

15-16 February 2021

COMETH Training course

From omics data
to tumor heterogeneity quantification

EIT Health is supported by the EIT,
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15 February 2021

Heterogeneity in cancer Does it matter?

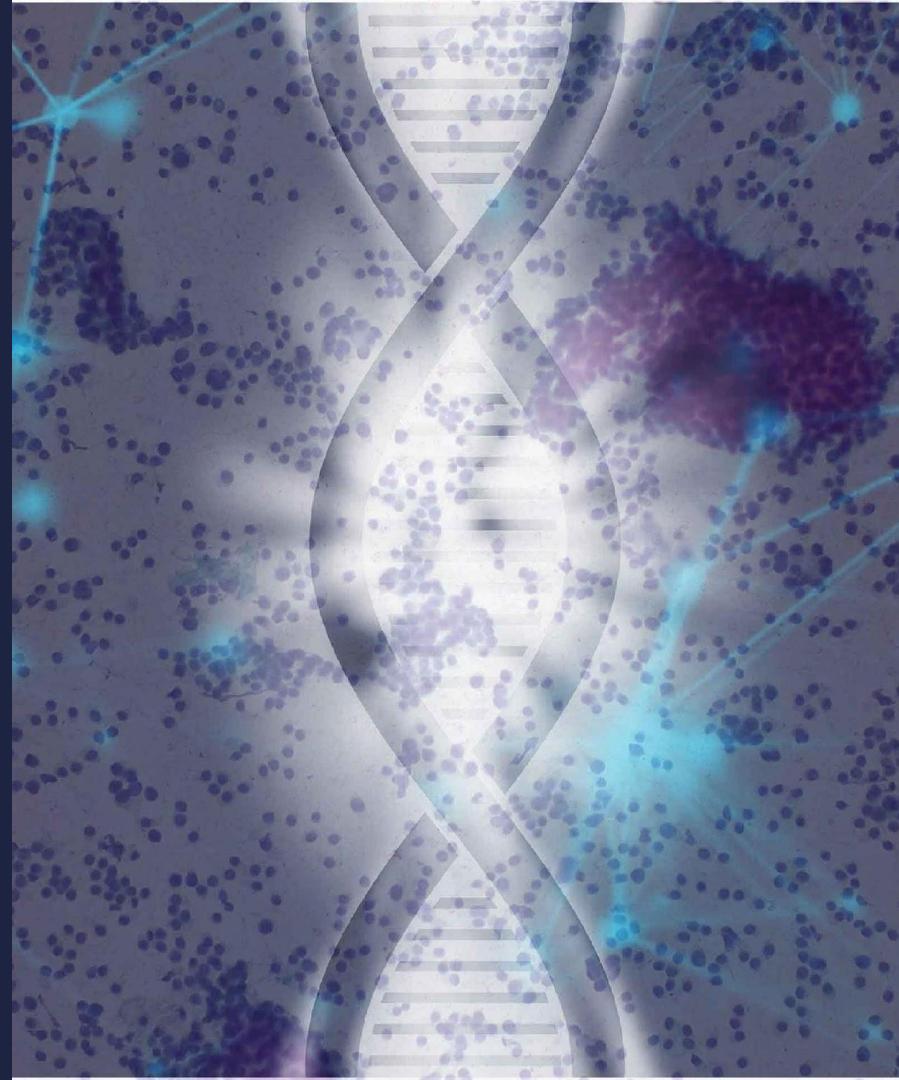
The example of pancreatic adenocarcinoma

Jerome Cros
Dpt of Pathology
Beaujon Hospital – Université de Paris
jerome.cros@aphp.fr



Study of tumor heterogeneity:

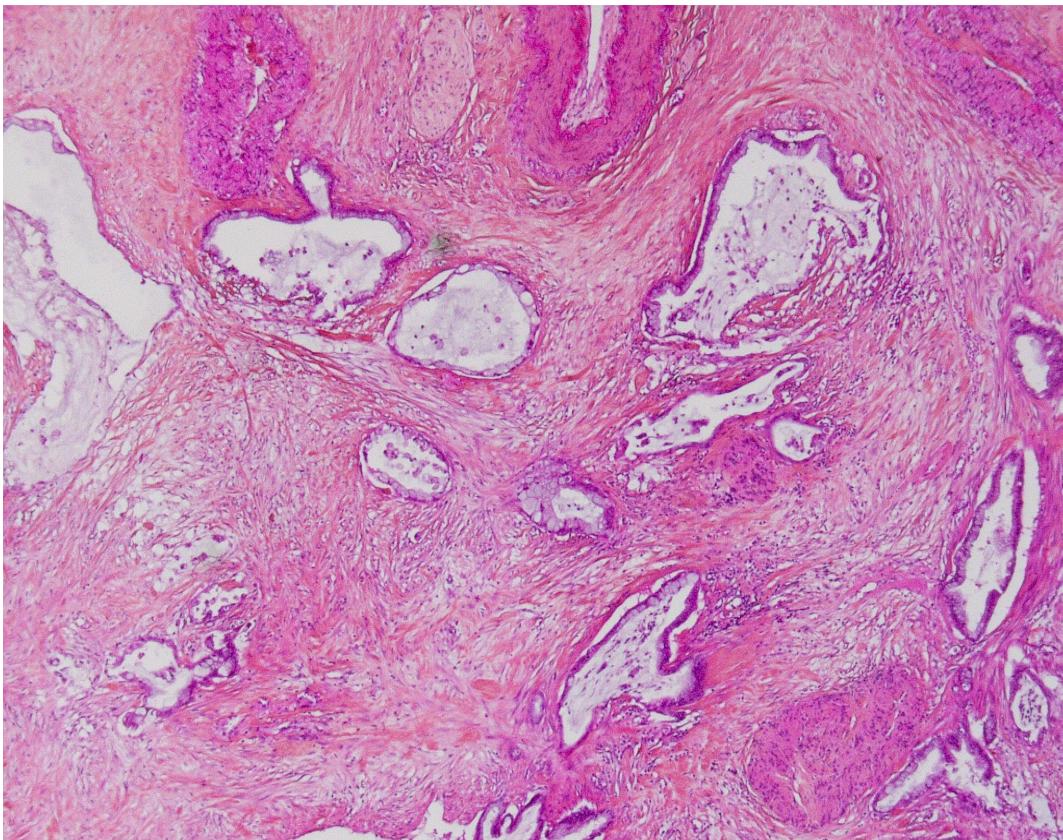
Issues with samples....



1. Beware of miss labeled samples when using public datasets++++

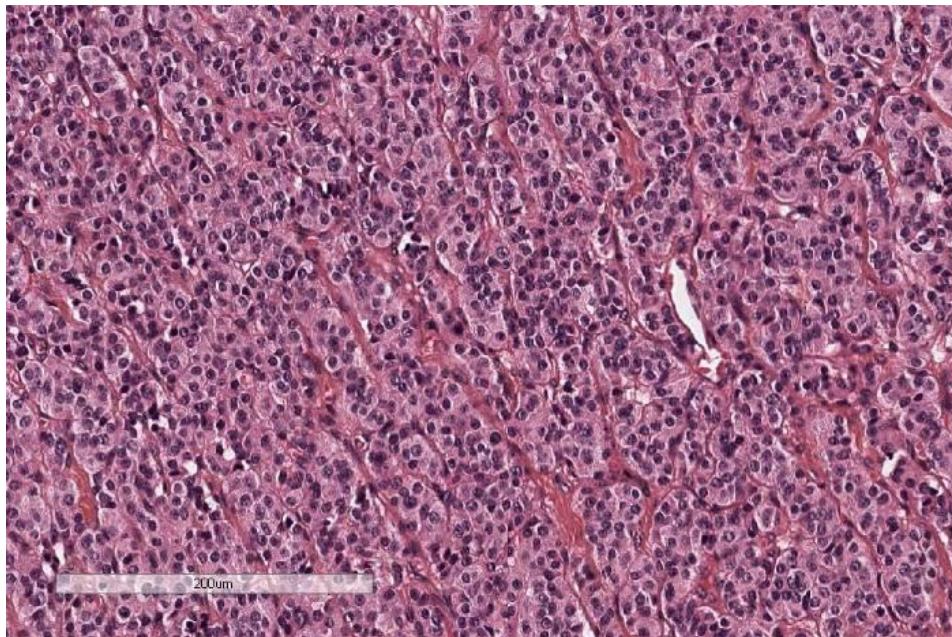
Pancreatic cancer

Adenocarcinoma (90%)



Pancreatic cancer

Adenocarcinoma (90%)

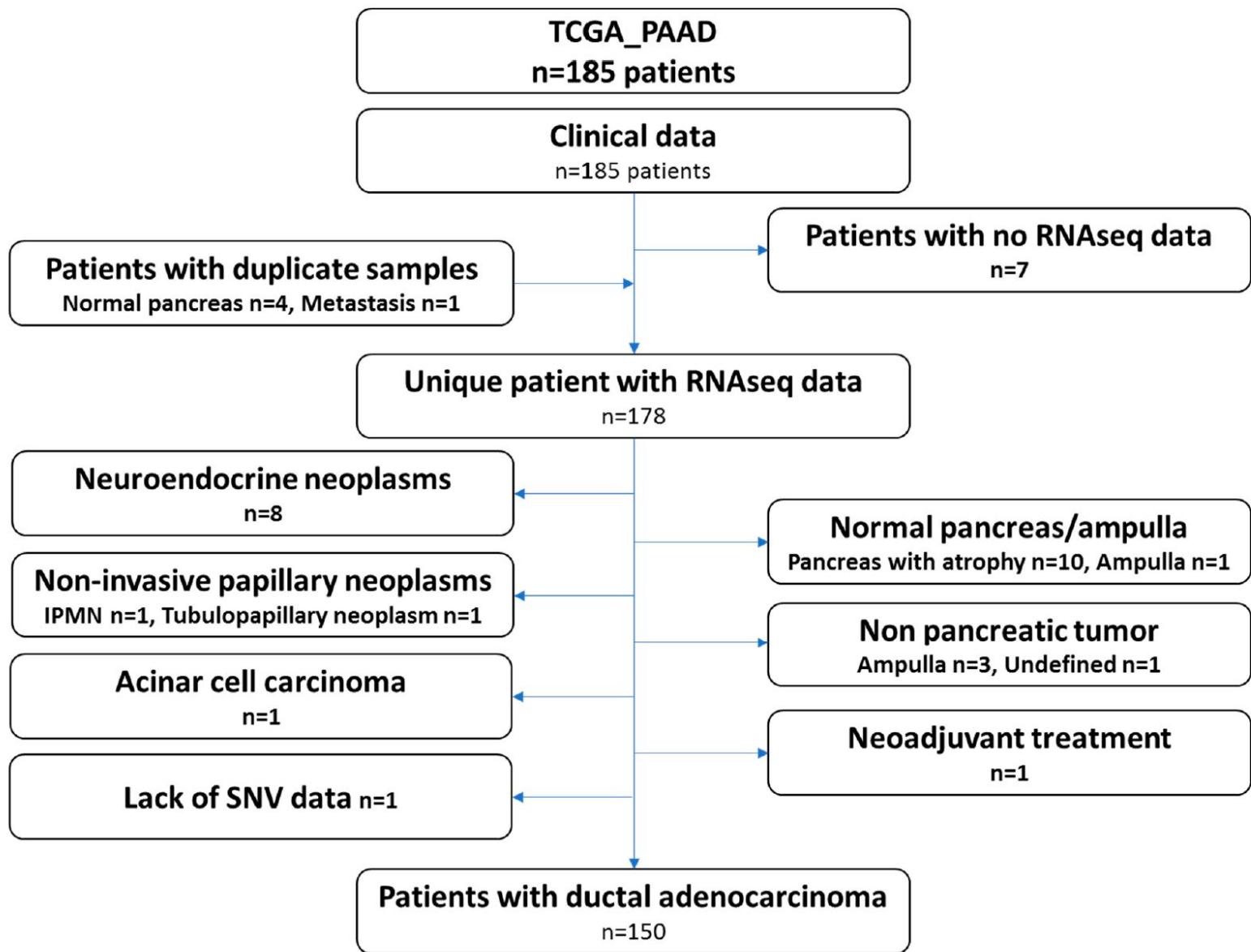


Neuroendocrine
lesions

Rare lesions
Acinar cell
carcinoma....

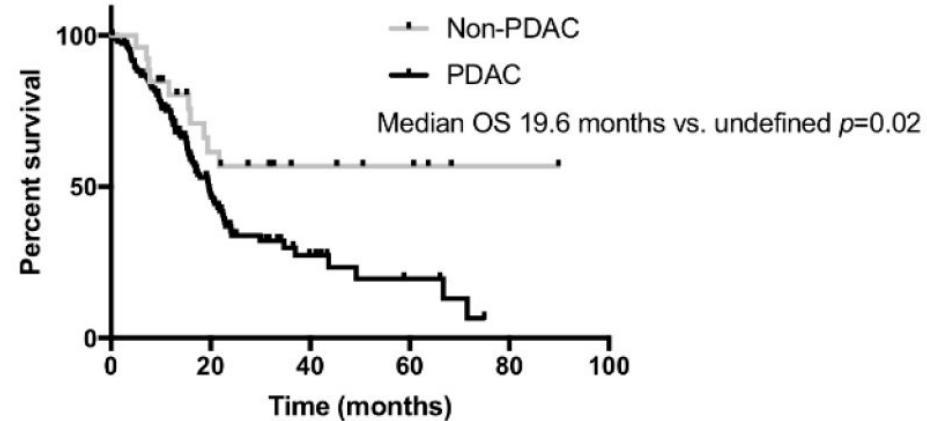
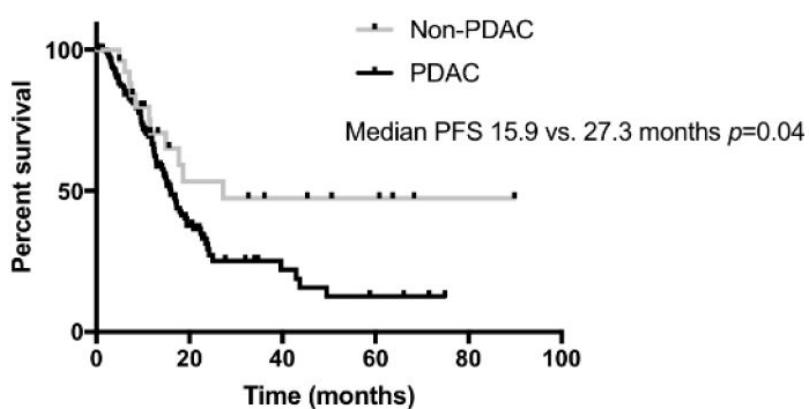
Completely different
morphology, biology....

1. Beware of miss labeled samples when using public datasets++++

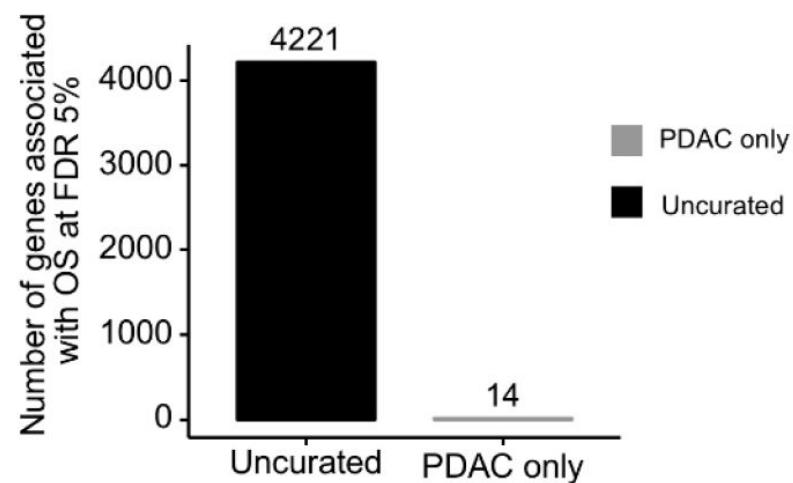
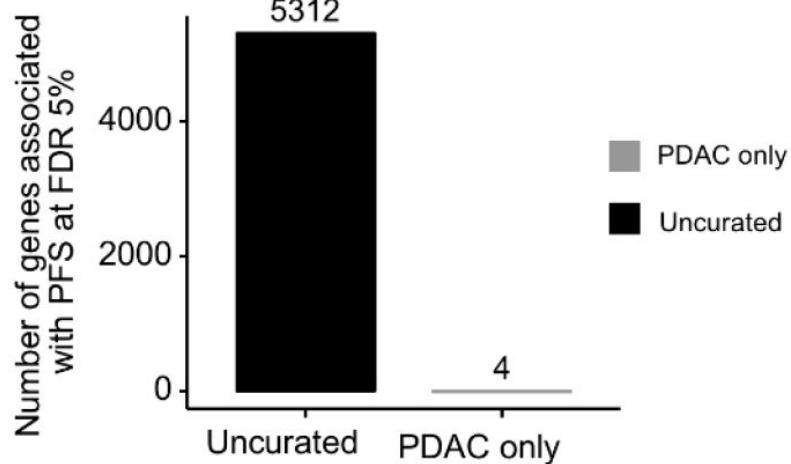


1. Beware of miss labeled samples when using public datasets++++

a

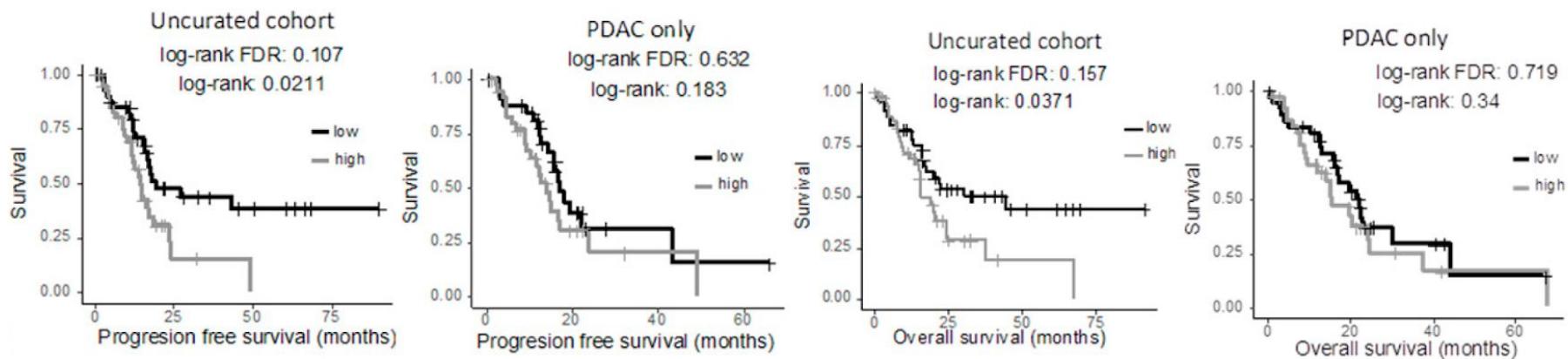
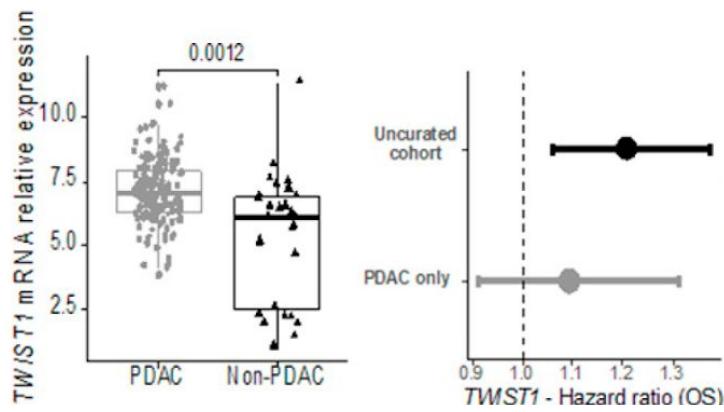


b



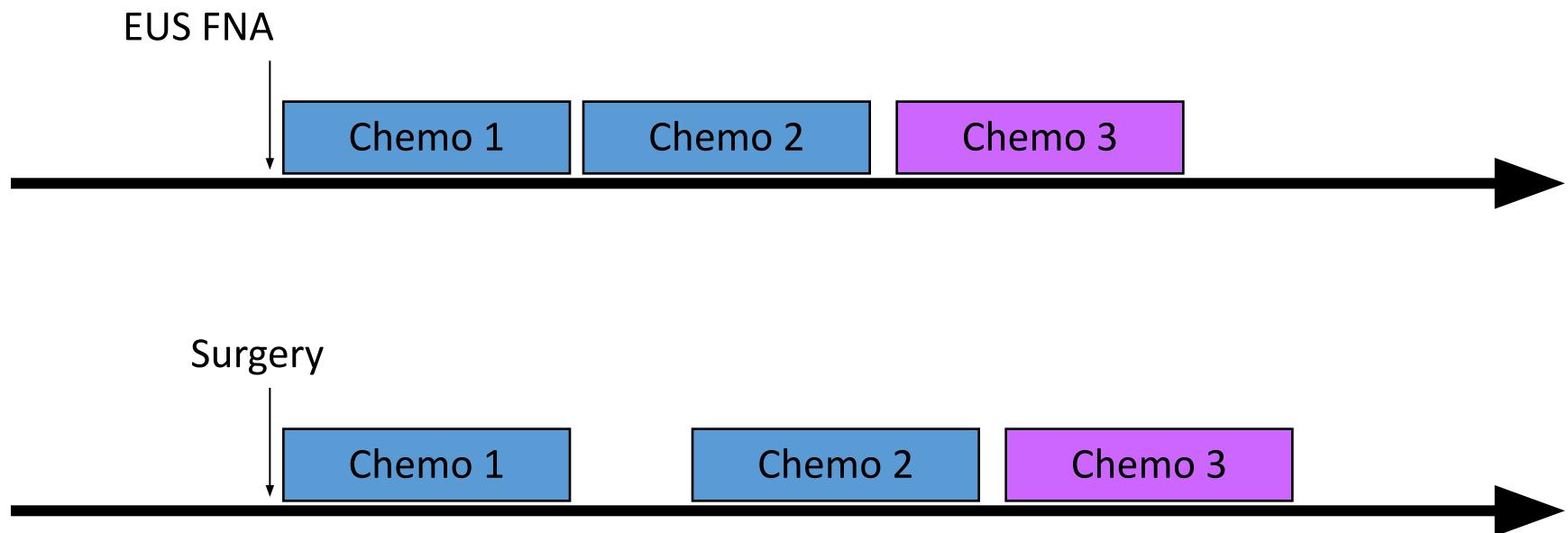
1. Beware of miss labeled samples when using public datasets++++

a



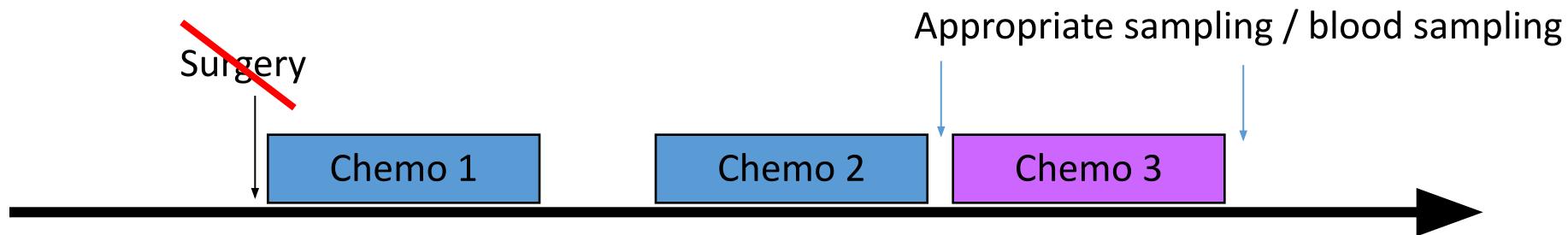
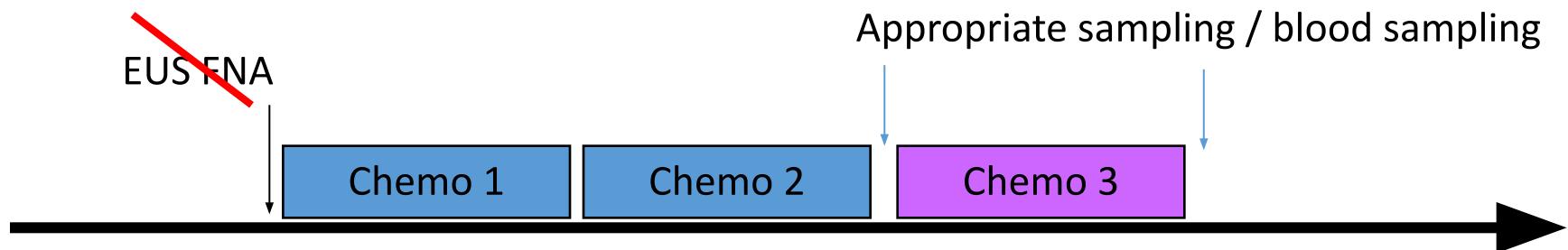
Many publications with the whole dataset... *Nat genet*, *Clin can res...*

2. Beware of the tissue samples used for biomarker/signature development



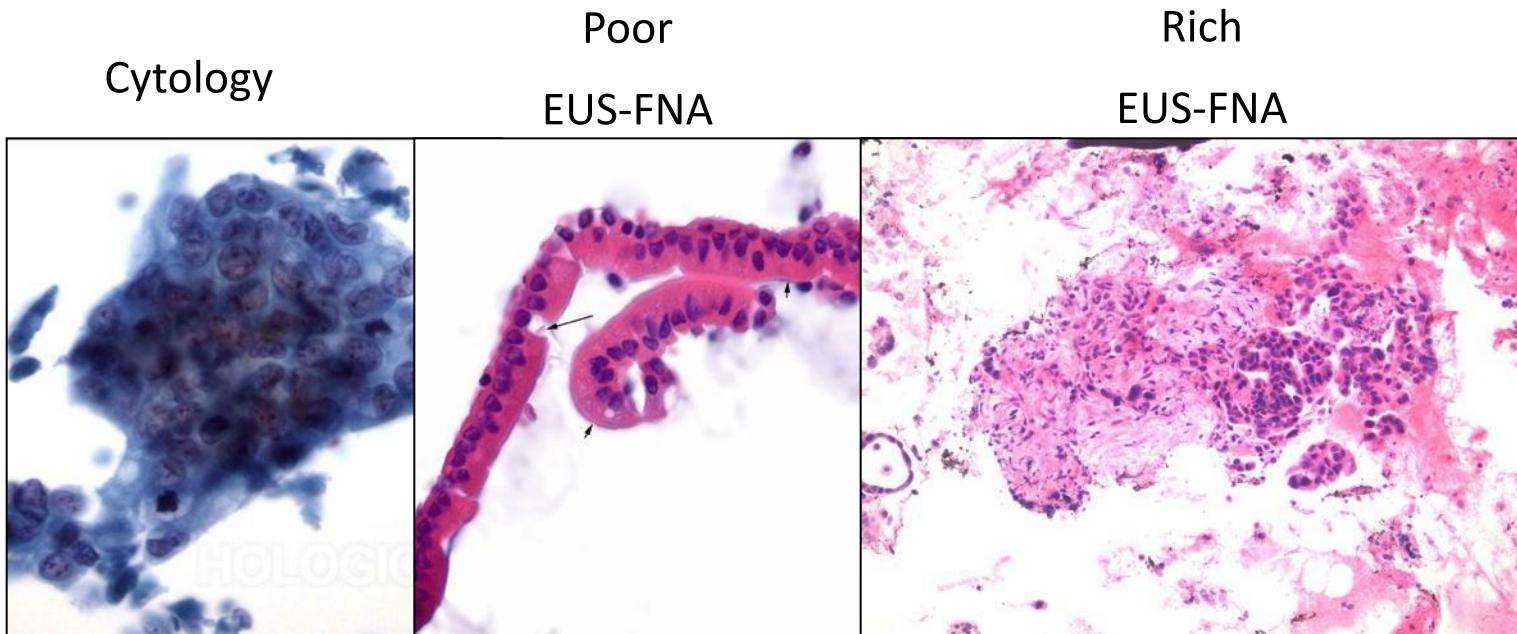
- Clonal selection induced by multiple therapies

2. Beware of the tissue samples used for biomarker/signature development



- Clonal selection induced by multiple therapies

3. What kind of tumor sample are accessible? How suitable are they?

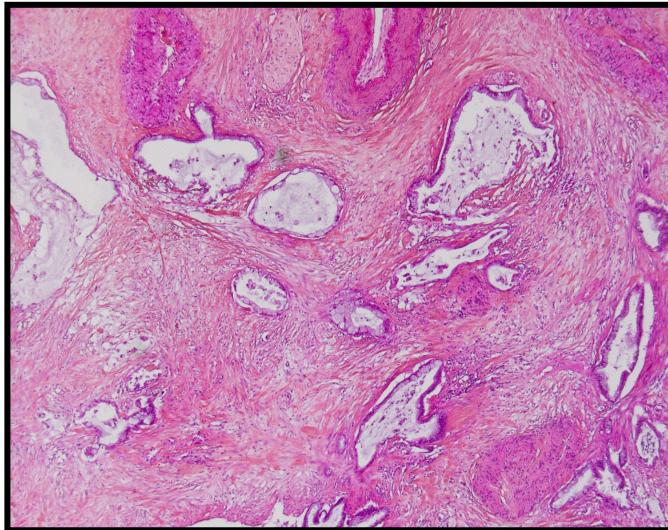


Protein on Tum cells	OK	OK	OK
Mutation on Tum cell	+/- OK	++/- OK	OK
Exp (mi)ARN	+/- OK (richness)	+/- OK (richness)	+/- OK (richness)
Protein in stroma	NON	NON	+/- OK

Liver biopsy++, true cut ++

3. What kind of tumor sample are accessible? How suitable are they?

Surgical specimen

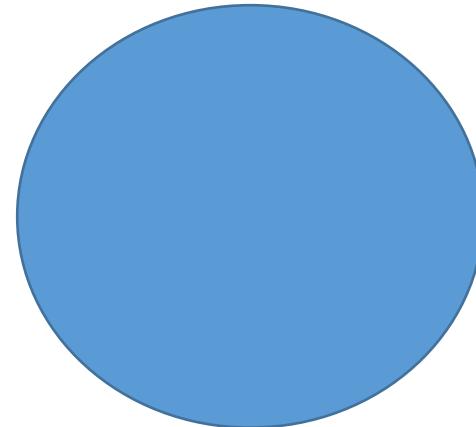


Tumor
+/- sphere
3cm diameter

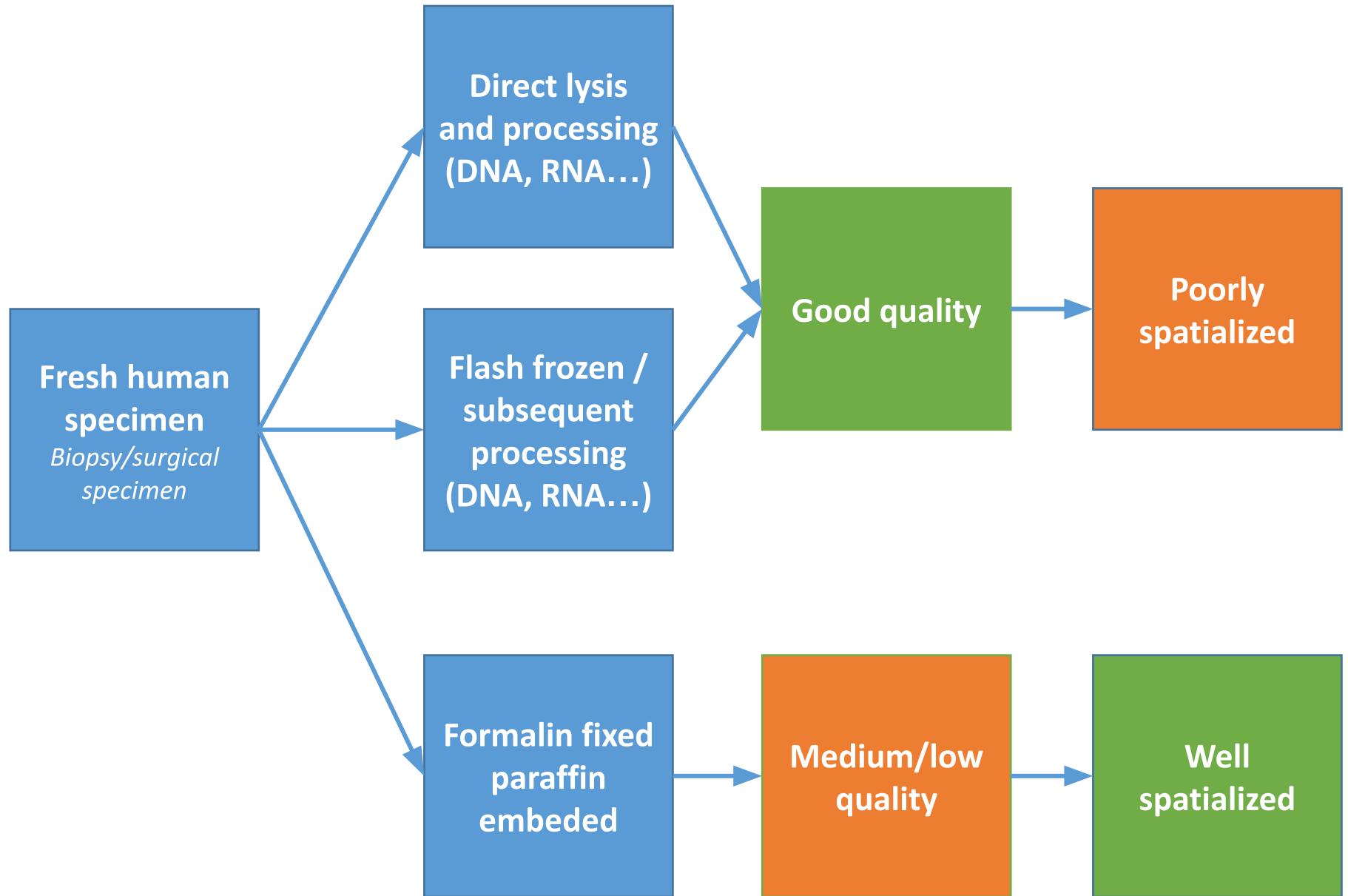
14cm^3

Frozen carrot
0.6cm wide
0.4 cm thick

0.11cm^3

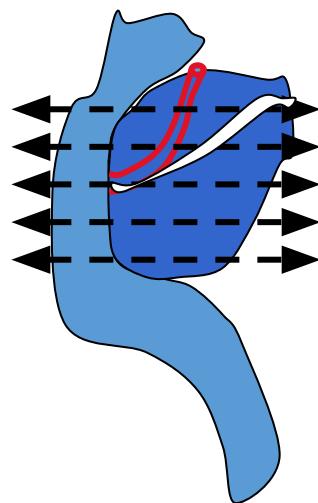


Protein on Tum cells	OK
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Exp (mi)ARN	OK
Protein in stroma	OK



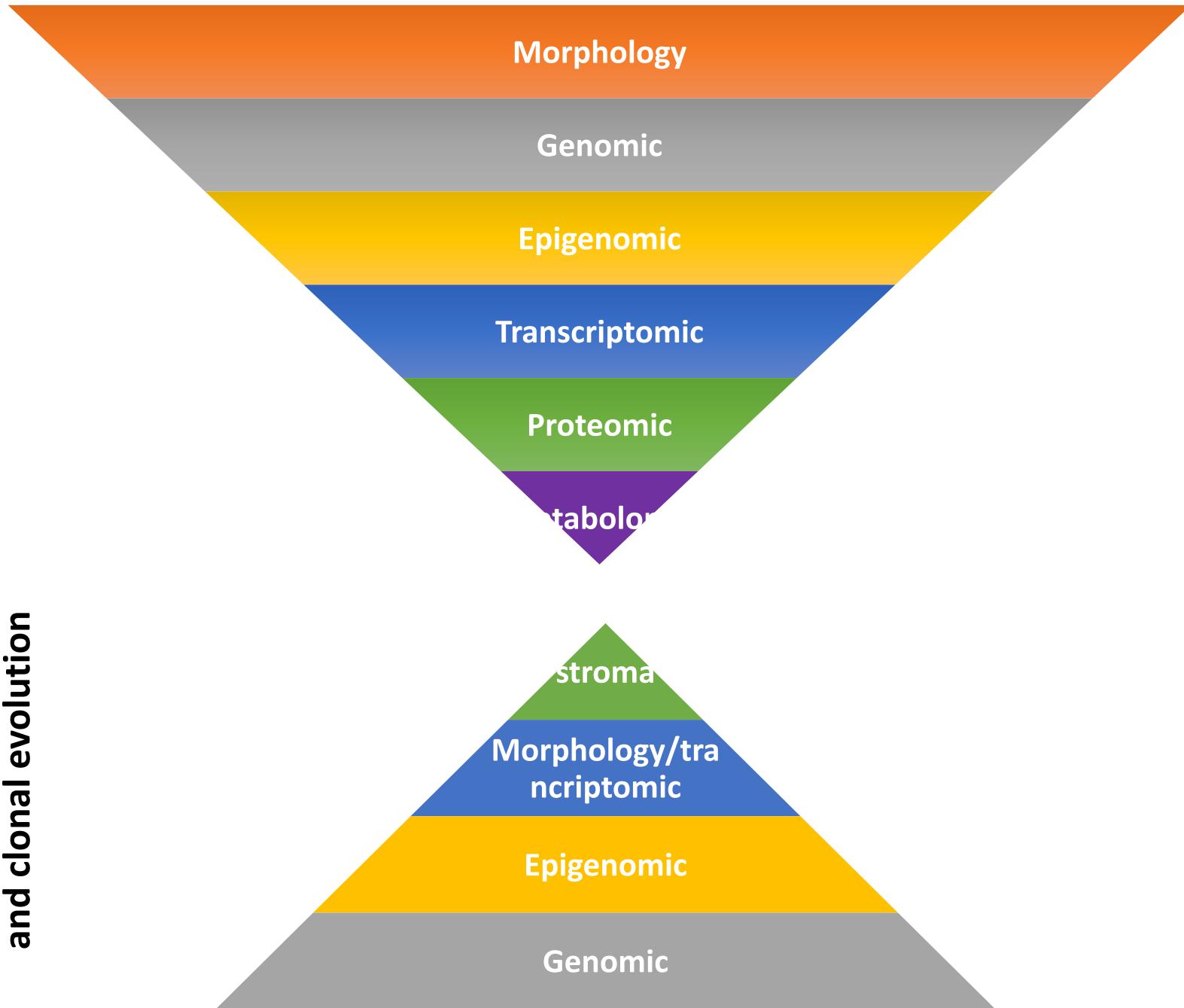
Spatialized sampling...

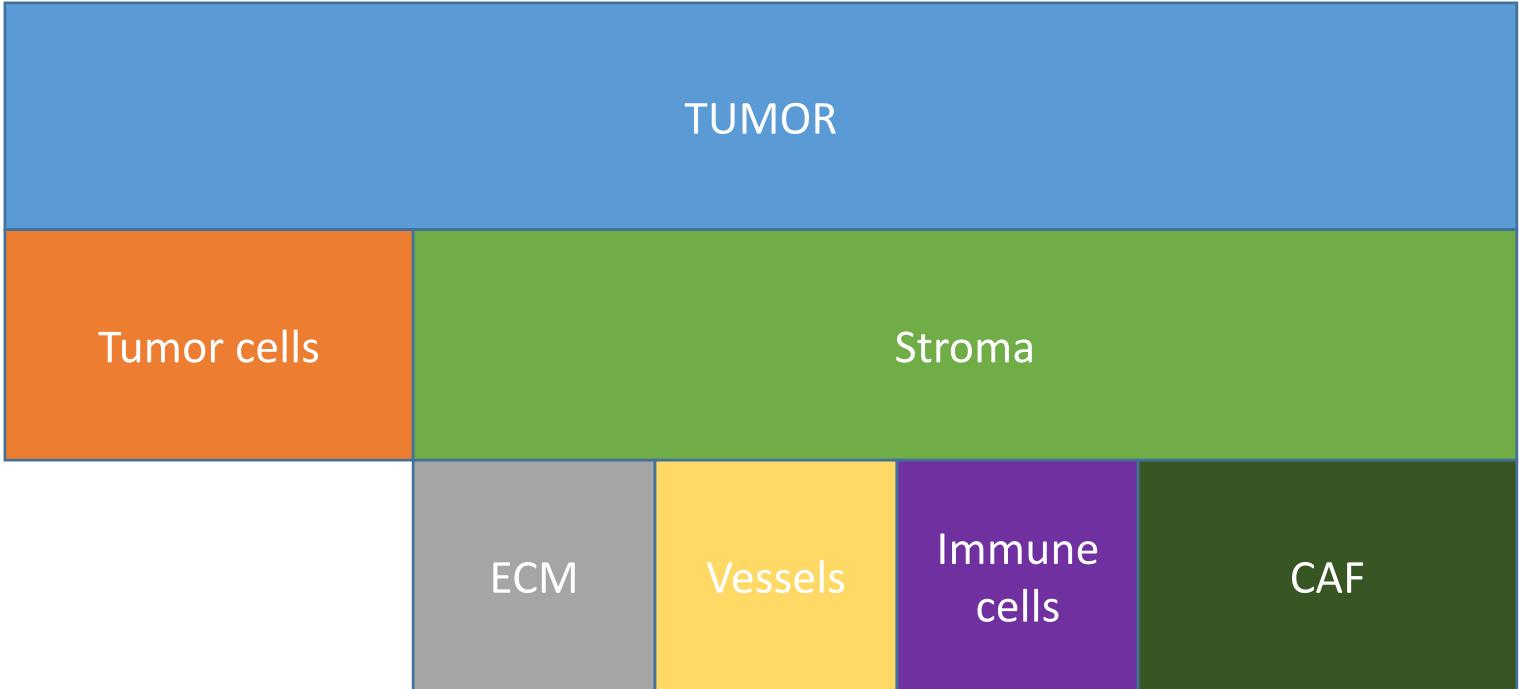
...yes but usually with formalin fixed paraffin embeded samples...



Inter-tumor heterogeneity

Intratumor heterogeneity
and clonal evolution

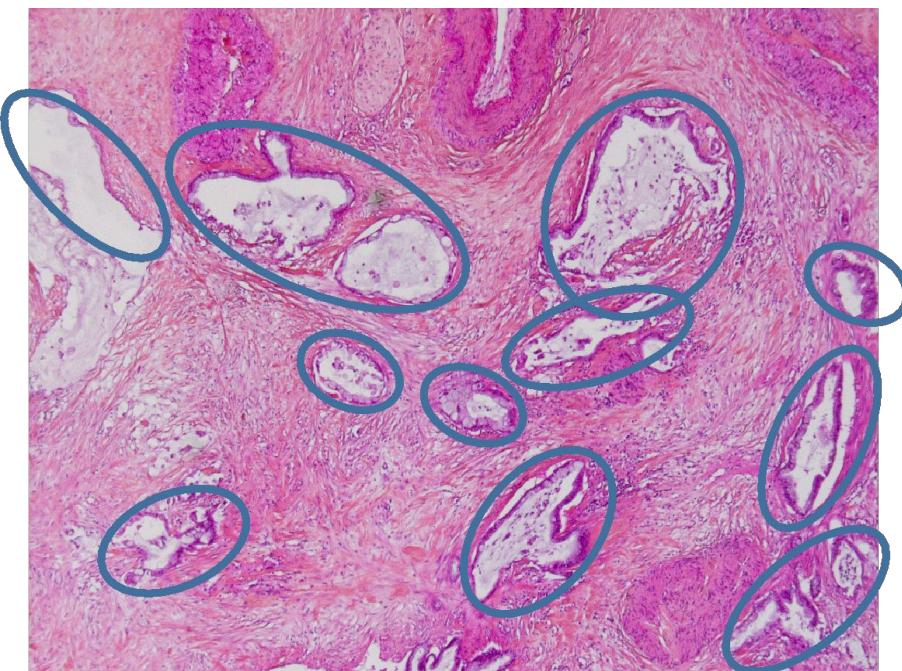




PANCREATIC ADENOCARCINOMA

Tumor cells

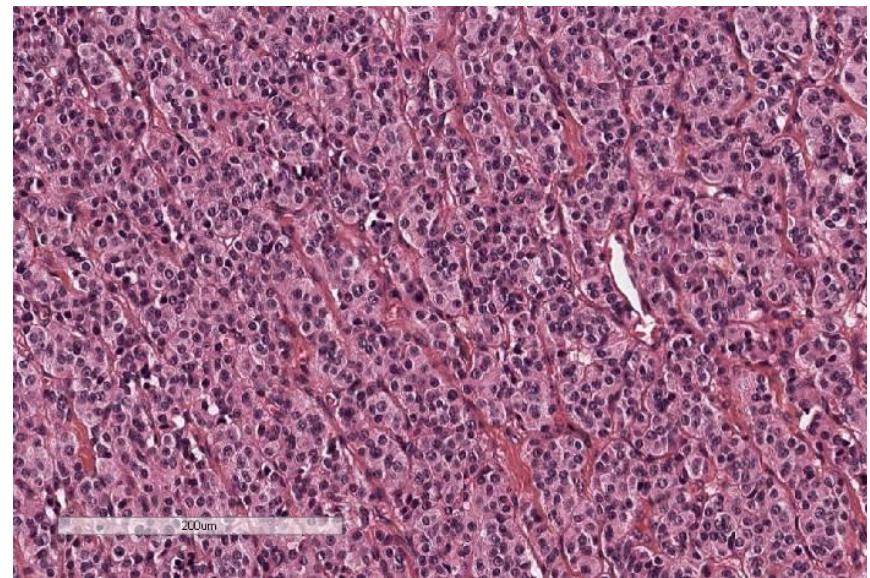
Stroma



PANCREATIC NEUROENDOCRINE TUMOR

Tumor cells

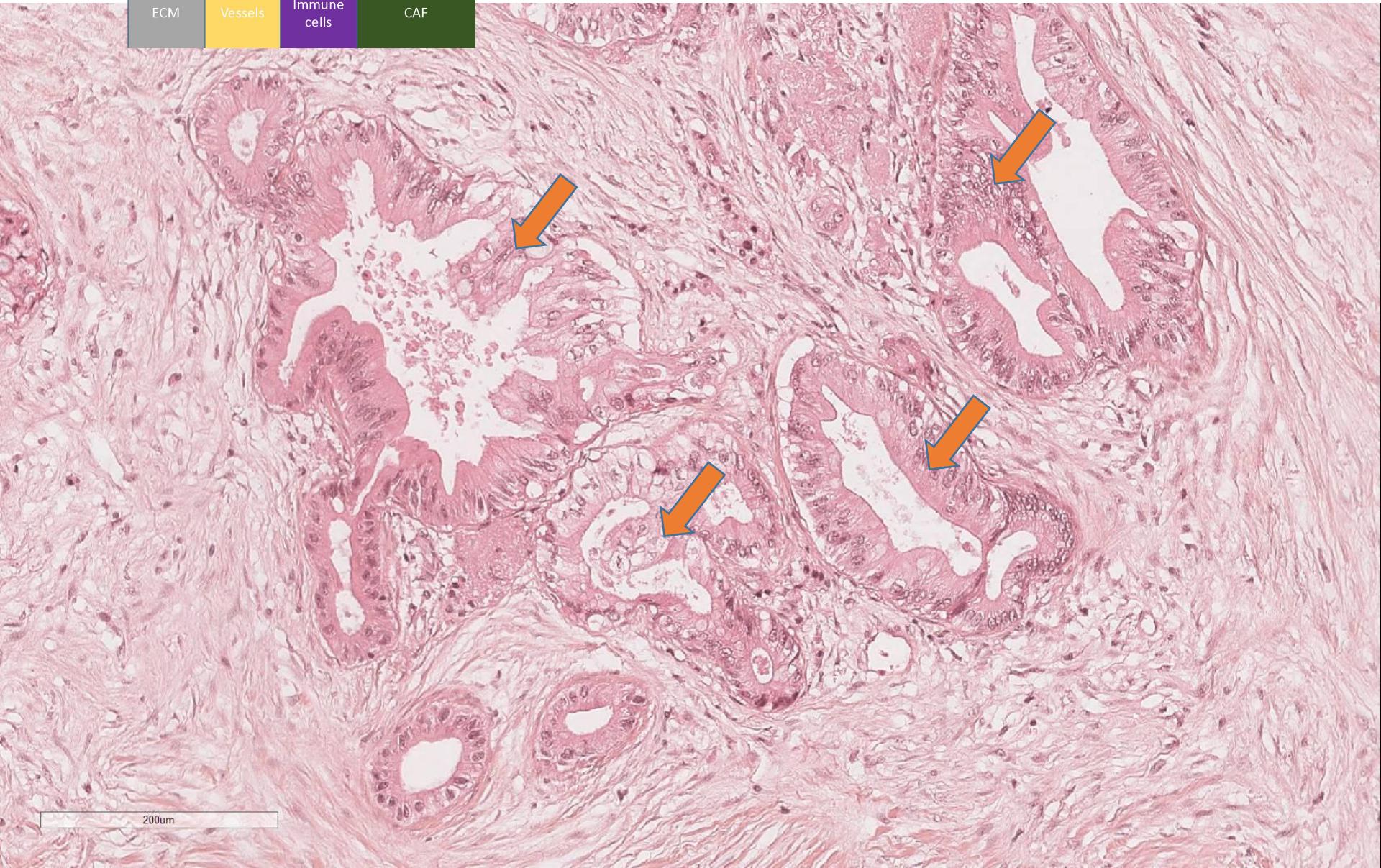
Stroma



TUMOR



Pancreatic adenocarcinoma

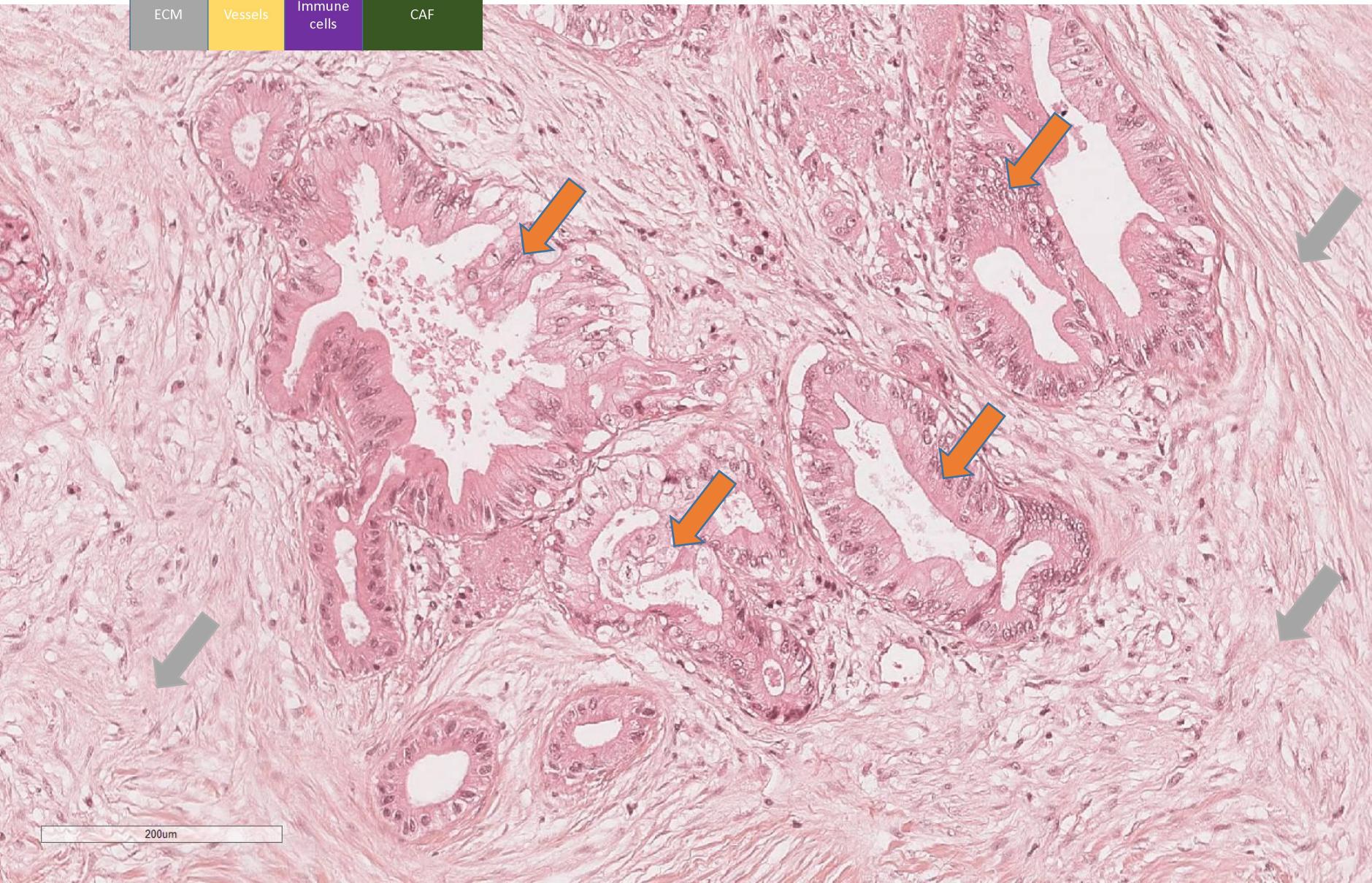


200um

TUMOR



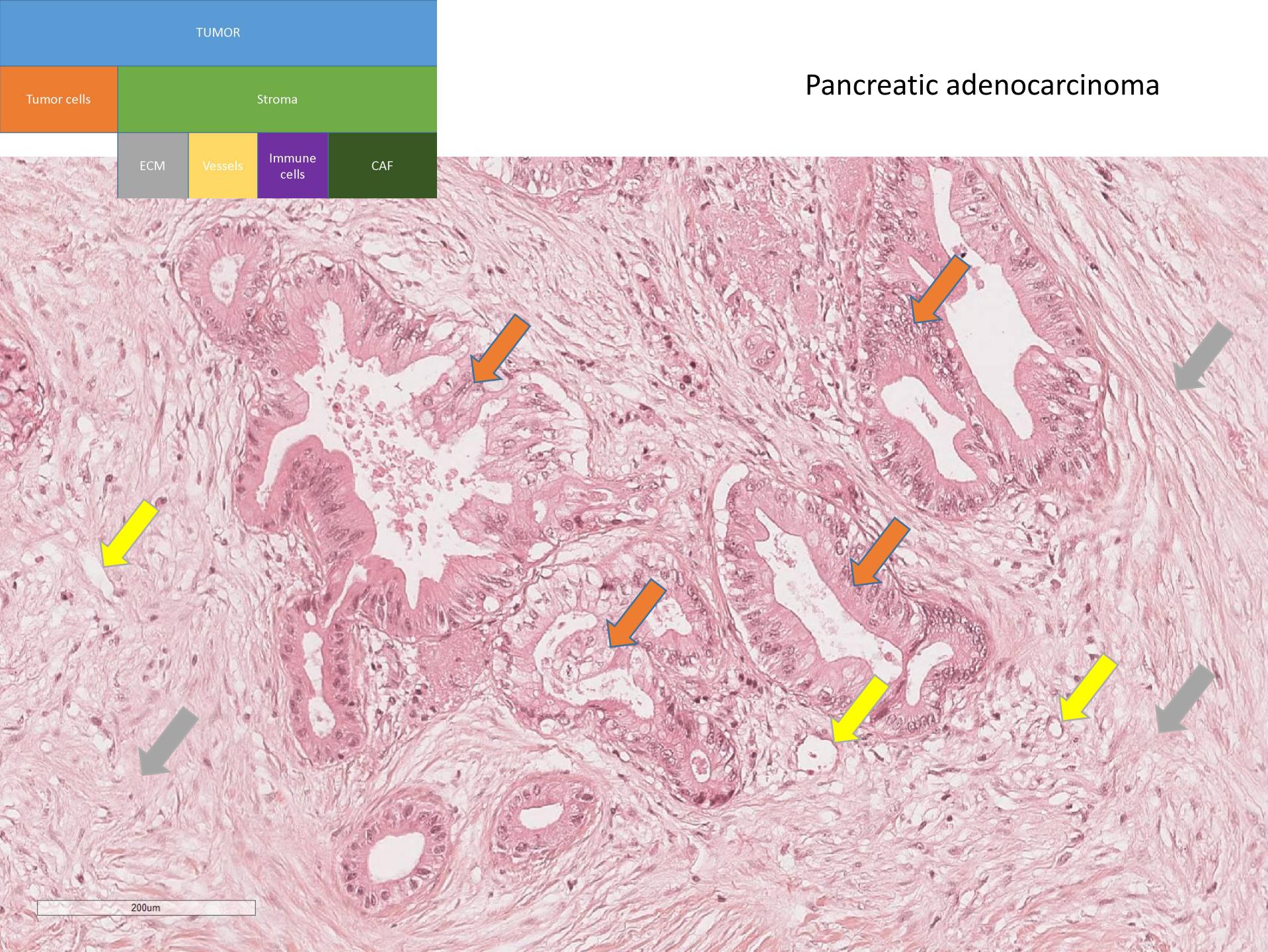
Pancreatic adenocarcinoma



200um

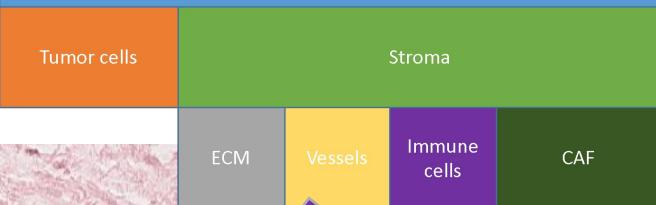
TUMOR

Pancreatic adenocarcinoma

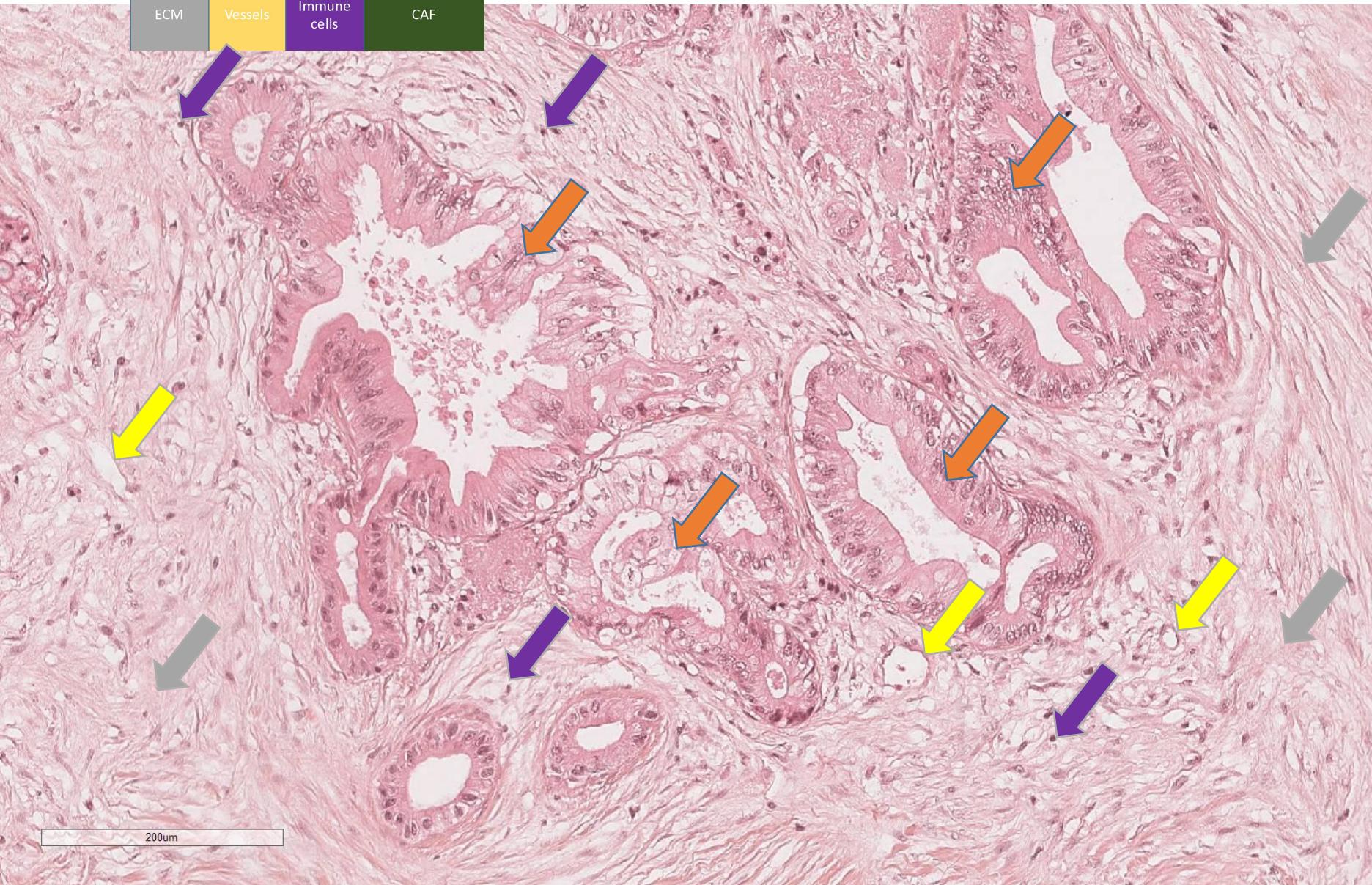


200um

TUMOR

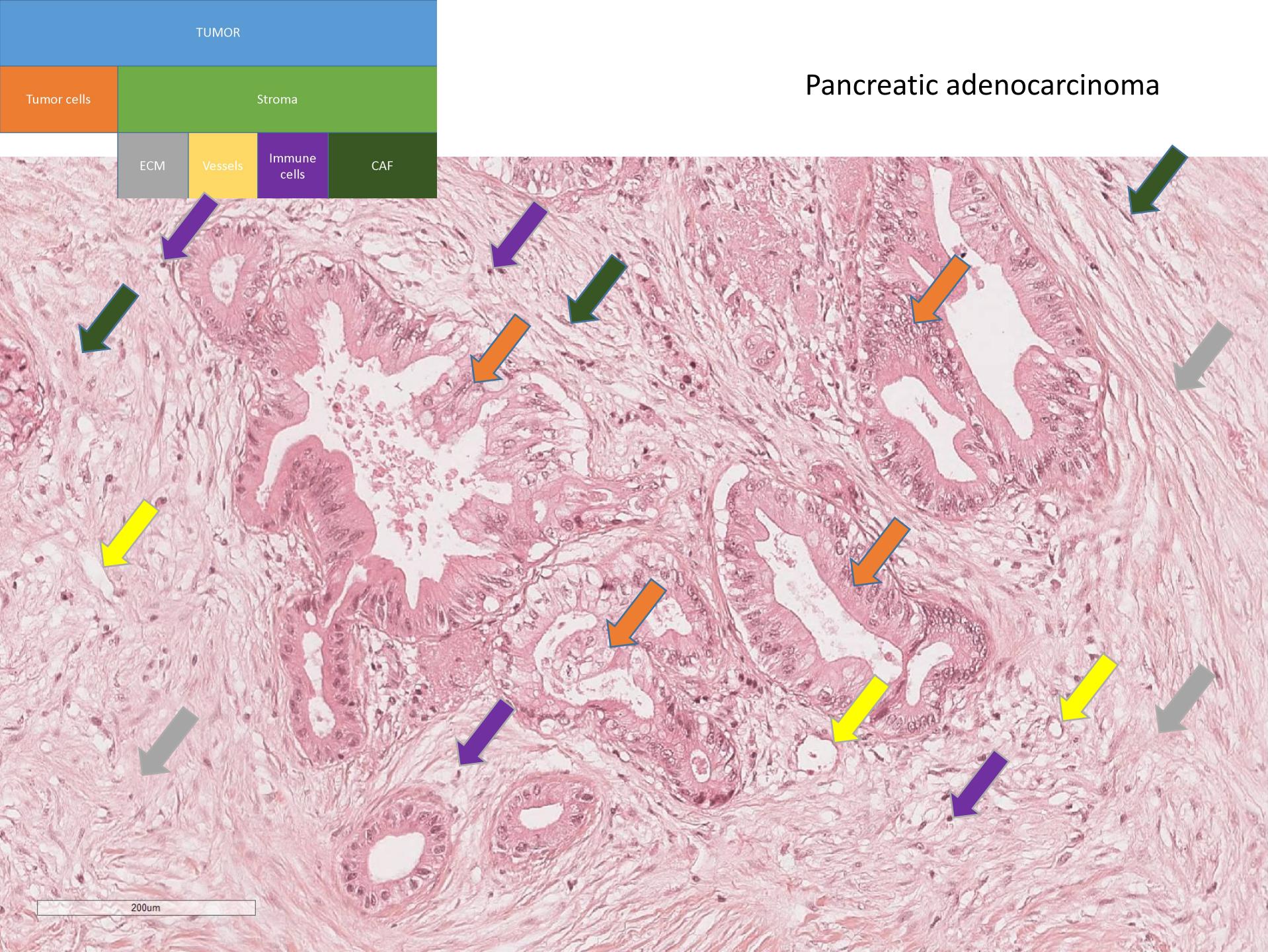


Pancreatic adenocarcinoma

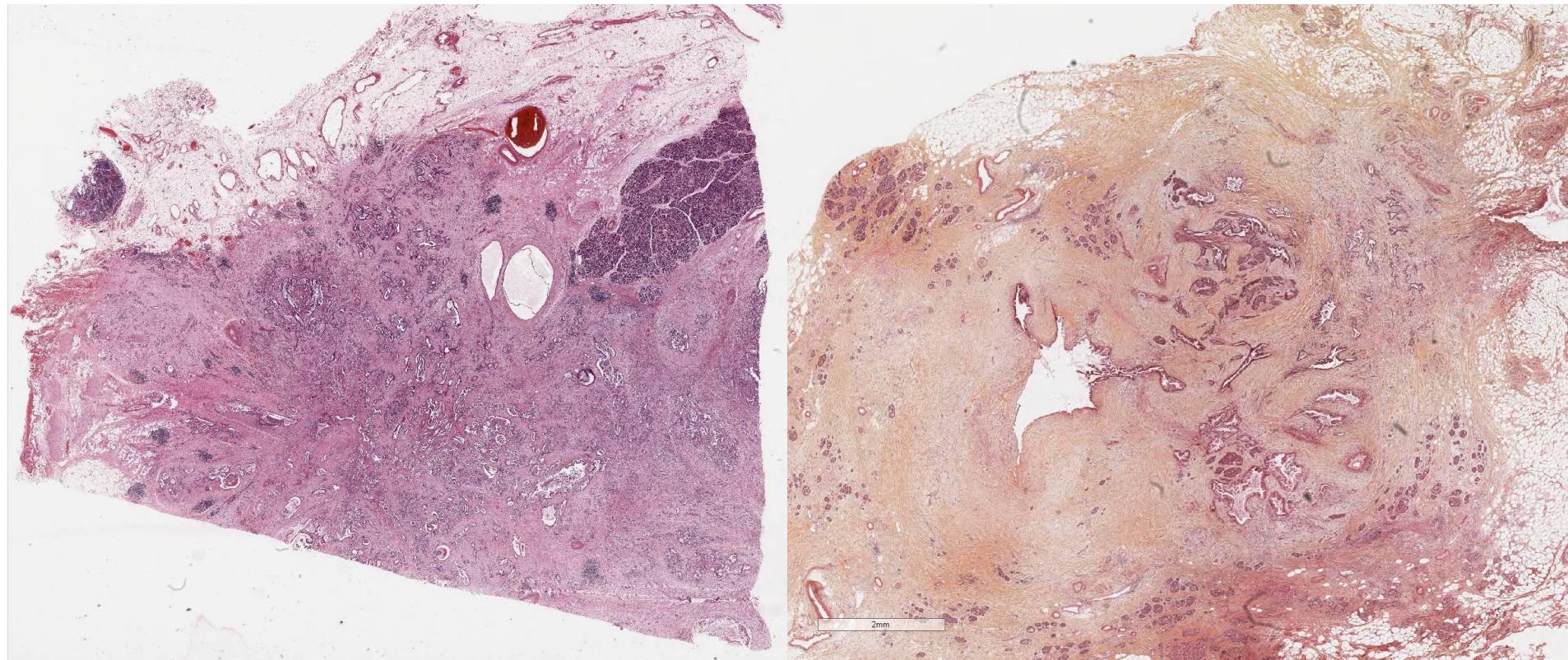


TUMOR

Pancreatic adenocarcinoma

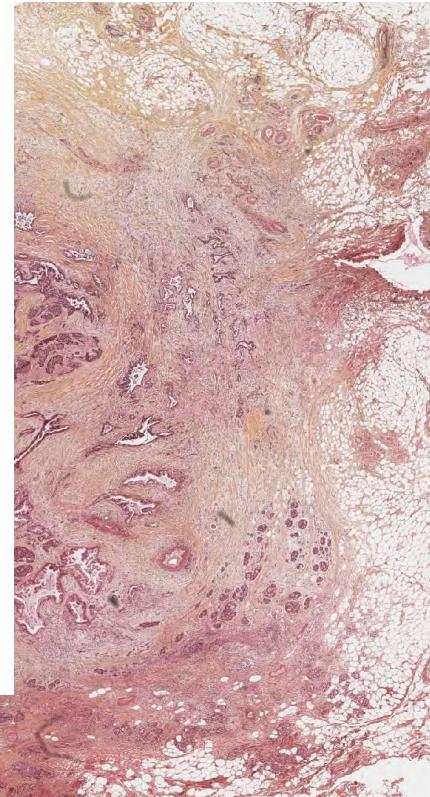
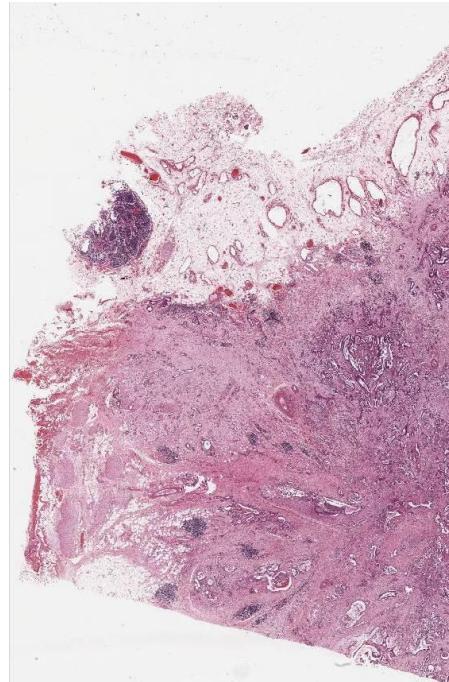
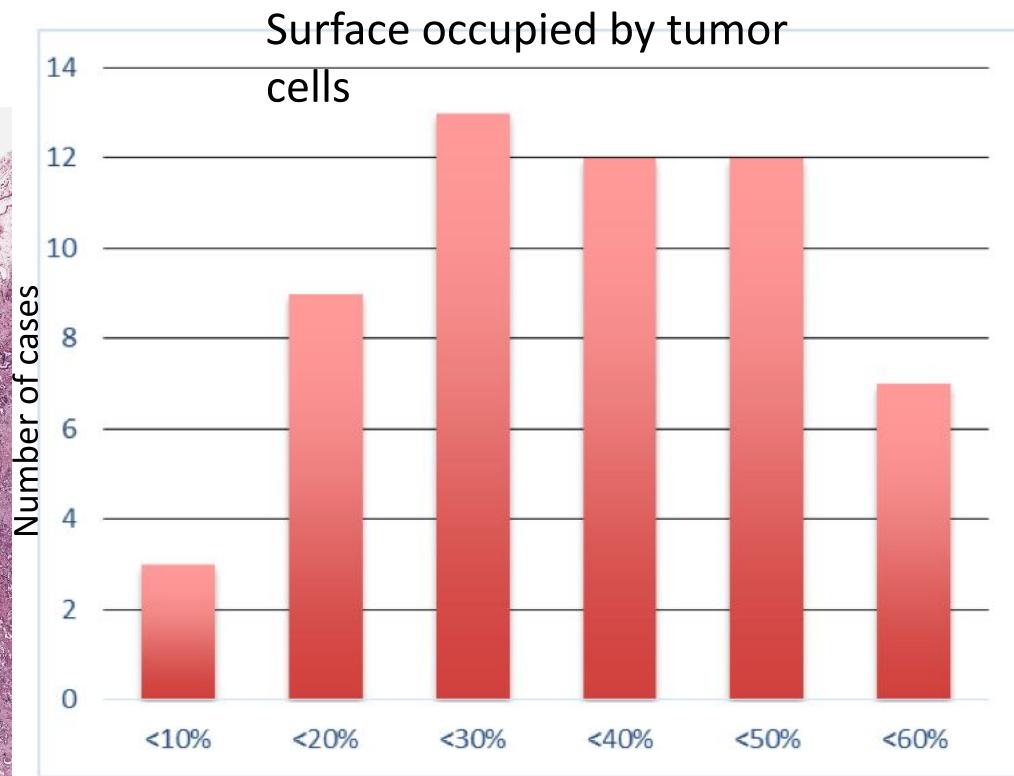


Inter-tumor morphological heterogeneity



Heterogeneity of the tumor cell – stroma ratio

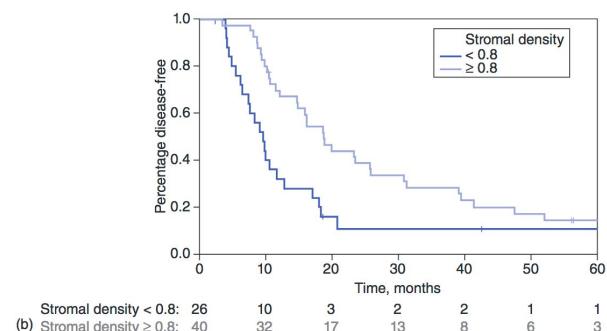
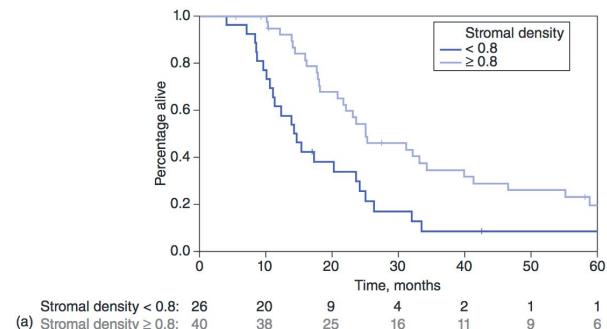
Inter-tumor morphological heterogeneity



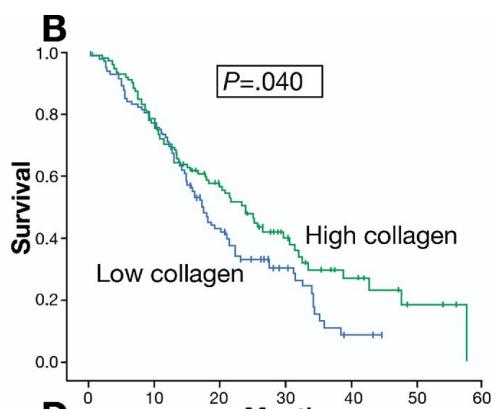
Heterogeneity of the tumor cells – stroma ratio

Inter-tumor morphological heterogeneity

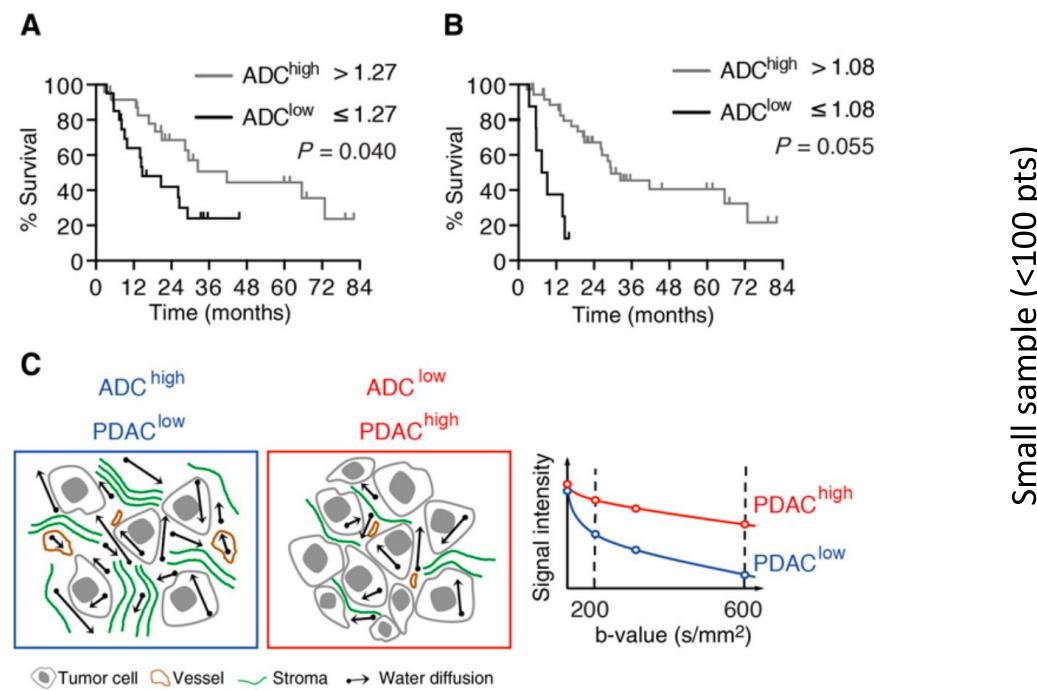
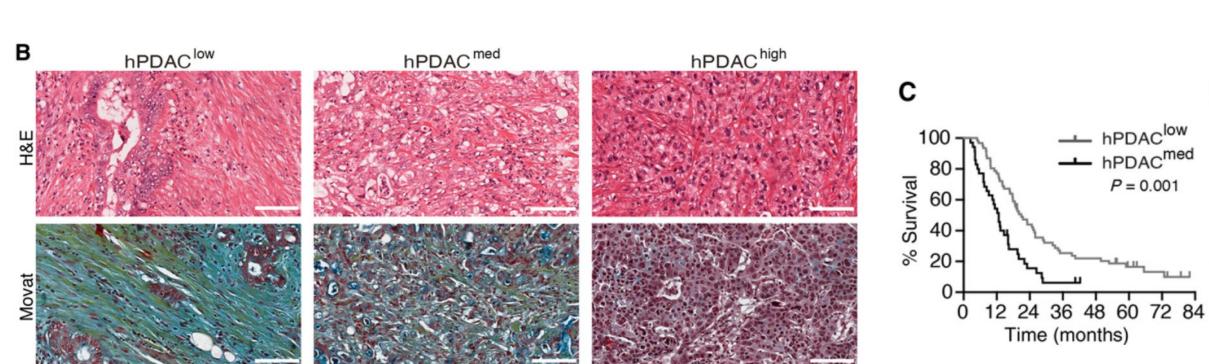
Heterogeneity = opportunity for biomarker development



Bever et al. HPB 2015, 292



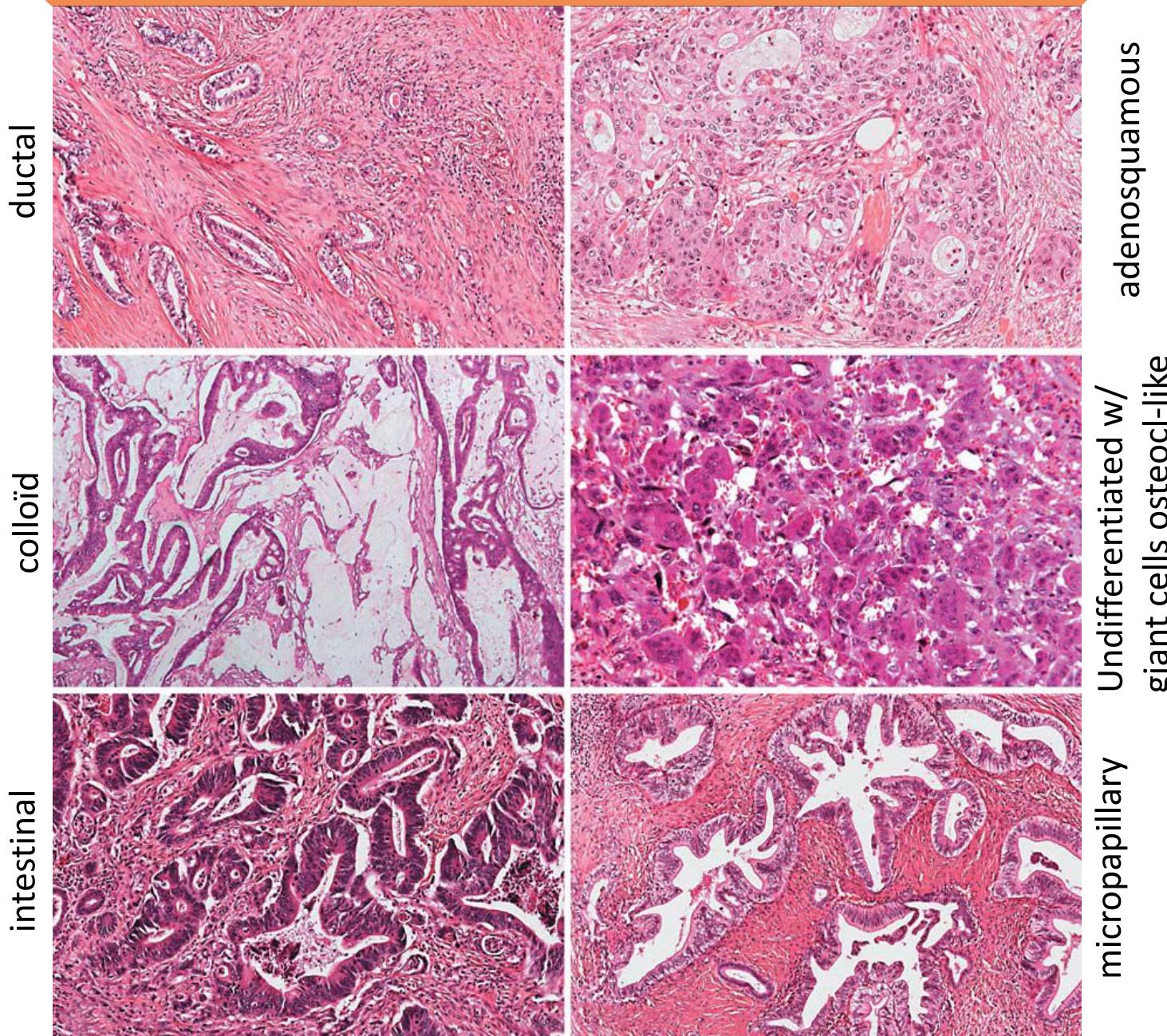
Erkan et al. Clin gas hep 2008



Small sample (<100 pts)

Heid et al. Clin can res 2017, 1461

Inter-tumor morphological heterogeneity



Inter-tumor morphological heterogeneity

Tumor type	Frequency	%	Type of associated IPMN	Median survival (months)
Conventional ductal adenocarcinoma	91	51.4	2 gastric	22.7
Combined ductal adenocarcinoma				
with cribriform component	17	9.6		28.7
with papillary component	17	9.6		13.9
with clear-cell component	16	9.0	1 pancreato-biliary	17.6
with complex component	12	6.7	1 gastric	10.7
with gyriform component	8	4.5		12.5
with micropapillary component	2	1.1		16.1
Variants and special carcinomas				
Adenosquamous carcinoma*	2	1.1		4.1
Colloidal/mucinous carcinoma*	2	1.1	1 intestinal	>64.3**
Medullary carcinoma*	1	0.5		>75.1**
Tubular carcinoma	3	1.7		>55.3**
Papillary carcinoma	6	3.4	2 pancreato-biliary, 1 intestinal, 1 gastric	20.6
All tumors	177	100		

NGS/IHC *KRAS/CDKN2A/SMAD4/TP53*

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More frequent *MYC amplification*?

NGS/IHC *KRAS/CDKN2A/SMAD4/TP53*

No major difference

Genomic

3 large datasets (ICGC (456 pts), TCGA (150 pts), Connor *et al.* (148 pts) – similar (boring) results

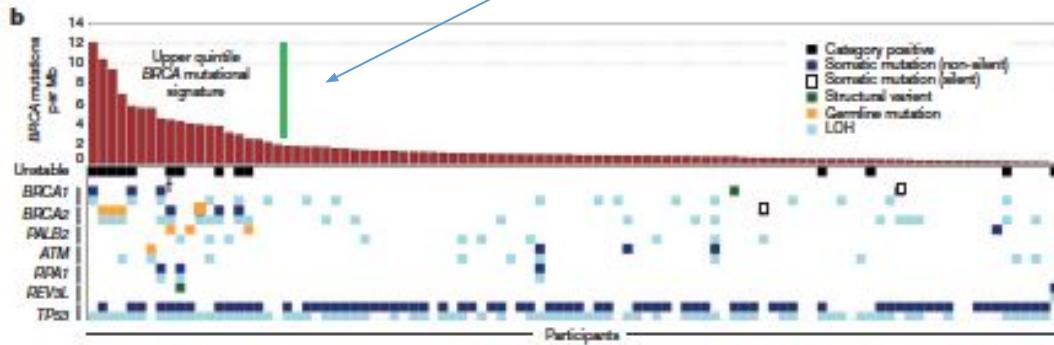
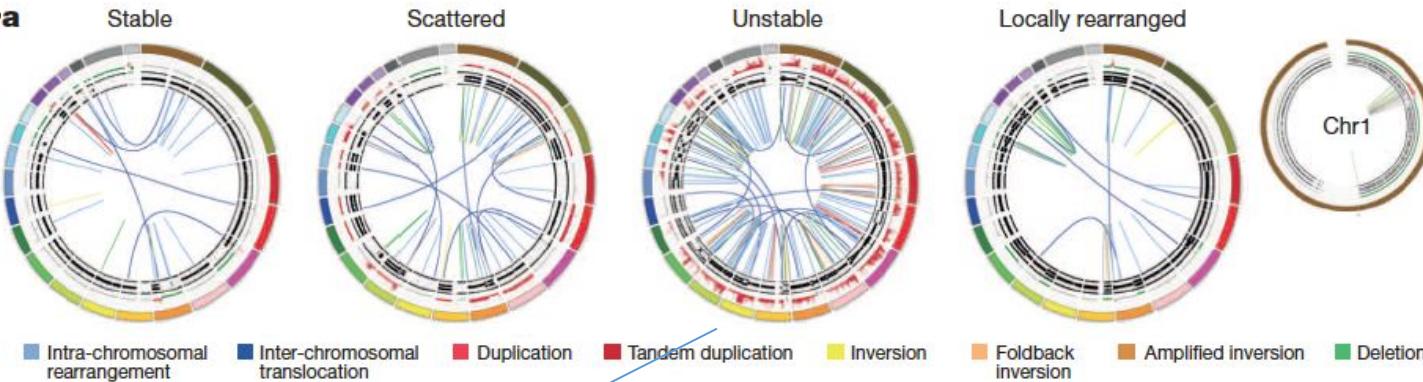
a



Genomic

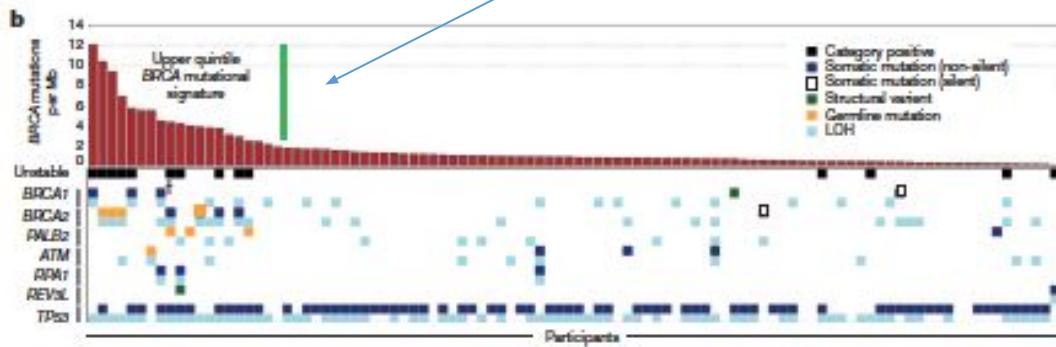
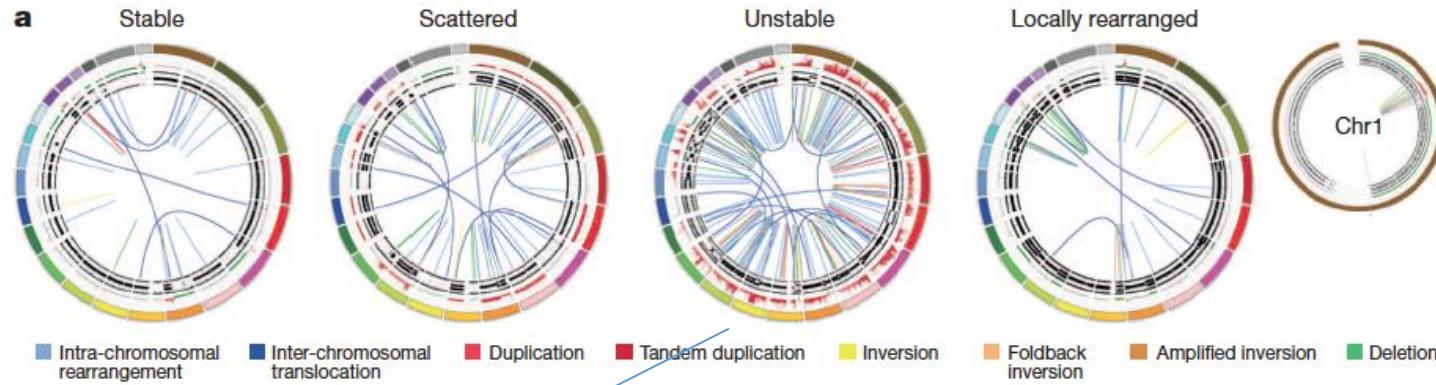
WG

S_a



Tricky in clinical practice...
...especially in samples with
poor tumor cell content

Genomic



Benefit from PARPi
in maintenance
therapy (POLO trial)

Signature	Discovery (n)	Replication (n)	ESPAc (n)	Population (%), 95% CI
Age Related	115*	59	NA	69.9% (64.2-75.6%)
DSBR, total	17**	10	NA	10.8% (6.98-14.7%)
DSBR, germline	9	2	NA	4.42% (1.9-6.97%)
DSBR, somatic	2	2	NA	1.6% (0.045-3.2%)
DSBR, occult	6	6	NA	4.8% (2.2-7.5%)
MMR	4	0	6	1.7% (0.65-2.7%)
Signature 8	16	20	NA	14.5% (10.1-18.8%)
APOBEC	1***	4	NA	2.0% (0.27-3.8%)
Signature 17	1	2	NA	1.2% (0-2.56%)
Total sample sizes	154	95	342	NA

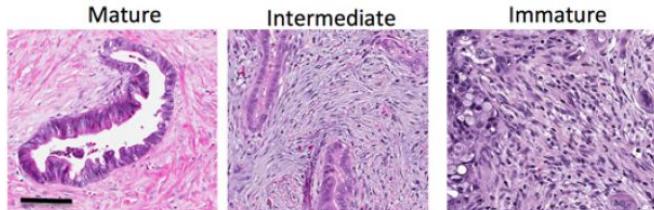
* there are 119 tumours from 115 cases in the Age Related discovery group

** there are 18 tumours from 17 cases in the DSBR discovery group

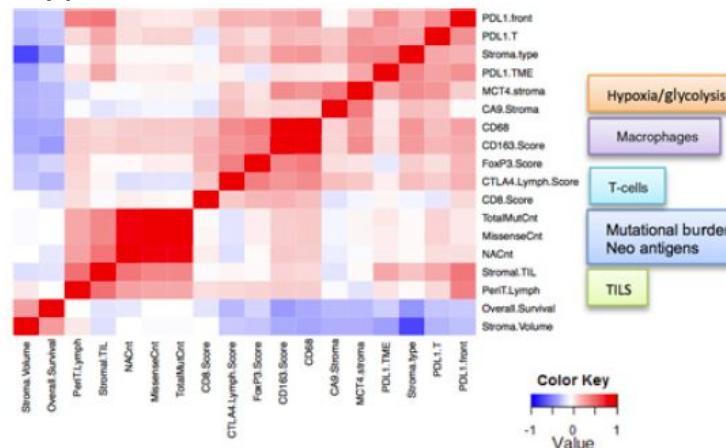
*** there are 2 tumours from 1 case in the APOBEC discovery group

Genomic

Type of stroma

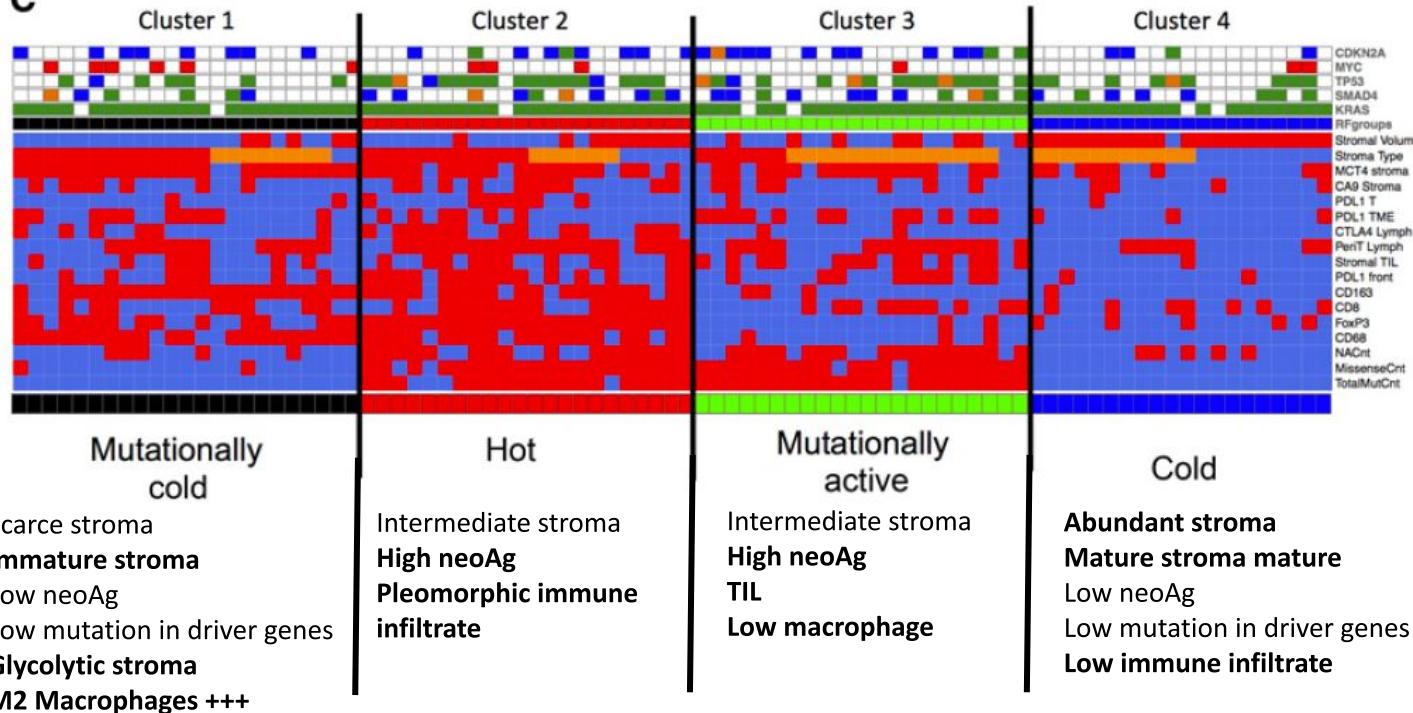


Type of immune infiltrate

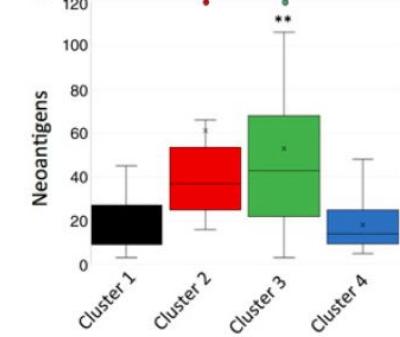


+
Mutationnal
profile
(WES)

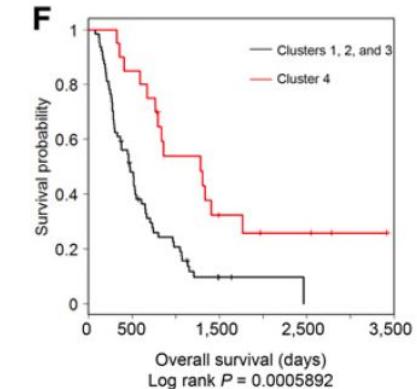
C



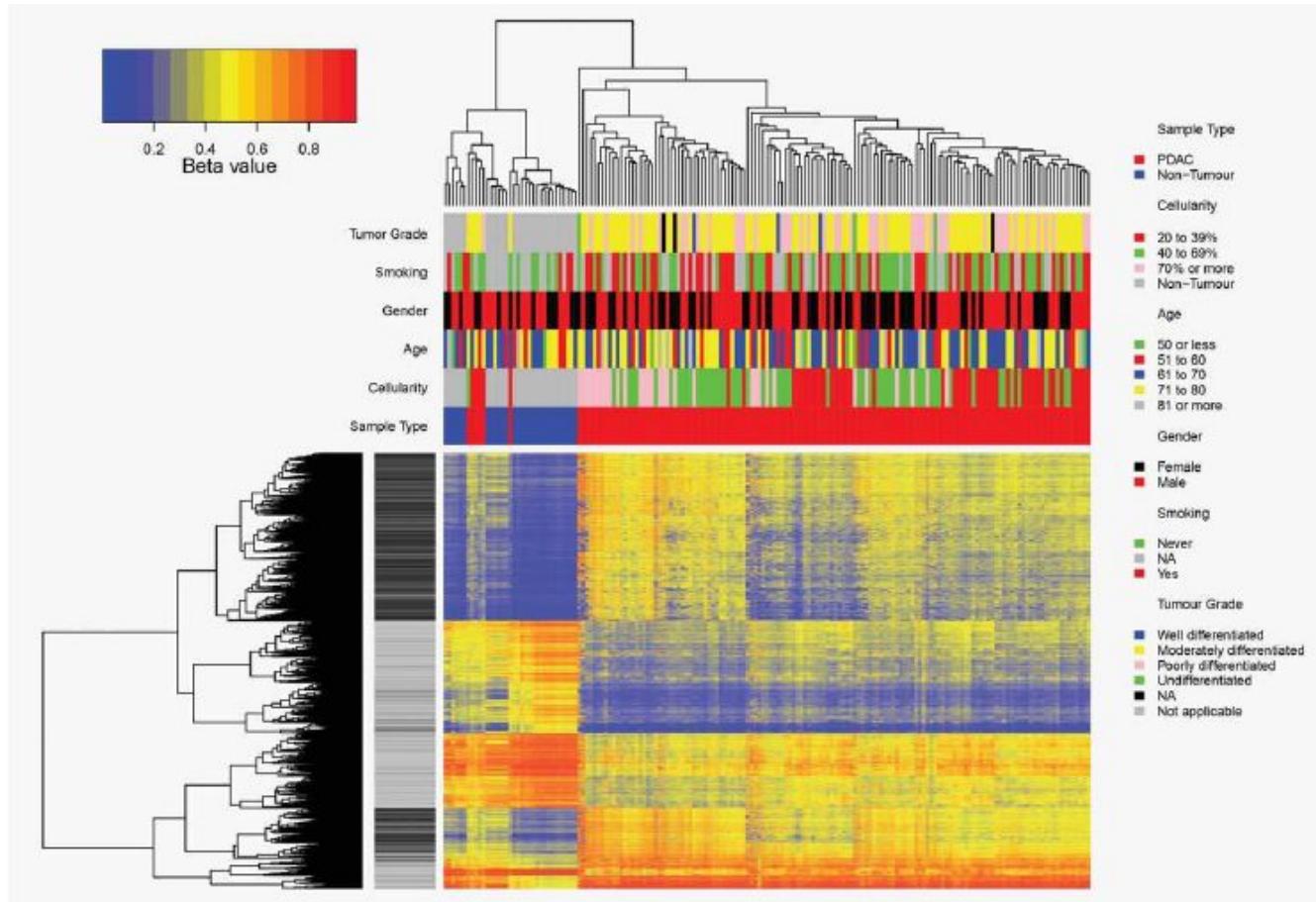
D



F



More studies are needed++, possible interest in diagnostic (better quality, circulating DNA)

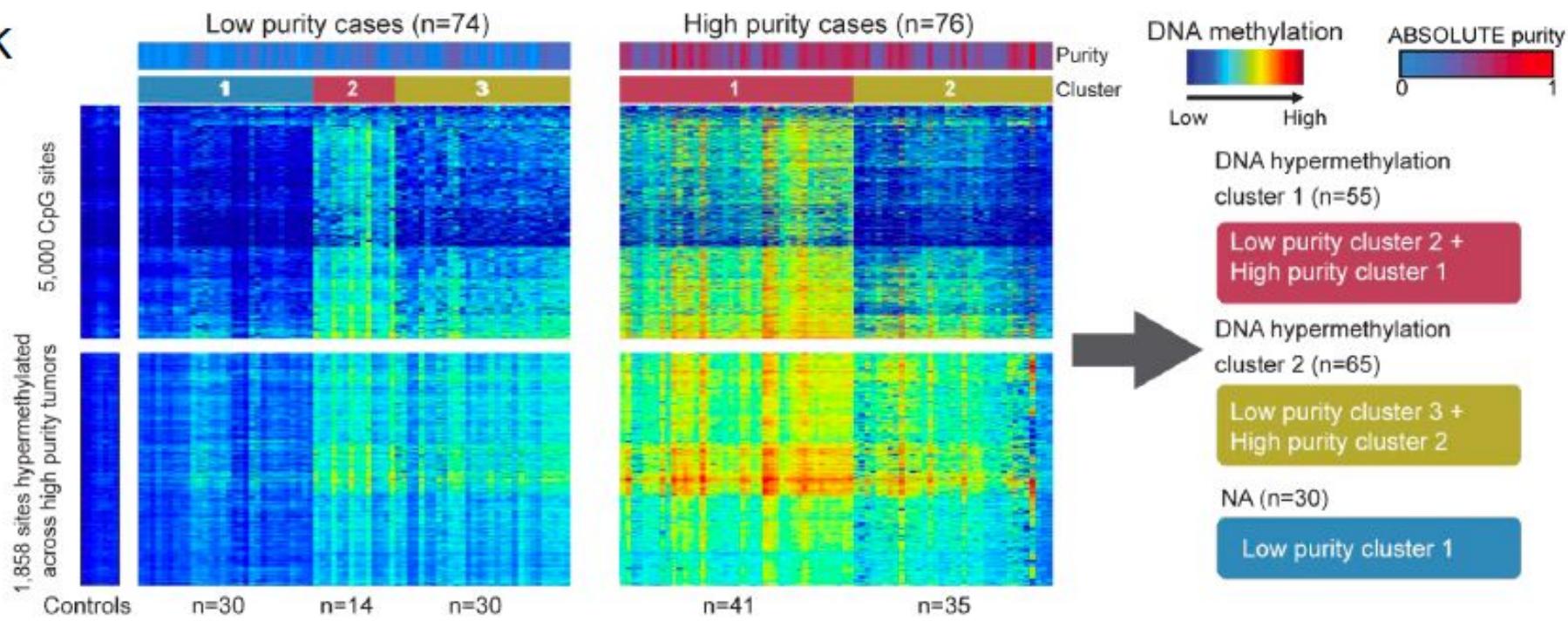


Nones *et al.* IJC 2014

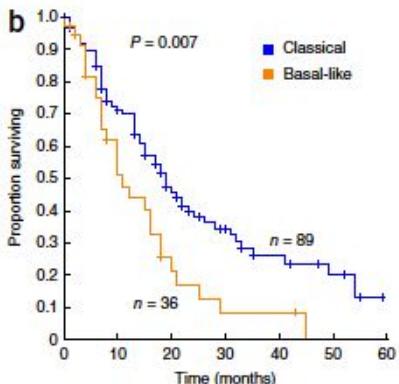
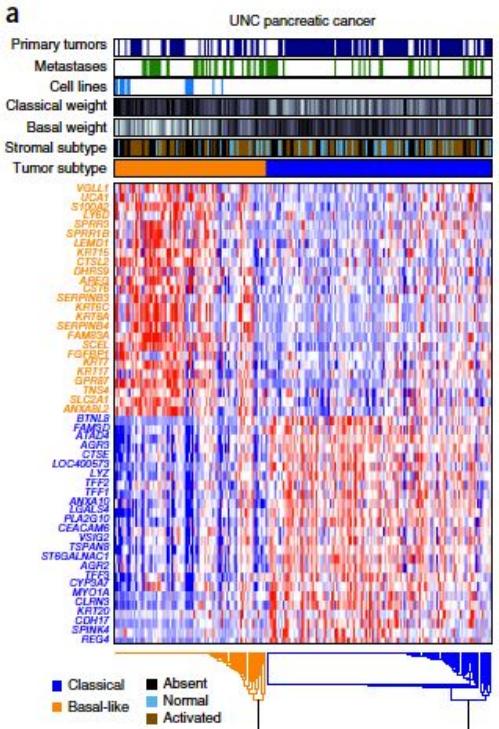
⬇ Axon guidance pathway genes *SLIT2*, *SLIT3*, *ROBO1*, *ROBO3*

⬆ MET

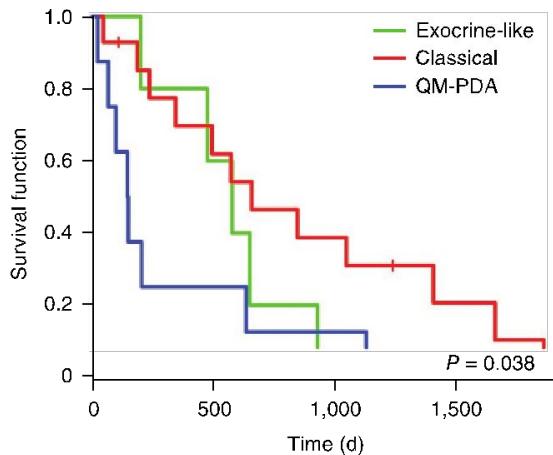
K



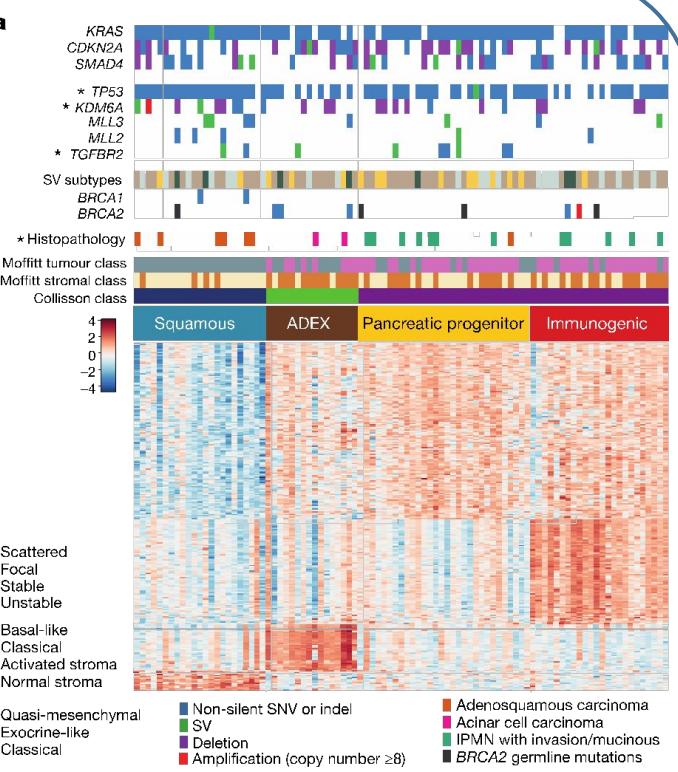
Moffit et al. *Nat Gen* 2015



b Collisson *et al.* *Nat Med* 2011

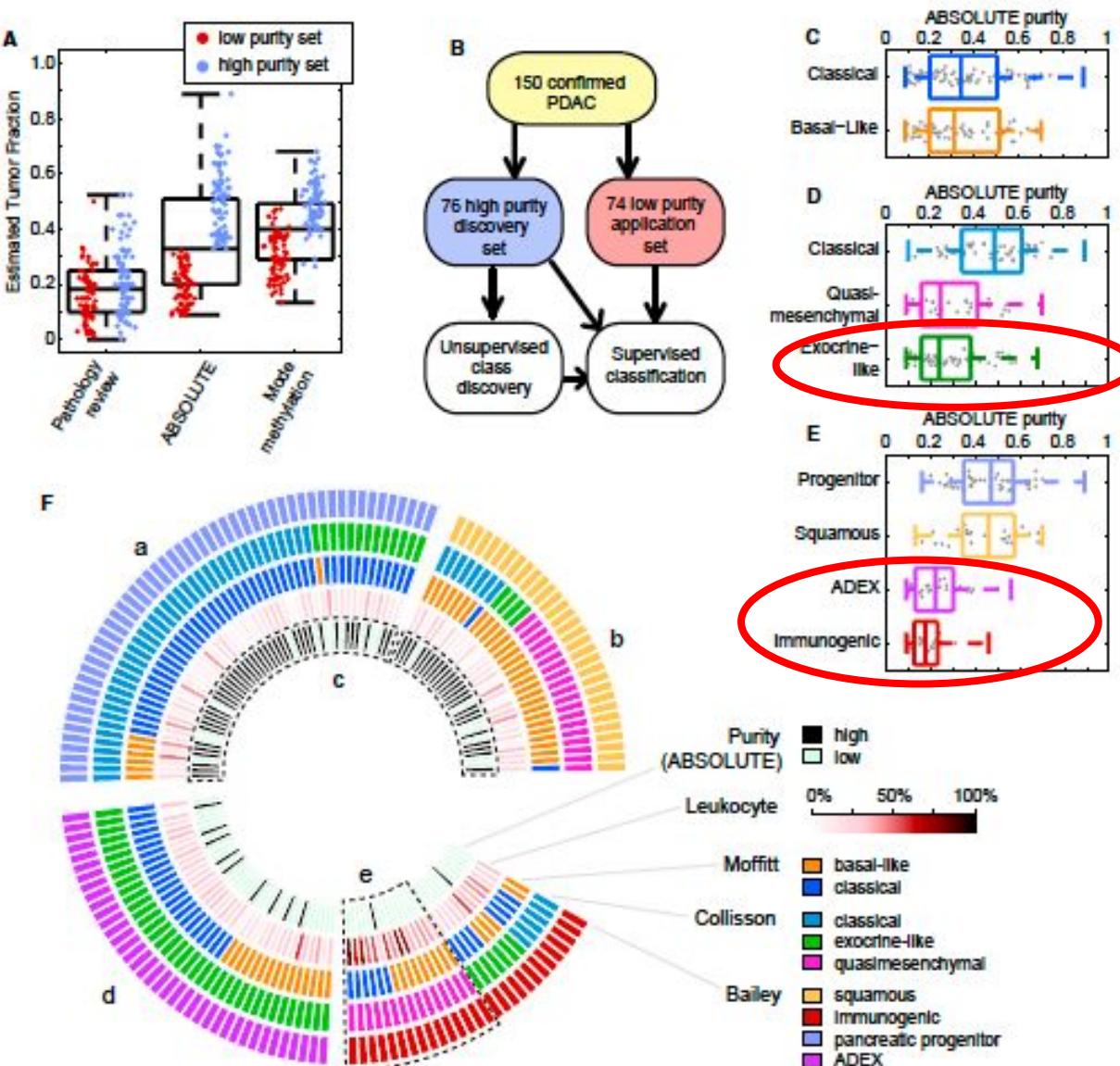


Transcriptomic



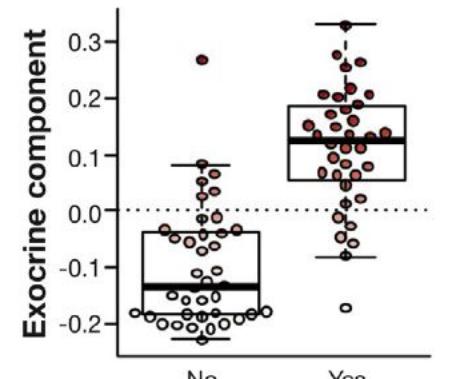
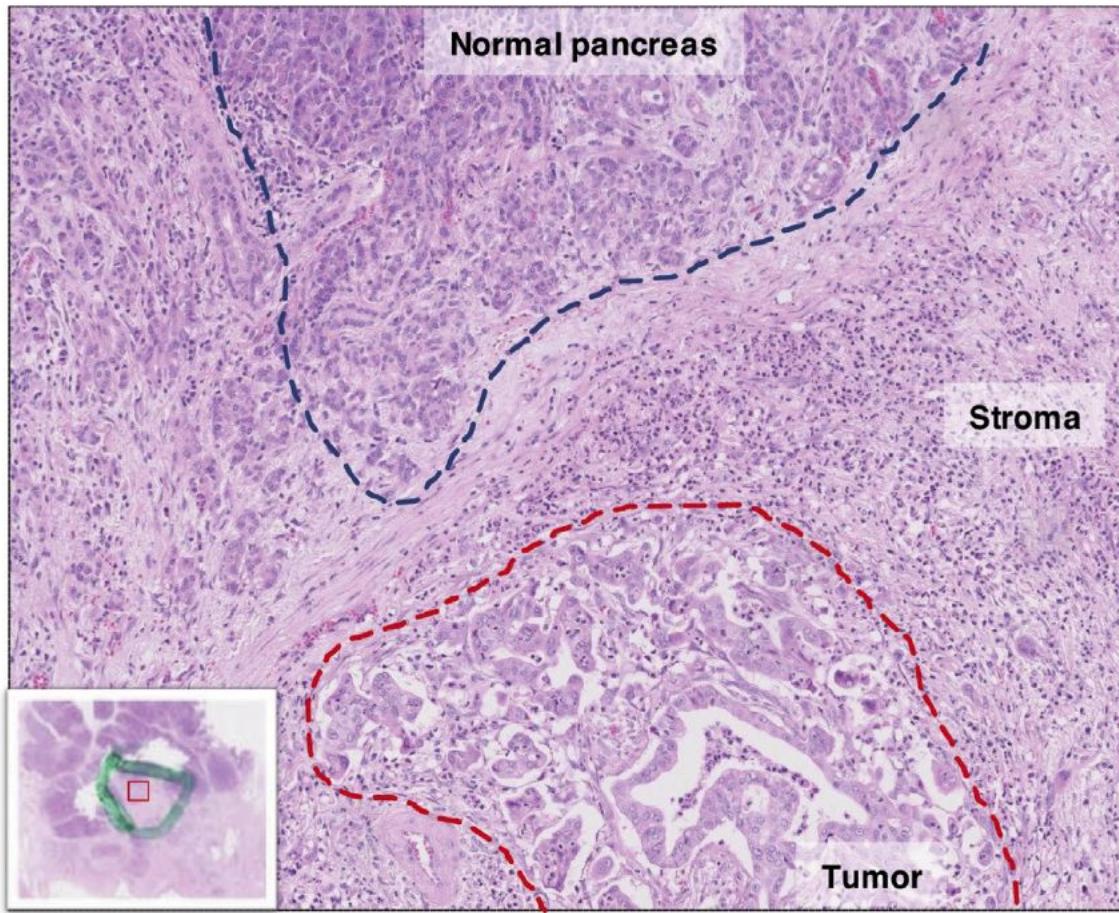
Bailey et al. *Nature* 2016

PDAC transcriptomic subtypes, how many? Do they all exist?

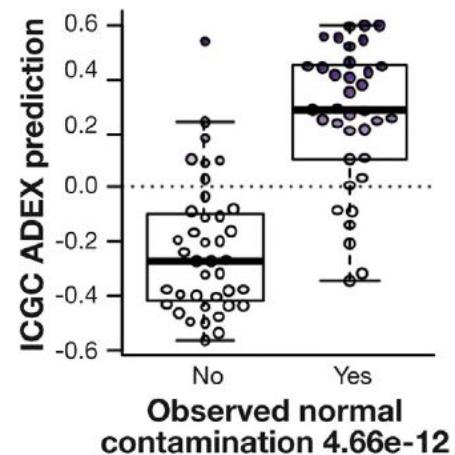


We try our best to give pure tumor area....

A

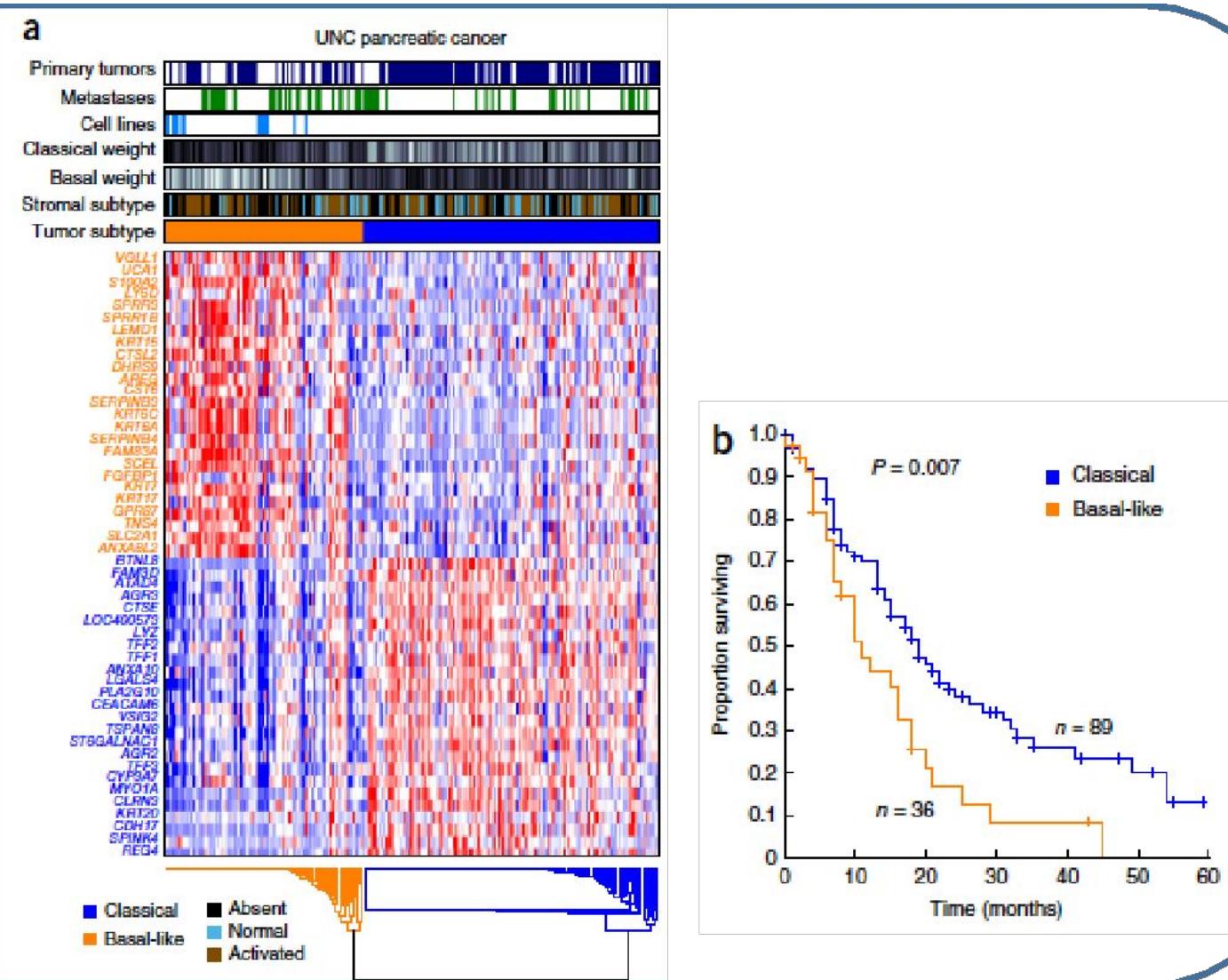


Observed normal
contamination $3.02e-12$



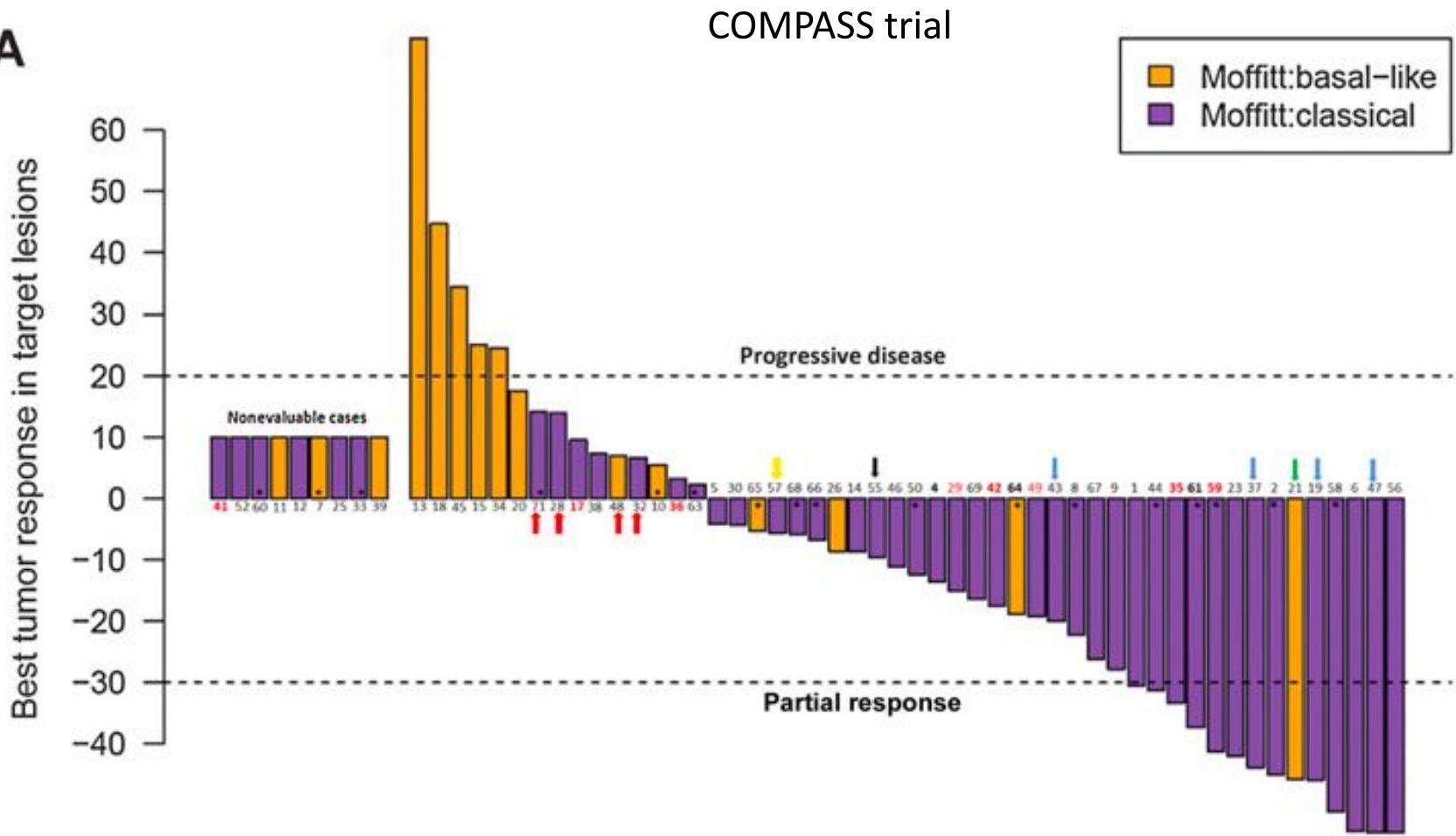
2 main transcriptomic tumor subtypes with different prognosis

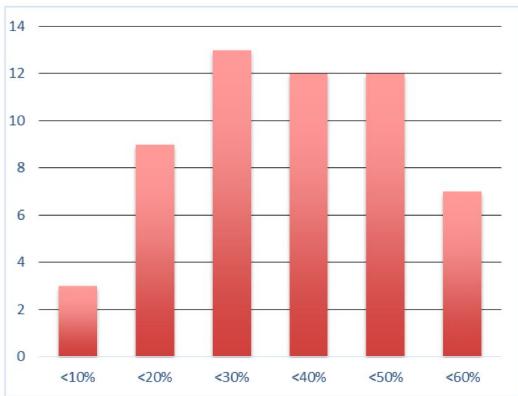
Moffitt et al. Nat Gen 2015



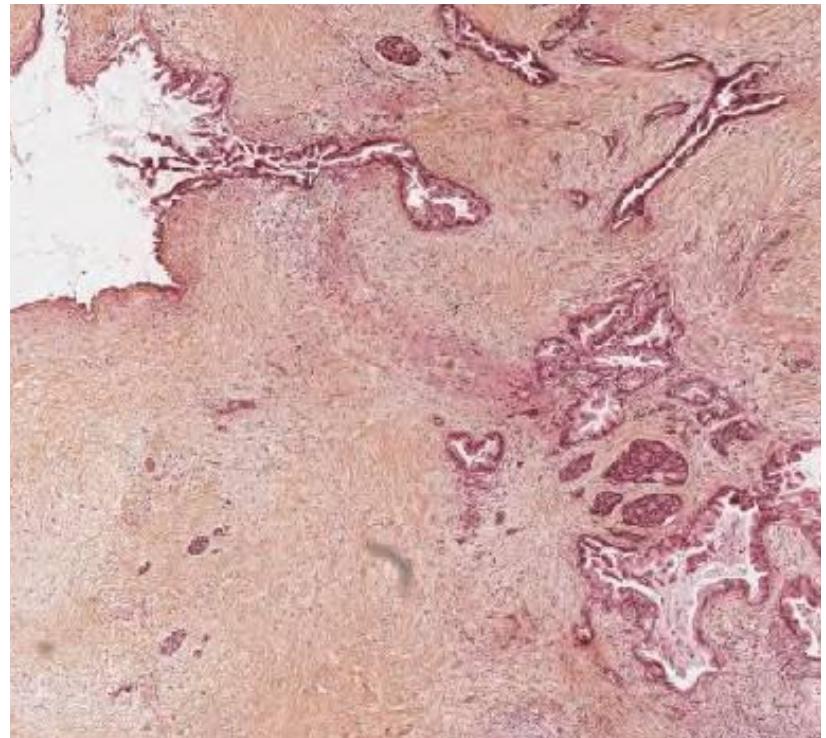
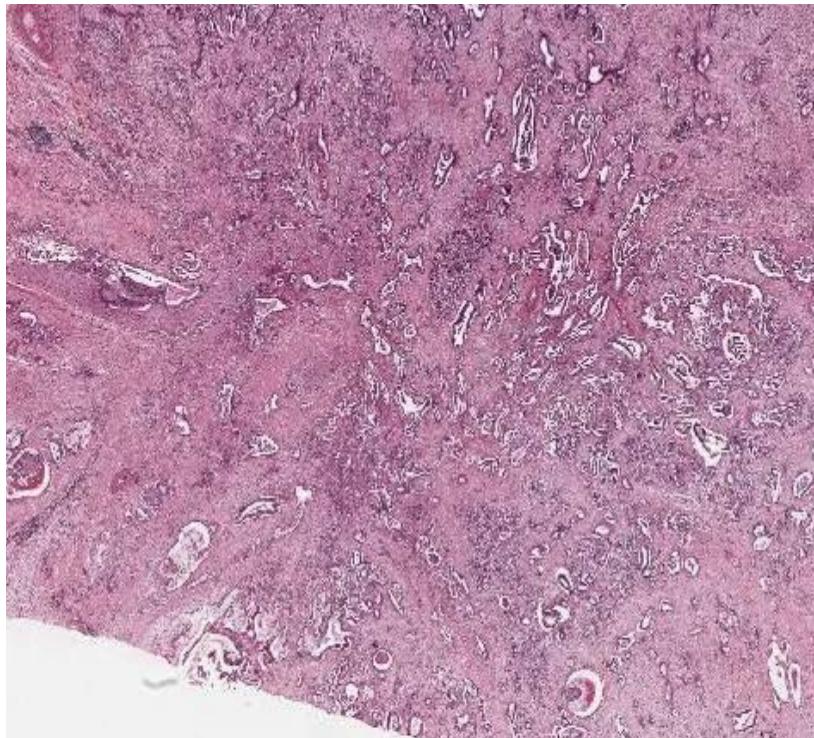
Molecular subtypes may have an important clinical utility++++

A





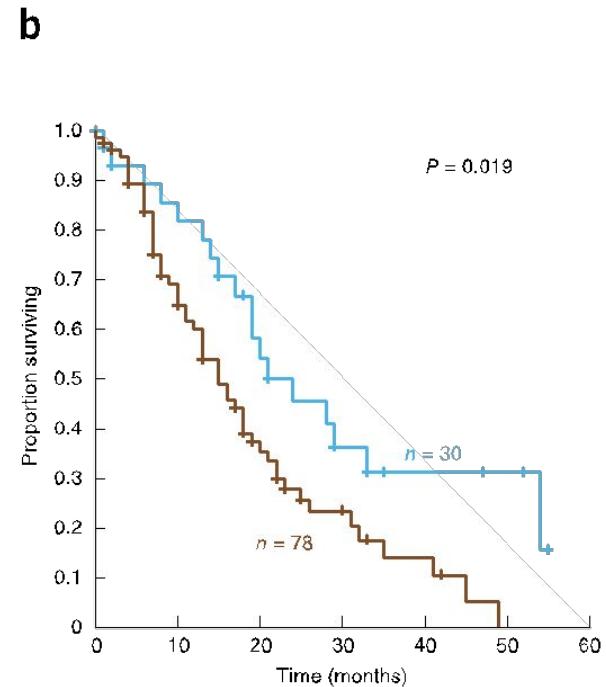
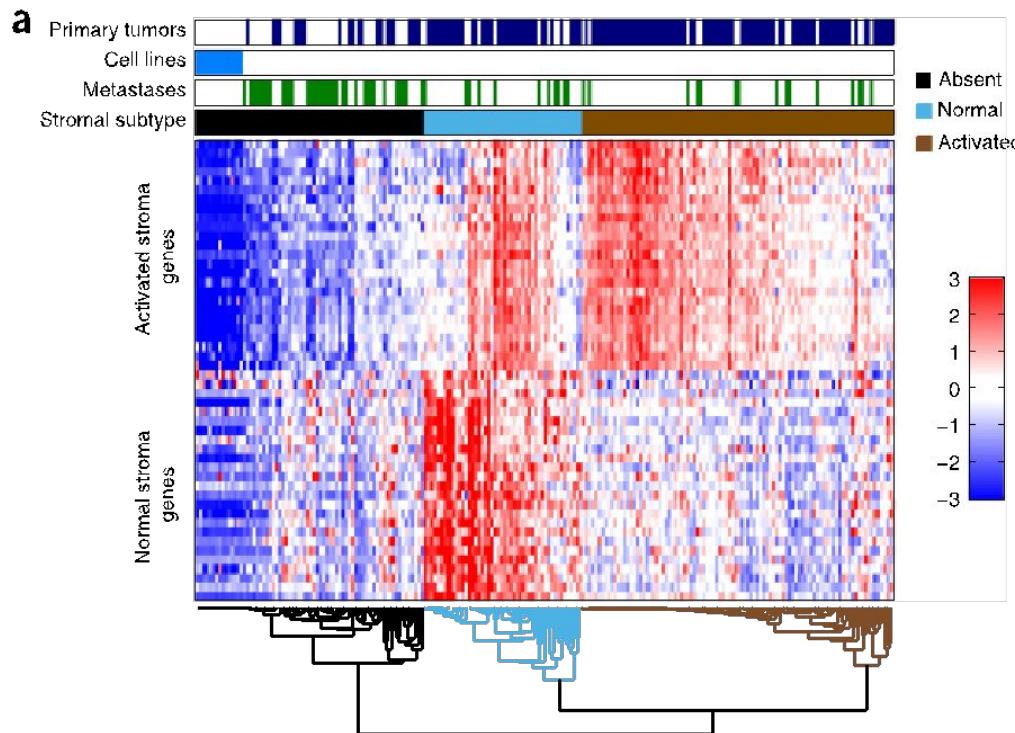
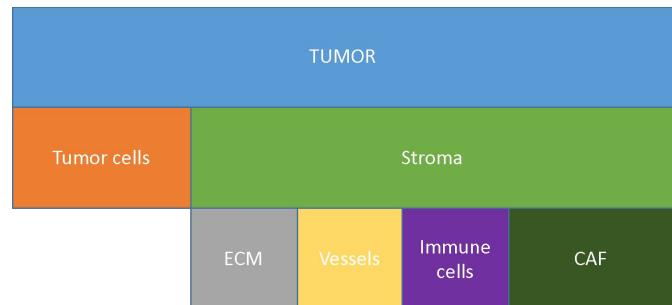
True challenge! How to define (clearly) the molecular subtype in samples with few tumor cells???



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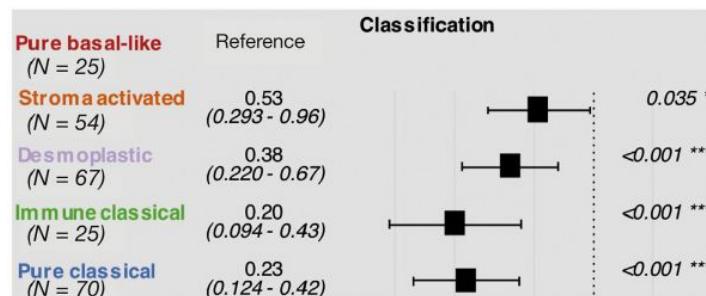
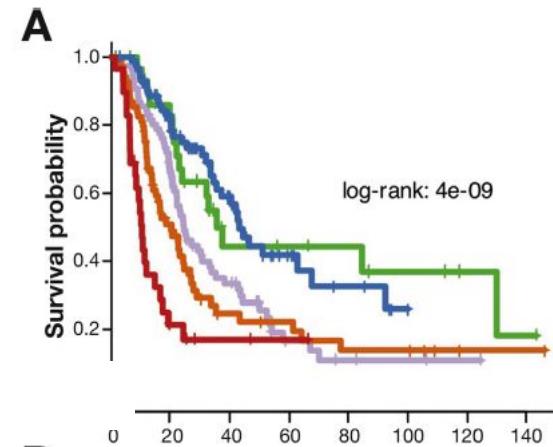
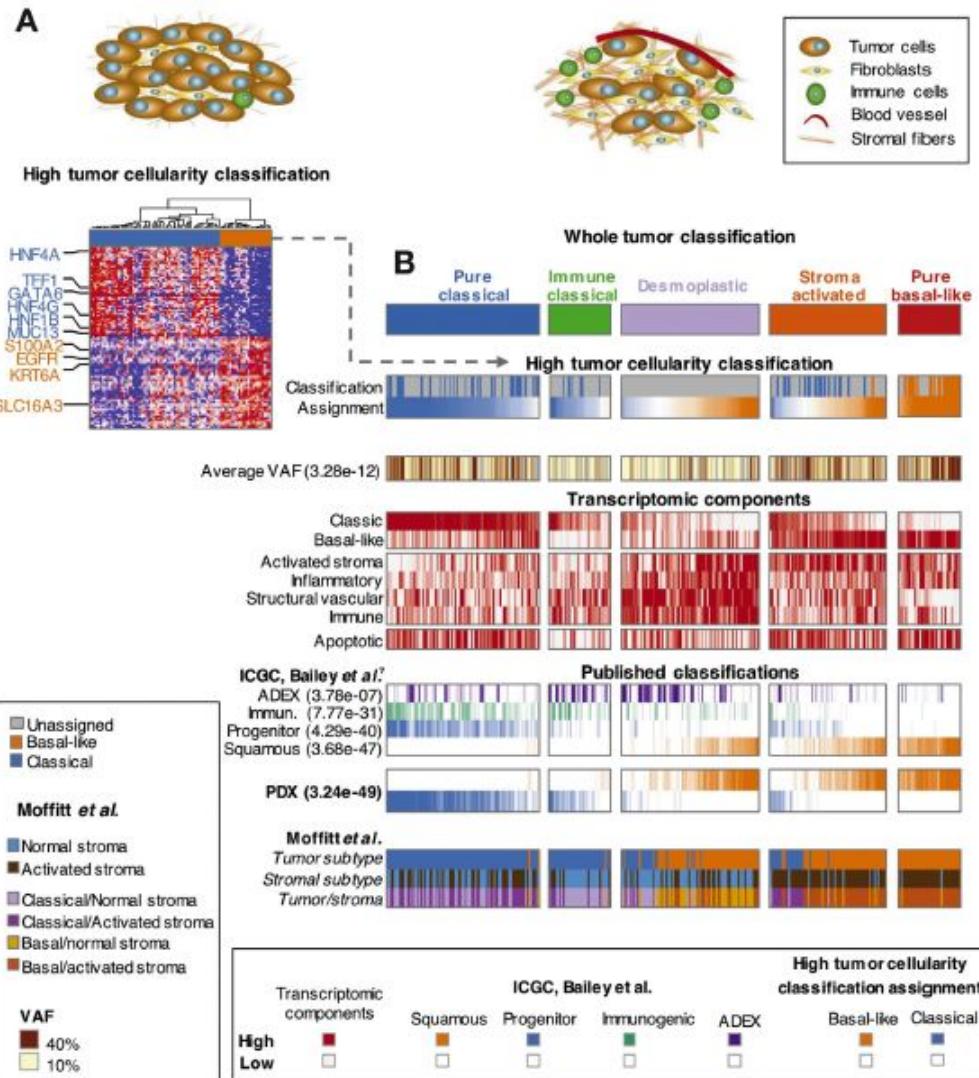
IHC markers

And the stroma is also heterogeneous...



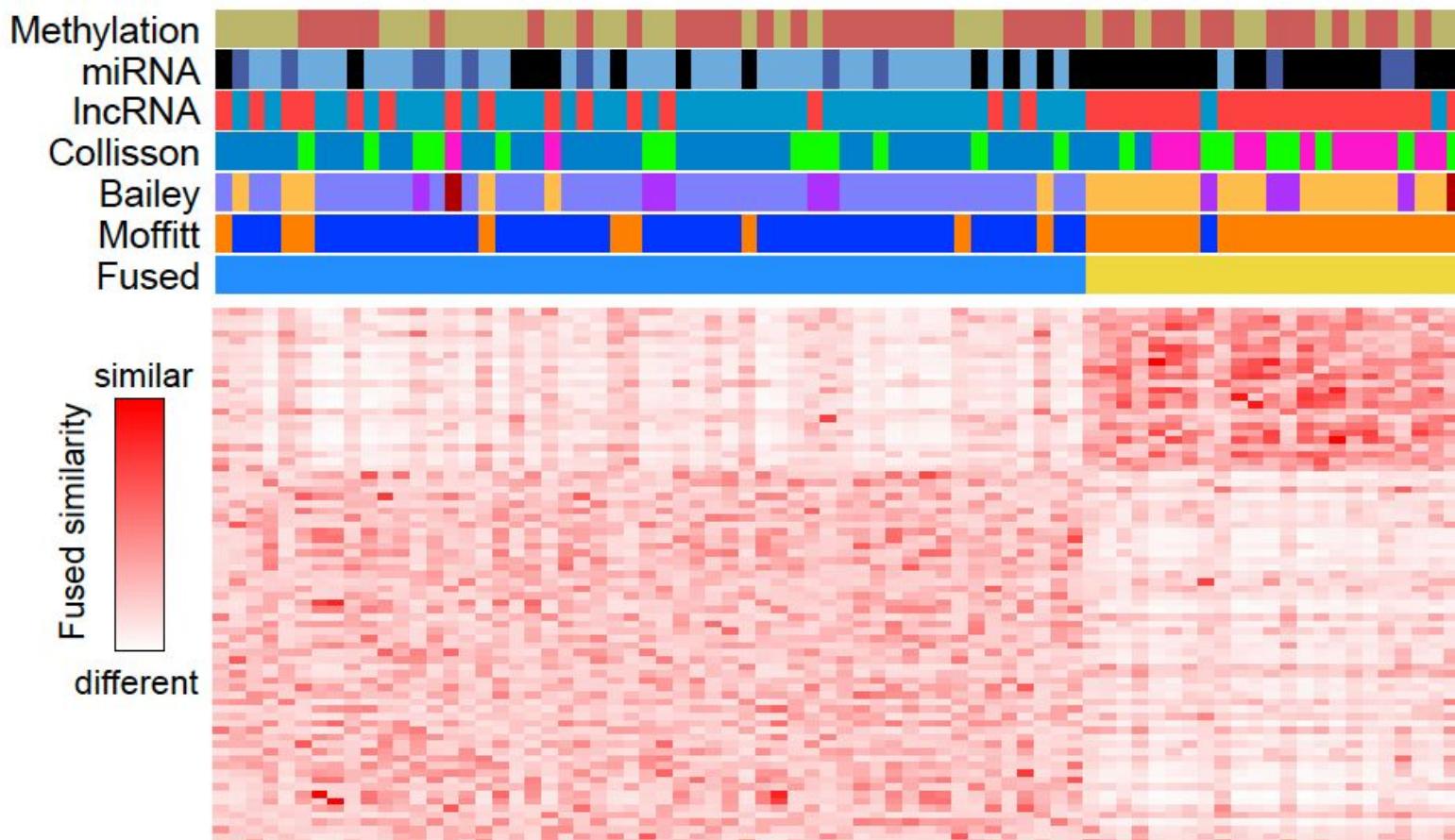
Moffitt et al. *Nature genetics* 2015

Integration of the heterogeneity of all the components....



Inter-tumor heterogeneity - conclusion

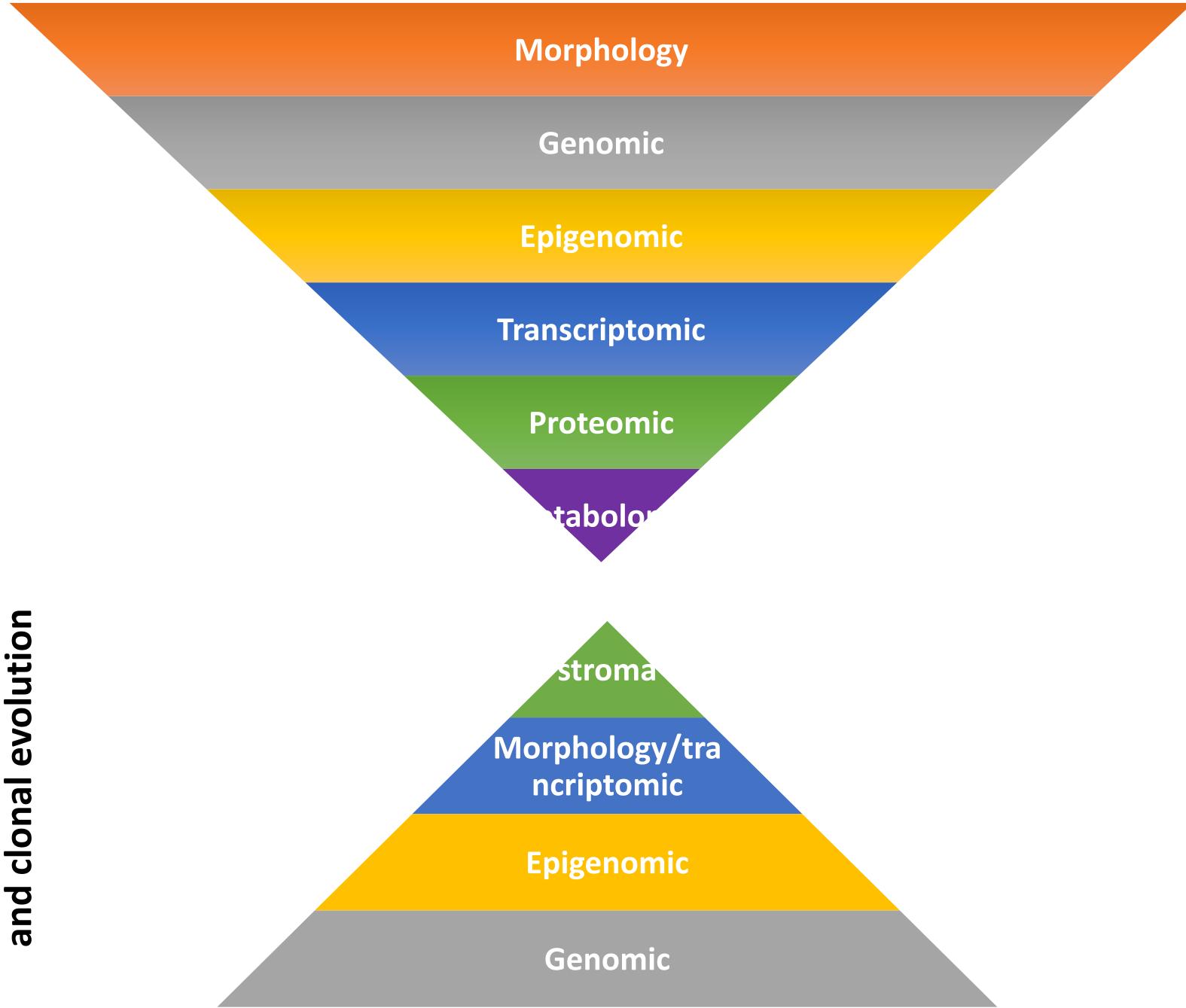
Major impact of purity (tumor cells) on classifications++++



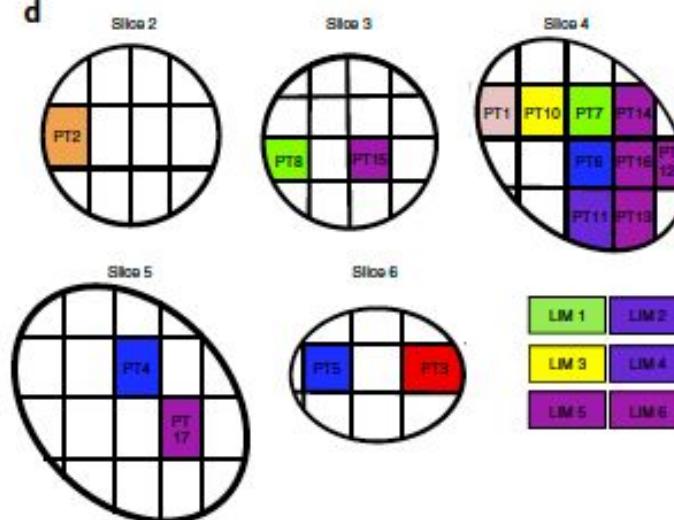
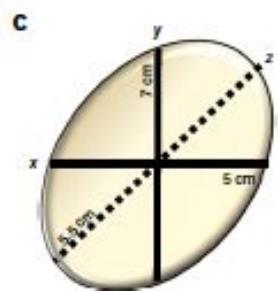
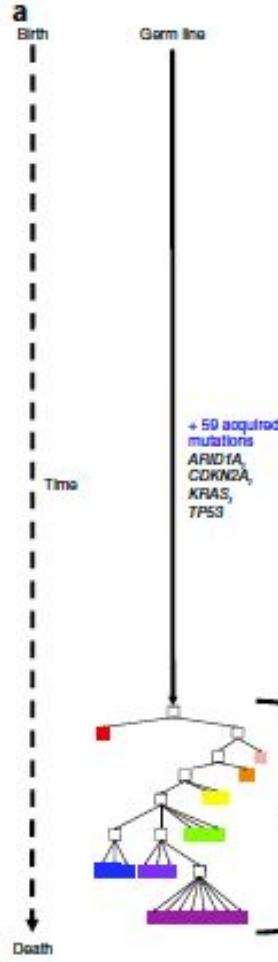
Methylation	miRNA	IncRNA	Collisson	Bailey	Moffitt
methylation-1	■ miRNA-1	■ IncRNA-1	■ Classical	■ ADEX	■ Basal-like
methylation-2	■ miRNA-2	■ IncRNA-2	■ Exocrine-like	■ Immunogenic	■ Classical
	■ miRNA-3		■ Quasimesenchymal	■ Progenitor	
				■ Squamous	

Inter-tumor heterogeneity

**Intratumor heterogeneity
and clonal evolution**



Intra-tumor heterogeneity - genomic

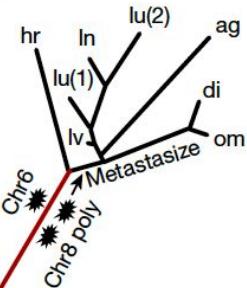
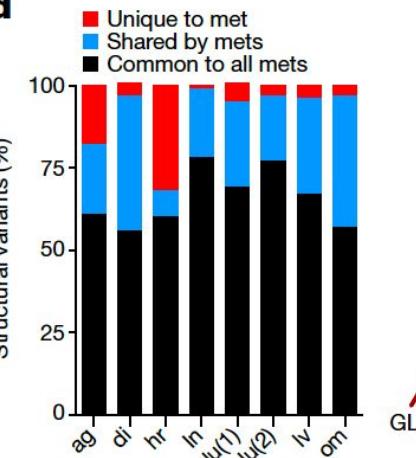


- Low heterogeneity of classical driver genes
- Most genomic events happen early
- No « metastasis » gene
- Physical and genomic spatialisation are different+++

Makohon-Moore *et al.* *Nat Gen* 2017
Yachida *et al.* *Nature* 2010

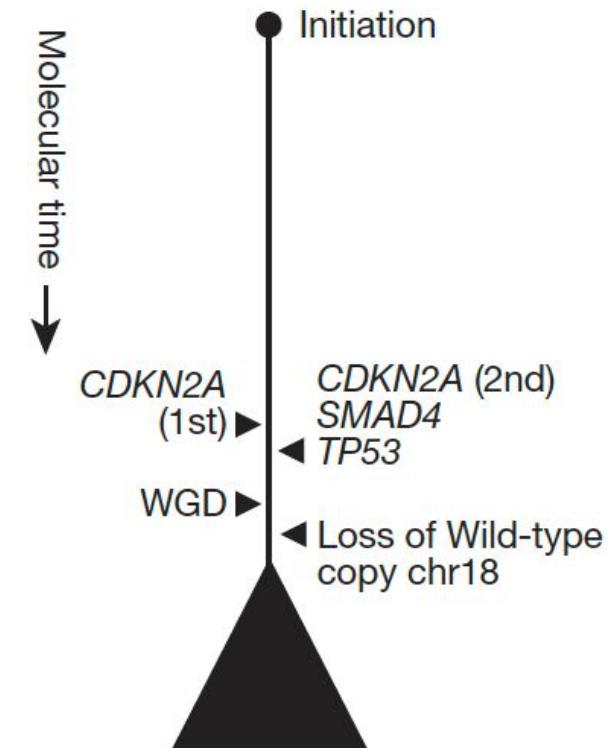
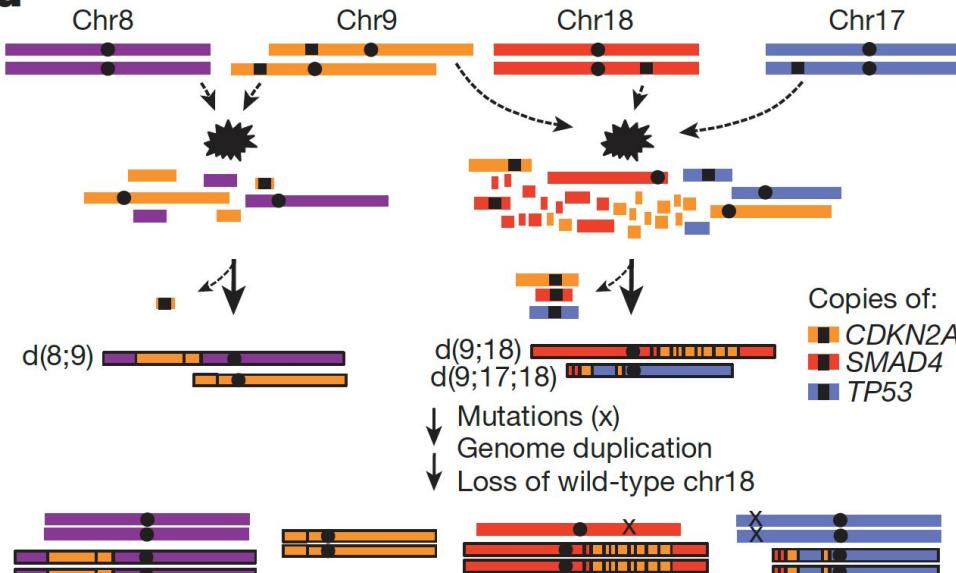
Intra-tumor heterogeneity - genomic

d

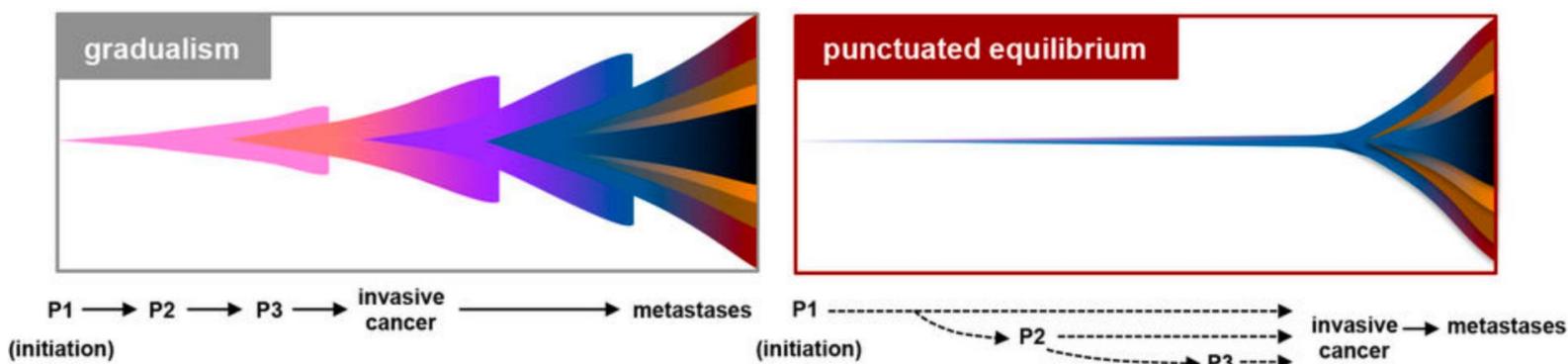
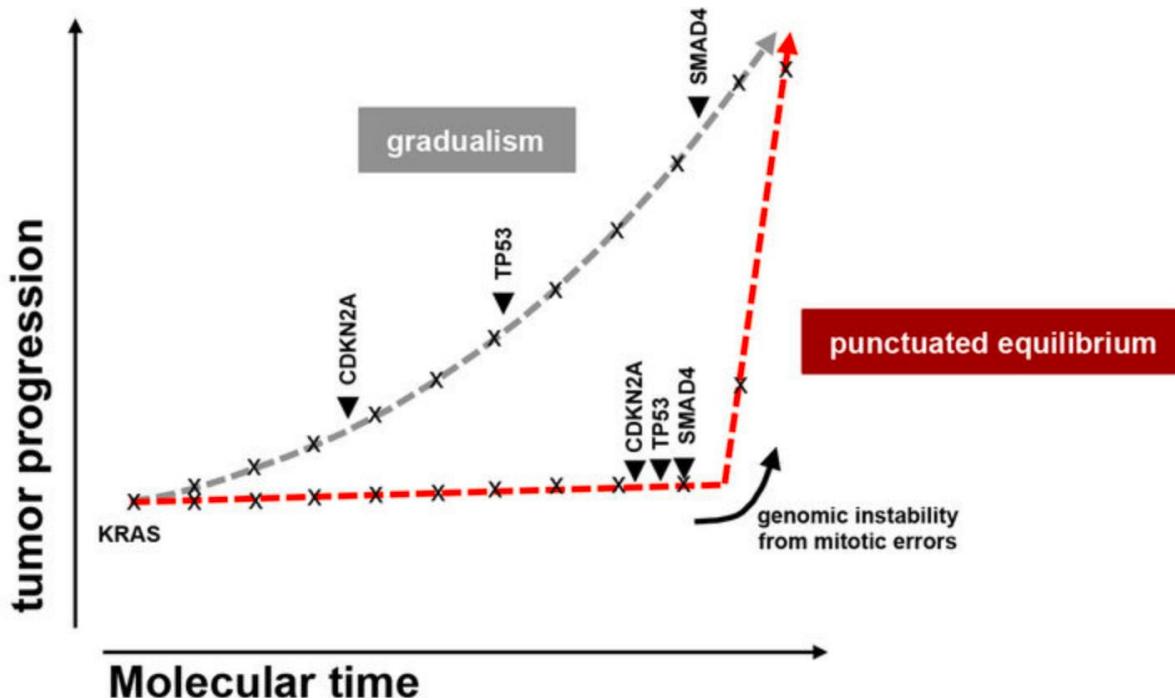


- Most genomic events happen before the first metastase
- 50% of tumours are not diploid (T ou H)
- Multiples simultaneous genomic alterations

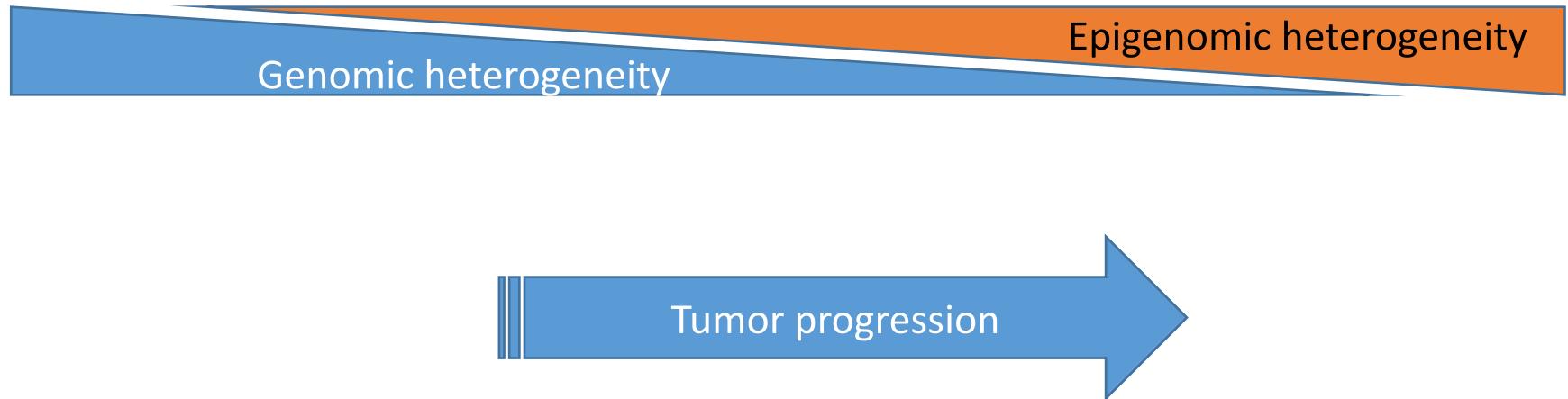
d



How to follow high risk patients???



Intra-tumor heterogeneity - epigenomic



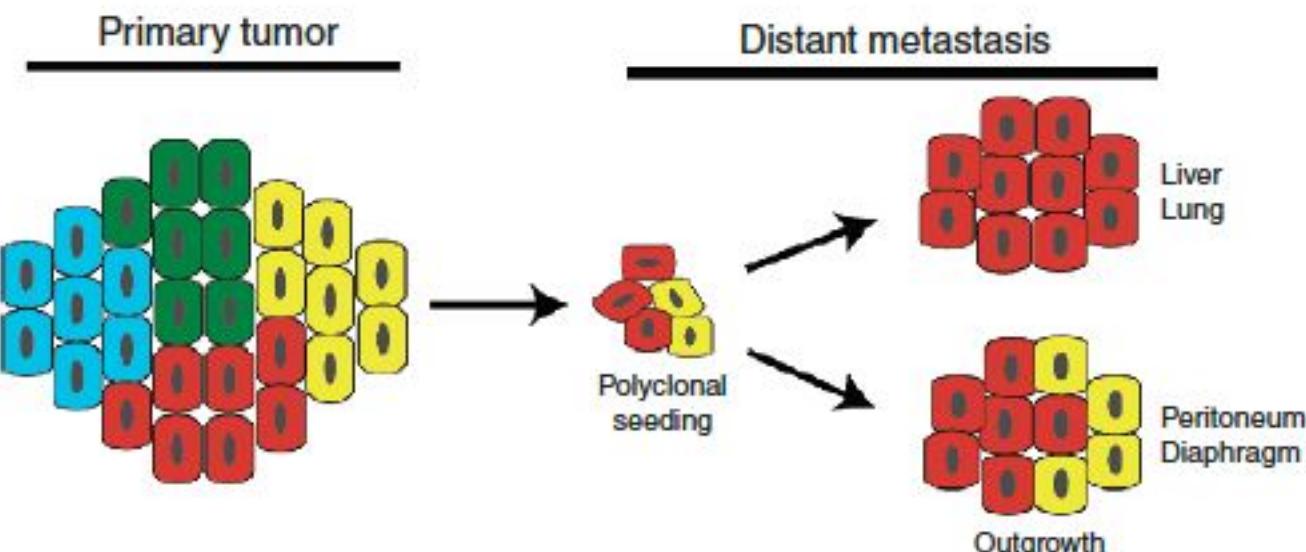
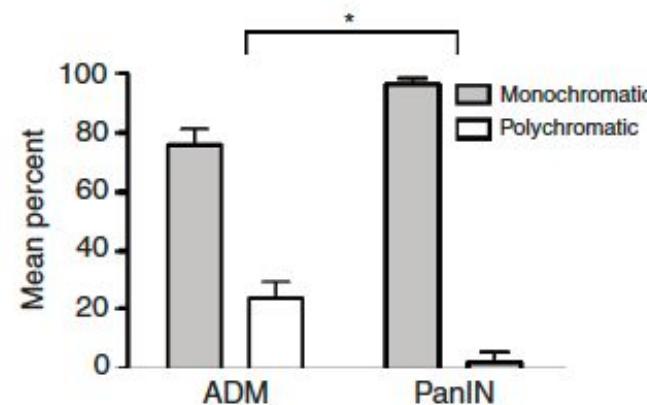
Genomic heterogeneity

Epigenomic heterogeneity

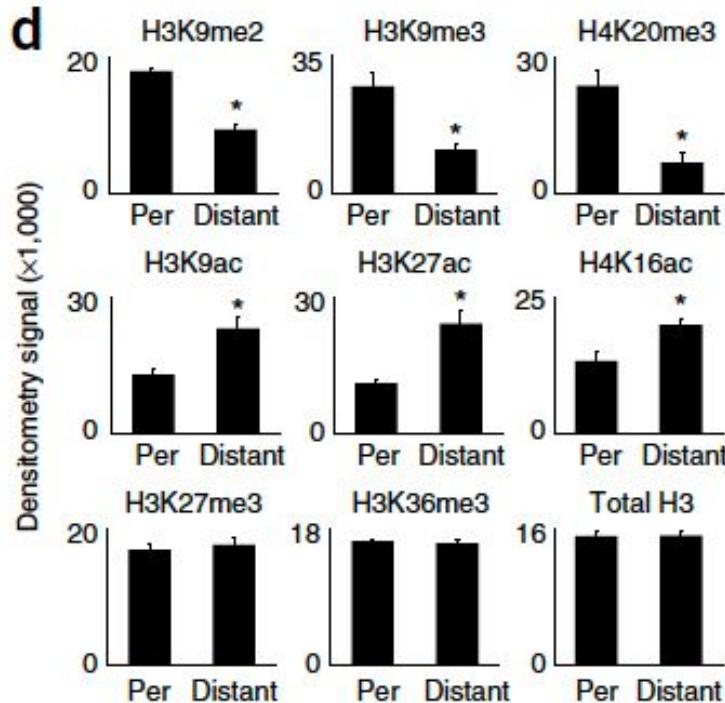
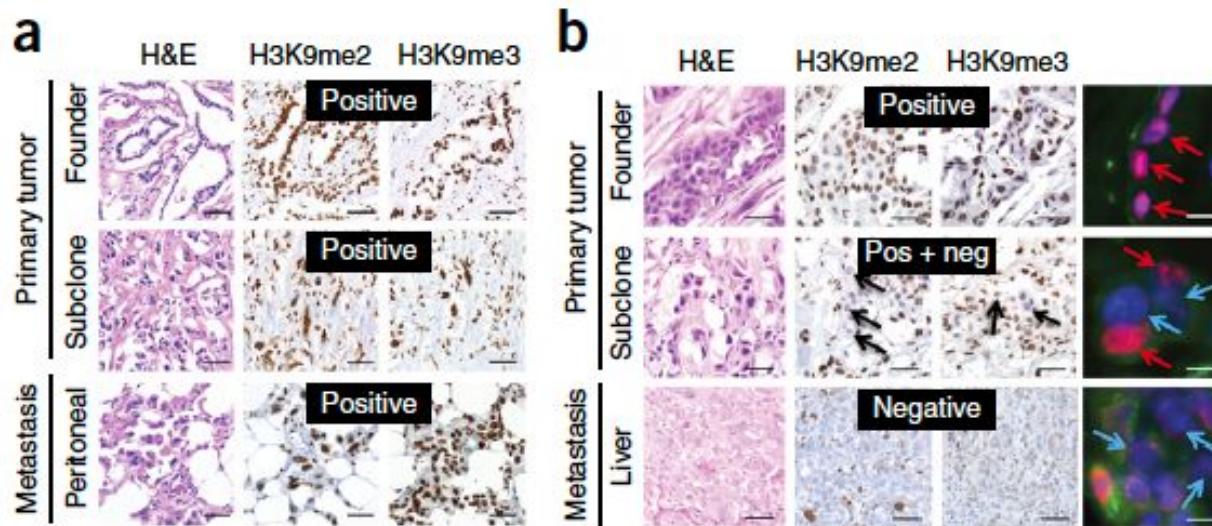
C

ADM and PanIN counts

Lesion	Monochromatic	Polychromatic	Total
ADM	149 (76%)	46 (24%)	195
PanIN	141 (97%)	4 (3%)	145

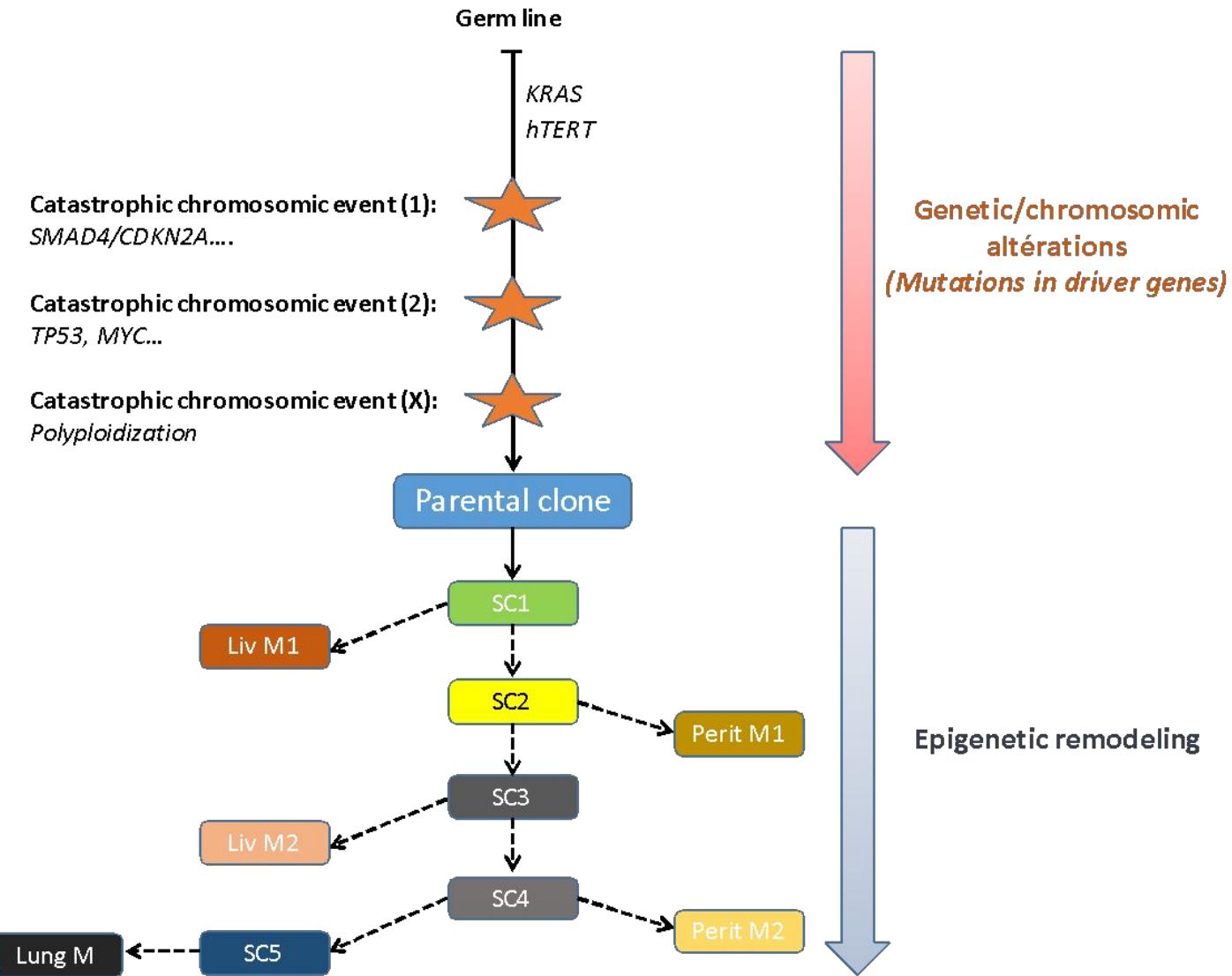


Intra-tumor heterogeneity - epigenomic



Genomic heterogeneity

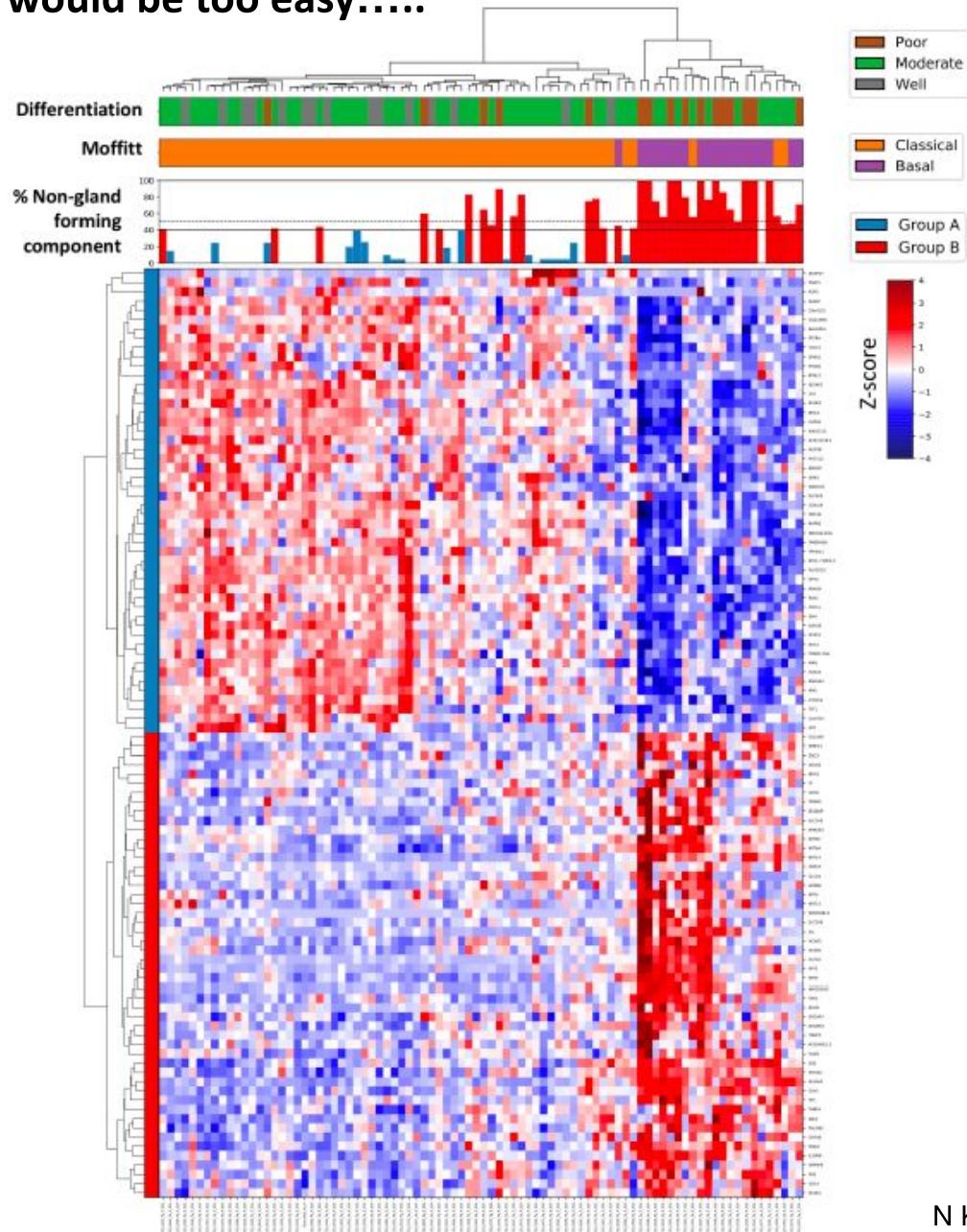
Epigenomic heterogeneity



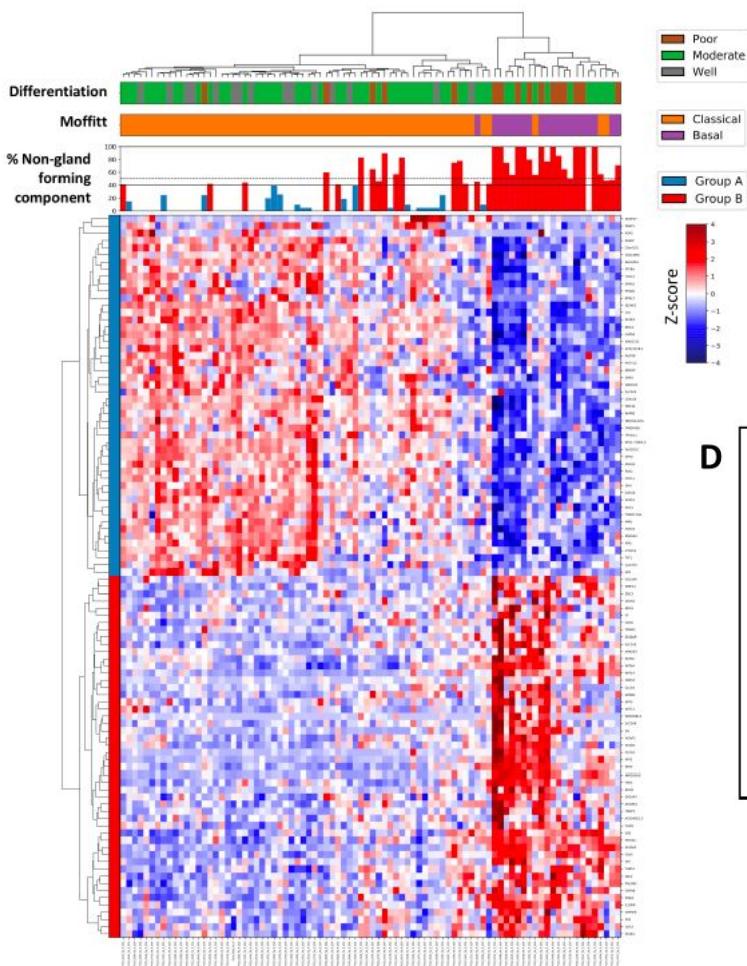
If tumor were pure, that would be too easy.....

A	"Gland forming" component	Patterns	
		Conventional	
		Tubulopapillary	
	"Non-gland forming" component	Patterns	
		Composite	
		Squamous	

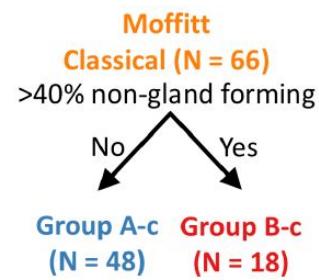
If tumor were pure, that would be too easy.....



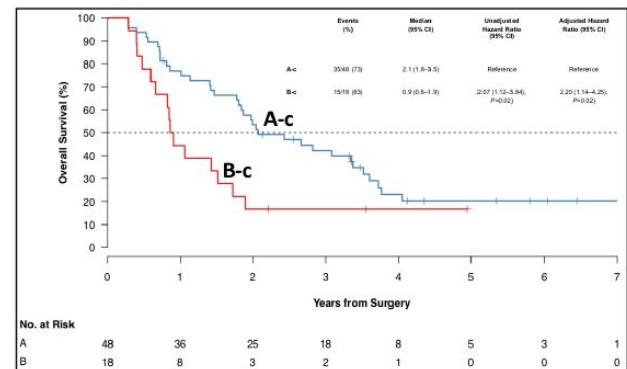
If tumor were pure, that would be too easy.....



D



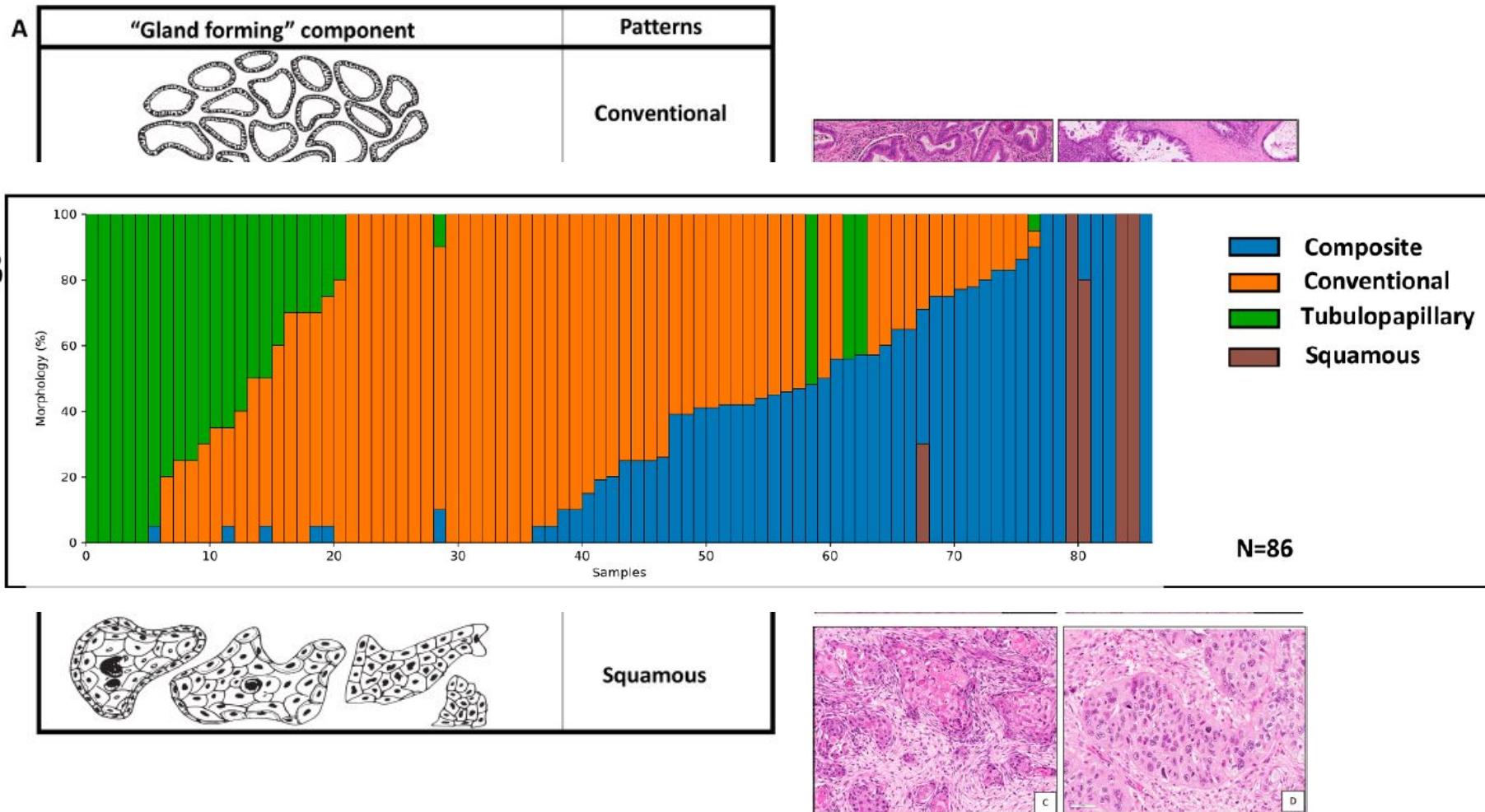
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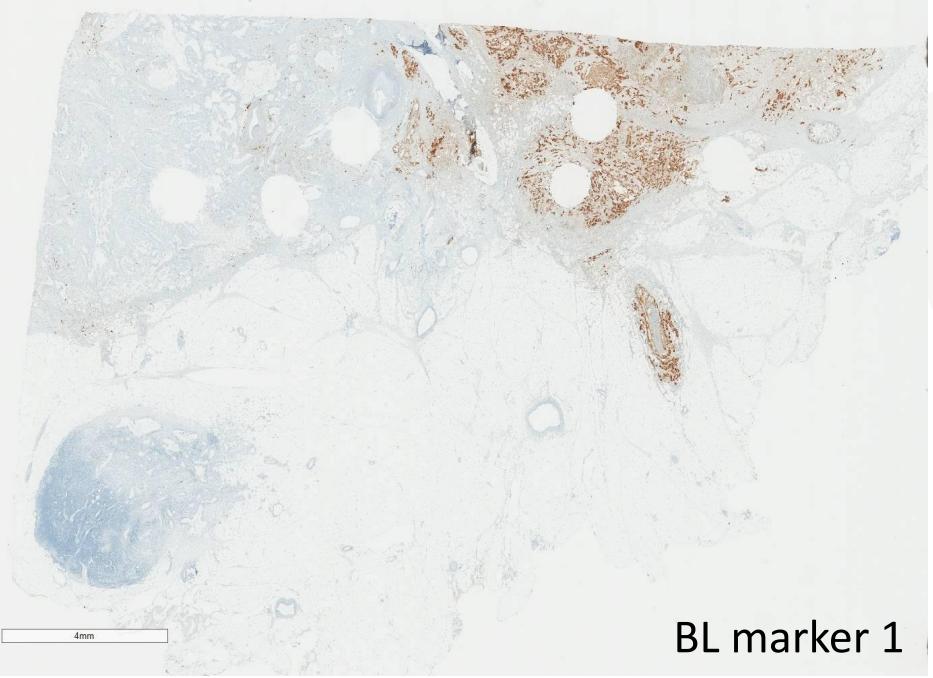


« classical » tumors with a basal like subpopulation?

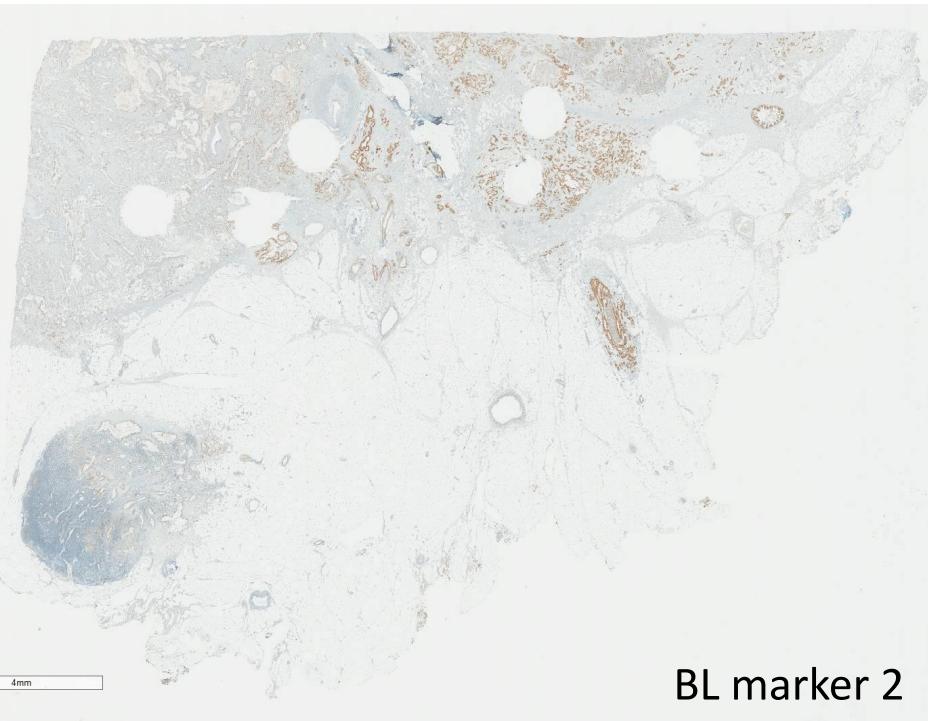
Intra-tumor heterogeneity - morphology

If tumor were pure, that would be too easy.....





BL marker 1

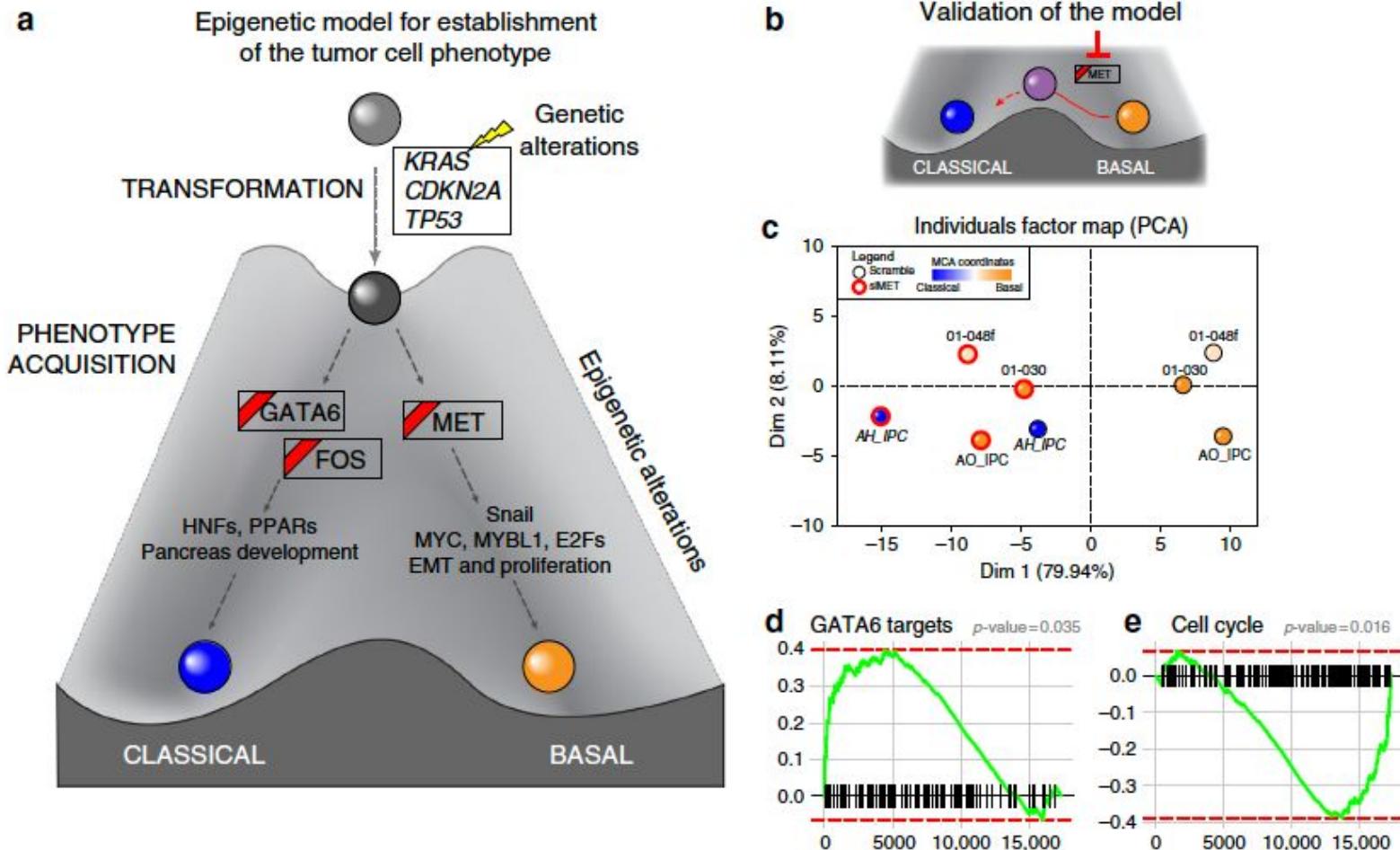


BL marker 2

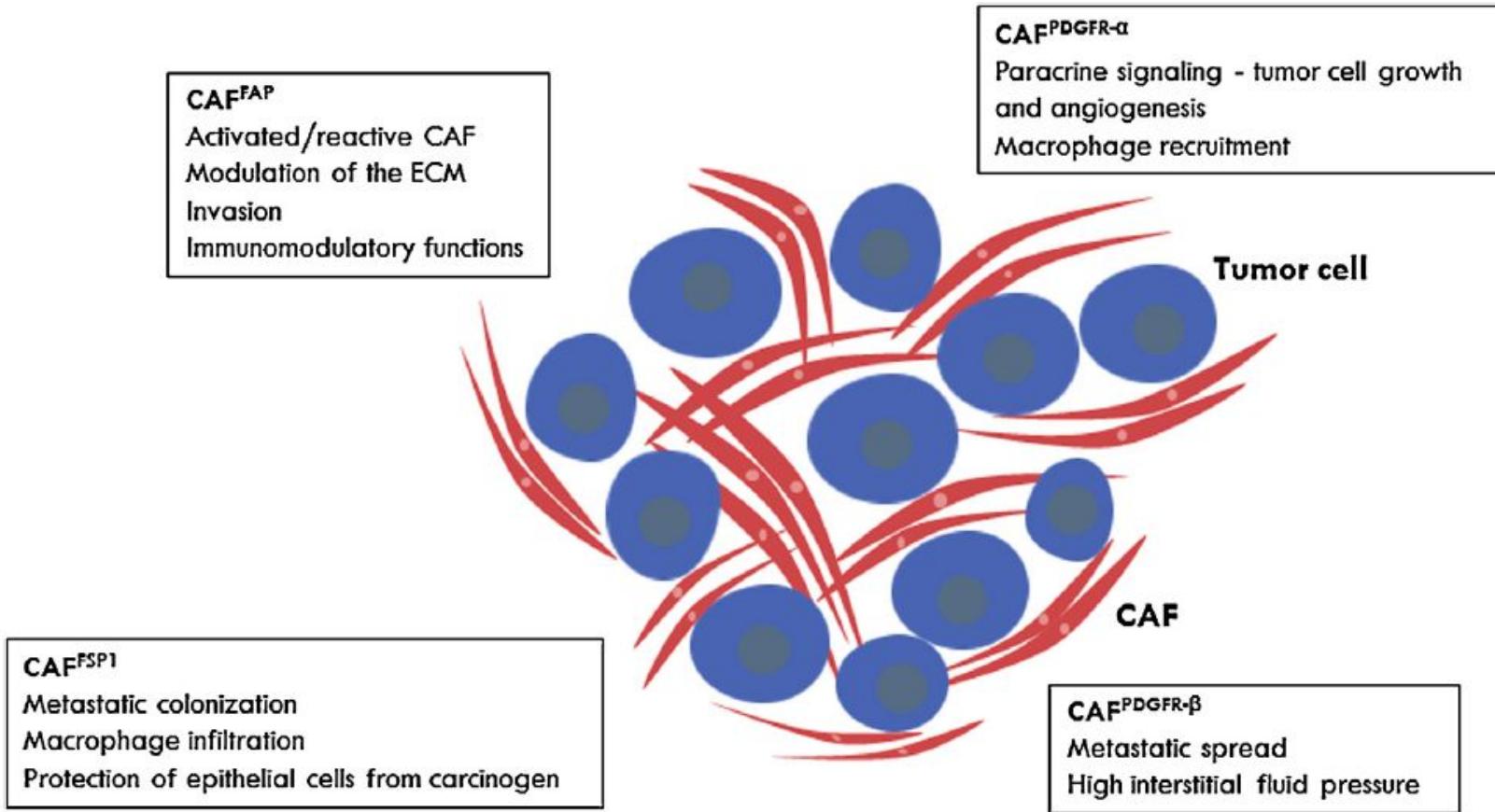


Class marker 1

Is there an epigenetic-driven plasticity between subtypes?

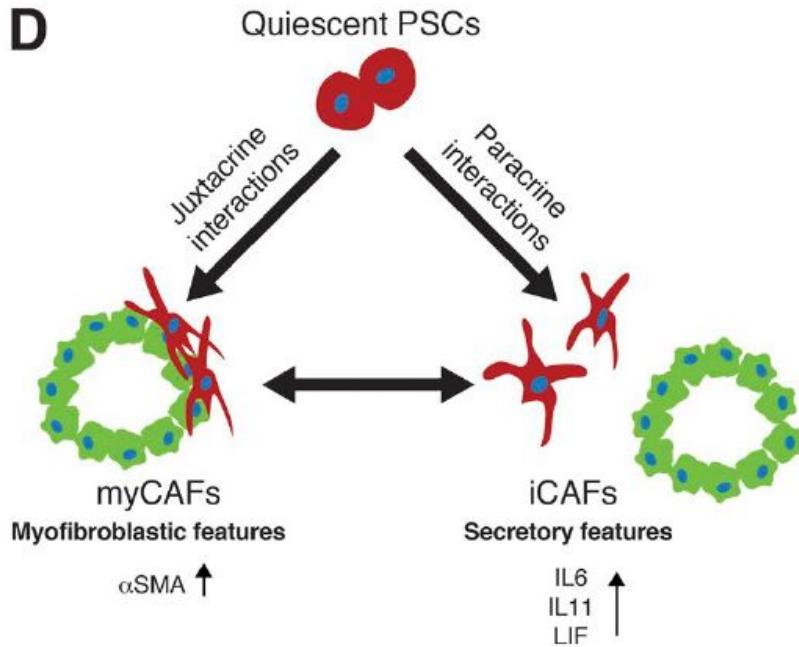


Intra-tumor heterogeneity - stroma

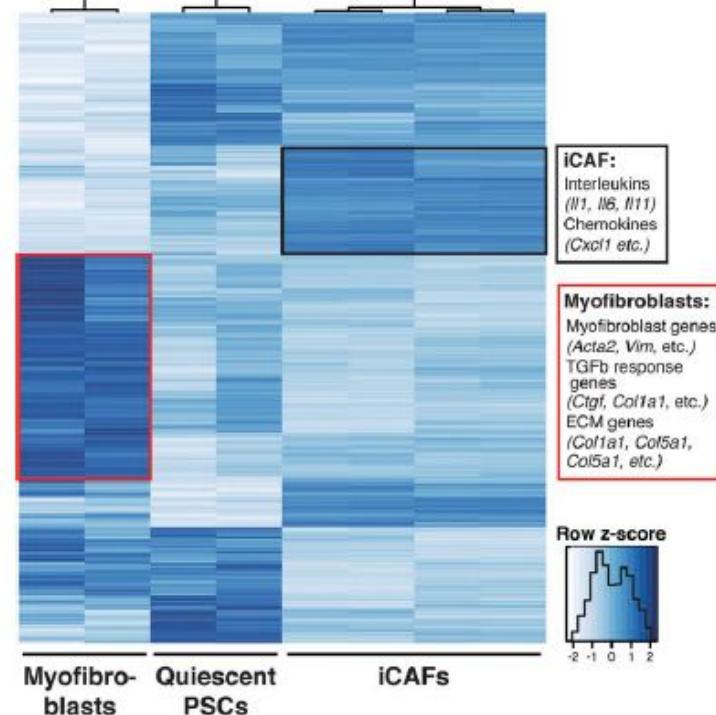


Intra-tumor heterogeneity - stroma

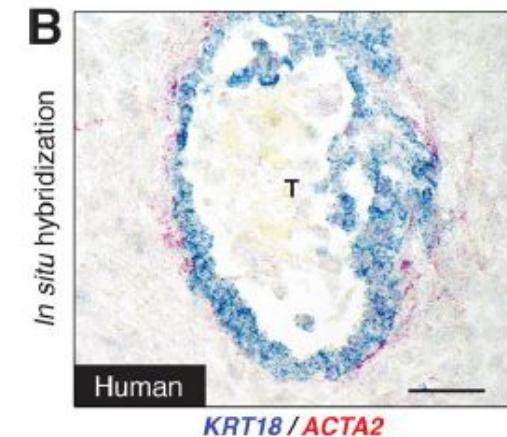
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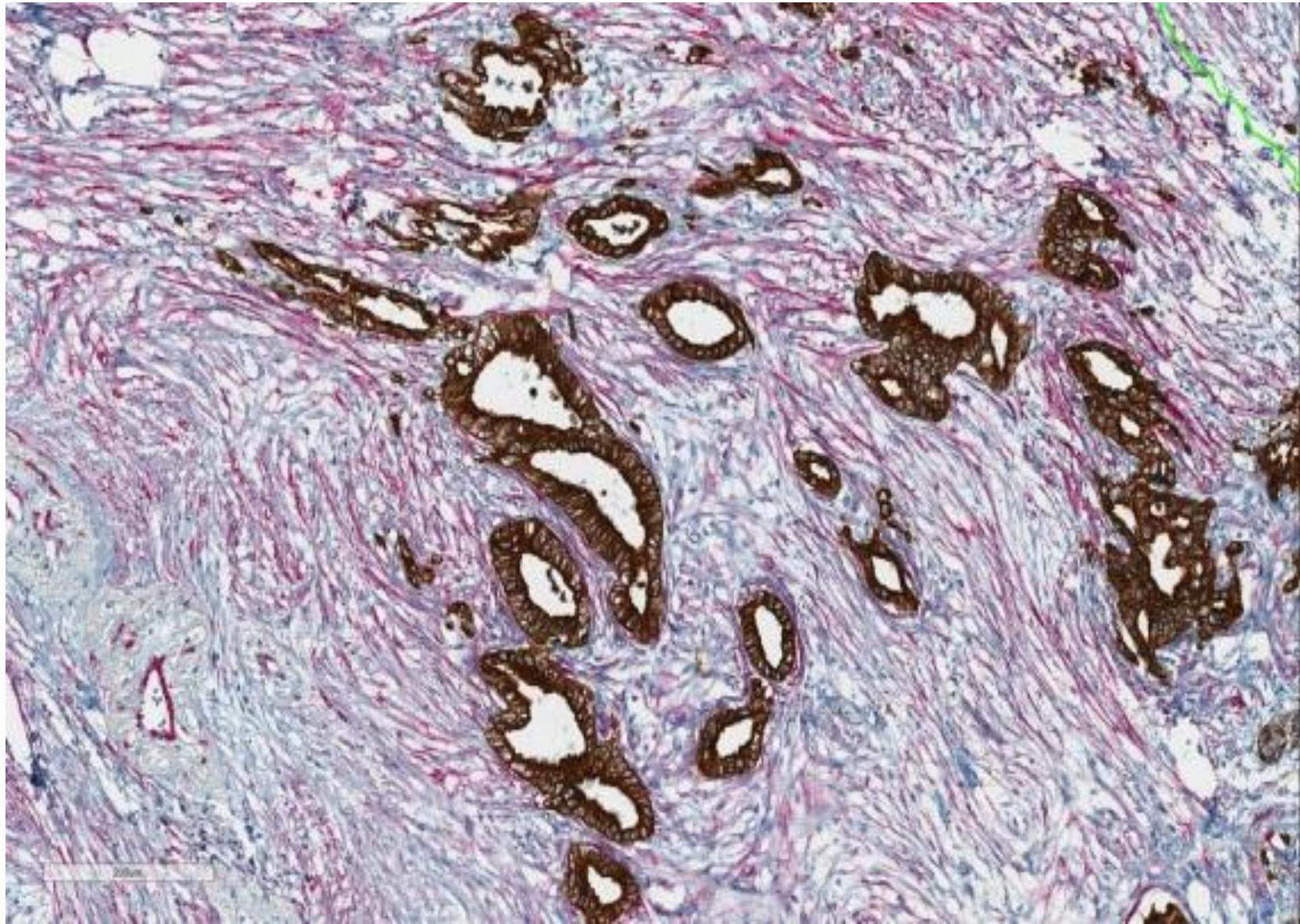
A



B



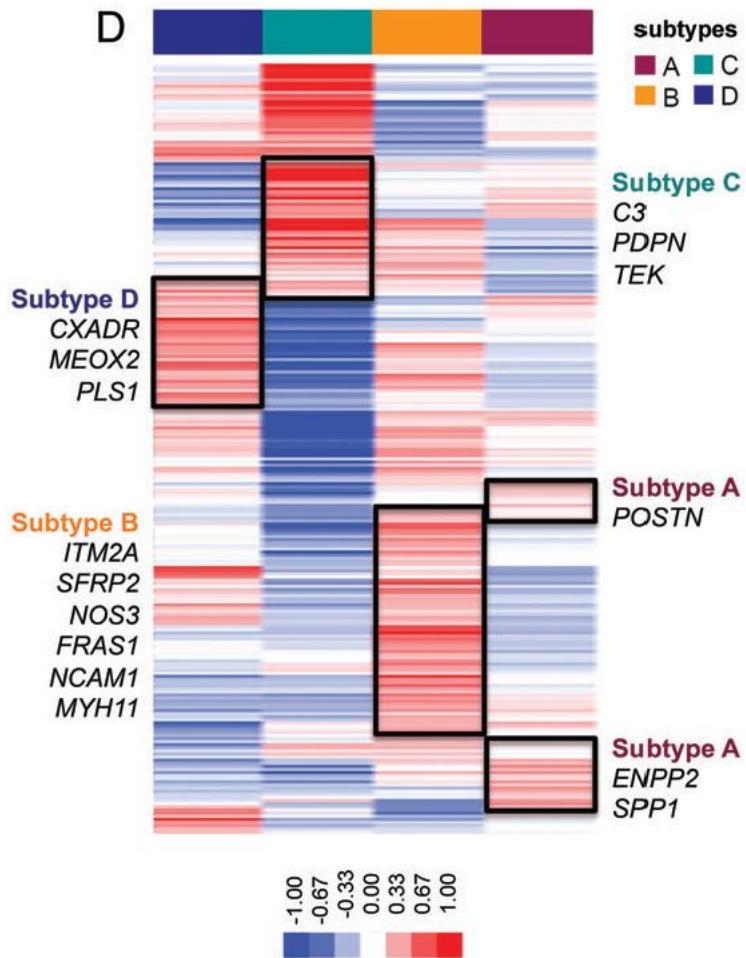
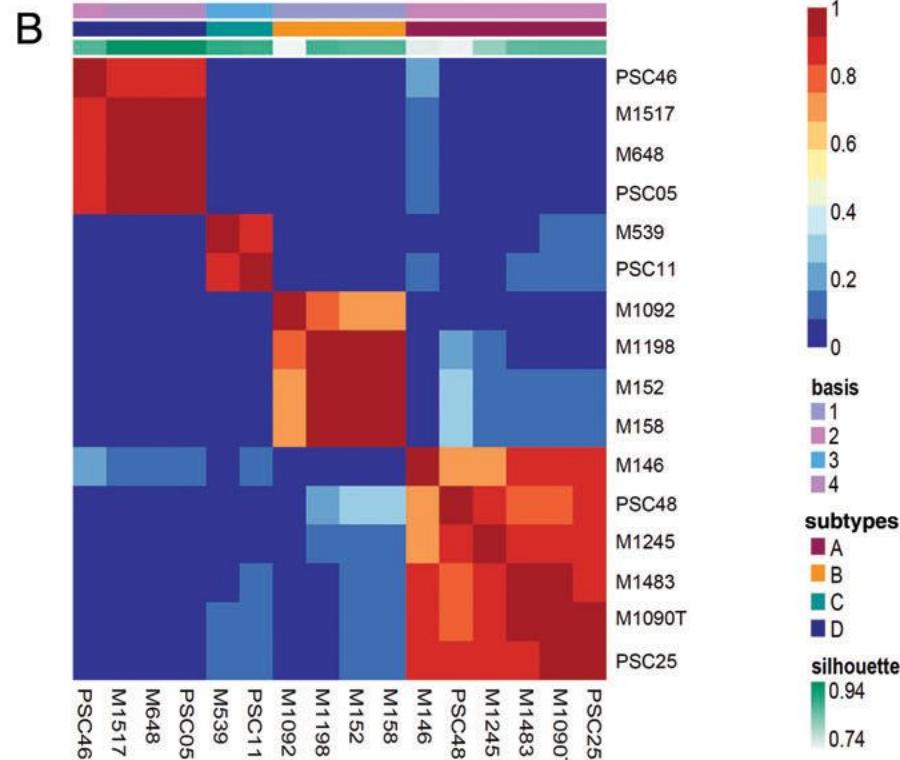
Intra-tumor heterogeneity - stroma



Intra-tumor heterogeneity - stroma

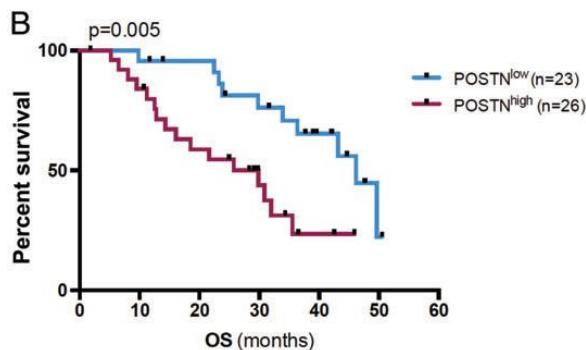
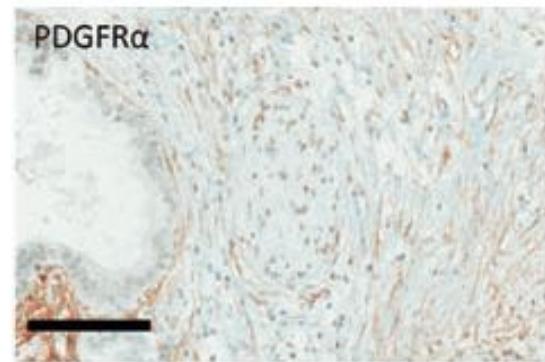
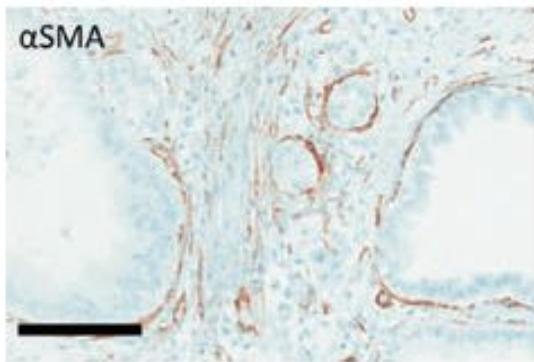
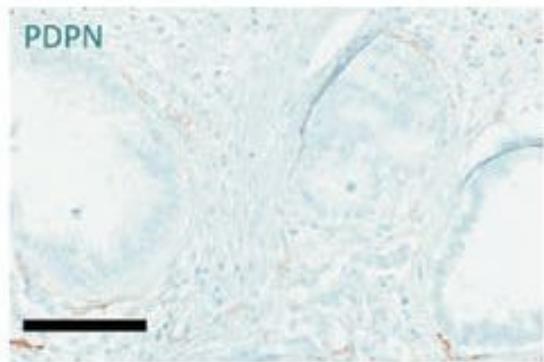
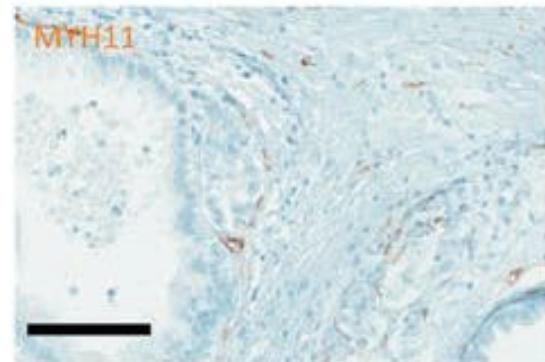
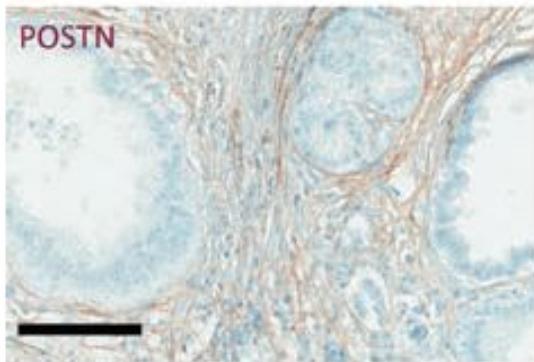
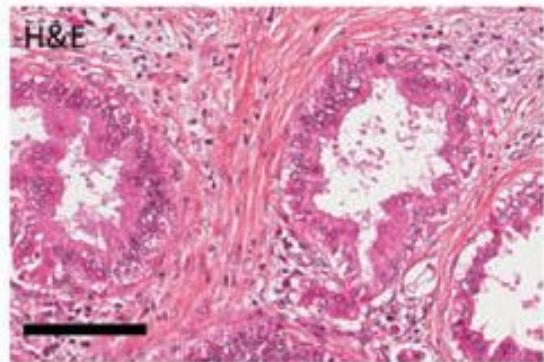
In humans, it is usually more complicated....

Patient-derived CAF



Intra-tumor heterogeneity - stroma

Multiple CAF subtype co-exist in the same tumor, albeit with different ratio and distribution?



Conclusion – PDAC heterogeneity

- Major inter-tumor heterogeneity, at multiple level
 - Therapeutic opportunity?
 - Well annotated samples++++
 - How to best define the subtype in routine practice?
-
- Epigenetic intratumor heterogeneity >> genetic
 - Probable massive spatial transcriptomic heterogeneity...
...of all components

GOOD LUCK!!!!!!!!!!!!!!



15 February 2021

Heterogeneity in cancer Does it matter?

The example of pancreatic adenocarcinoma

Jerome Cros
Dpt of Pathology
Beaujon Hospital – Université de Paris
jerome.cros@aphp.fr

