

15-16 February 2021

COMETH Training course

From omics data

to tumor heterogeneity quantification

EIT Health is supported by the EIT,
a body of the European Union



15 February 2021

Heterogeneity in cancer

Does it matter?

The example of pancreatic adenocarcinoma

Jerome Cros

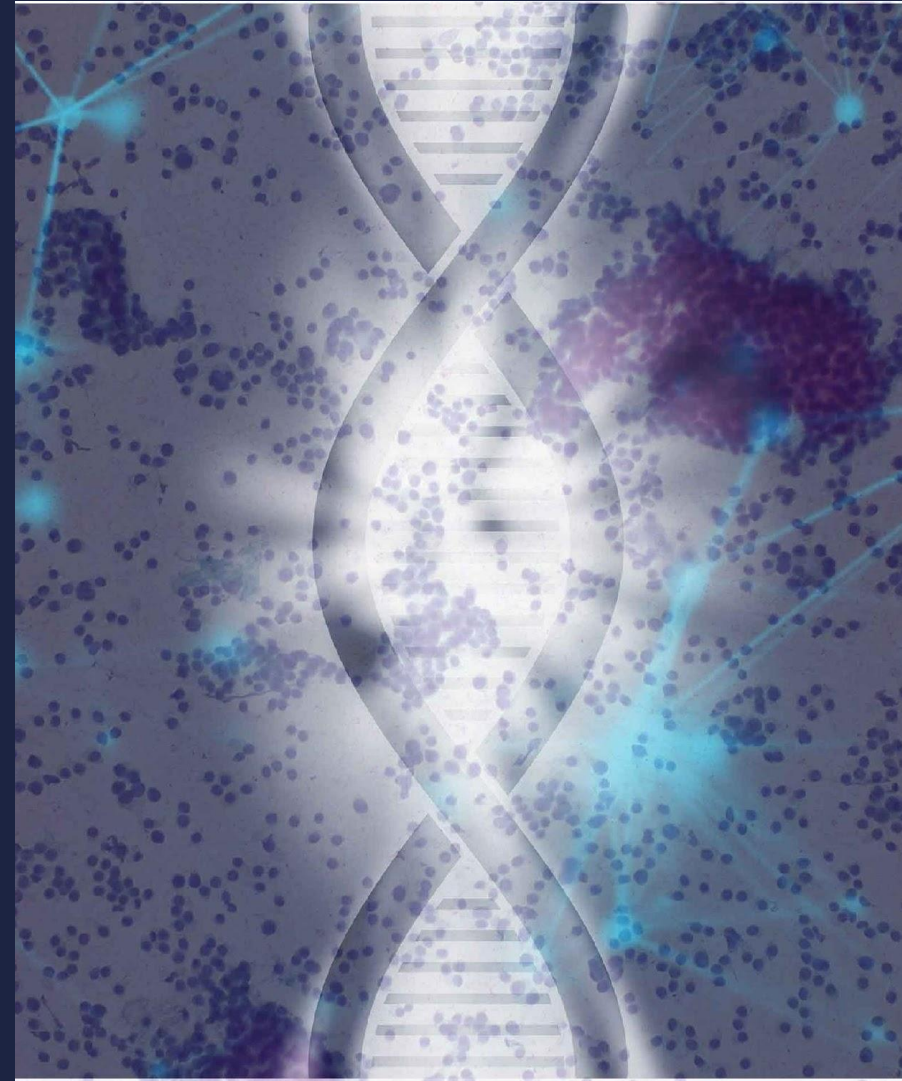
Dpt of Pathology

Beaujon Hospital – Université de Paris

jerome.cros@aphp.fr



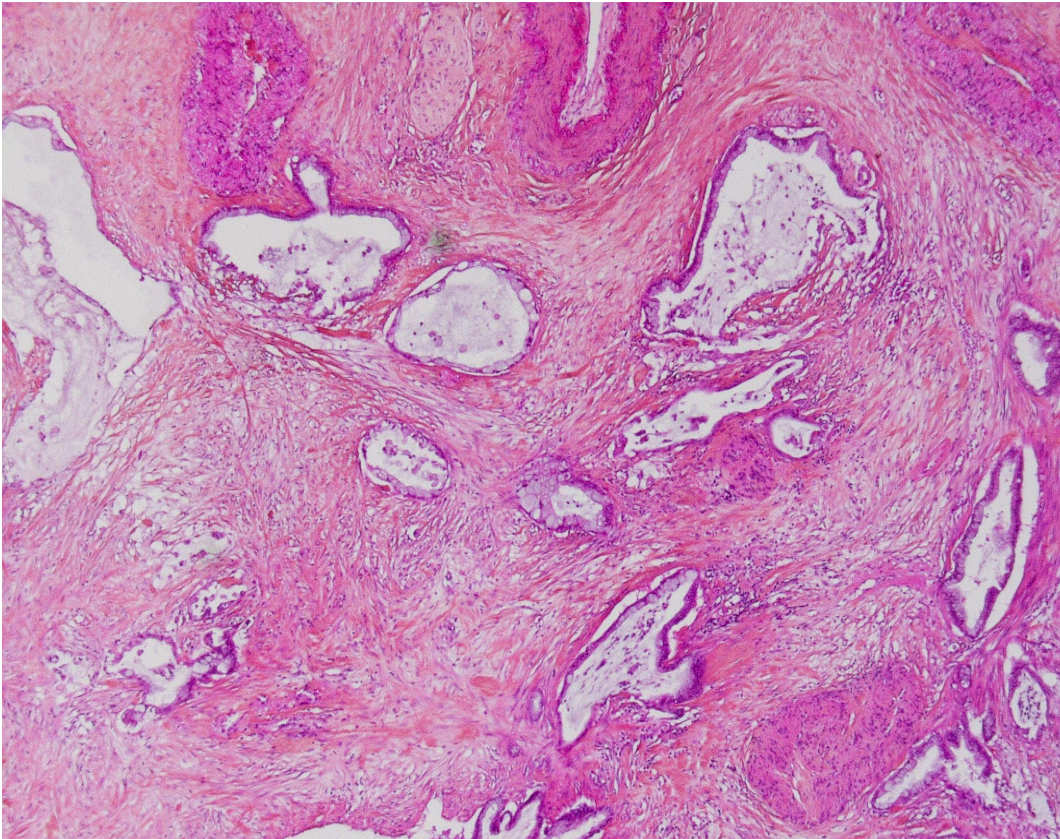
Study of tumor heterogeneity: Issues with samples....



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

Pancreatic cancer

Adenocarcinoma (90%)

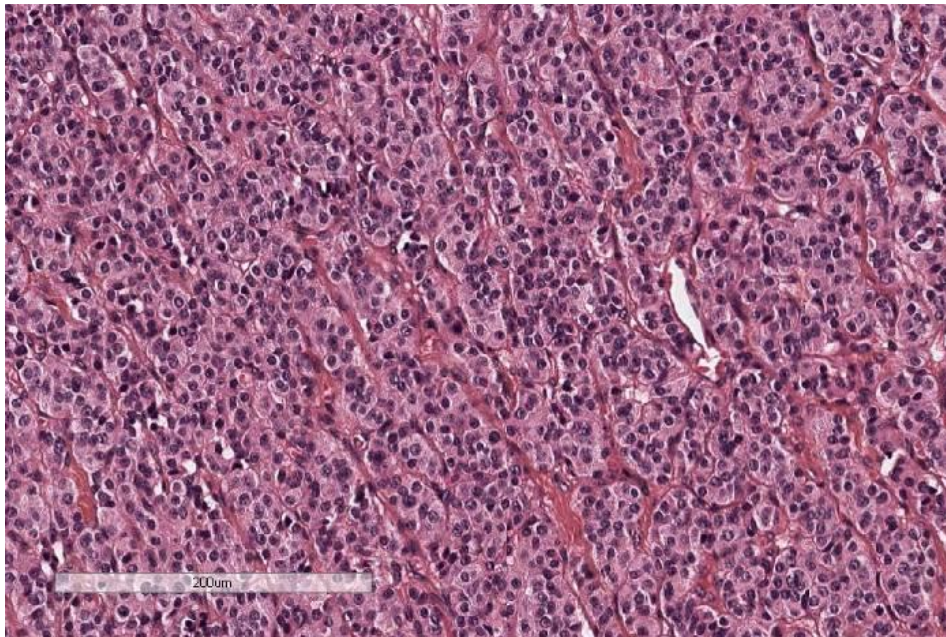


Pancreatic cancer

Adenocarcinoma (90%)

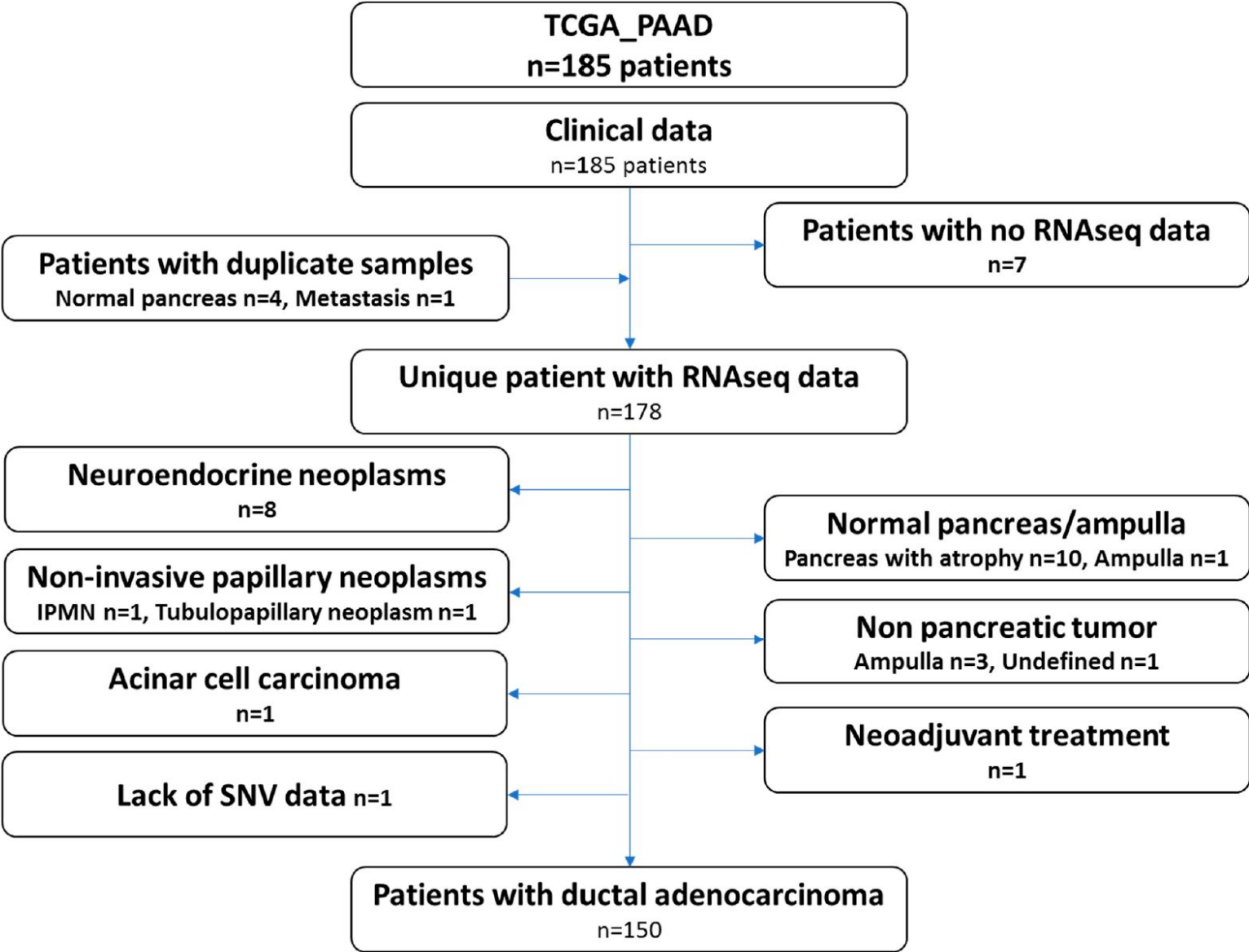
Neuroendocrine
lesions

Rare lesions
Acinar cell
carcinoma....



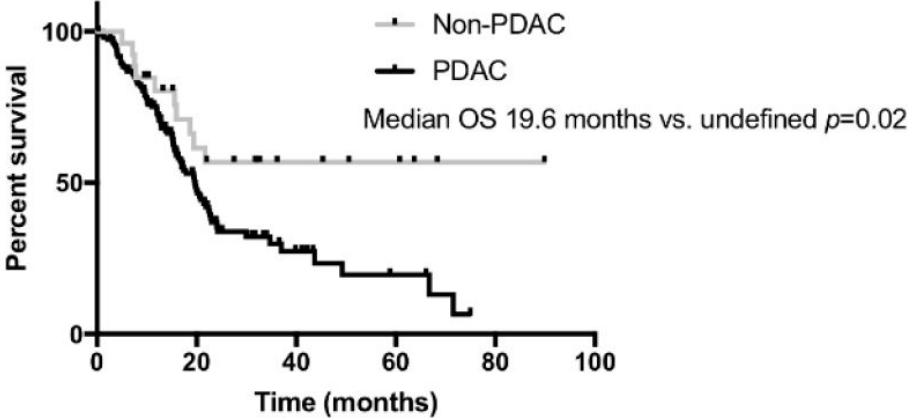
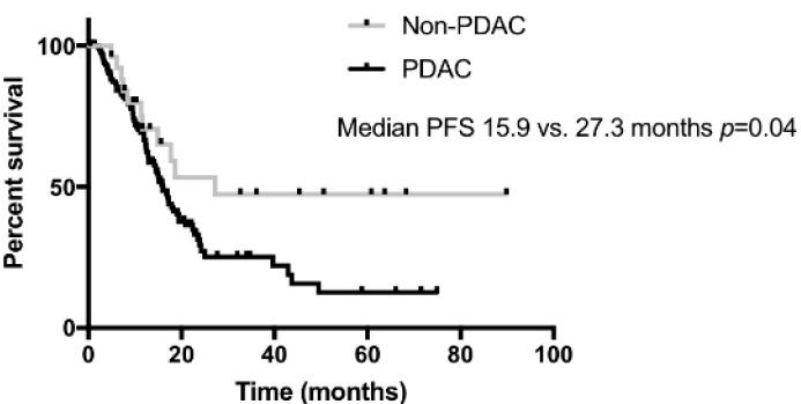
Completely different
morphology, biology....

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

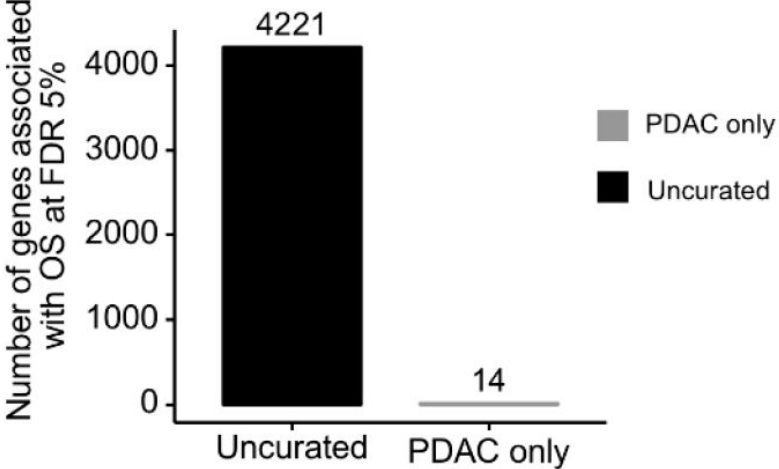
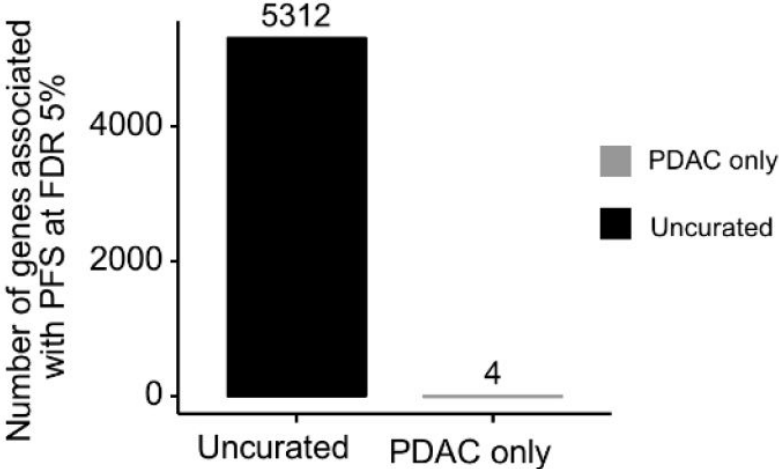


1. Beware of miss labeled samples when using public datasets+++++

a

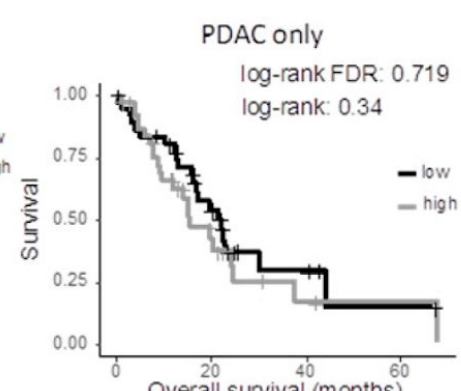
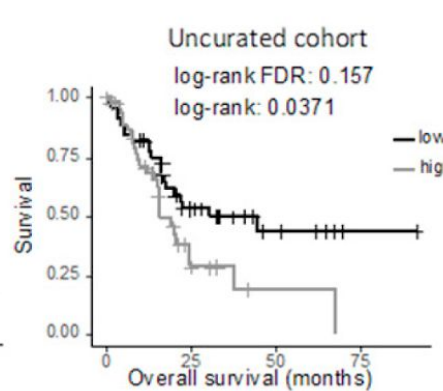
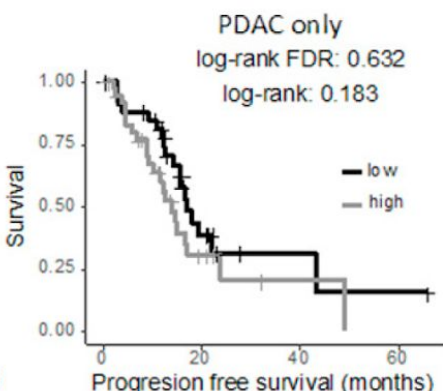
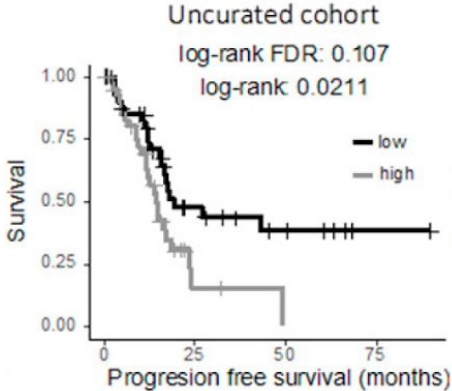
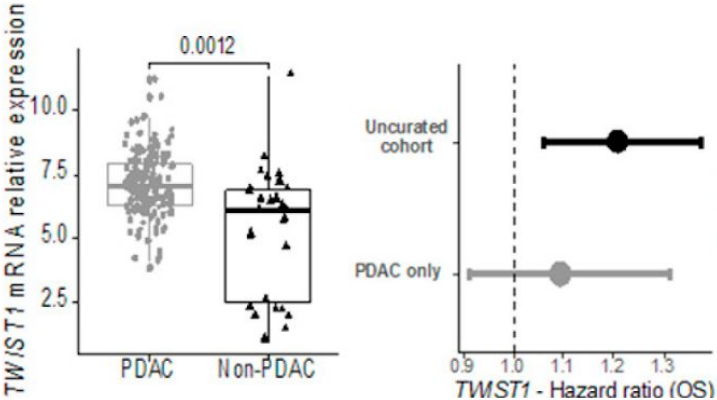


b



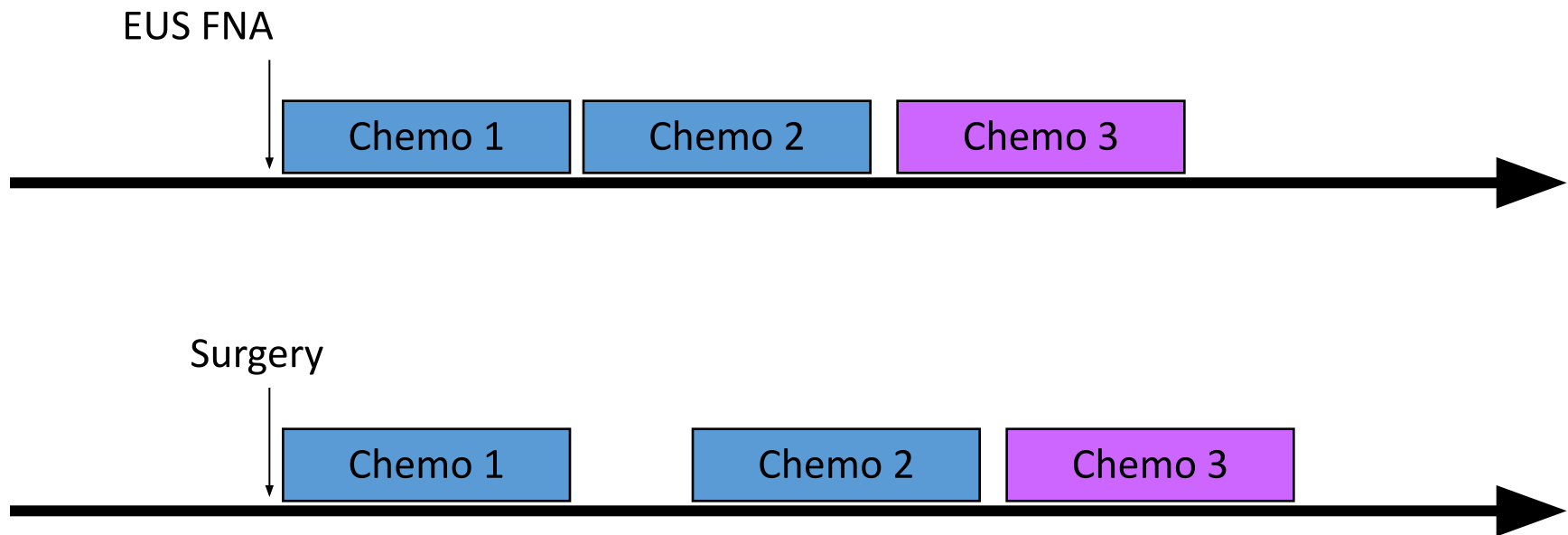
1. Beware of miss labeled samples when using public datasets+++++

a



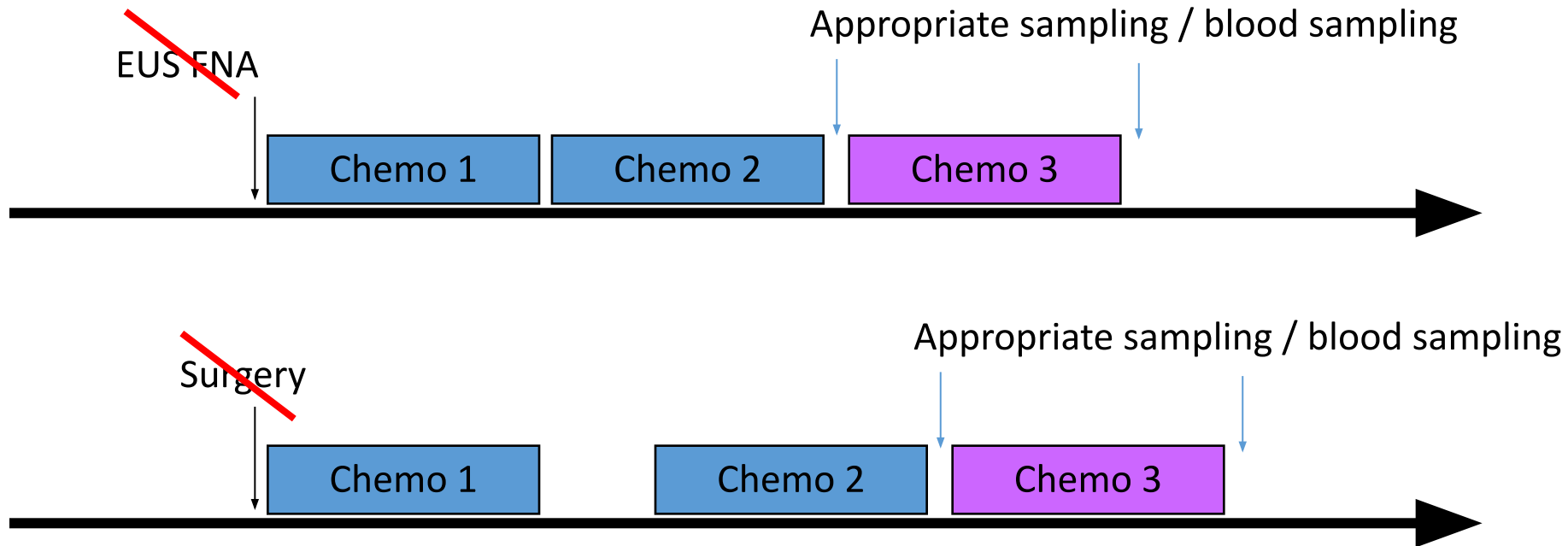
Many publications with the whole dataset... *Nat genet, Clin can res...*

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



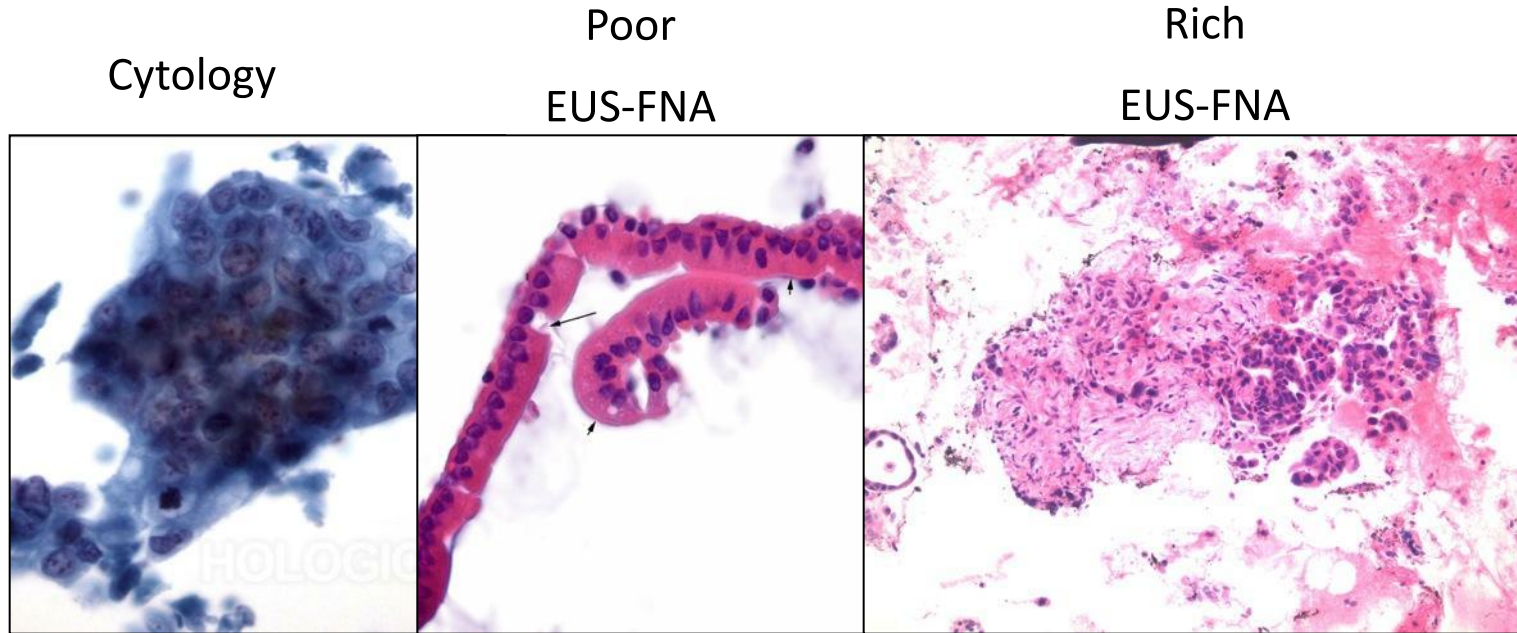
- Clonal selection induced by multiple therapies

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?

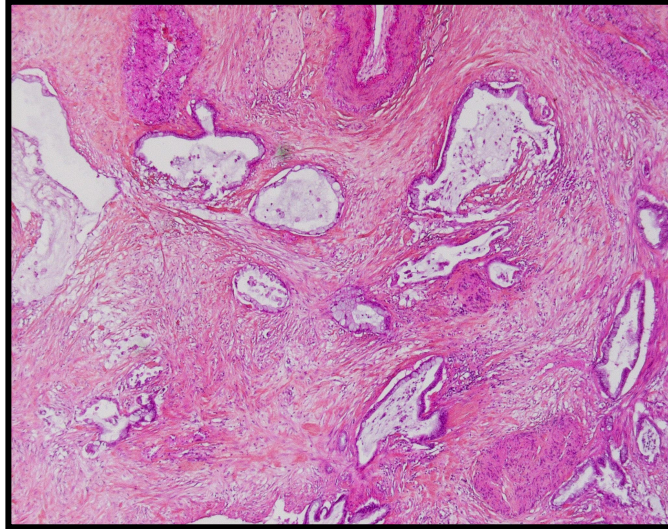


Protein on Tum cells	OK	OK	OK
Mutation on Tum cell	+/- OK	++/- OK	OK
Exp (mi)ARN	+/- OK (richness)	+/- OK (richness)	+/- OK (richness)
Protein in stroma	NON	NON	+/- OK

Liver biopsy+++ , true cut +++

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

Surgical specimen

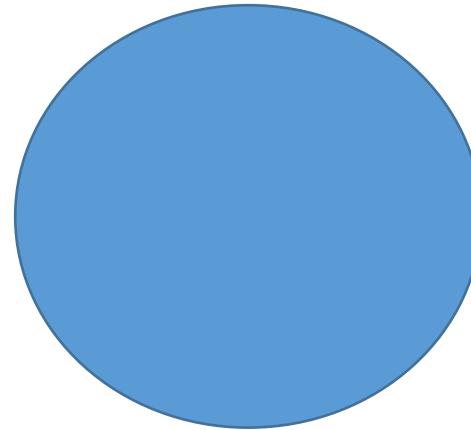


Tumor
+/- sphere
3cm diameter

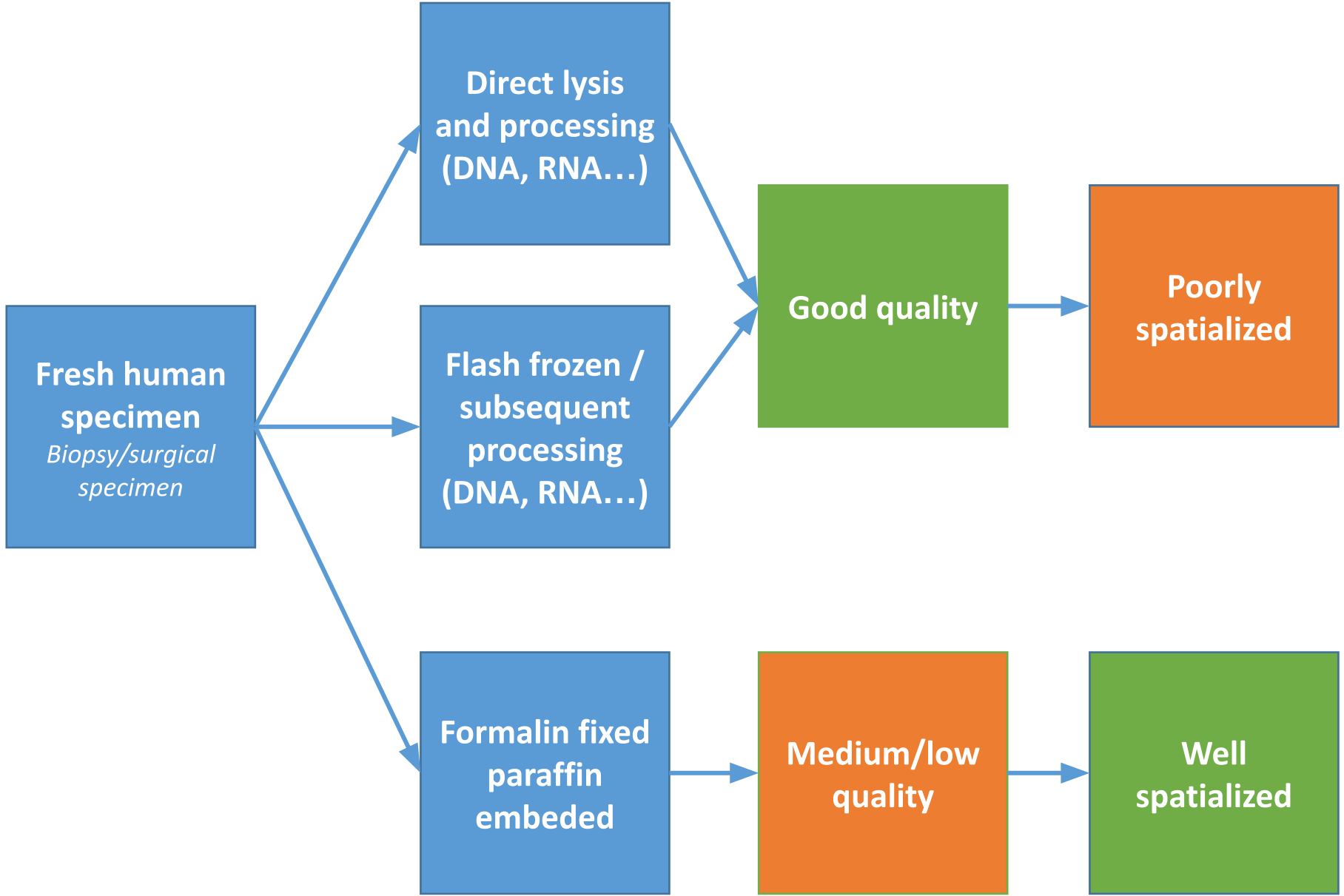
Frozen carrot
0.6cm wide
0.4 cm thick

14cm³

0.11cm³



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



Fresh human specimen
Biopsy/surgical specimen

Direct lysis and processing (DNA, RNA...)

Flash frozen / subsequent processing (DNA, RNA...)

Formalin fixed paraffin embedded

Good quality

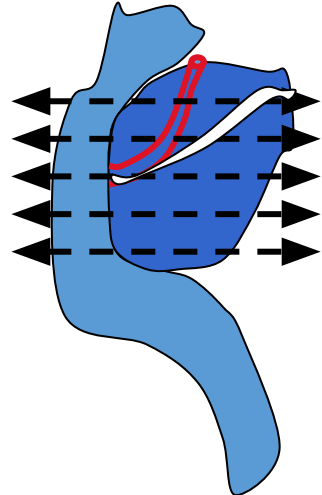
Medium/low quality

Poorly spatialized

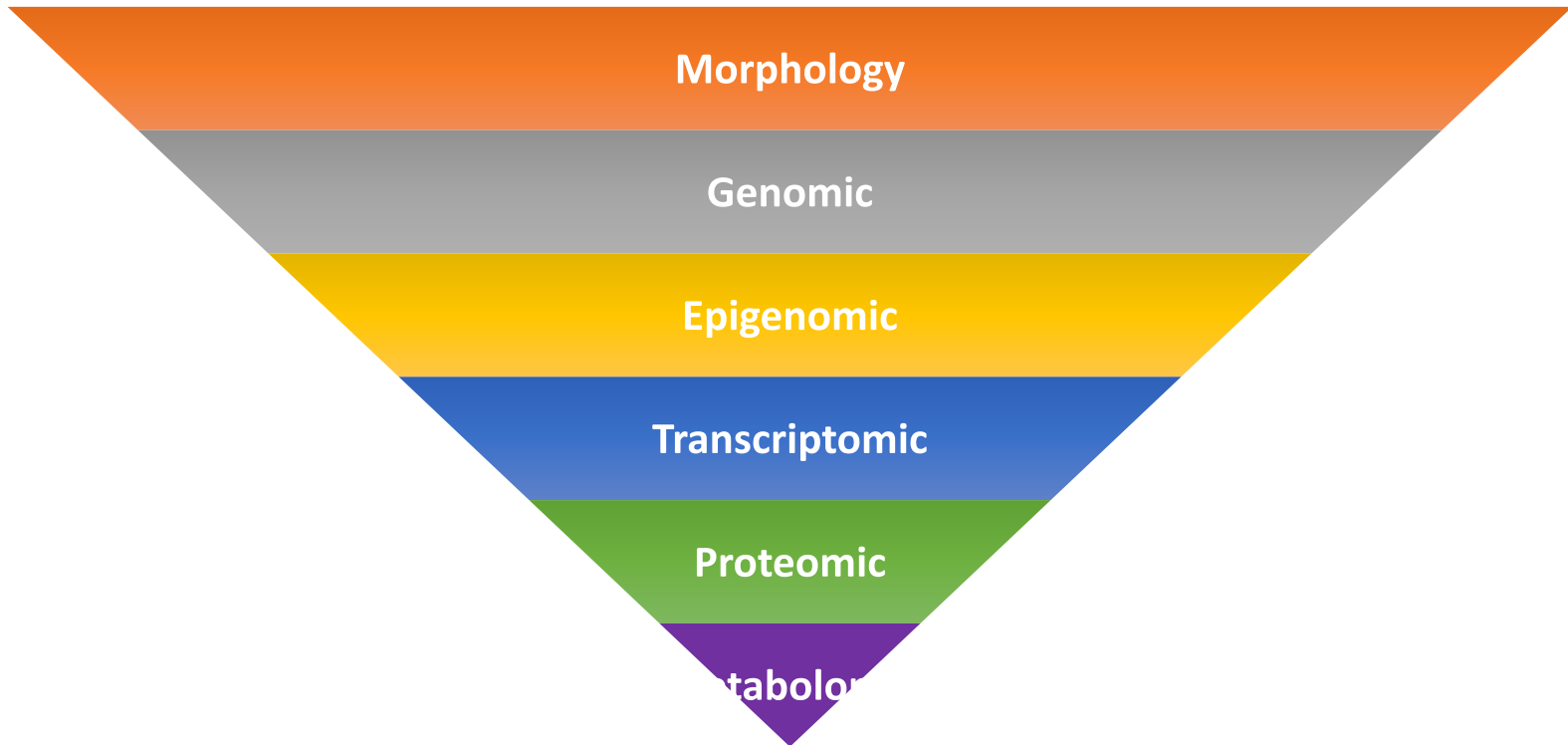
Well spatialized

Spatialized sampling...

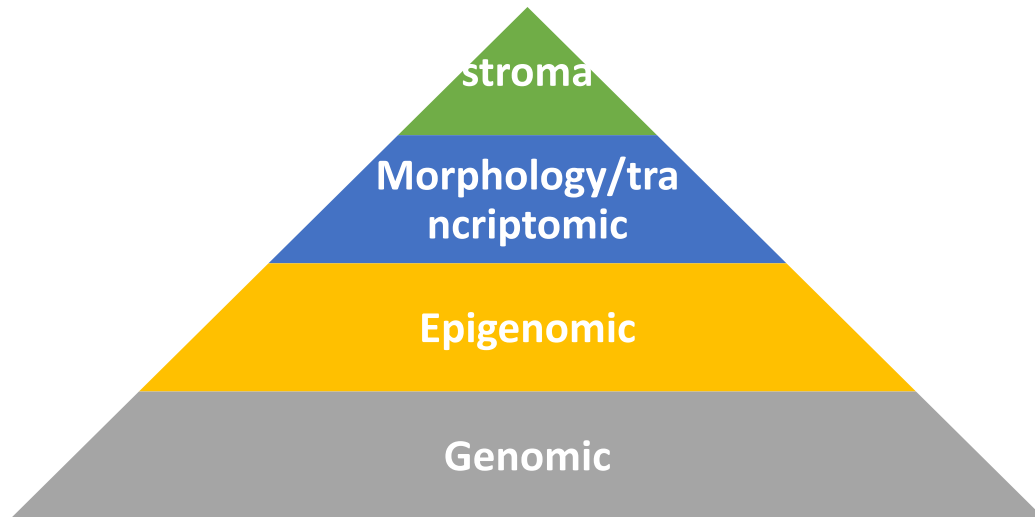
...yes but usually with formalin fixed paraffin embedded samples...

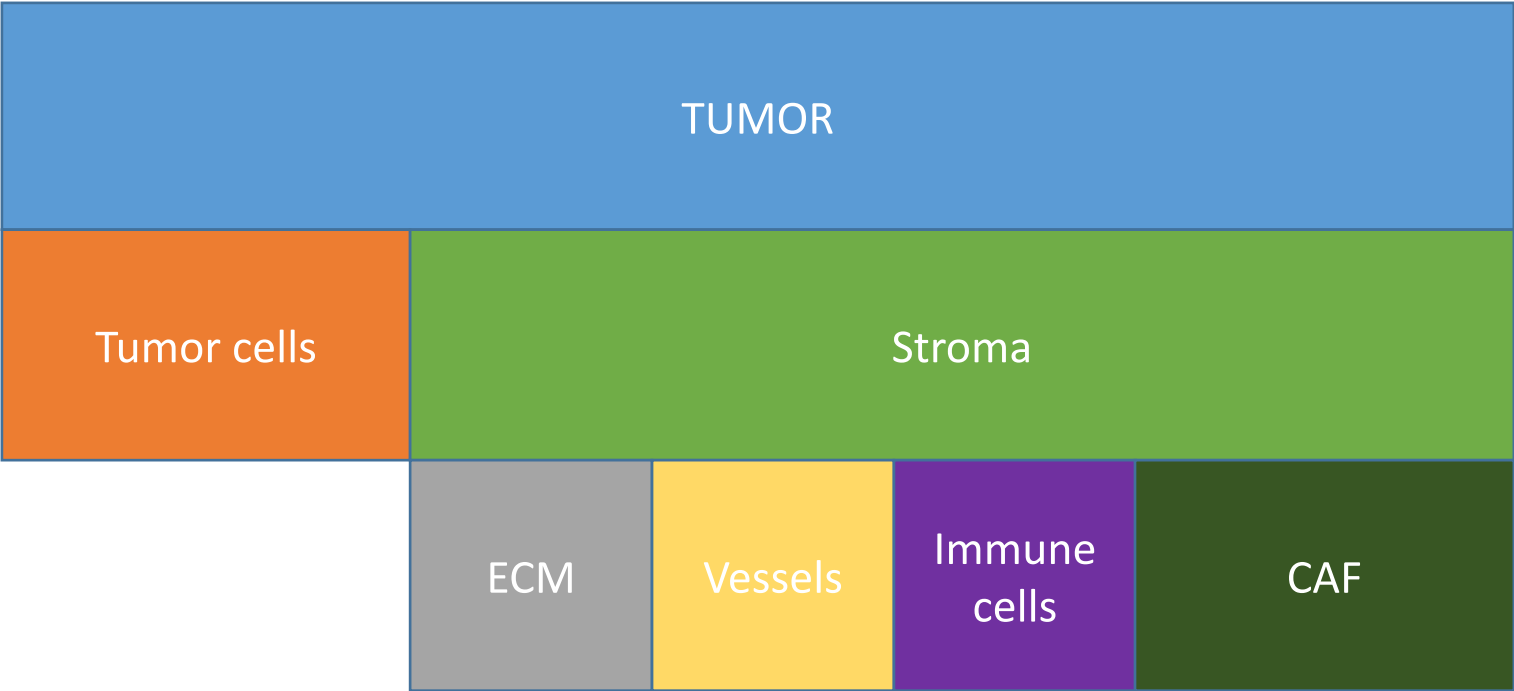


Inter-tumor heterogeneity



**Intratumor heterogeneity
and clonal evolution**

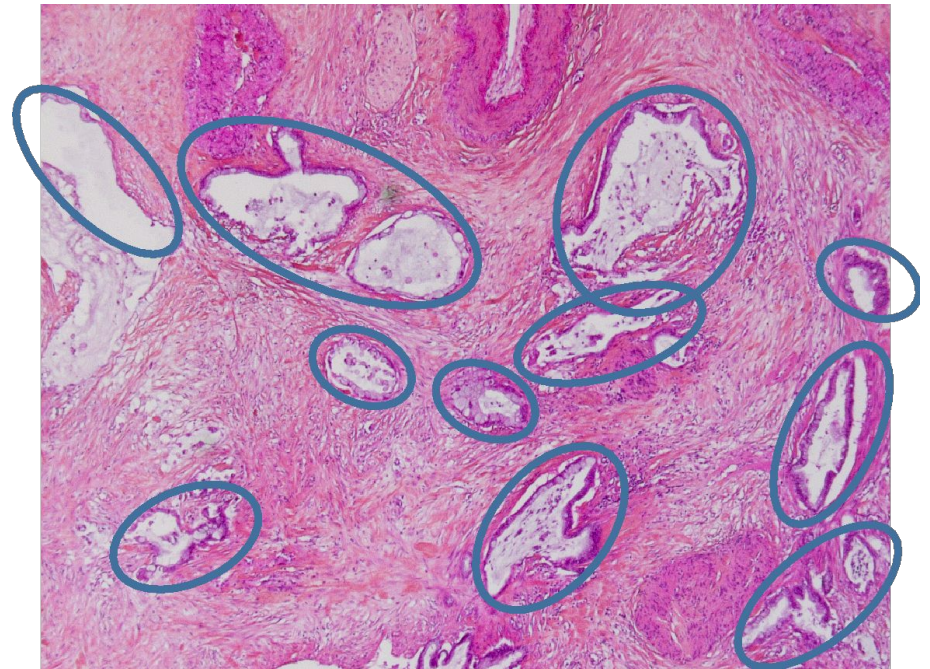




PANCREATIC ADENOCARCINOMA

Tumor cells

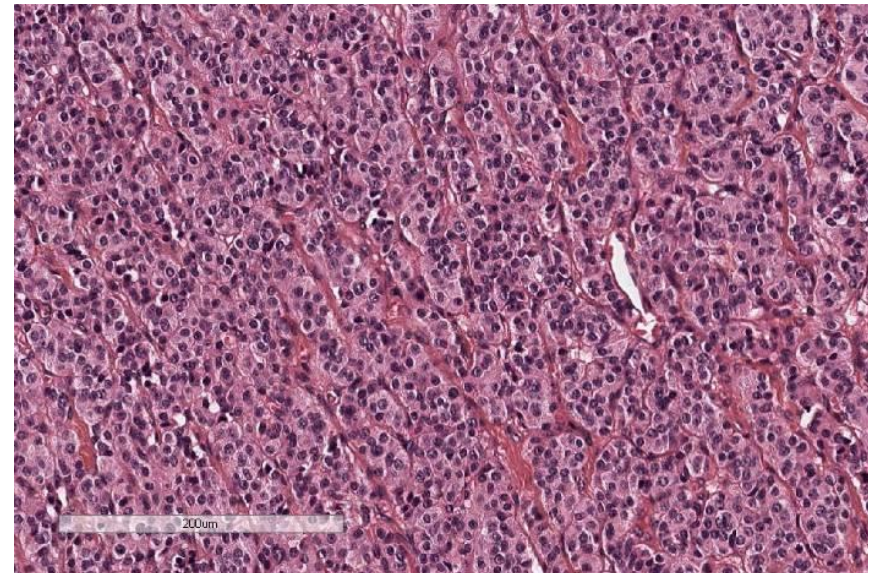
Stroma



PANCREATIC NEUROENDOCRINE TUMOR

Tumor cells

Stroma



TUMOR

Tumor cells

Stroma

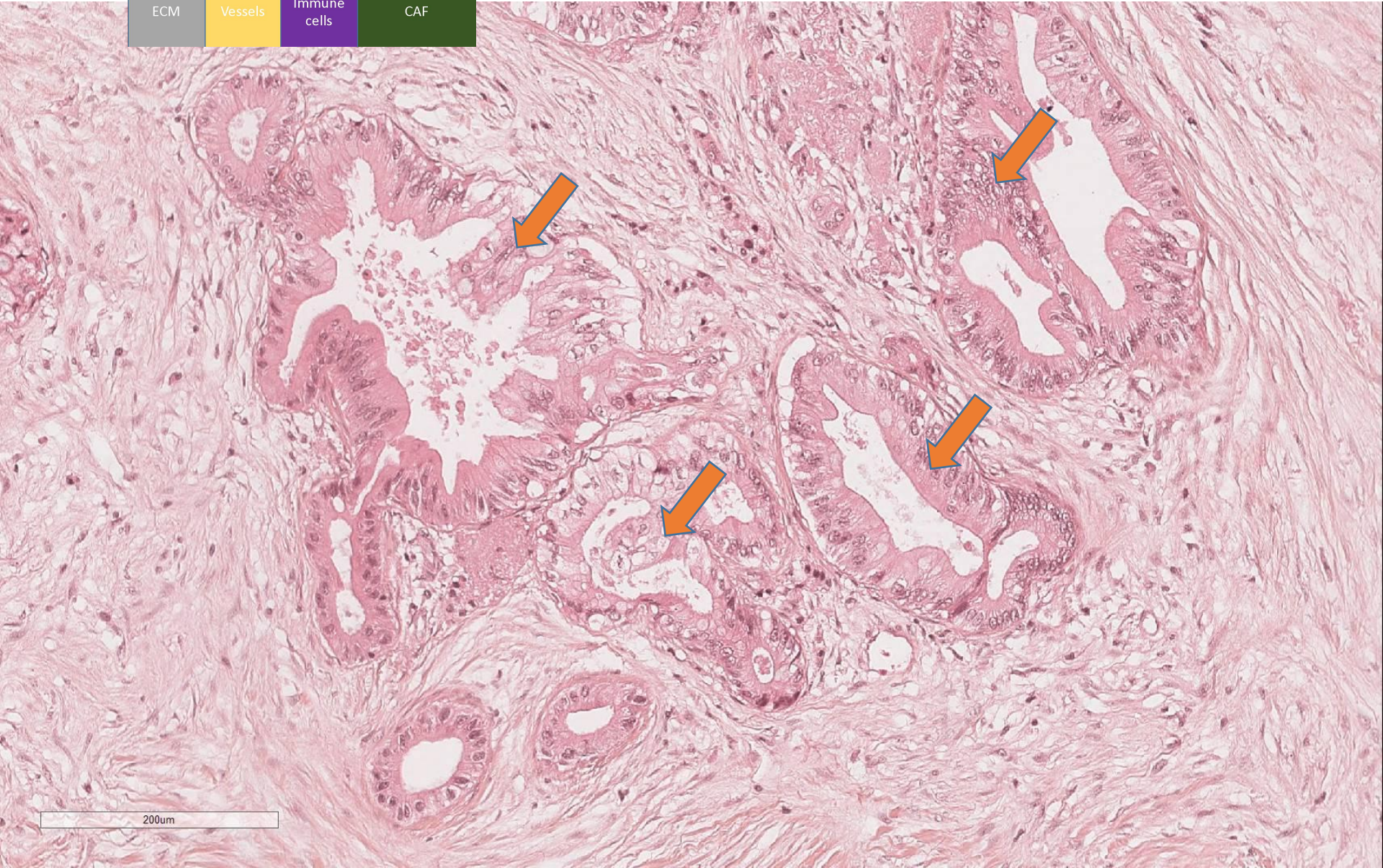
ECM

Vessels

Immune cells

CAF

Pancreatic adenocarcinoma



200um

TUMOR

Tumor cells

Stroma

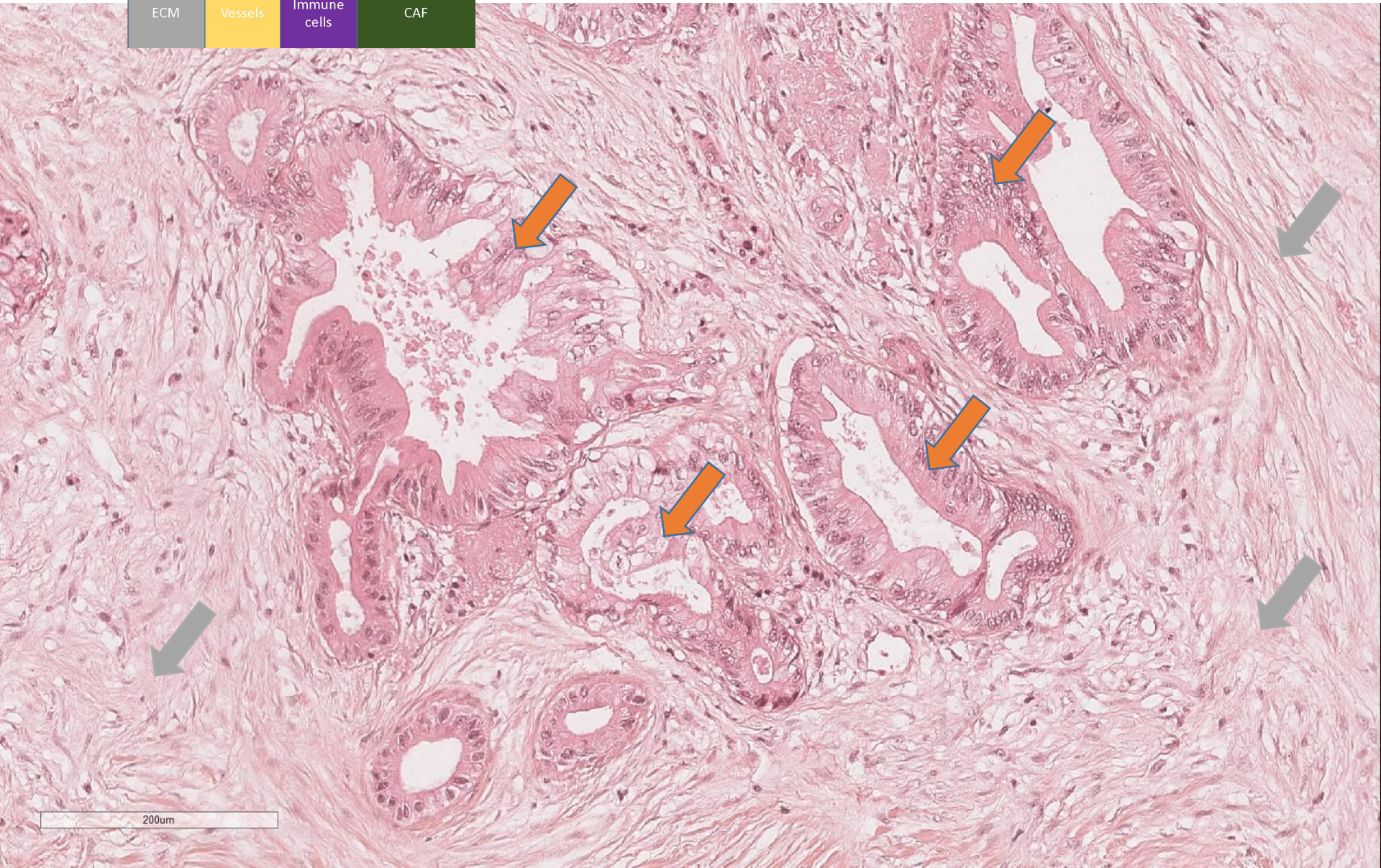
ECM

Vessels

Immune cells

CAF

Pancreatic adenocarcinoma



200um

TUMOR

Tumor cells

Stroma

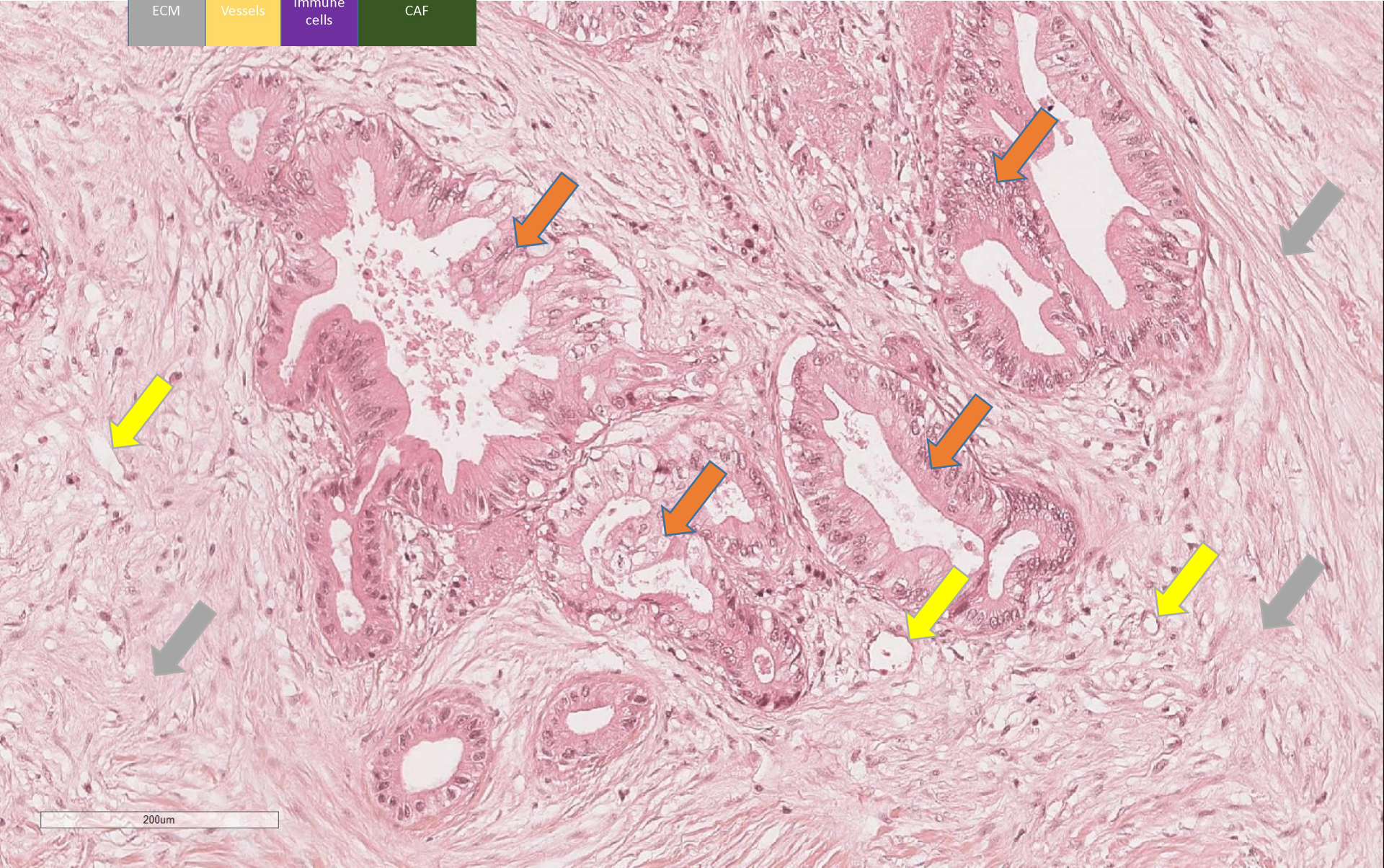
ECM

Vessels

Immune cells

CAF

Pancreatic adenocarcinoma



200um

TUMOR

Tumor cells

Stroma

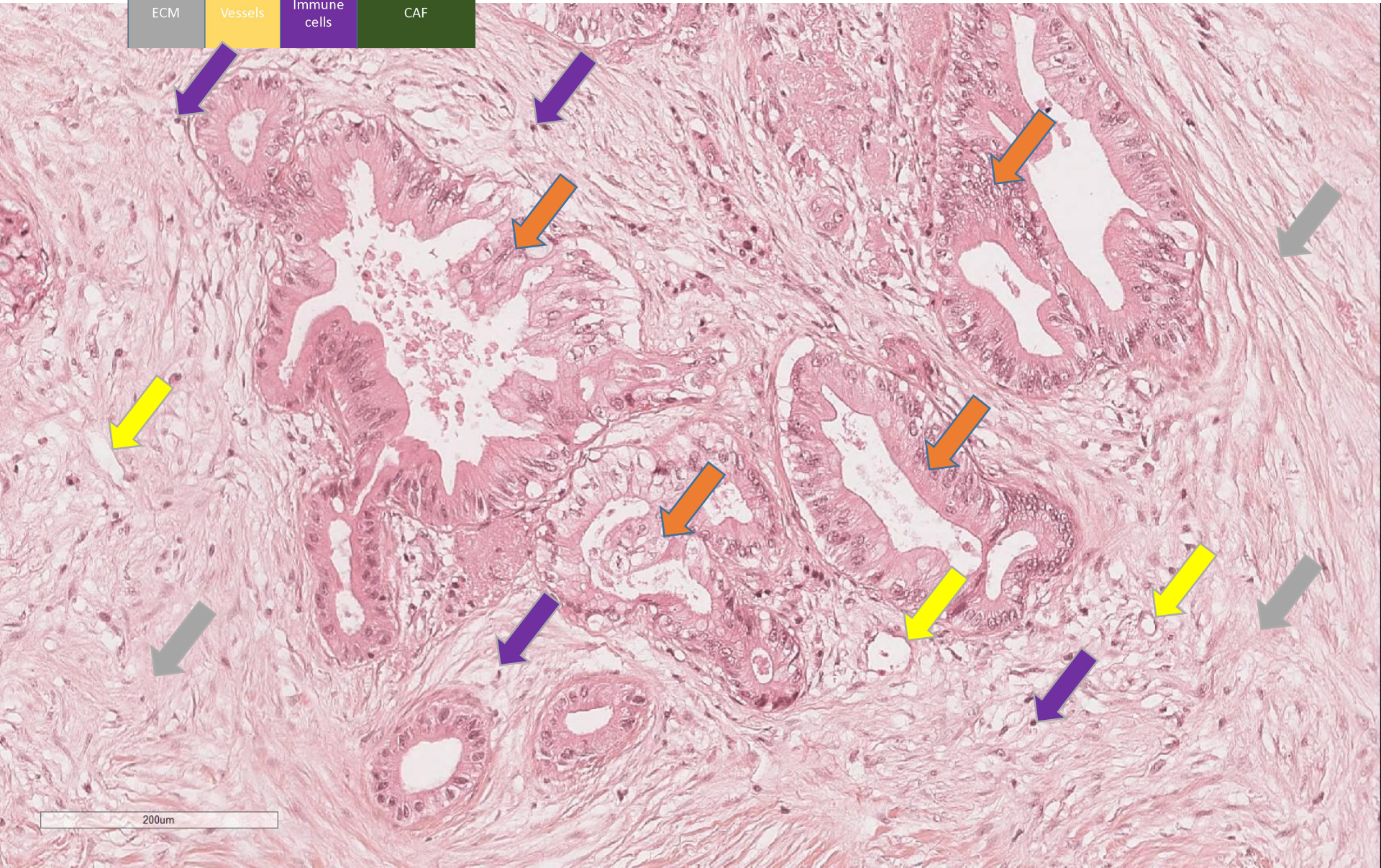
ECM

Vessels

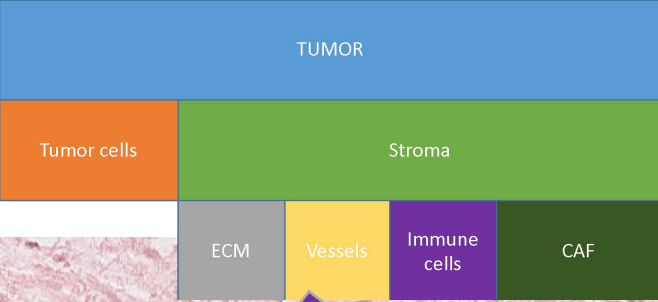
Immune cells

CAF

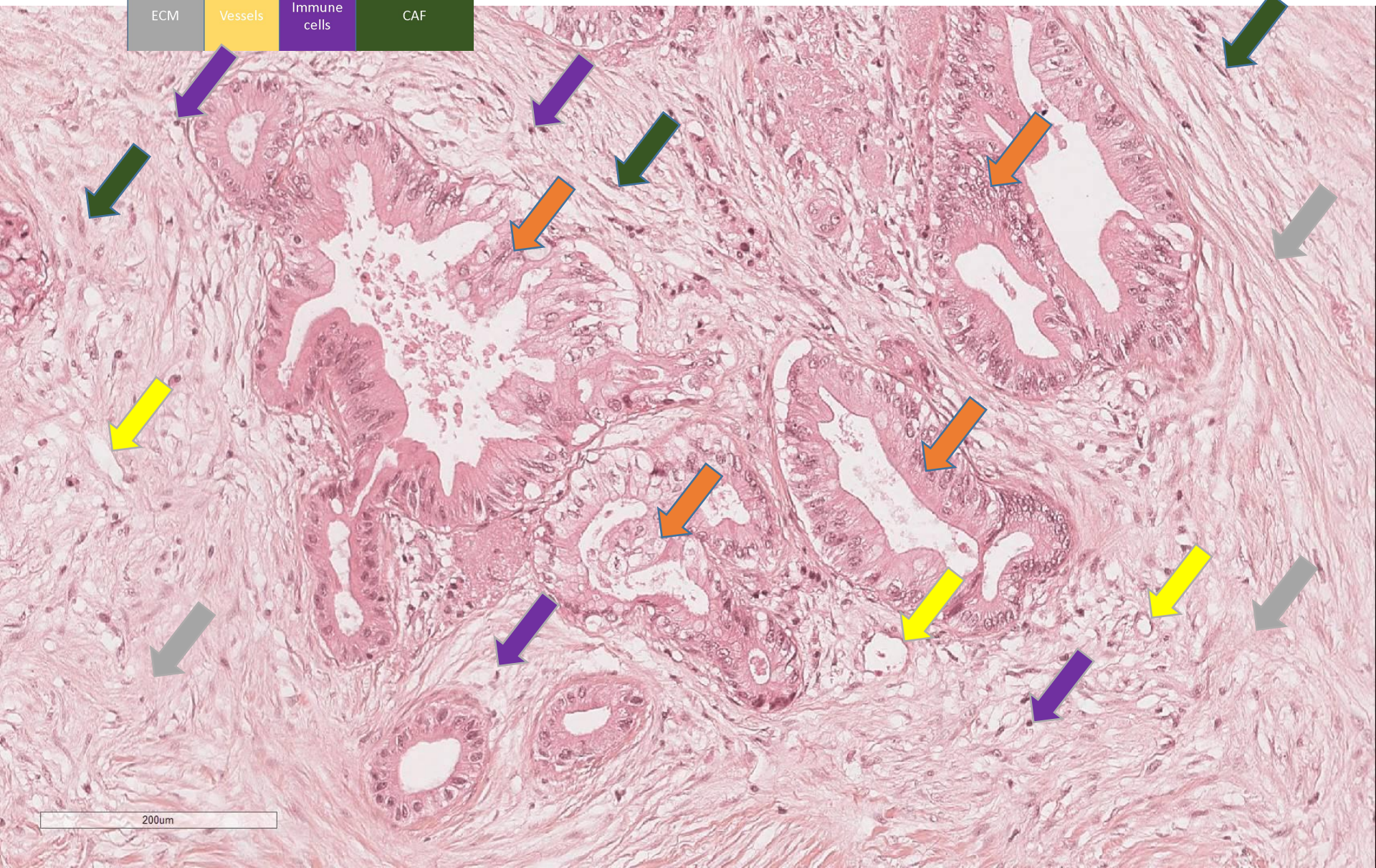
Pancreatic adenocarcinoma



200um

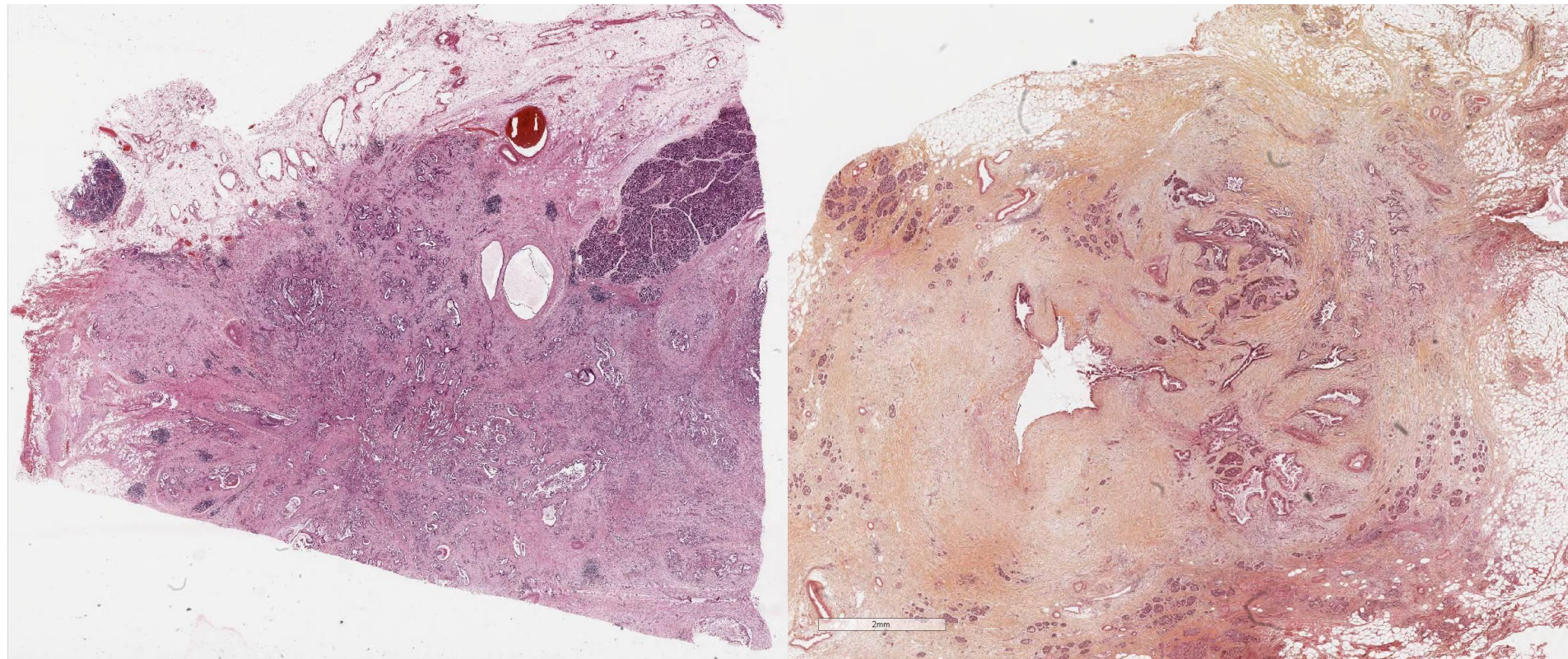


Pancreatic adenocarcinoma



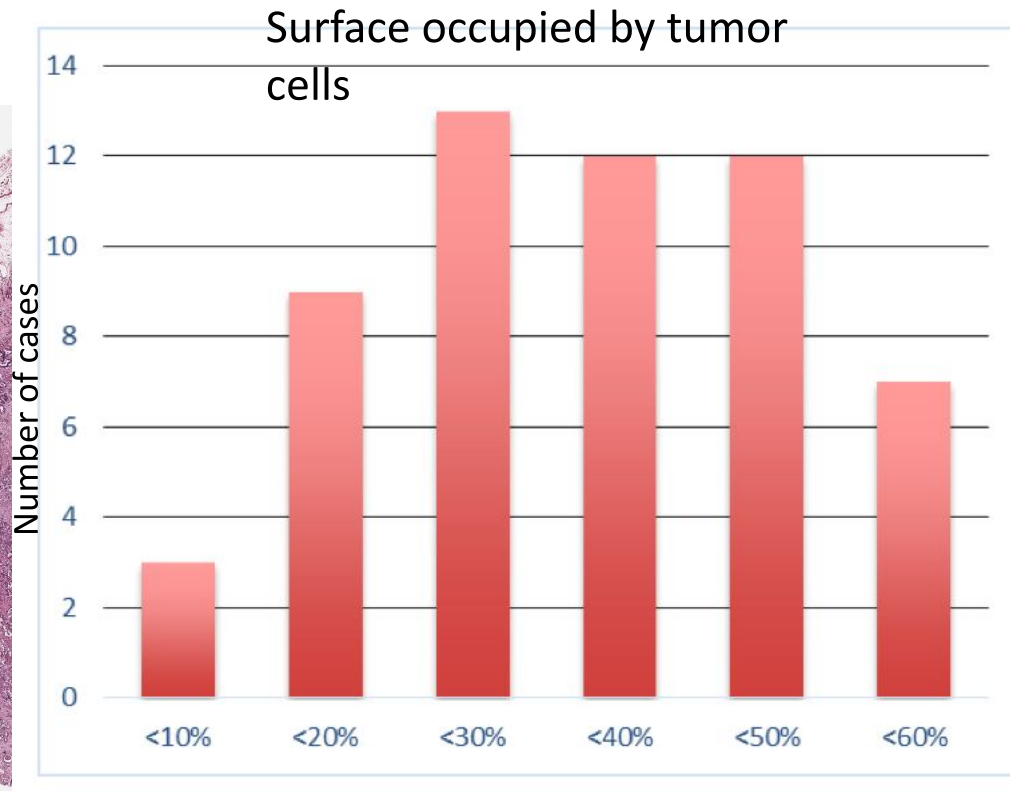
200um

Inter-tumor morphological heterogeneity

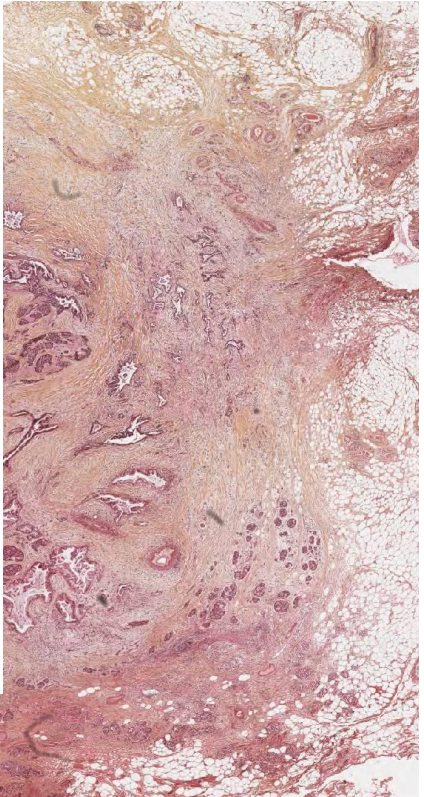
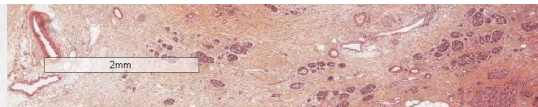
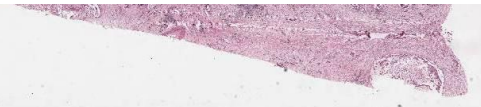
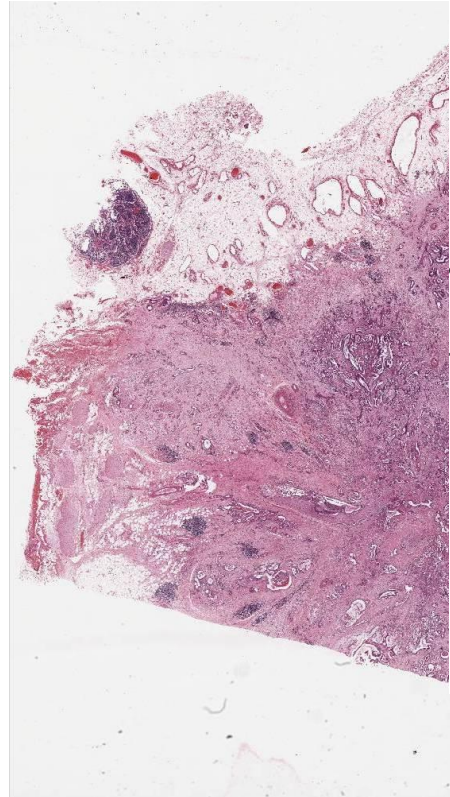


Heterogeneity of the tumor cell – stroma ratio

Inter-tumor morphological heterogeneity

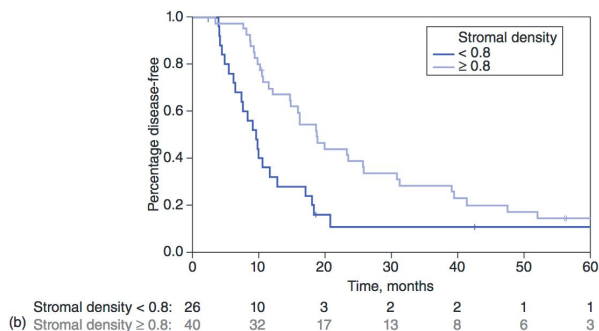
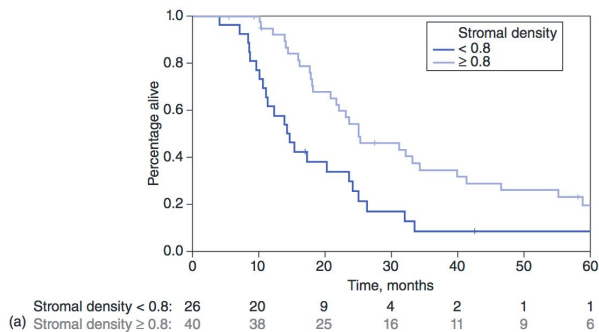


Heterogeneity of the tumor cells – stroma ratio

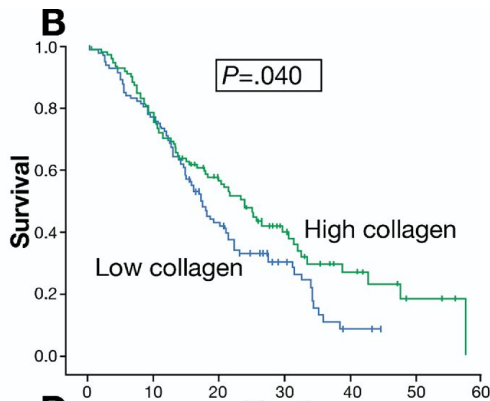


Inter-tumor morphological heterogeneity

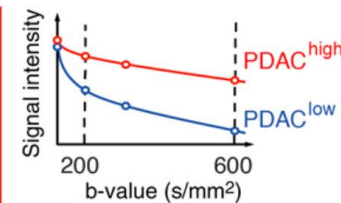
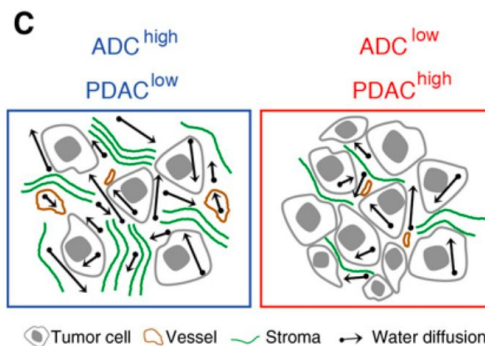
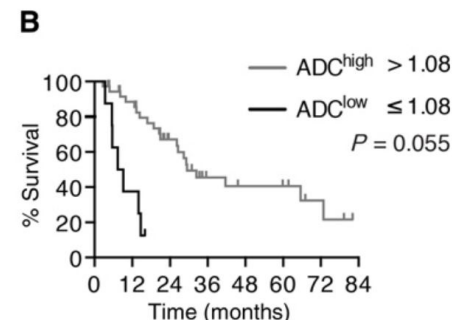
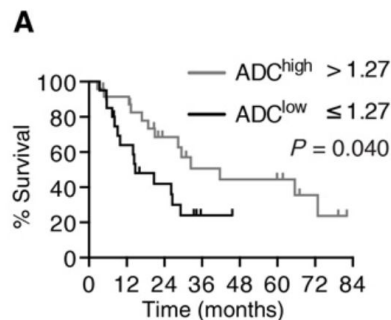
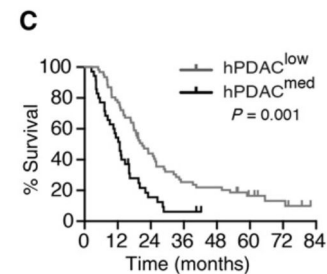
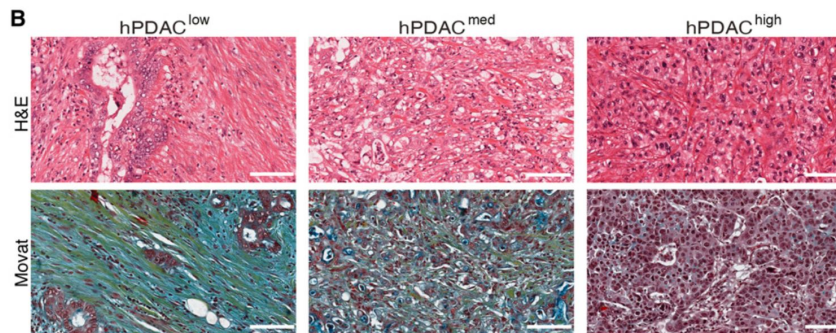
Heterogeneity = opportunity for biomarker development



Bever et al. HPB 2015, 292



Erkan et al. Clin gas hep 2008

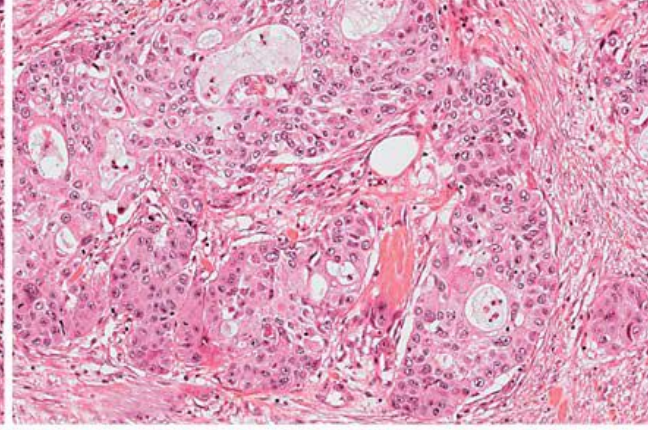
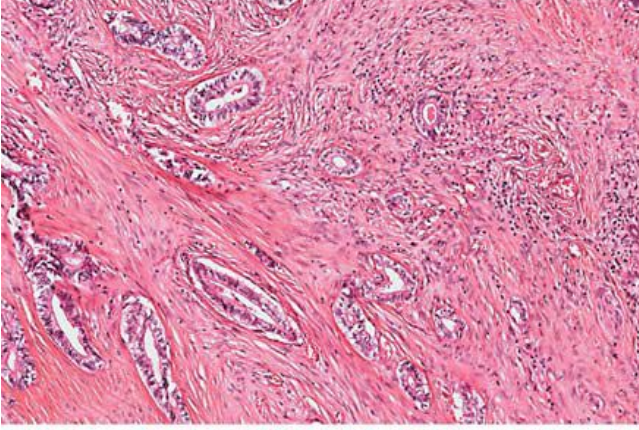


Heid et al. Clin can res 2017, 1461

Small sample (<100 pts)

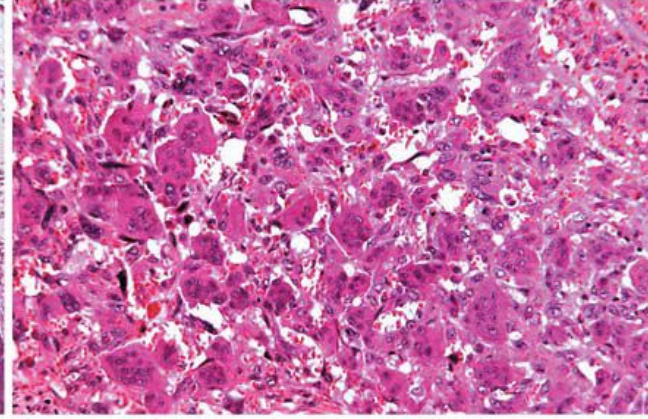
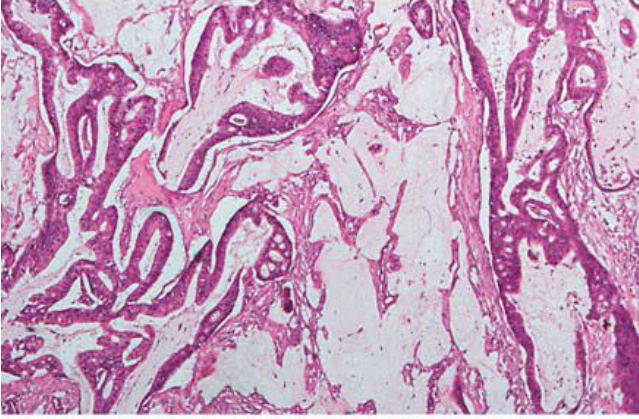
Inter-tumor morphological heterogeneity

ductal



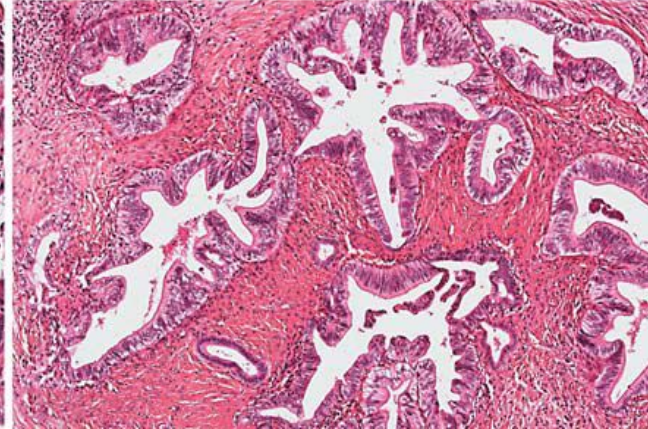
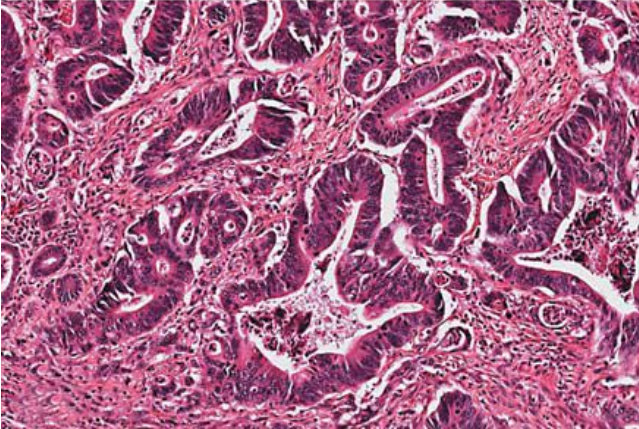
adenosquamous

colloid



Undifferentiated w/
giant cells osteoclast-like

intestinal



micropapillary

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*

Inter-tumor morphological heterogeneity

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More frequent *MYC amplification*?



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

No major difference

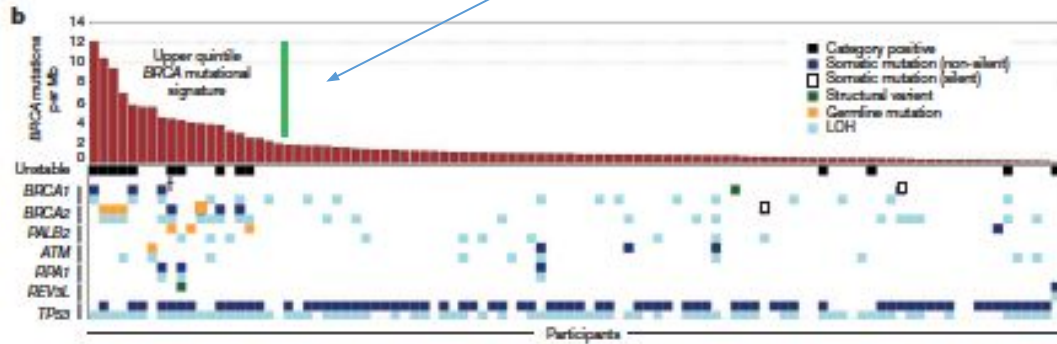
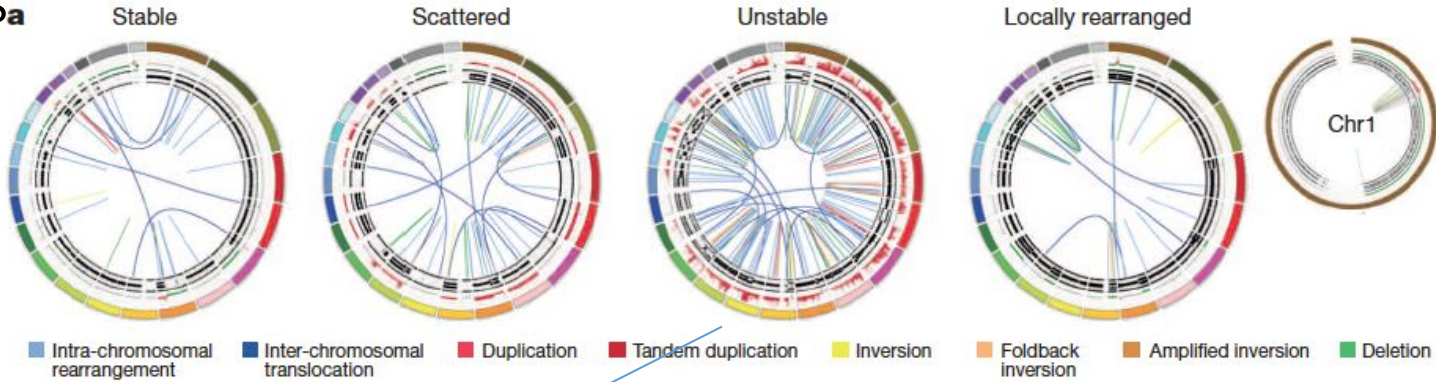
Genomic

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



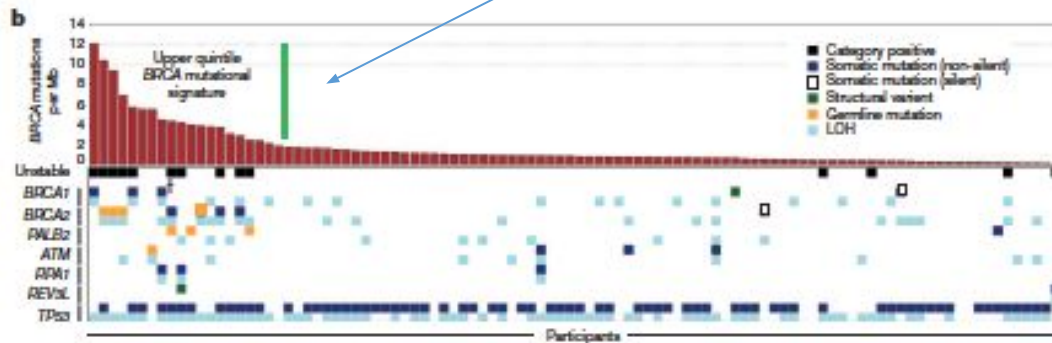
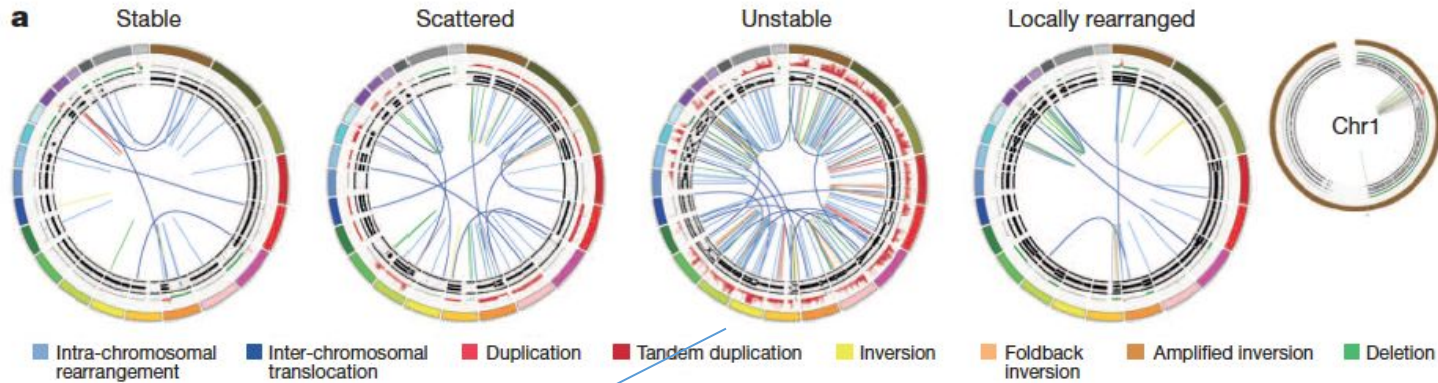
WG

Sa



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

Genomic



Benefit from PARPi in maintenance therapy (POLO trial)

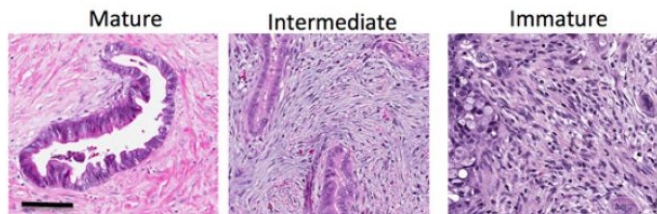
Signature	Discovery (n)	Replication (n)	ESPAC (n)	Population (%; 95% CI)
Age-Related	115*	59	NA	69.9% (64.2-75.6%)
DSBR, total	17**	10	NA	10.8% (6.98-14.7%)
DSBR, germline	9	2	NA	4.42% (1.9-6.97%)
DSBR, somatic	2	2	NA	1.6% (0.045-3.2%)
DSBR, occult	6	6	NA	4.8% (2.2-7.5%)
MMR	4	0	6	1.7% (0.65-2.7%)
Signature 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

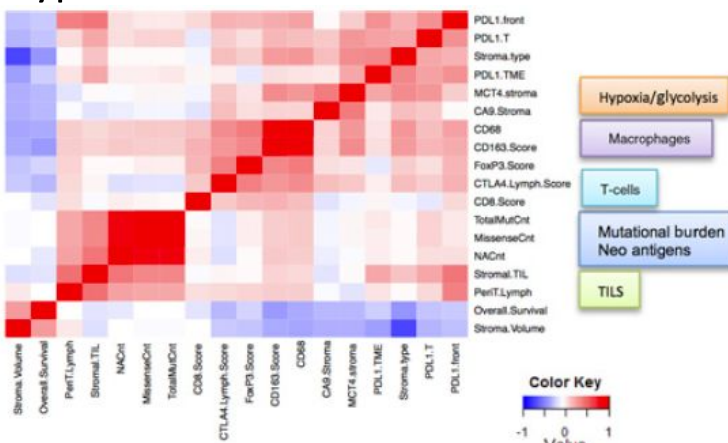
** there are 18 tumours from 17 cases in the DSBR discovery group

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

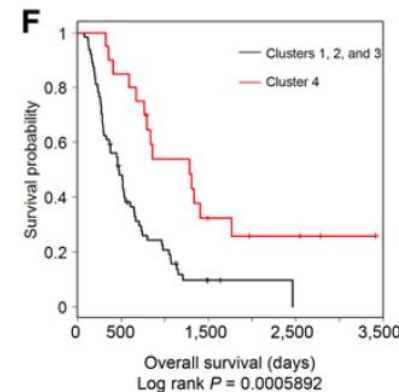
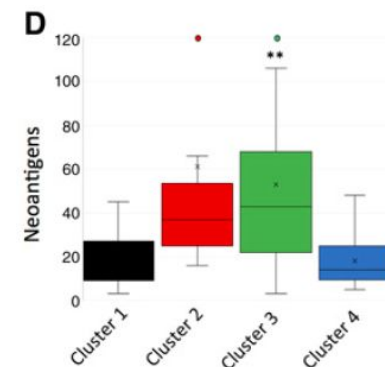
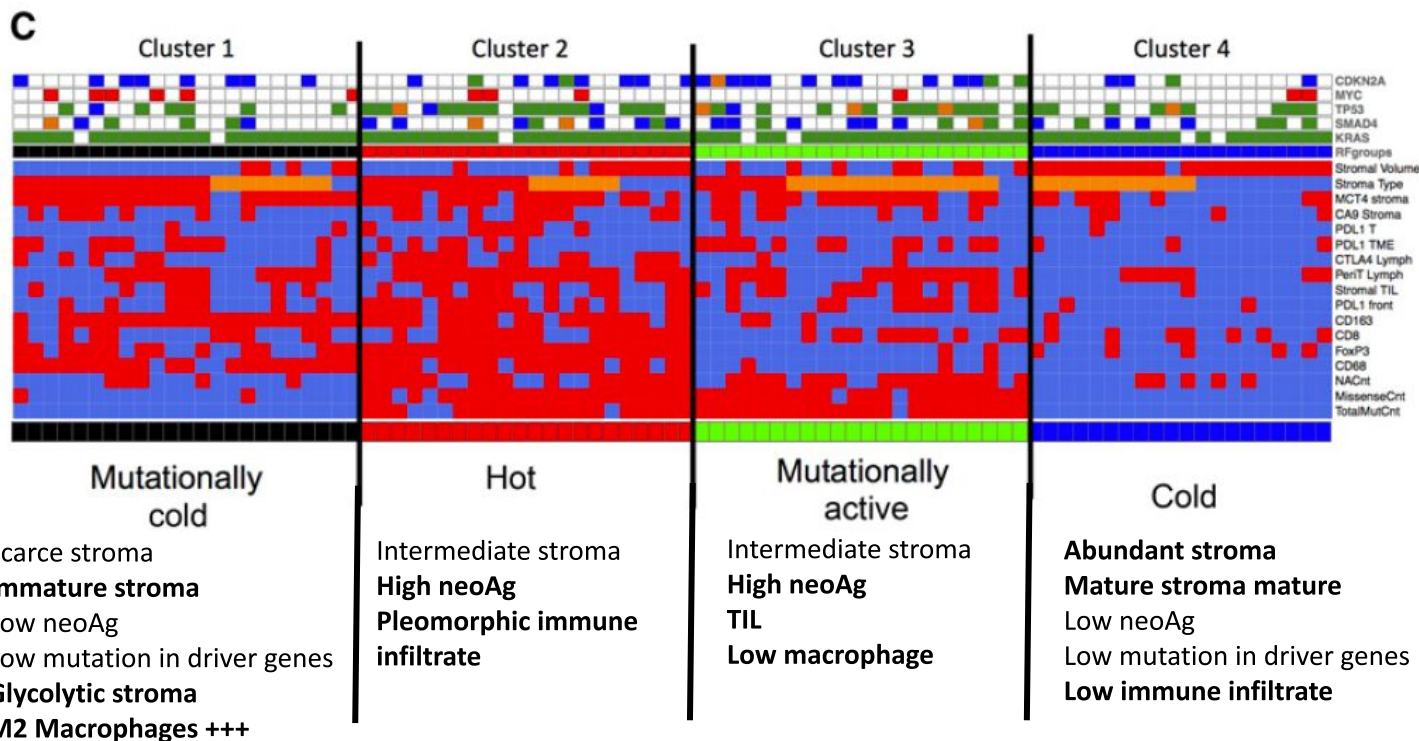
Type of stroma



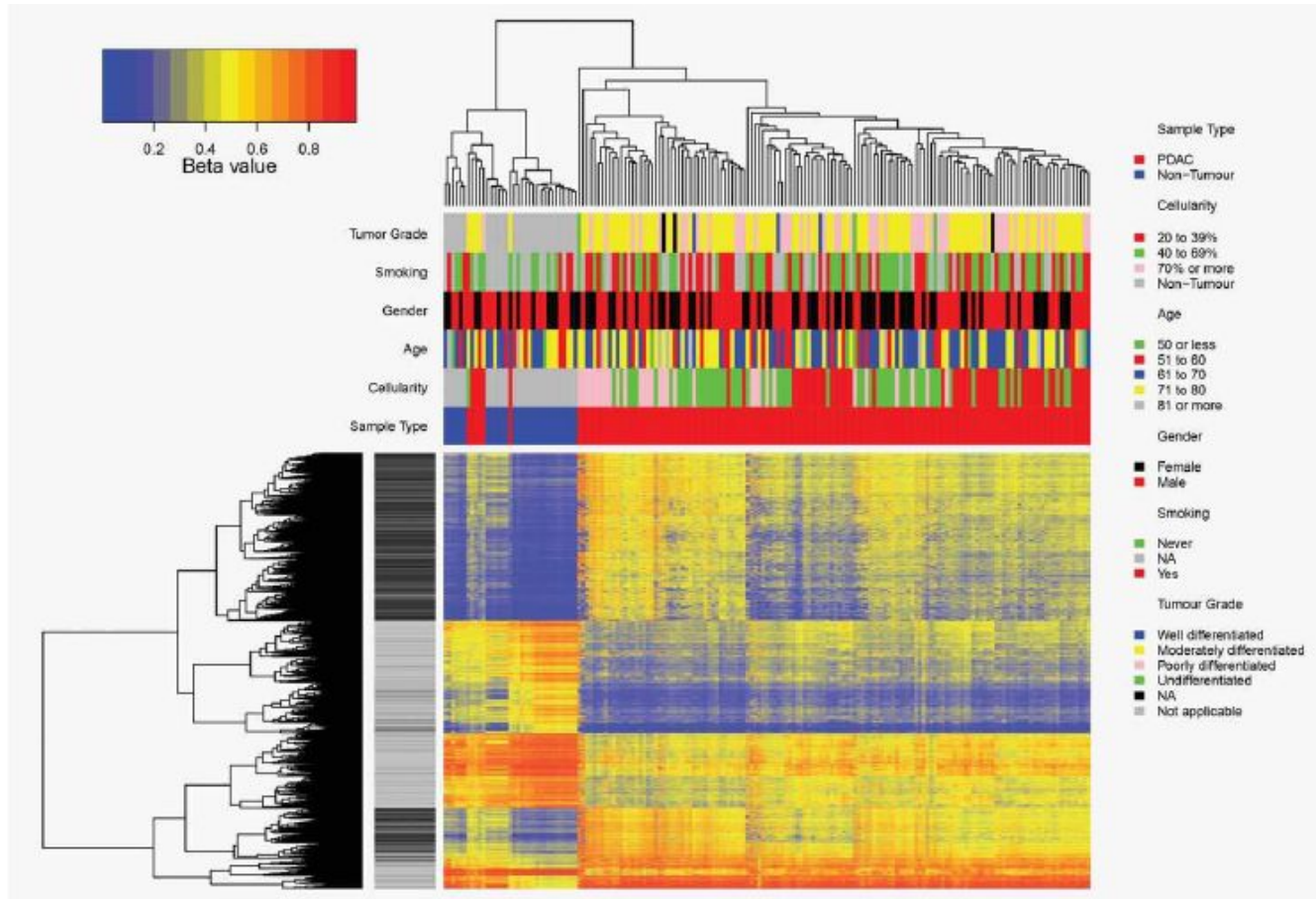
Type of immune infiltrate



+
Mutational profile (WES)



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

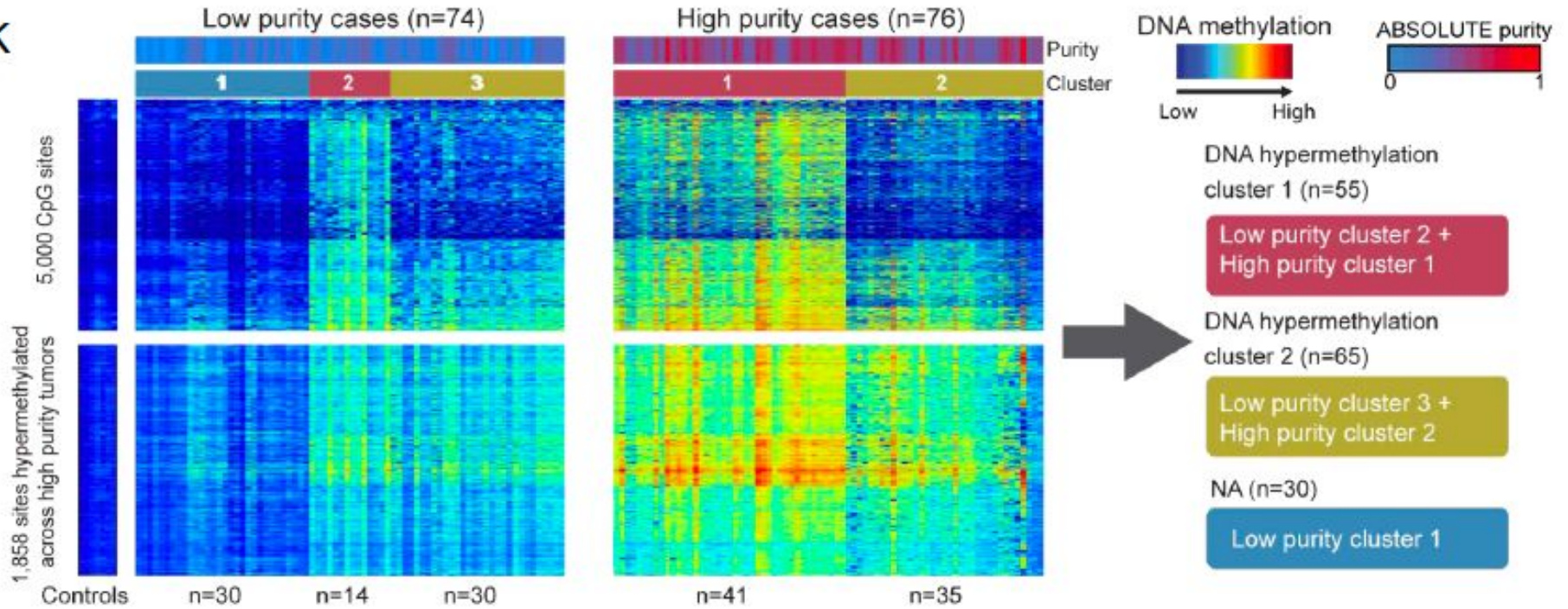


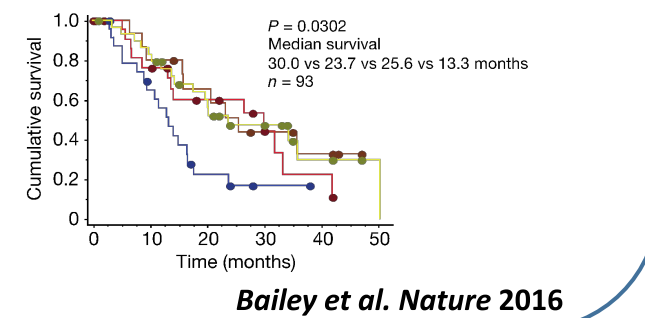
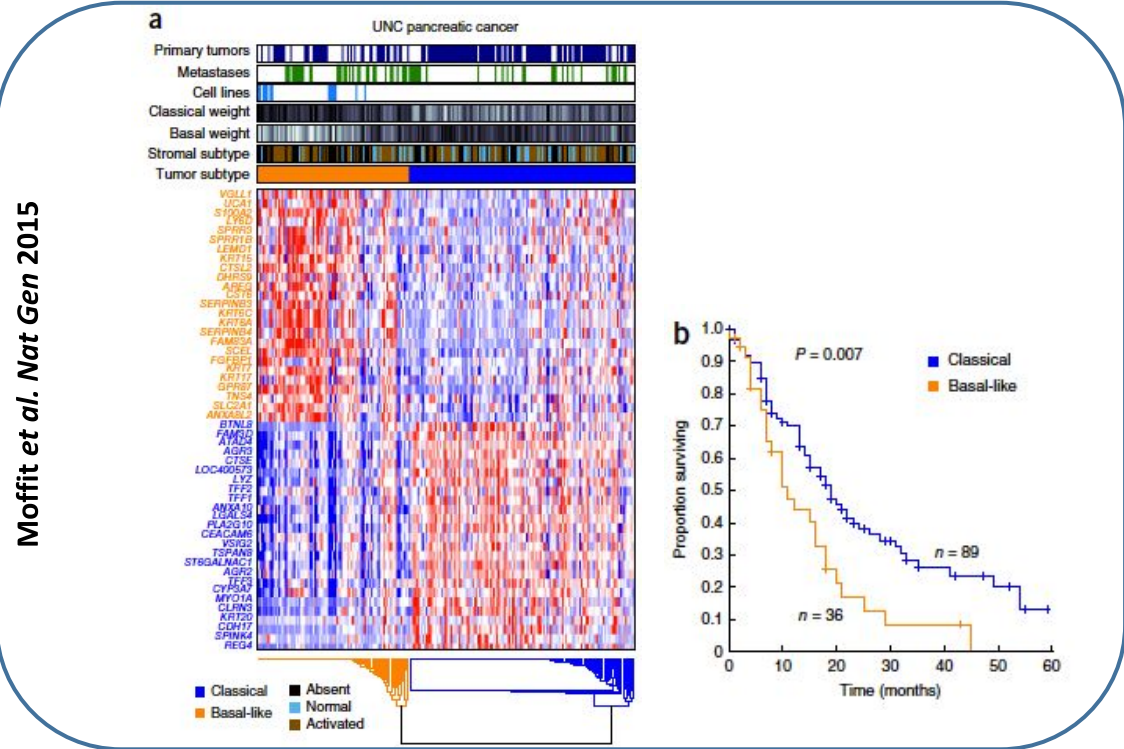
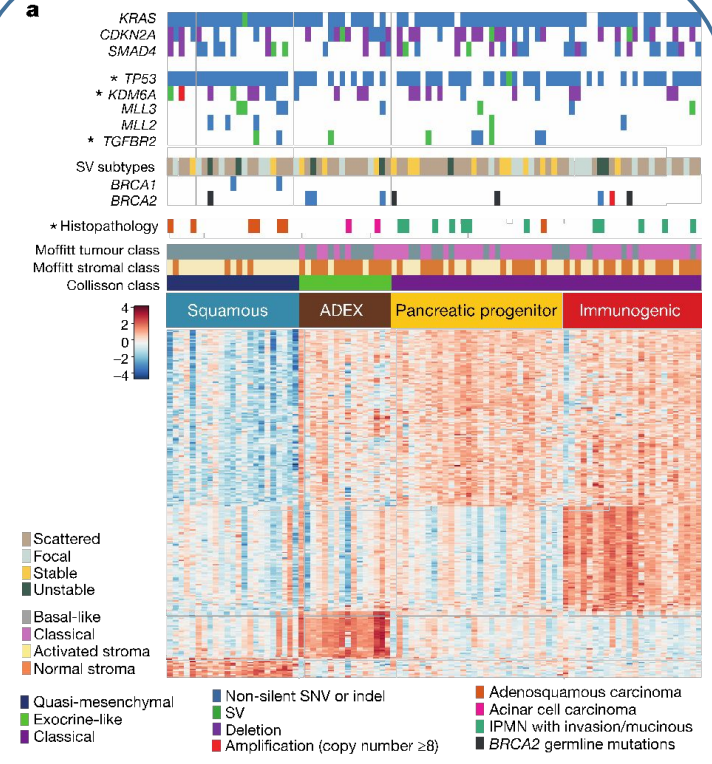
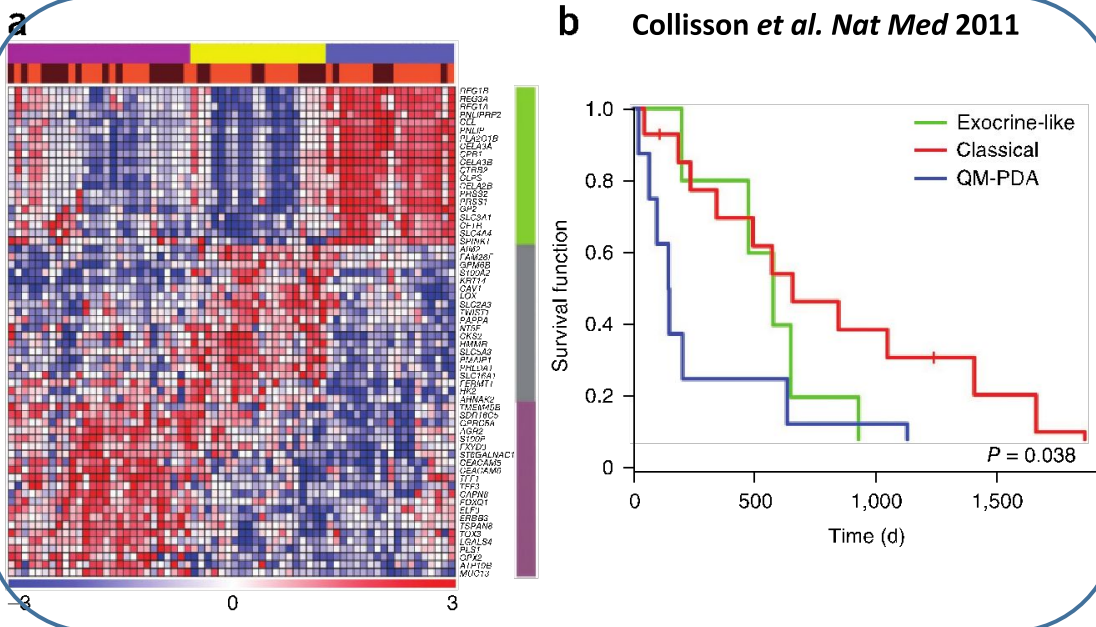
Nones et al. IJC 2014

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

↑ *MET*

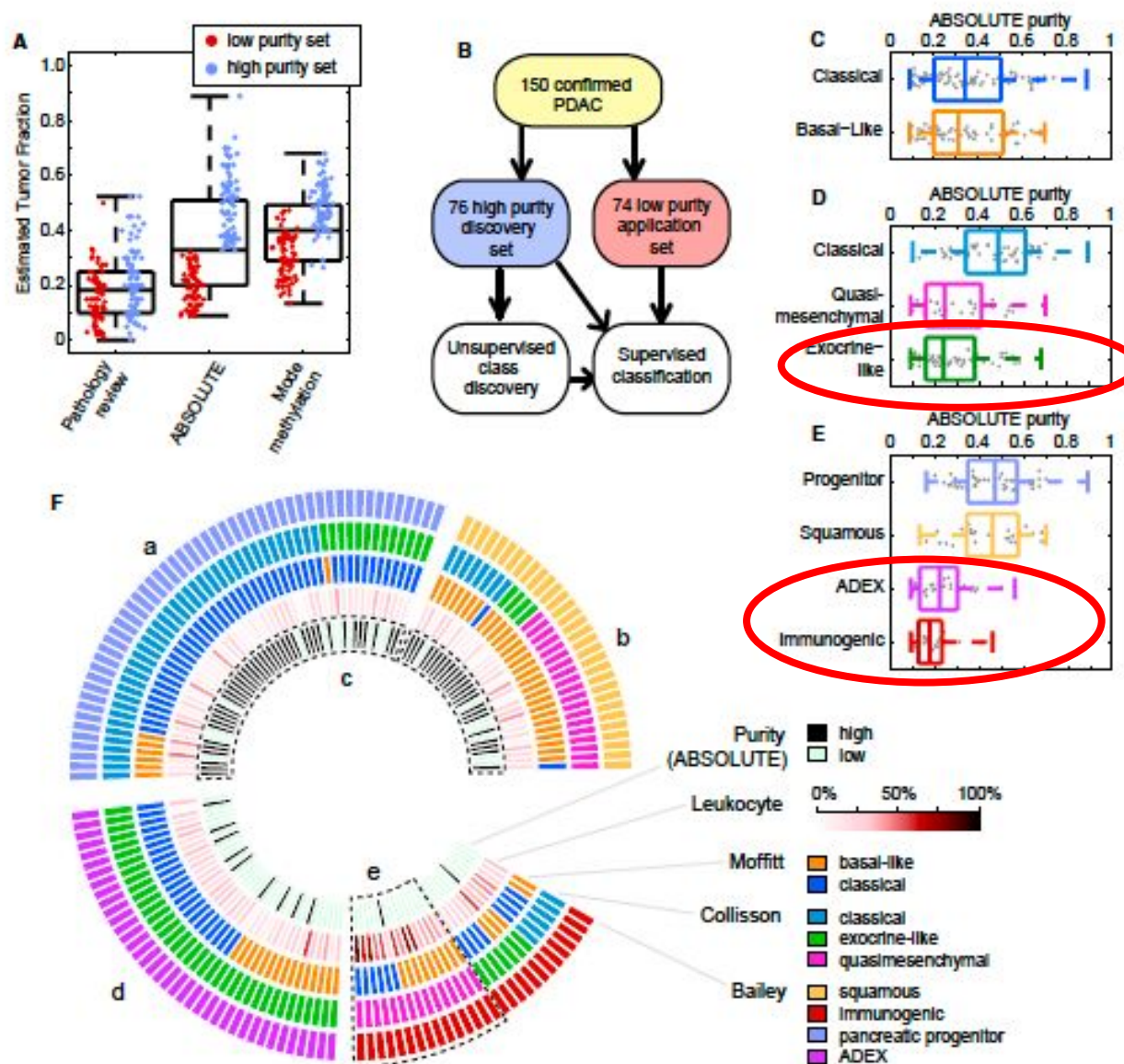
K



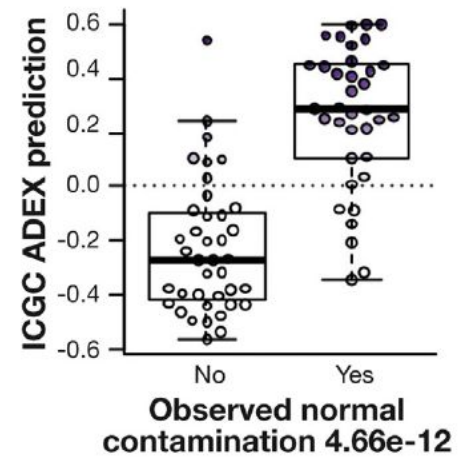
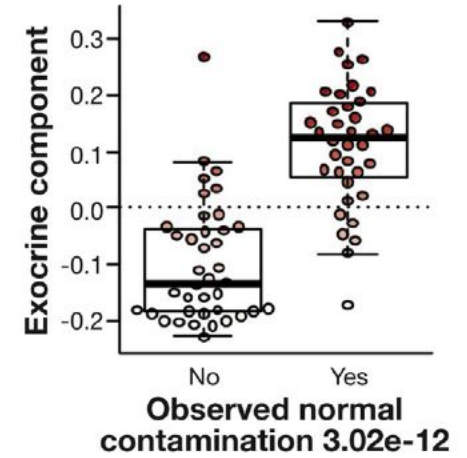
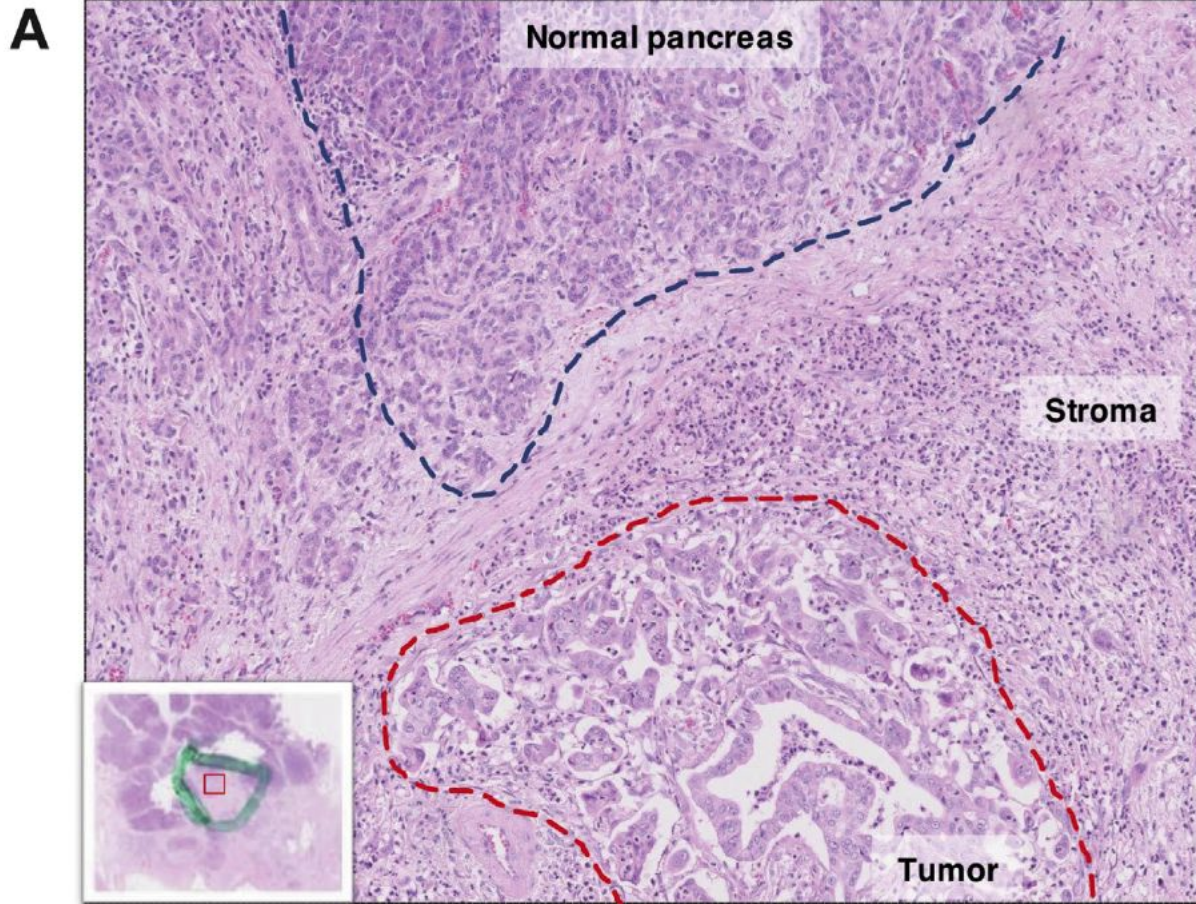


Moffitt et al. Nat Gen 2015

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

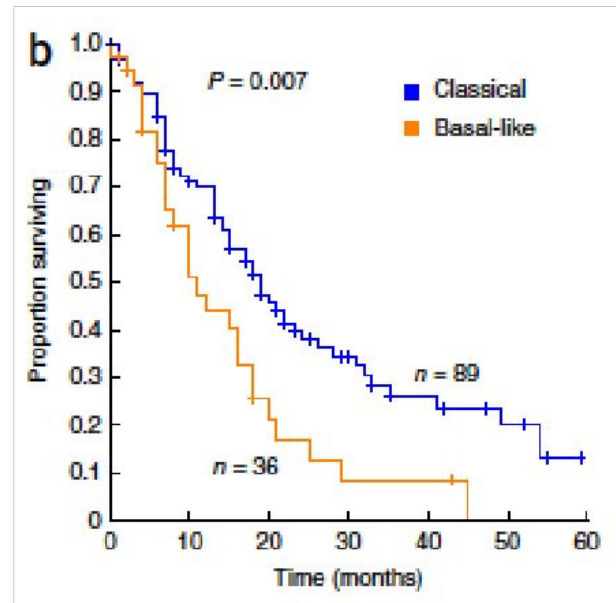
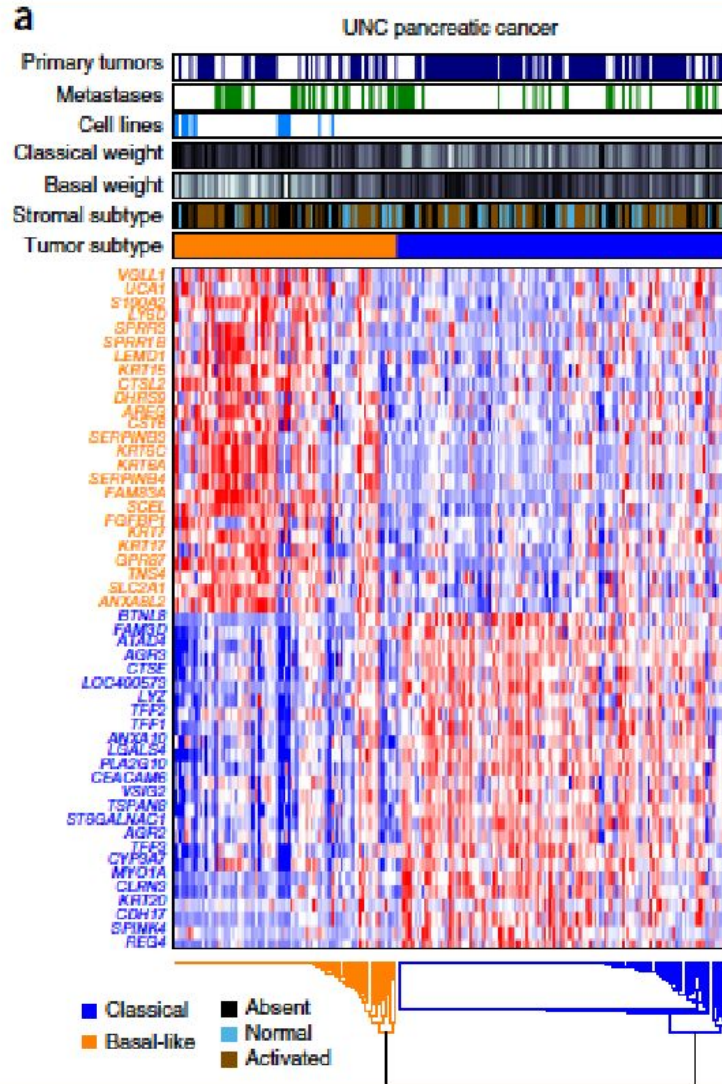


We try our best to give pure tumor area....

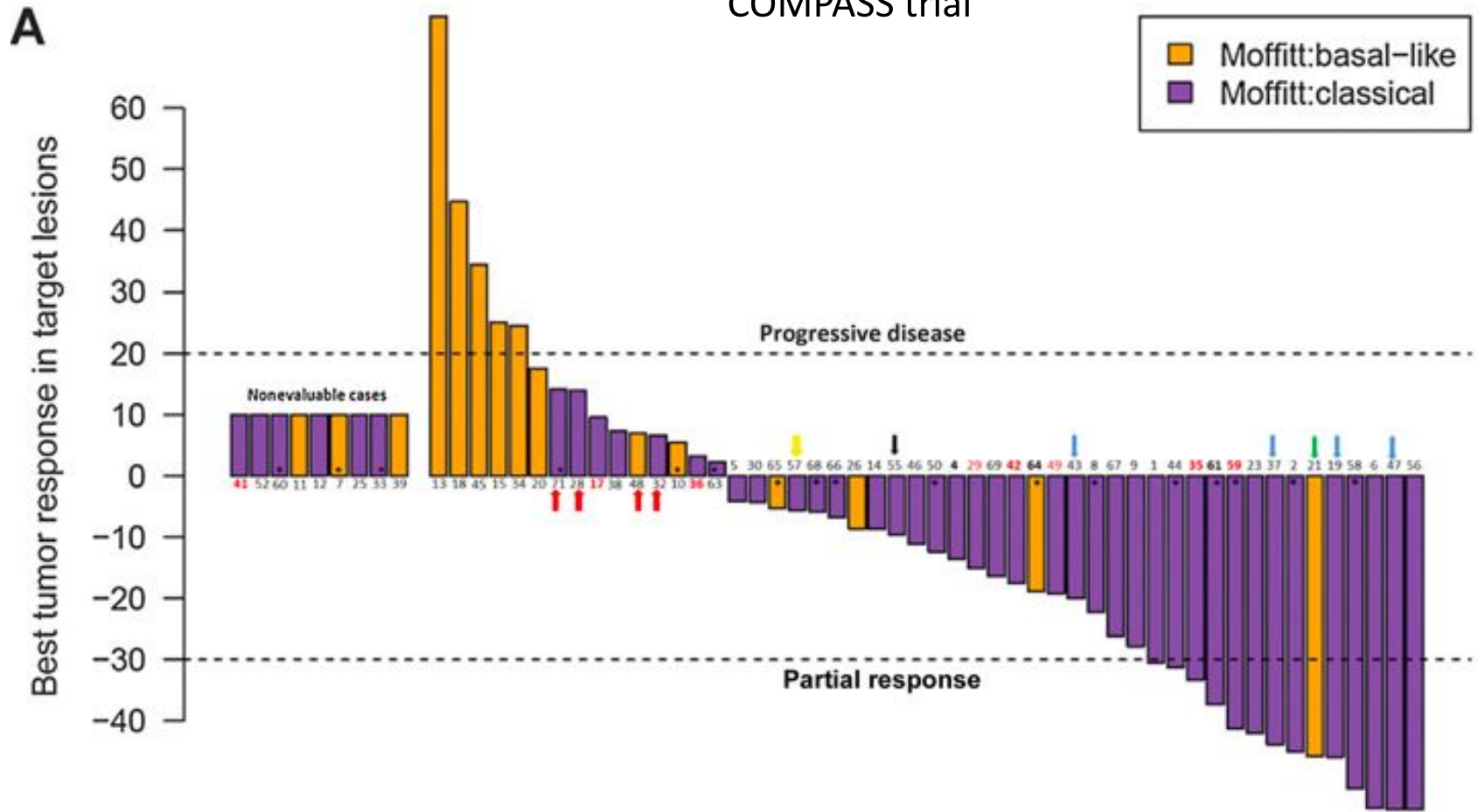


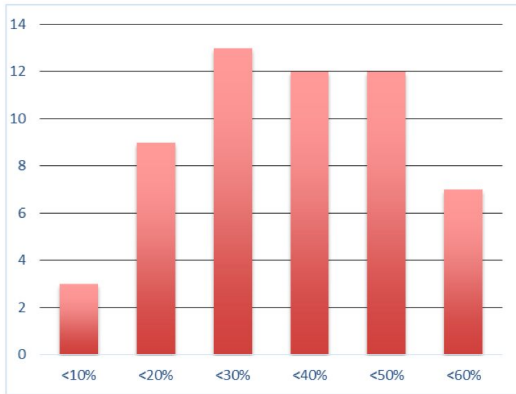
2 main transcriptomic tumor subtypes with different prognosis

Moffitt et al. Nat Gen 2015

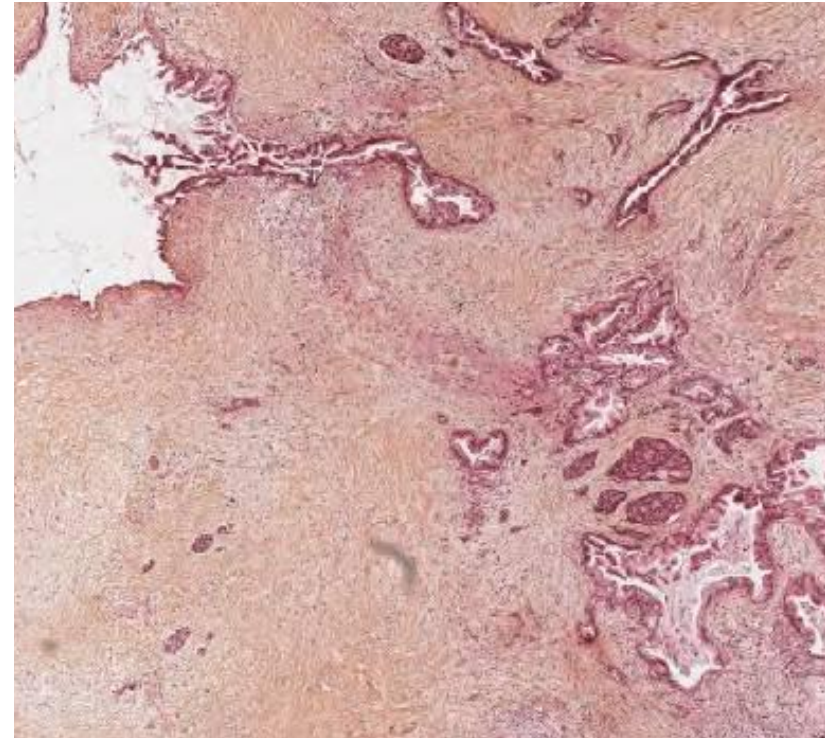
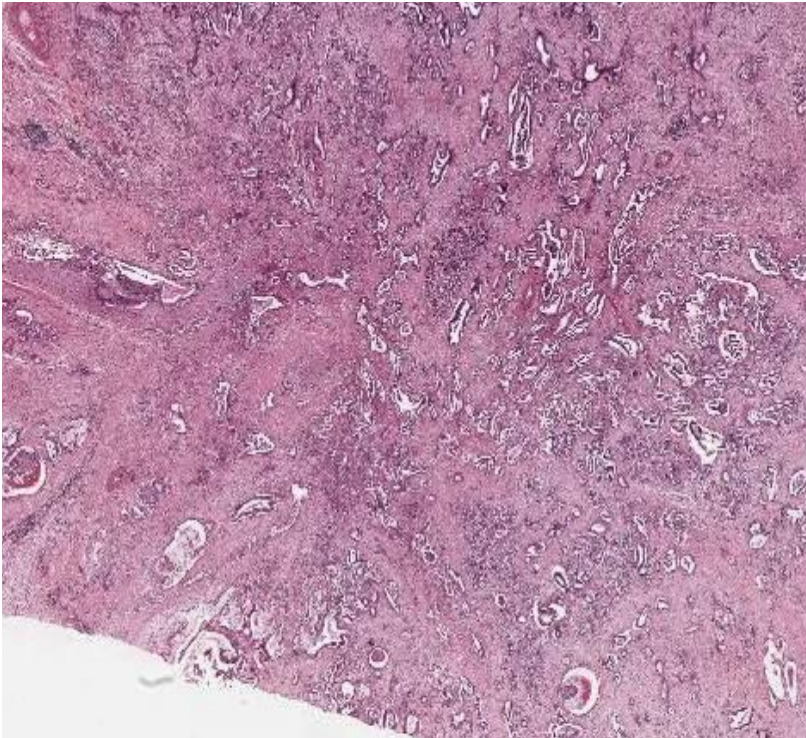


Molecular subtypes may have an important clinical utility++++





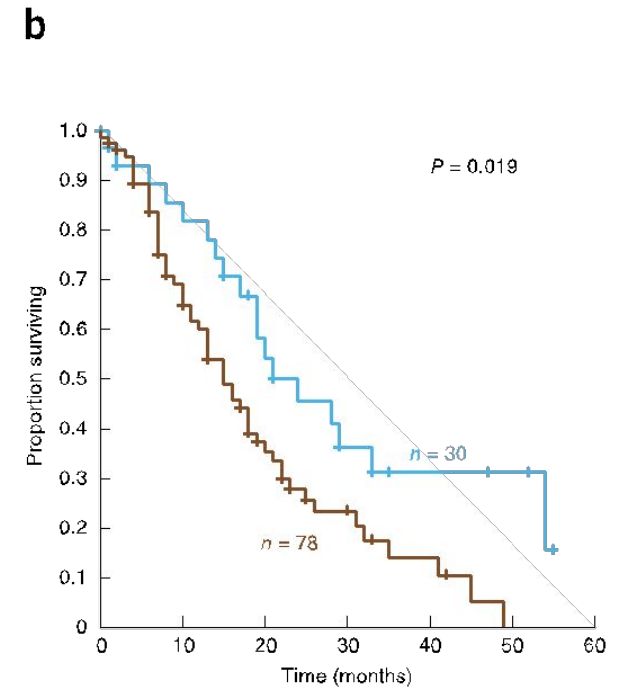
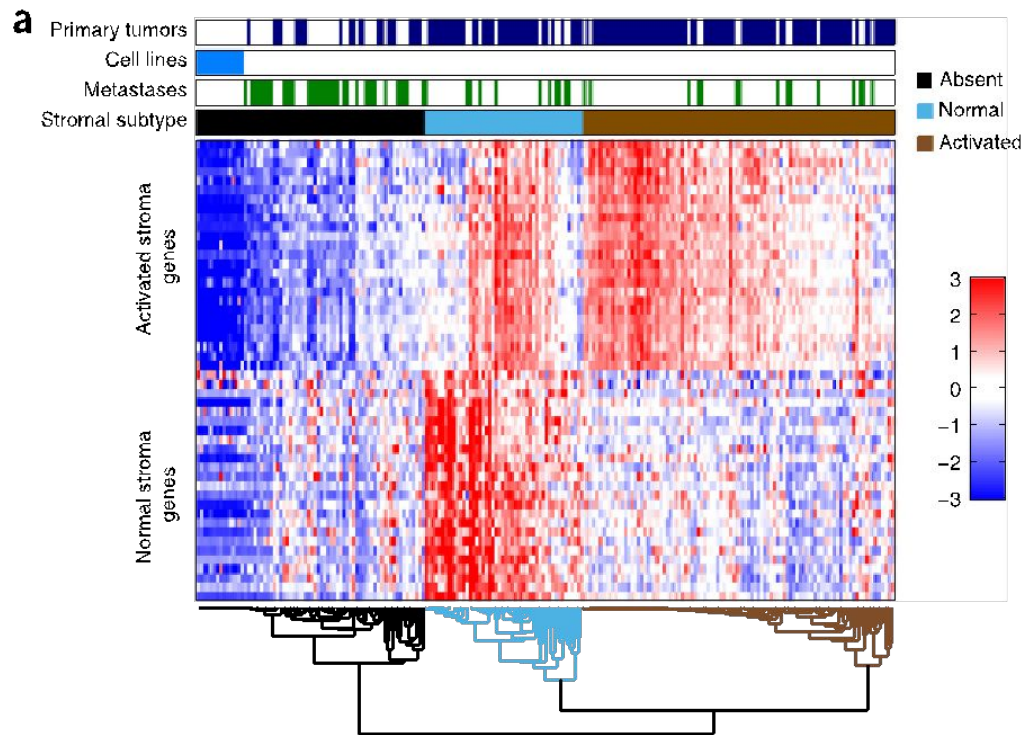
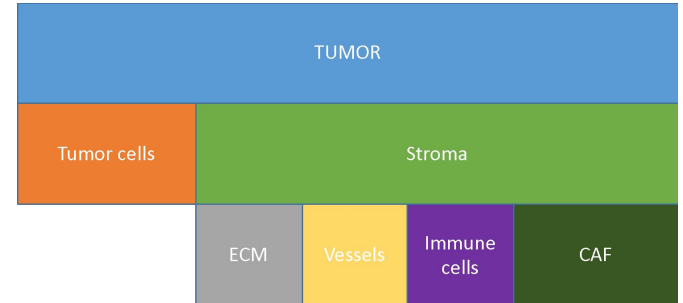
True challenge! How to define (clearly) the molecular subtype in samples with few tumor cells???



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

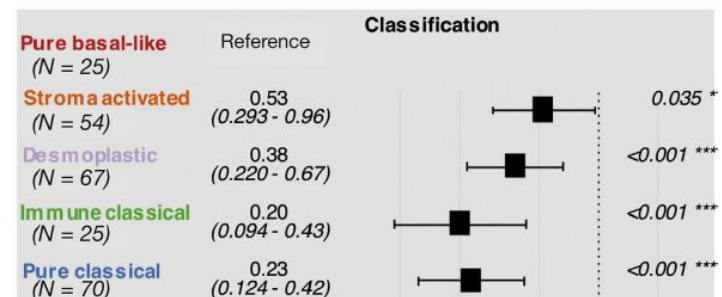
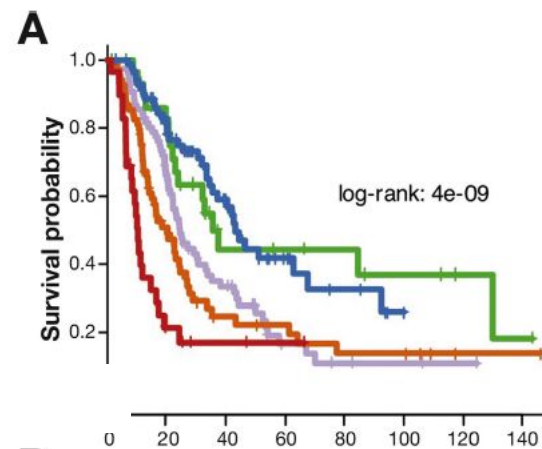
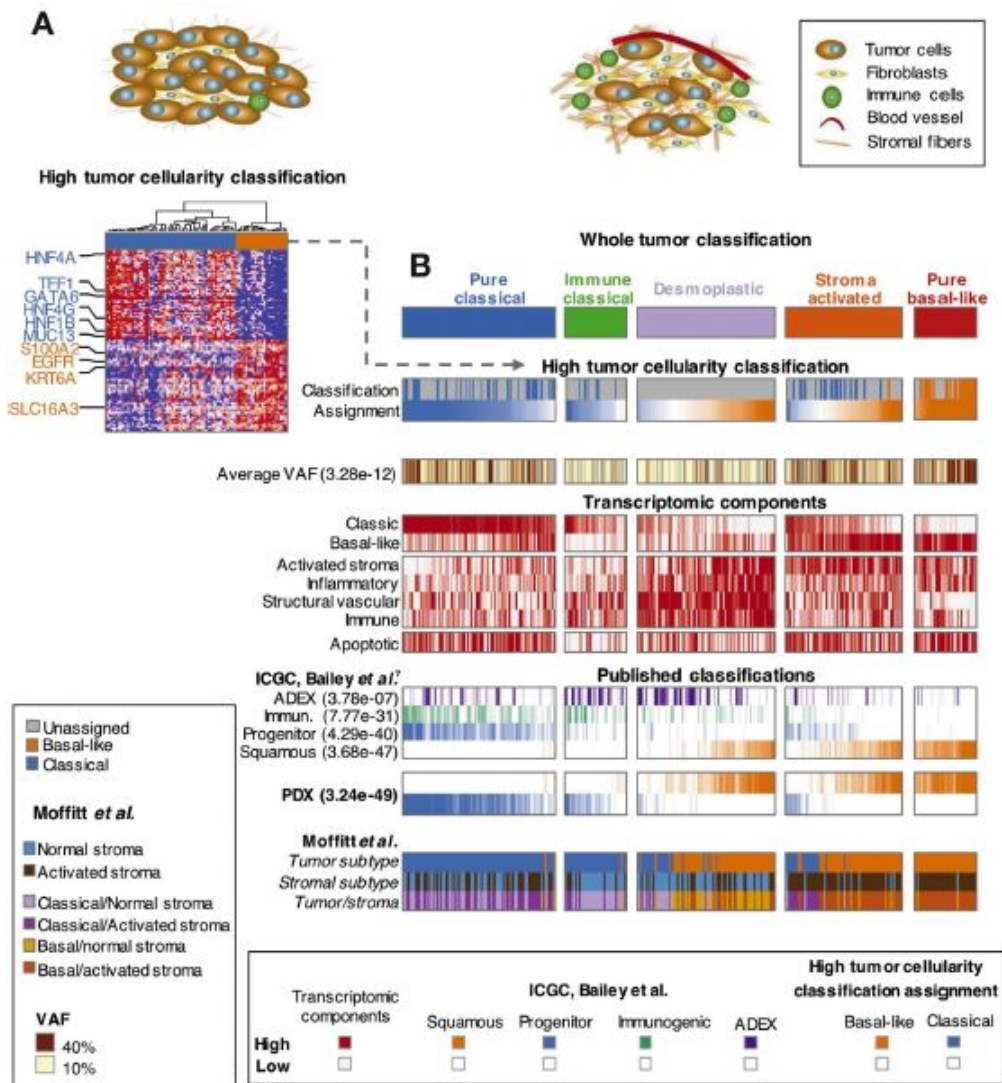
IHC markers

And the stroma is also heterogeneous...



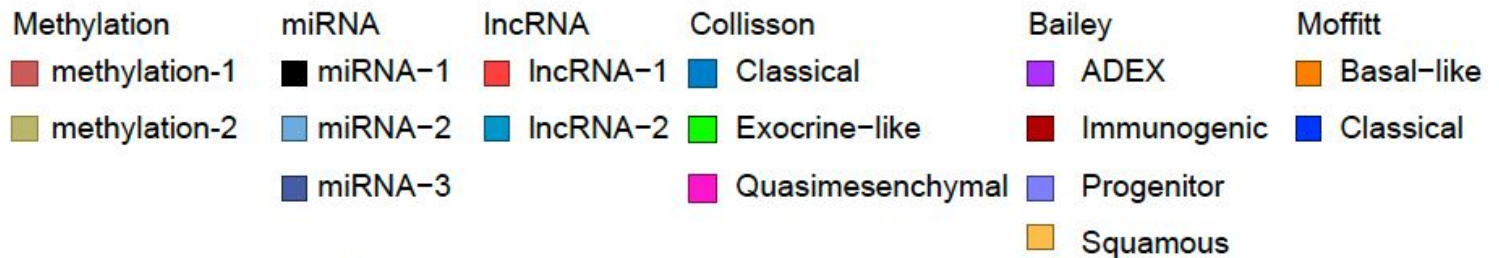
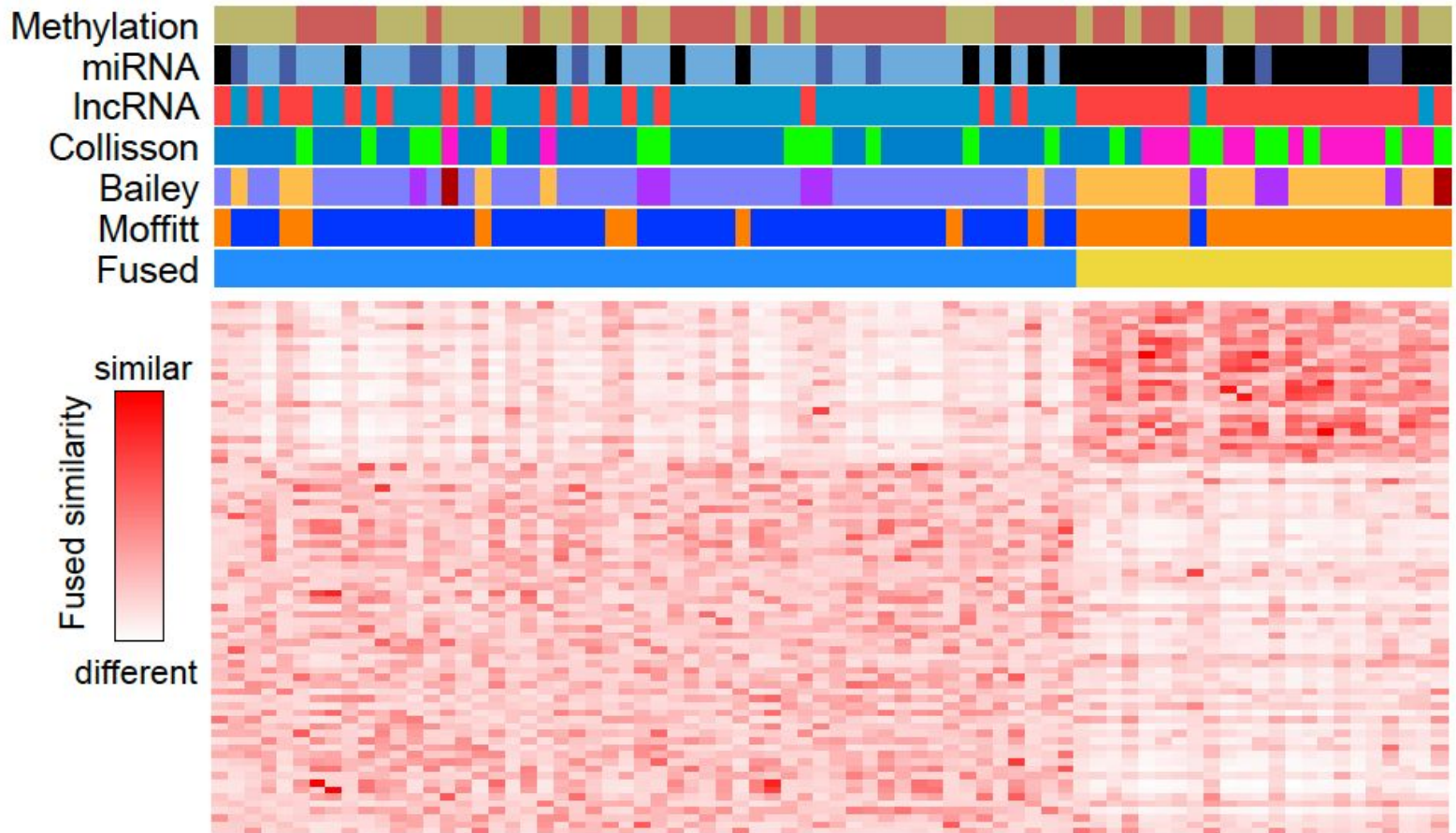
Moffitt et al. Nature genetics 2015

Intergration of the heterogeneity of all the components....

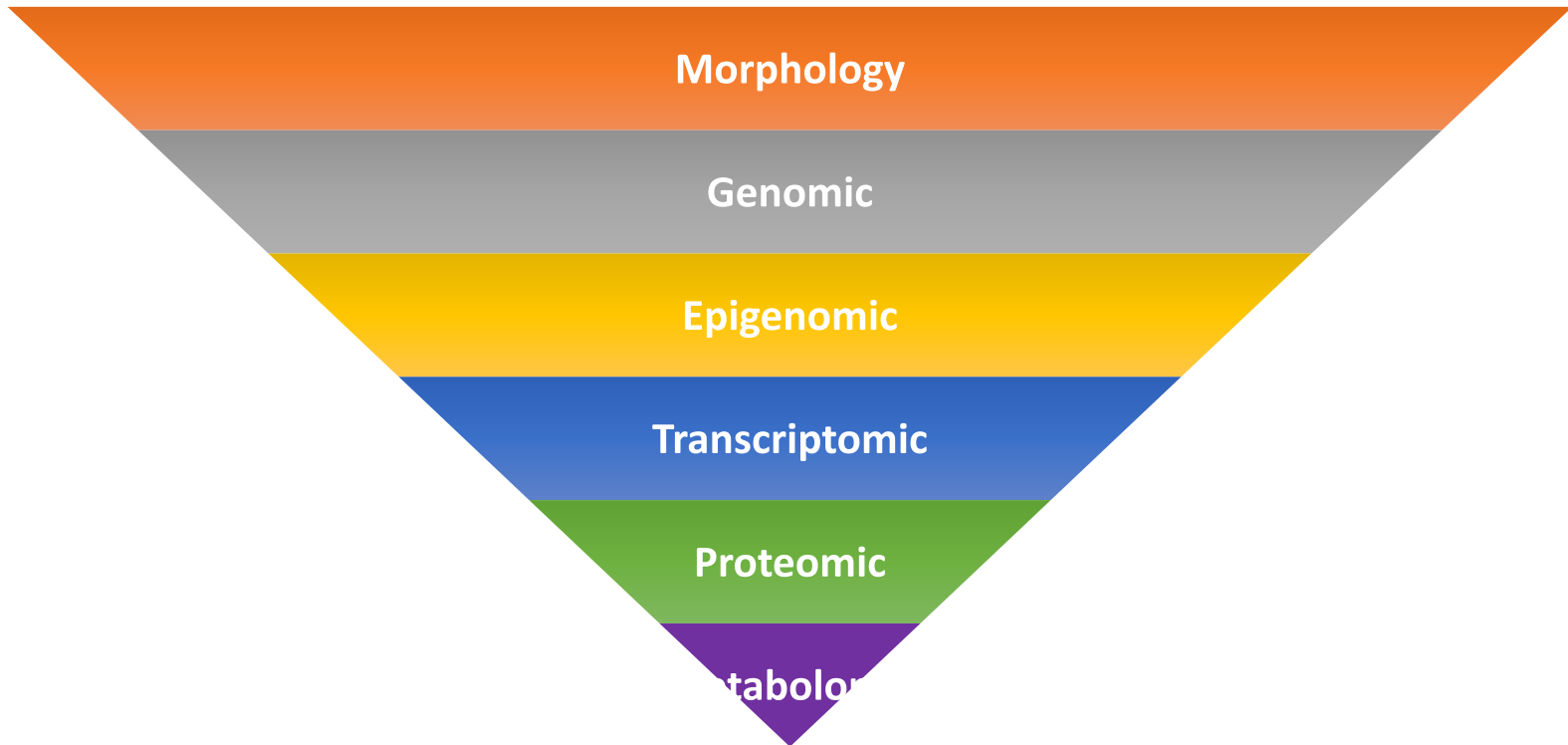


Inter-tumor heterogeneity - conclusion

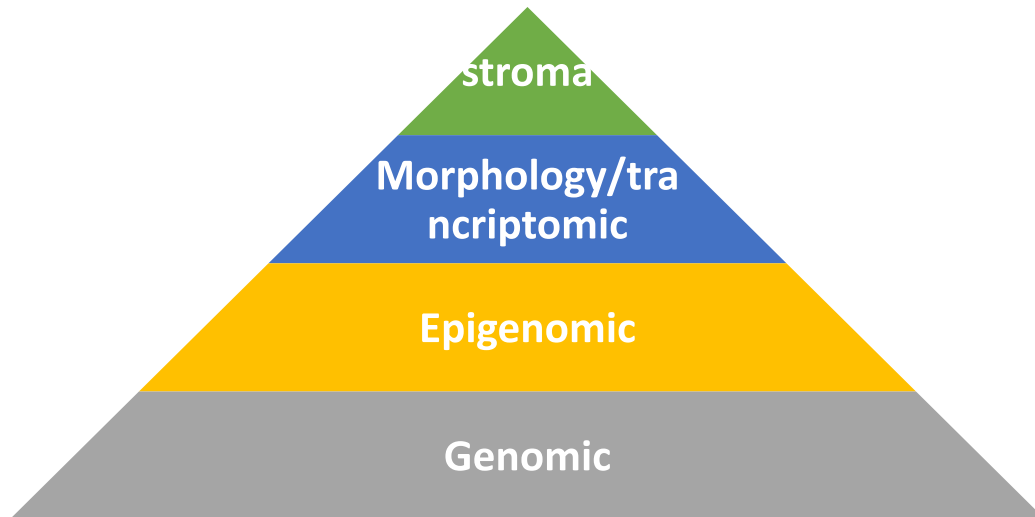
Major impact of purity (tumor cells) on classifications++++



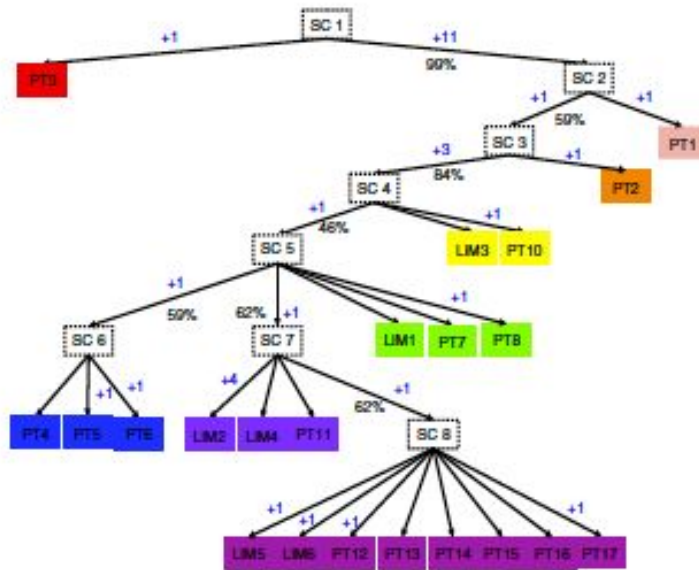
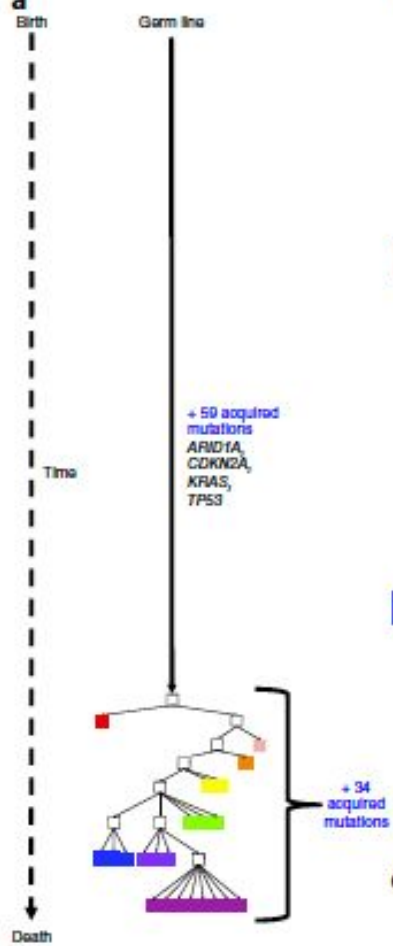
Inter-tumor heterogeneity



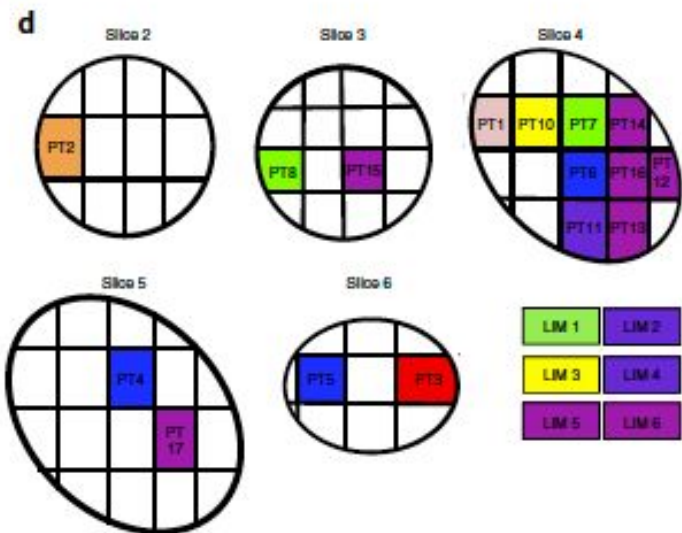
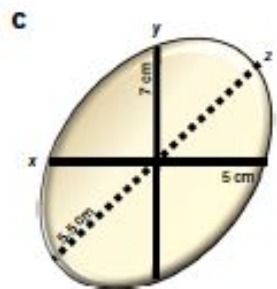
**Intratumor heterogeneity
and clonal evolution**



Intra-tumor heterogeneity - genomic

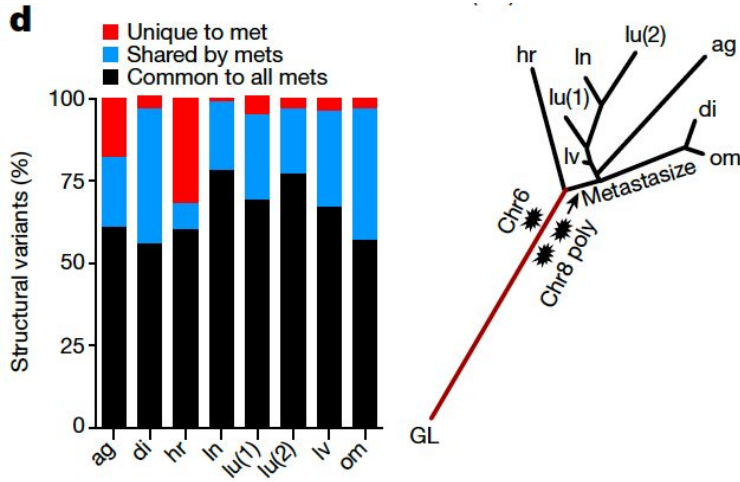


- Low heterogeneity of classical driver genes
- Most genomic events happen early
- No « metastasis » gene
- Physical and genomic spatialisation are different+++

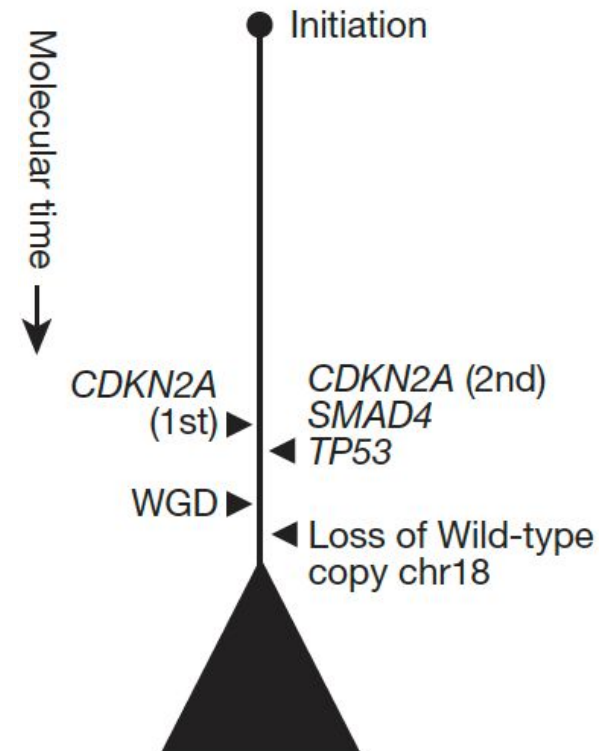
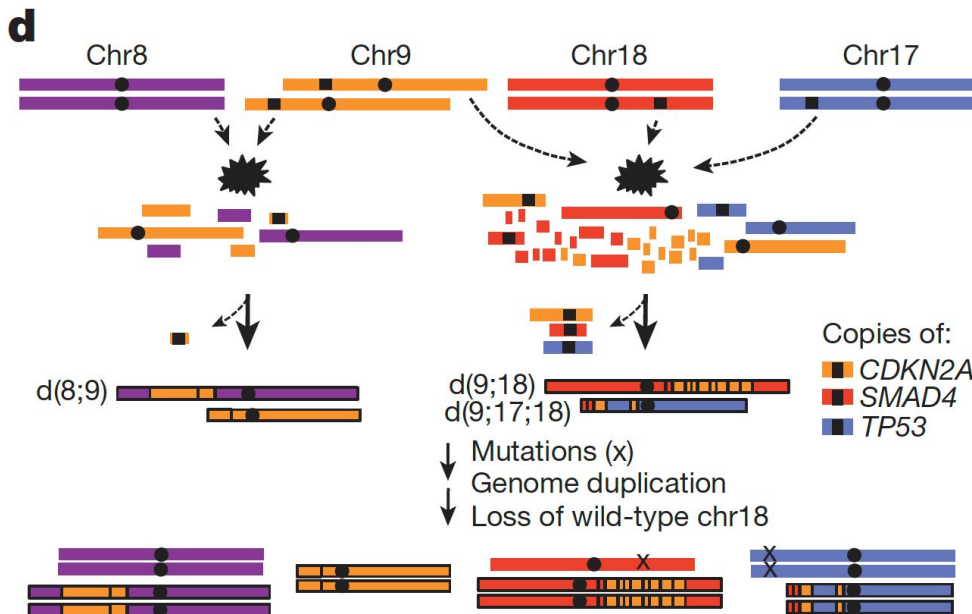


Makohon-Moore *et al. Nat Gen* 2017
 Yachida *et al. Nature* 2010

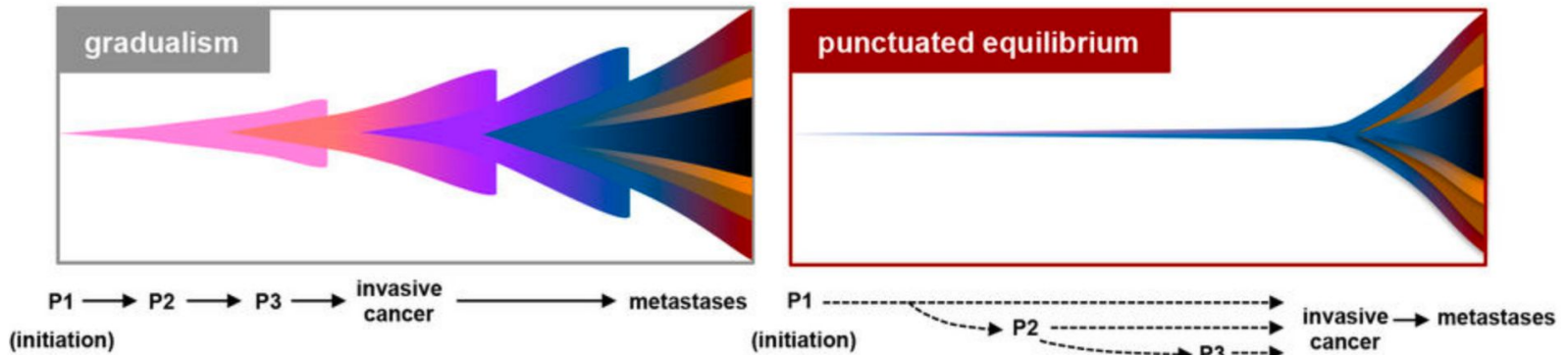
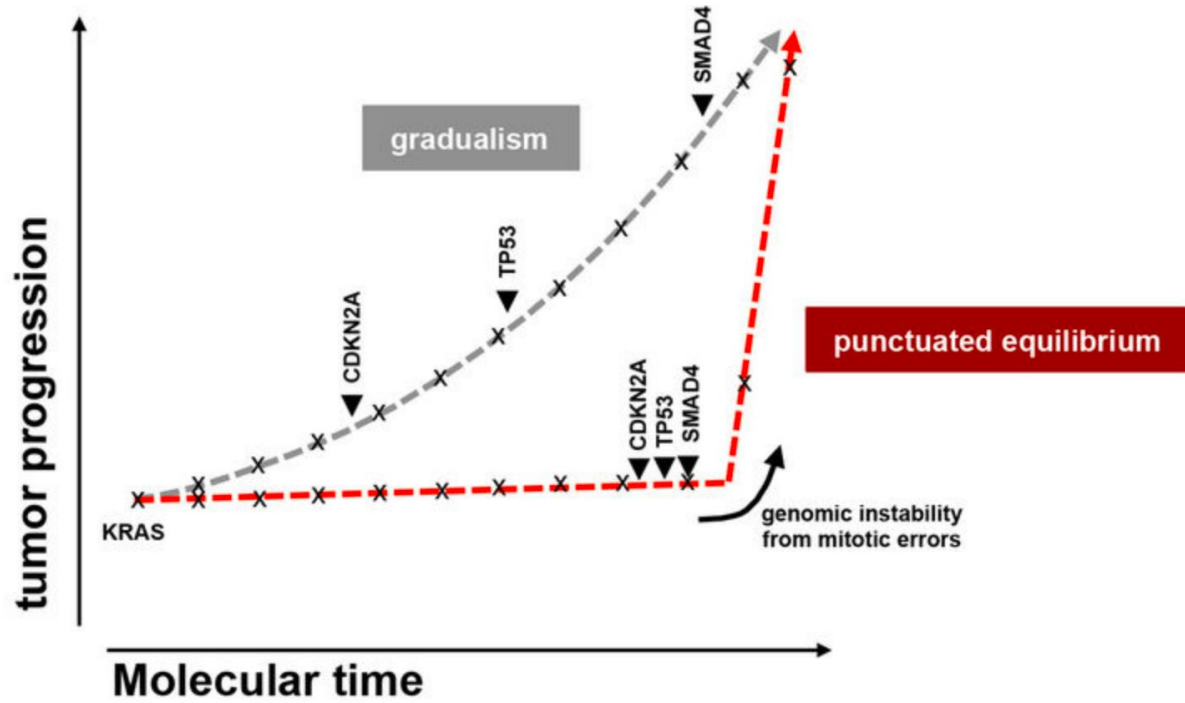
Intra-tumor heterogeneity - genomic



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



How to follow high risk patients???



Intra-tumor heterogeneity - epigenomic



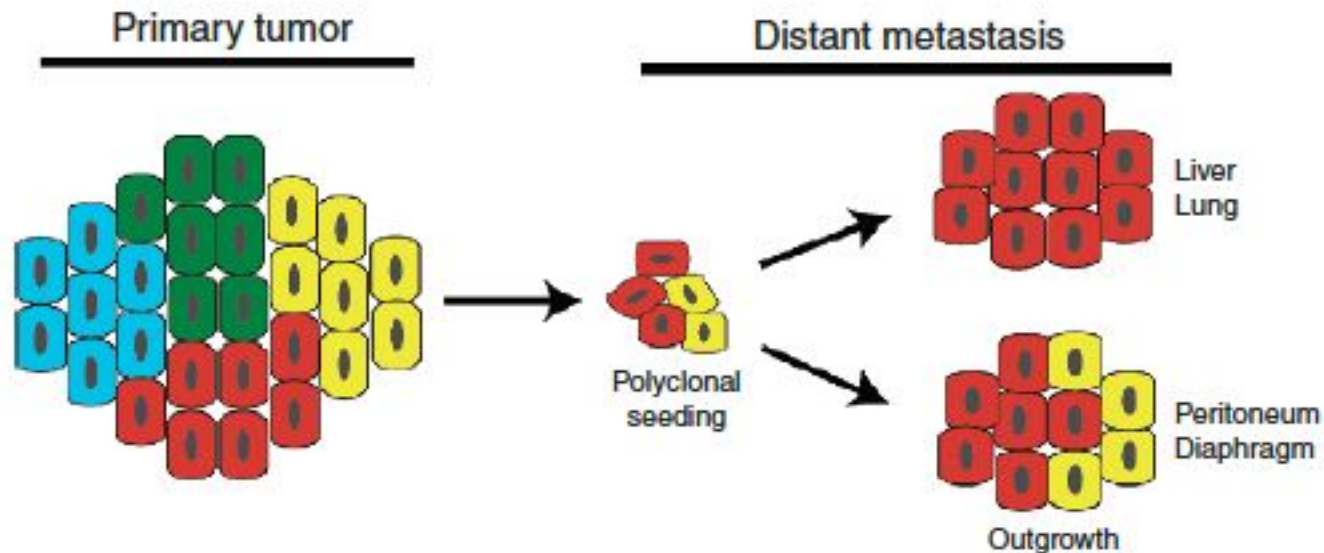
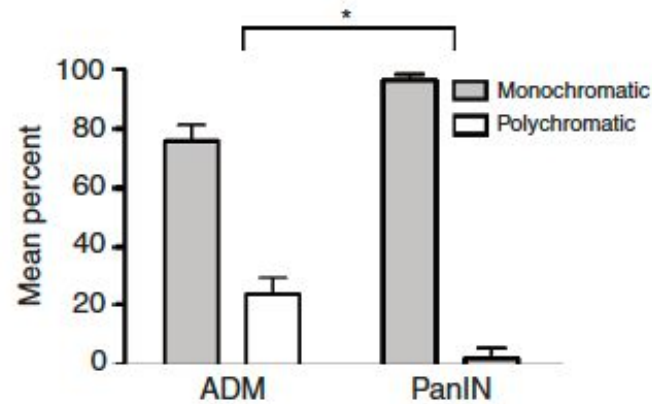
Genomic heterogeneity

Epigenomic heterogeneity

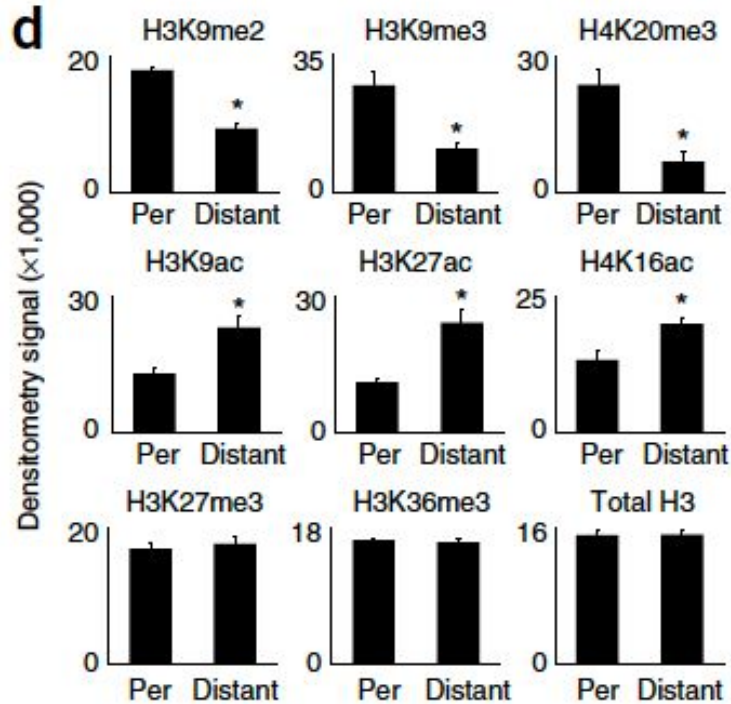
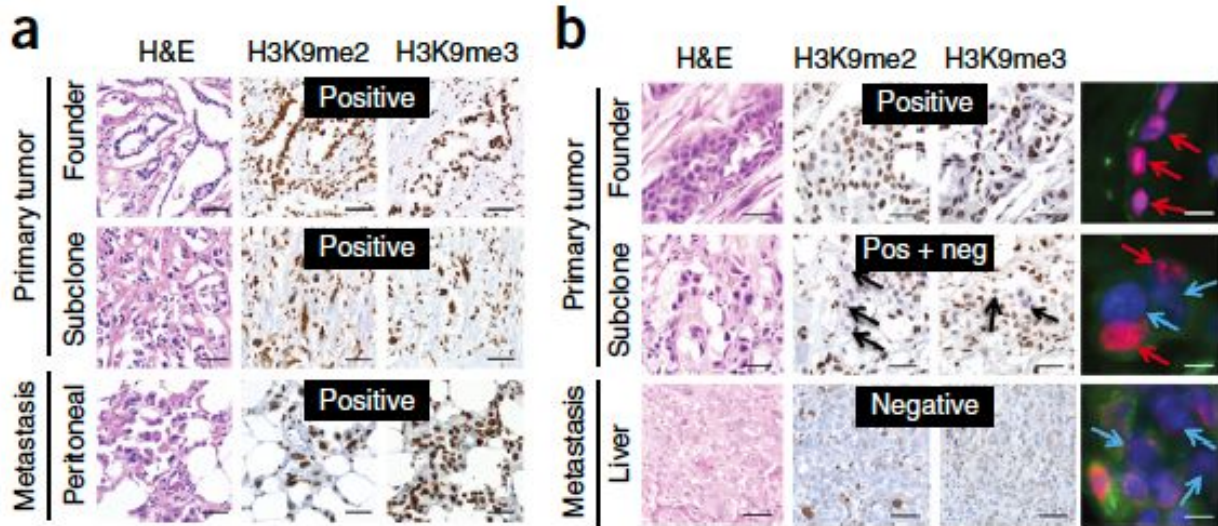
C

ADM and PanIN counts

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



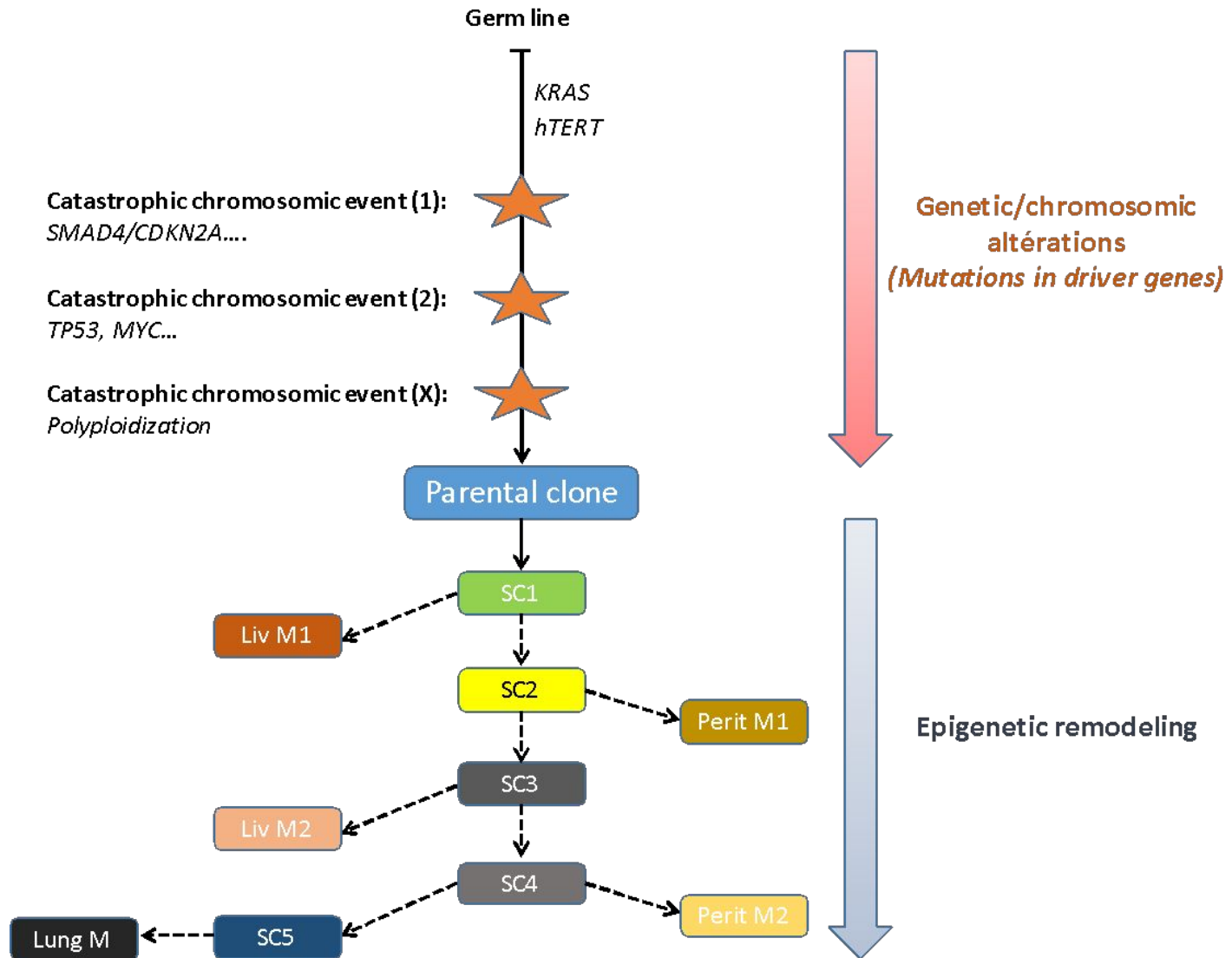
Intra-tumor heterogeneity - epigenomic



Intra-tumor heterogeneity - epigenomic

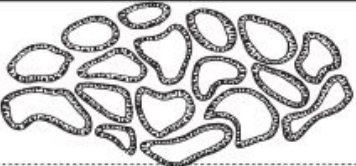
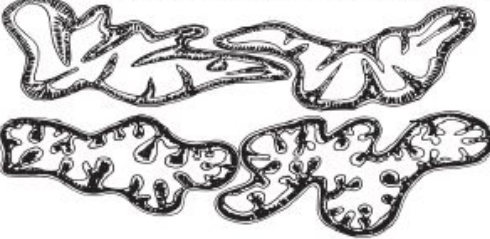


Genomic heterogeneity

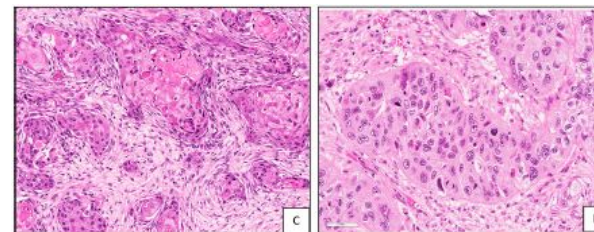
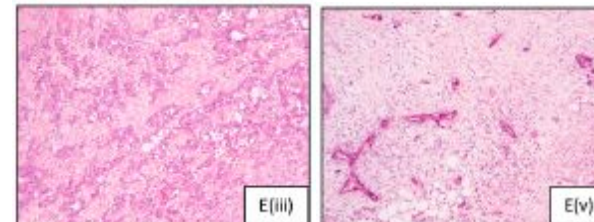
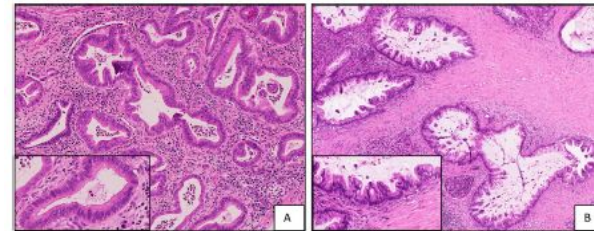
Epigenomic heterogeneity



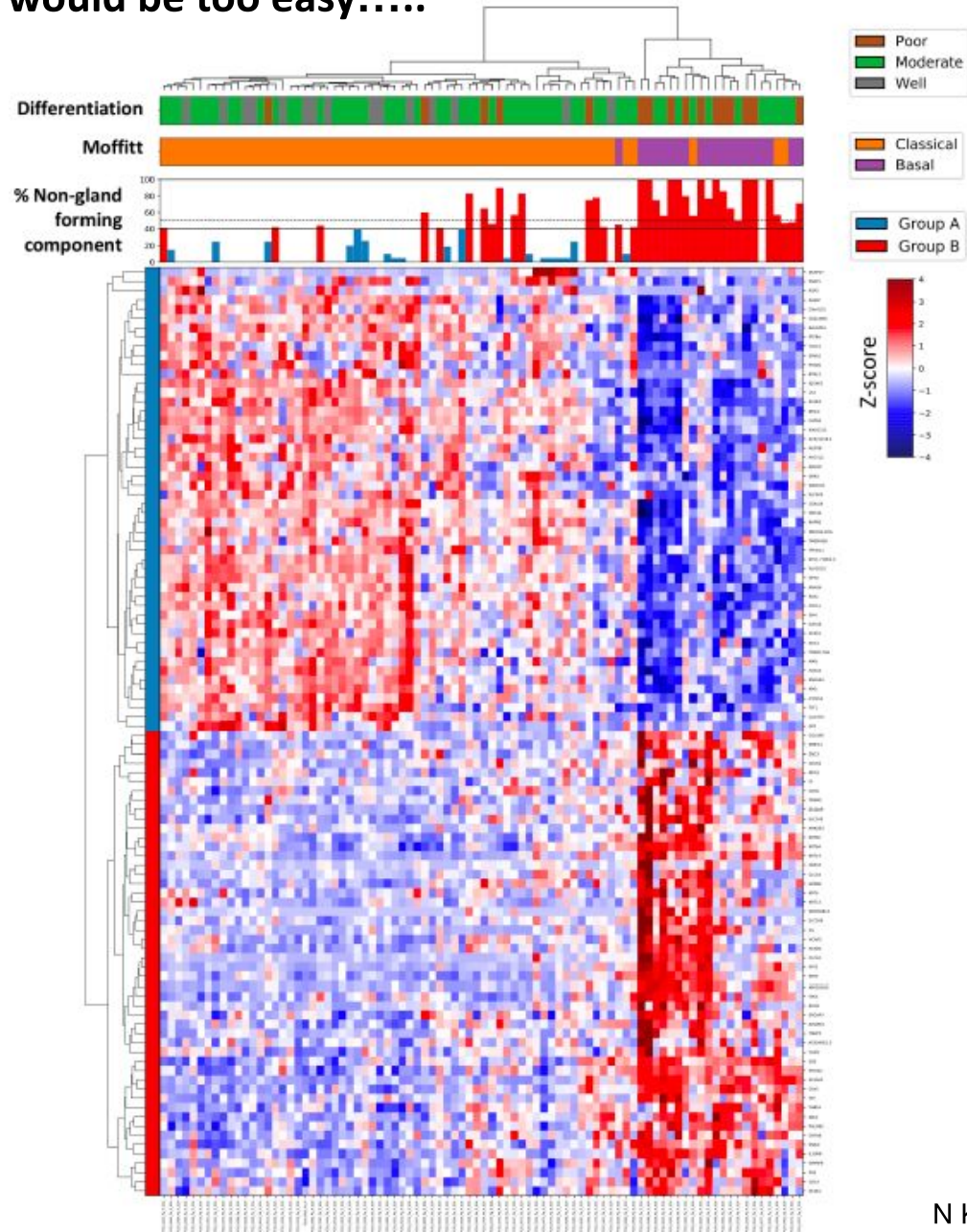
If tumor were pure, that would be too easy.....

A

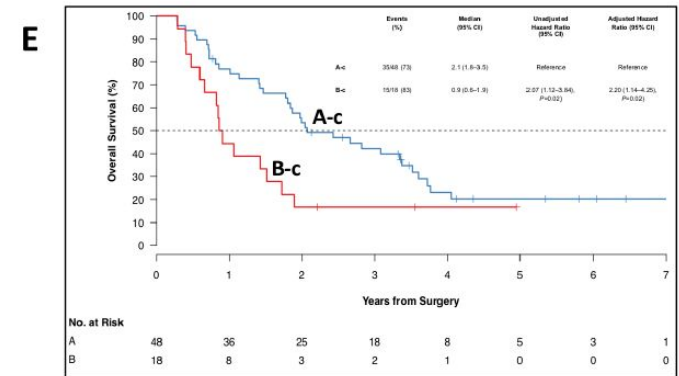
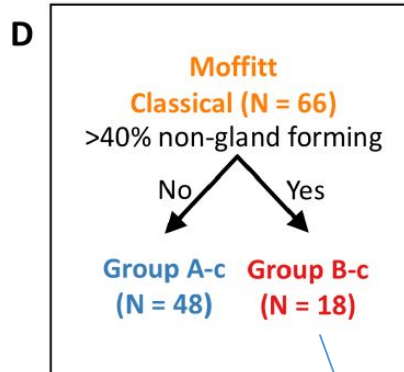
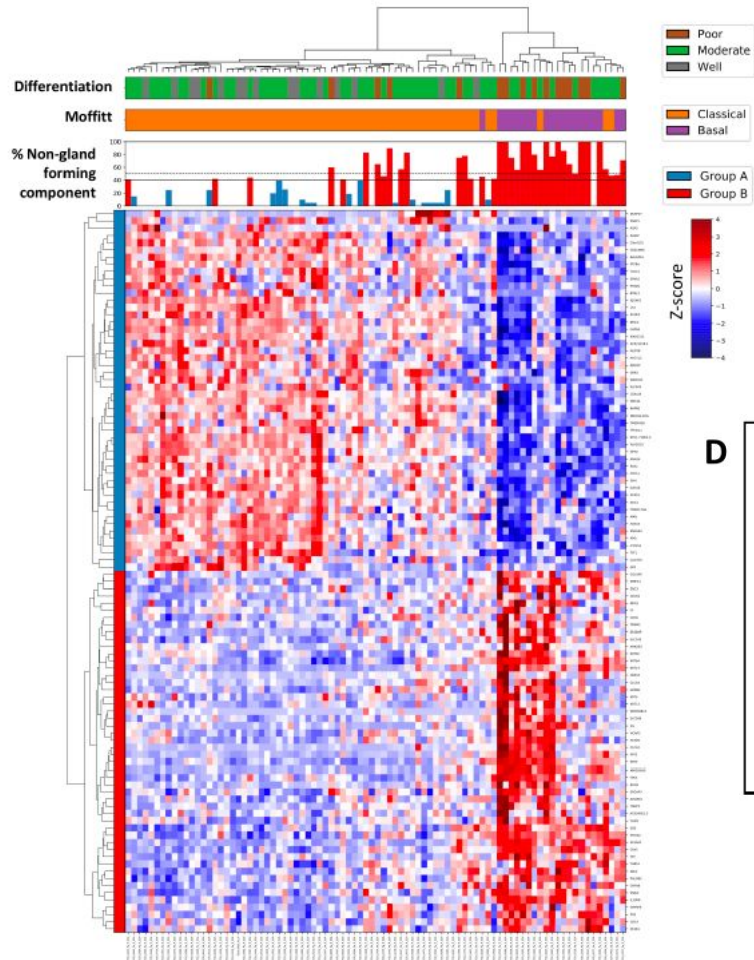
"Gland forming" component	Patterns
	Conventional
	Tubulopapillary
"Non-gland forming" component	Patterns
	Composite
	Squamous



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

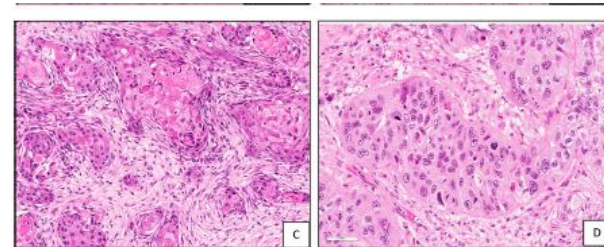
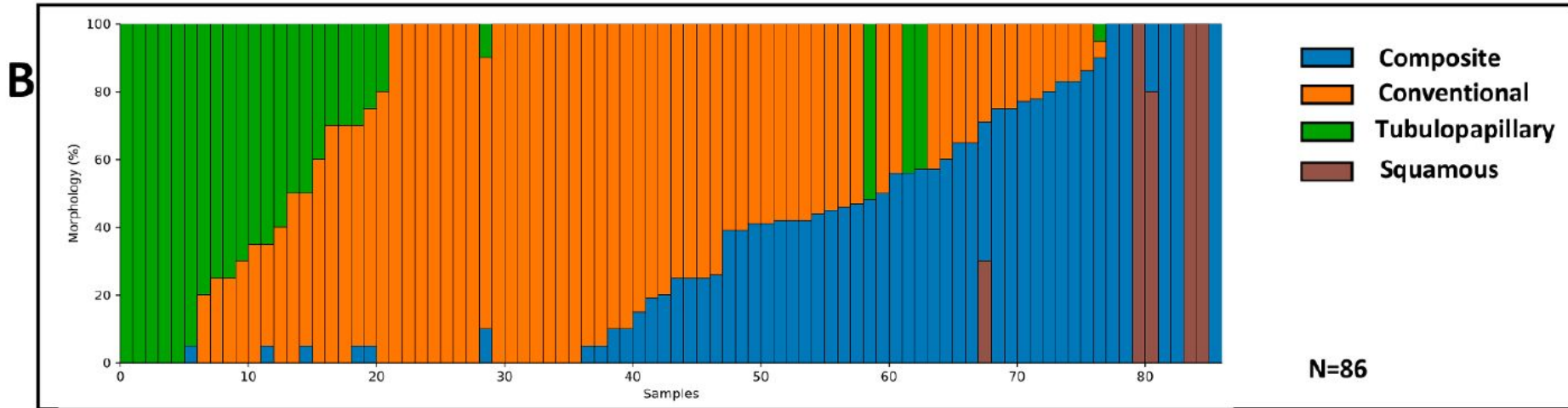
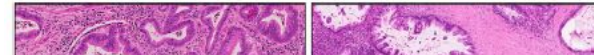
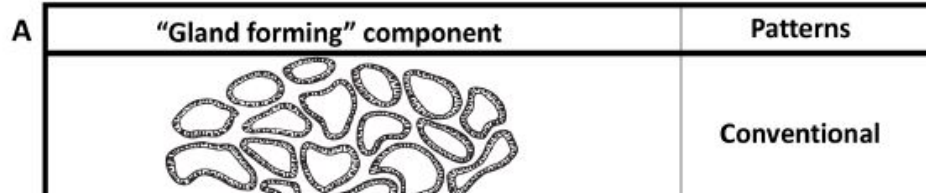


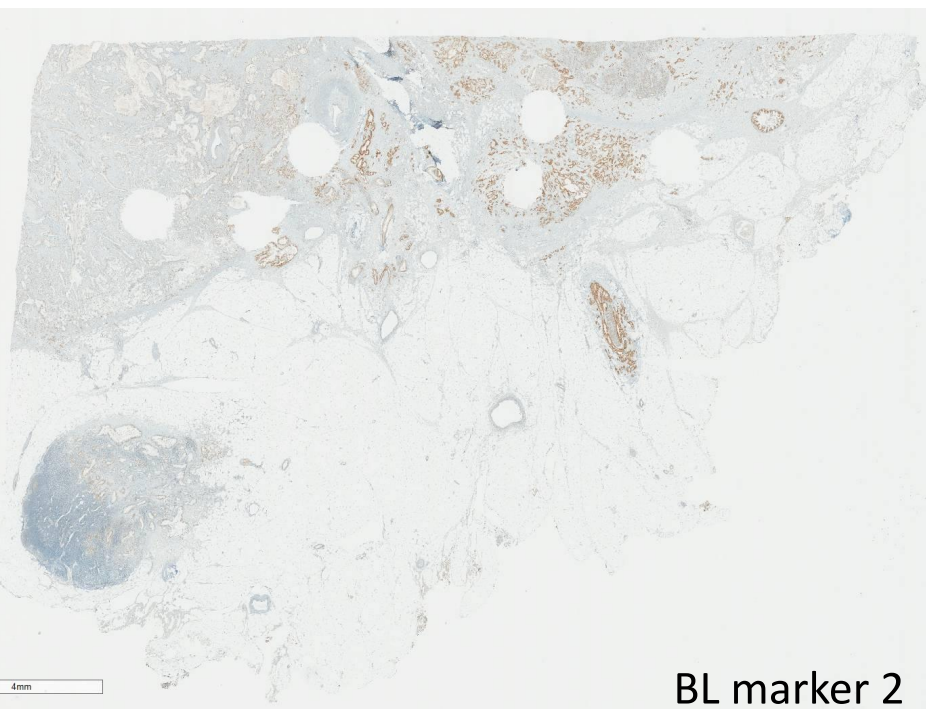
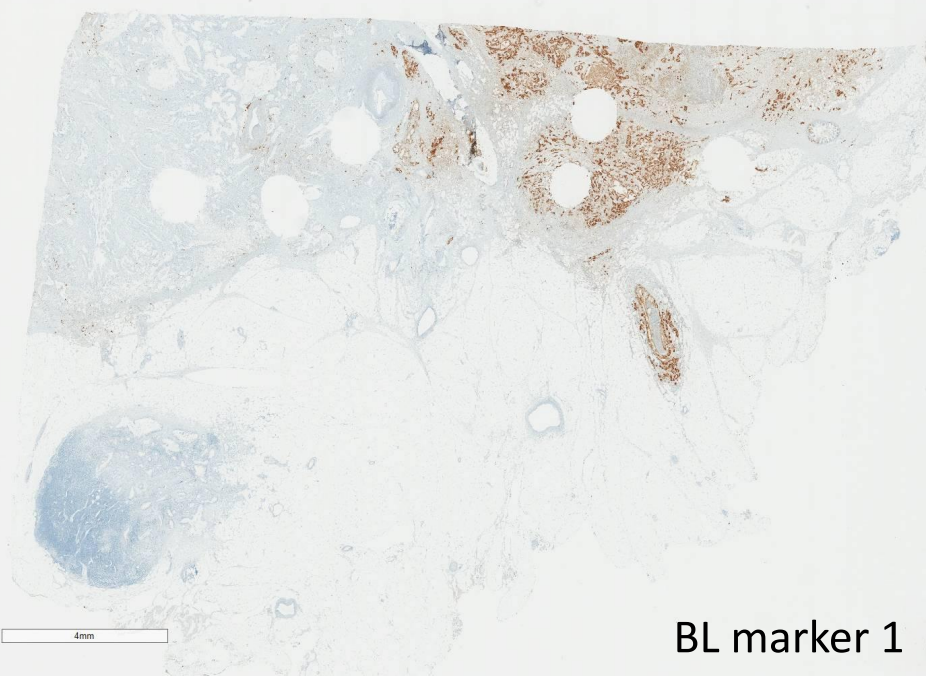
If tumor were pure, that would be too easy.....



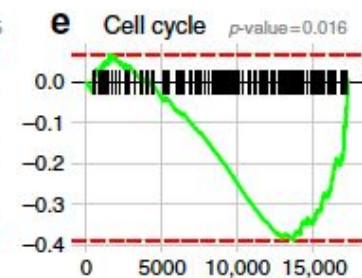
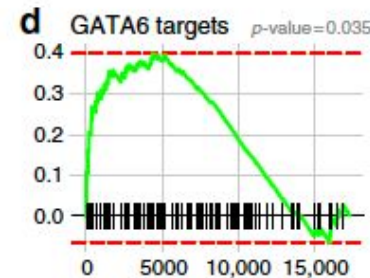
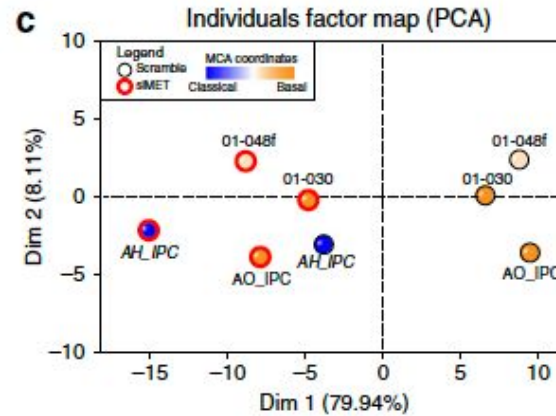
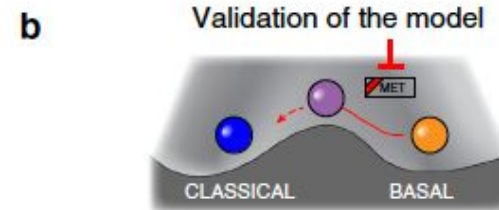
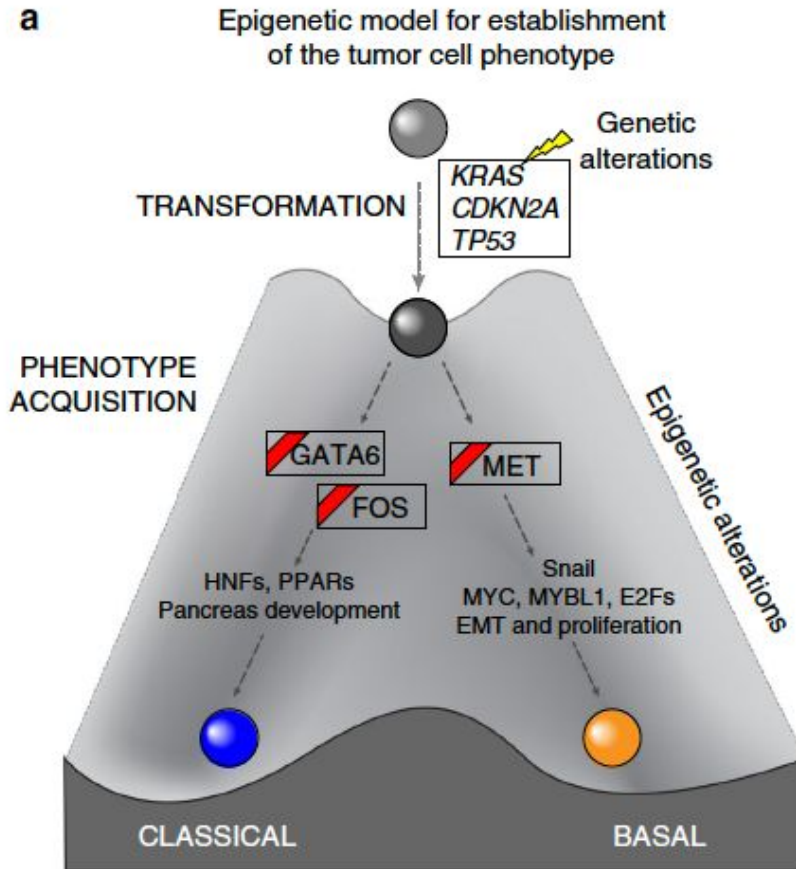
« classical » tumors with a basal like subpopulation?

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



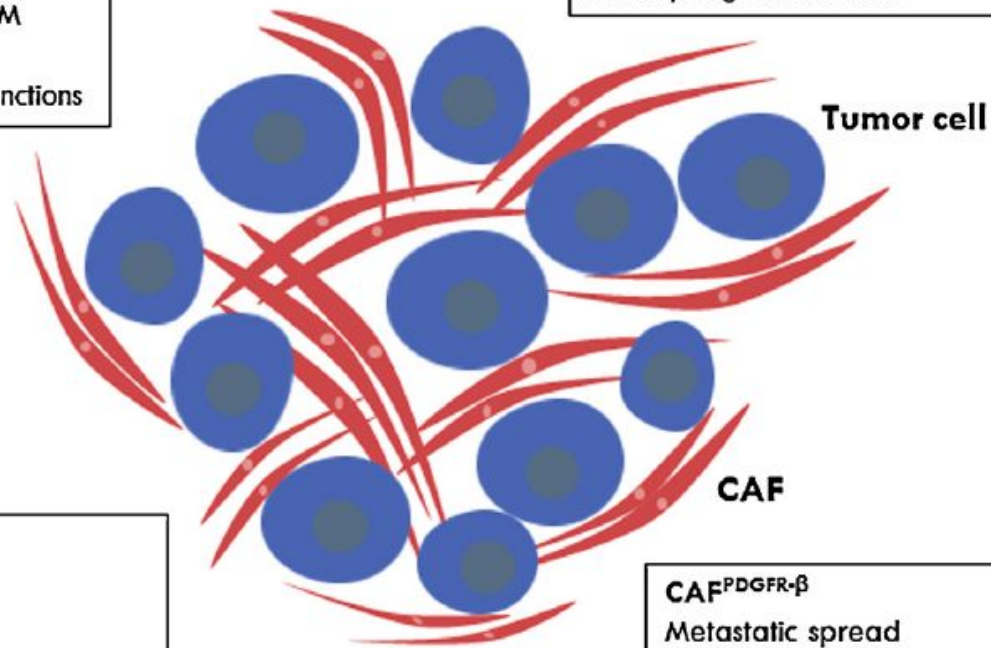


Is there an epigenetic-driven plasticity between subtypes?



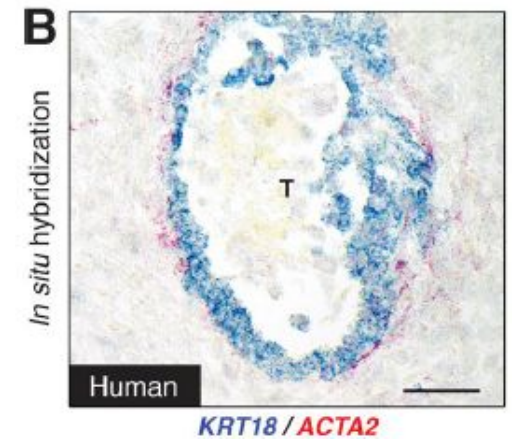
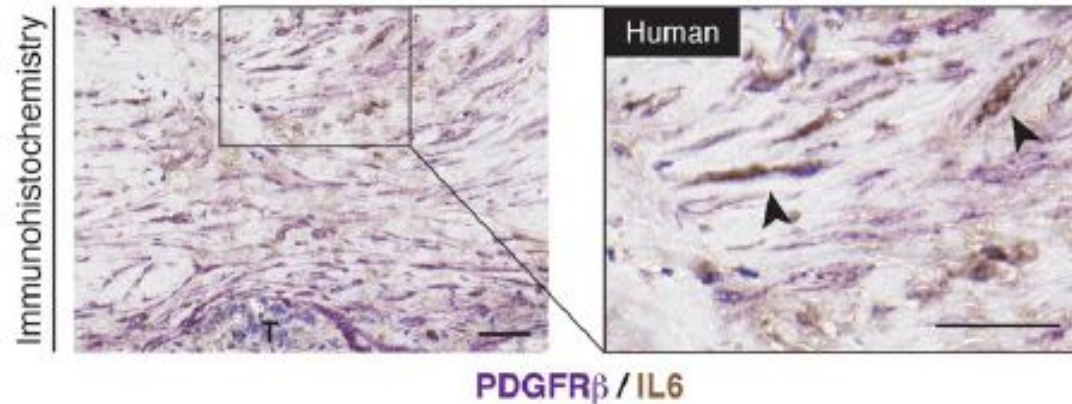
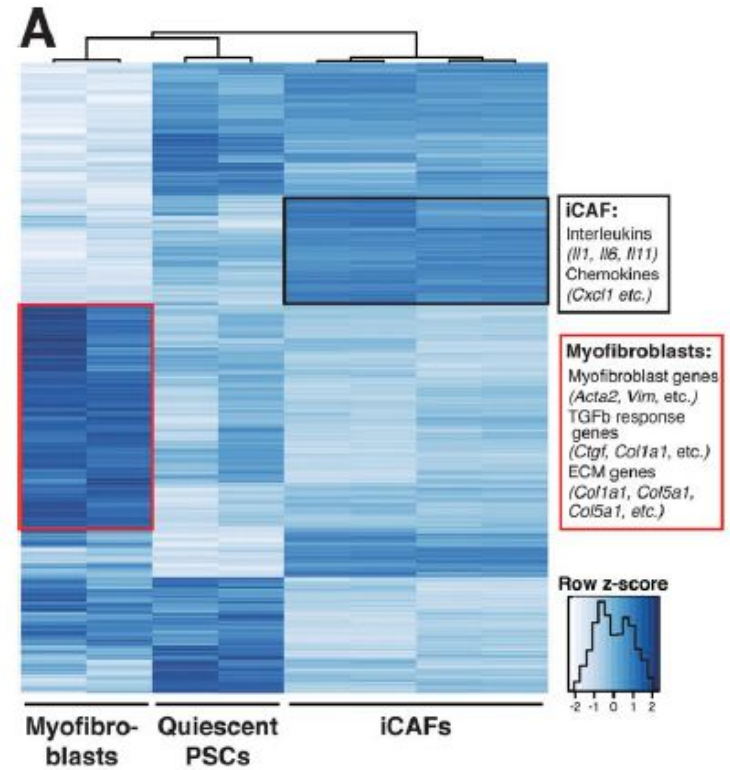
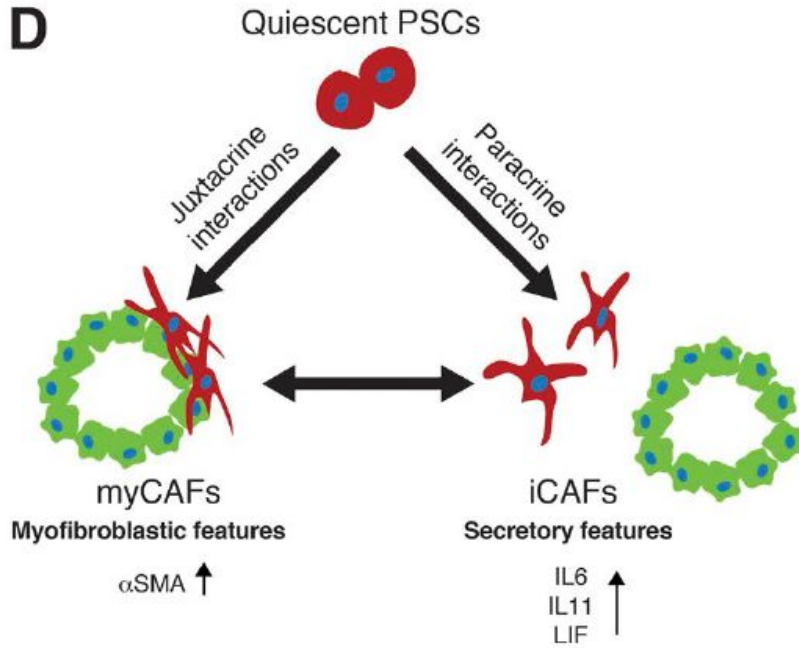
CAF^{FAP}
Activated/reactive CAF
Modulation of the ECM
Invasion
Immunomodulatory functions

CAF^{PDGFR-α}
Paracrine signaling - tumor cell growth and angiogenesis
Macrophage recruitment

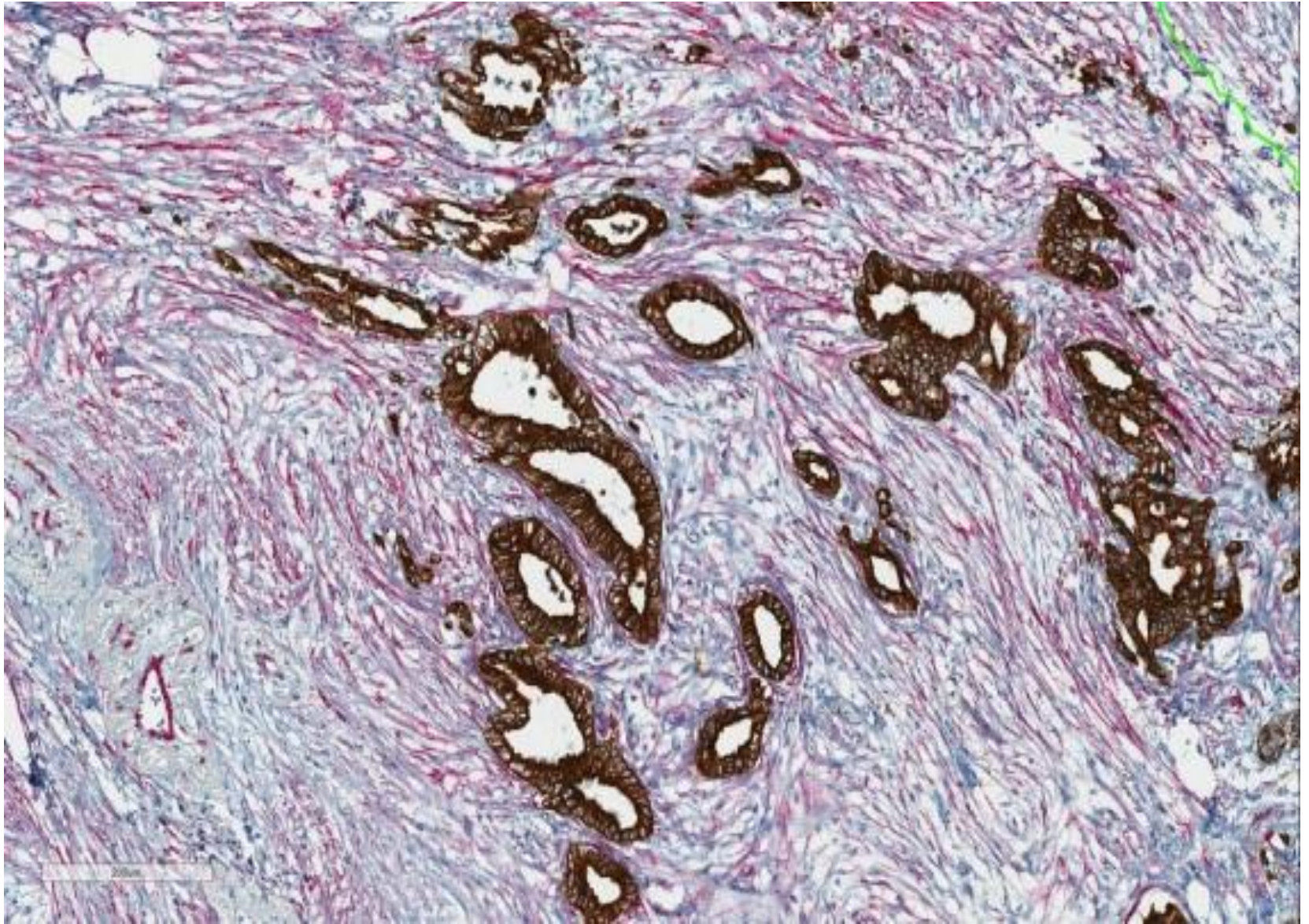


CAF^{FSP1}
Metastatic colonization
Macrophage infiltration
Protection of epithelial cells from carcinogen

CAF^{PDGFR-β}
Metastatic spread
High interstitial fluid pressure

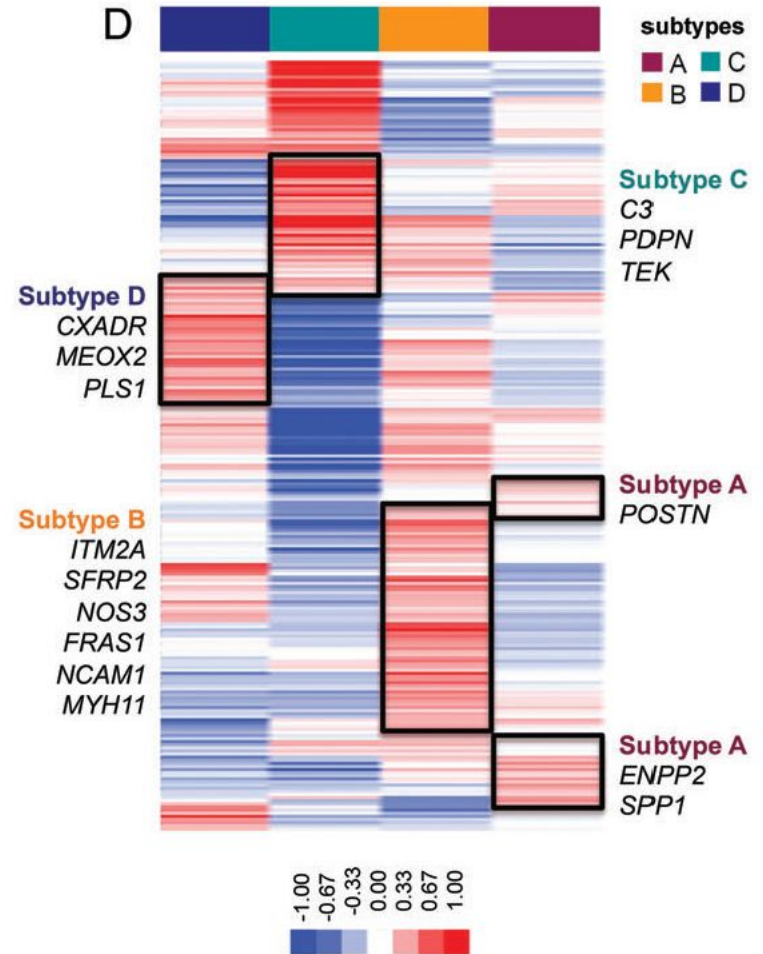
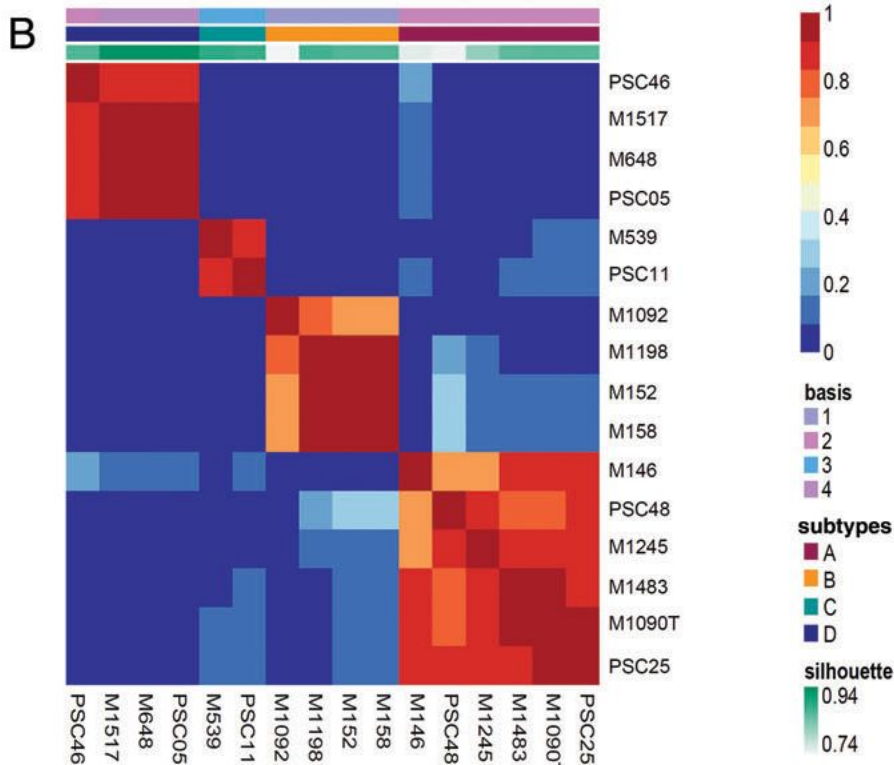


Intra-tumor heterogeneity - stroma

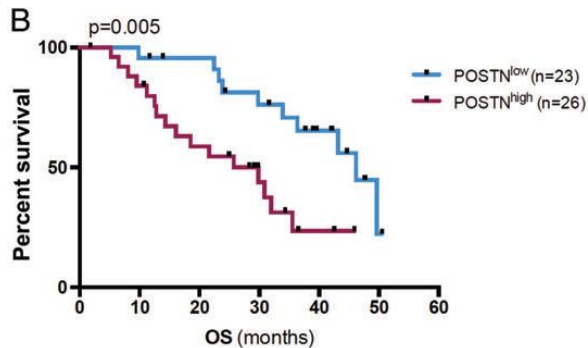
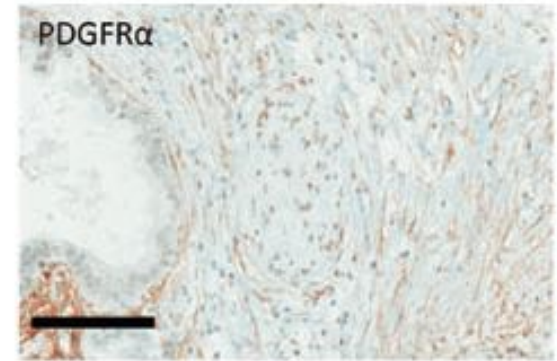
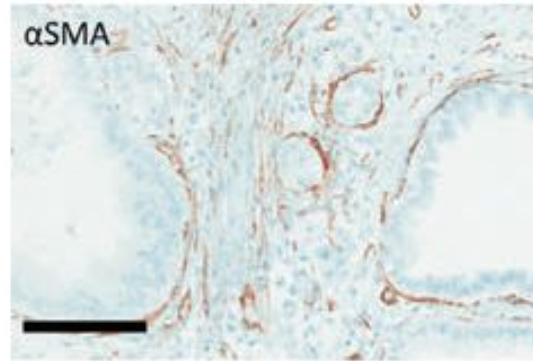
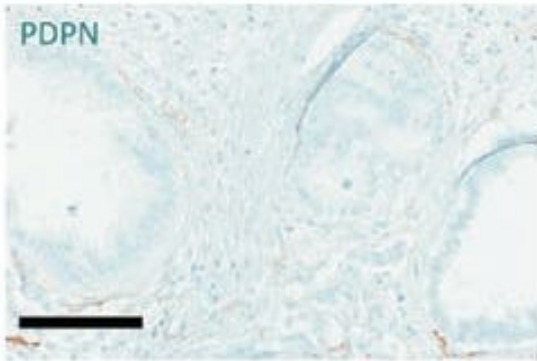
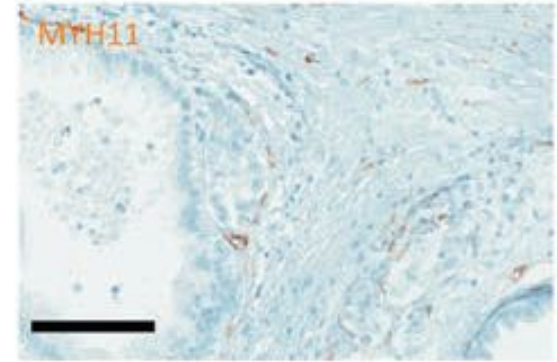
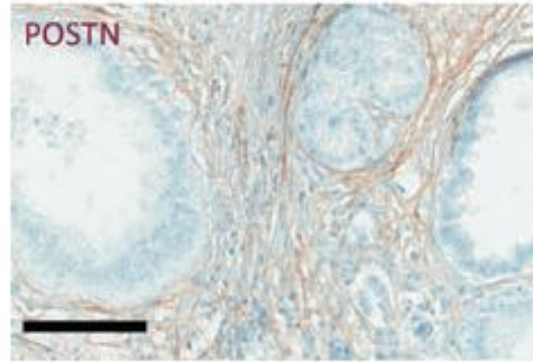
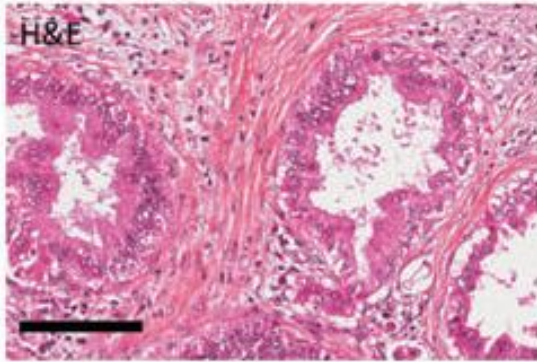


In humans, it is usually more complicated....

Patient-derived CAF



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

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Beaujon Hospital – Université de Paris

jerome.cros@aphp.fr

