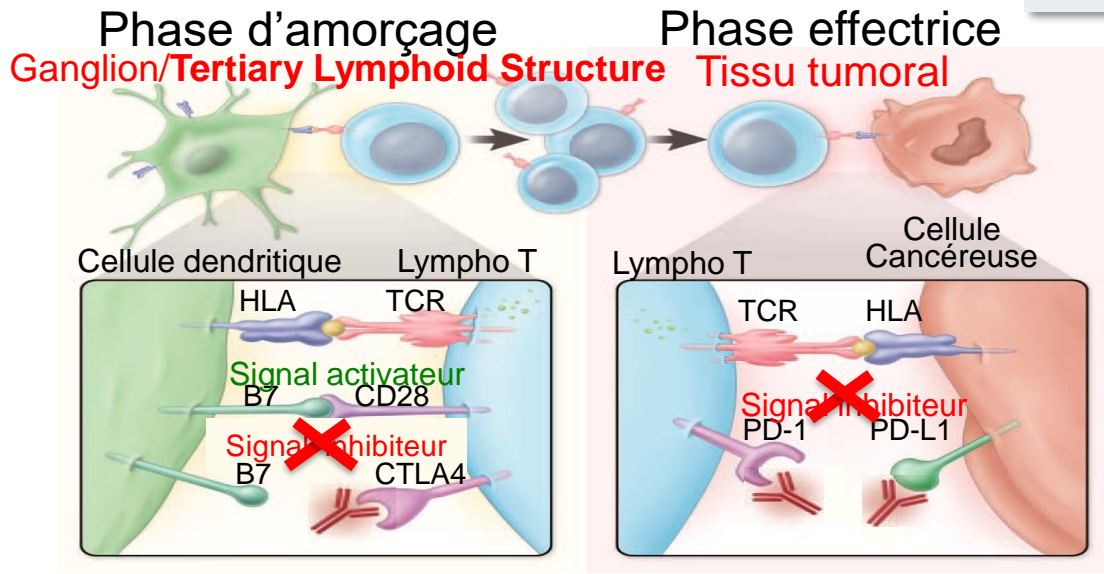
Dr. J. Allison Dr. T. Honjo

# Le ciblage des points de contrôle immunitaires des lympho T cytotoxiques



Anticorps **Anti-PD-1** ciblent les cellules T infiltrant la tumeur (TILs)  
 Anticorps **Anti-PD-L1** ciblent les cellules tumorales

Anticorps **Anti-CTLA-4** ciblent les lymphocytes T périphériques des ganglions

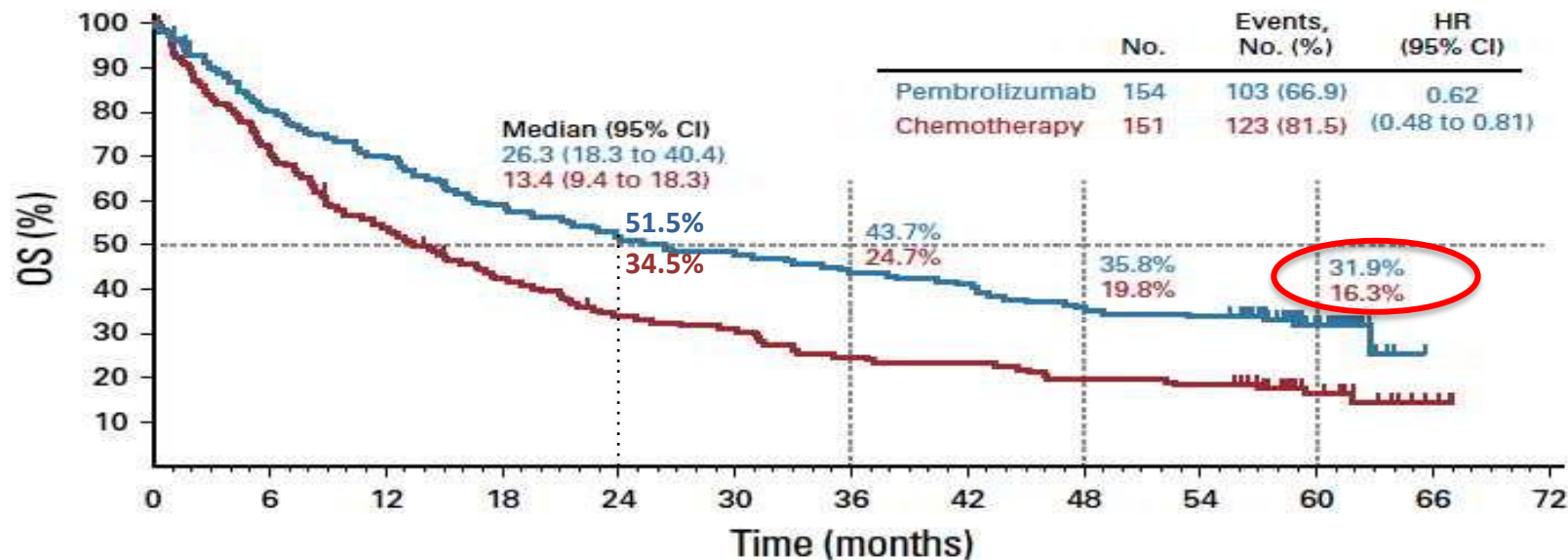


PD-1	CTLA-4
Régule l'activité des cellules T effectrices dans les tissus et la tumeur	Régule les étapes précoces de l'activation des lympho T
Exprimé par toutes les cellules sanguines	Exprimé exclusivement par les lympho T
Expression induite c/o les autres lymphocytes: B et cellules NK...	

# KN-024: 2<sup>ème</sup> update, JCO, avril 2021

Median fu= 59.9 months

Adéno+ Epi,  $\geq 50\%$  PD-L1



No. at risk:

Pembrolizumab	154	121	106	89	78	73	66	62	54	51	20	0	0
Chemotherapy	151	108	80	61	48	44	35	33	28	26	13	3	0

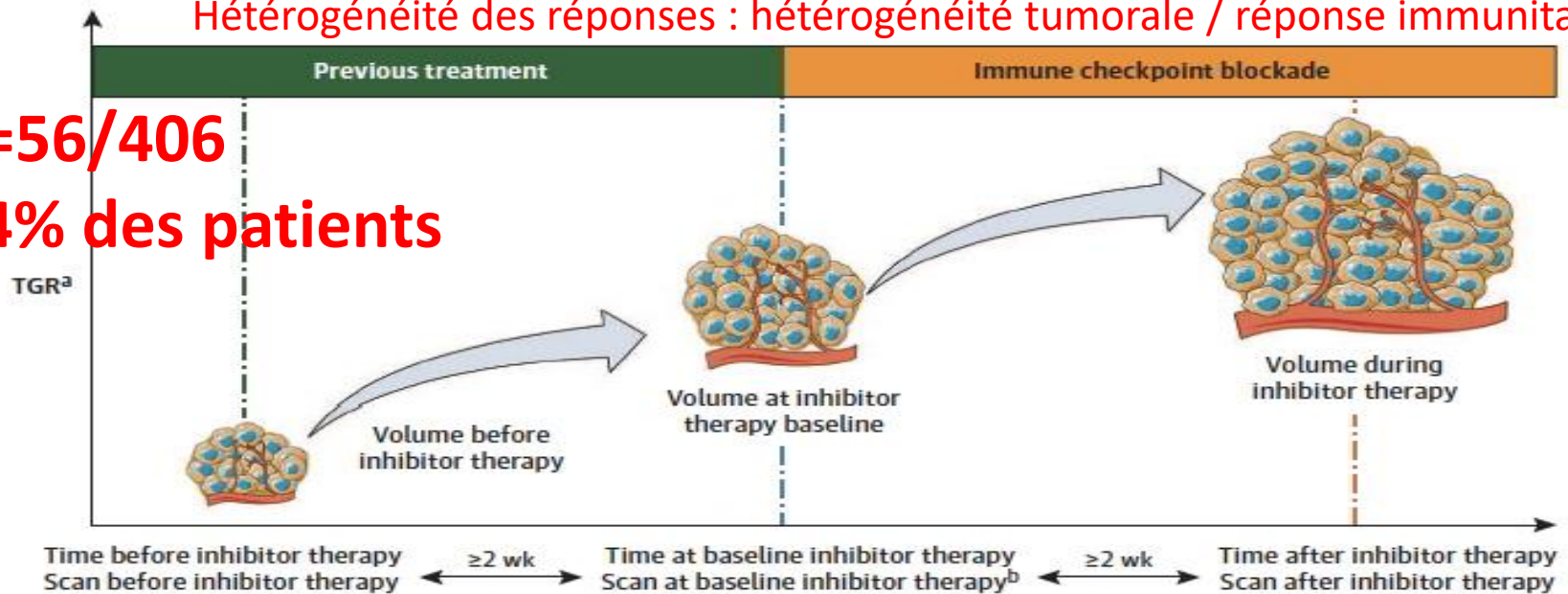
## Recul à 5 ans

# Hyperprogressive Disease in Patients With Advanced Non-Small Cell Lung Cancer Treated With PD-1/PD-L1 Inhibitors or With Single-Agent Chemotherapy

Roberto Ferrara, MD; Laura Mezquita, MD, PhD; Matthieu Texier, MSc; Jihene Lahmar, MD; Clarisse Audigier-Valette, MD; Laurent Tessonier, MD; Julien Mazieres, MD, PhD; Gerard Zalcman, MD, PhD; Solenn Brosseau, MD; Sylvestre Le Moulec, MD; Laura Leroy, MD; Boris Duchemann, MD; Corentin Lefebvre, MD; Remi Veillon, MD; Virginie Westeel, MD, PhD; Serge Koscielny, MSc; Stephane Champiat, MD; Charles Ferté, MD, PhD; David Planchard, MD, PhD; Jordi Remon, MD; Marie-Eve Boucher, MD; Anas Gazzah, MD; Julien Adam, MD, PhD; Emilio Bria, MD; Giampaolo Tortora, MD, PhD; Jean-Charles Soria, MD, PhD; Benjamin Besse, MD, PhD; Caroline Caramella, MD

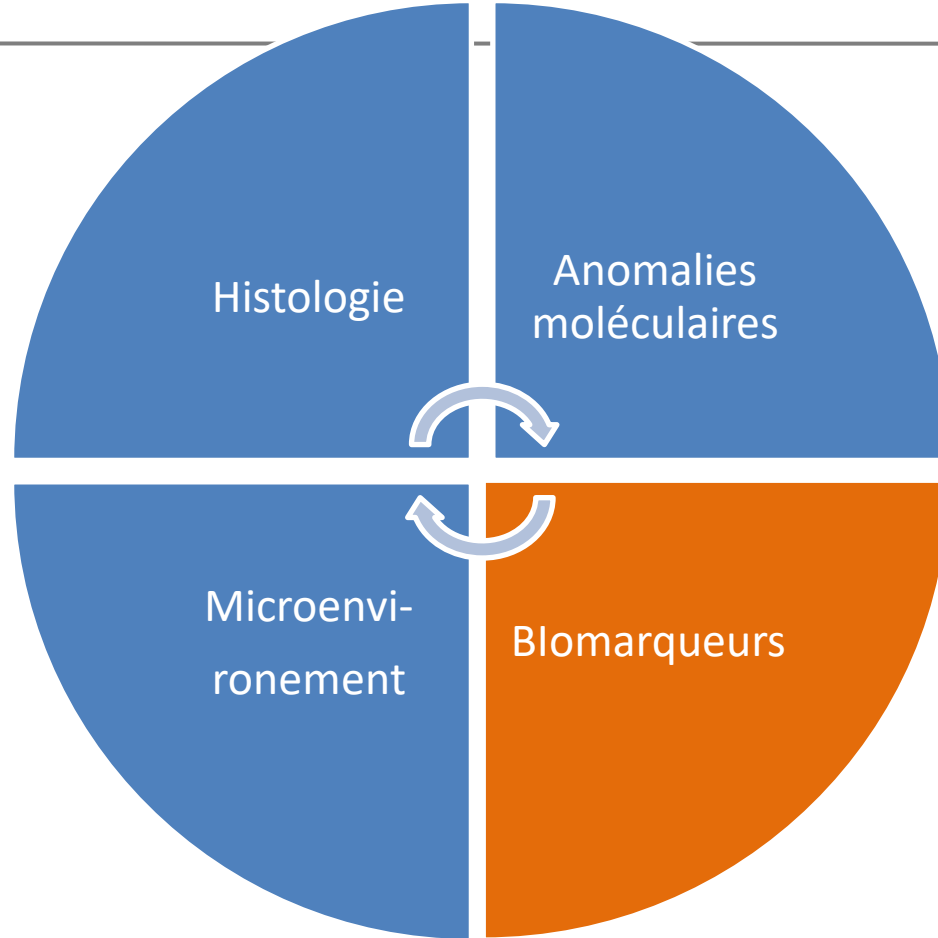
Hétérogénéité des réponses : hétérogénéité tumorale / réponse immunitaire ?

n=56/406  
14% des patients



# Hétérogénéité tumorale

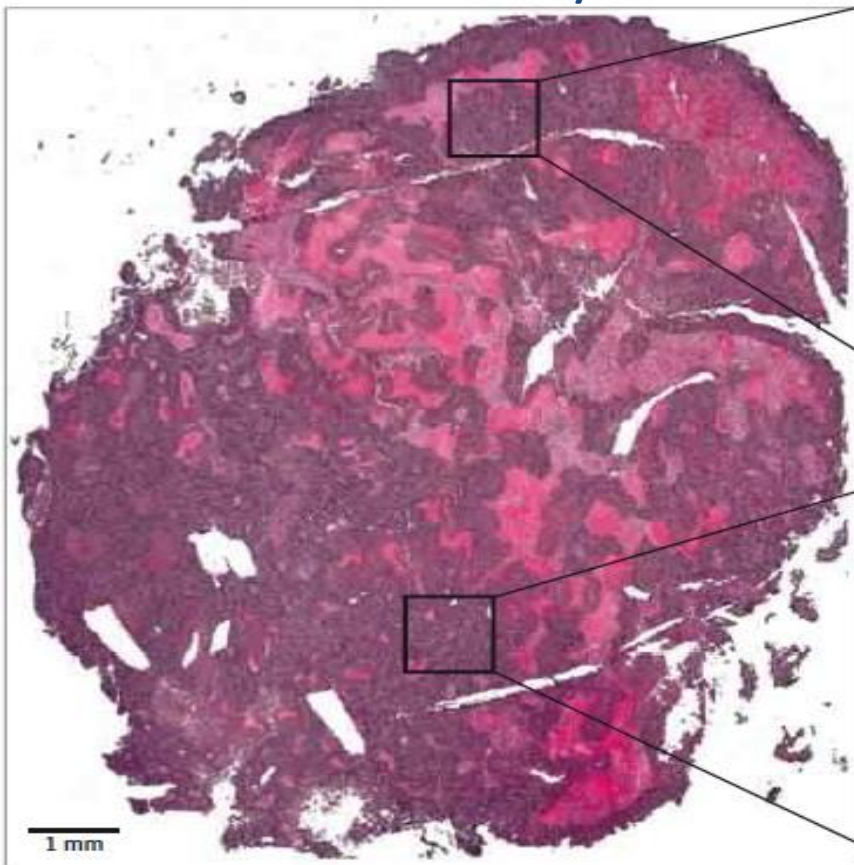
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# Hétérogénéité spatiale de l'expression de PD-L1

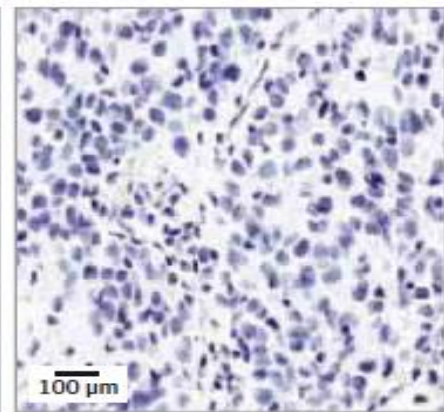
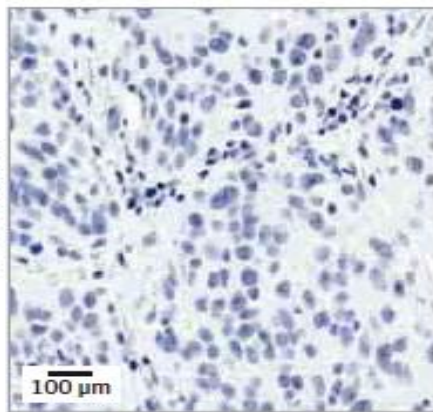
Idem infiltration CD3+/CD8+



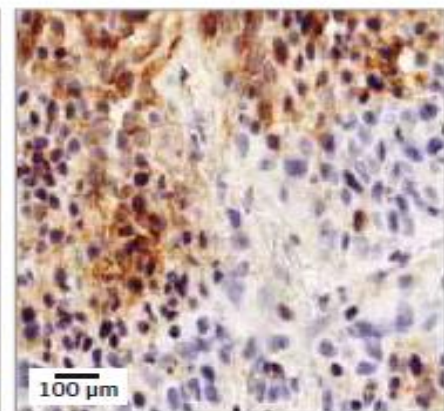
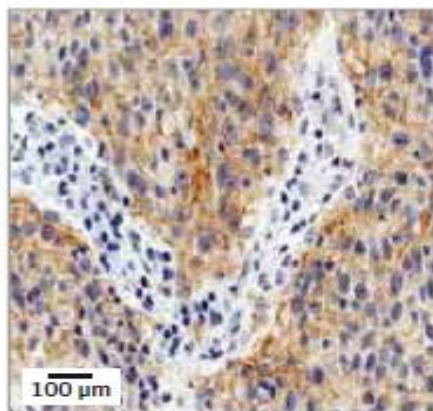
E1L3N antibody

SP142 antibody

PD-L1 Negative



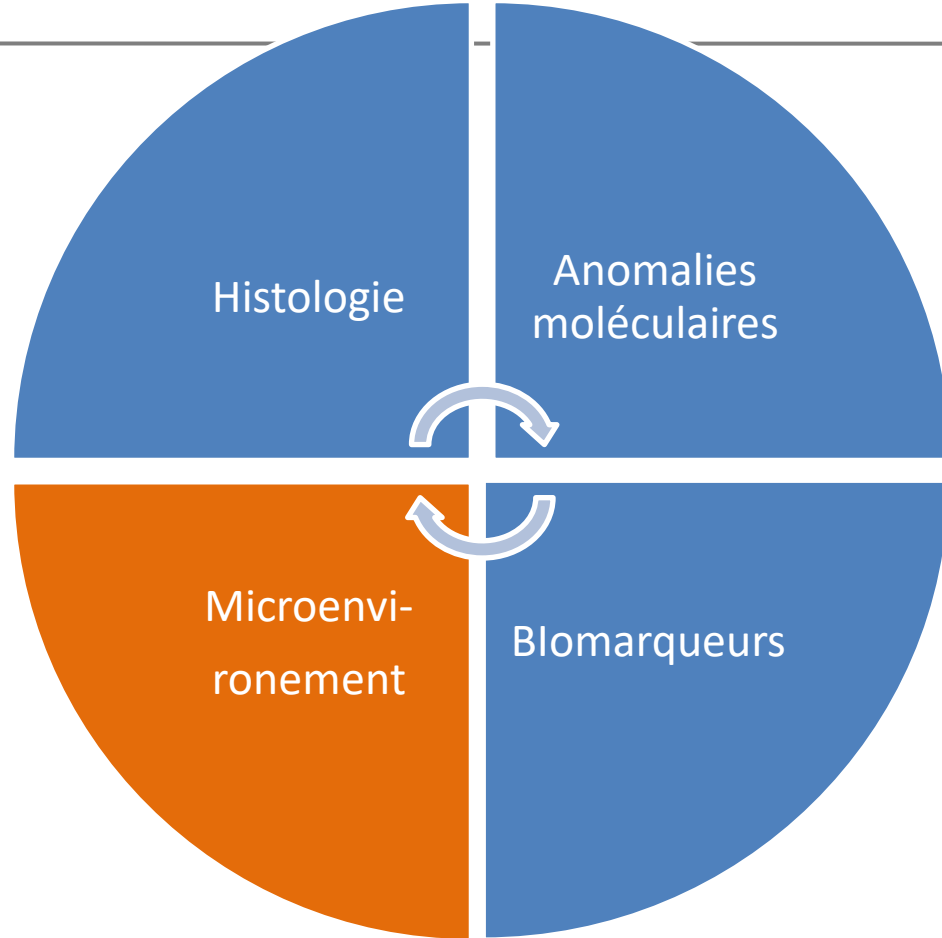
PD-L1 Positive



Mais à ce jour les moins mauvais des marqueurs prédictifs....

# Hétérogénéité tumorale

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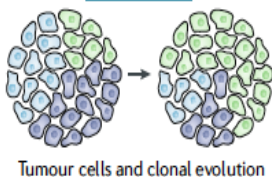




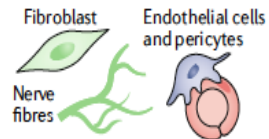
# le microenvironnement tumoral est hétérogène au plan architectural et cellulaire...dépendant du site tumoral

## Tumour component

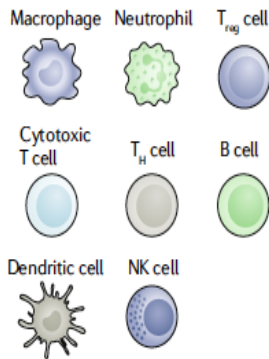
### Tumour cells



### Stromal cells



### Immune cells



## Mechanism and outcome

Mechanism and outcome	Tissue specificity	Proposed therapeutics
Growth factors	Universal	Chemotherapy
Hormone dependence	Unique	Hormone blockers
Oncogene dependence	Unique	Kinase inhibitors
HLA machinery loss	Universal	IFN $\gamma$
IFN $\gamma$ responsiveness	Universal	NA
Chemokines	Universal	Blocking monoclonals
Antigens	Unique	Vaccines

## Physical access barrier

Mechanism and outcome	Tissue specificity	Proposed therapeutics
Physical access barrier	Unique	Radiation therapy or anti-TGF $\beta$
Immunomodulation	Unique	NA
Neurotransmitters	Unique	$\beta$ -Blockers

## Suppressive cytokines

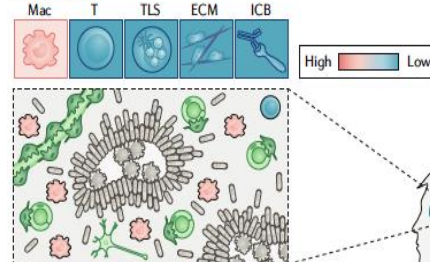
Mechanism and outcome	Tissue specificity	Proposed therapeutics
Suppressive cytokines	Universal	Blocking monoclonals
Metabolic depletion	Universal	Small molecules
High myeloid density	Unique	Blocking monoclonals
High granulocyte density	Unique	Blocking monoclonals
Exhaustion	Universal	Checkpoint blockade
Cytotoxicity	Universal	Checkpoint agonists
Pro-inflammatory cytokines	Universal	Checkpoint agonists
High lymphoid density	Unique	Checkpoints or cytokines
TLS organization	Unique	Chemokines
Cross-presentation	Universal	FLT3L or monoclonal agonists

Poor immune recognition and outcome

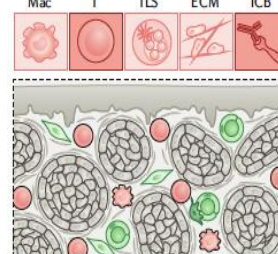
Favourable immune recognition and outcome

Already approved modalities

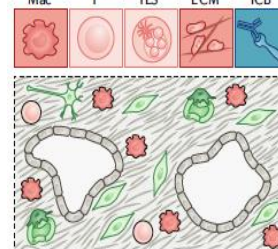
## a Glioblastoma



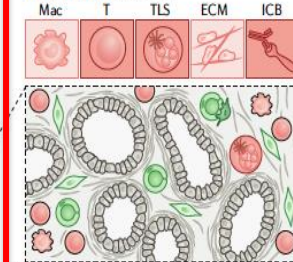
## b Skin melanoma



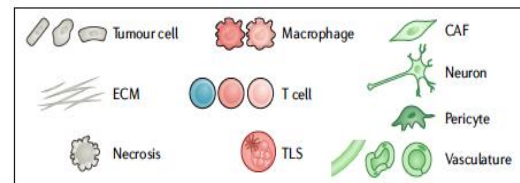
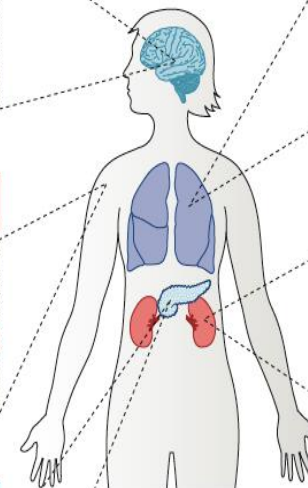
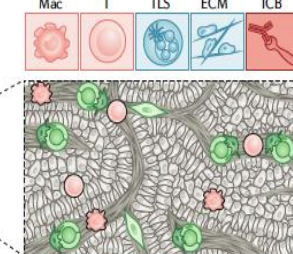
## c Pancreatic ductal adenocarcinoma



## d Lung adenocarcinoma

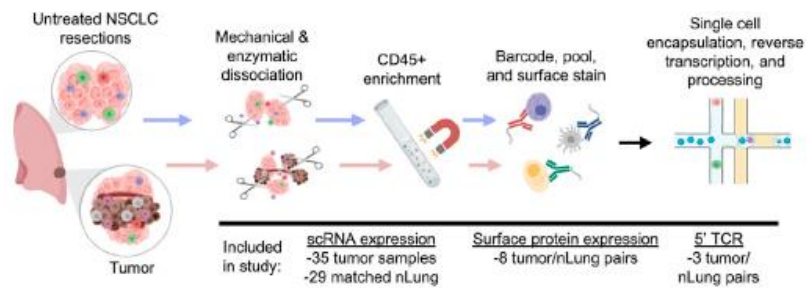


## e Renal cell carcinoma (clear cell)

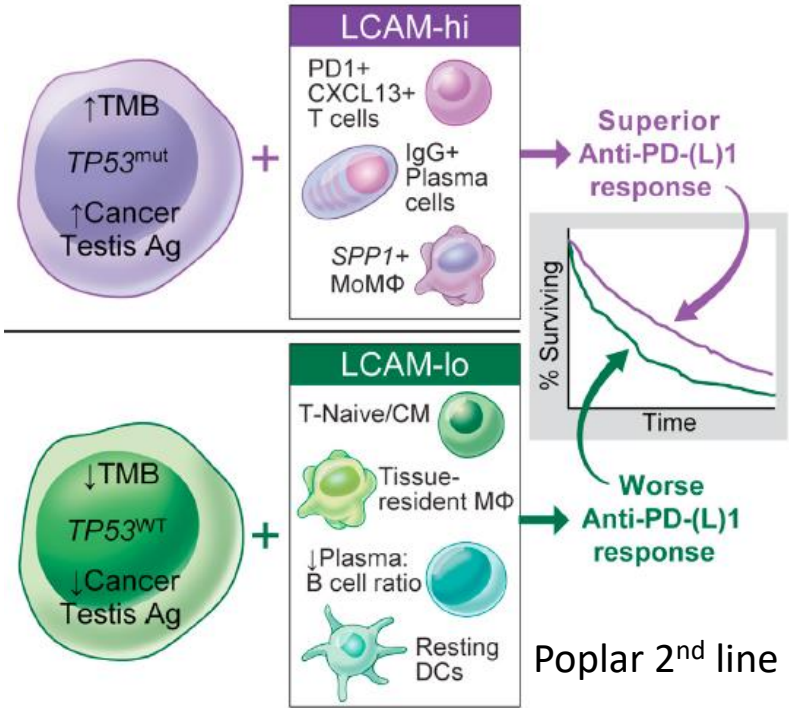


# Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification

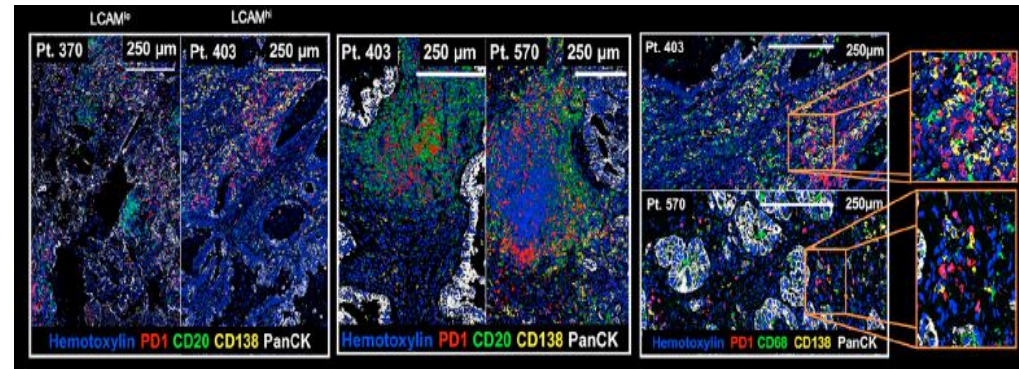
**Cancer Cell** Leader AM et al. 39, 1594–1609, December 13, 2021



## Lung Cancer Immune Activation Module



Infiltration Plasmocytes => sensibilité anti-PD(L)1



H. Salmon, U932 Inserm

Poplar 2<sup>nd</sup> line phase 2 trial: Atezo vs. Doc

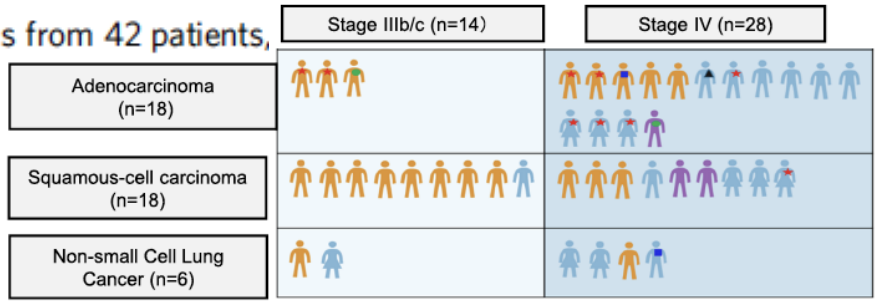




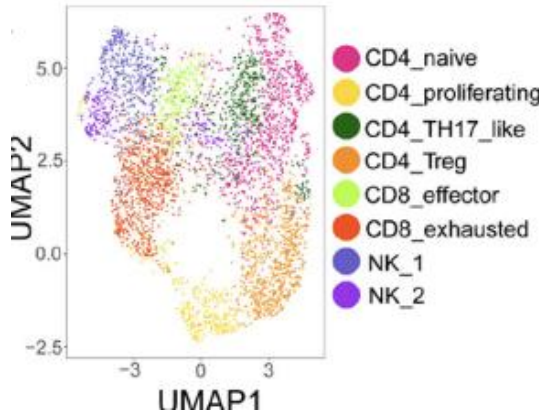
# Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer

- Non-smoker (n=18)
- Former-smoker (n=3)
- Smoker (n=21)
- ★ EGFR mutation (n=9)
- ALK fusion (n=2)
- Her2 mutation (n=2)
- ▲ RET fusion (n=1)

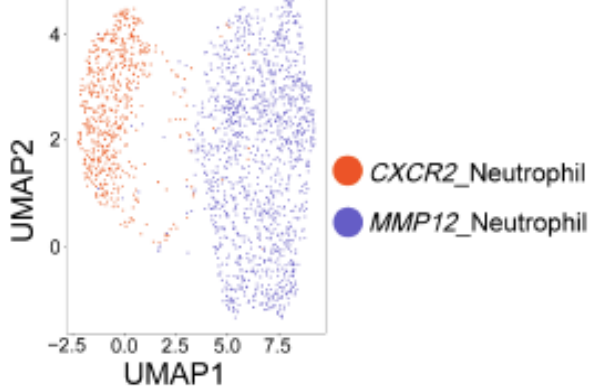
90,406 cells from 42 patients,



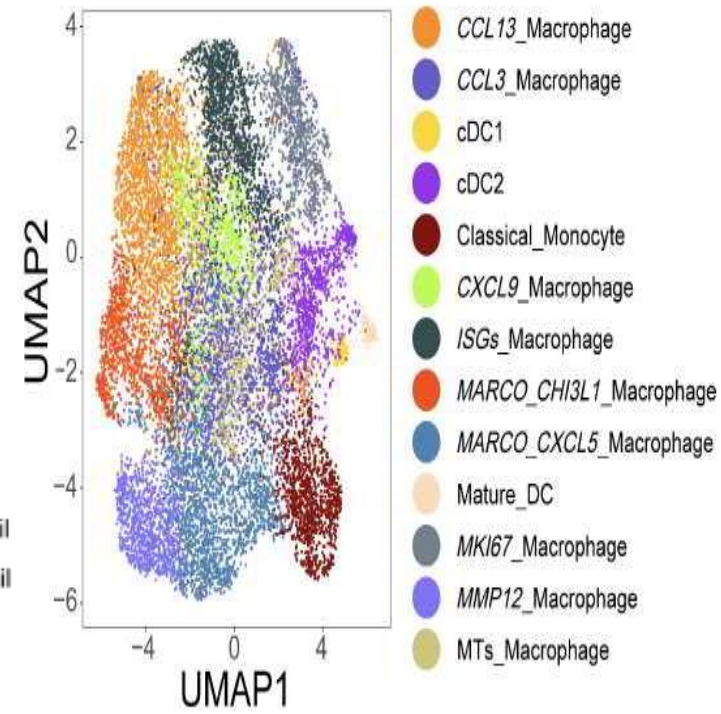
6 clusters T-cell + 2 NK



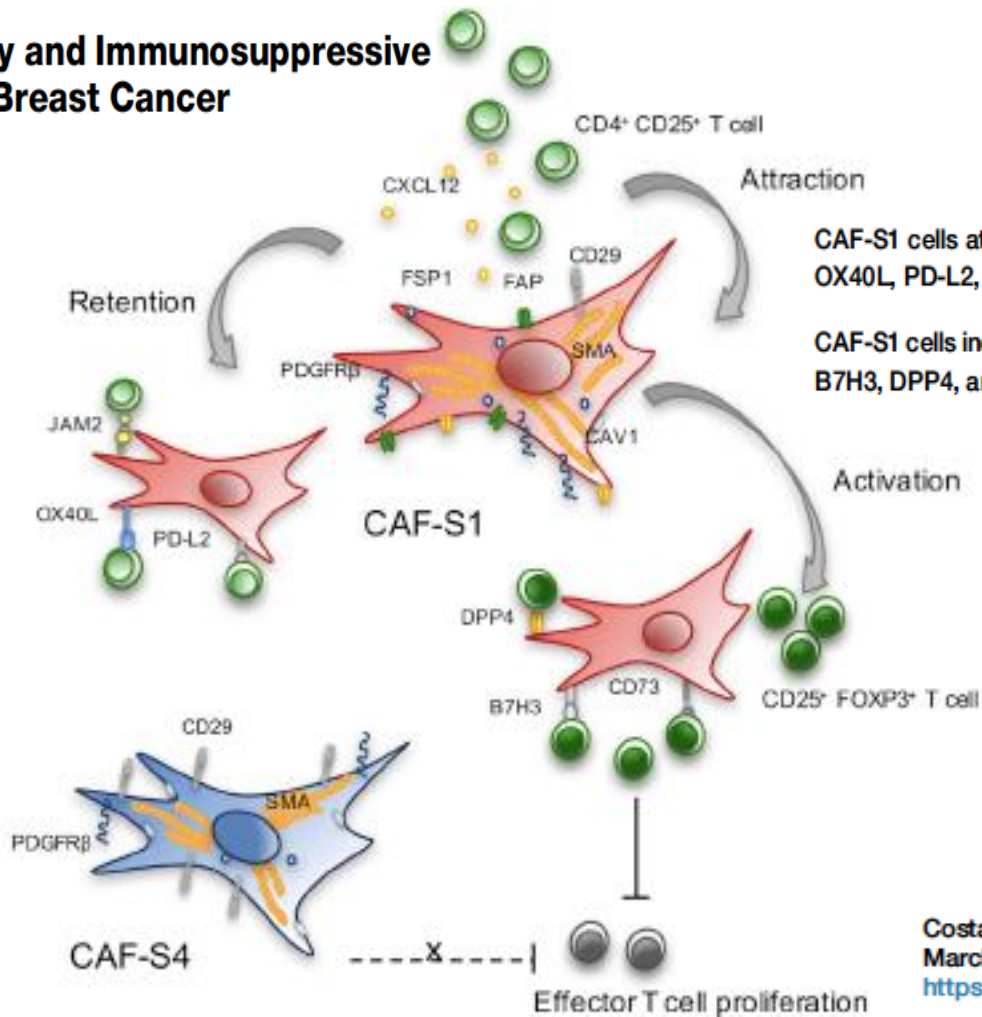
2 clusters neutrophil



10 clusters de macroph  
3 clusters dendritic



Bulk RNAseq  
K sein, ovaire



CAF-S1 cells attract and retain CD4<sup>+</sup>CD25<sup>+</sup> T cells through OX40L, PD-L2, and JAM2

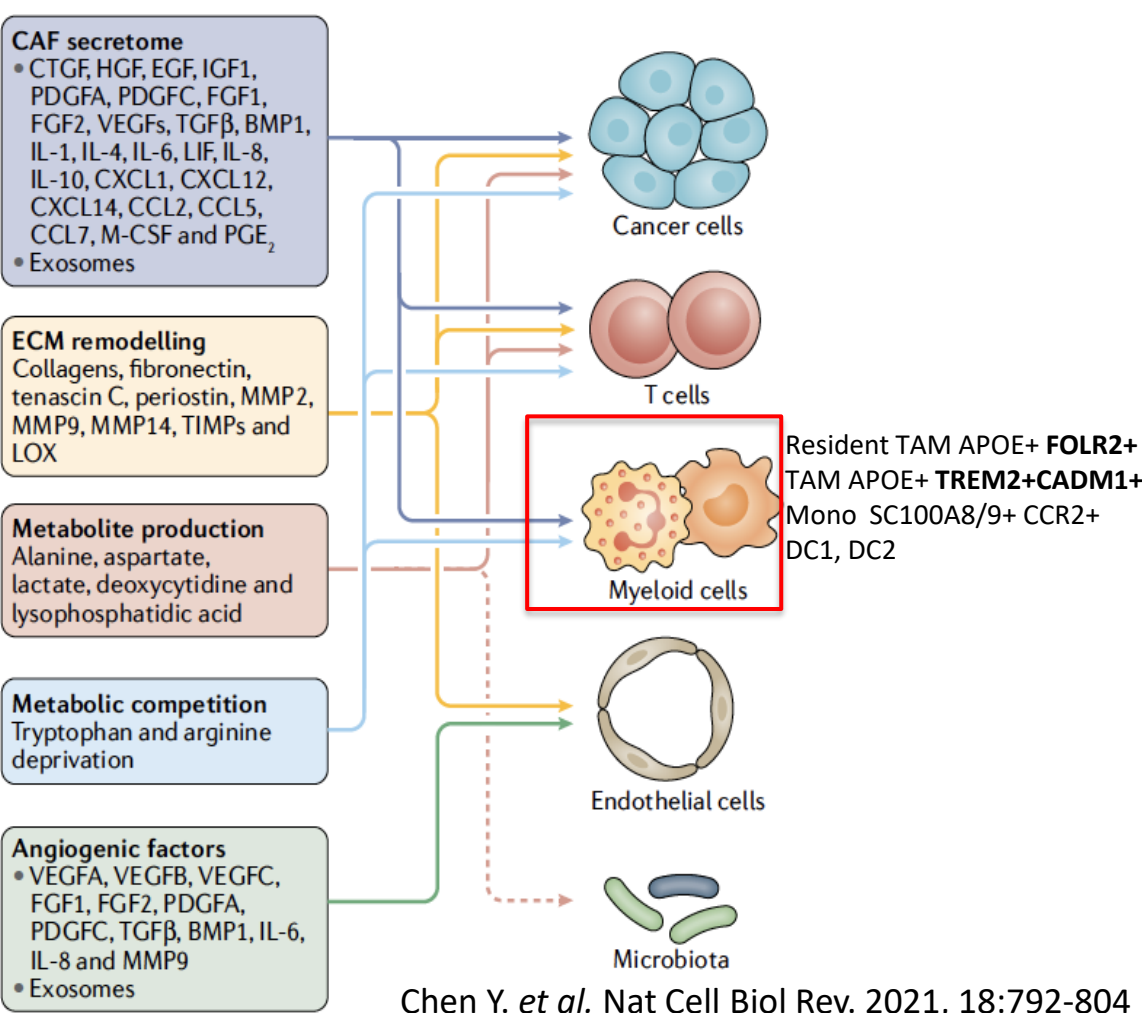
CAF-S1 cells increase CD25<sup>+</sup>FOXP3<sup>+</sup> T lymphocytes, through B7H3, DPP4, and CD73

# Les CAFs grands organisateurs du micro-environnement tumoral ?



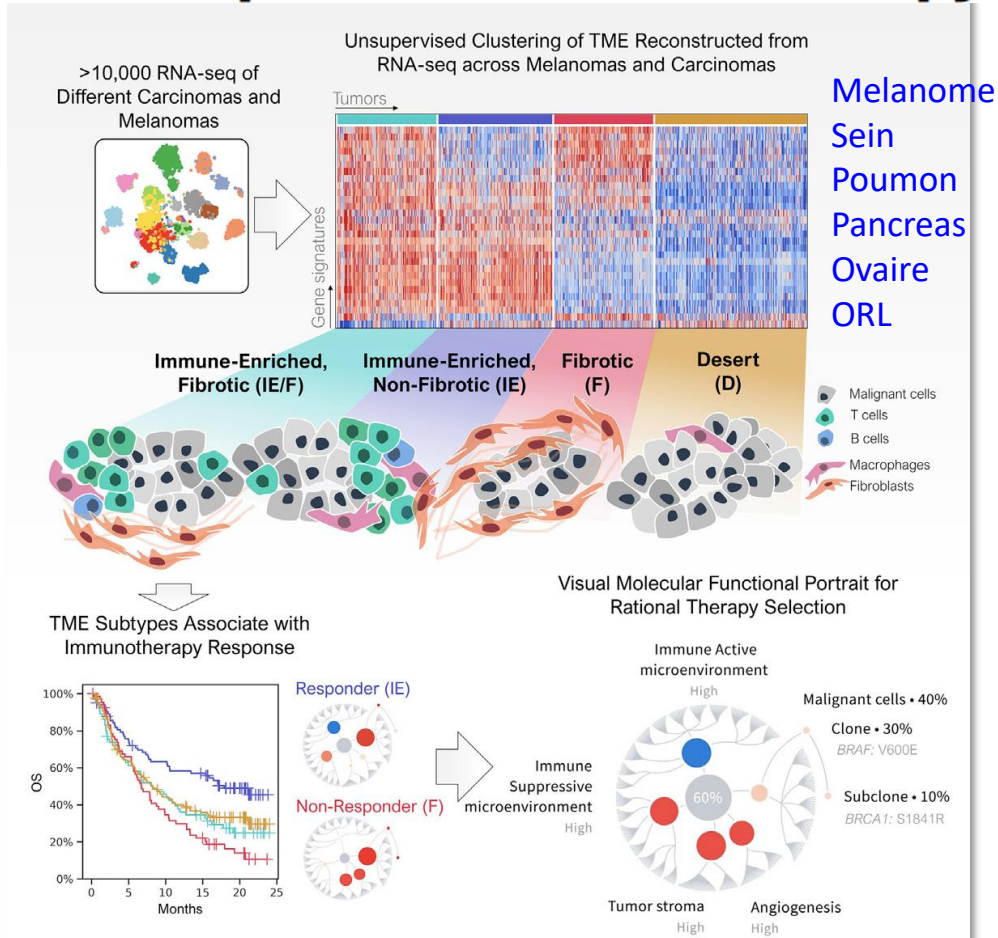
Manquent souvent 2 dimensions au  
ScRNAseq:

- La morphologie
- Les relations inter-cellulaires au sein de la tumeur (spatiales)



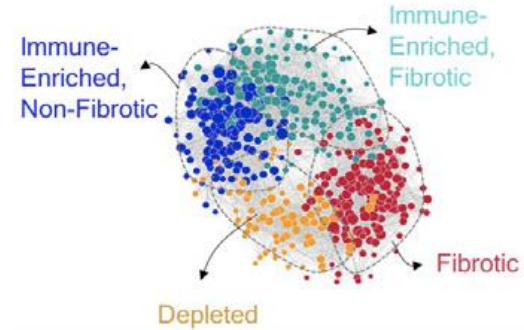


# Conserved pan-cancer microenvironment subtypes predict response to immunotherapy

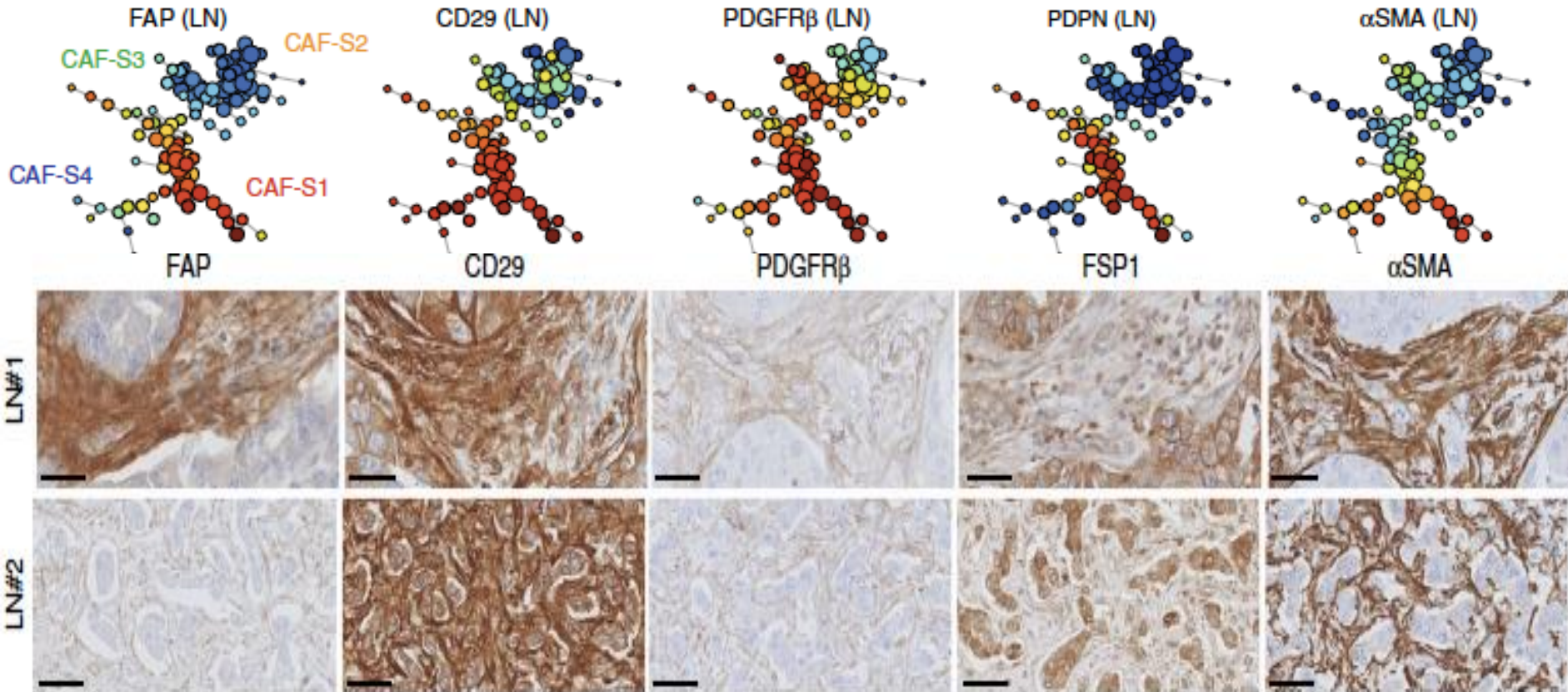


4 signatures transcriptomiques de microenvironnements tumoraux  
(cohortes de découverte= mélanomes)

Based on 29 Gene Expression Signatures Comprising TME and Malignant Cell Properties



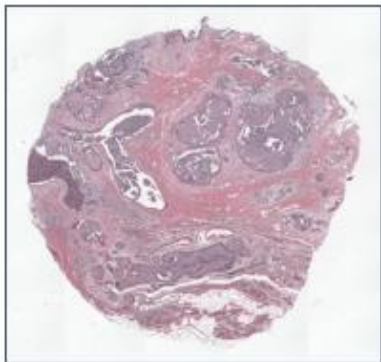
# L'hétérogénéité du ME tumoral appréciée par l'IHC de routine ? marqueurs sous-types transcriptomiques de CAFs (K sein)



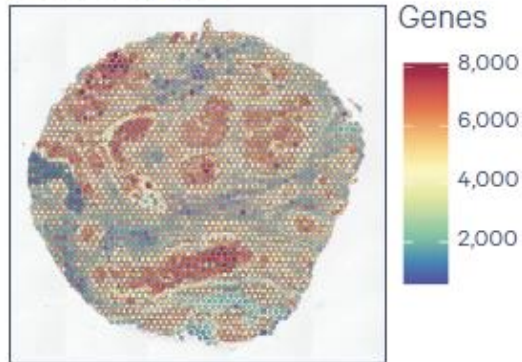


# L'hétérogénéité du ME tumoral appréciée par la transcriptomique spatiale

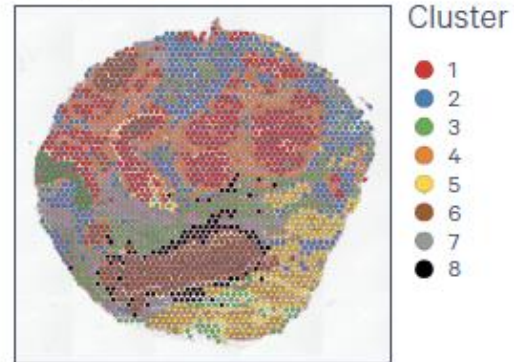
A. H&E



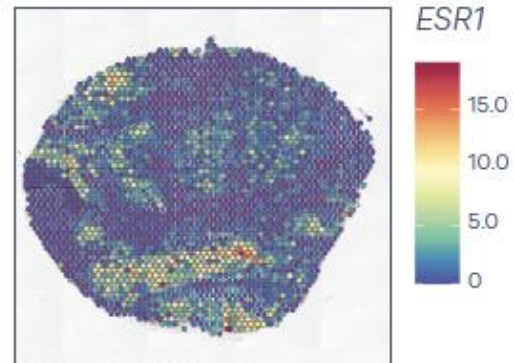
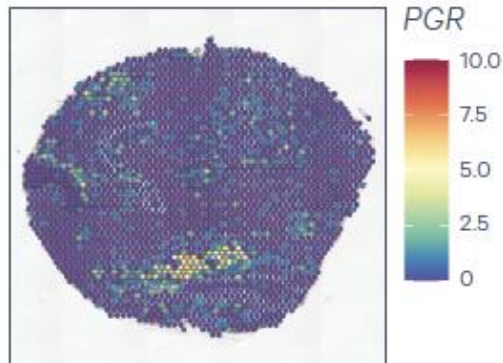
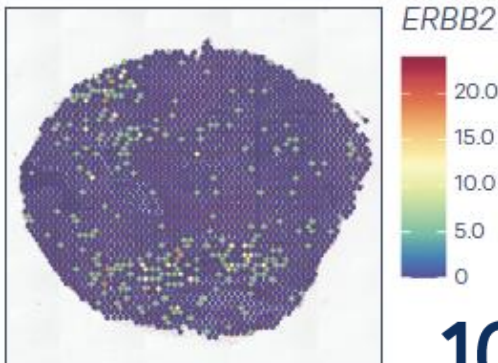
B. Total genes



C. Spot clusters



D. Three key breast cancer biomarkers



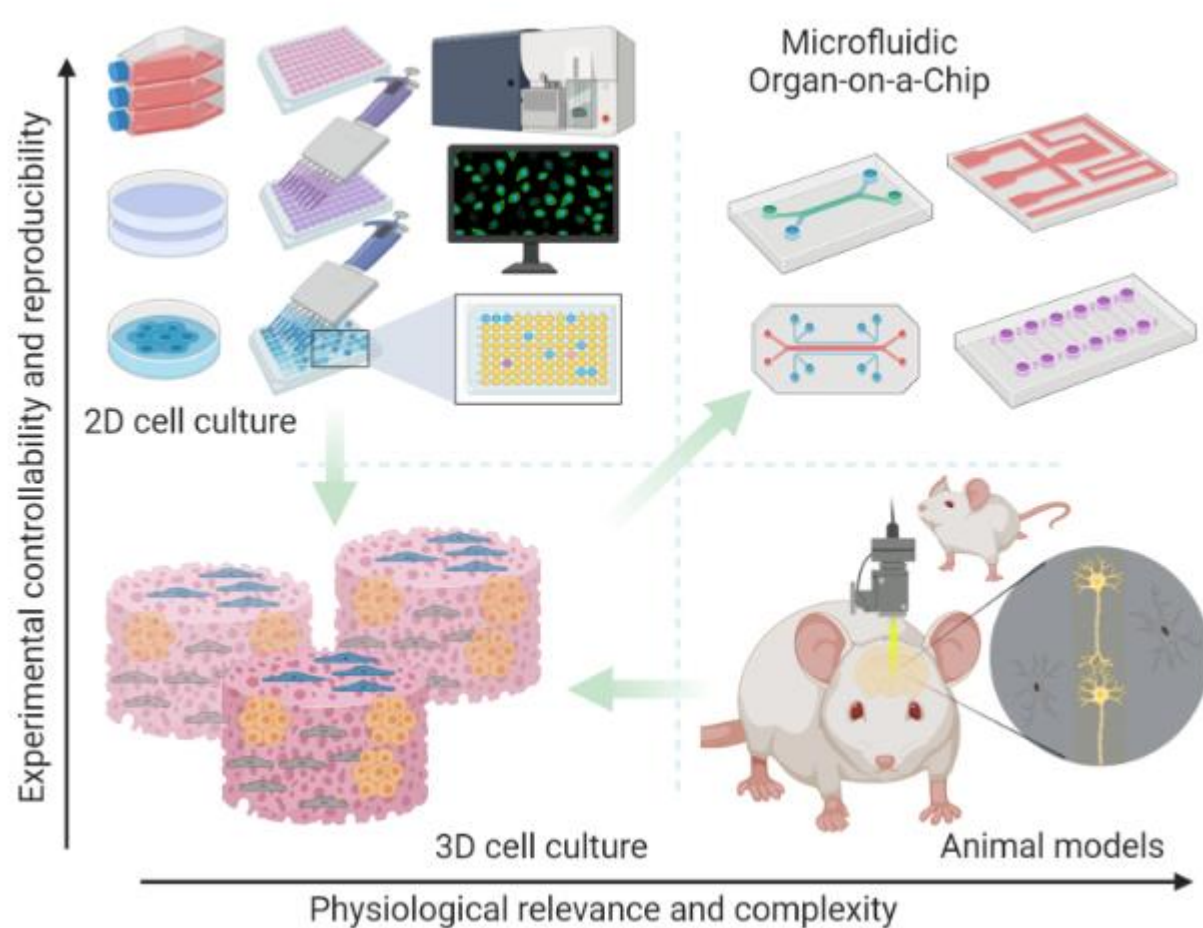
10x  
GENOMICS

1 spot # 10-12 cellules (≠ScRNAseq)

Visium Spatial™

Modèles précliniques pour étudier  
le rôle de l'hétérogénéité

# Comment reconstituer expérimentalement l'hétérogénéité tumorale ?



## 2D :

- + : Rapide et peu coûteux
- : Pas de représentation du MTE

## 3D :

- + : Représentation du MTE
- : Non contrôlable

## Modèles animaux :

- + : Représentation du MTE
- : Grande variabilité inter-espèces
- : Pas de système immunitaire...

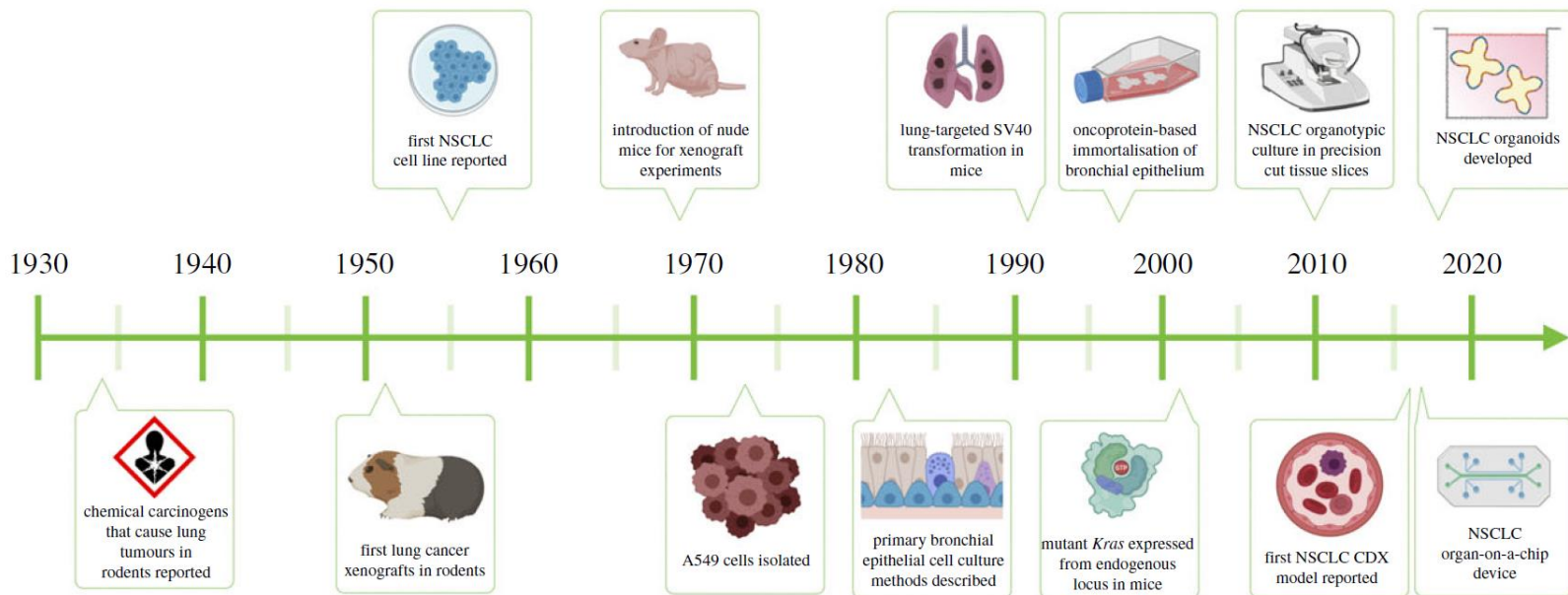
## Systèmes microfluidiques (chip)

- 3D
- Systèmes cellulaires **autologues avec les cellules immunes du patient**
- Miniaturisation ( $10^4$  cellules)
- **Compartmentalisation**



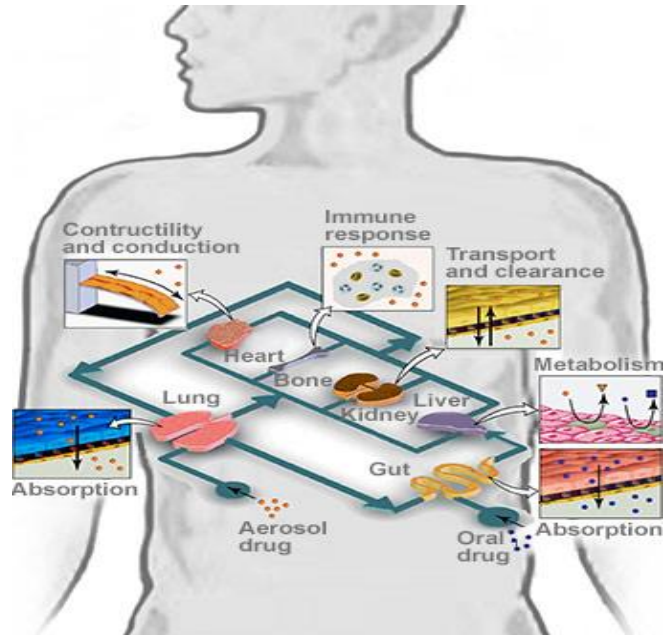
# Progress towards non-small-cell lung cancer models that represent clinical evolutionary trajectories

Robert E. Hynds<sup>1,2</sup>, Kristopher K. Frese<sup>3,4</sup>, David R. Pearce<sup>1</sup>, Eva Grönroos<sup>2</sup>  
Caroline Dive<sup>3,4</sup> and Charles Swanton<sup>1,2</sup>

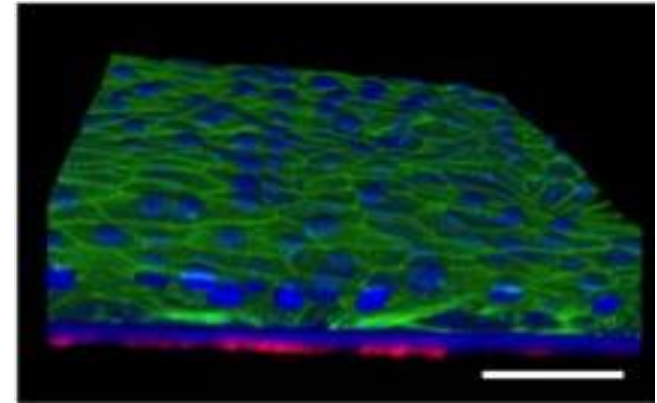
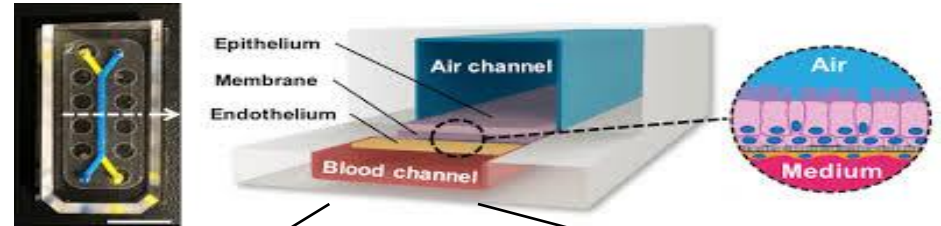


# Organs-on-chip

**Organs-on-chip** (OOC) are 3D co-cultures, within engineered microfluidic devices, that recapitulate the microarchitecture and functions of living human organs.



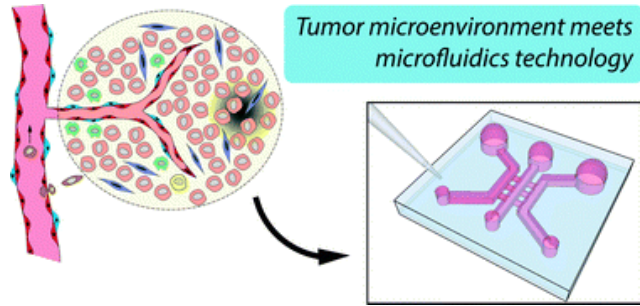
## Lung-on-chip







Wyss Institut, Harvard

Ingber DE *et al.*

# Organ-on-a-chip technology for oncology research (tumor-on-chip): our proof of concept (2015-2018)



Tumor microenvironment meets microfluidics technology

-  Tumor Cell
-  Inflammatory Cells
-  Fibroblast
-  Endothelial Cell

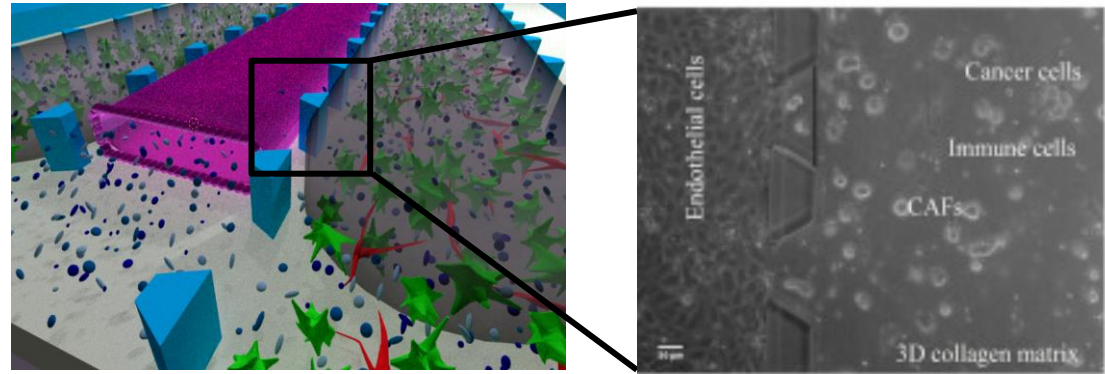
Young EW., 2013. *Integrative Biology*

**Cell Reports** 25, 3884–3893, December 26, 2018

## Dissecting Effects of Anti-cancer Drugs and Cancer-Associated Fibroblasts by On-Chip Reconstitution of Immunocompetent Tumor Microenvironments

Marie Nguyen,<sup>1,2,11</sup> Adele De Ninno,<sup>3,9,11</sup> Arianna Mencattini,<sup>4,11</sup> Fanny Mermet-Meillon,<sup>1,2,11</sup> Giulia Fornabaio,<sup>1,2</sup> Sophia S. Evans,<sup>1,2</sup> Méli ssande Cossutta,<sup>1,2</sup> Yasmine Khira,<sup>1,2</sup> Weijing Han,<sup>1,2</sup> Philémon Sirven,<sup>1,8</sup> Floriane Pelon,<sup>1,8</sup> Davide Di Giuseppe,<sup>4</sup> Francesca Romana Bertani,<sup>3</sup> Annamaria Gerardino,<sup>3</sup> Ayako Yamada,<sup>1,6,7</sup> Stéphanie Descroix,<sup>1,6,7</sup> Vassili Soumelis,<sup>1,5</sup> Fatima Mechta-Grigoriou,<sup>1,8</sup> Gérard Zalcman,<sup>1,2,10</sup> Jacques Camonis,<sup>1,2</sup> Eugenio Martinelli,<sup>4,12</sup> Luca Businaro,<sup>3,12</sup> and Maria Carla Parrini<sup>1,2,12,13,\*</sup>

## HER2+ breast cancer immunocompetent TME recapitulation



- Recapitulating an anti-tumoral ADCC (antibody-dependent cell-mediated cytotoxicity) immune response by trastuzumab.
- Cancer-associated fibroblasts (CAFs) antagonized the effects of trastuzumab.

Nguyen M. *et al.* Cell Rep. 2018

De la biologie à la clinique

Que se passe t'il chez nos patients ?

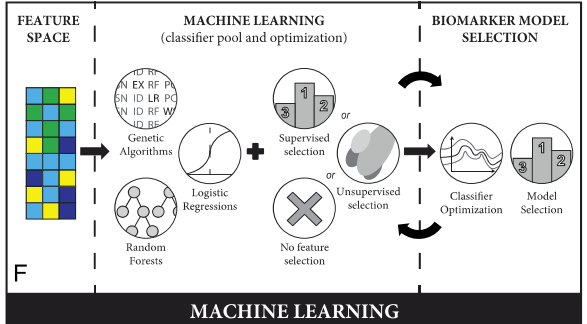
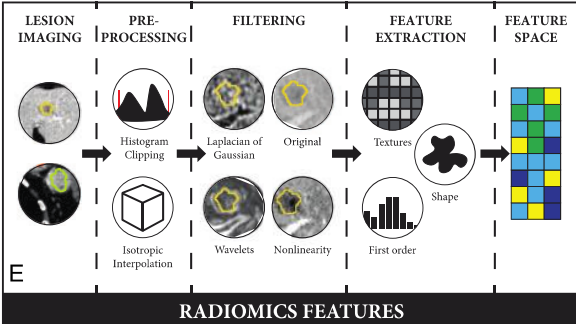
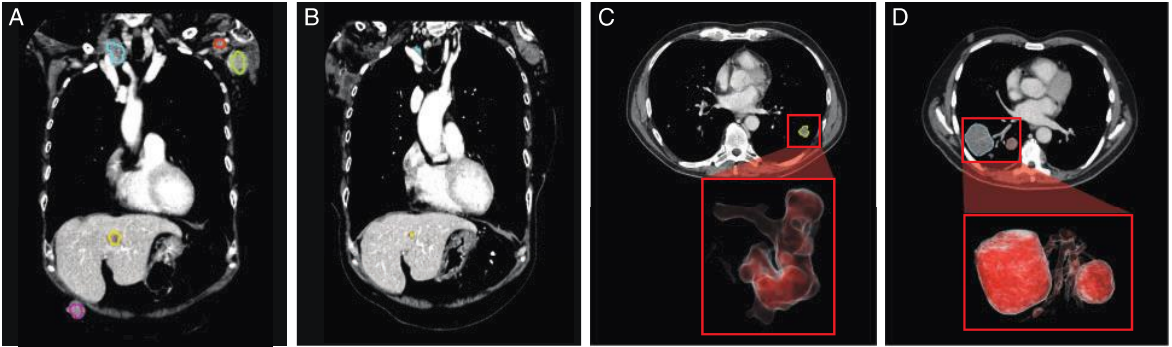
Peut-on évaluer l'hétérogénéité tumorale chez nos patients dans leur suivi clinique?

**RADIOMICS**



# Radiomics

Highman et al 2015

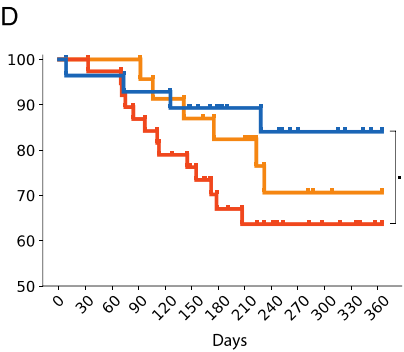
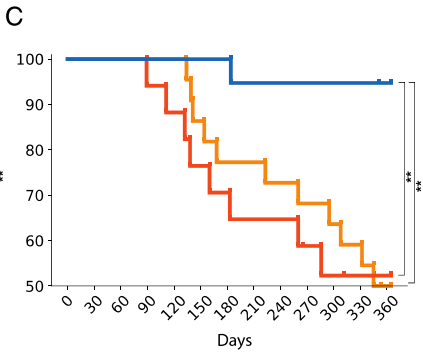
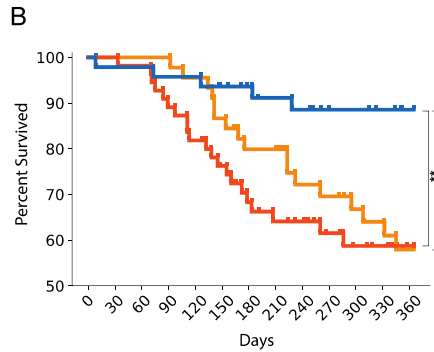
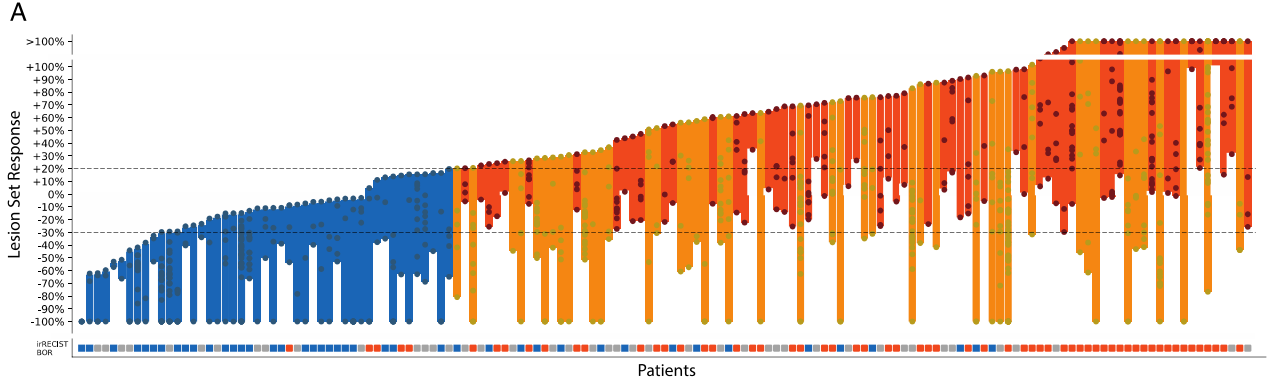


Trebeschi, Ann of Oncol, 2019

# Radiomics

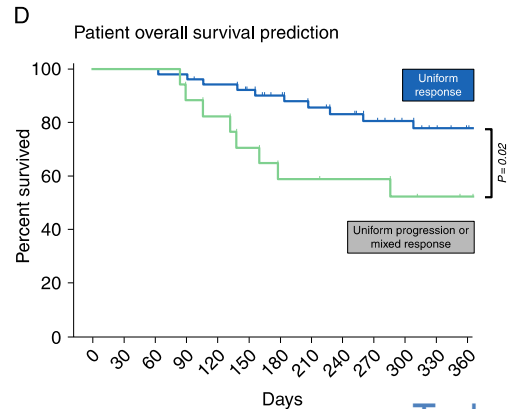
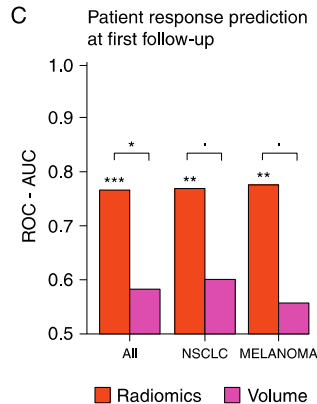
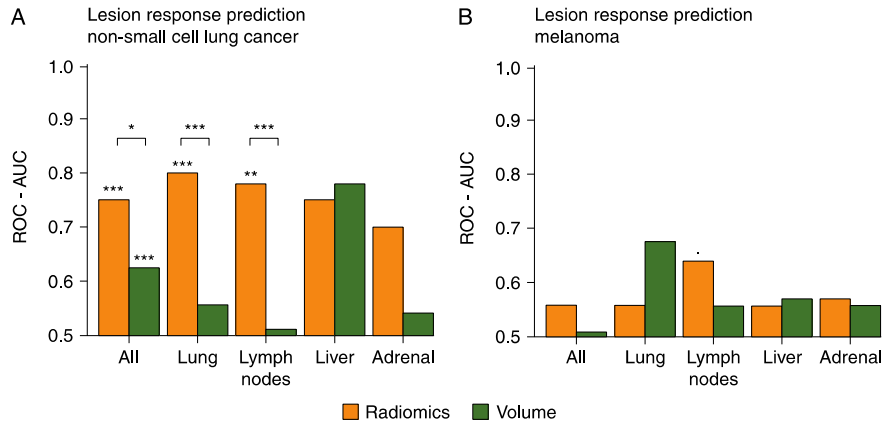
- non-invasive procedure performed routinely in patients with cancer
- visualisation and assessment of the whole tumour as well as any sites of metastatic disease
- better assessment of any heterogeneity that exists within or between tumours driving differential prognoses and responses to treatment
- opportunities to escalate or change treatment, or consider multimodality treatment options, such as the addition of stereotactic ablative body radiotherapy (SABR) for oligoprogressive disease.

# Radiomics



Uniform Progression    Mixed    Uniform Response

# Radiomics



# Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA

*Abbosh et al. has shown that primary tumour volumes pre surgery correlate with ctDNA*

<https://doi.org/10.1038/s41586-023-05776-4>

Received: 6 April 2022

Accepted: 30 January 2023

Published online: 13 April 2023

 Check for updates

Christopher Abbosh<sup>1,101</sup>✉, Alexander M. Frankell<sup>1,2,101</sup>, Thomas Harrison<sup>3,101</sup>, Judit Klisstk<sup>4,5,6,101</sup>, Aaron Garnett<sup>3,101</sup>, Laura Johnson<sup>3</sup>, Selvaraju Veeriah<sup>1</sup>, Mike Moreau<sup>3</sup>, Adrian Chesh<sup>3</sup>, Tafadzwa L. Chaunzwa<sup>7,8</sup>, Jakob Weiss<sup>7,8,9</sup>, Morgan R. Schroeder<sup>3</sup>, Sophia Ward<sup>1,2,10</sup>, Kristiana Grigoriadis<sup>1,2,11</sup>, Aamir Shahpurwalla<sup>3</sup>, Kevin Litchfield<sup>1,12</sup>, Clare Puttick<sup>1,2,11</sup>, Dhruva Biswas<sup>1,2,13</sup>, Takahiro Karasaki<sup>1,2,14</sup>, James R. M. Black<sup>1,11</sup>, Carlos Martínez-Ruiz<sup>1,11</sup>, Maise Al Bakir<sup>1,2</sup>, Oriol Pich<sup>2</sup>, Thomas B. K. Watkins<sup>2</sup>, Emilla L. Lim<sup>1,2</sup>, Ariana Huebner<sup>1,2,11</sup>, David A. Moore<sup>1,2,15</sup>, Nadia Godin-Heymann<sup>16</sup>, Anne L'Hernault<sup>16</sup>, Hannah Bye<sup>16</sup>, Aaron Odell<sup>3</sup>, Paula Roberts<sup>3</sup>, Fabio Gomes<sup>17</sup>, Akshay J. Patel<sup>18</sup>, Elizabeth Manzano<sup>1</sup>, Crispin T. Hiley<sup>1,2</sup>, Nicolas Carey<sup>19</sup>, Joan Riley<sup>19</sup>, Daniel E. Cook<sup>2</sup>, Darren Hodgson<sup>16</sup>, Daniel Stetson<sup>20</sup>, J. Carl Barrett<sup>20</sup>, Roderik M. Kortlever<sup>21</sup>, Gerard I. Evan<sup>21</sup>, Allan Hackshaw<sup>22</sup>, Robert D. Daber<sup>3</sup>, Jacqui A. Shaw<sup>19</sup>, Hugo J. W. L. Aerts<sup>7,8,23</sup>, Abel Licon<sup>3</sup>, Josh Stahl<sup>3</sup>, Marlam Jamal-Hanjan<sup>1,14,24</sup>, TRACERx Consortium\*, Nicolai J. Birkbak<sup>1,2,4,5,6,102</sup>, Nicholas McGranahan<sup>1,11,102</sup>✉ & Charles Swanton<sup>1,2,24,102</sup>✉

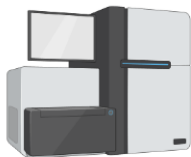


**103 patients**  
with relapse of  
disease

ctDNA Sample  
Processing

Sample analysis  
(Multi-step)

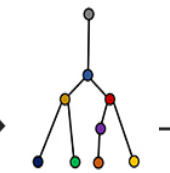
214 longitudinal ctDNA plasma samples (28 patients)...up to Mortuary and autopsy !



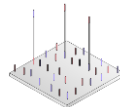
Whole exome  
sequencing



Identification of  
tumour-specific  
alterations



Resolving clonal and  
sub-clonal phylogeny

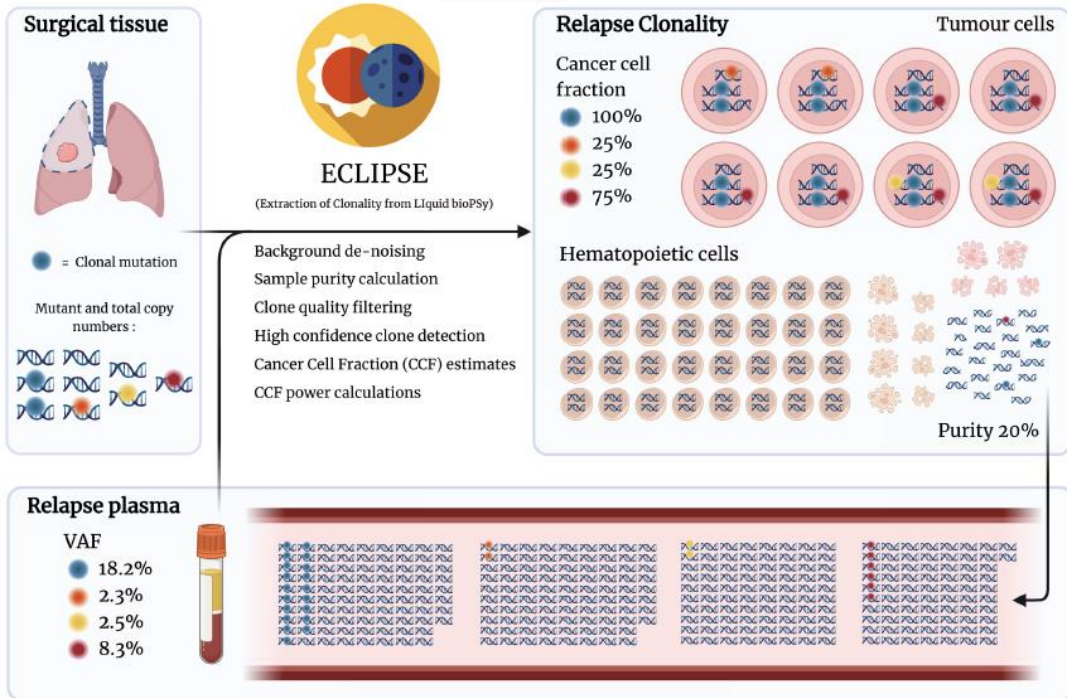


Personalised  
amplicon panels

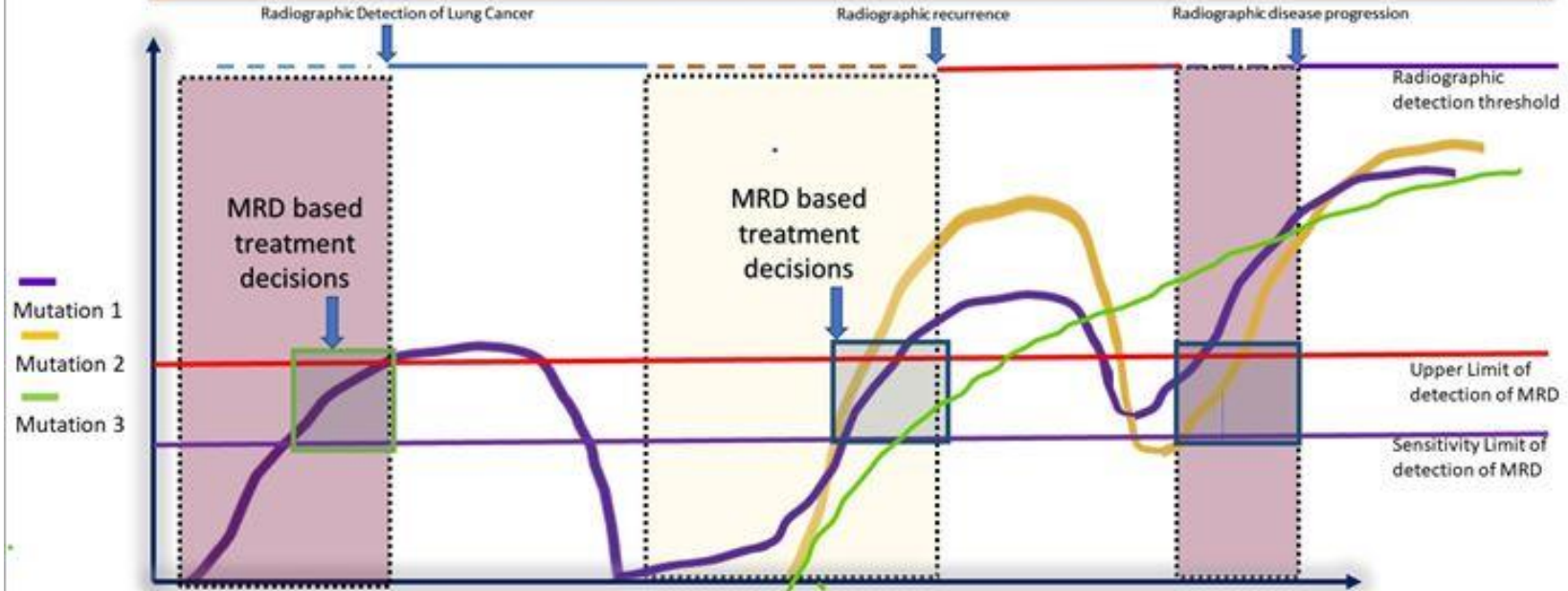


ECLIPSE  
(Extraction of  
Clonality from  
Liquid bioPSE)

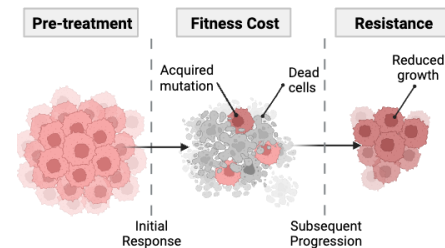
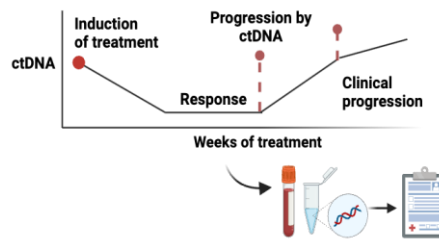
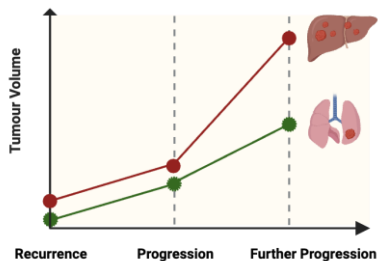
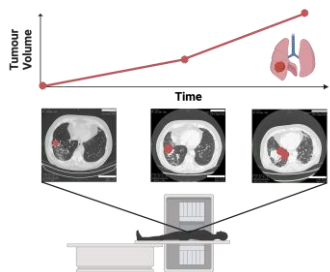
C. Abbosh *et al.*  
Nature 2023



# Moving ctDNA toward clinical action ?



# Abstr. # 209: Tracking cancer progression using longitudinal imaging & ctDNA



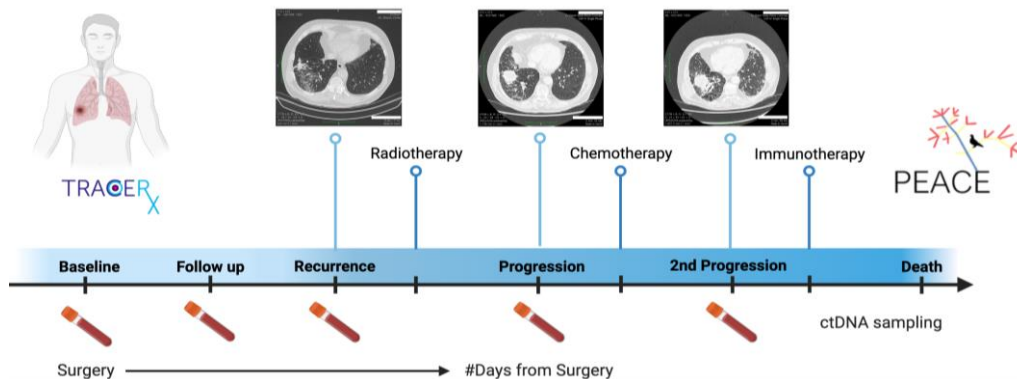
Can we infer tumour growth using imaging?

Does lesion growth differ between patients and organs?

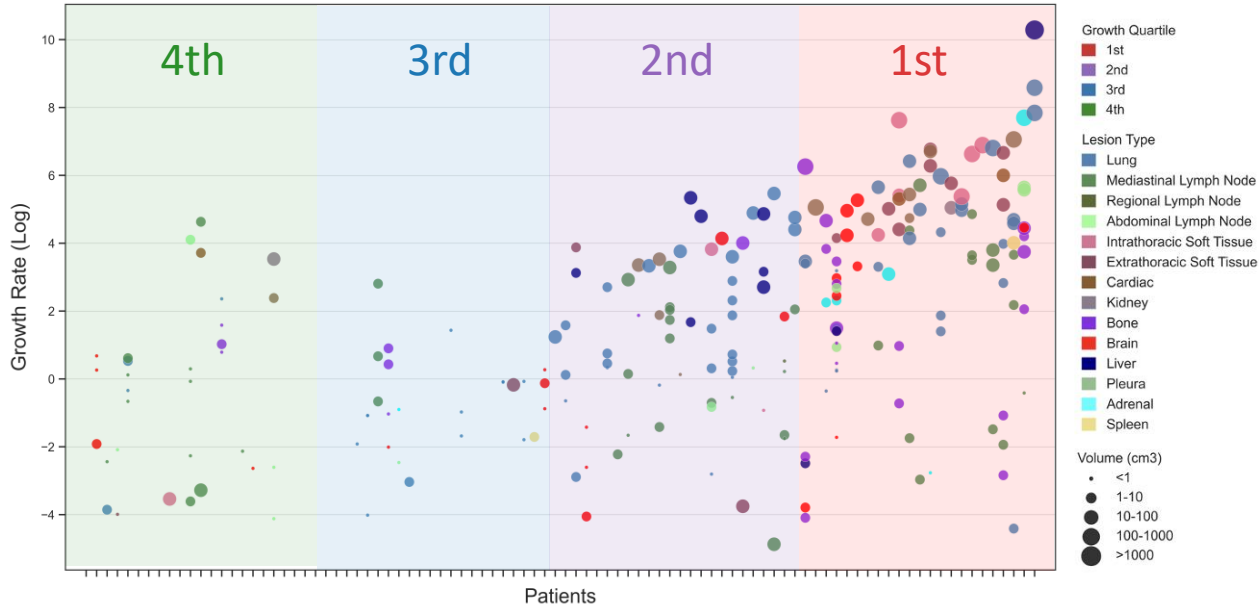
Does tumour volume correlate with ctDNA?

Can imaging identify treatment resistant lesions?

214 longitudinal ctDNA plasma samples (28 patients)



3D tumour volumes for 341 metastases over serial imaging scans.



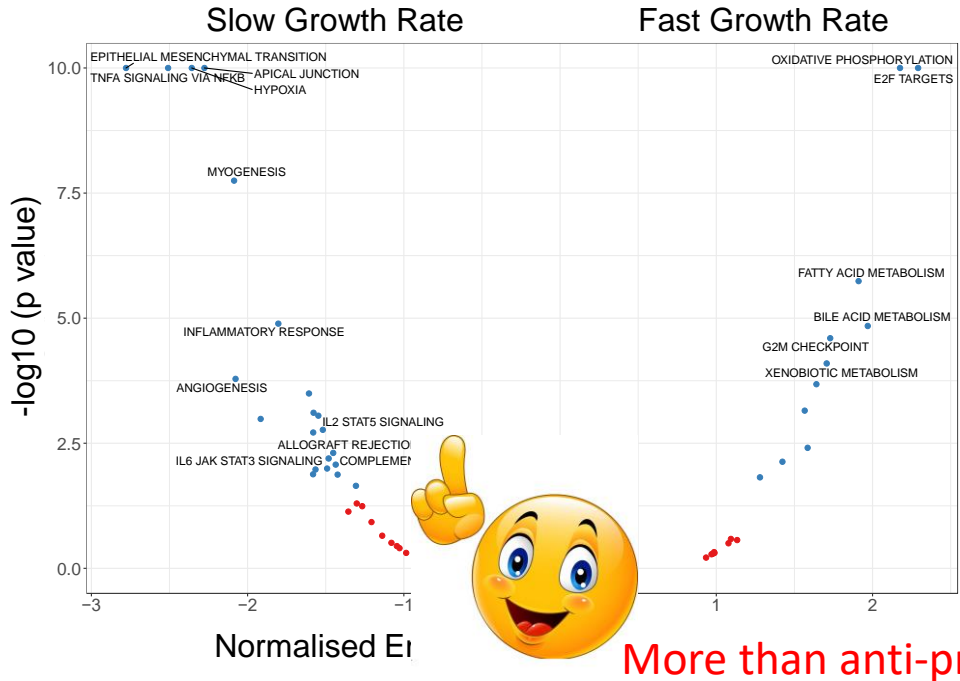
*cf.* RECIST v1.1:

- Response in one site
  - Progression in another site
- = Progression



Different lesions have different growth speed: **heterogeneity** within the different anatomical sites

RNA sequencing data of metastases sampled at autopsy which also had volumetric measurements, n=13



“Lesions with a high growth rate overexpressed signaling pathways associated with proliferation “



E2F targets  
G2M checkpoints  
Xenobiotic metabolism

“low growth rate lesions overexpressed pathways associated with the **immune system**.”

EMT, Hypoxia, apical junction  
Inflammatory response, angiogenesis, IL2/STAT

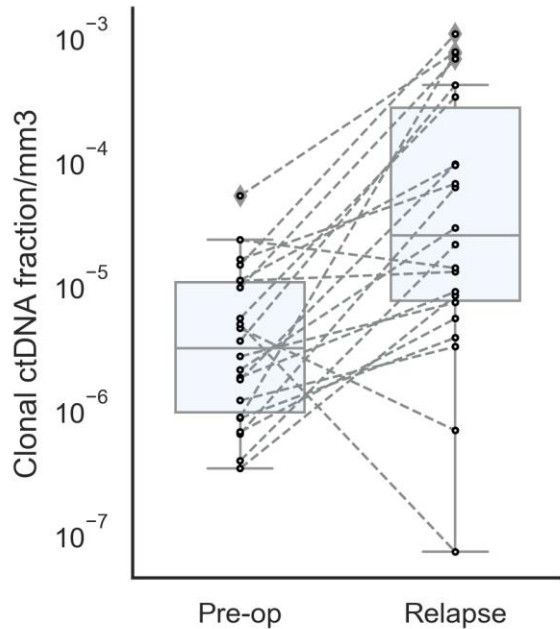


## ctDNA correlates with total volume post relapse for **the majority** of patients

22 patients with paired pre-op ctDNA

⇒ calculate the ctDNA shedded per unit of tumour volume.

⇒ ctDNA shedded at disease relapse with total disease volume at relapse.

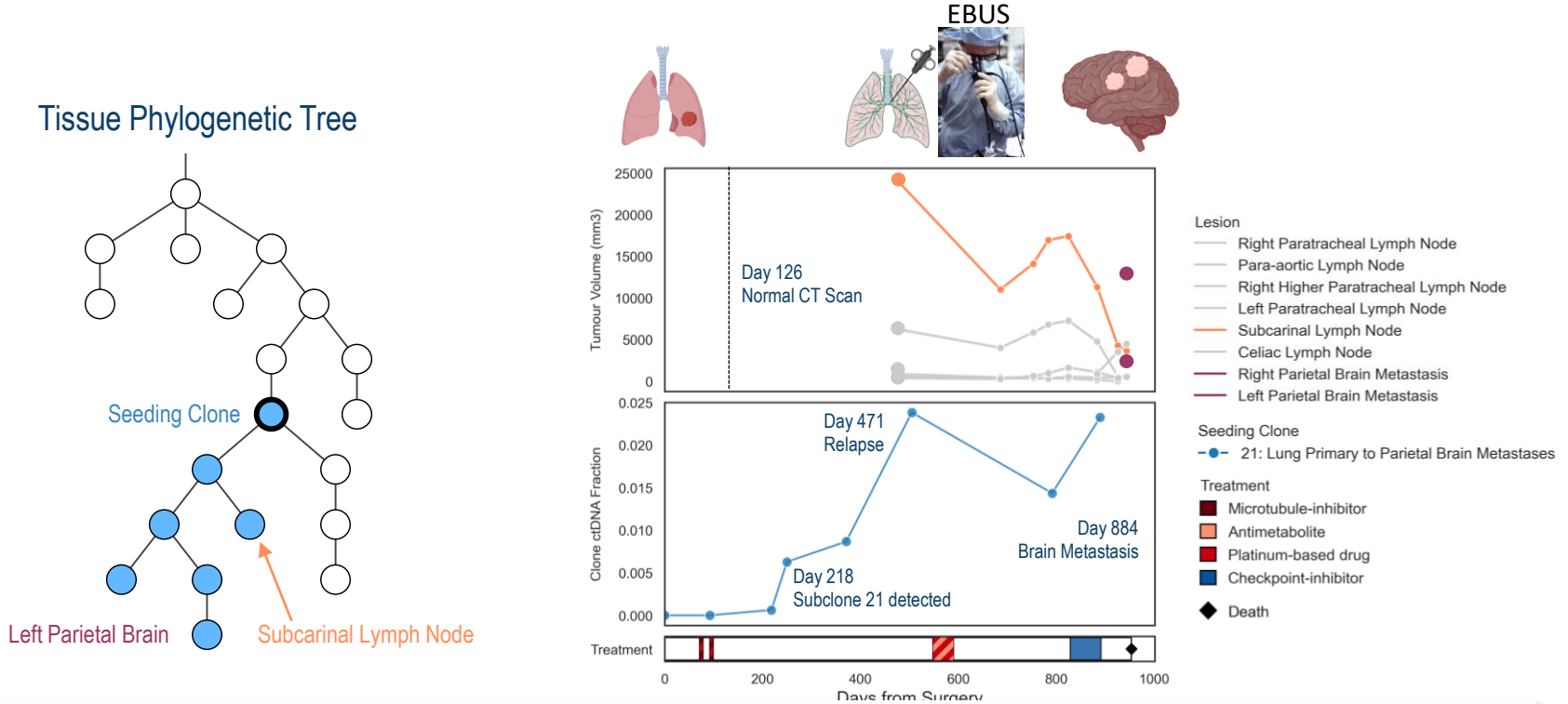


Wilcoxon  $p < 0.0003$

Level of ctDNA shedded per unit of tumour volume **increased at disease relapse** compared to pre-surgery, while disease volume at relapse could be lower than pre-surgery => **Micrometastatic disease ?**

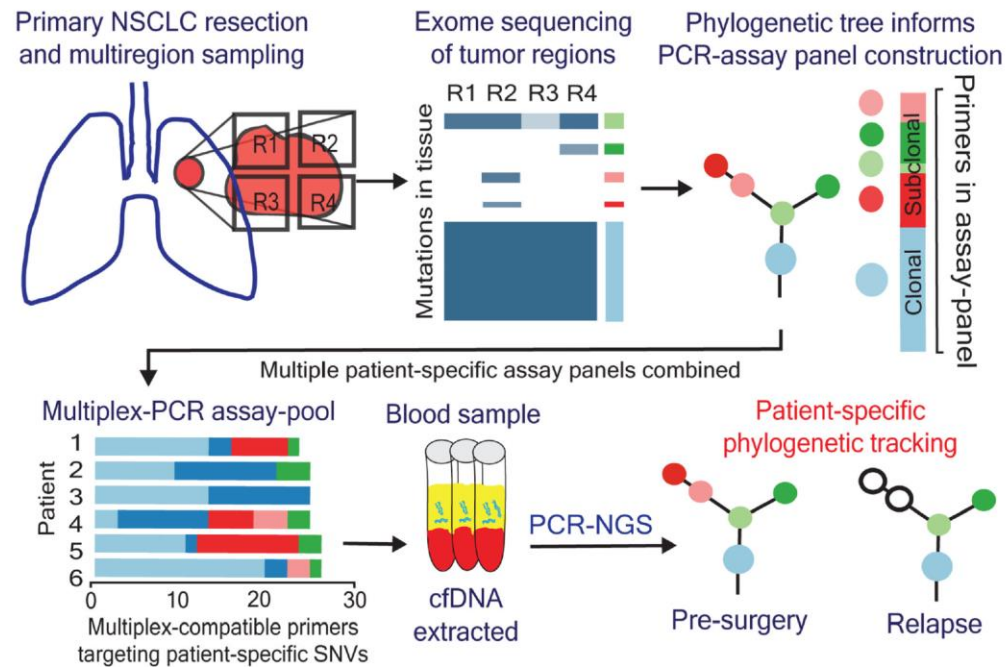
**ctDNA correlates with total volume longitudinally post-disease relapse**, supporting its use as a marker for measuring cancer progression.

# The seeding subclone can be detected and tracked in ctDNA in advance of radiological disease relapse

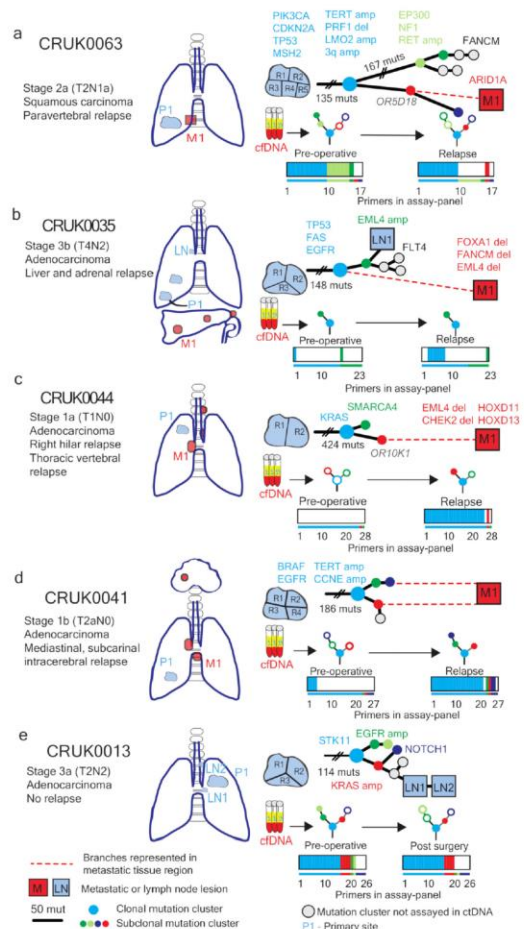
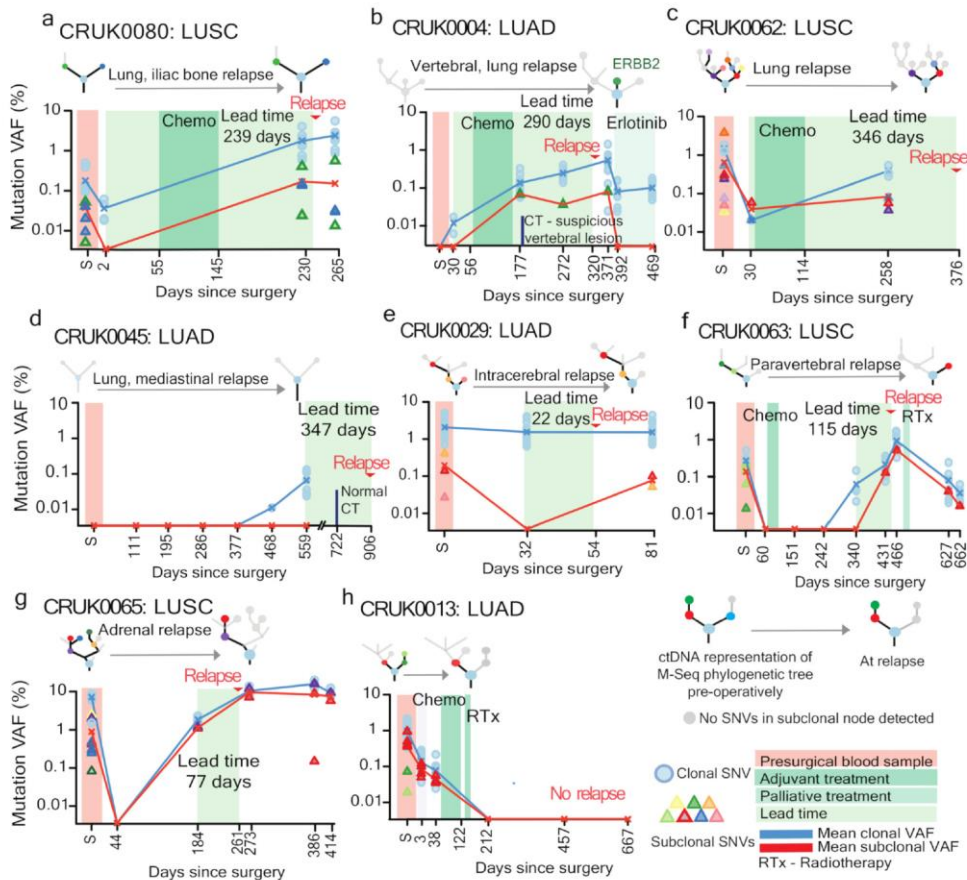


**Longitudinal imaging combined with DNA sequencing and ctDNA tracking can accurately reconstruct the natural history of cancer evolution.**

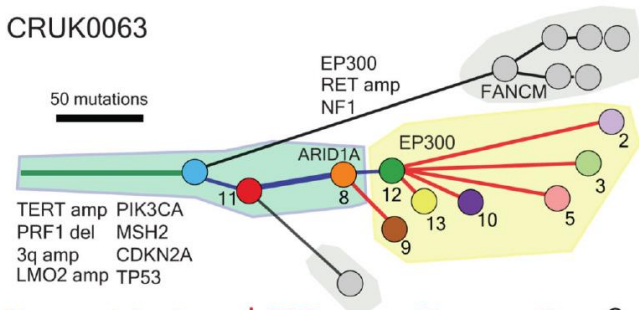
## Phylogenetic ctDNA analysis depicts early stage lung cancer evolution



# Post-operative ctDNA detection predicts and characterizes NSCLC relapse



a CRUK0063



Ubiquitous mutations  
Mutations unique to primary tumor  
Shared mutations in metastatic samples  
Private mutations in metastatic samples

Metastases

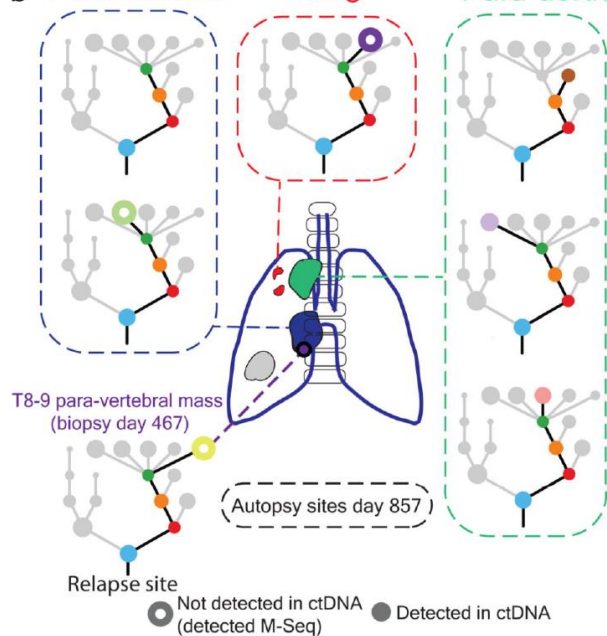
Primary

Common to metastases and primary

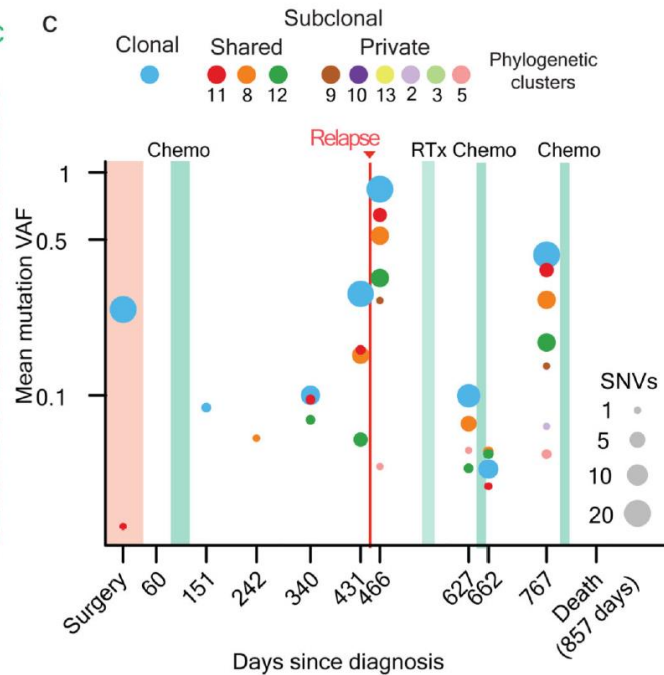
b Para-vertebral

Lung

Para-aortic

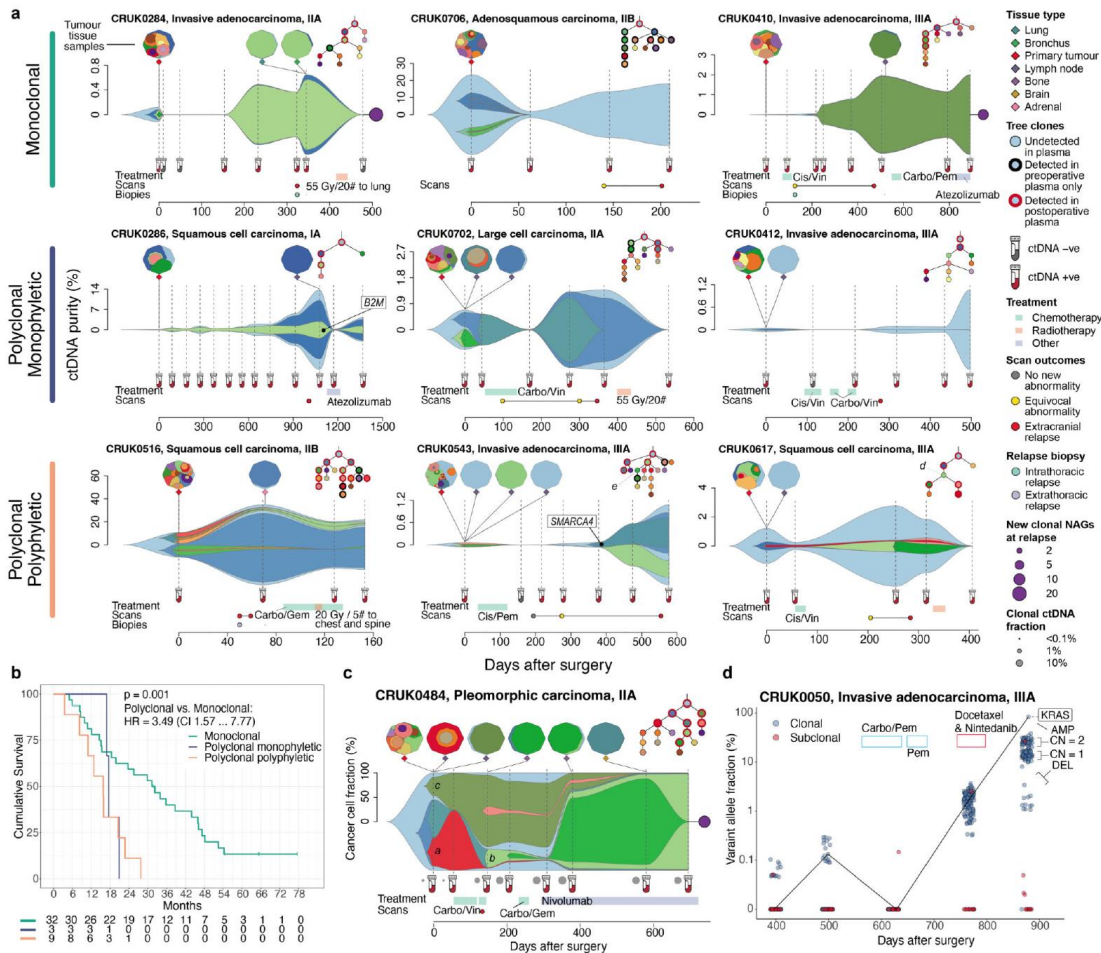


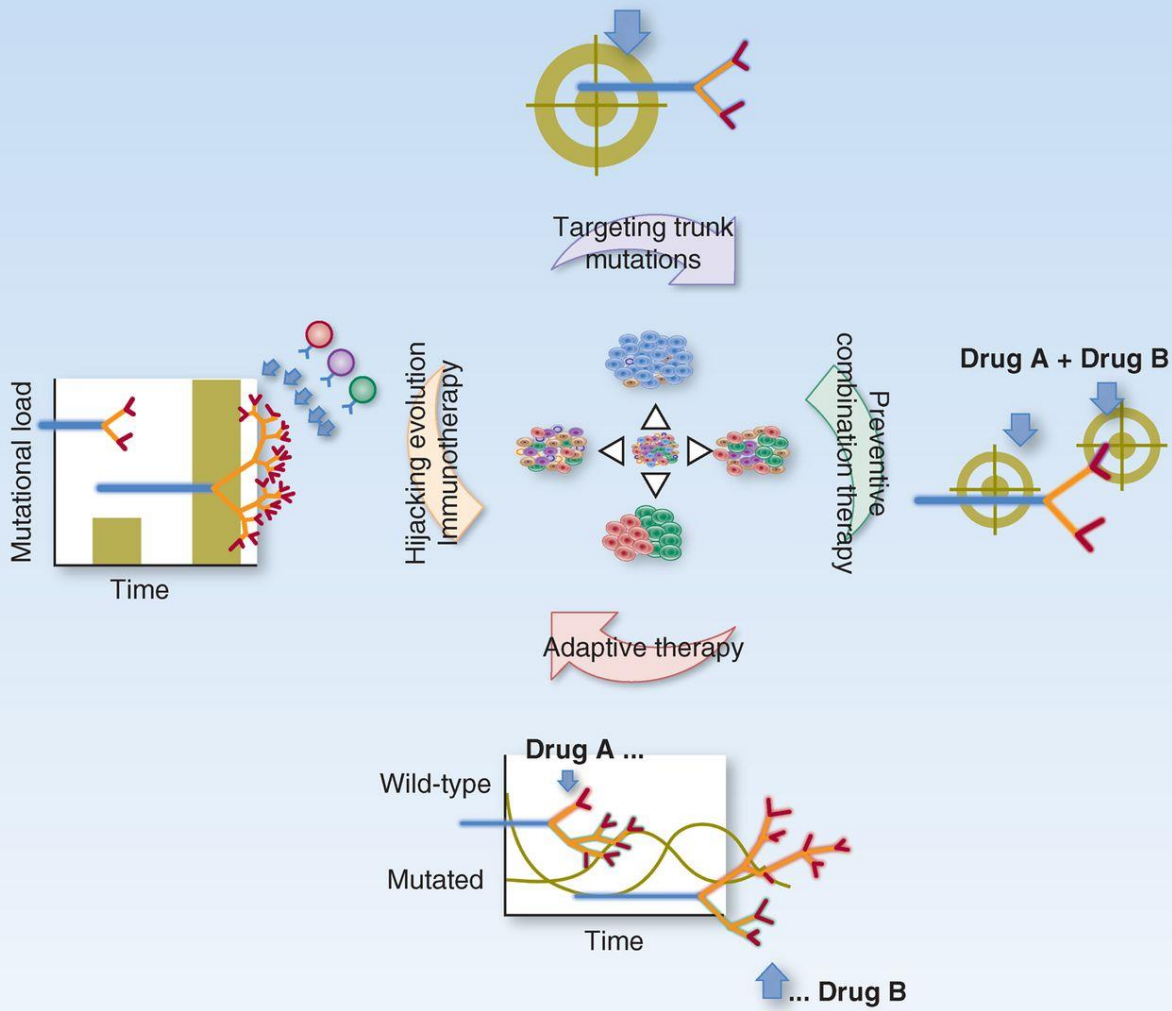
c





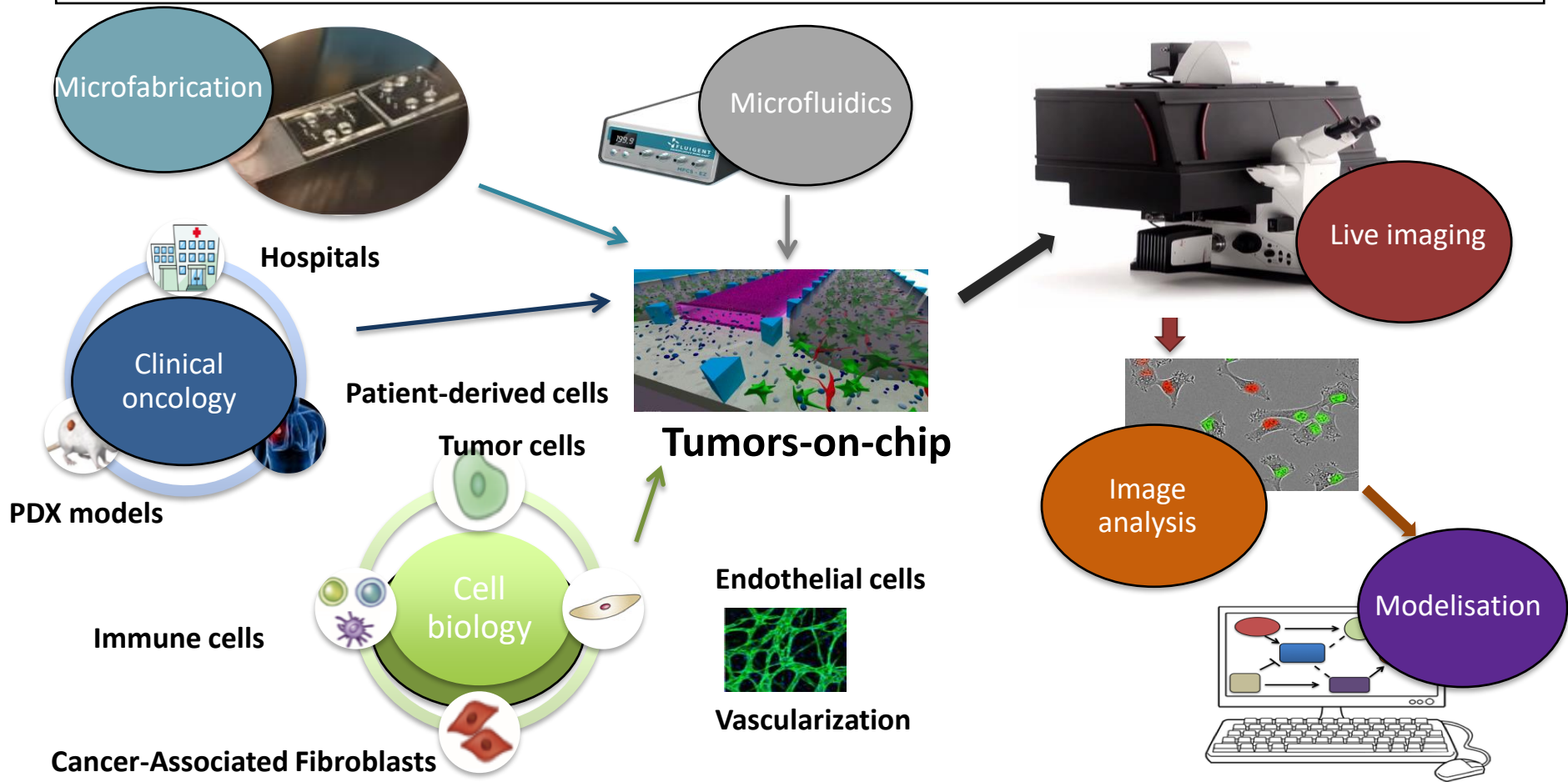
# Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA





Utiliser les connaissances de l'hétérogénéité tumorale pour traiter nos patients

# A multidisciplinary approach for studying TME heterogeneity



# Remerciements

**Pour les diapositives empruntées**

**G rard Zalcman**

**Manon Carr **