

Heterogeneity in pancreatic adenocarcinoma

Does it happen? Is it important?

Jerome Cros

Dpt of Pathology – INSERM U1149

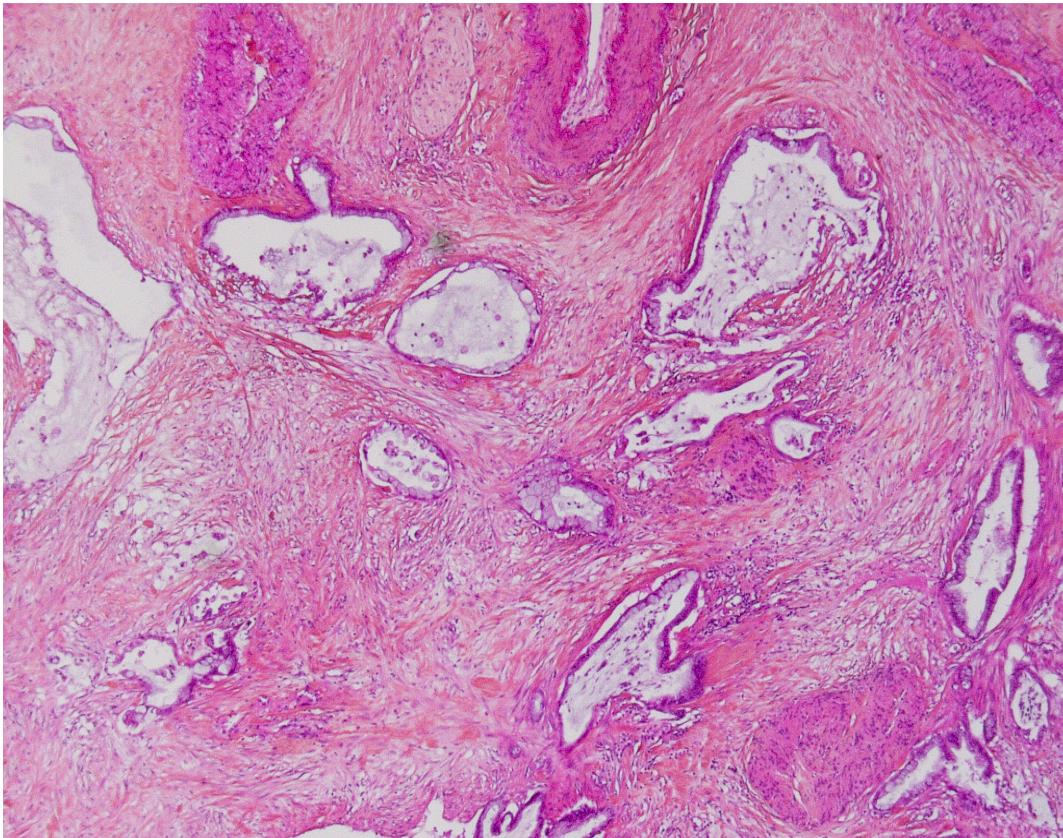
Beaujon Hospital, Paris, France

Study of tumor heterogeneity and issues with samples....

1. Beware of pancreatic non-PDAC tumor when using public datasets++++

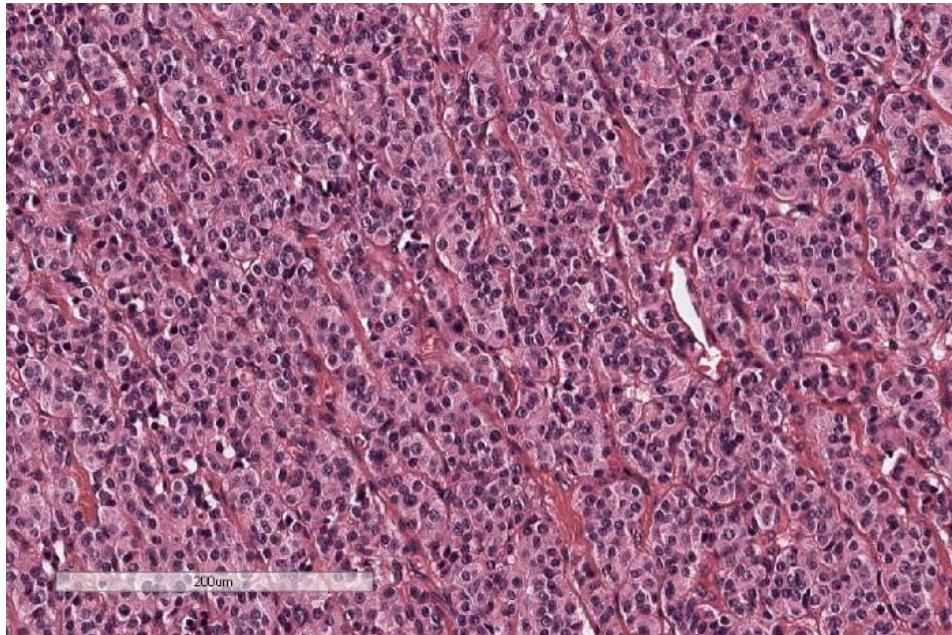
Pancreatic cancer

Adenocarcinoma (90%)



Pancreatic cancer

Adenocarcinoma (90%)

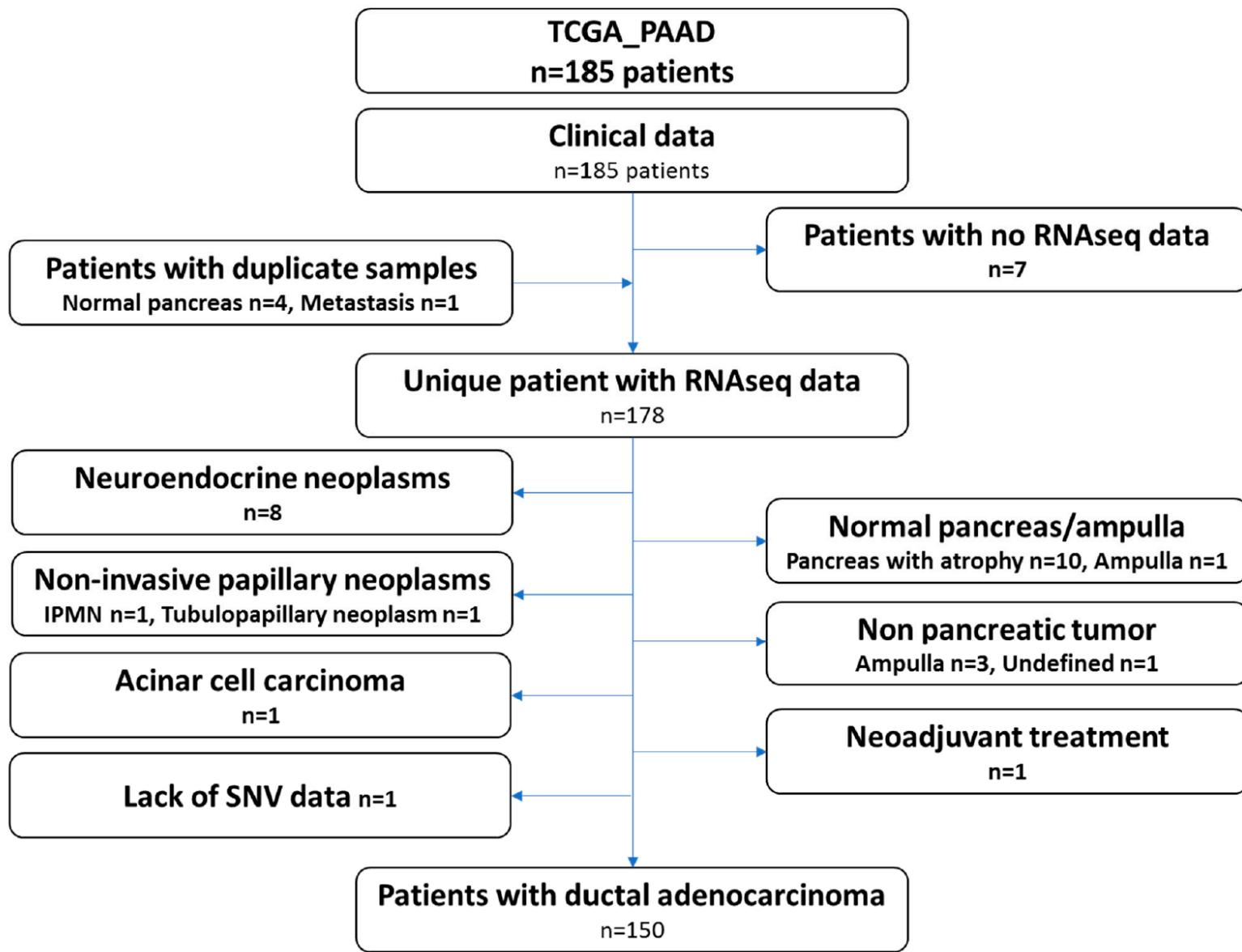


Neuroendocrine
lesions

Rare lesions
Acinar cell
carcinoma....

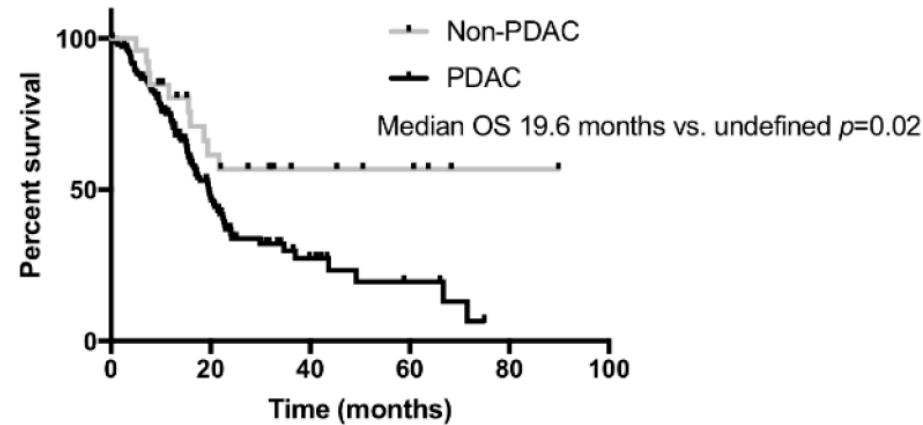
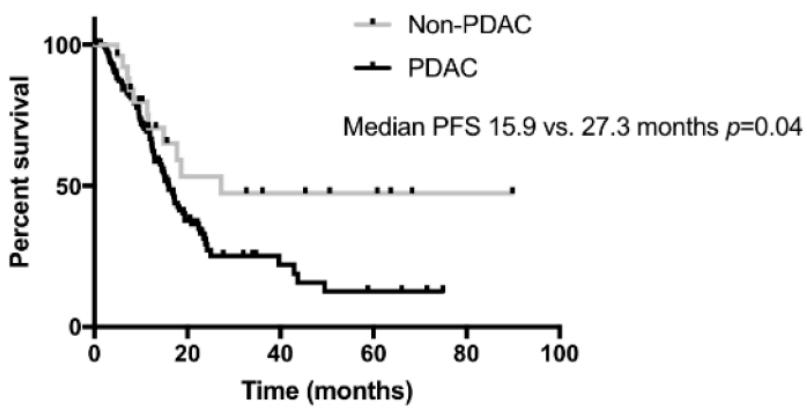
Completely different
morphology, biology....

Beware of pancreatic non-PDAC tumor when using public datasets++++

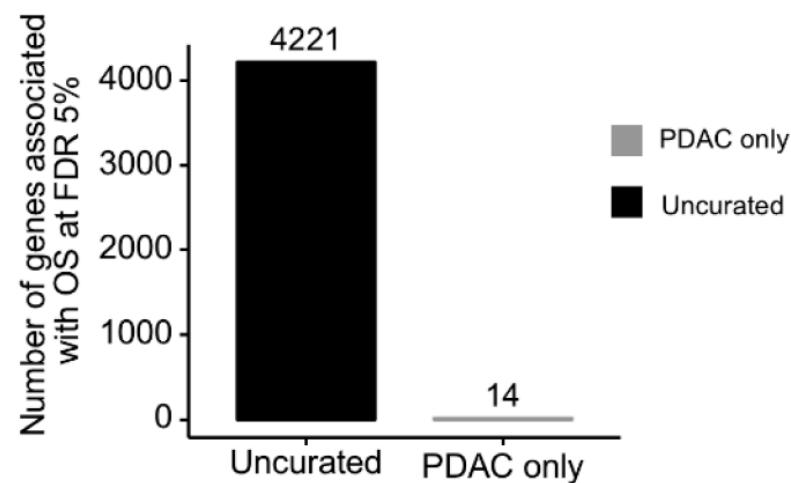
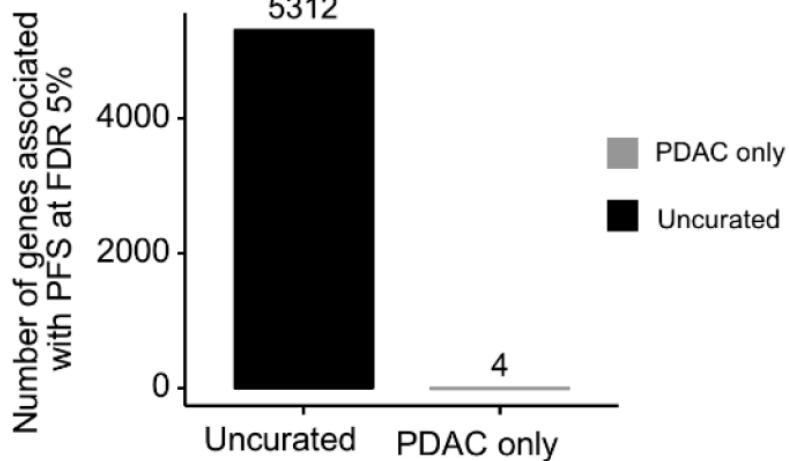


Beware of pancreatic non-PDAC tumor when using public datasets++++

a

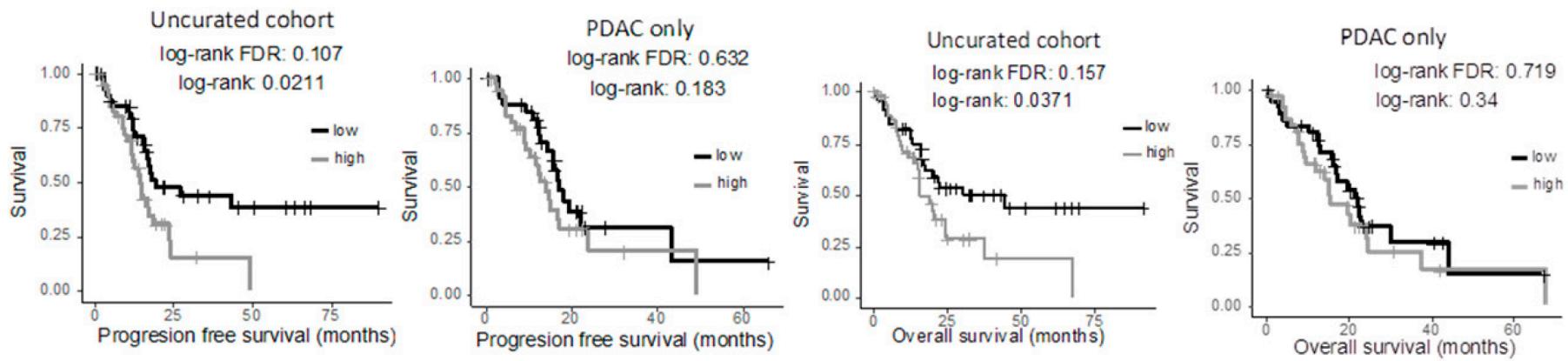
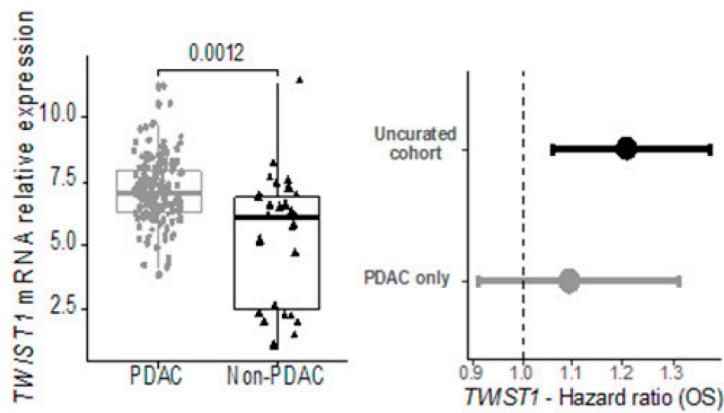


b

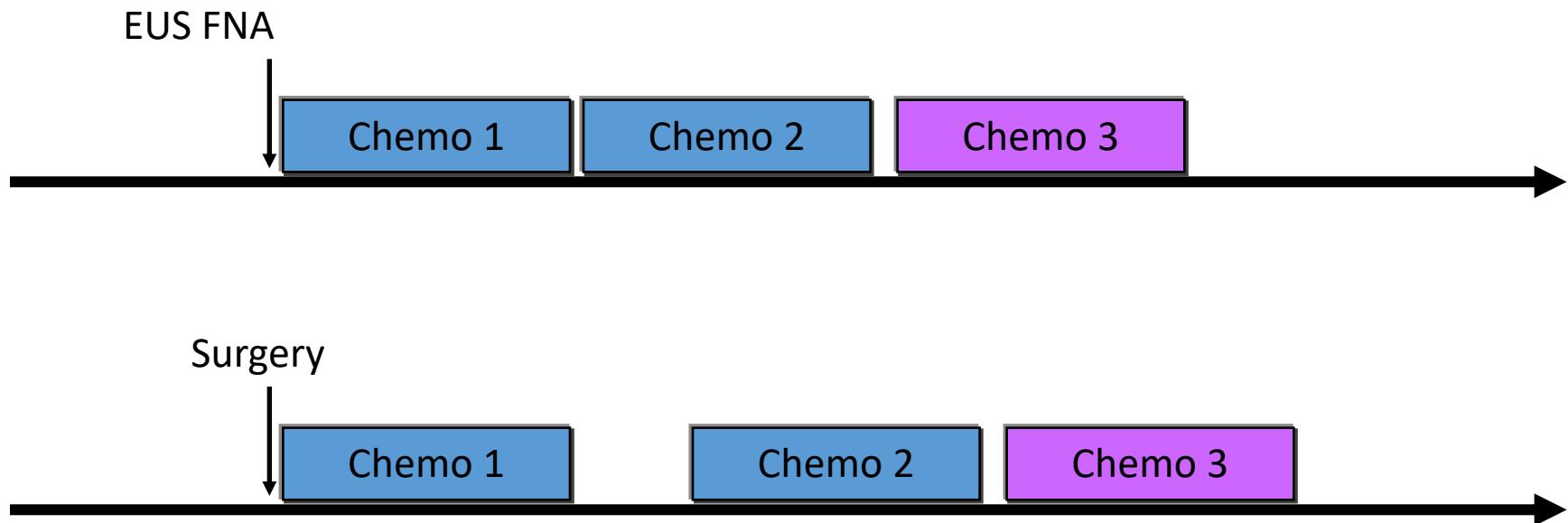


Beware of pancreatic non-PDAC tumor when using public datasets++++

a

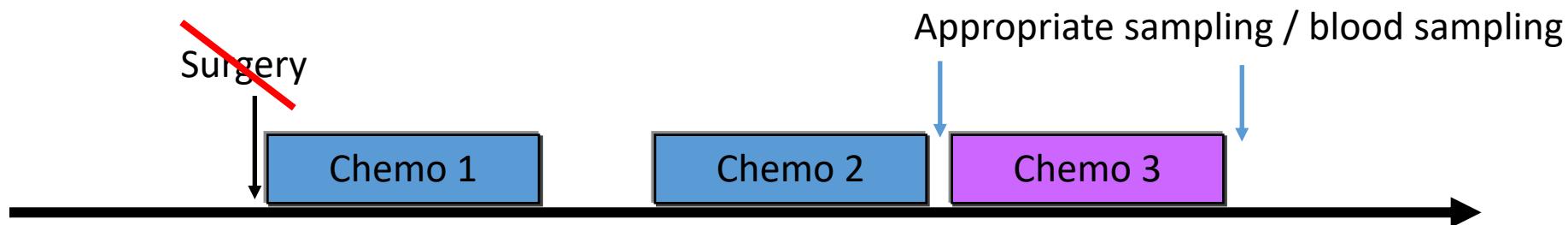
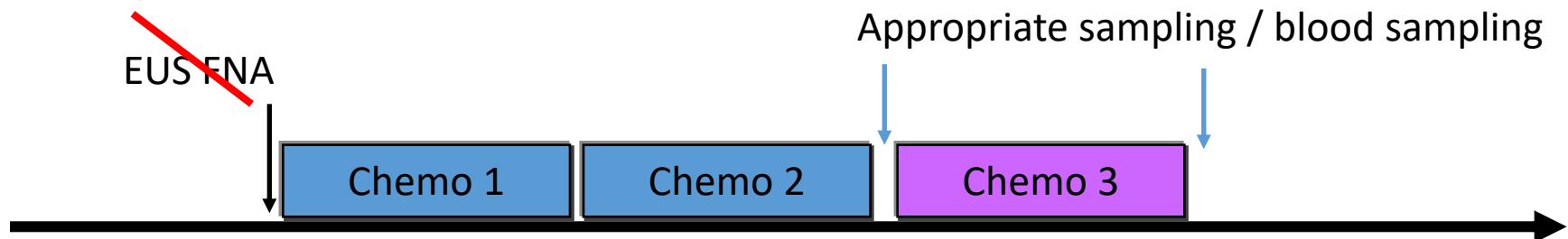


2. Beware of the tissue samples used for biomarker/signature development



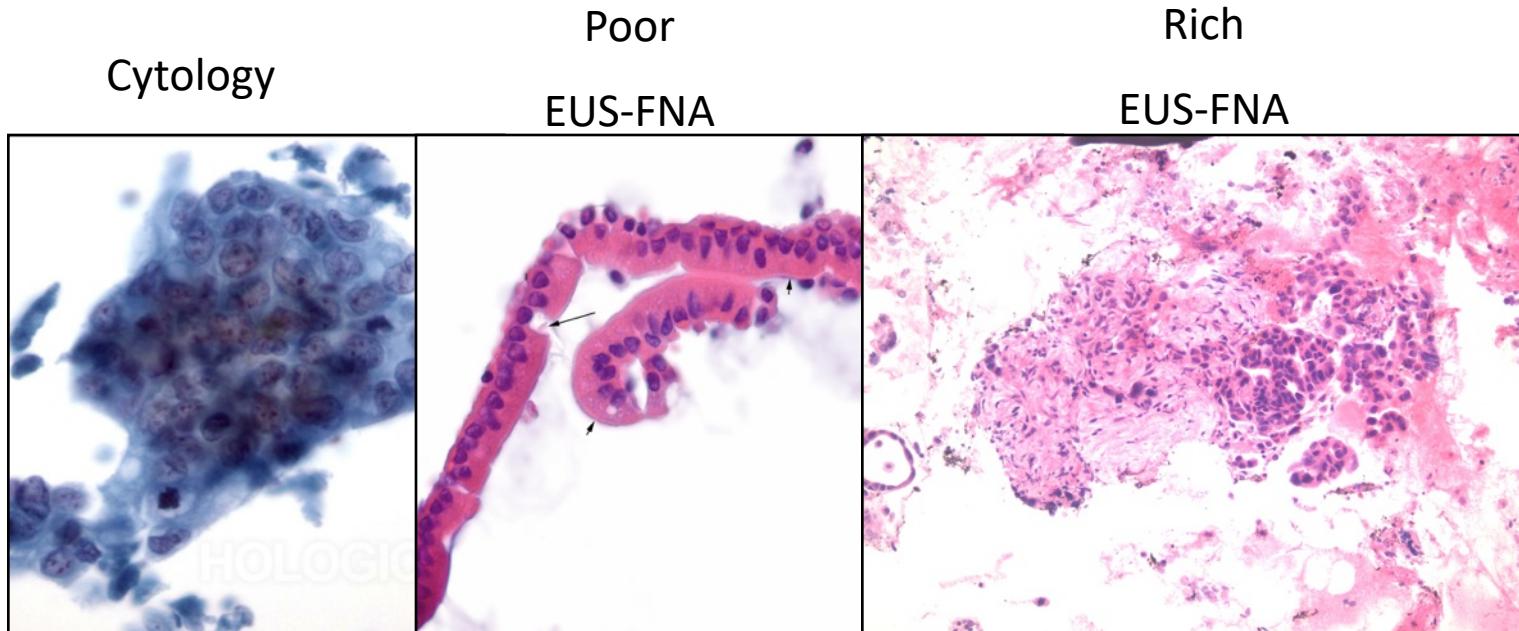
- Clonal selection induced by multiple therapies

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?

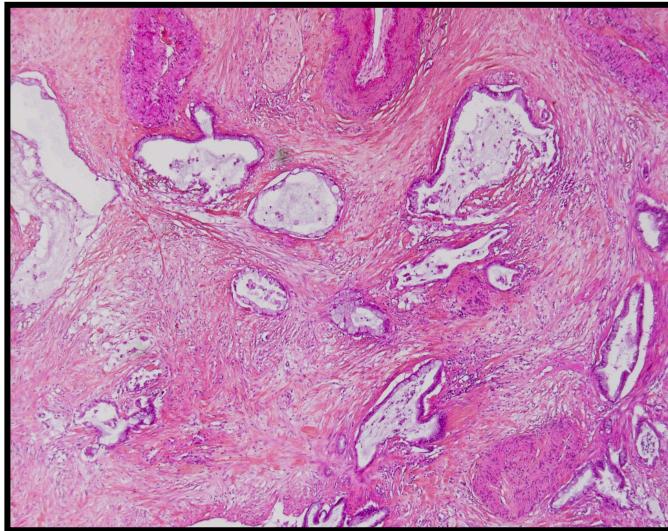


Protein on Tum cells	OK	OK	OK
Mutation on Tum cell	+/- OK	++/- OK	OK
Exp (mi)ARN	+/- OK (richness)	+/- OK (richness)	+/- OK (richness)
Protein in stroma	NON	NON	+/- OK

Liver biopsy++, true cut +++

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

Surgical specimen

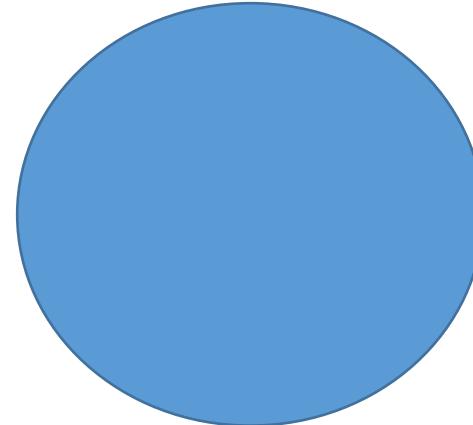


Tumor
+/- sphere
3cm diameter

14cm^3

Frozen carrot
0.6cm wide
0.4 cm thick

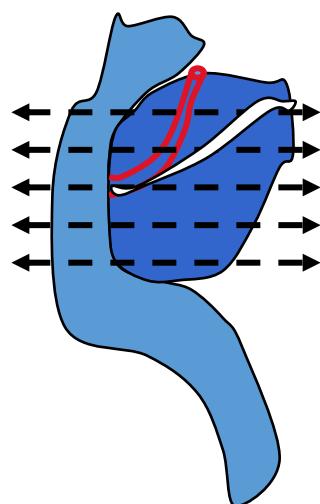
0.11cm^3



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

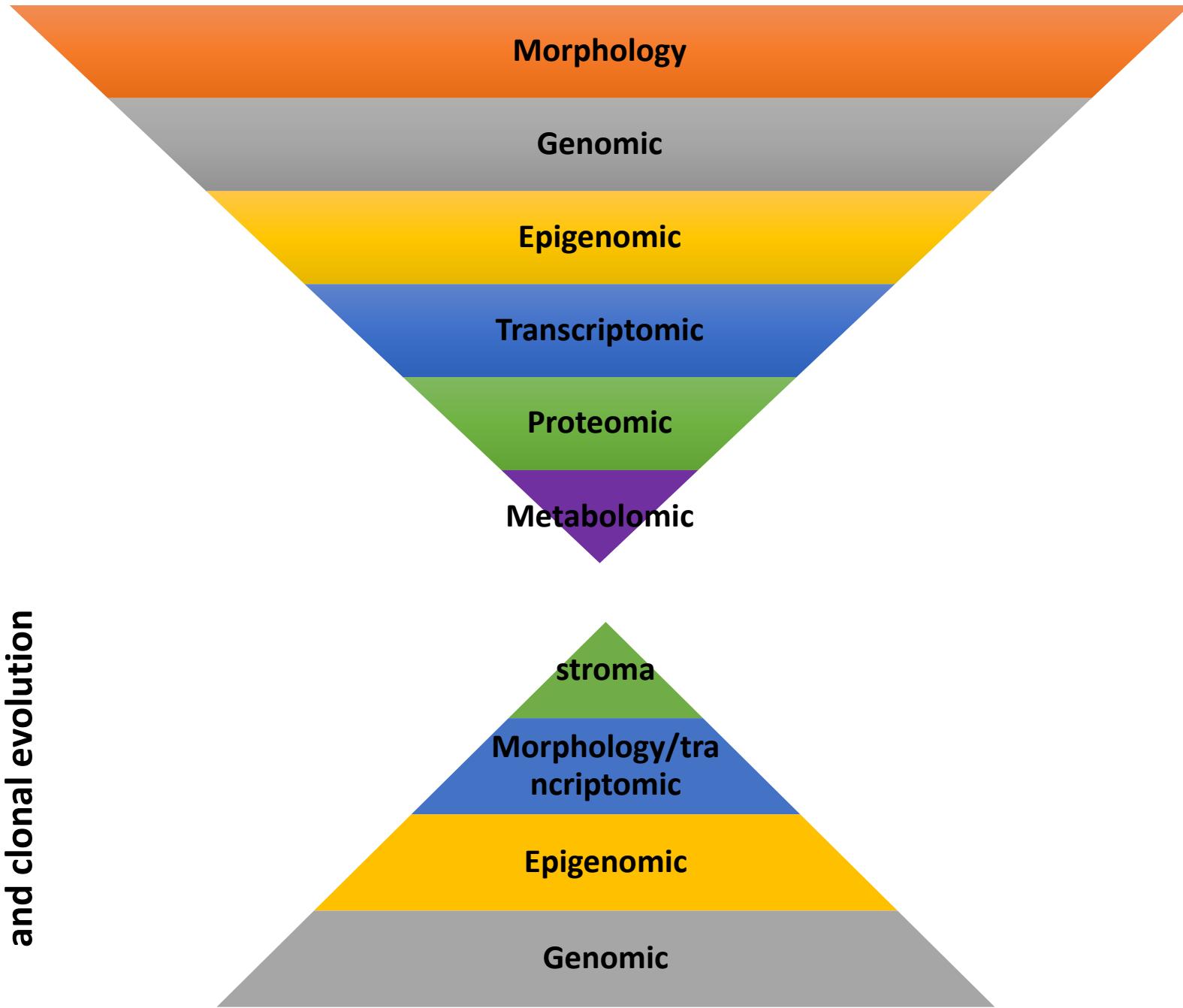
Spatialized sampling...

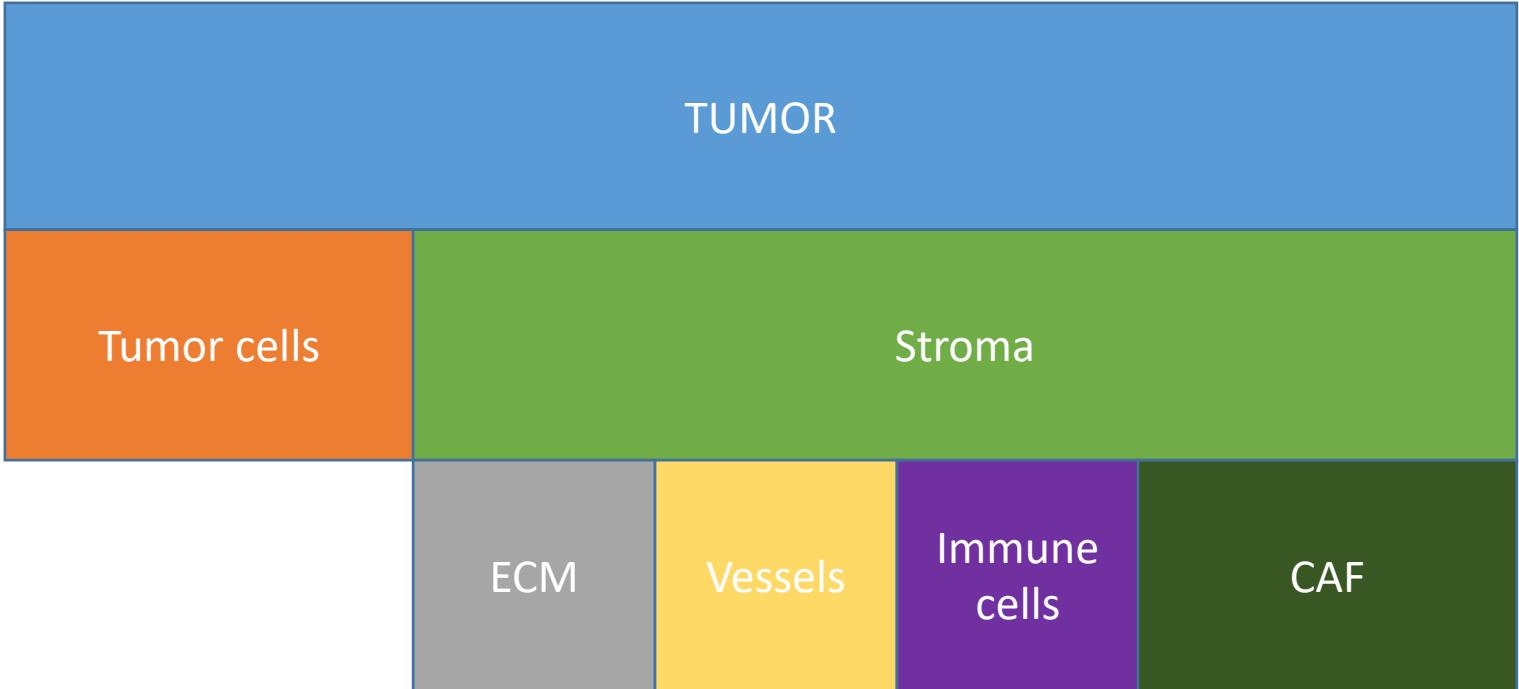
...yes but usually with formalin fixed paraffin embeded samples...



Inter-tumor heterogeneity

Intratumor heterogeneity
and clonal evolution

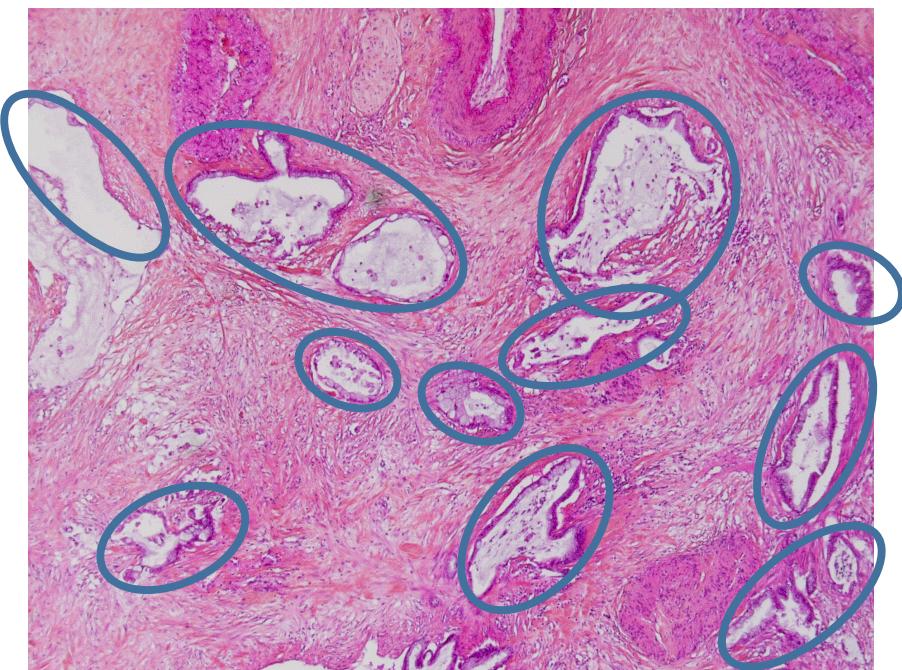




PANCREATIC ADENOCARCINOMA

Tumor cells

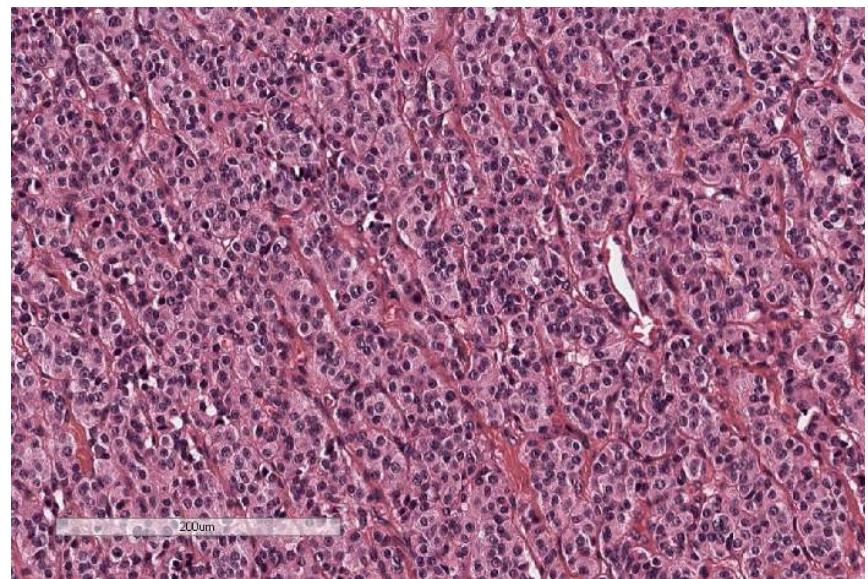
Stroma



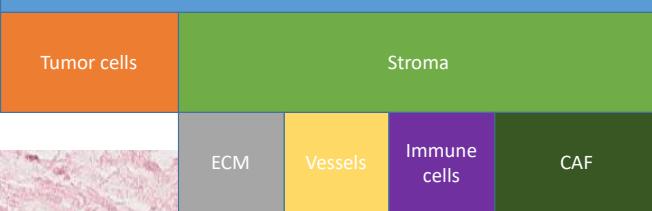
PANCREATIC NEUROENDOCRINE TUMOR

Tumor cells

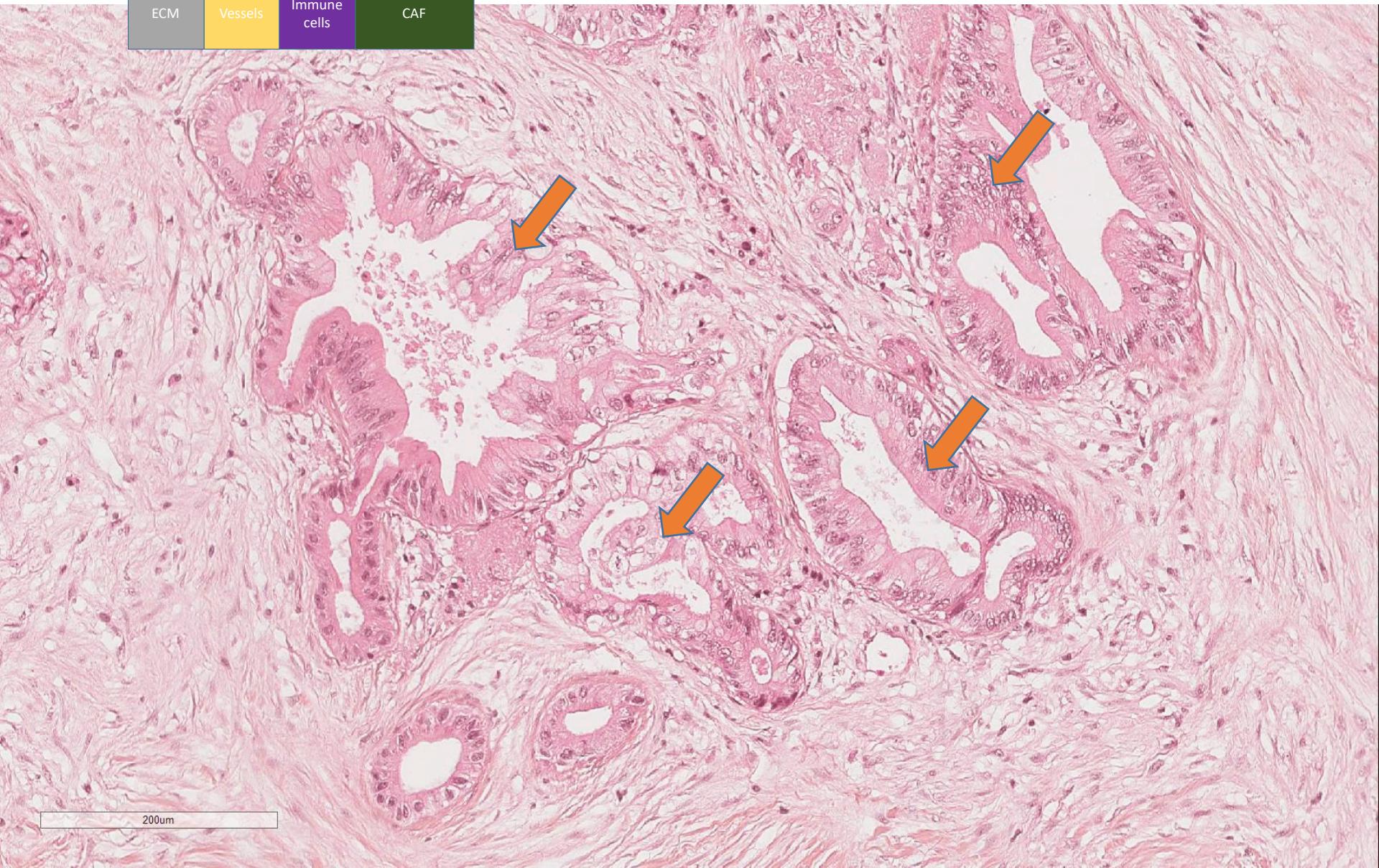
Stroma



TUMOR

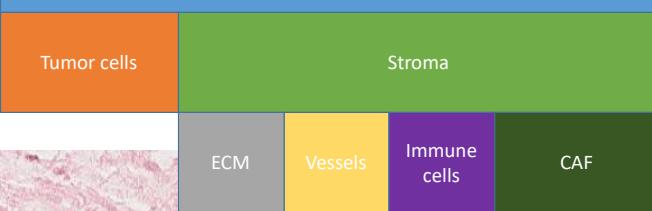


Pancreatic adenocarcinoma

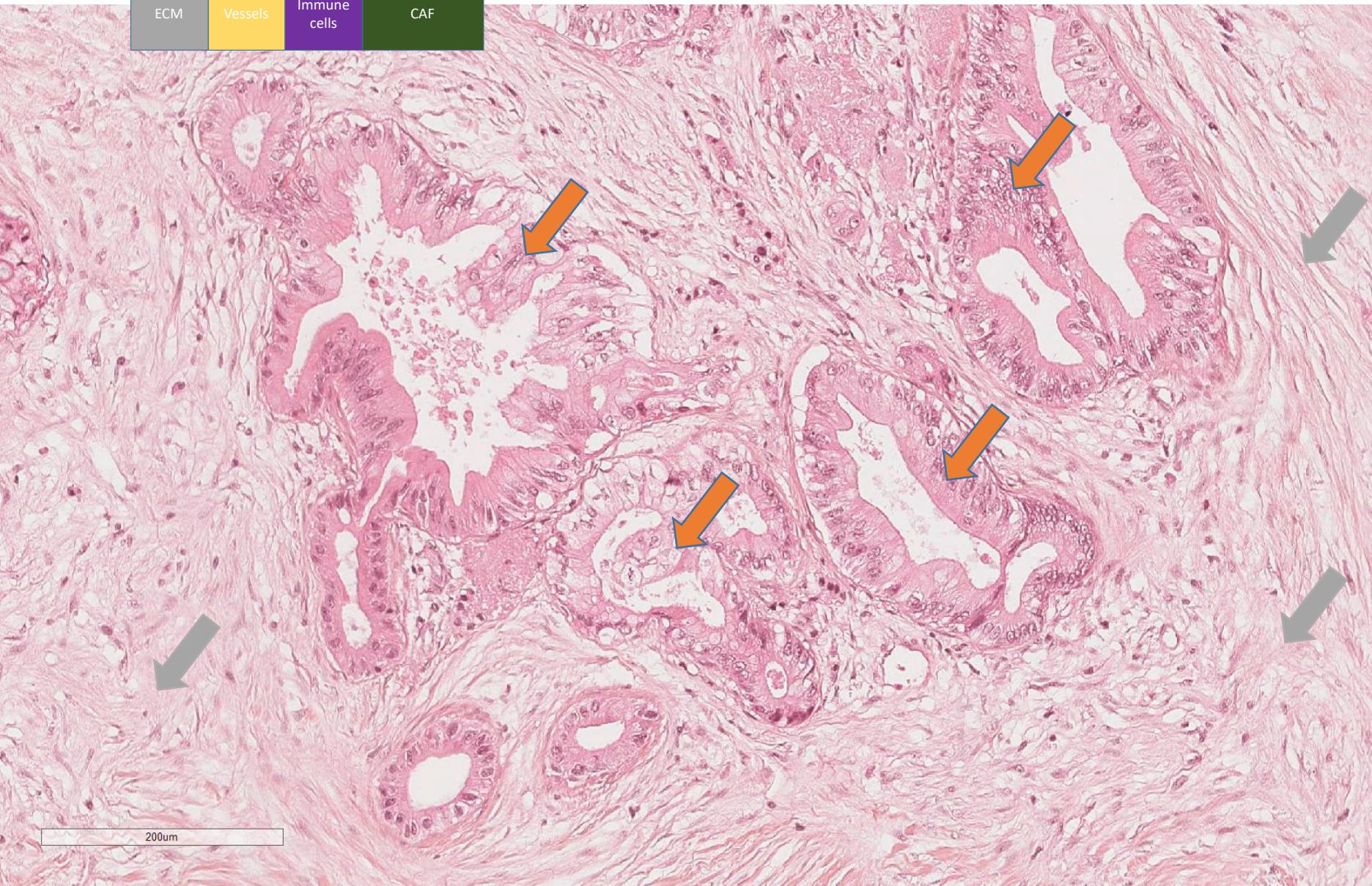


200um

TUMOR

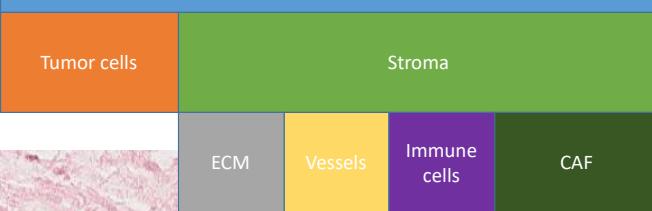


Pancreatic adenocarcinoma

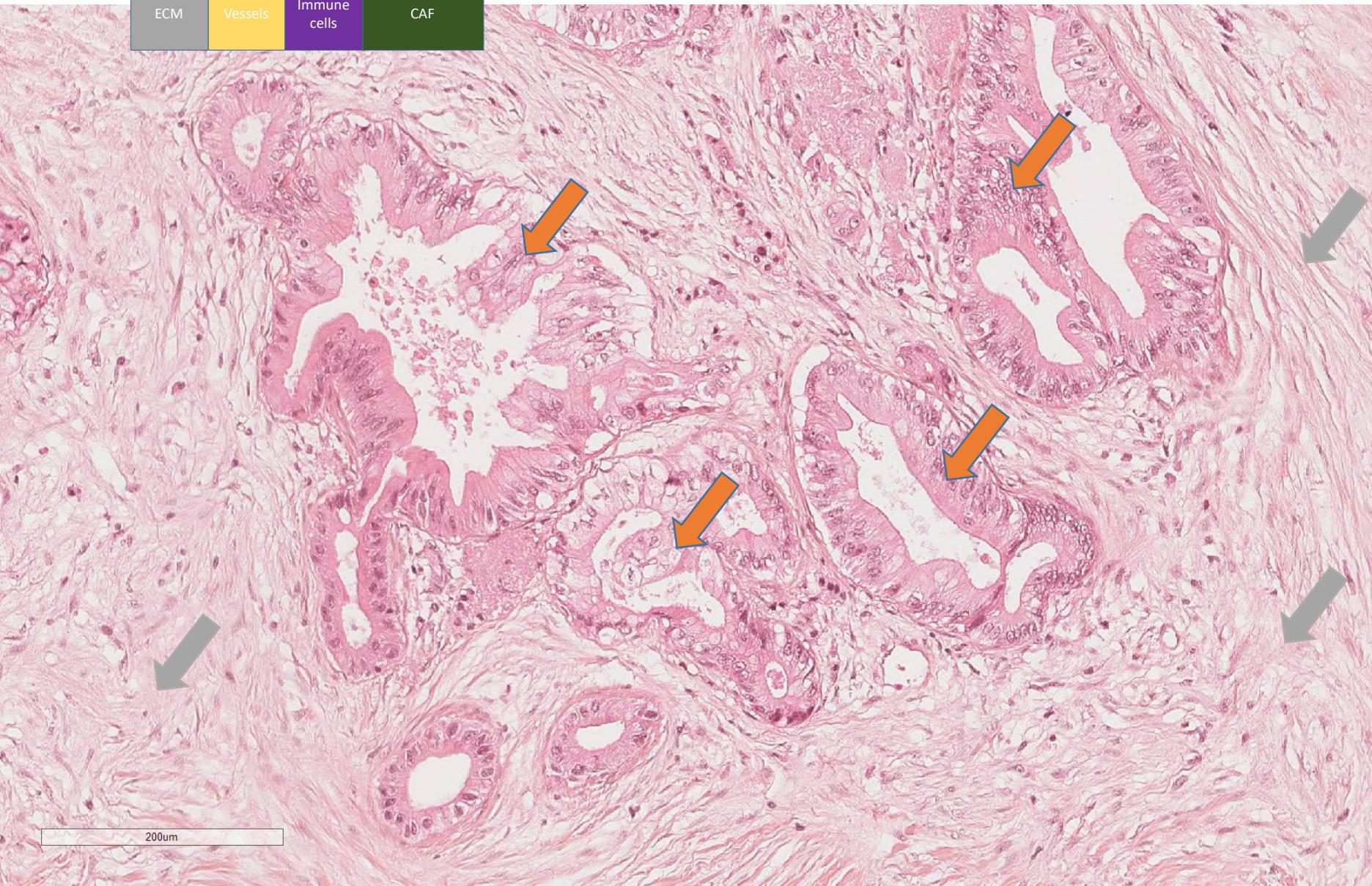


200um

TUMOR



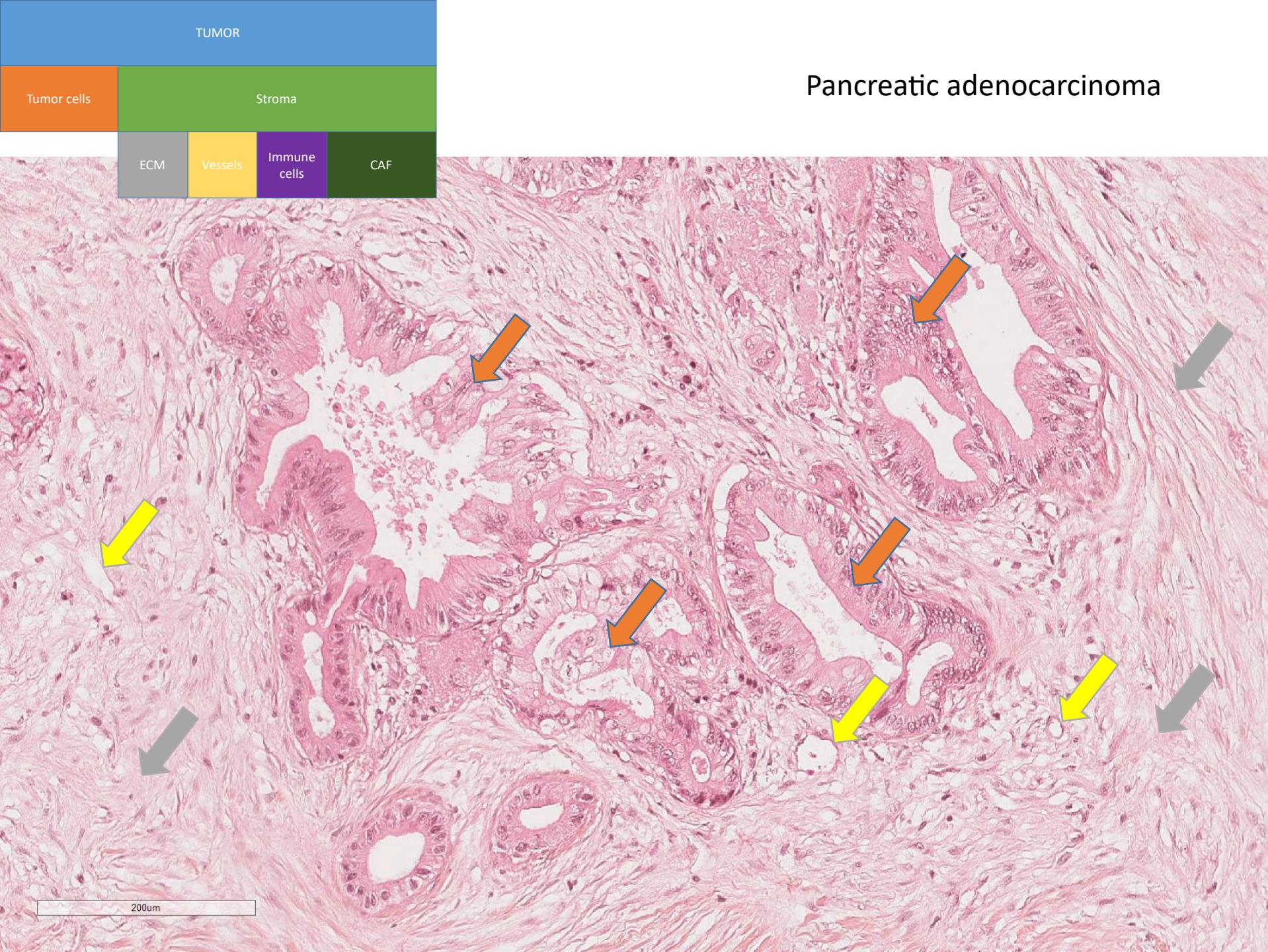
Pancreatic adenocarcinoma



200um

TUMOR

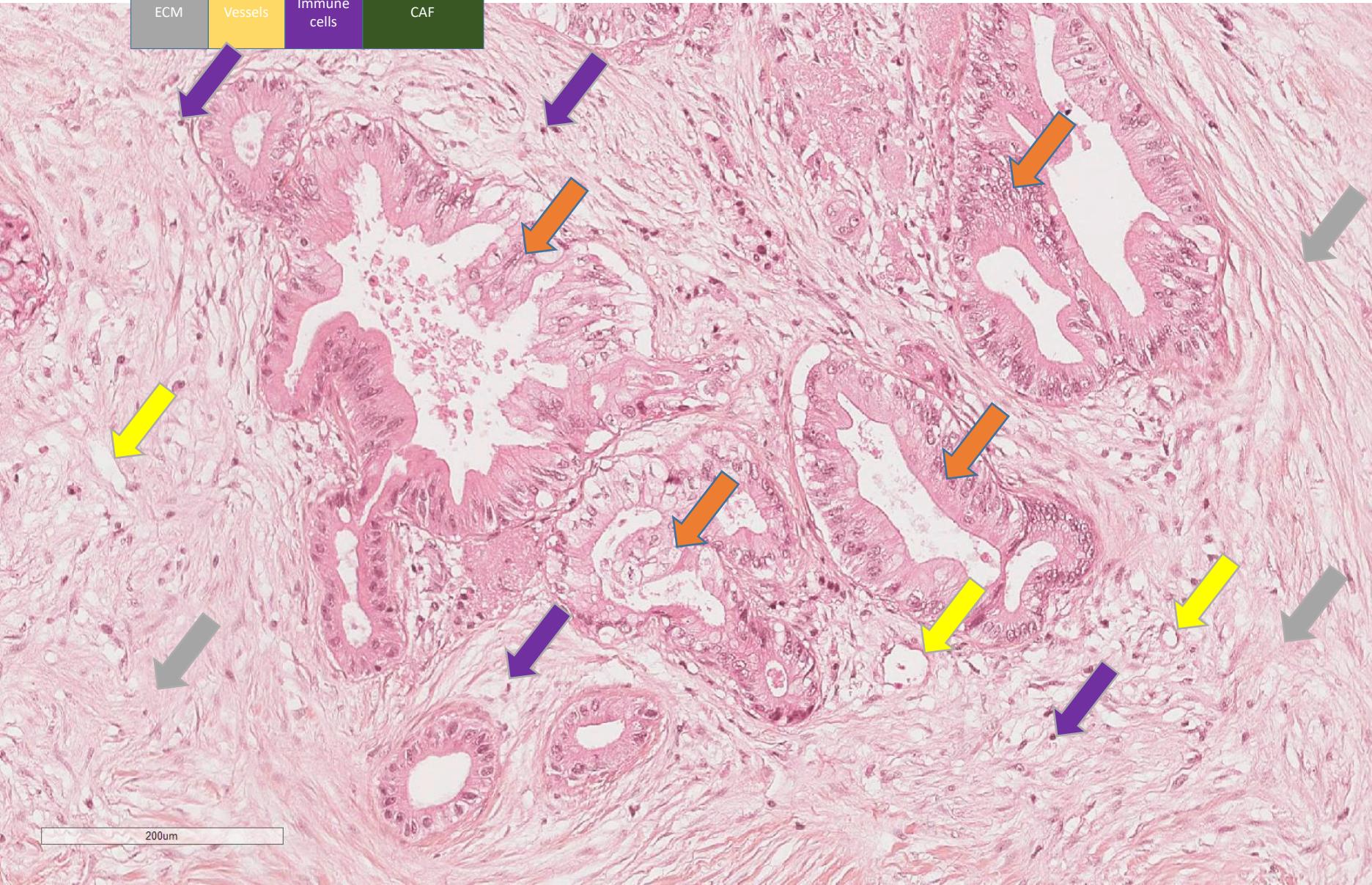
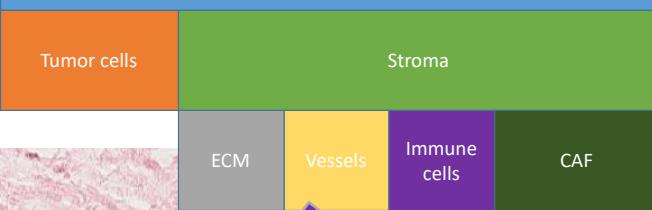
Pancreatic adenocarcinoma



200um

TUMOR

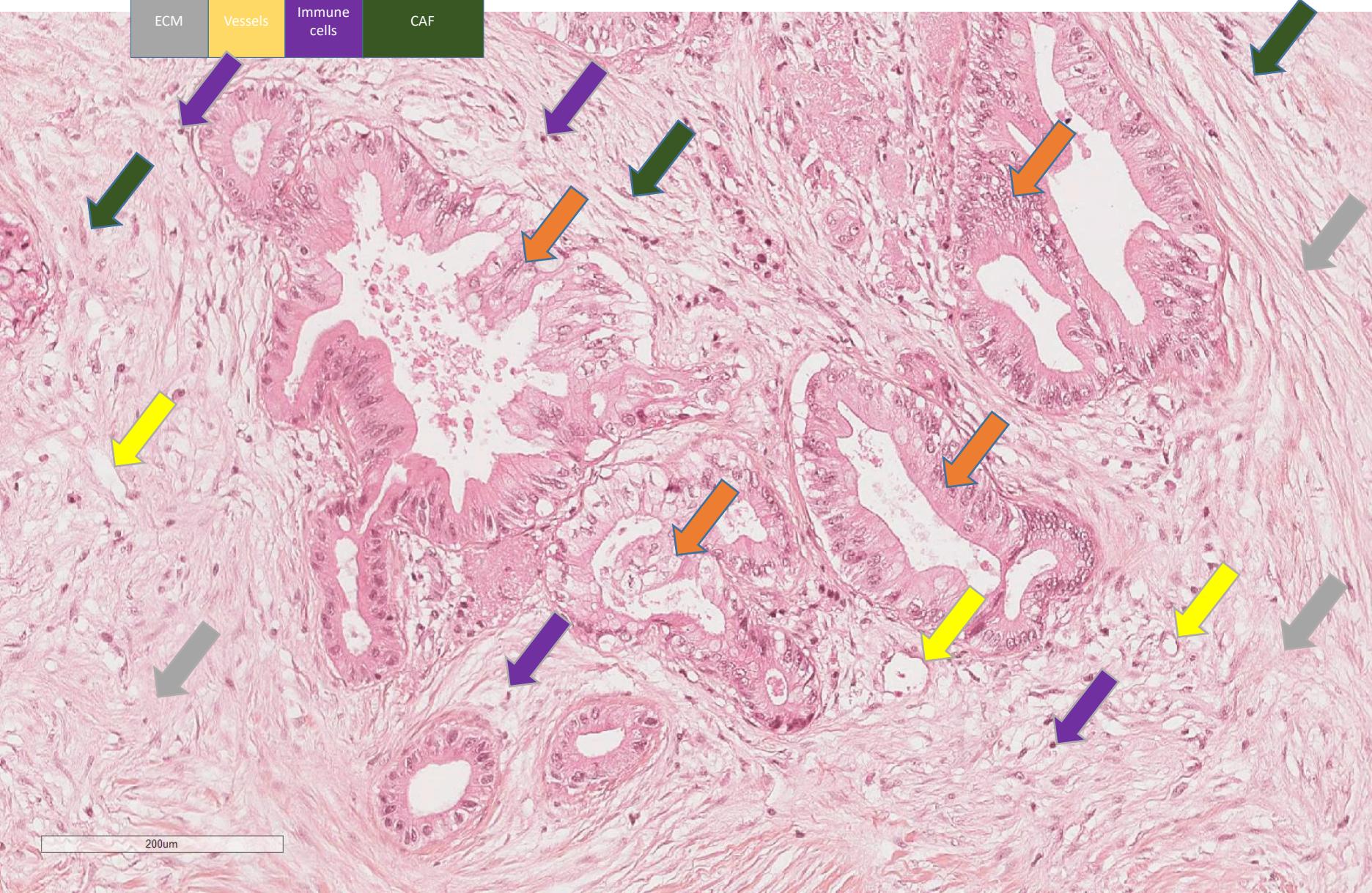
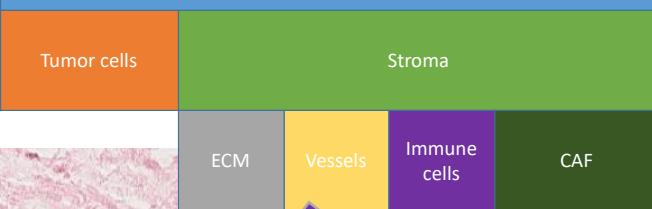
Pancreatic adenocarcinoma



200um

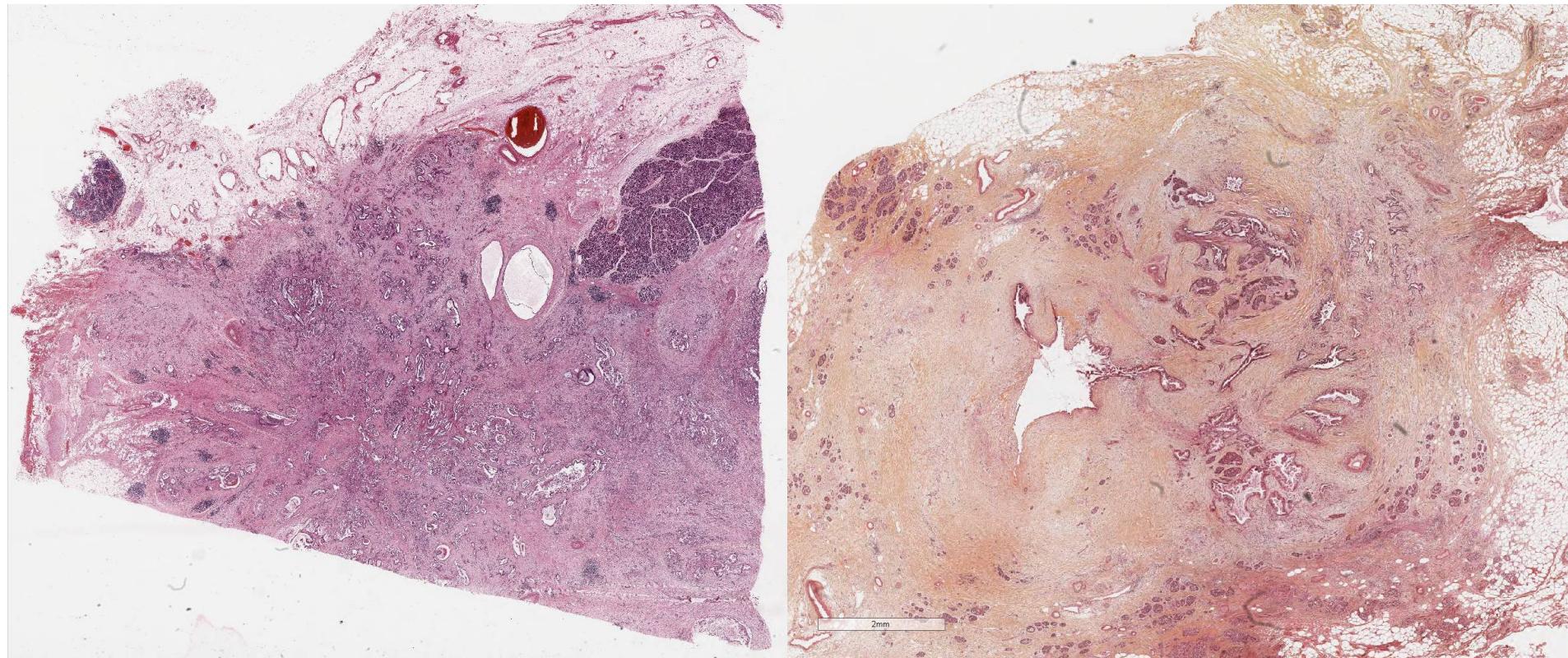
TUMOR

Pancreatic adenocarcinoma



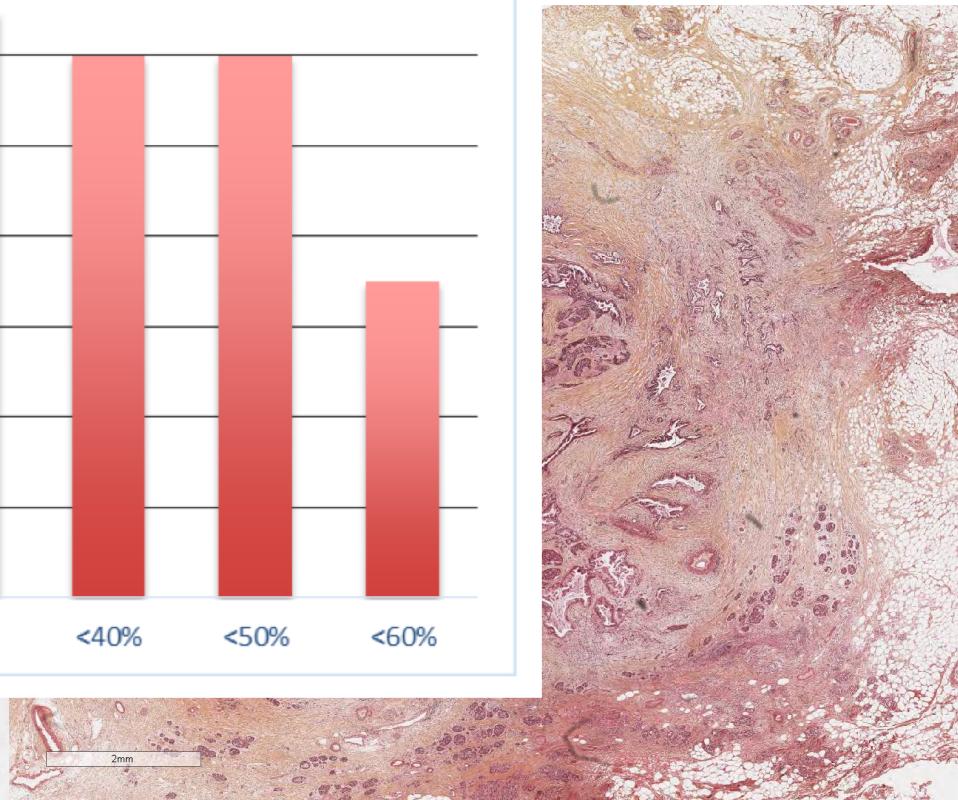
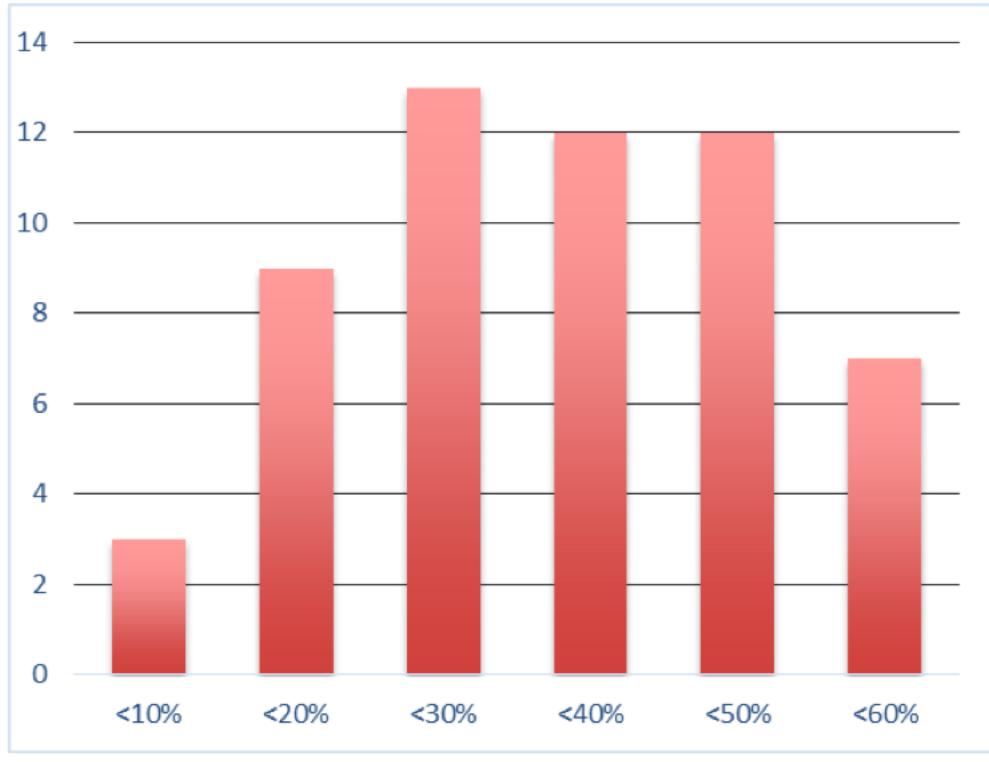
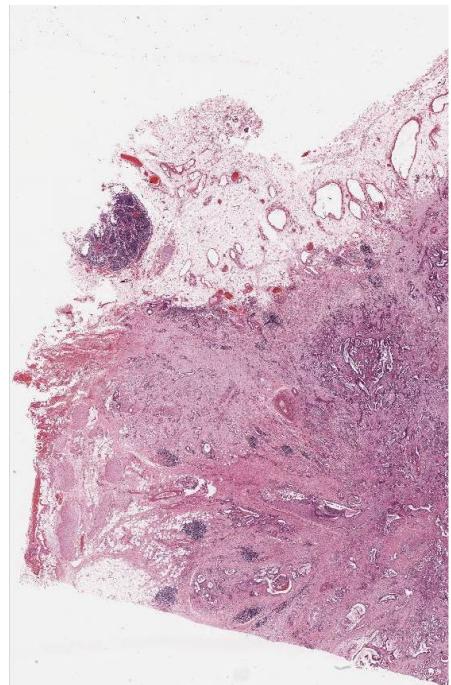
200um

Inter-tumor morphological heterogeneity



Heterogeneity of the tumor cell – stroma ratio

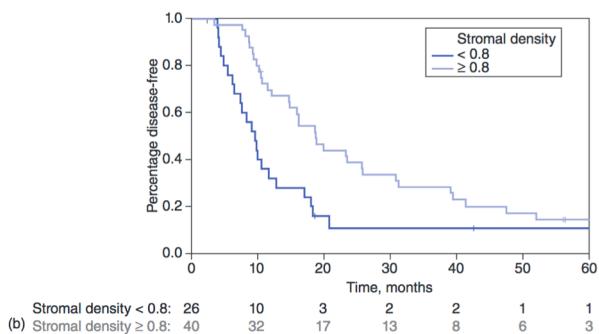
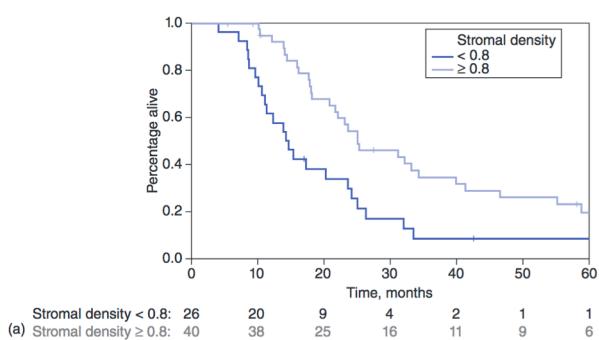
Inter-tumor morphological heterogeneity



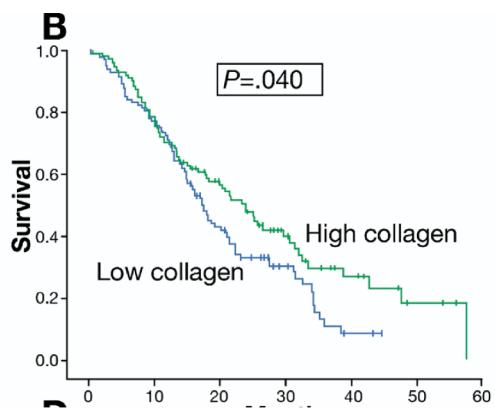
Heterogeneity of the tumor cells – stroma ratio

Inter-tumor morphological heterogeneity

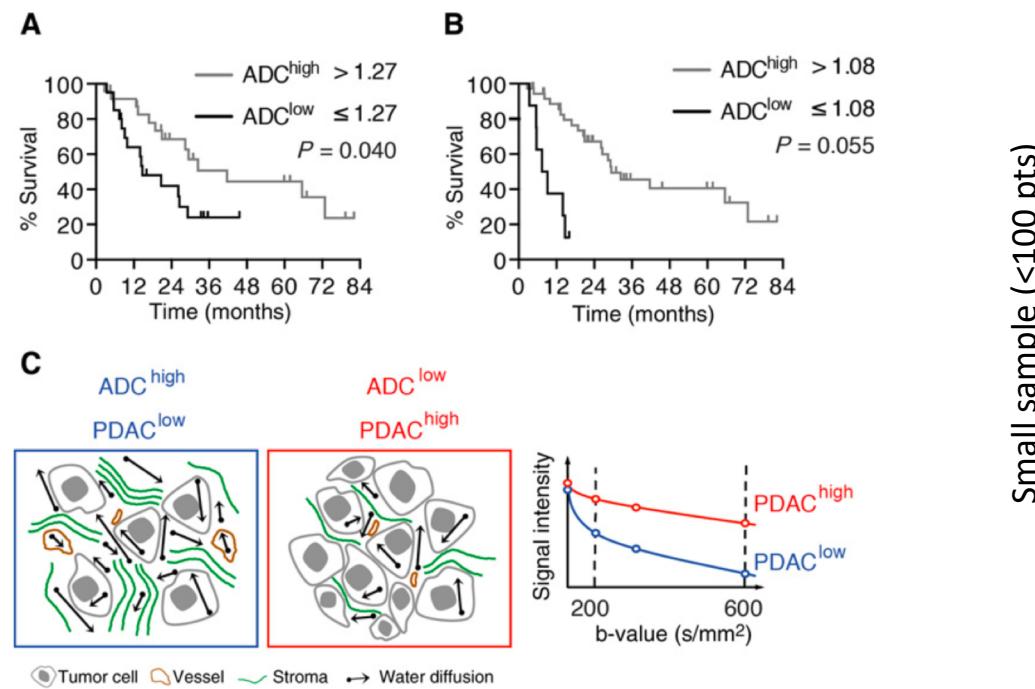
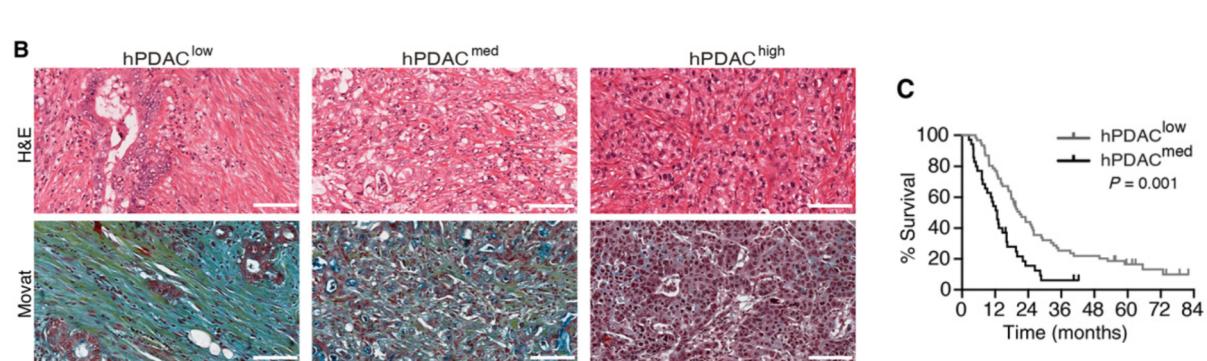
Stroma abundance—pronostic role?



Bever et al. HPB 2015, 292



Erkan et al. Clin gas hep 2008

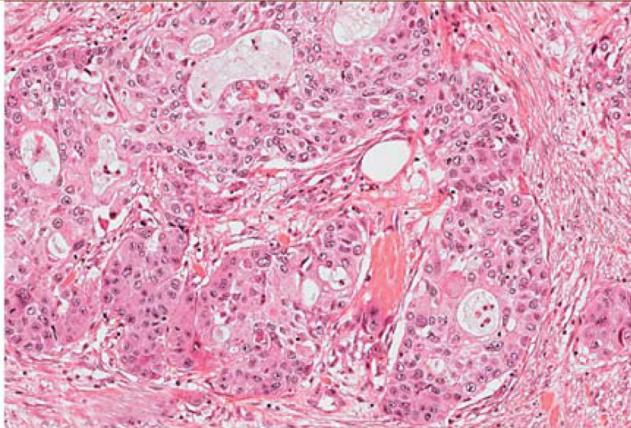
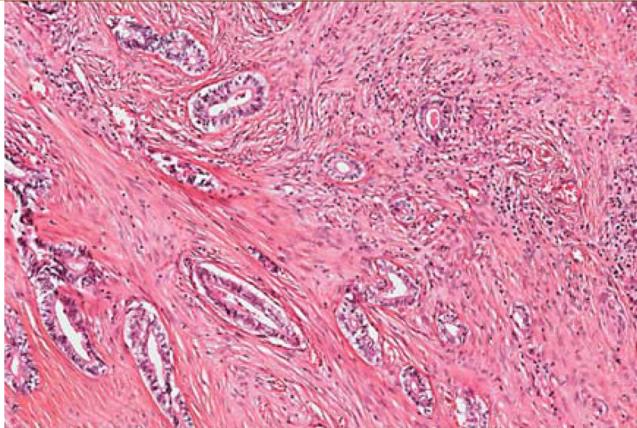


Small sample (<100 pts)

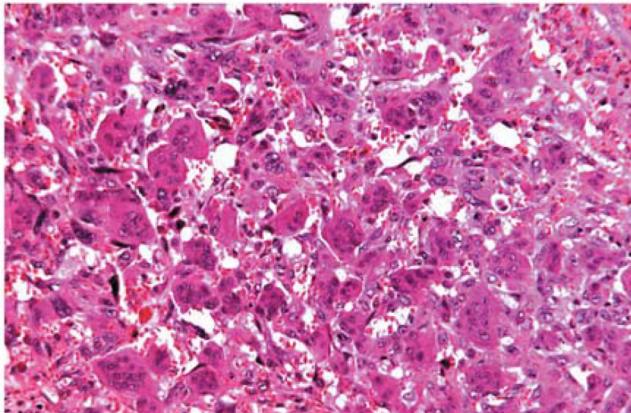
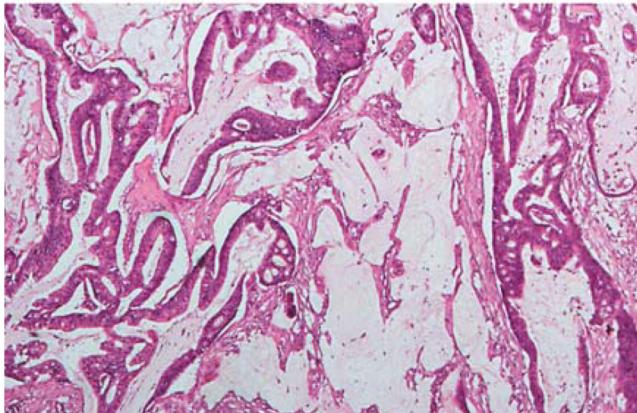
Heid et al. Clin can res 2017, 1461

Inter-tumor morphological heterogeneity

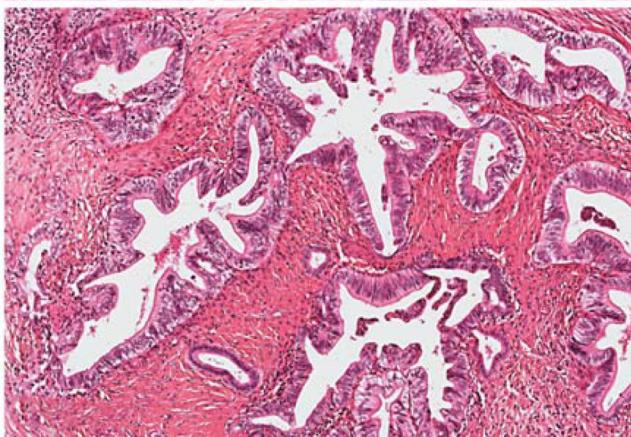
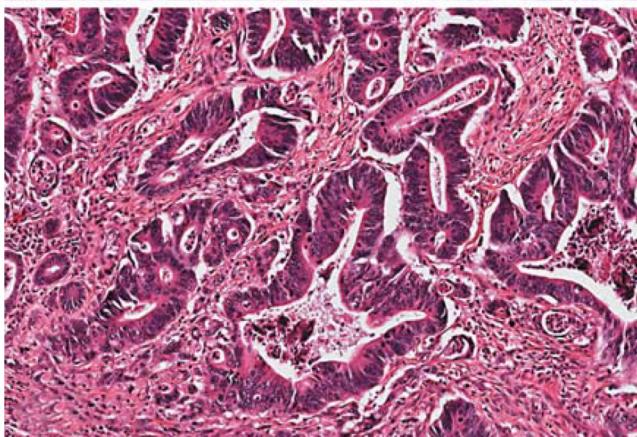
ductal



colloid



intestinal



micropapillary Undifferentiated w/
 giant cells osteocl-like
 adenosquamous

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*?

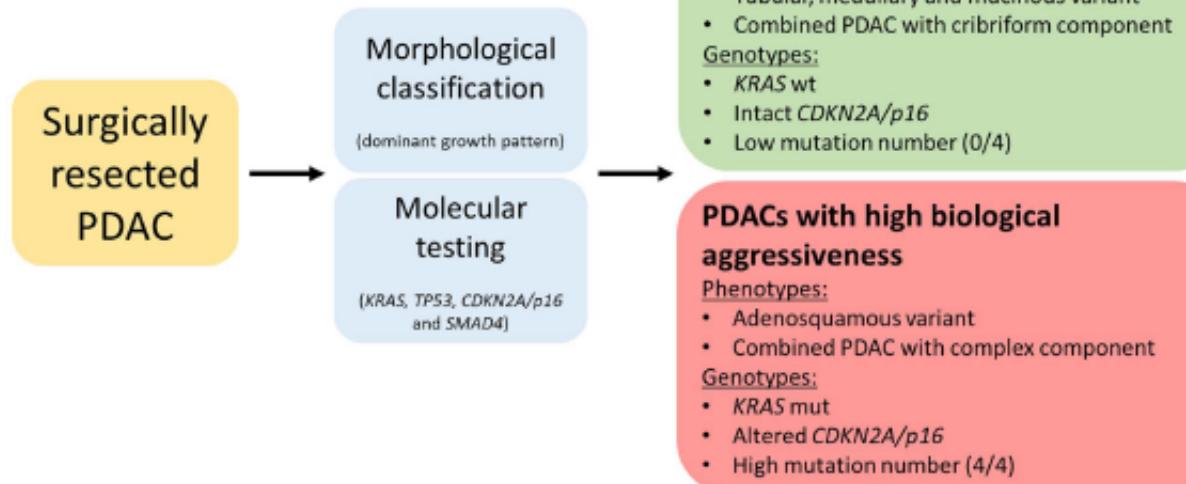
NGS/IHC *KRAS/CDKN2A/SMAD4/TP53*

No major difference

Inter-tumor morphological heterogeneity

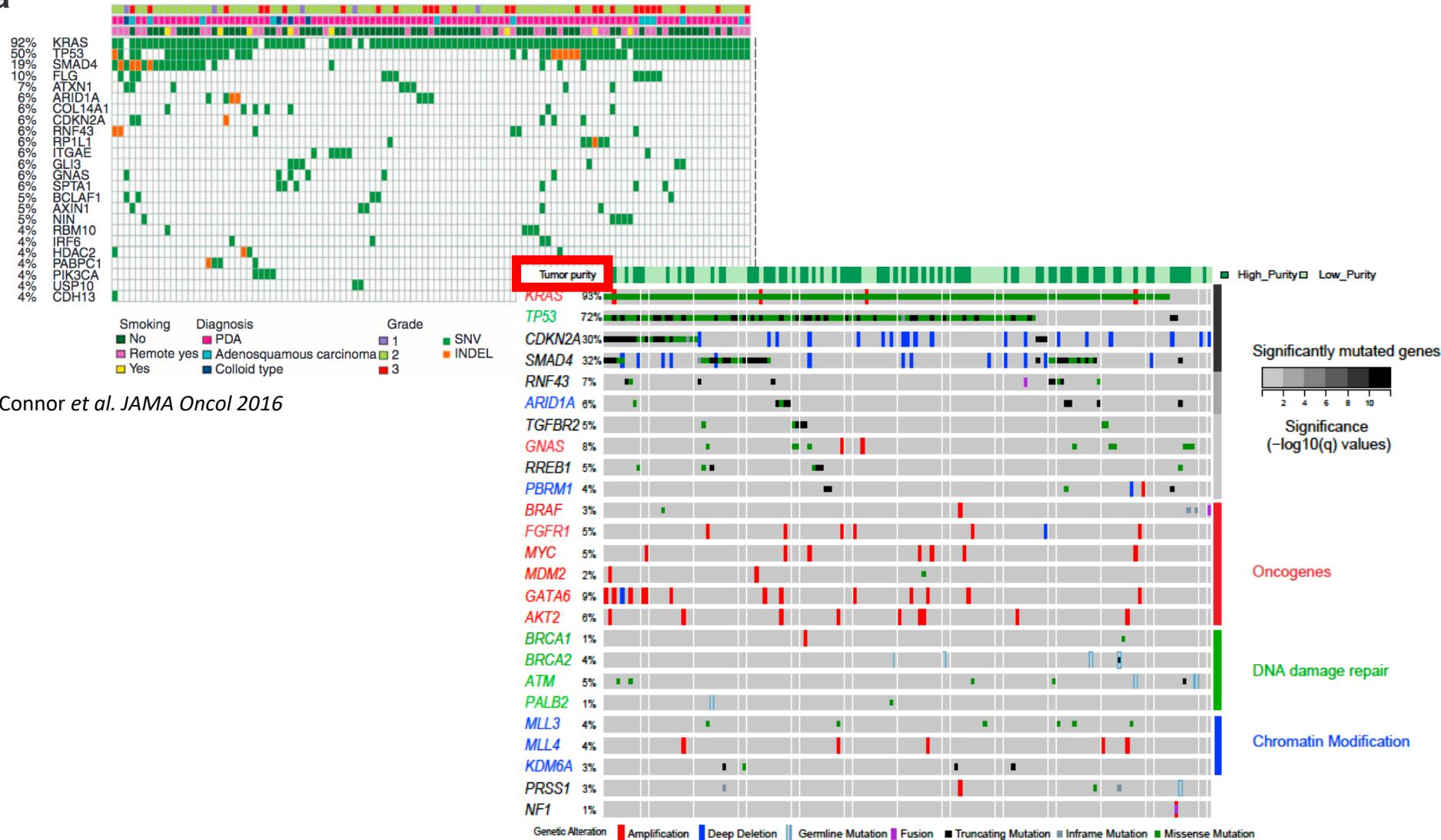
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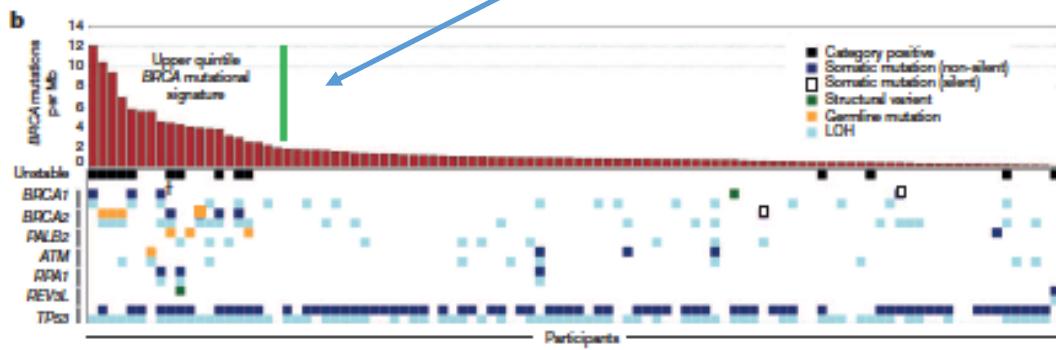
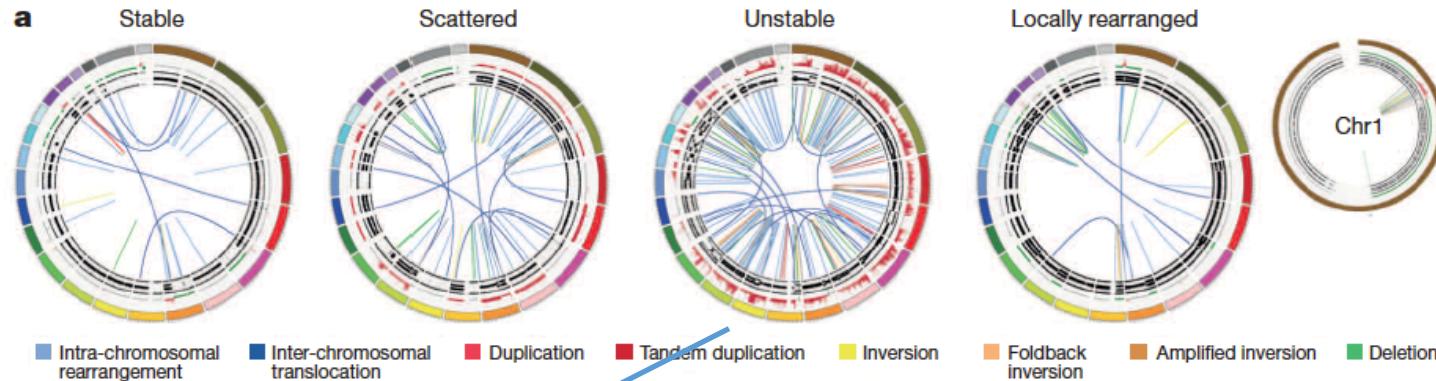


3 large datasets (ICGC (456 pts), TCGA (150 pts), Connor *et al.* (148 pts) – similar results

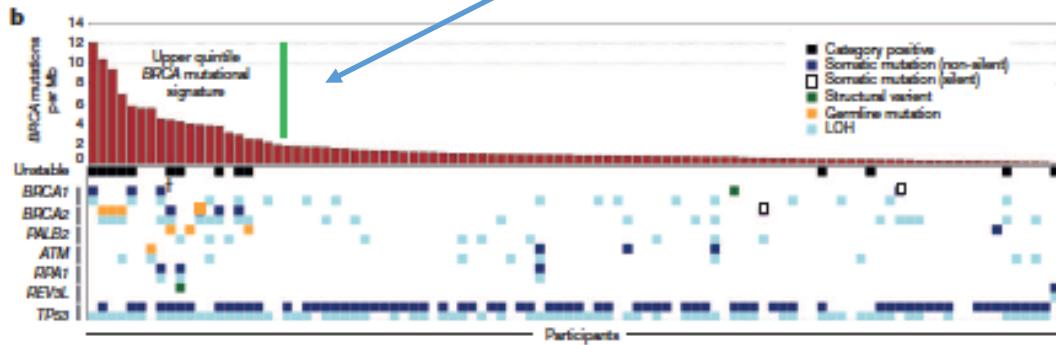
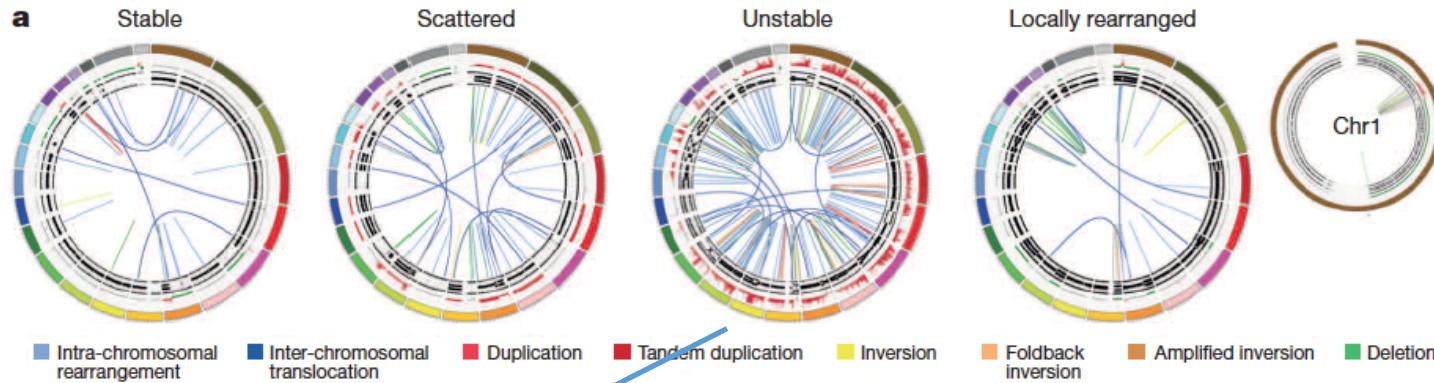
a



Genomic



Genomic



Benefit from PARPi
in maintenance
therapy (POLO trial)

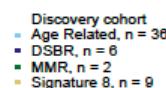
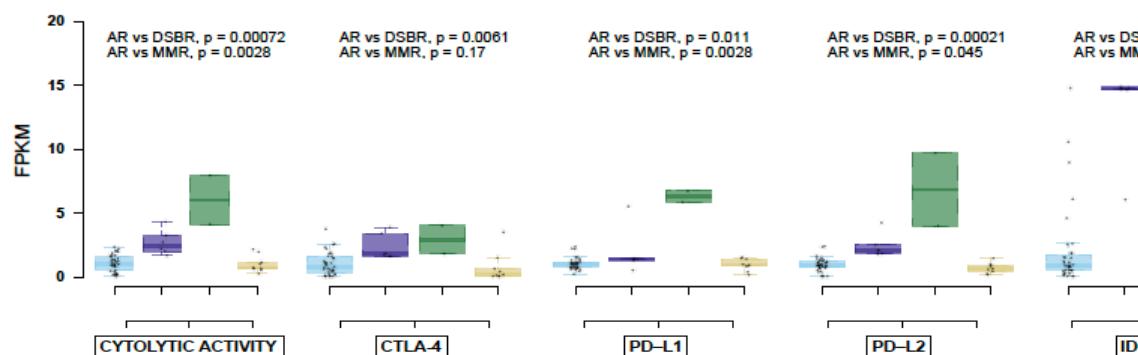
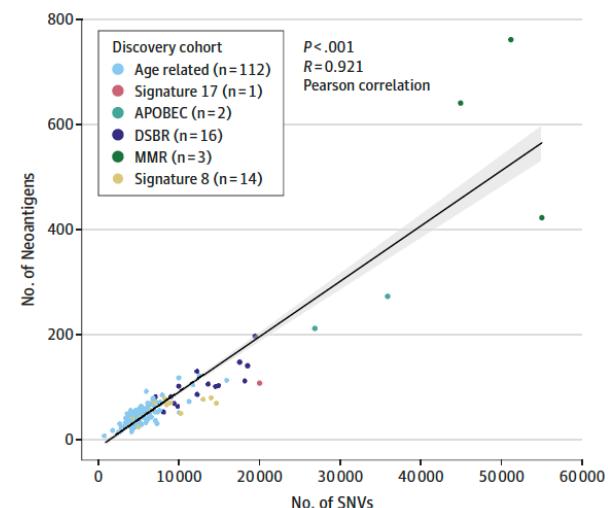
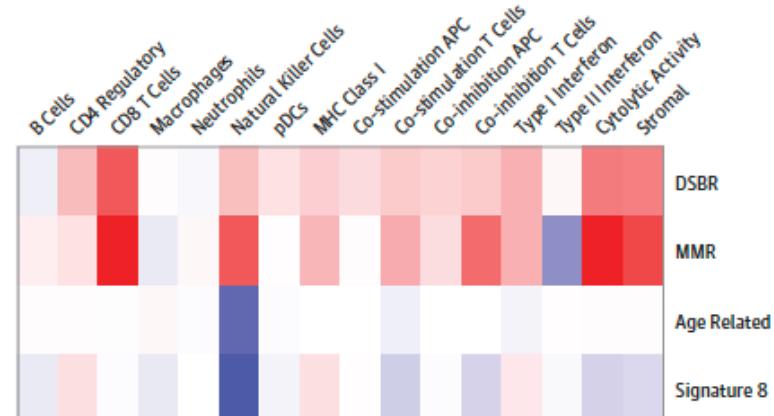
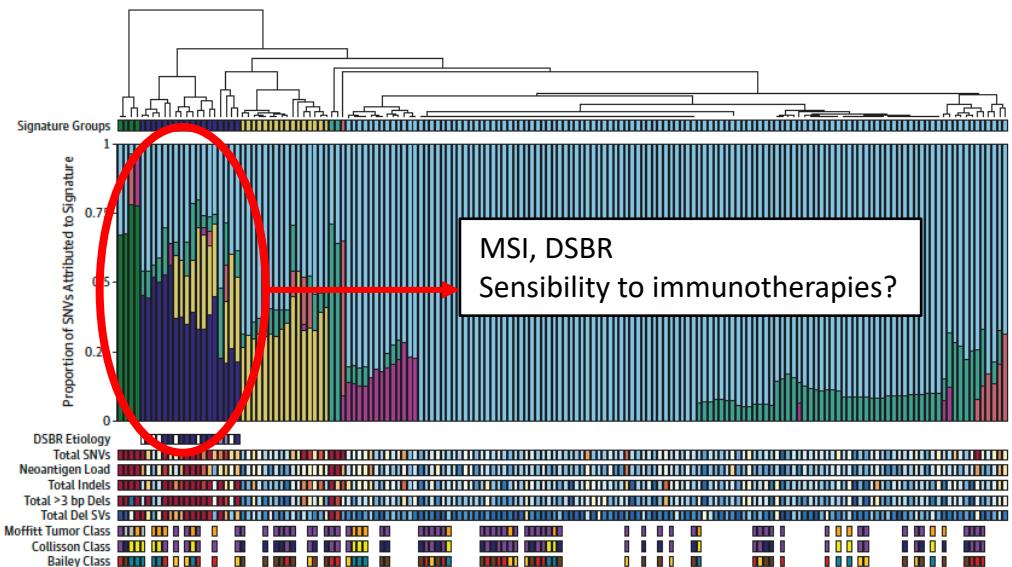
Signature	Discovery (n)	Replication (n)	ESPC (n)	Population (%), 95% CI
Age Related	115*	59	NA	69.9% (64.2-75.6%)
DSBR, total	17**	10	NA	10.8% (6.98-14.7%)
DSBR, germline	9	2	NA	4.42% (1.9-6.97%)
DSBR, somatic	2	2	NA	1.6% (0.045-3.2%)
DSBR, occult	6	6	NA	4.8% (2.2-7.5%)
MMR	4	0	6	1.7% (0.65-2.7%)
Signature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

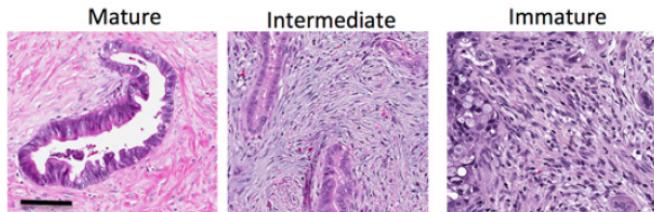
*** there are 2 tumours from 1 case in the APOBEC discovery group

Genomic

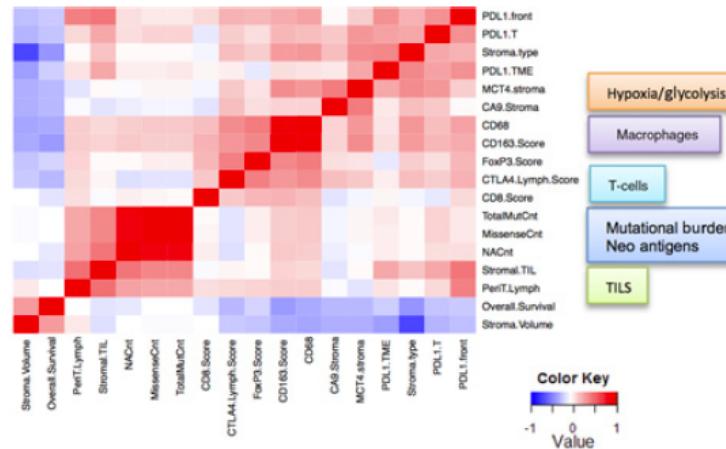


Genomic

Type of stroma

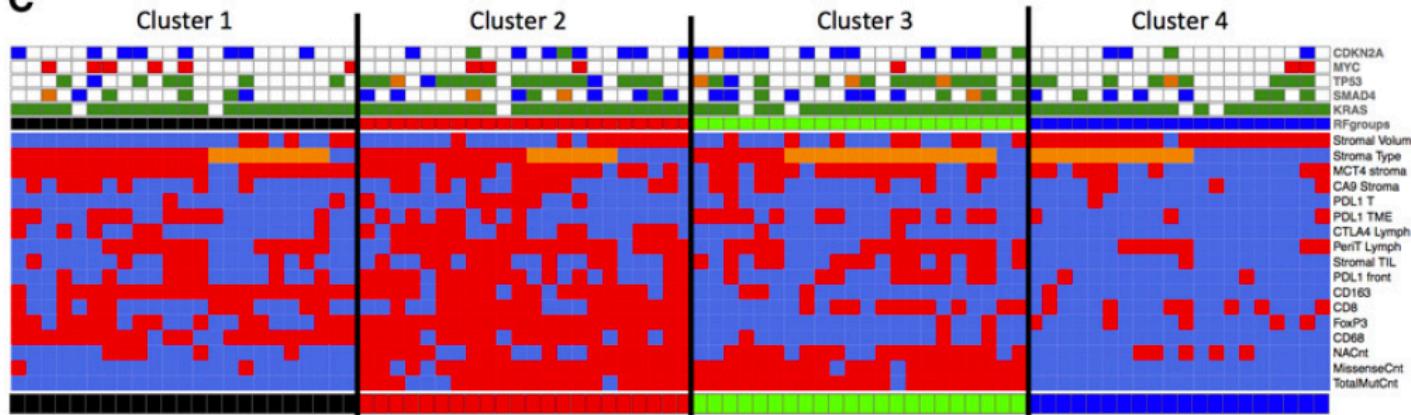


Type of immune infiltrate



+
Mutationnal
profile
(WES)

C



Mutationally
cold

Scarce stroma
Immature stroma
Low neoAg
Low mutation in driver genes
Glycolytic stroma
M2 Macrophages +++

Hot

Intermediate stroma
High neoAg
Pleomorphic immune infiltrate

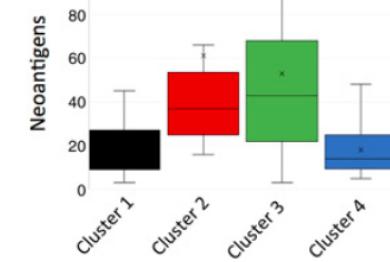
Mutationally
active

Intermediate stroma
High neoAg
TIL
Low macrophage

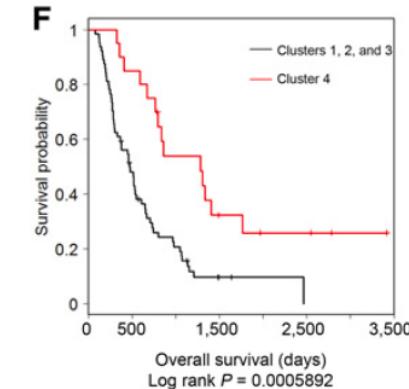
Cold

Abundant stroma
Mature stroma mature
Low neoAg
Low mutation in driver genes
Low immune infiltrate

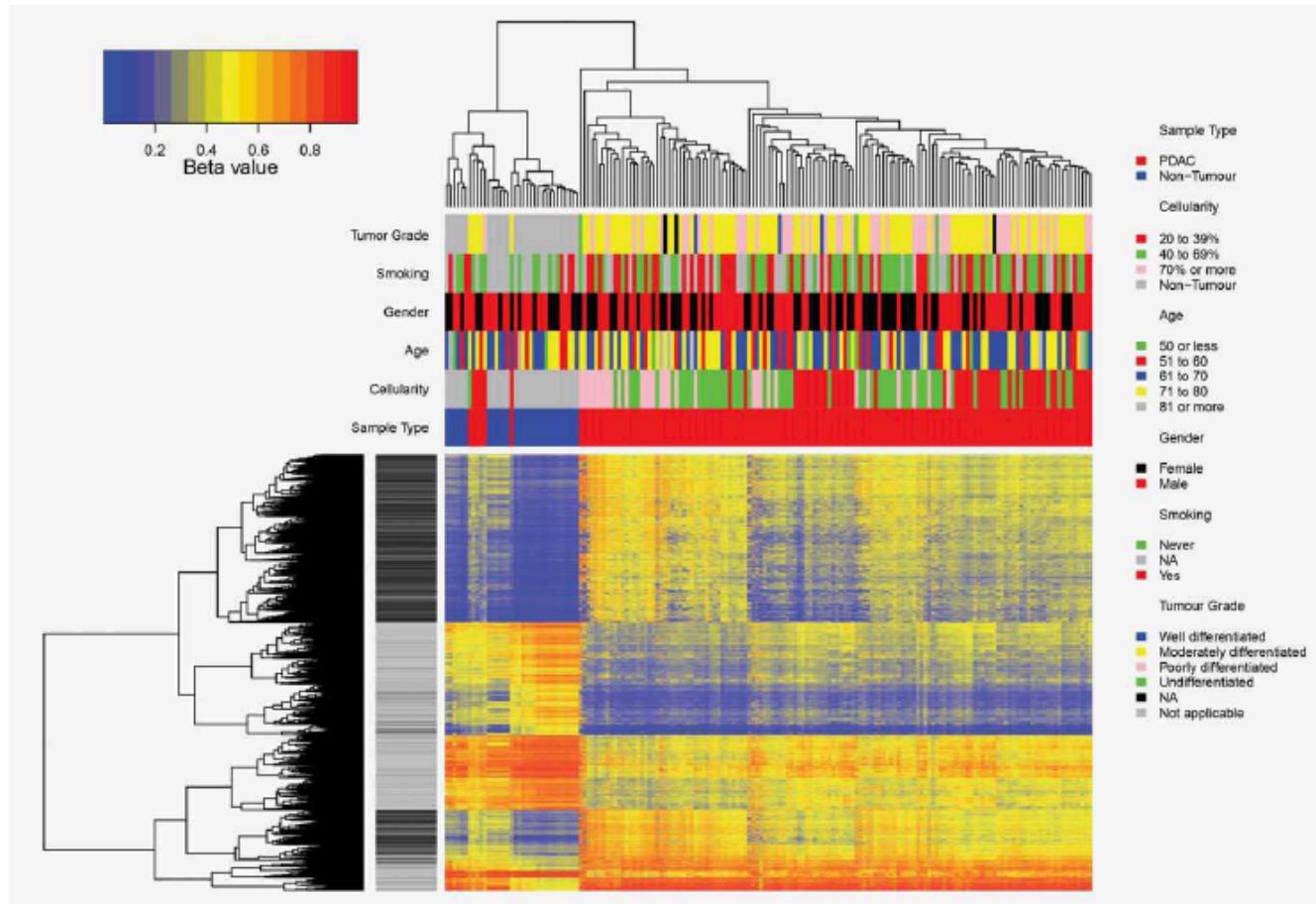
D



F



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

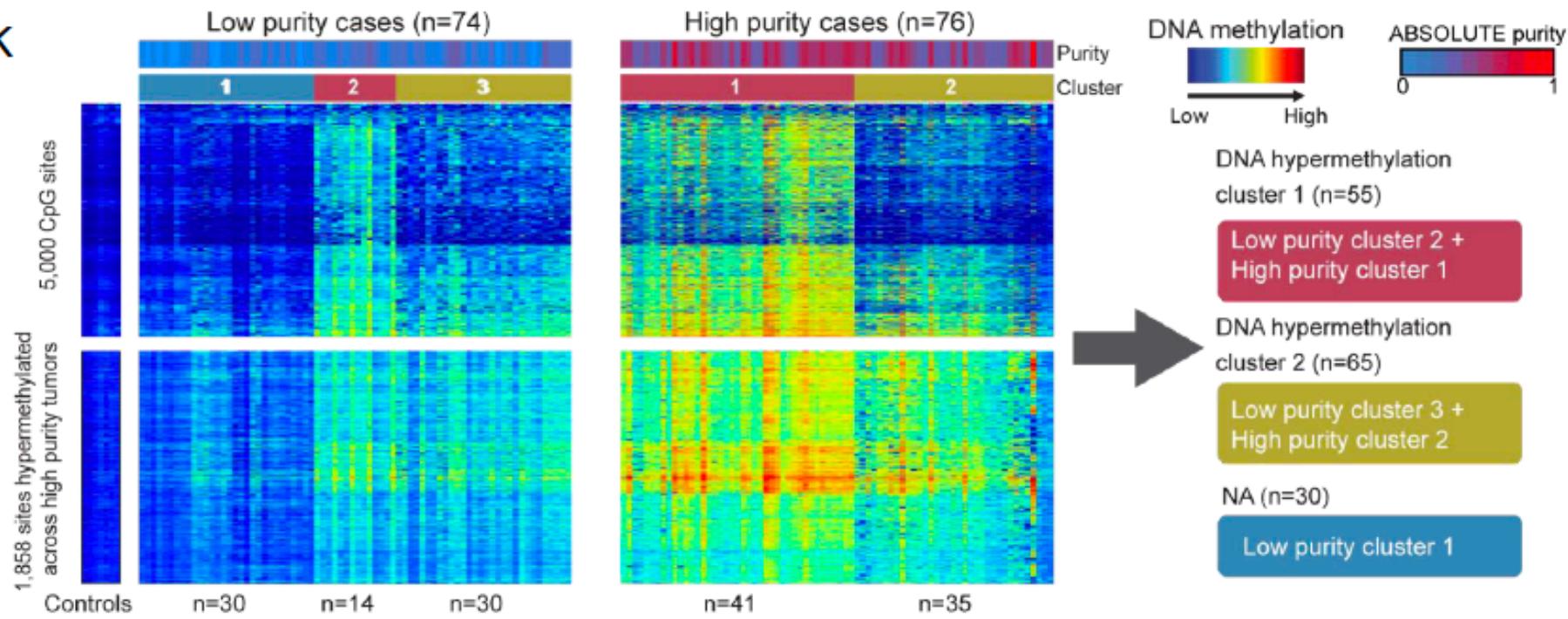


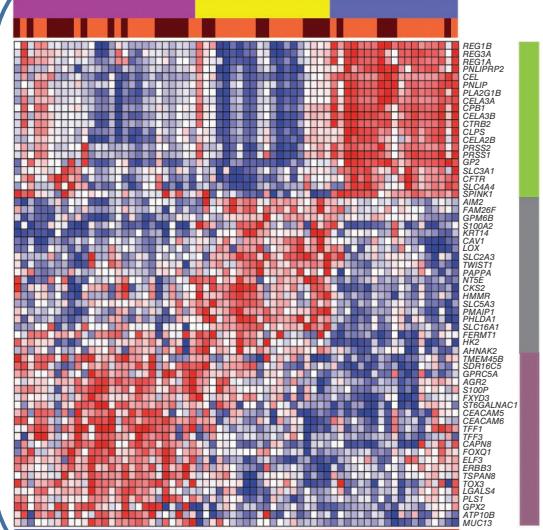
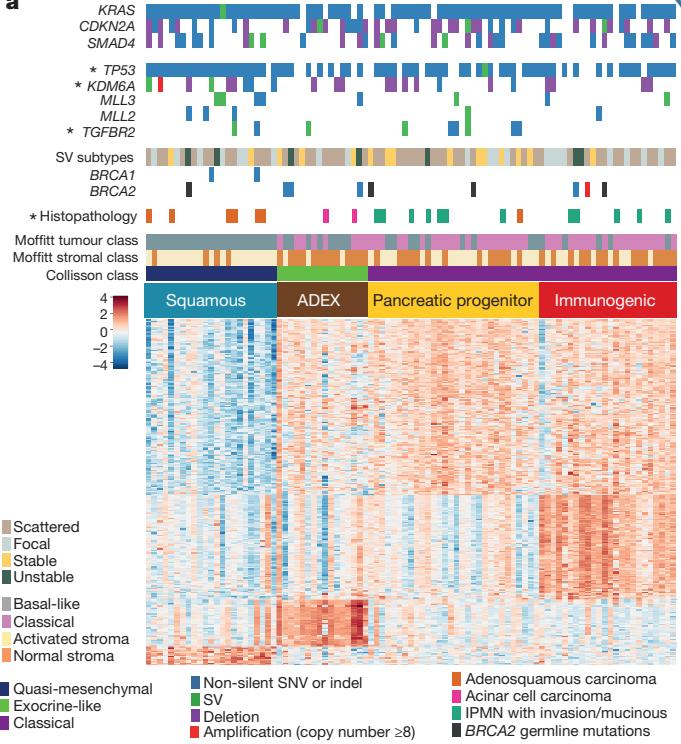
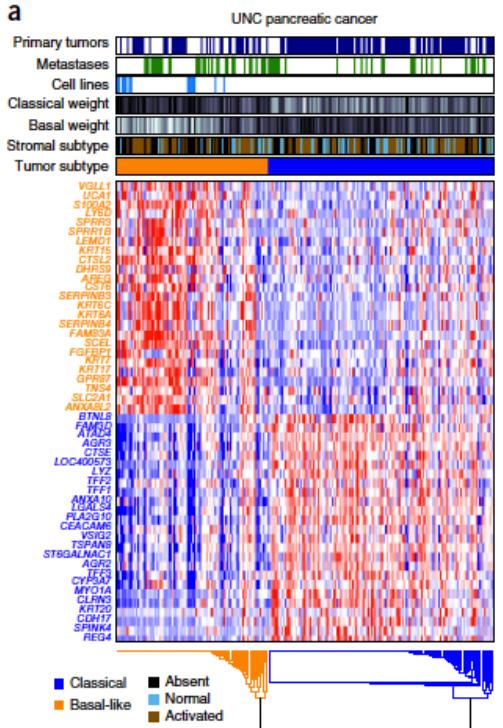
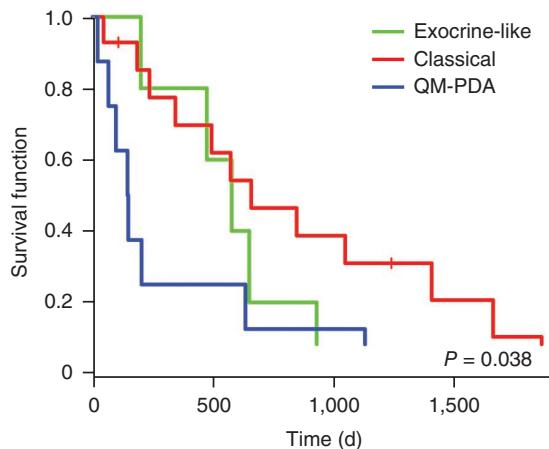
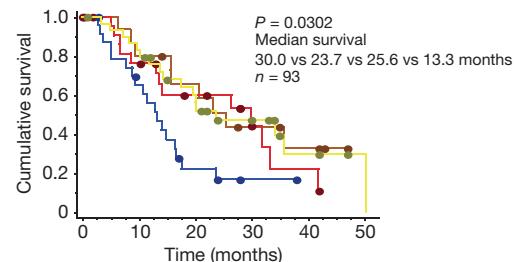
Nones *et al.* IJC 2014

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

↑ MET

K

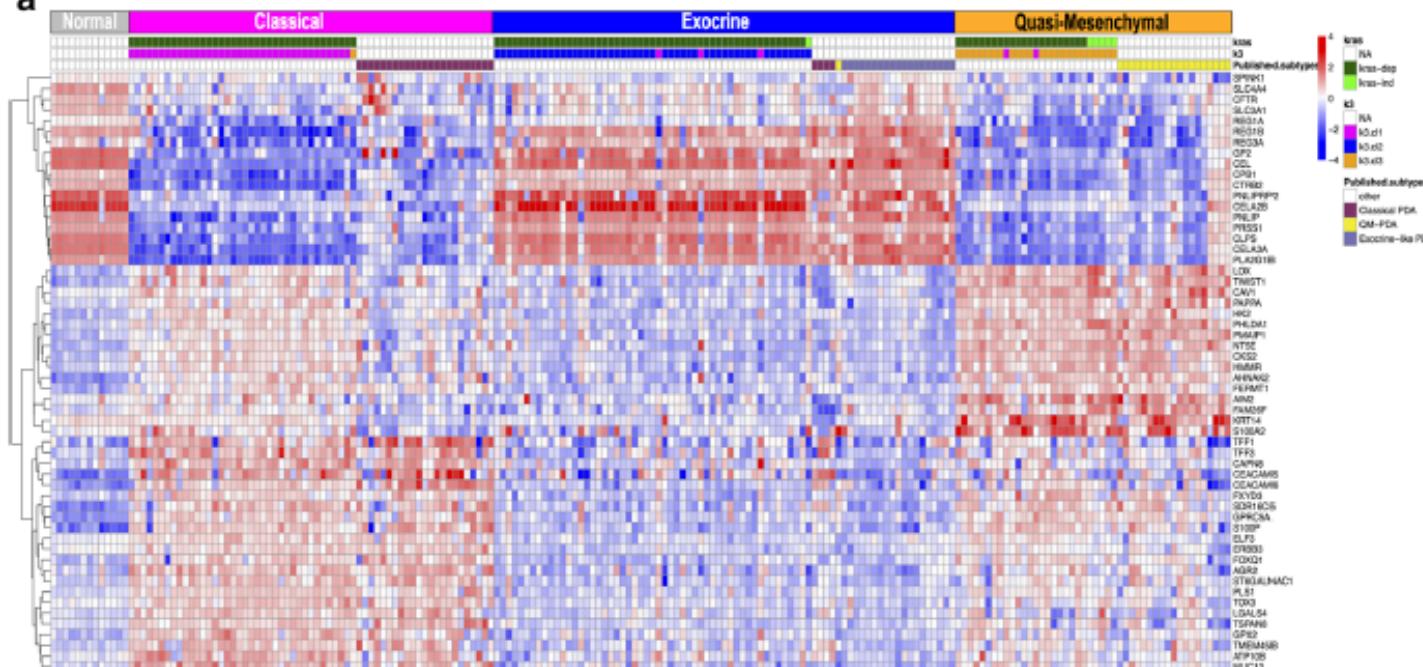


**b Collisson et al. Nat Med 2011****Bailey et al. Nature 2016**

Transcriptomic

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

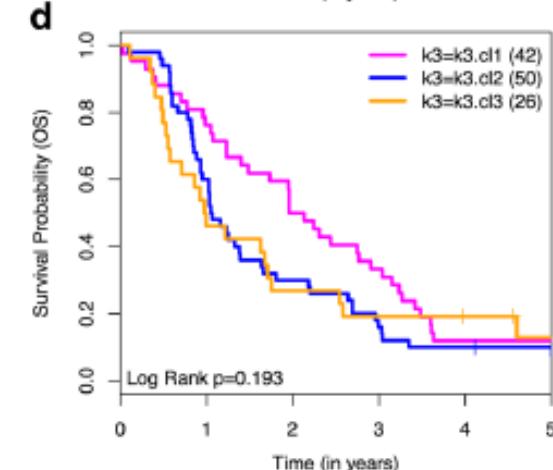
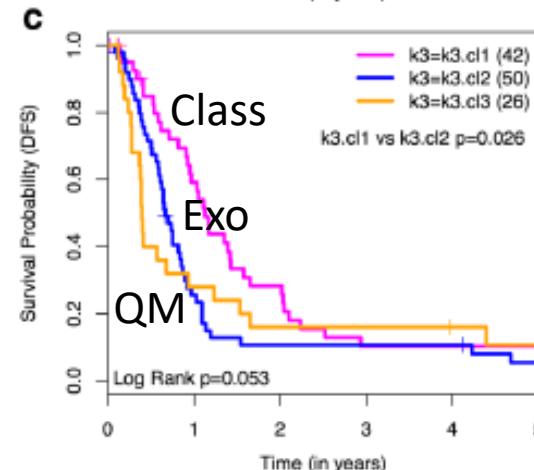
a



b

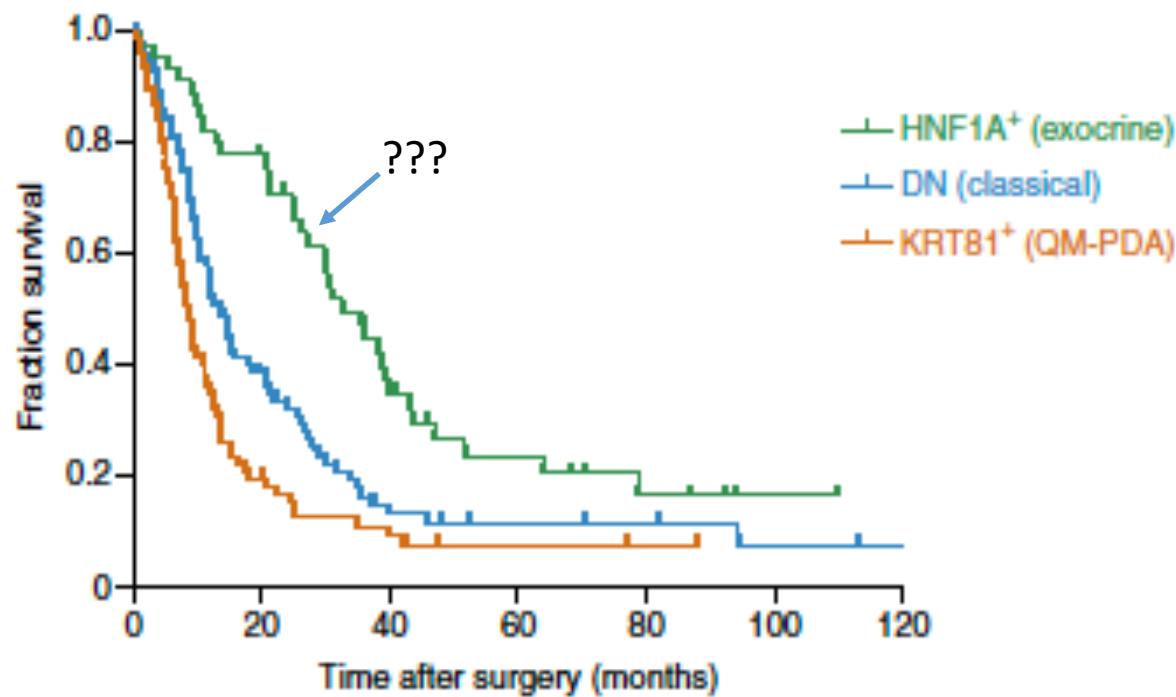
	k3.cl1	k3.cl2	k3.cl3
classical	23	4	0
Exocrine	0	19	0
QM-PDA	0	1	19
k2.cl1	38	0	26
k2.cl2	4	50	0

118pts

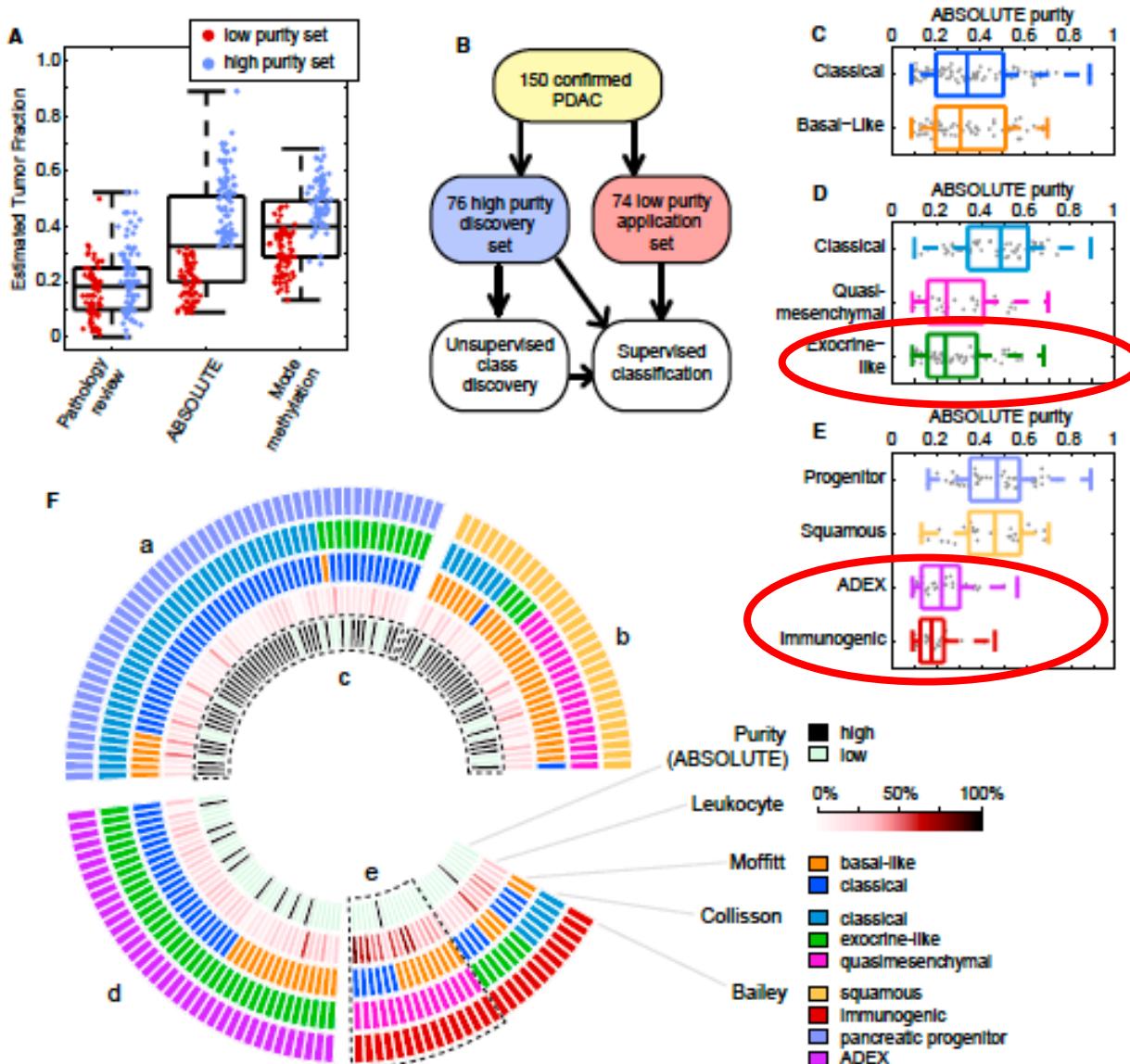


PDAC transcriptomic subtypes, how many? Do they all exist?

231 patients

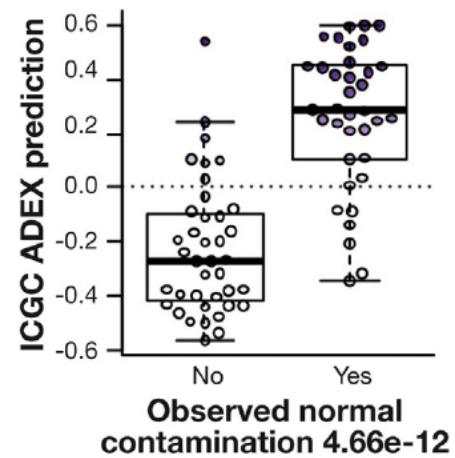
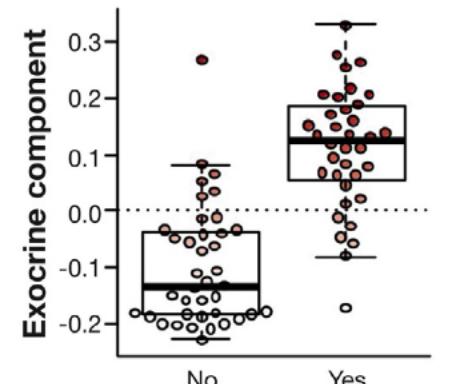
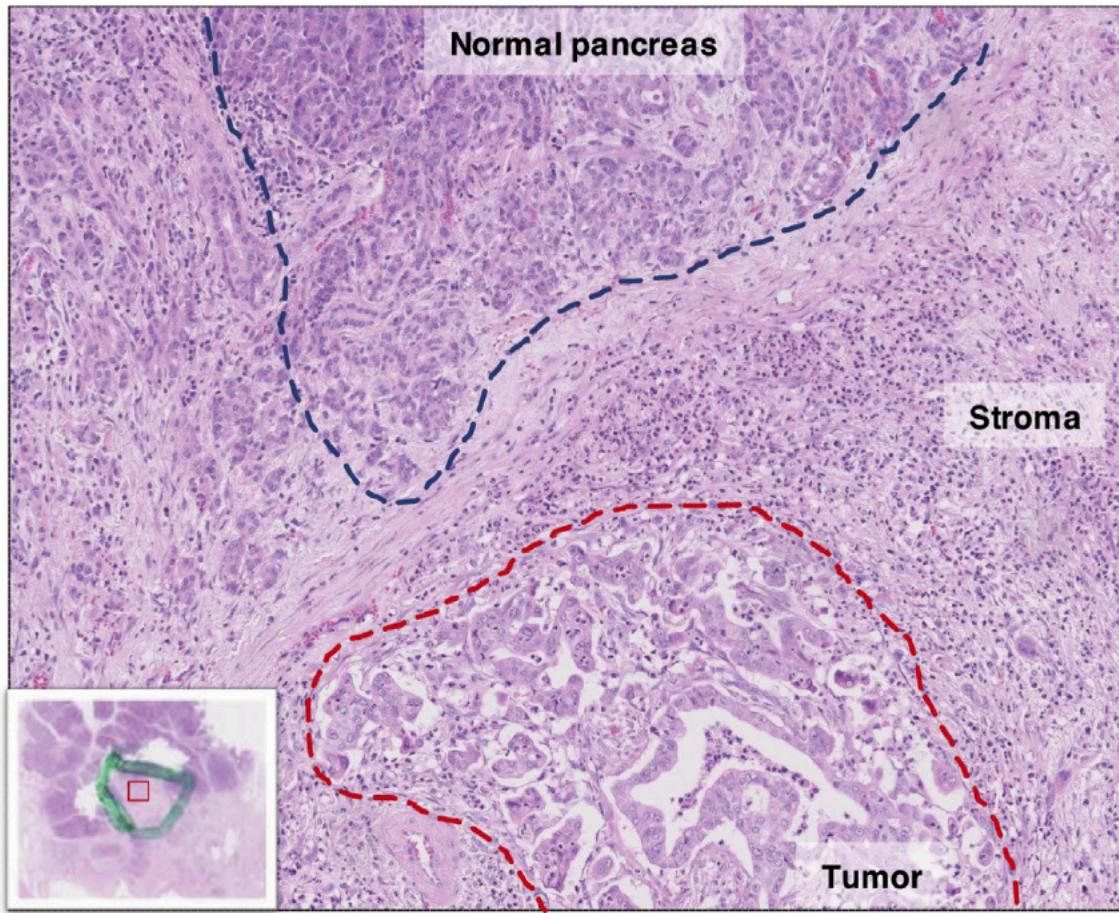


PDAC transcriptomic subtypes, how many? Do they all exist?



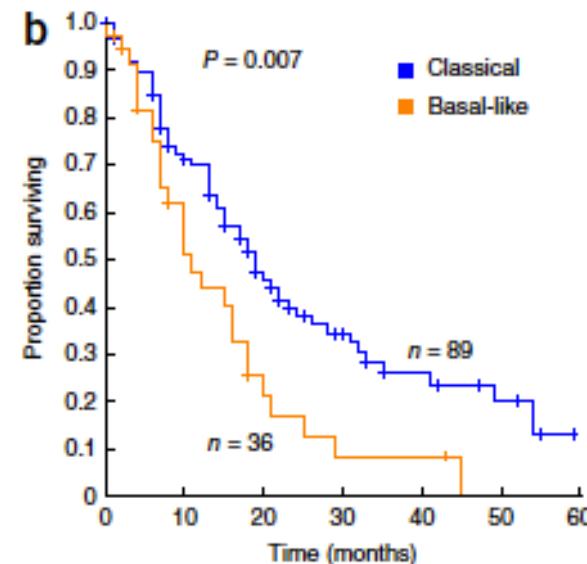
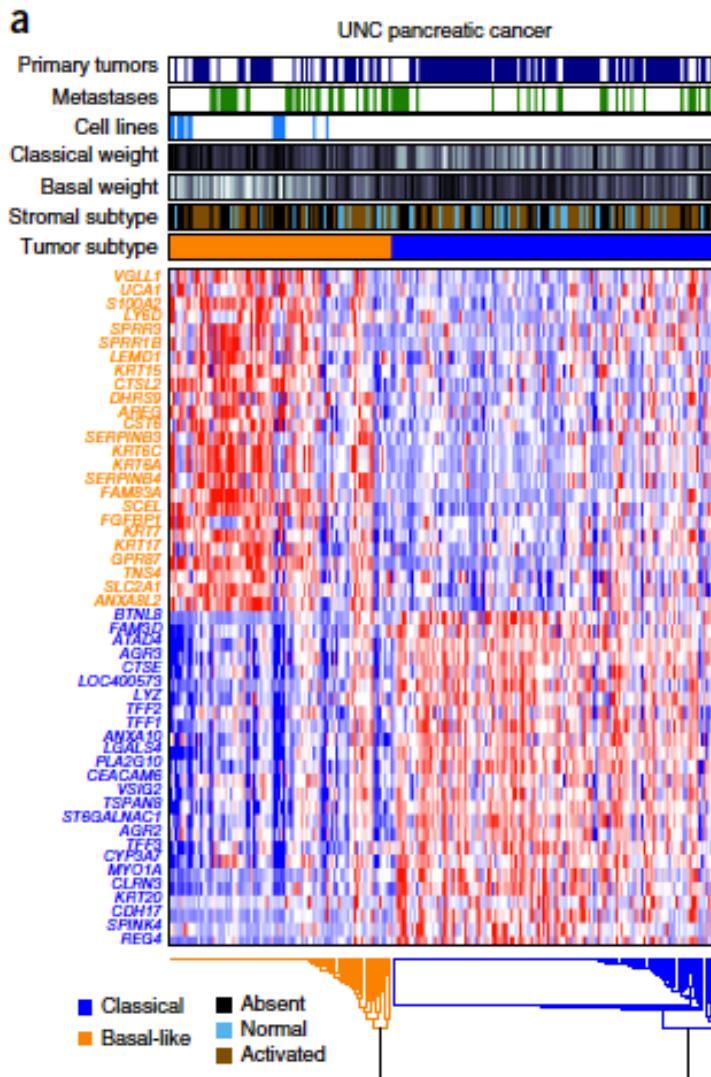
We try our best to give you pure tumor area....

A



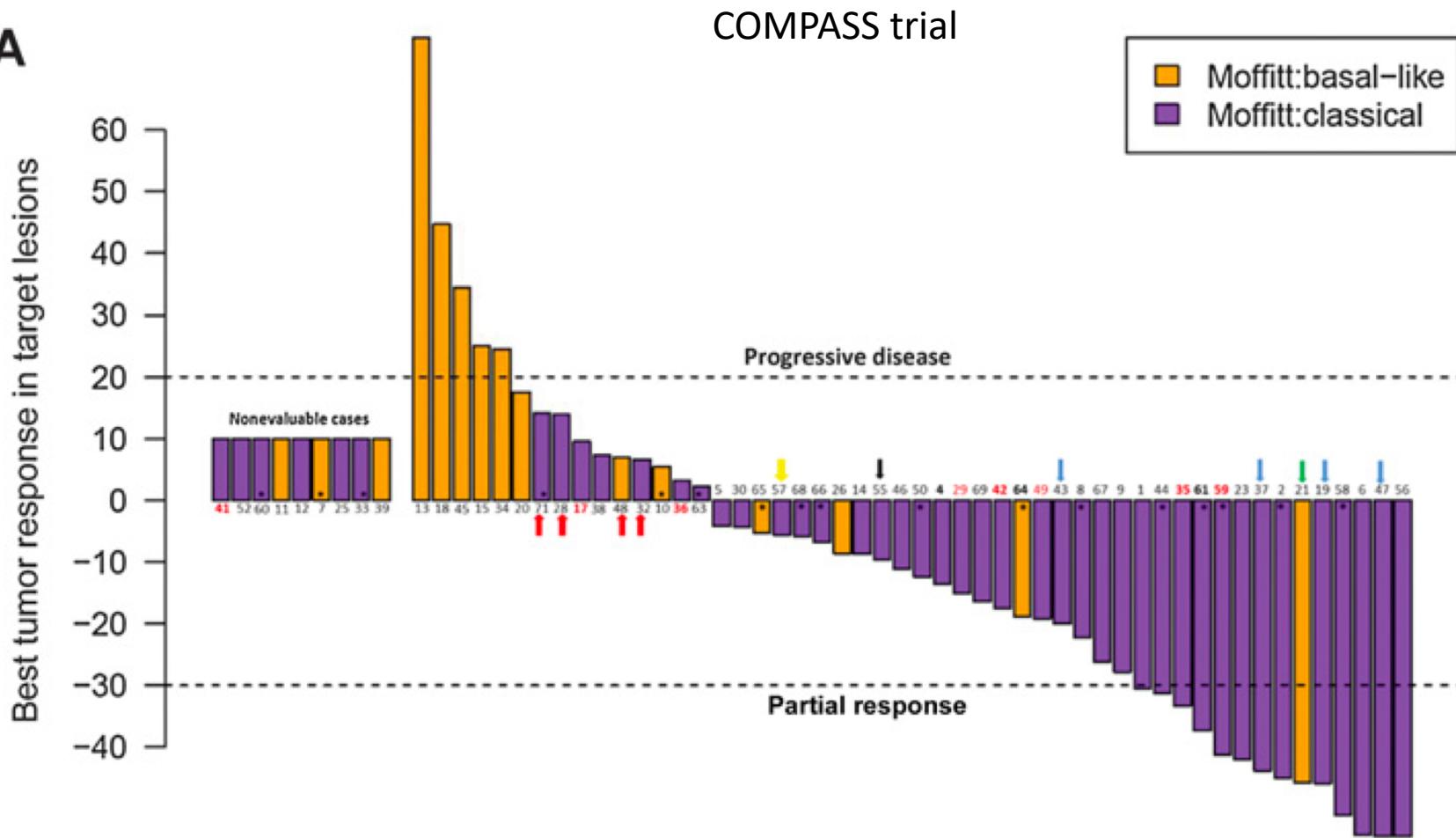
2 main transcriptomic tumor subtypes with different prognosis

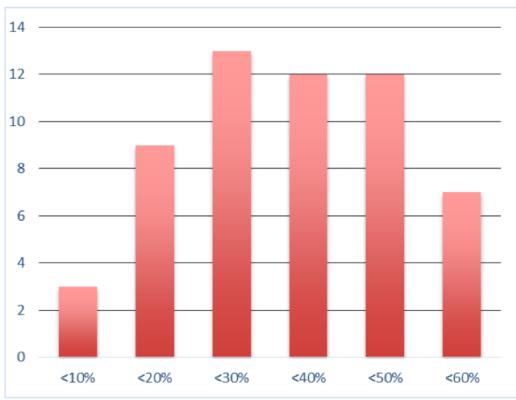
Moffit et al. Nat Gen 2015



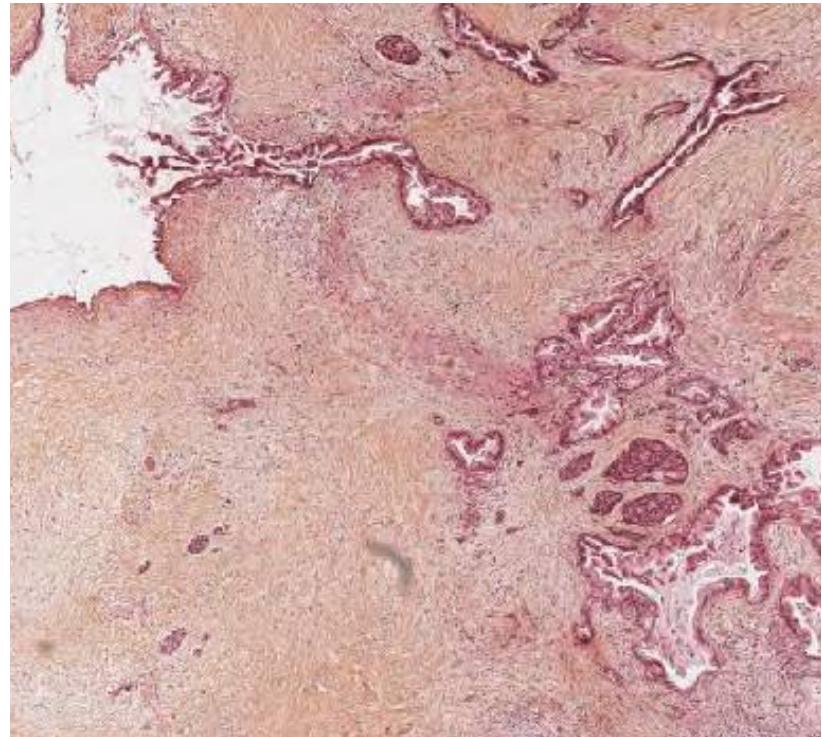
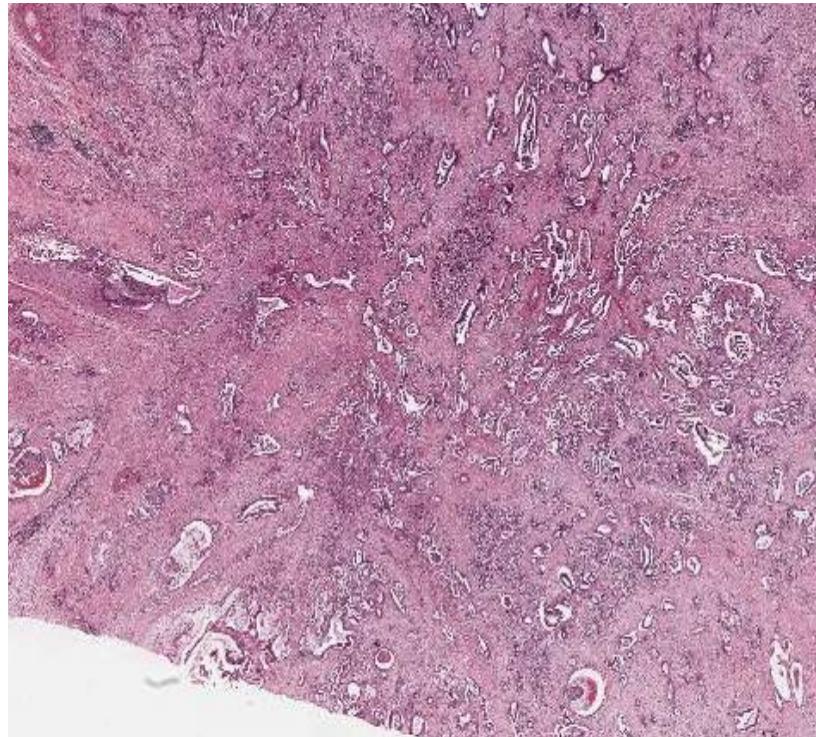
Molecular subtypes may have an important clinical utility++++

A

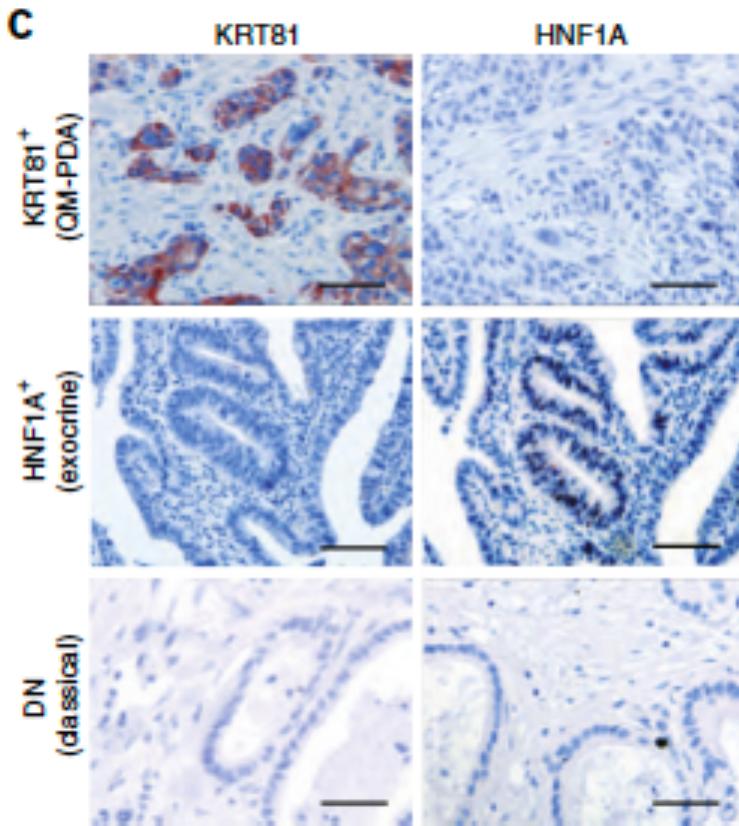




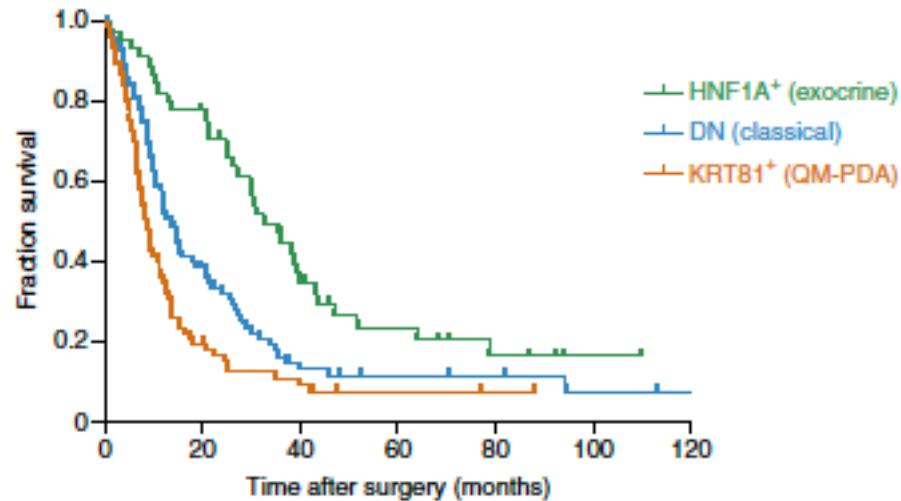
True challenge! How to define (clearly) the molecular subtype in samples with few tumor cells???



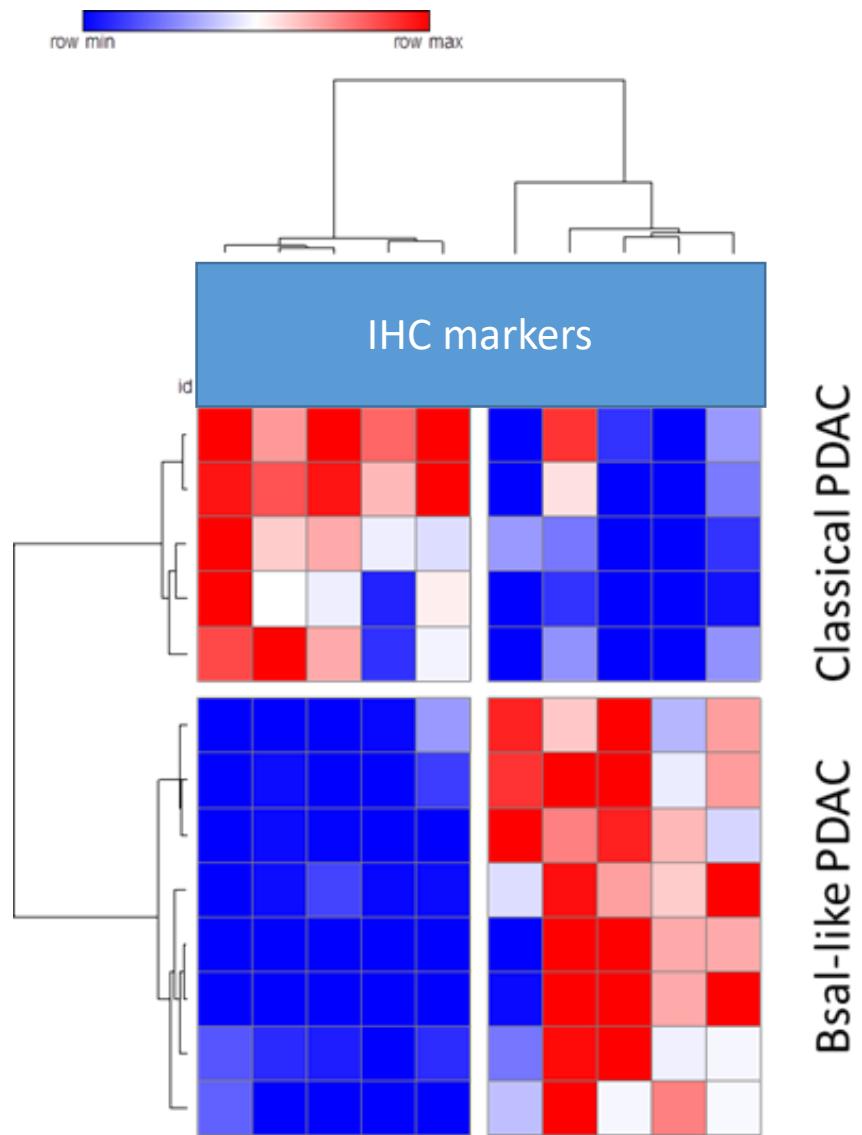
True challenge! How to define (clearly) the molecular subtype in samples with few tumor cells???



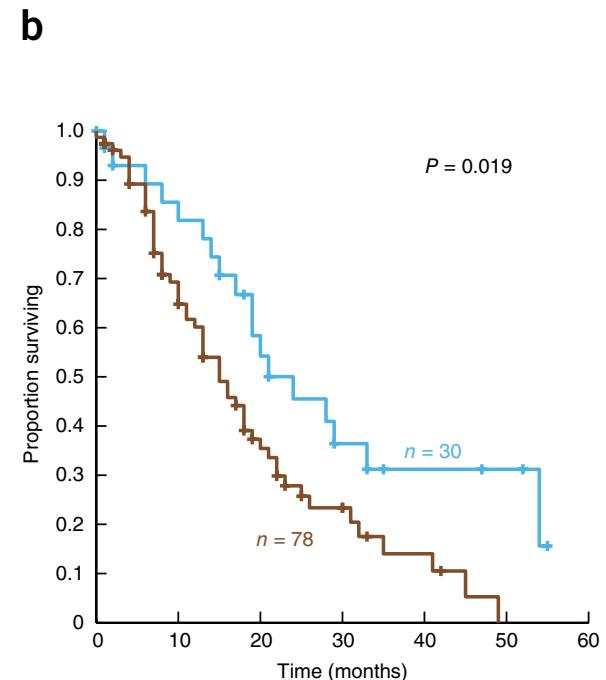
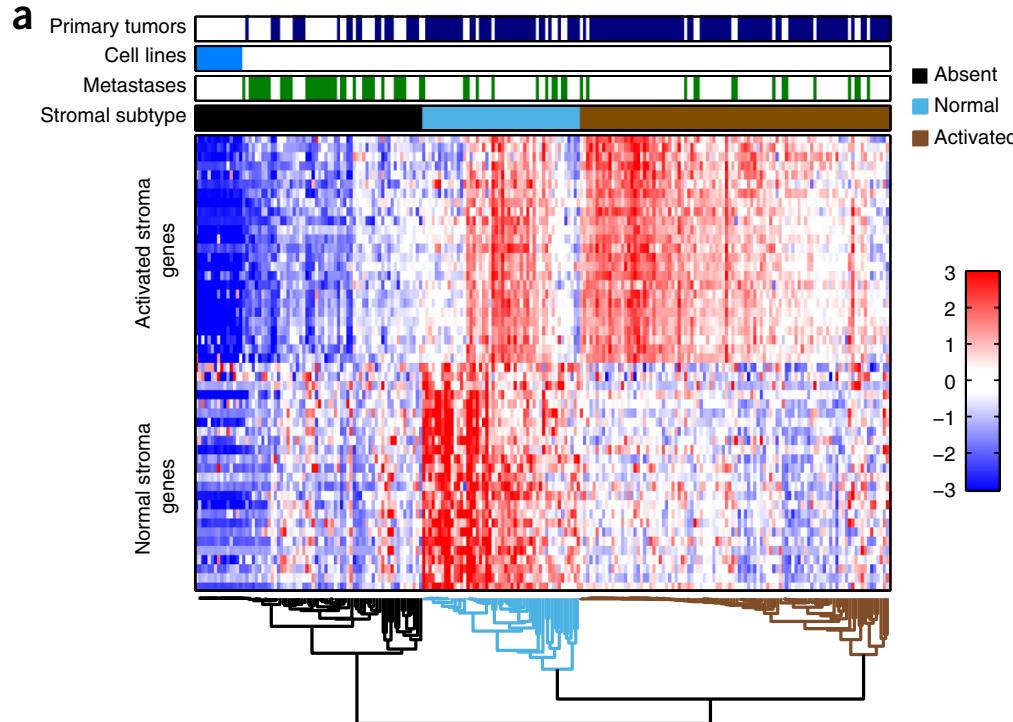
What do we do with double +?



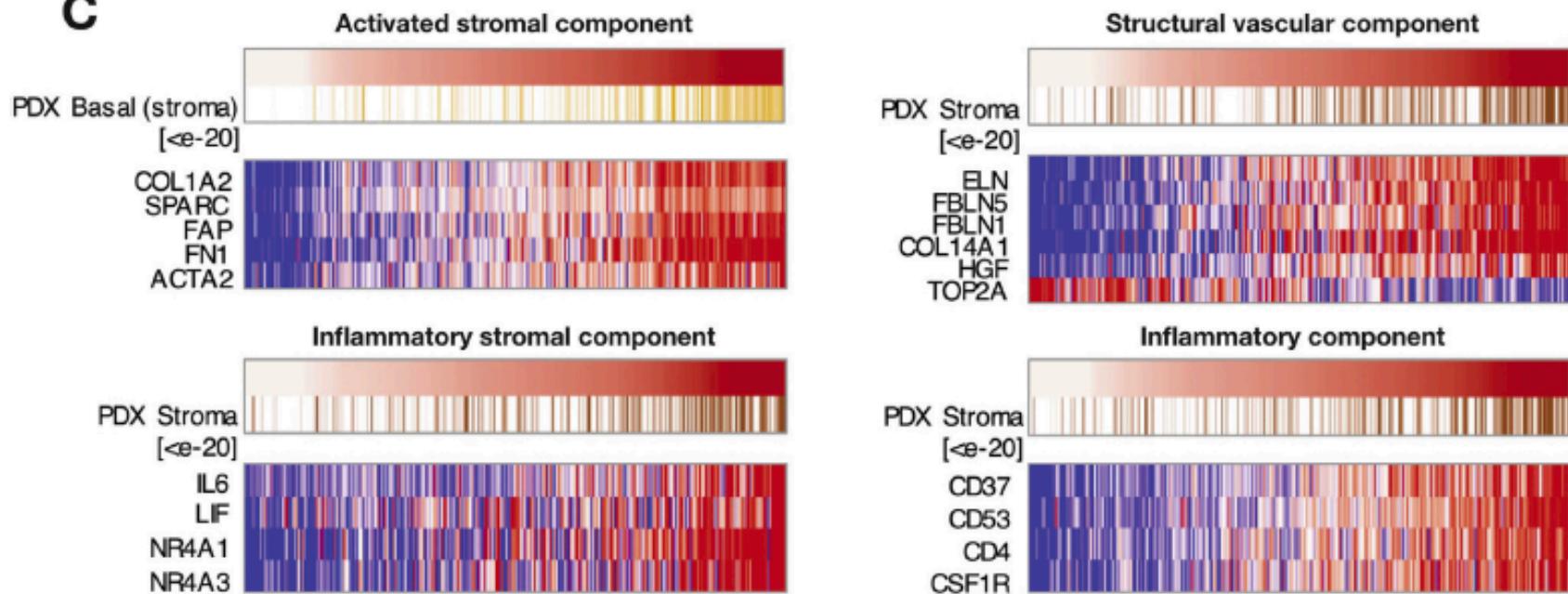
True challenge! How to define (clearly) the molecular subtype in samples with few tumor cells???



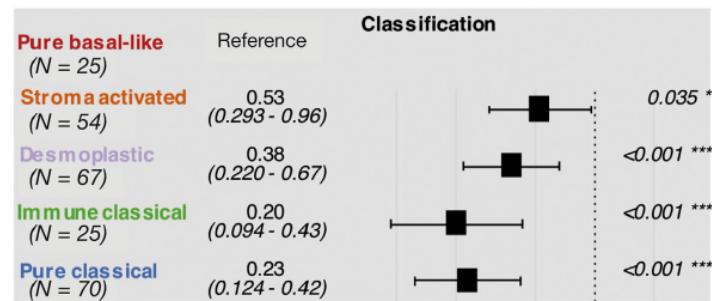
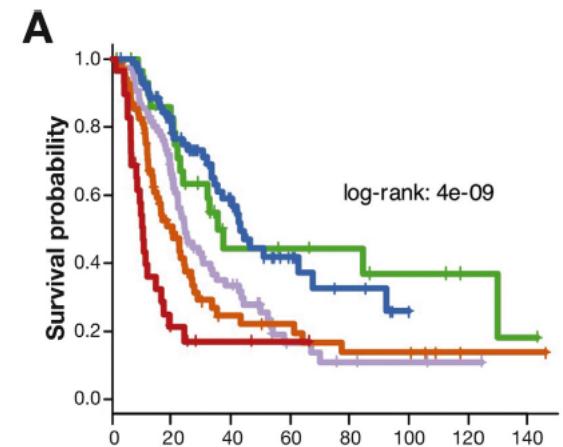
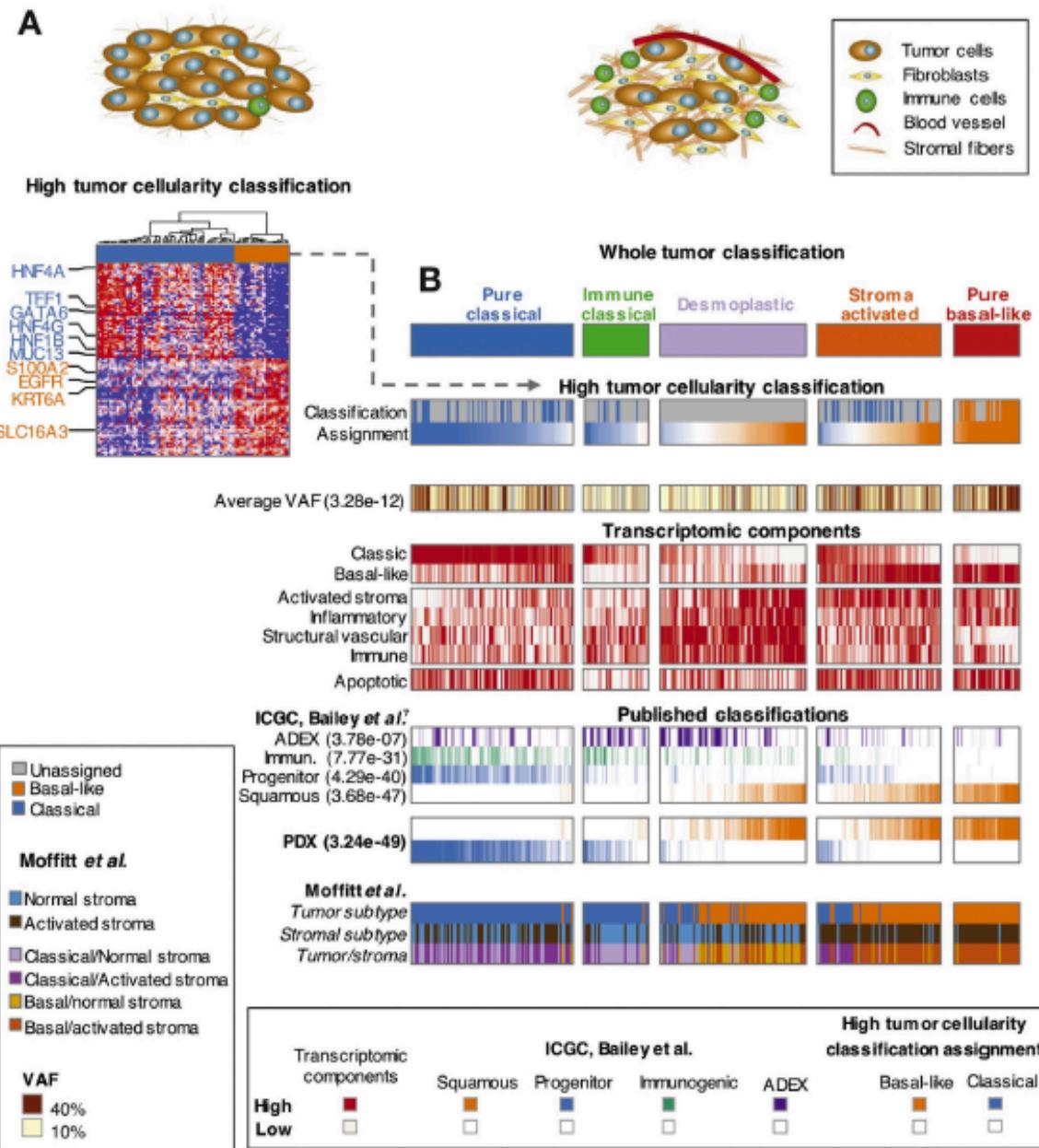
And the stroma is also heterogeneous...



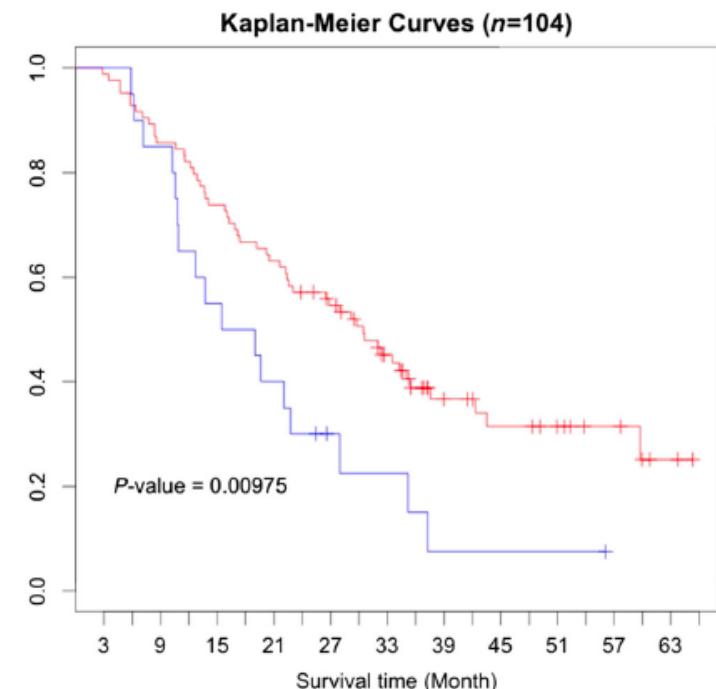
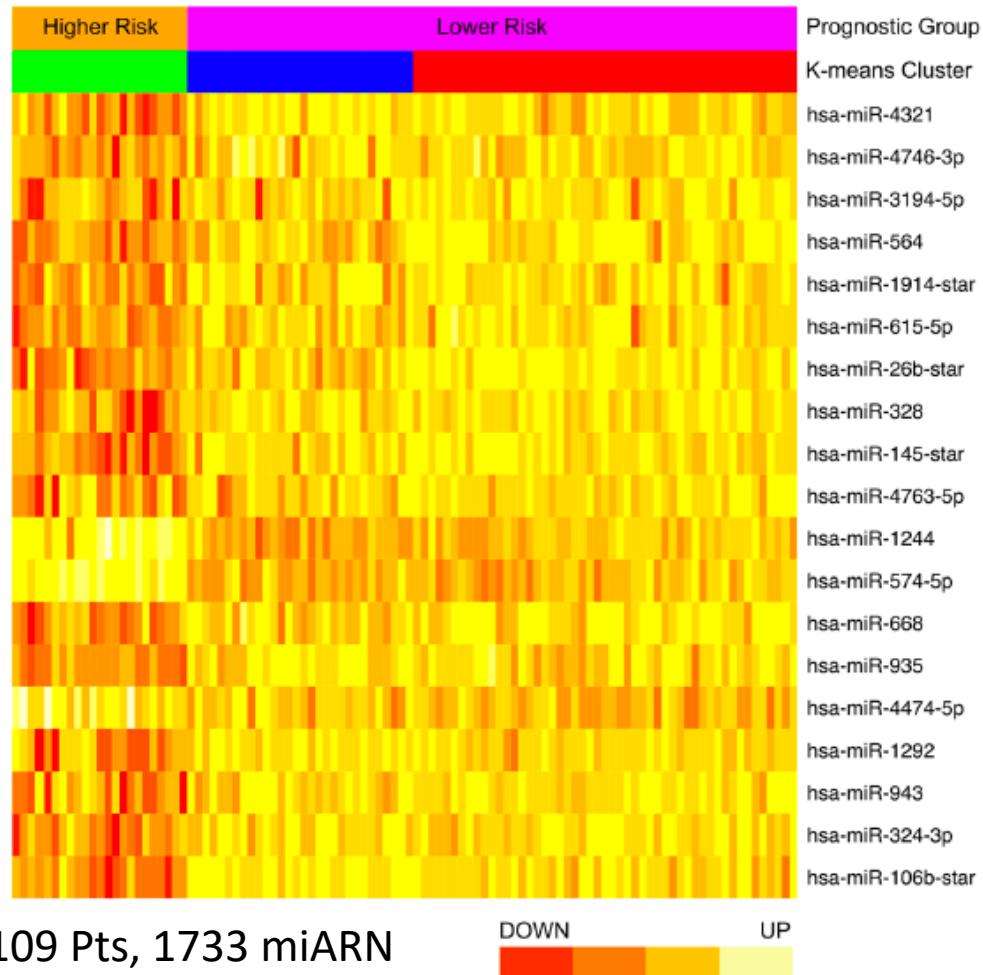
Moffitt et al. *Nature genetics* 2015

C

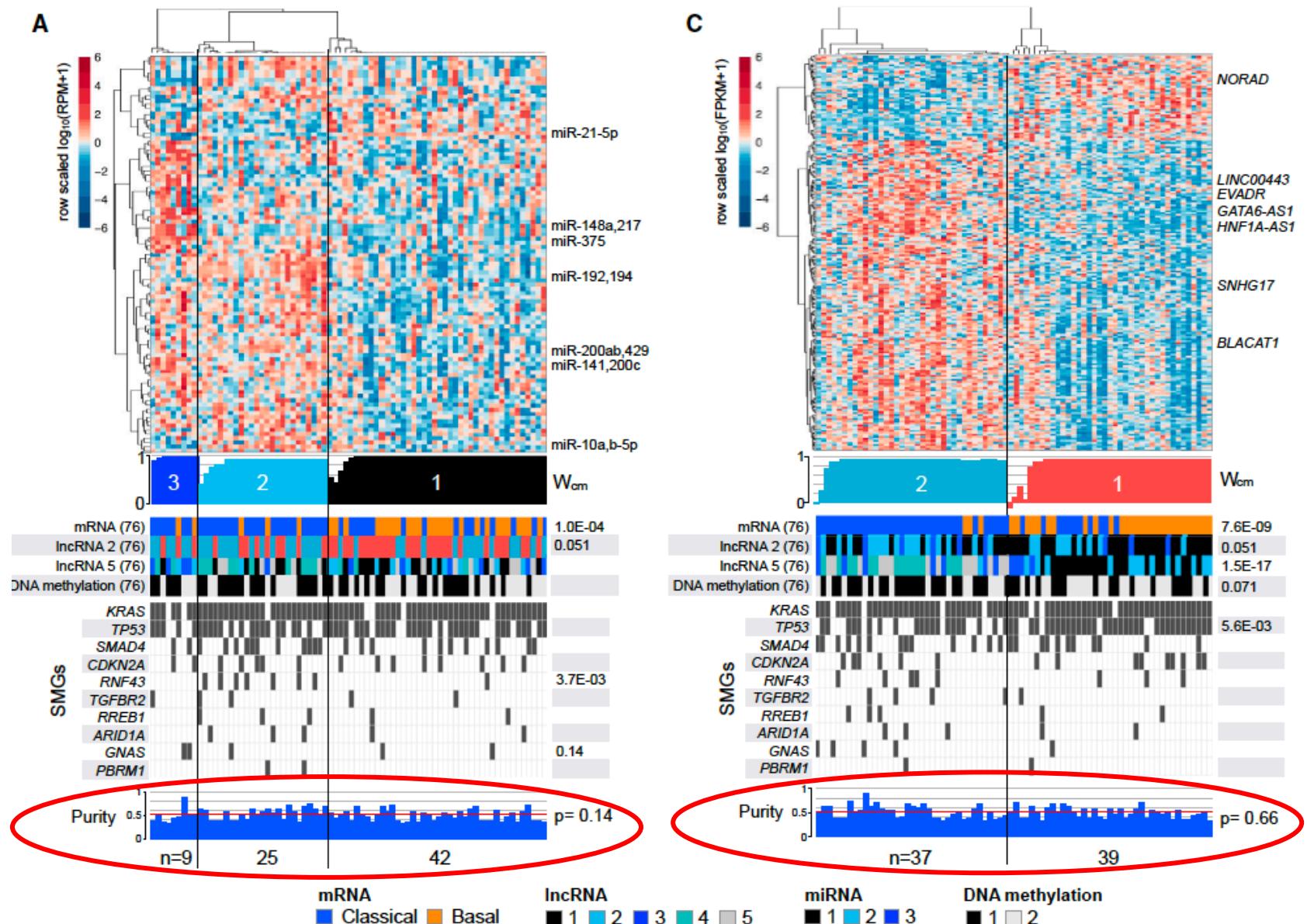
Transcriptomic



There is also heterogeneity in non coding RNA...

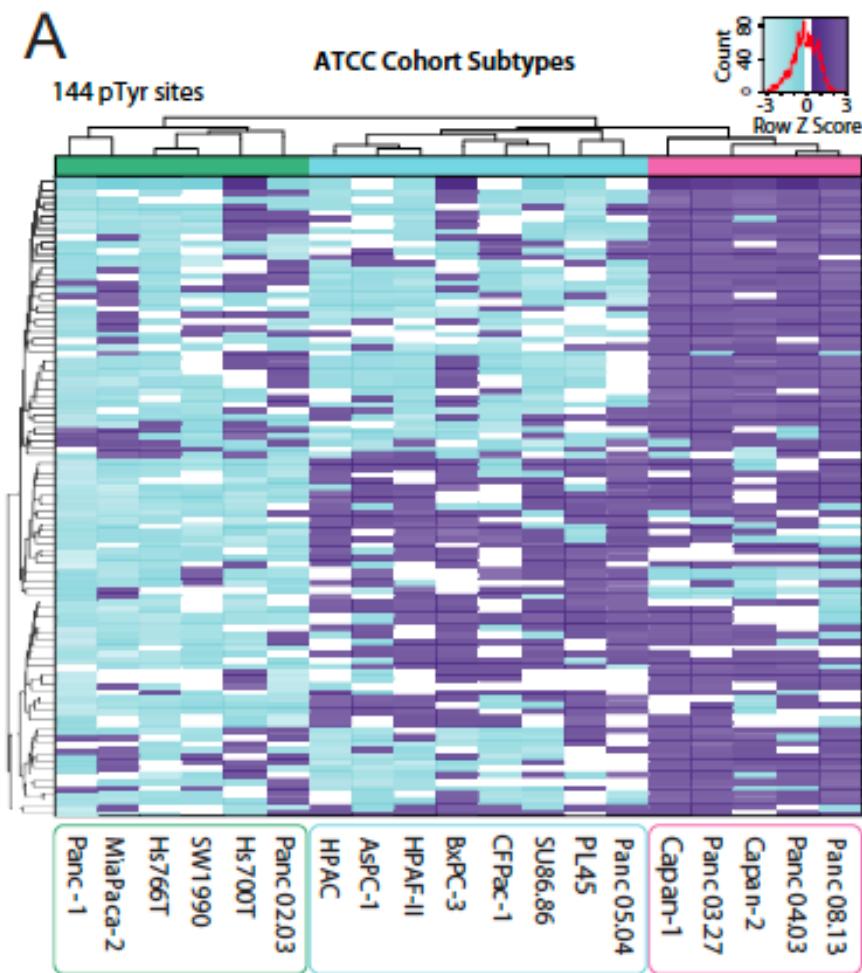
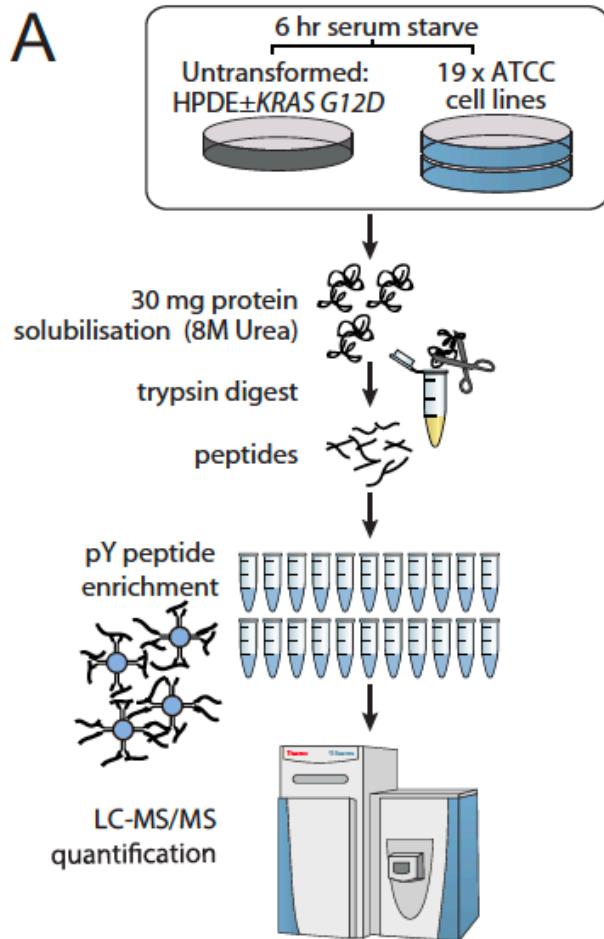


There is also heterogeneity in long non coding RNA...



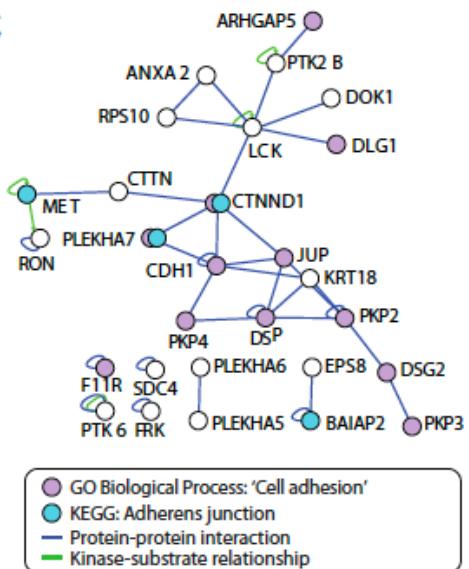
Very few studies!

Phosphoproteome heterogeneity....(cell lines).....

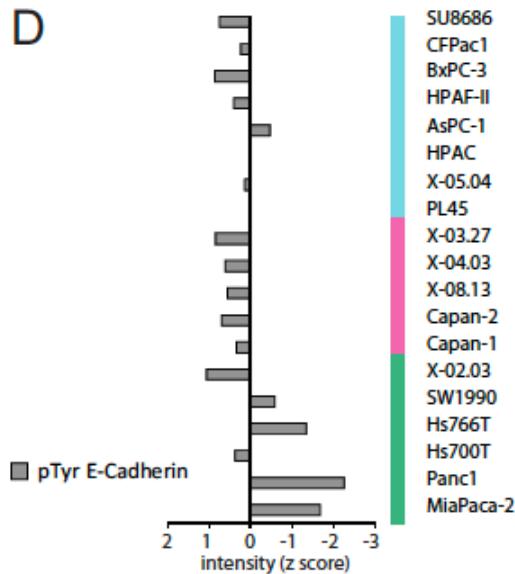


Phosphoproteome heterogeneity....(cell lines).....

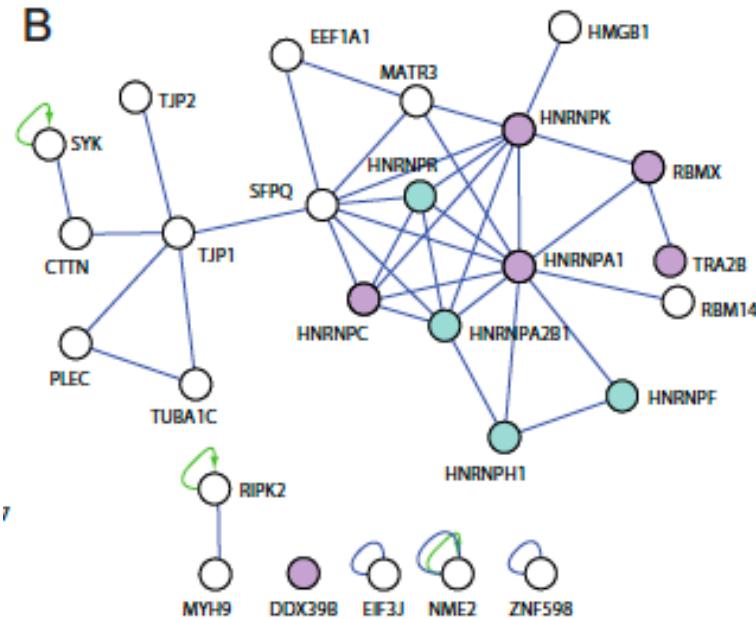
C



D

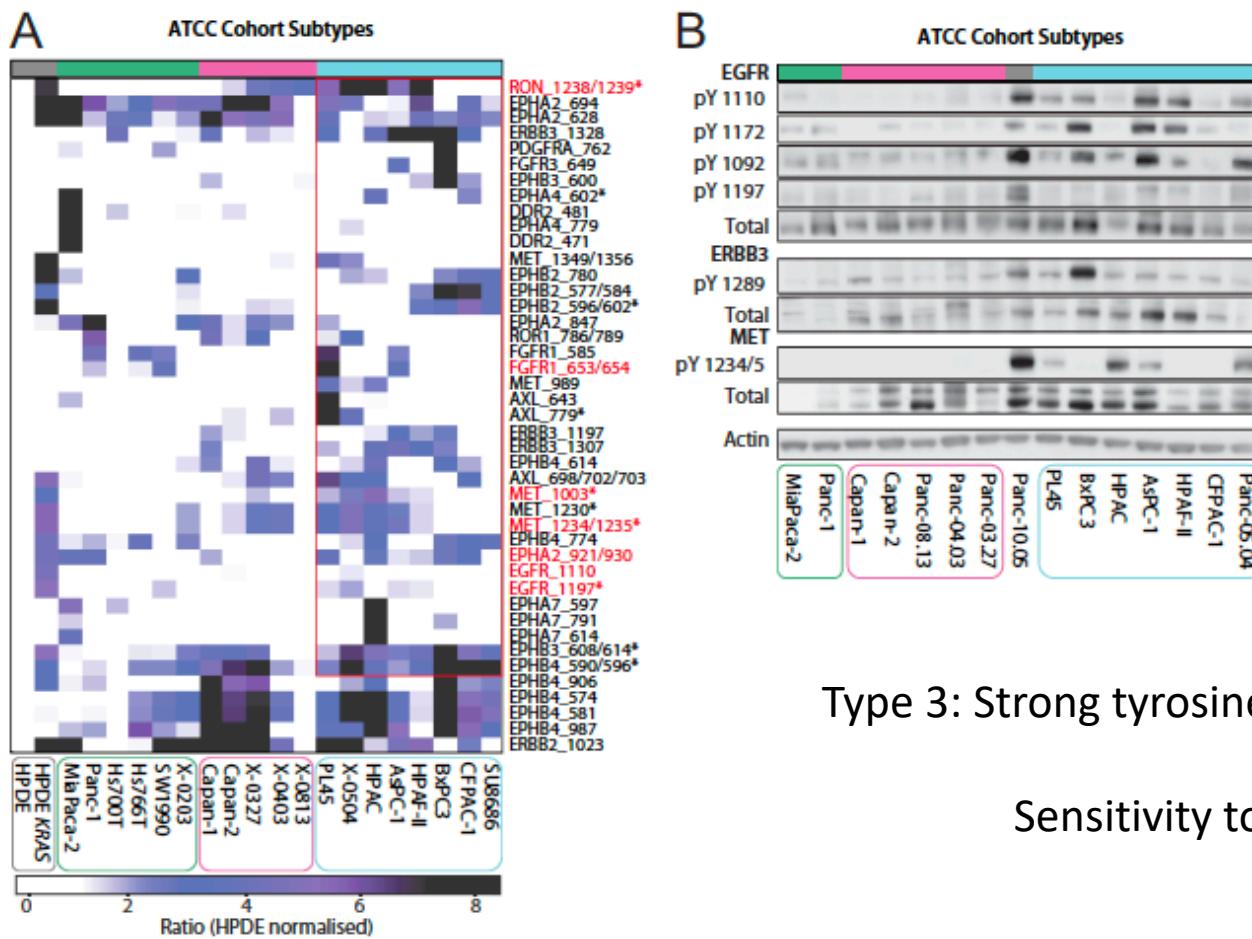


Type 1: EMT, low global phosphorylation



Type 2: Gene processing

Proteomic

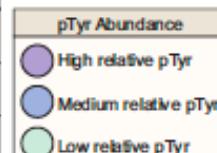


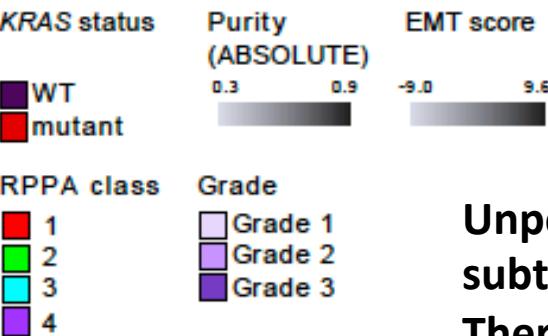
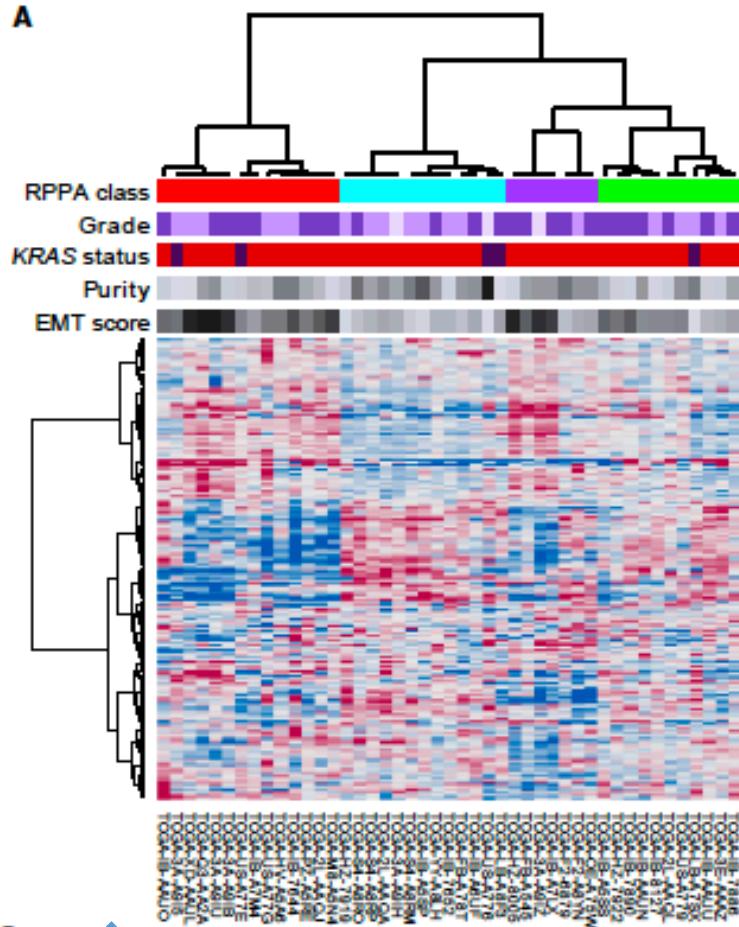
Type 3: Strong tyrosine kinase activity

Sensitivity to TKi?

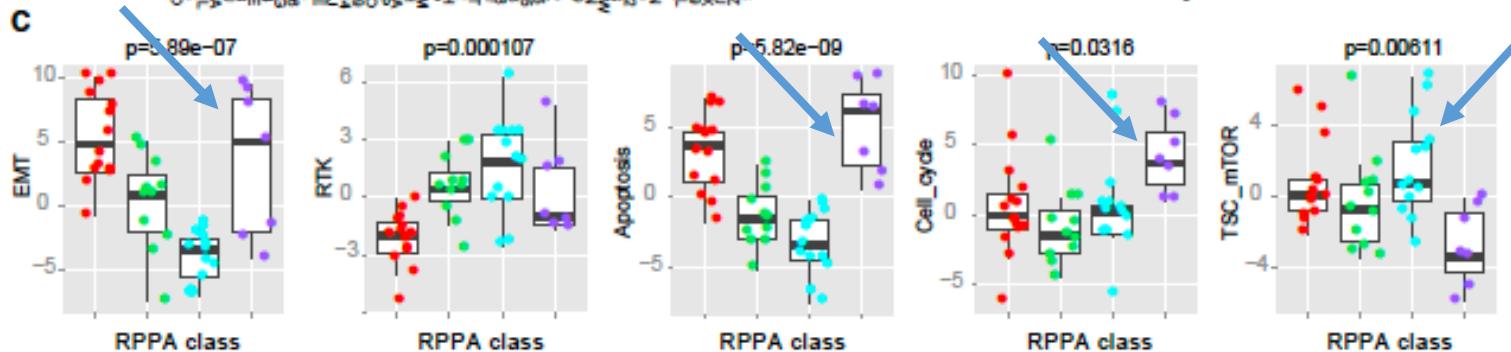
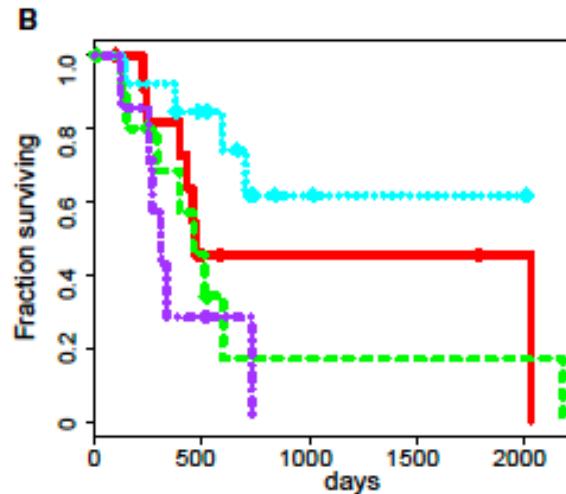
C

Subgroup 1 "Low pTyr"	Subgroup 2 "Mixed"	Subgroup 3 "RTK enriched"	Origin	Protein & pTyr Sites
●	●	●	RTKs	EGFR pTyr 1092/1172
●	●	●	RTKs	ERBB3 pTyr 1307/1328, RON pTyr 1238/1239 MET pTyr 1003
●	●	●	8 site classifier	BAIAP2 pTyr 337, CTNND1 pTyr 174/904, PKP2 pTyr 166, PKP3 pTyr 84
●	●	●	33 site classifier	DSP pTyr 28, LSR pTyr 406, SHB pTyr 114



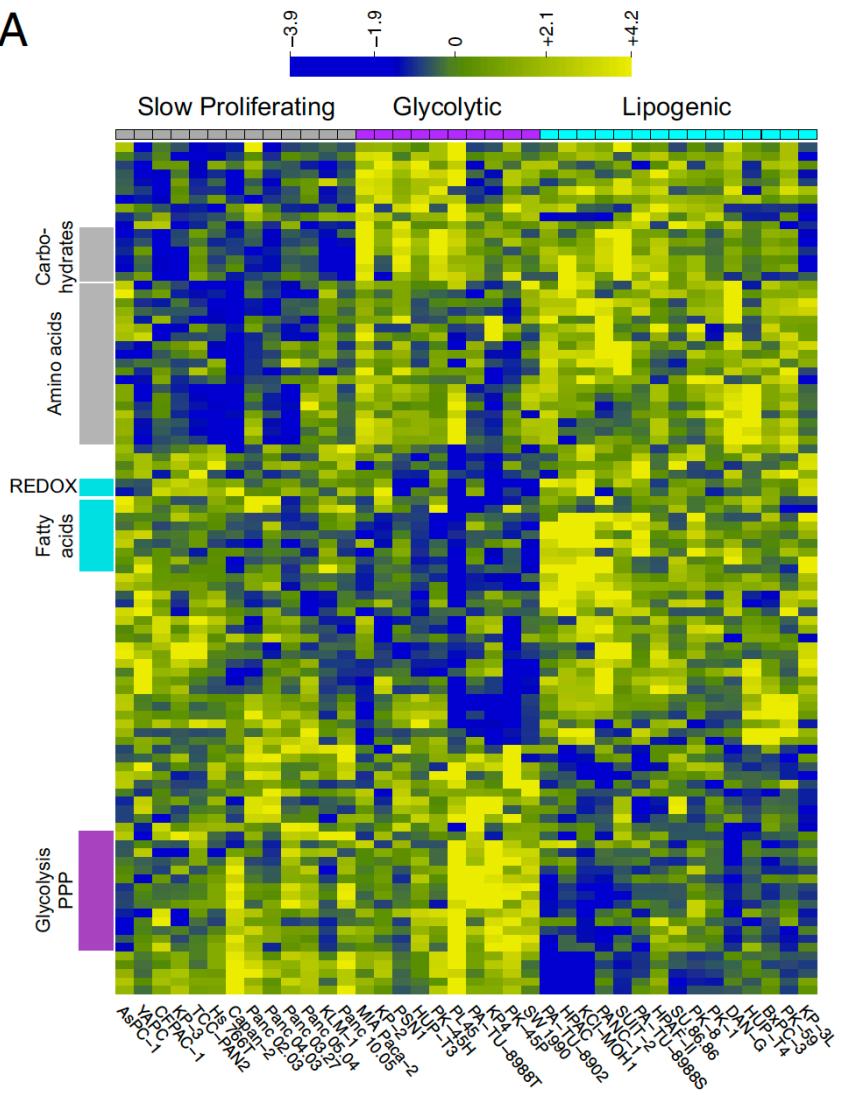


Unperfect overlap with the subtypes...
Therapeutic opportunities?

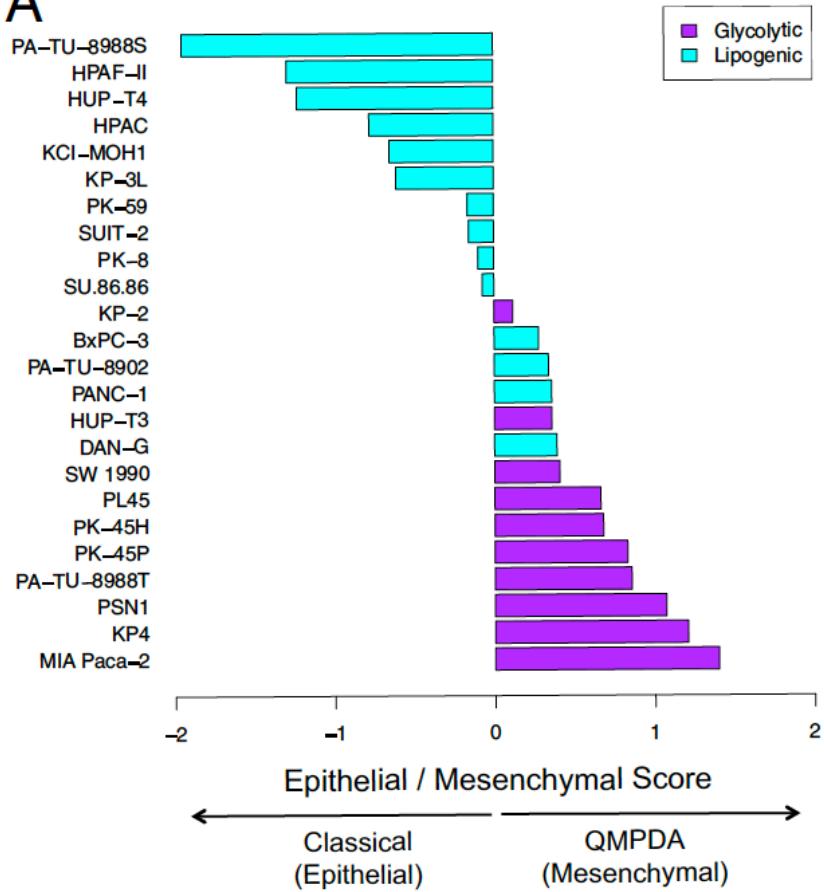


Critical in PDAC growth. Numerous publications on individual mechanisms++++

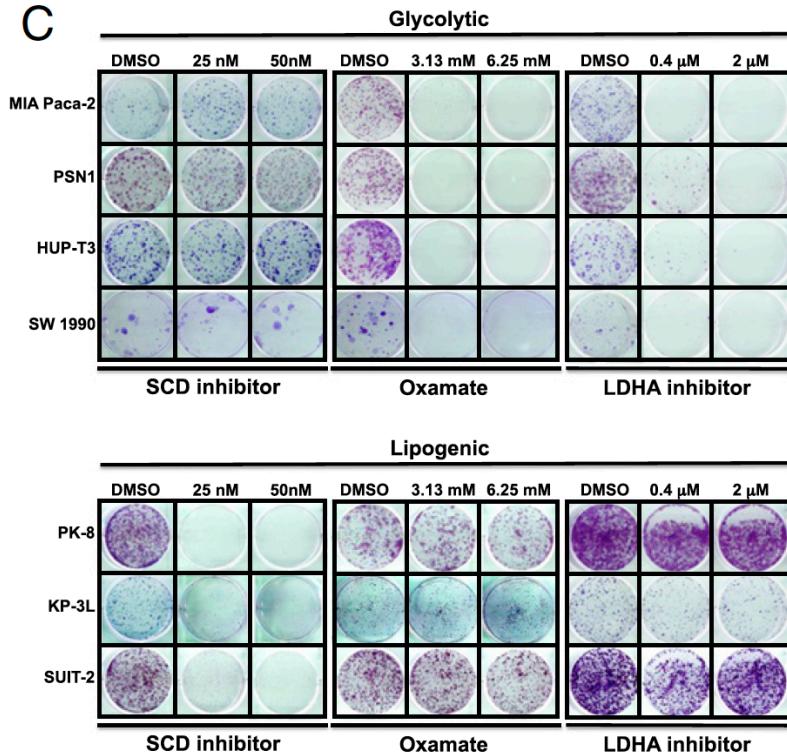
A



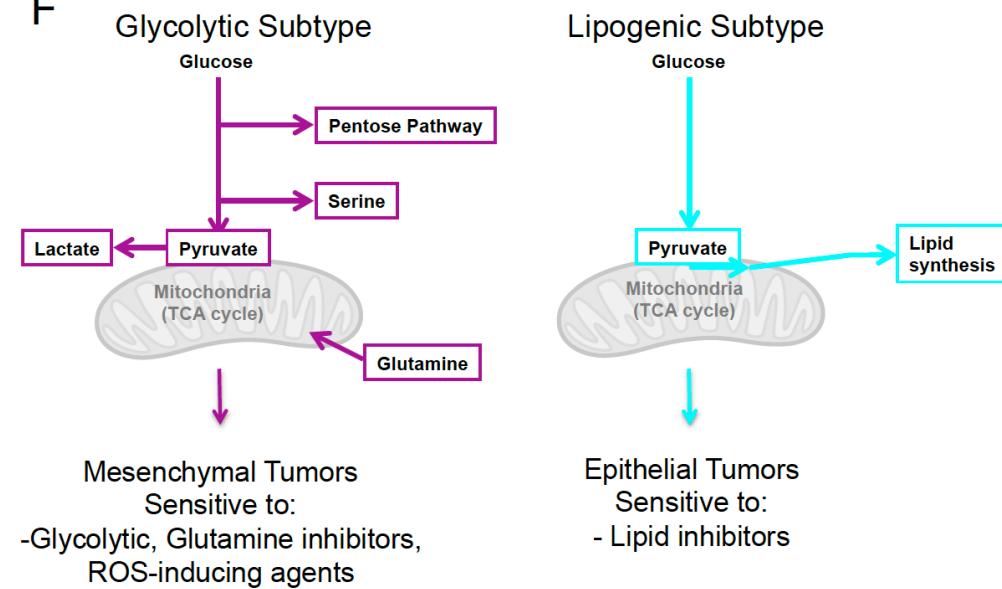
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C



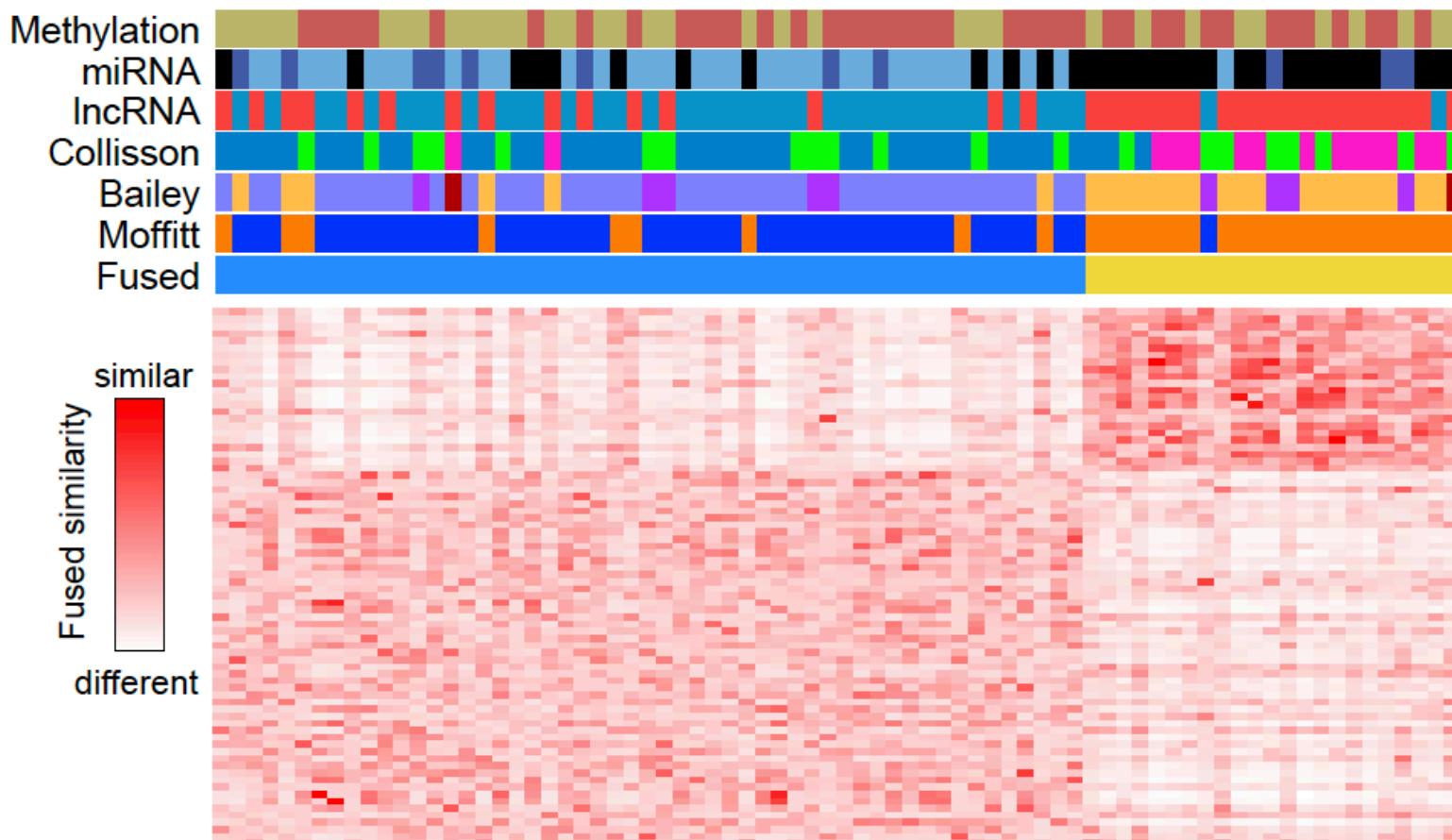
F



Human tumors ?

Inter-tumor heterogeneity - conclusion

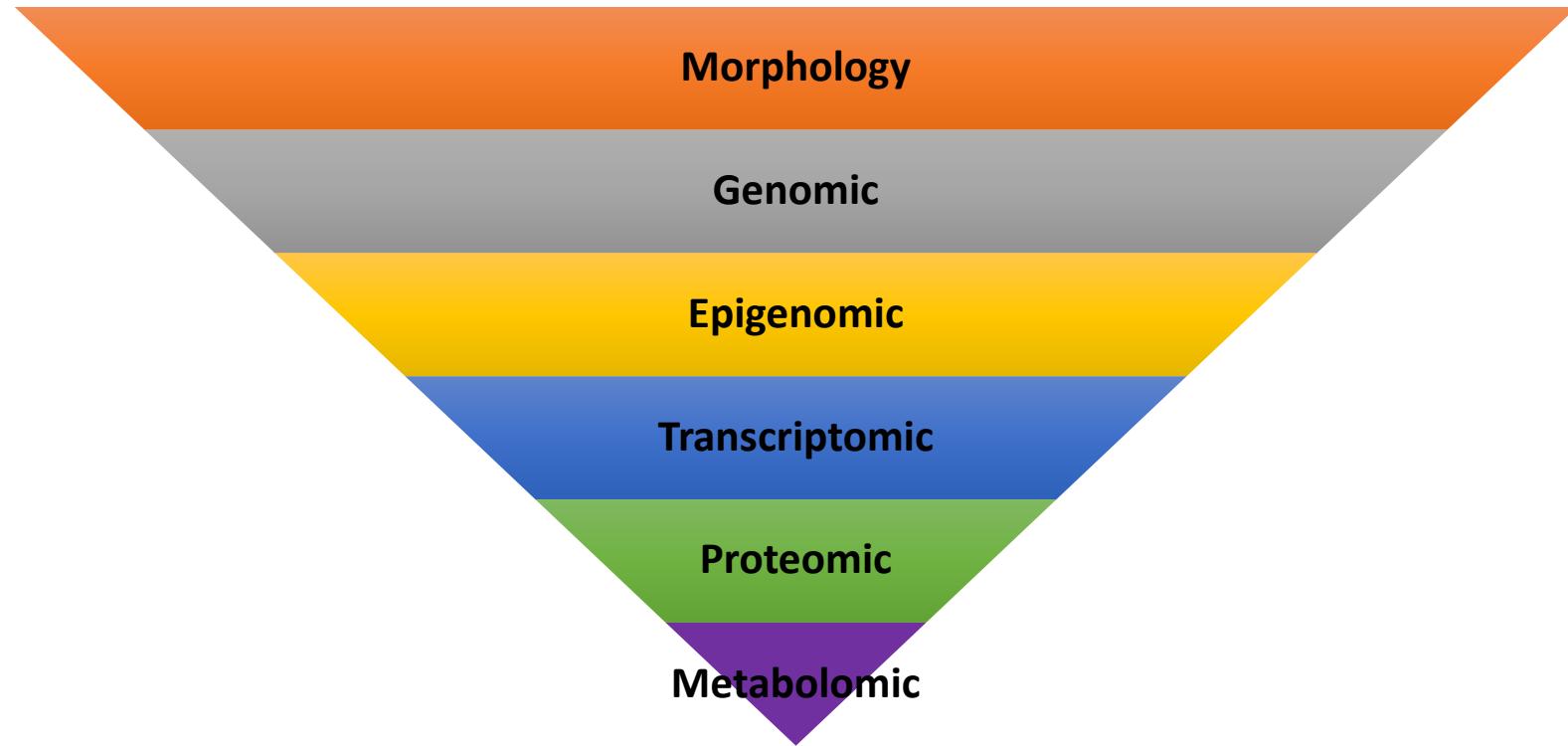
Major impact of purity (tumor cells) on classifications++++



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



Morphology

Genomic

Epigenomic

Transcriptomic

Proteomic

Metabolomic

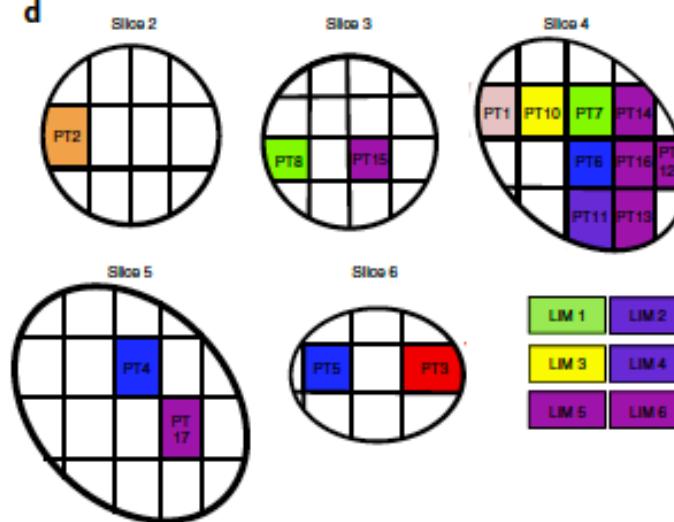
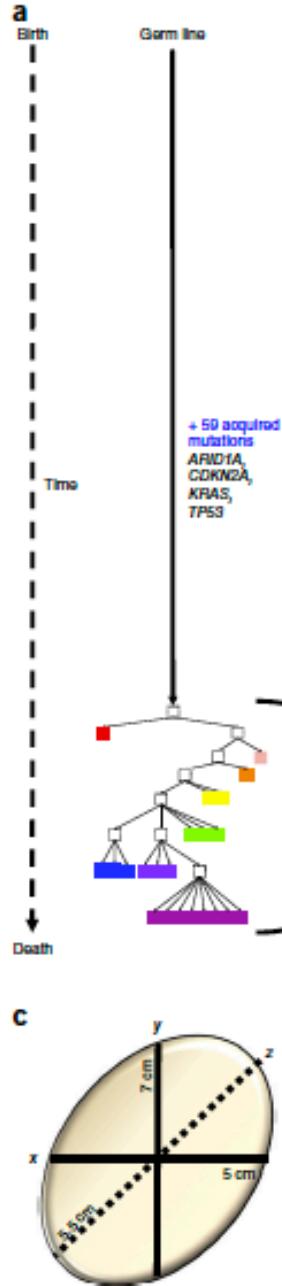
stroma

Morphology/transcriptomic

Epigenomic

Genomic

Intra-tumor heterogeneity - genomic

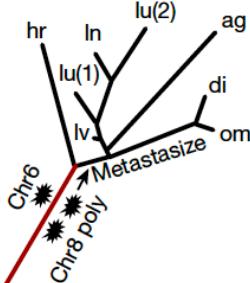
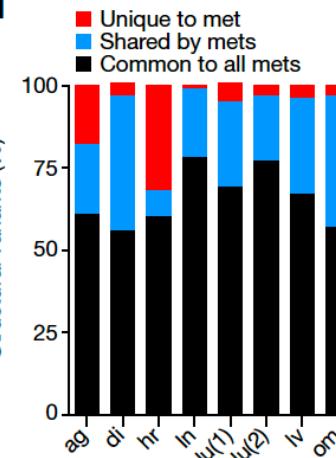


- Low heterogeneity of classical driver genes
- Most genomic events happen early
- No « metastasis » gene
- Physical and genomic spatialisation are different+++

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

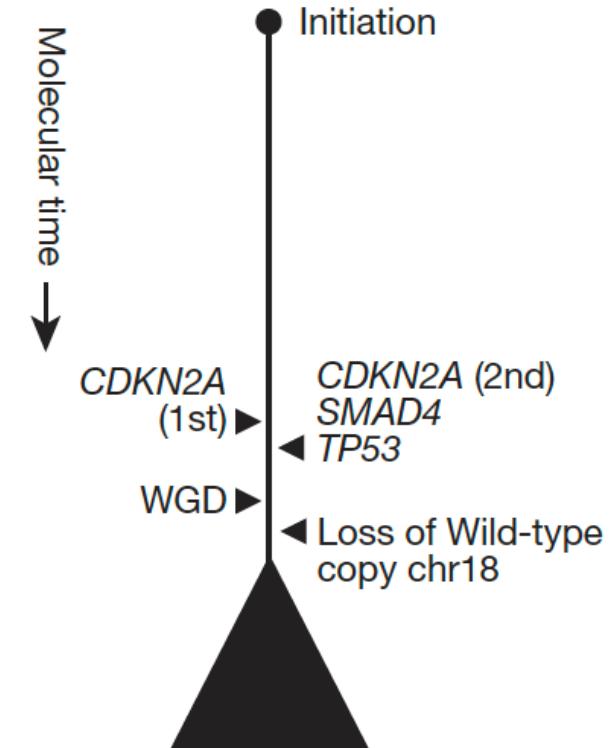
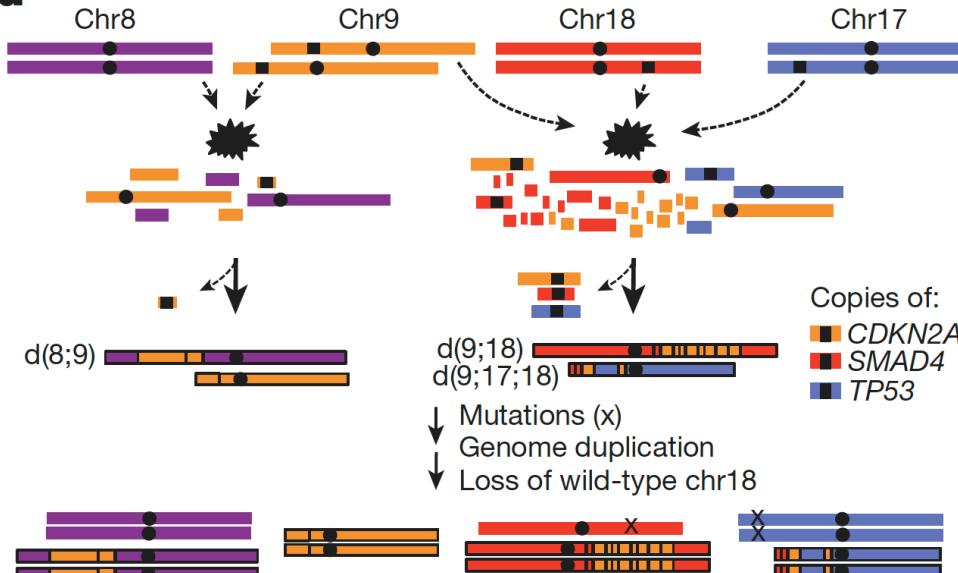
Intra-tumor heterogeneity - genomic

d

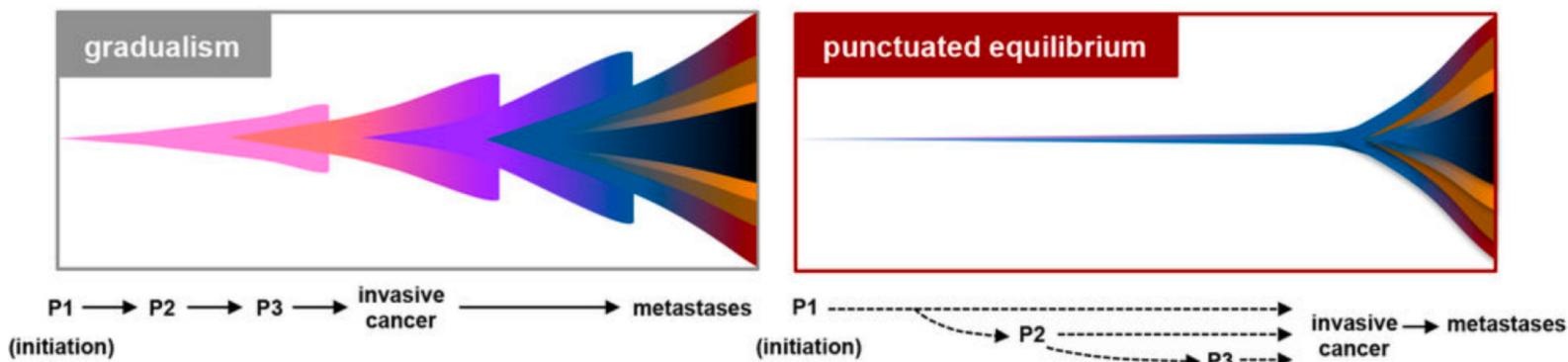
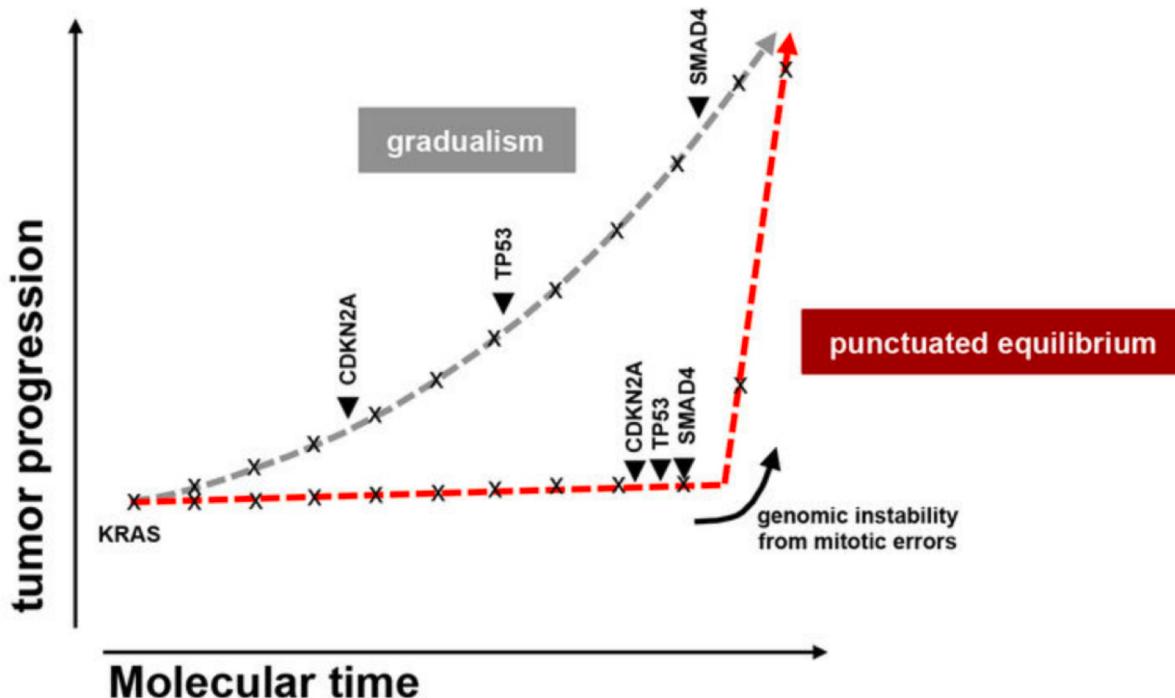


- Most genomic events happen before the first metastase
- 50% of tumours are not diploid (T ou H)
- Multiples simultaneous genomic alterations

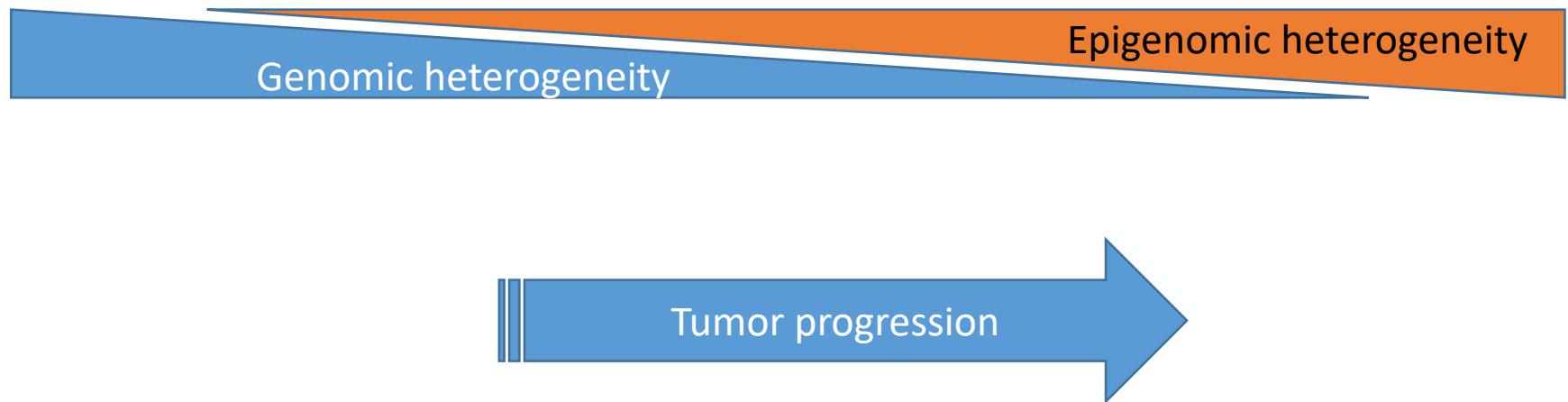
d



How to follow high risk patients???



Intra-tumor heterogeneity - epigenomic



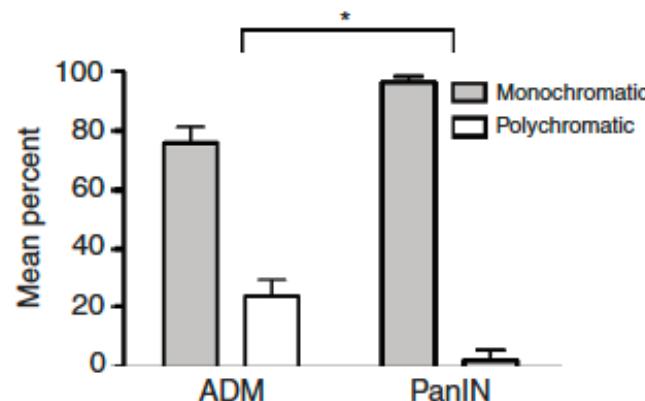
Genomic heterogeneity

Epigenomic heterogeneity

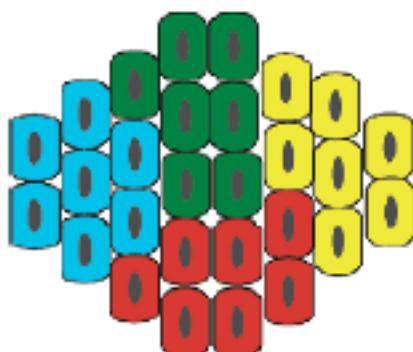
C

ADM and PanIN counts

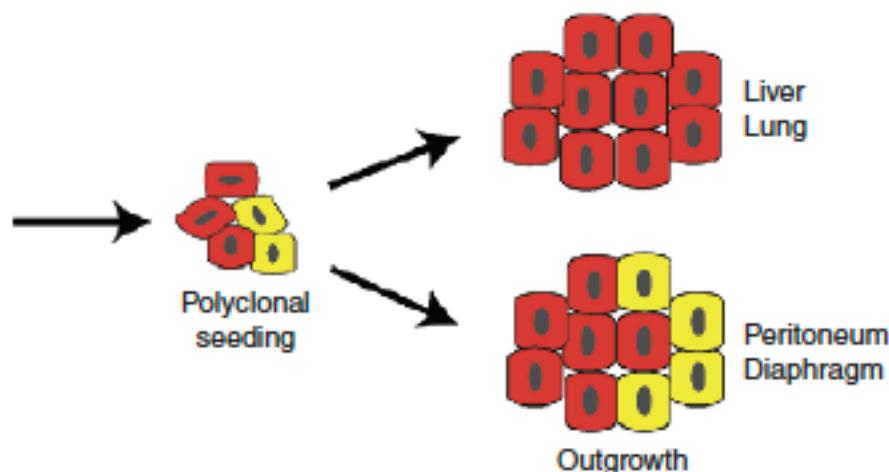
Lesion	Monochromatic	Polychromatic	Total
ADM	149 (76%)	46 (24%)	195
PanIN	141 (97%)	4 (3%)	145



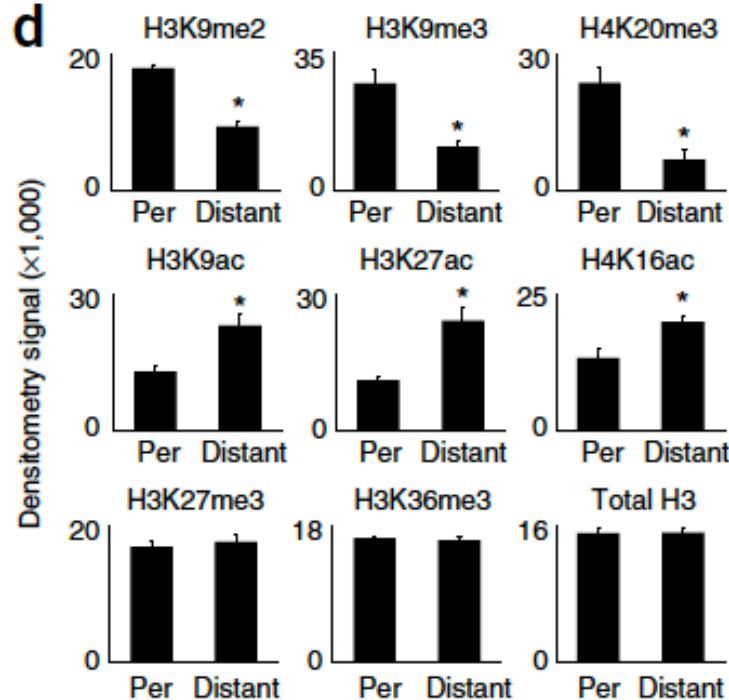
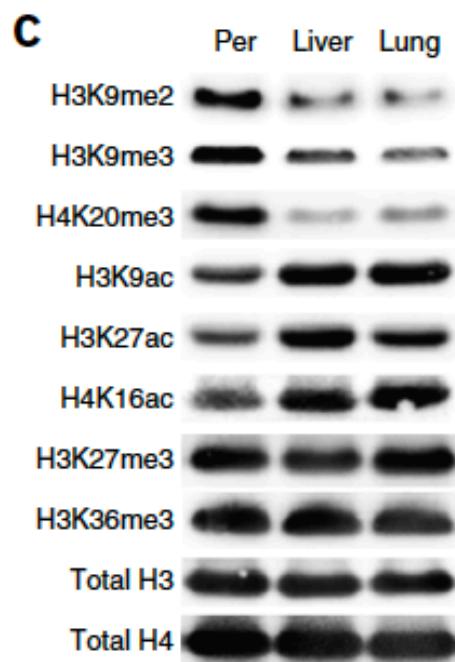
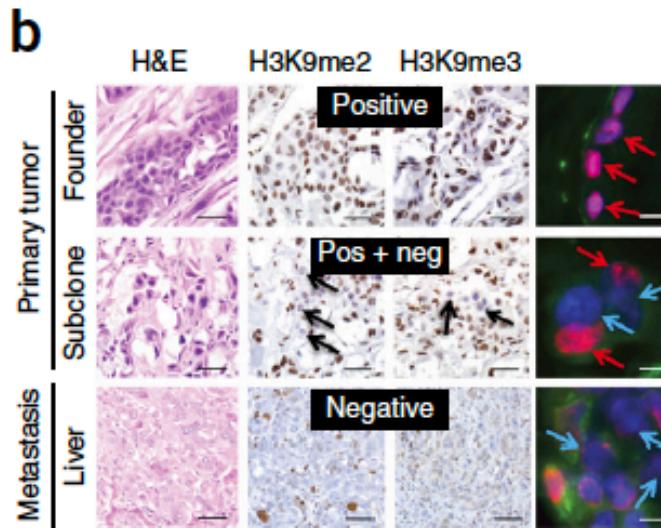
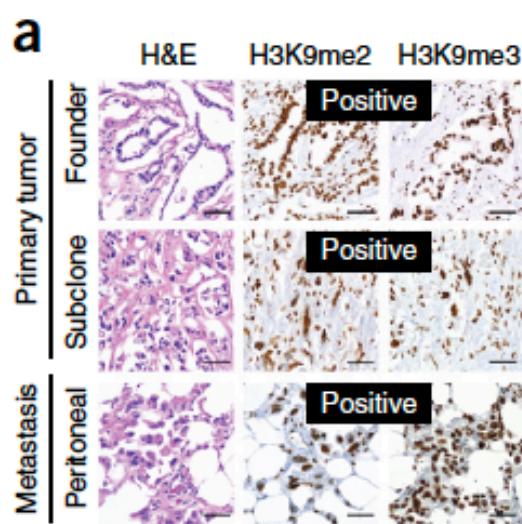
Primary tumor



Distant metastasis

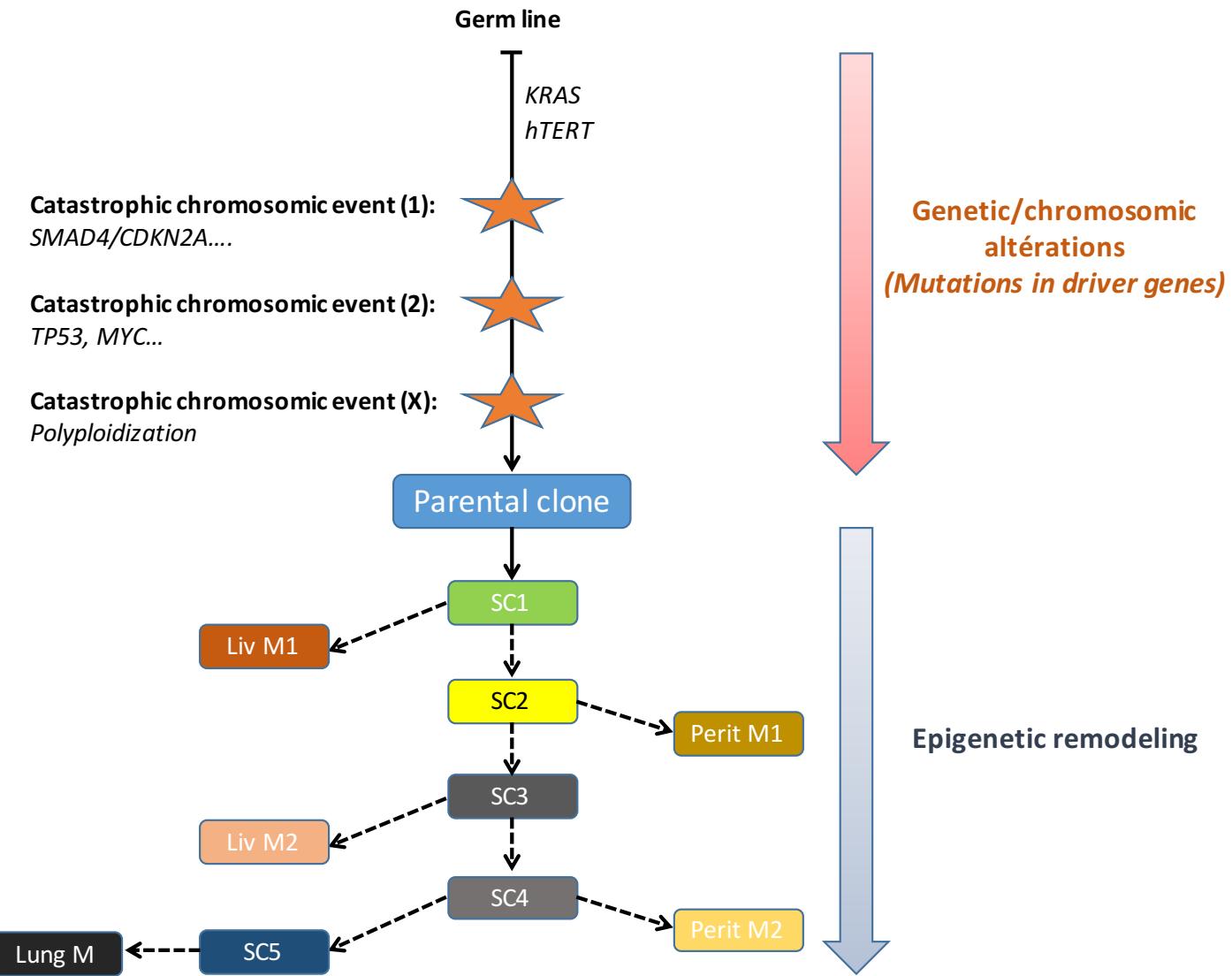


Intra-tumor heterogeneity - epigenomic



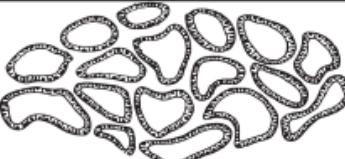
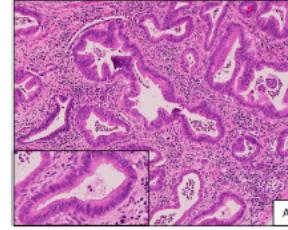
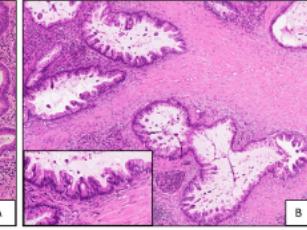
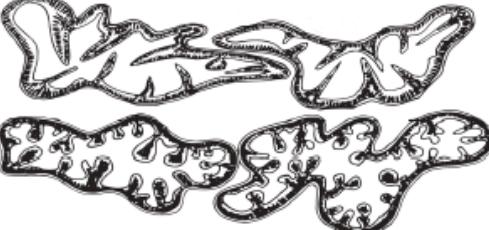
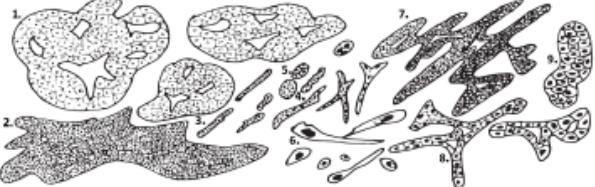
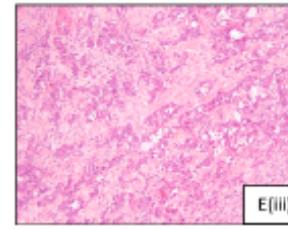
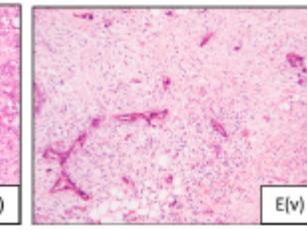
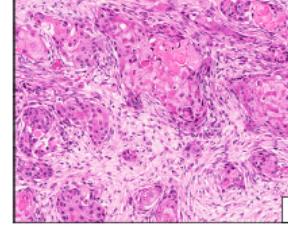
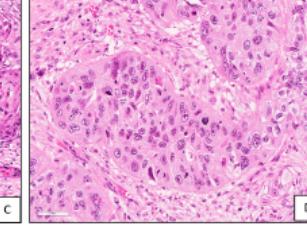
Genomic heterogeneity

Epigenomic heterogeneity



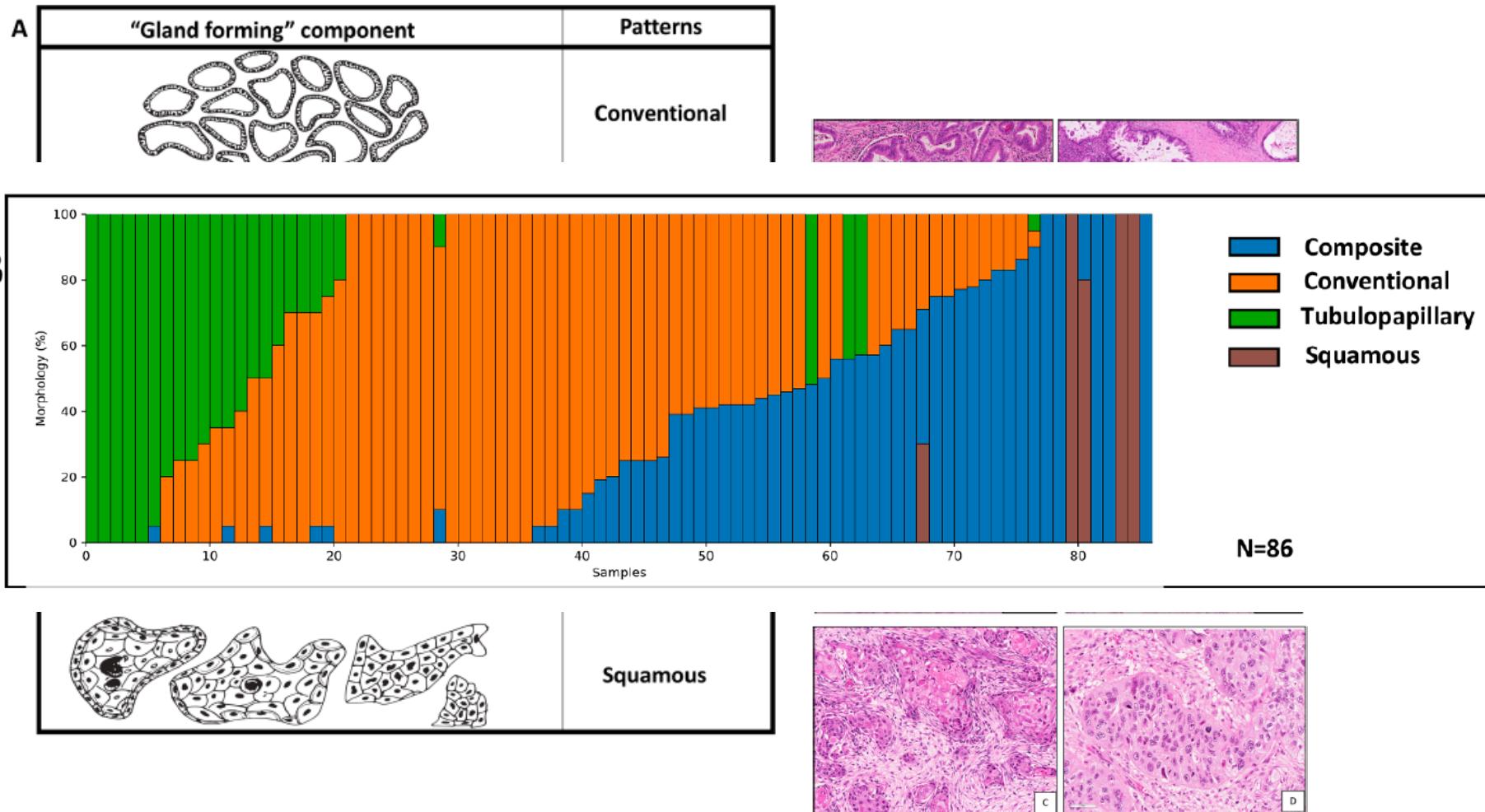
Intra-tumor heterogeneity - morphology

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

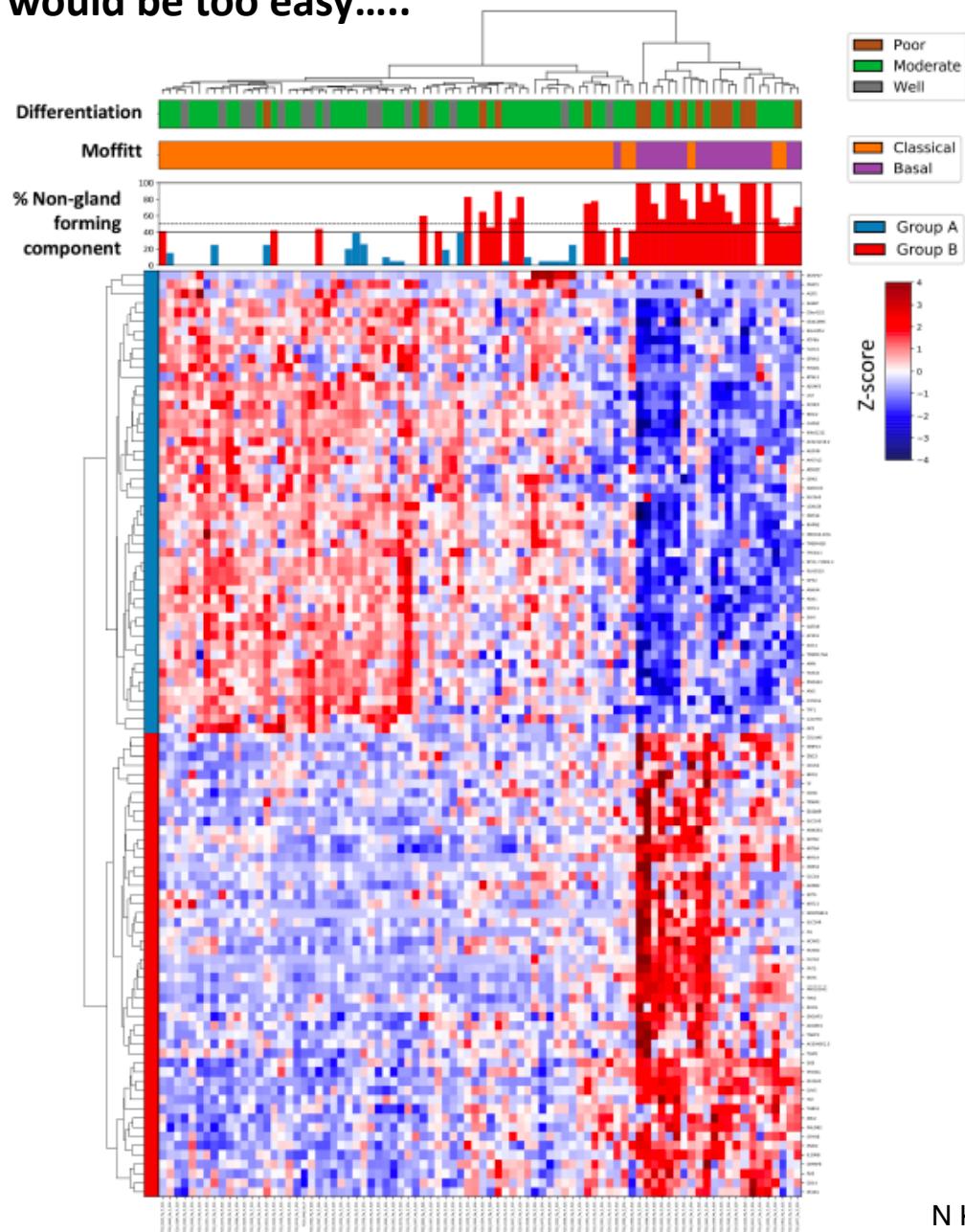
A	"Gland forming" component	Patterns	
		Conventional	 
		Tubulopapillary	
	"Non-gland forming" component	Patterns	
		Composite	 
		Squamous	 

Intra-tumor heterogeneity - morphology

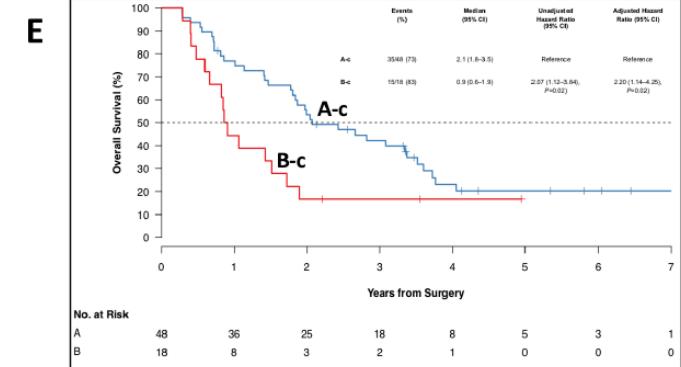
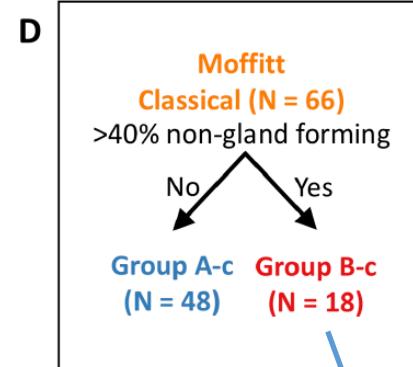
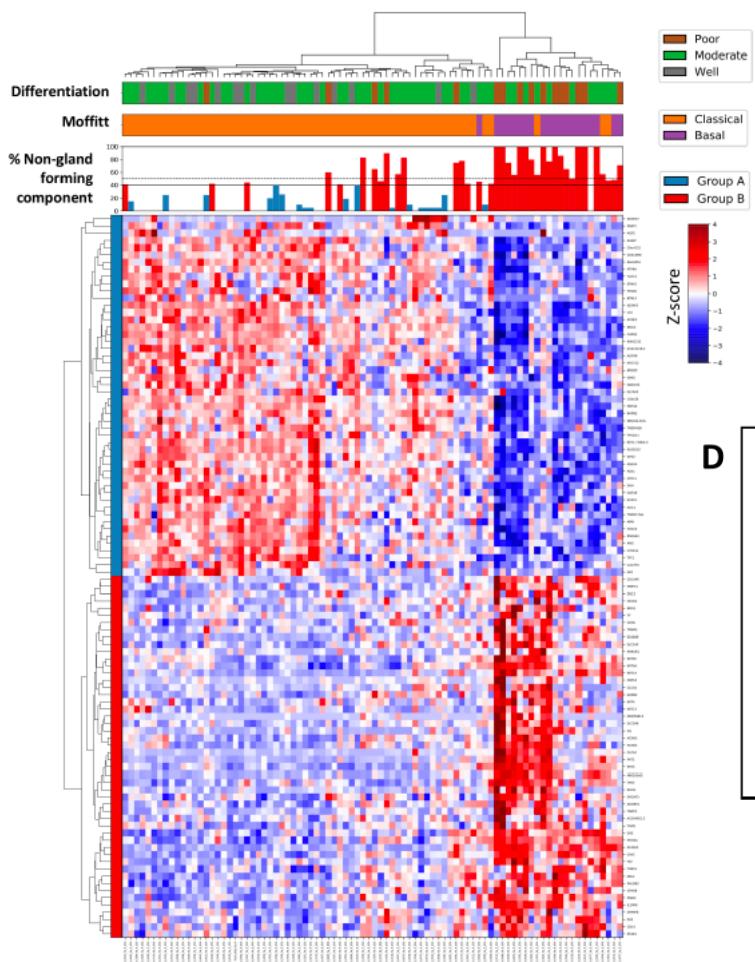
If tumor were pure, that would be too easy.....



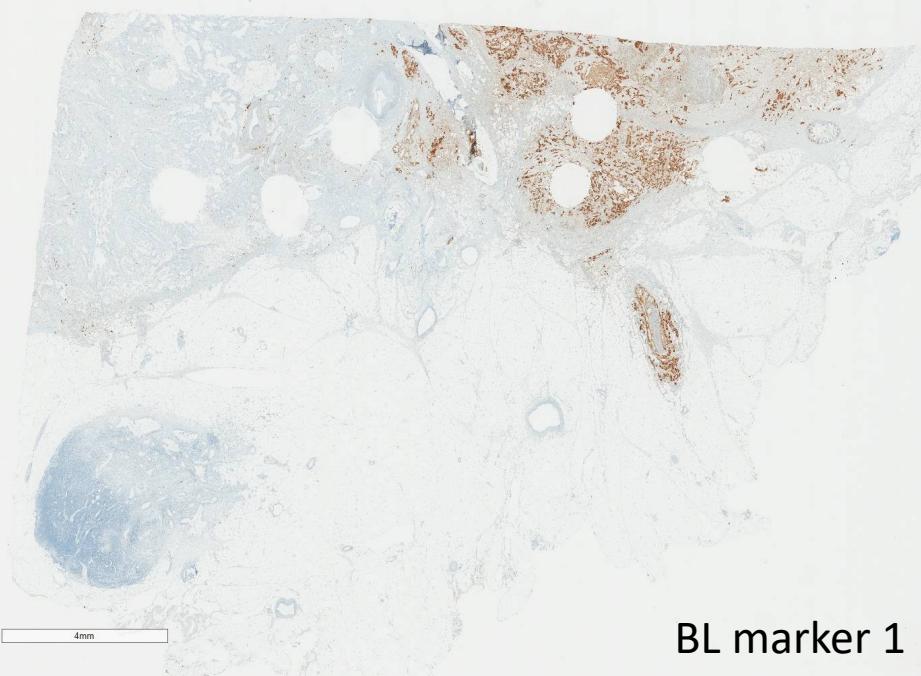
If tumor were pure, that would be too easy.....



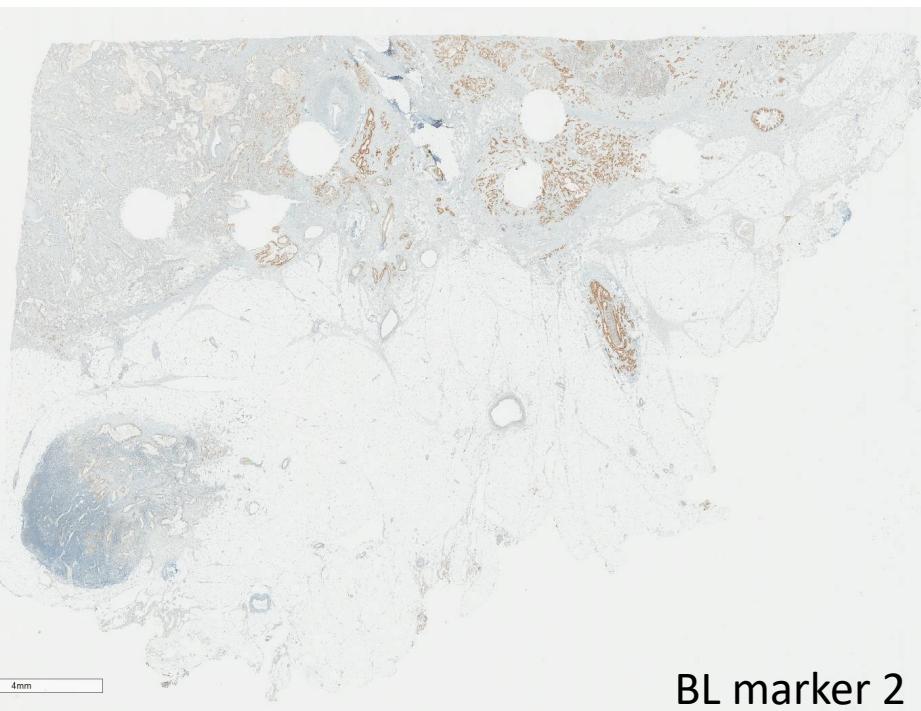
If tumor were pure, that would be too easy.....



« classical » tumors with a basal like subpopulation?



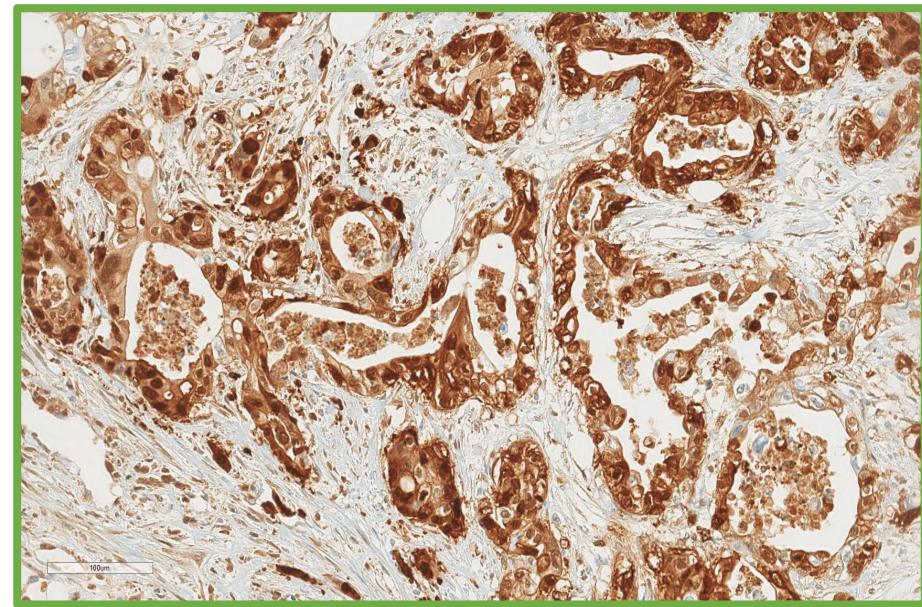
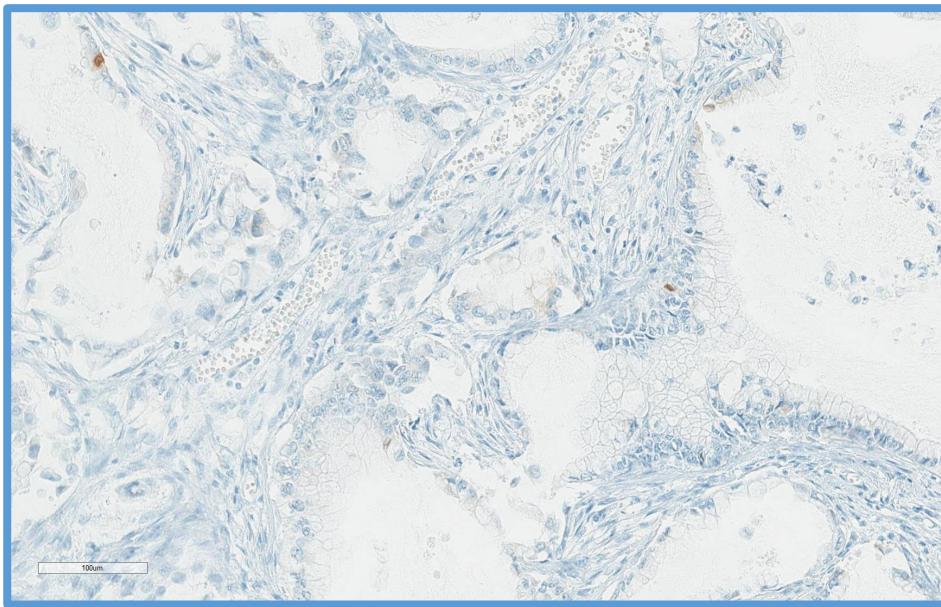
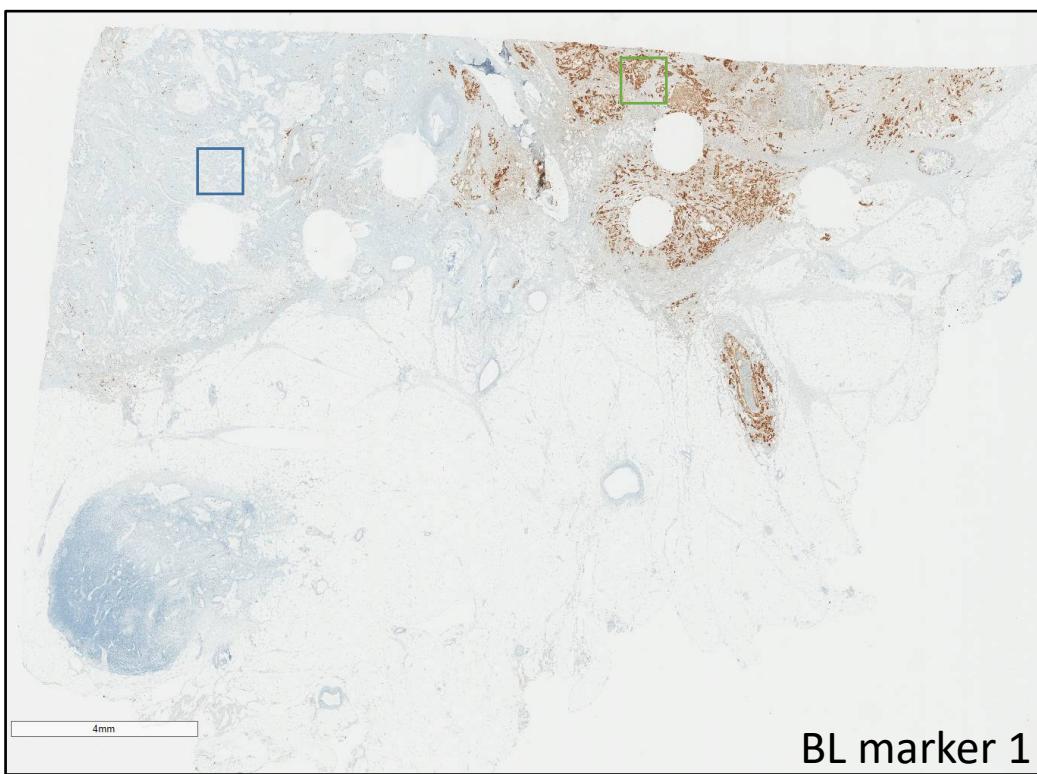
BL marker 1



BL marker 2

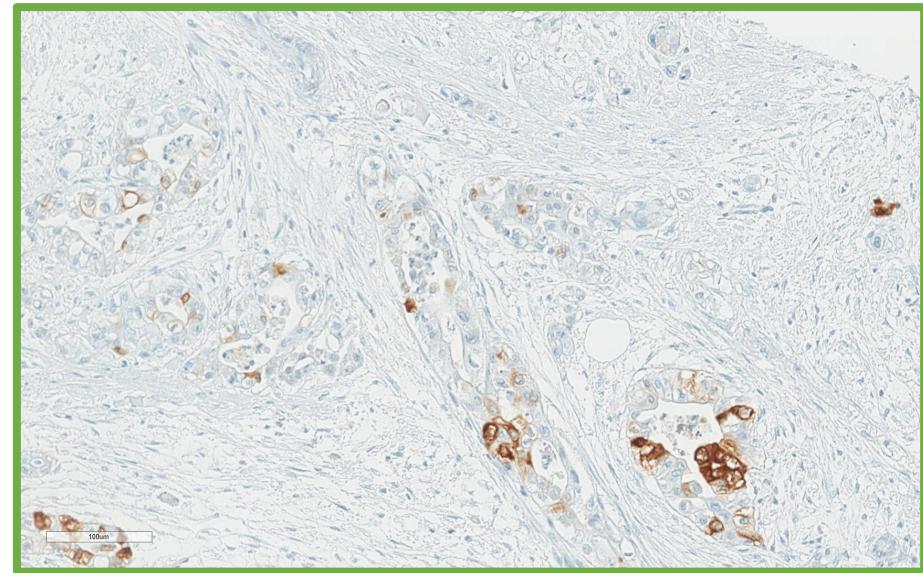
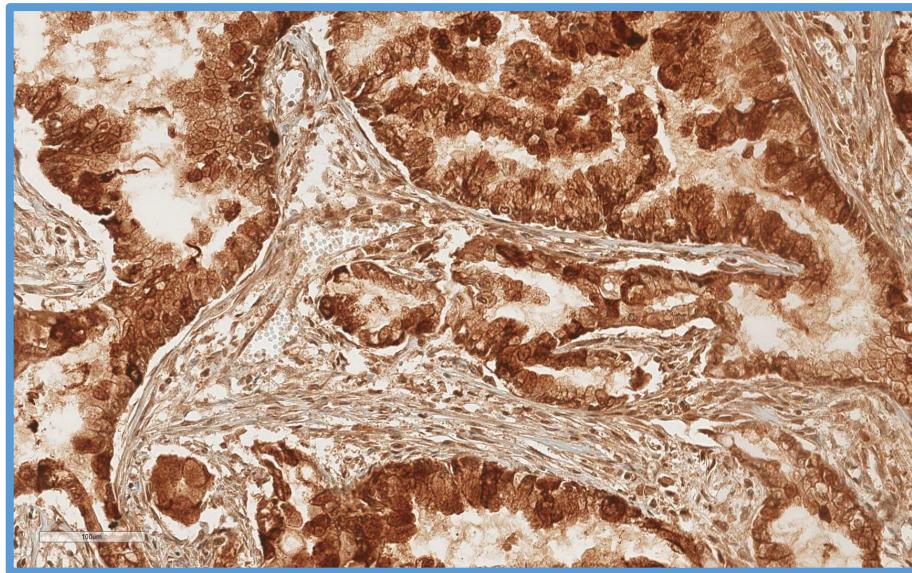


Class marker 1

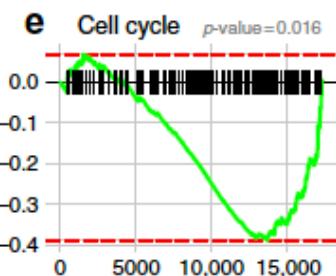
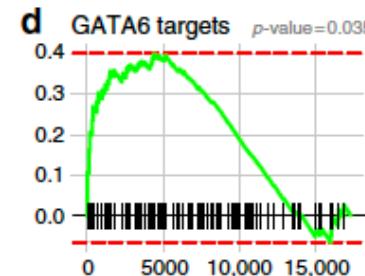
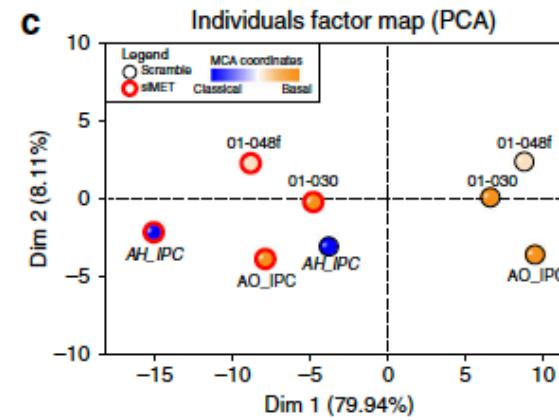
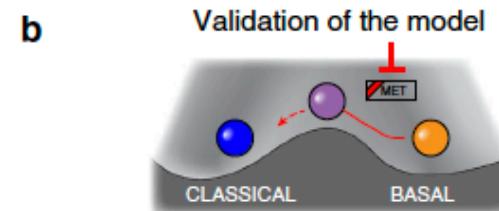
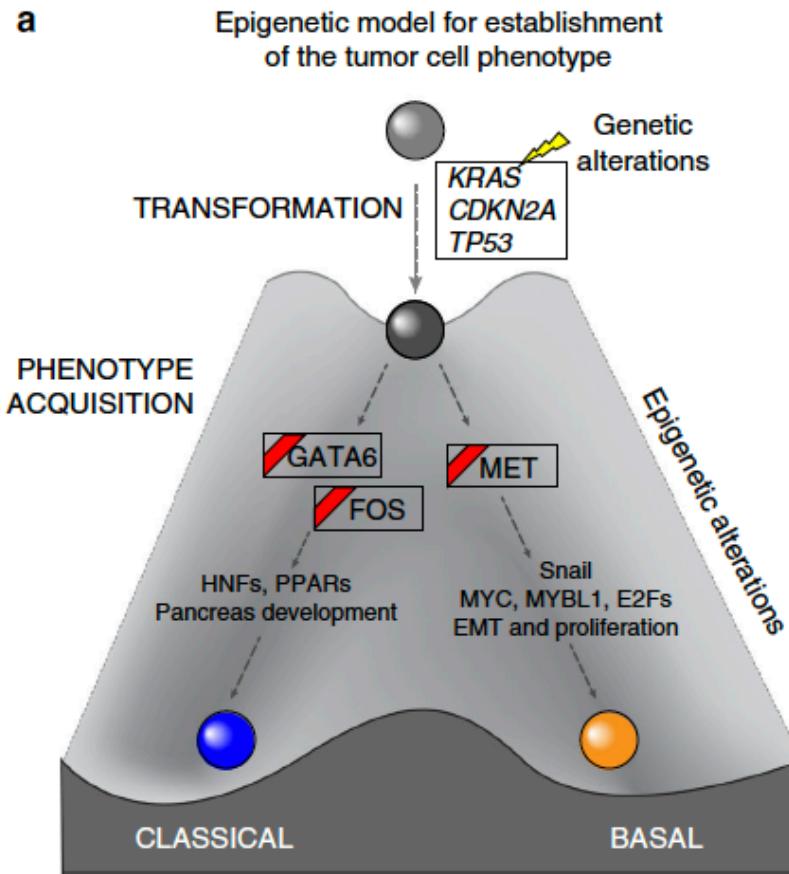




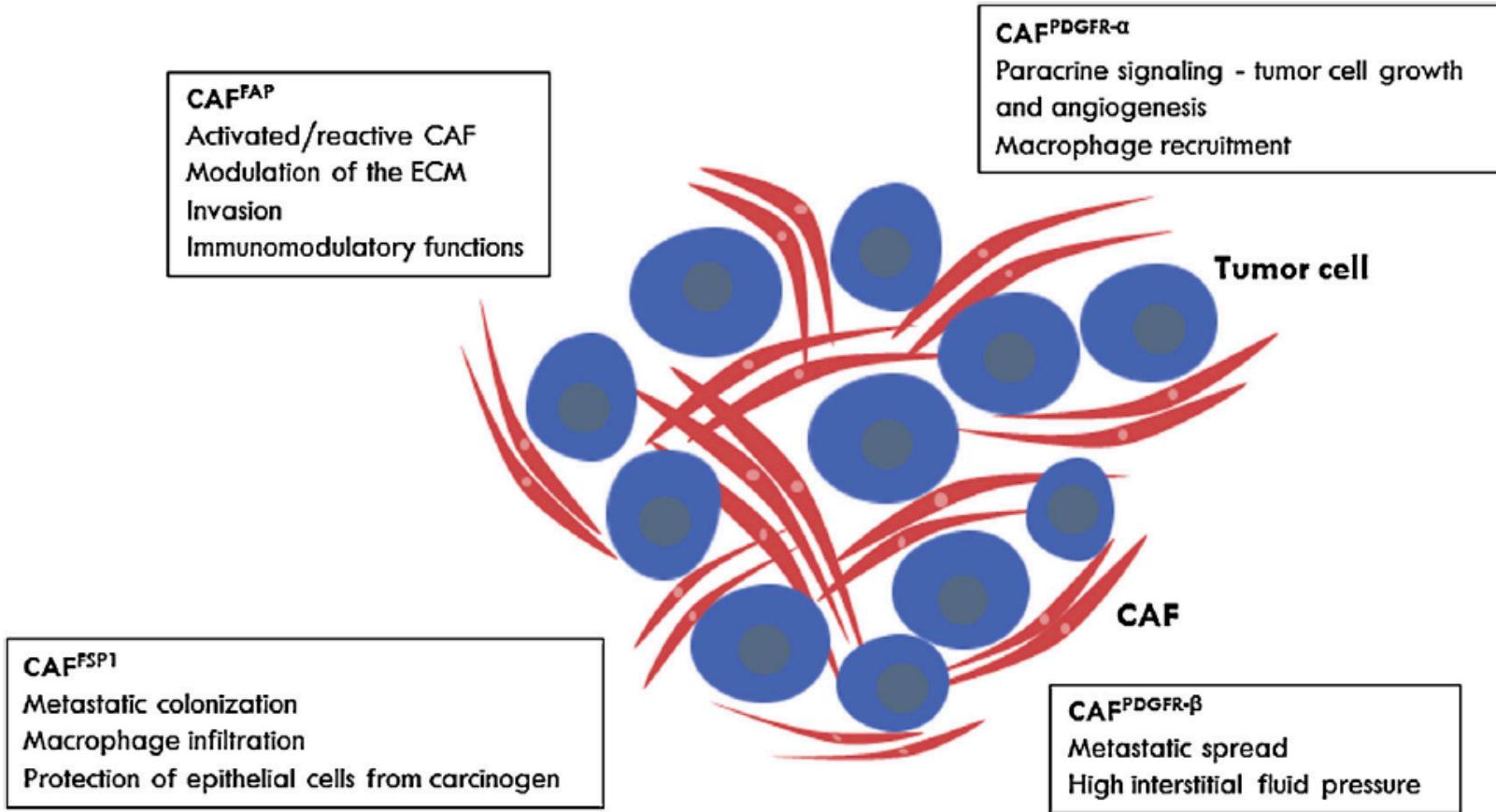
Classical marker 1



Is there an epigenetic-driven plasticity between subtypes?

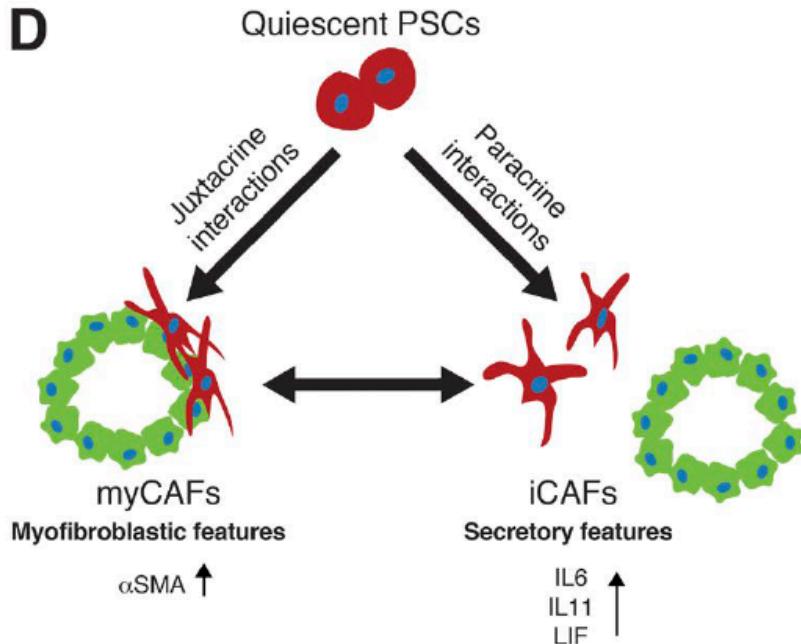


Intra-tumor heterogeneity - stroma

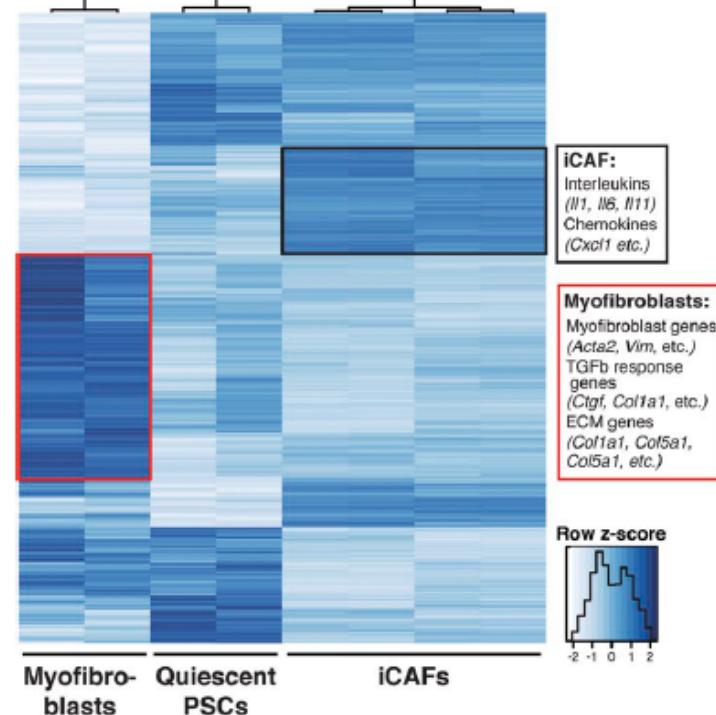


Intra-tumor heterogeneity - stroma

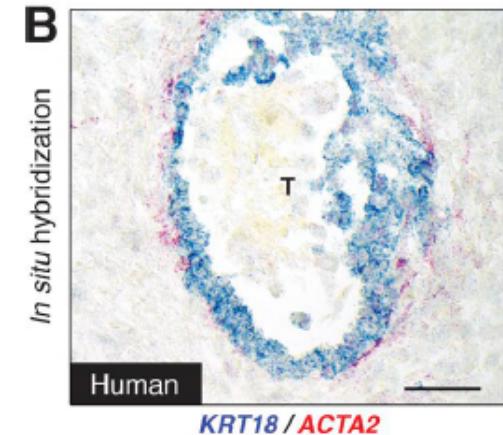
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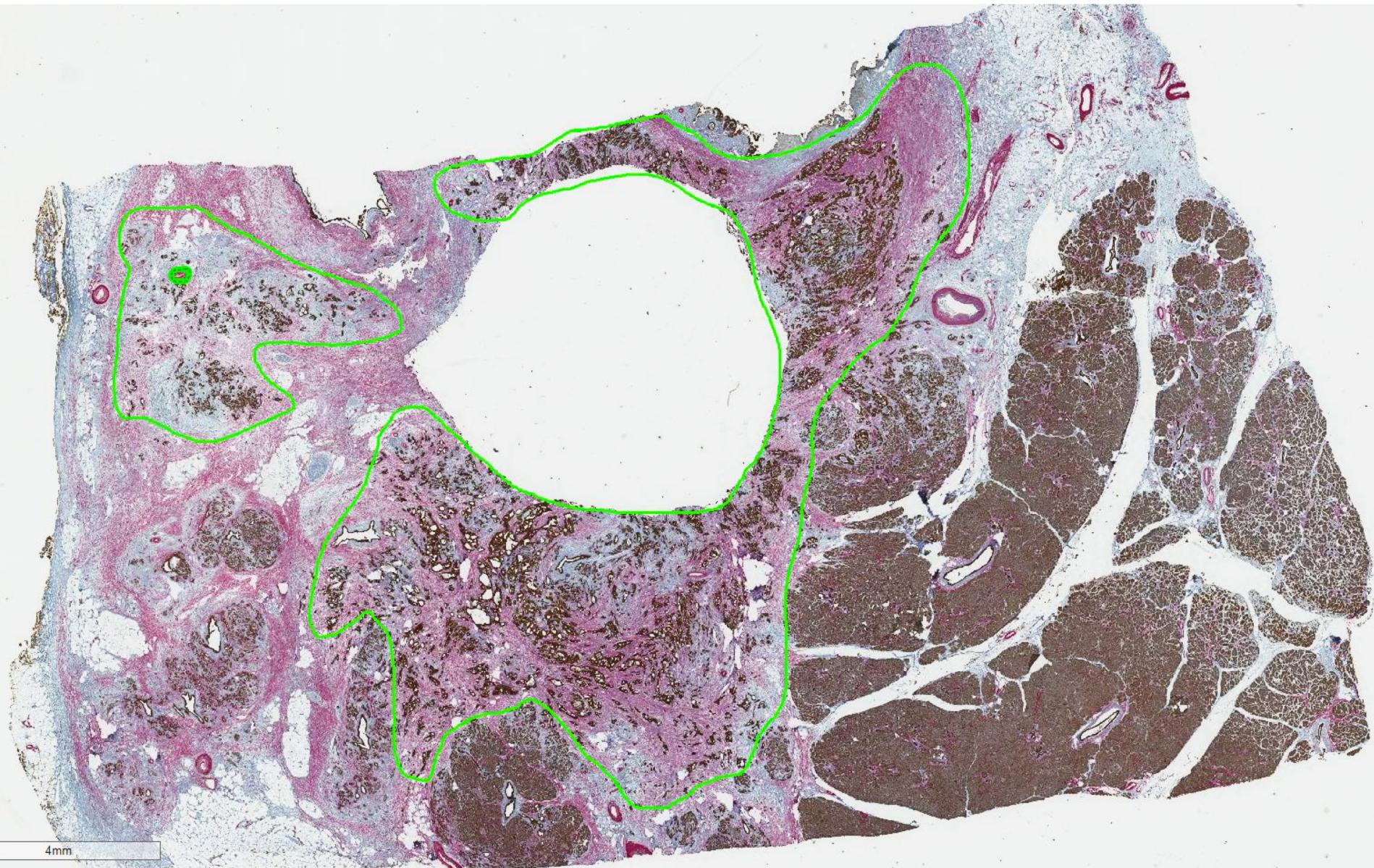
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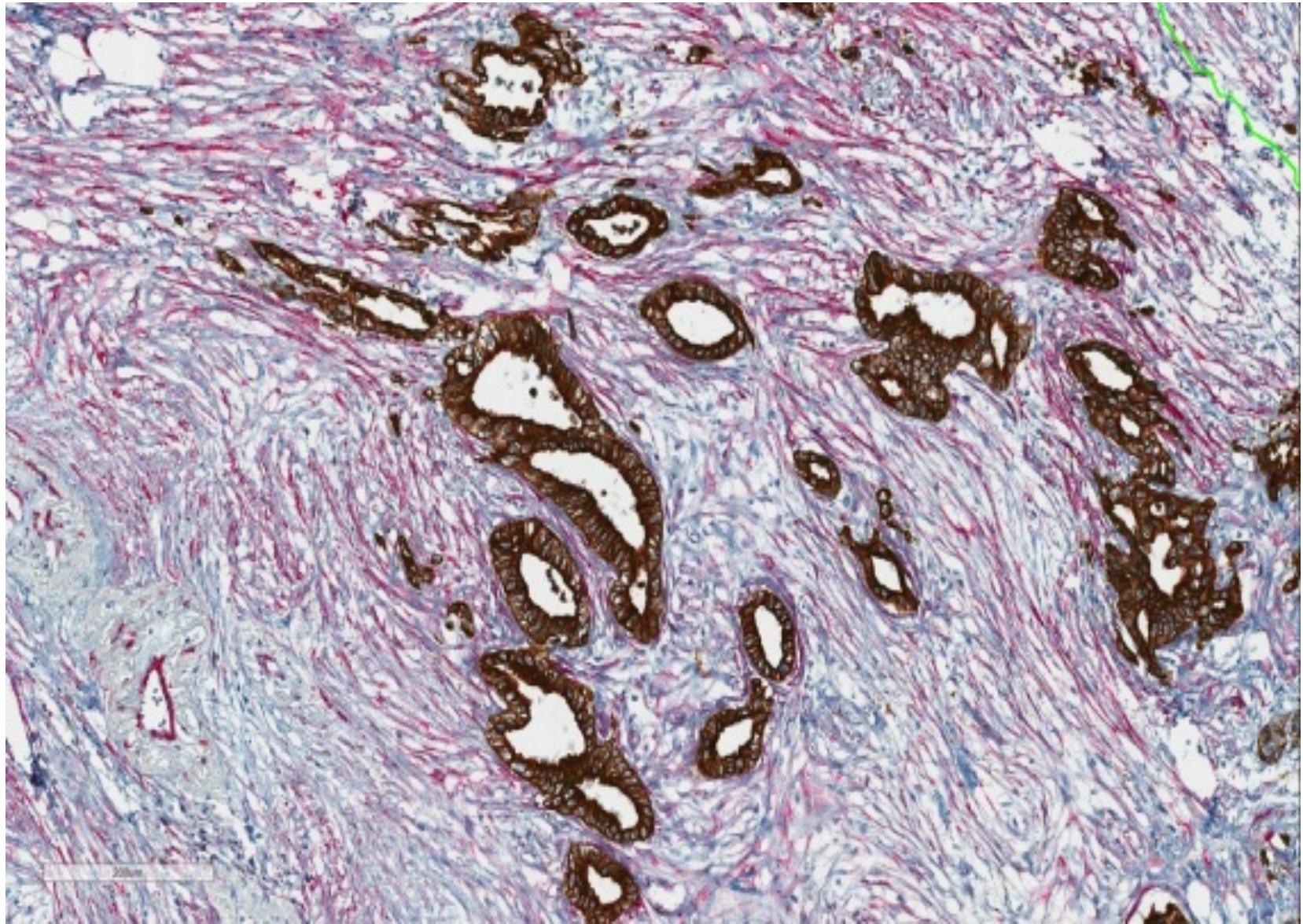
B



Intra-tumor heterogeneity - stroma



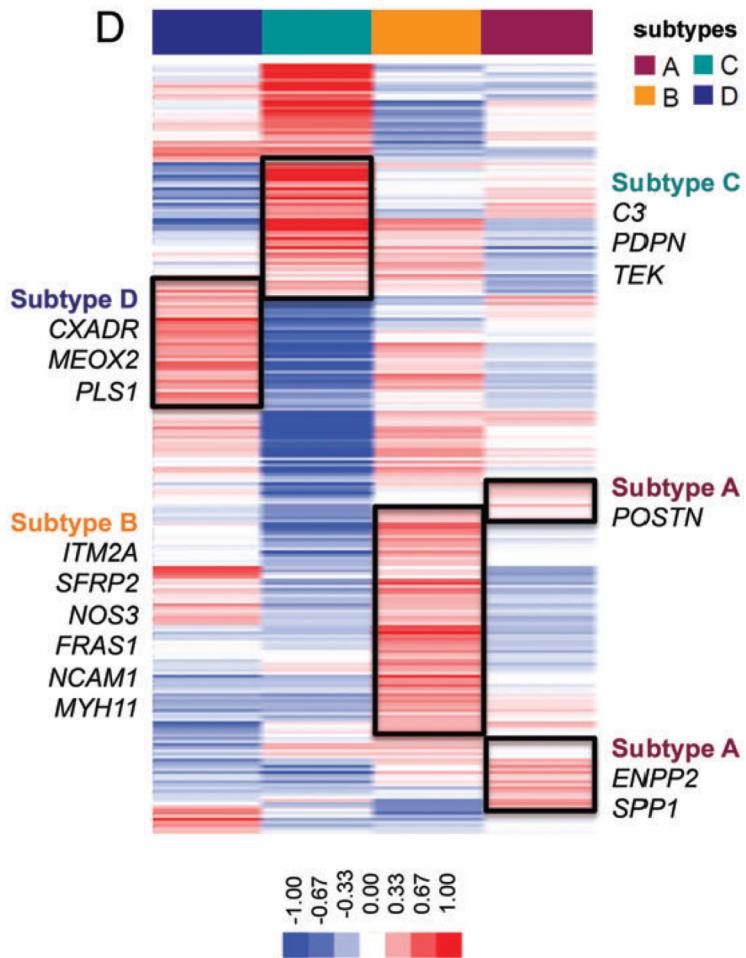
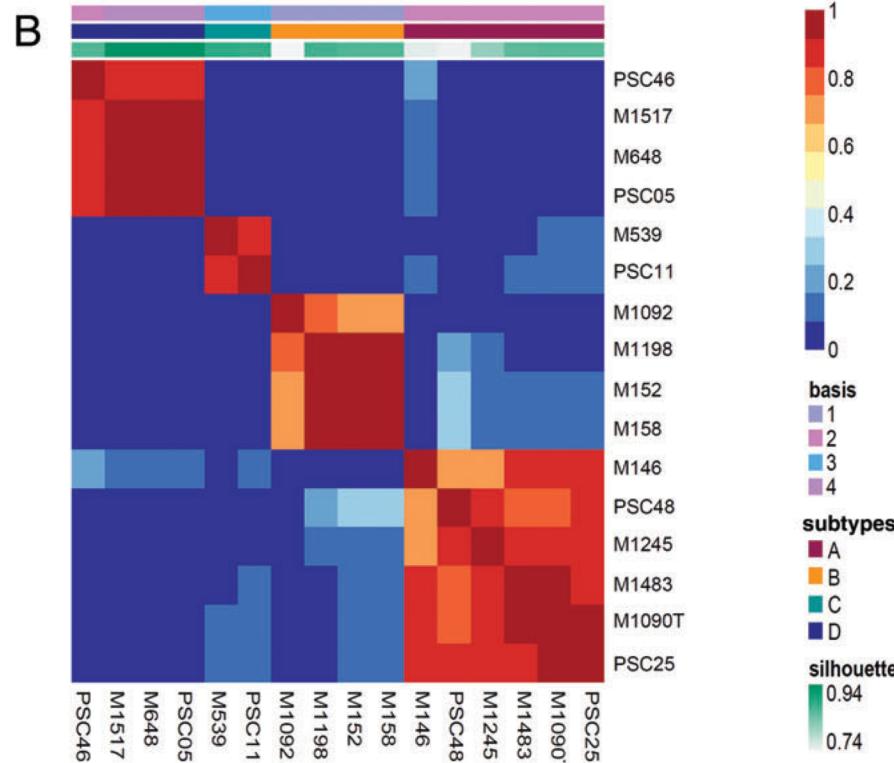
Intra-tumor heterogeneity - stroma



Intra-tumor heterogeneity - stroma

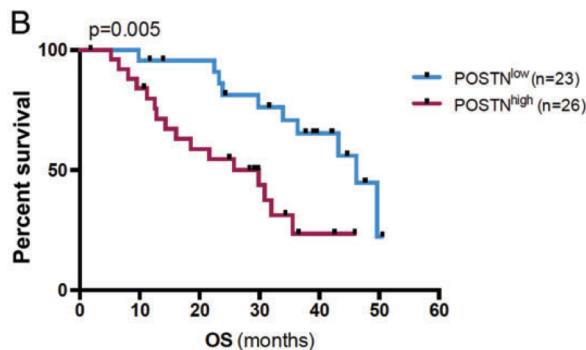
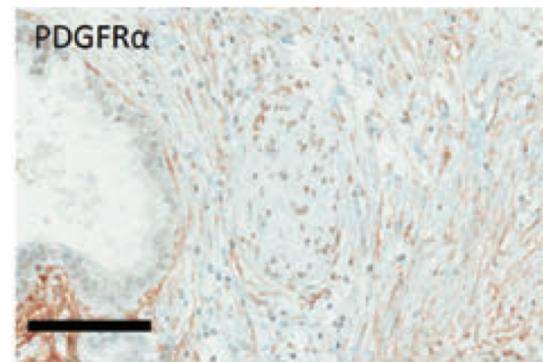
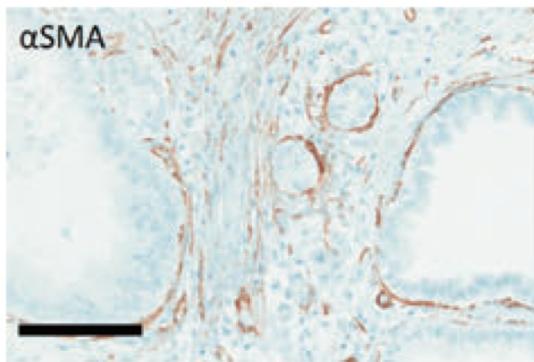
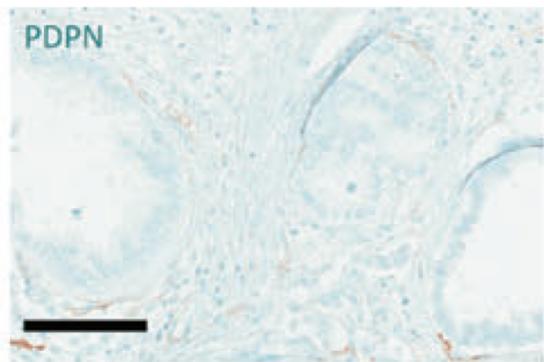
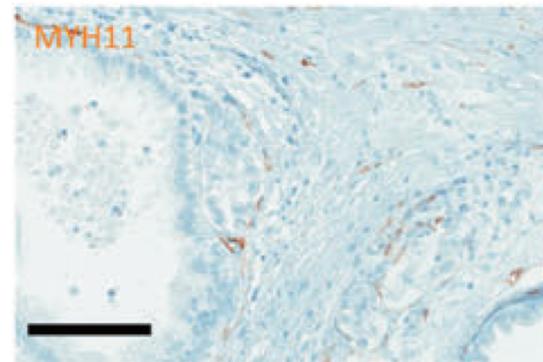
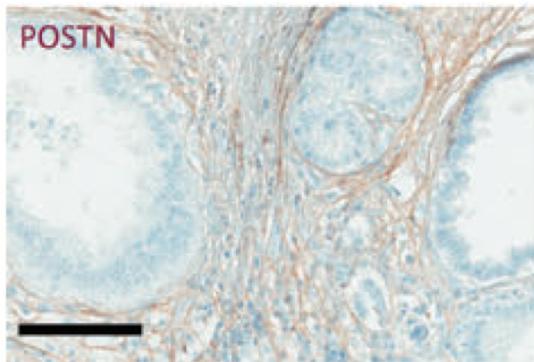
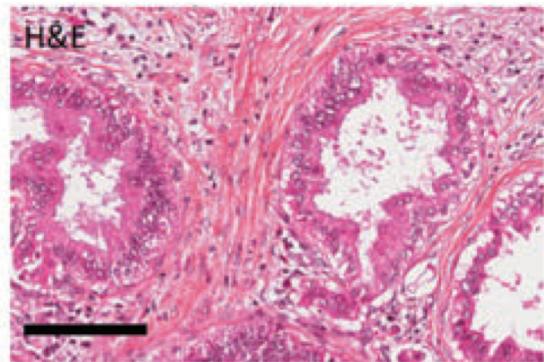
In humans, it may be a bit more complicated....

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?
 - 2 tumor subtypes, how many stroma subtypes....?
 - How to best define the subtype in routine practice?
-
- Most genomic events happen early
 - Epigenetic intratumor heterogeneity >> genetic
 - Probable massive spatial transcriptomic heterogeneity....

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



Heterogeneity in pancreatic adenocarcinoma

Does it happen? Is it important?

Jerome Cros

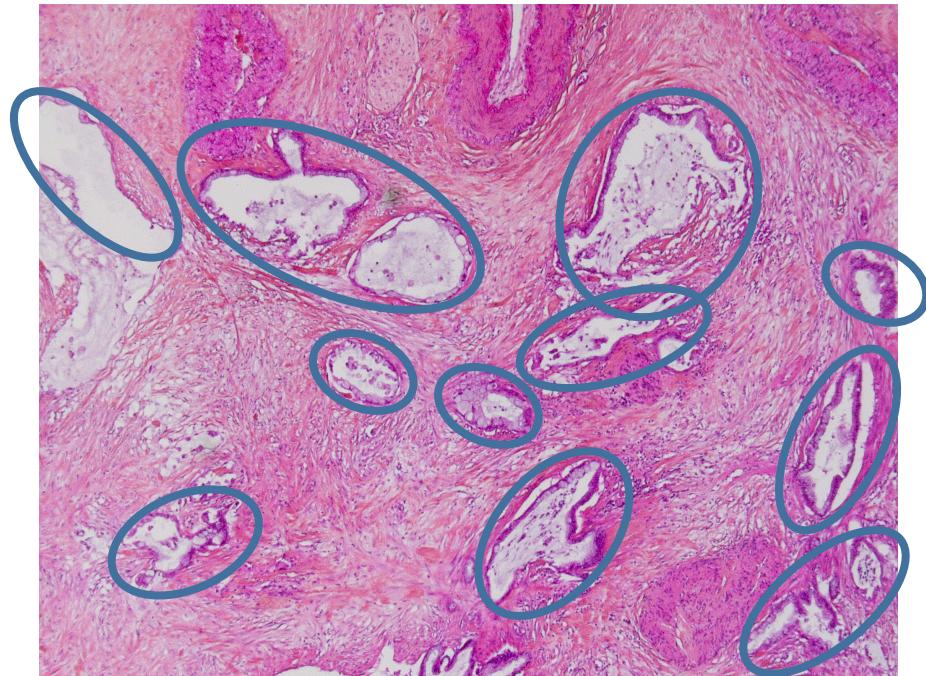
Dpt of Pathology – INSERM U1149

Beaujon Hospital, Paris, France

PANCREATIC ADENOCARCINOMA

Tumor cells

Stroma



PANCREATIC NEUROENDOCRINE TUMOR

Tumor cells

Stroma