Heterogeneity in pancreatic adenocarcinoma

Does it happen? Is it important?

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CRI CENTRE de RECHERCHE sur l'INFLAMMATION

unity Addressing Unmet Needs through Innovation In hepaTology and gastroenterologY

Inserm

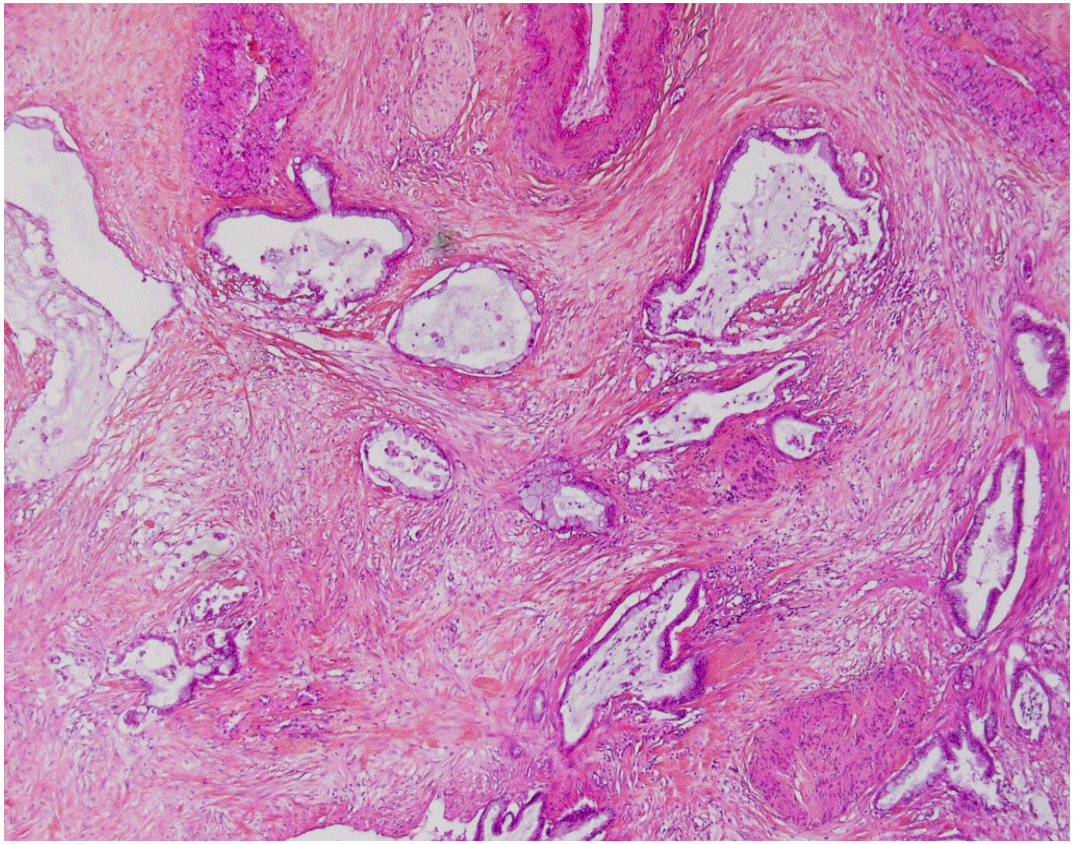
HÔPITAUX UNIVERSITAIRES PARIS NORD VAL DE SEINE Beaujon

Study of tumor heterogeneity and issues with samples….

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

Pancreatic cancer

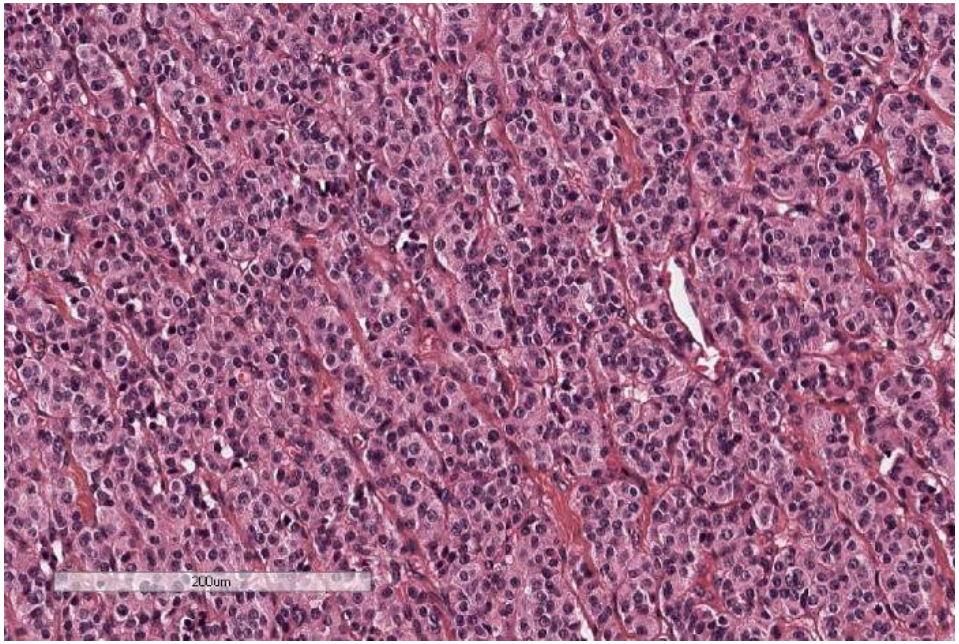
Adenocarcinoma (90%)



Pancreatic cancer

Adenocarcinoma (90%)

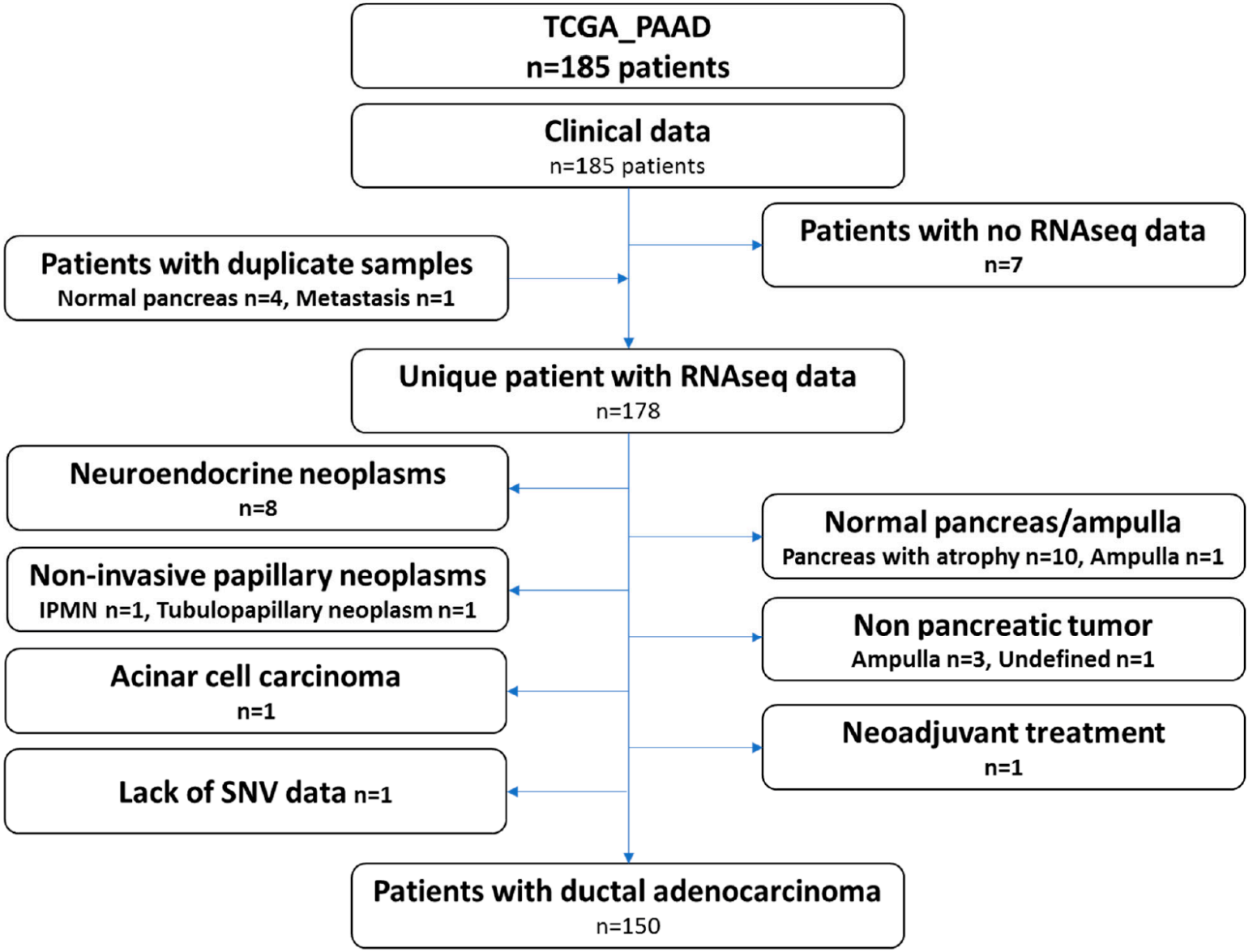
Neuroendocrine lesions



Rare lesions Acinar cell carcinoma….

Completely different morphology, biology….

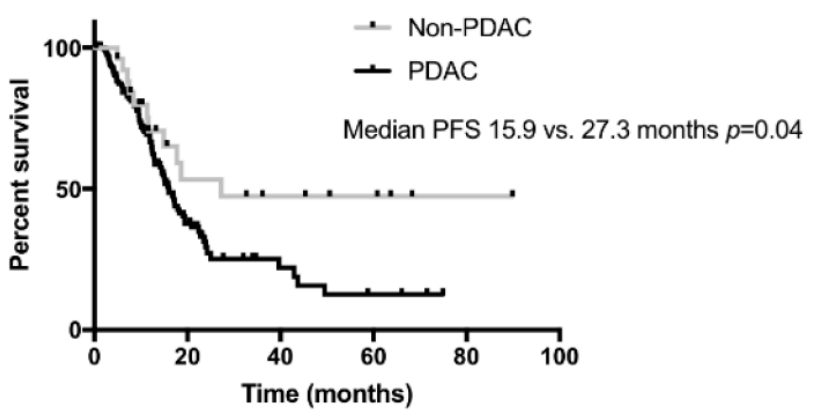
Beware of pancreatic non-PDAC tumor when using public datasets+++++

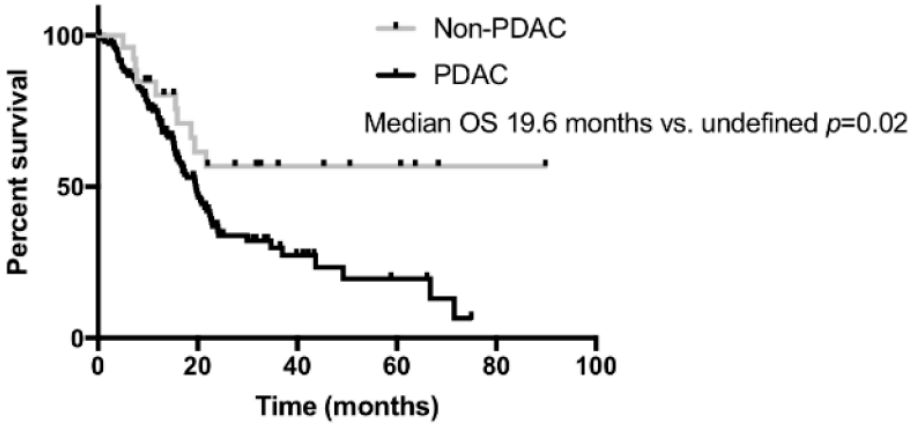


Nicolle *et al. Cancers* 2019

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

a





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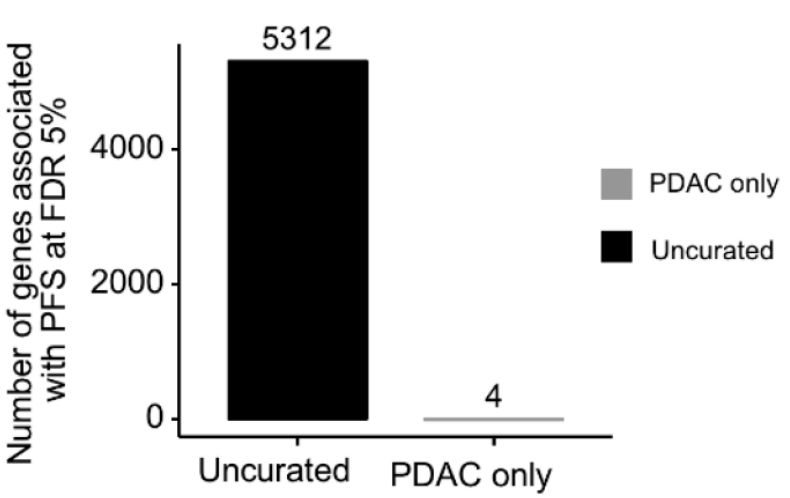

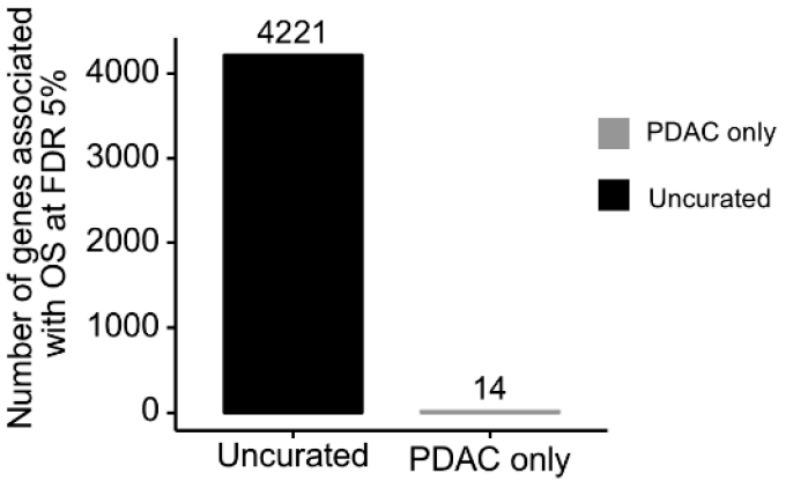

Nicolle *et al. Cancers* 2019

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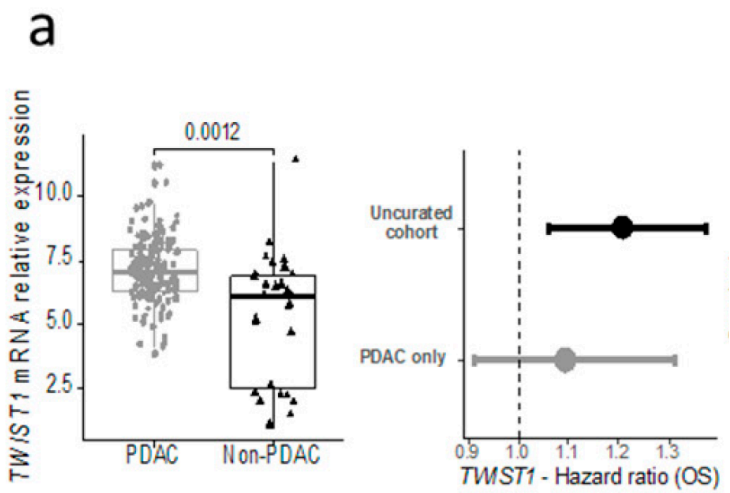
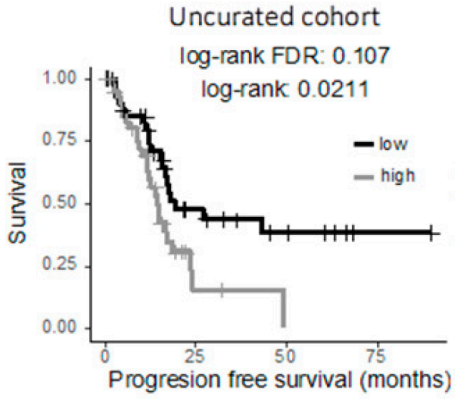
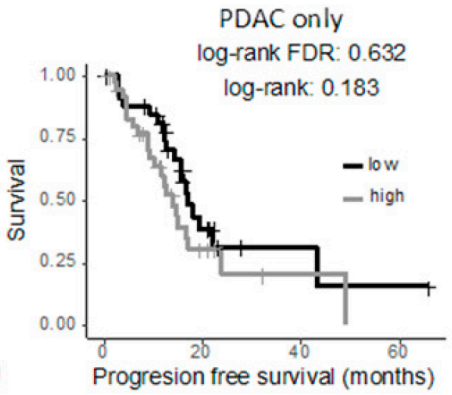
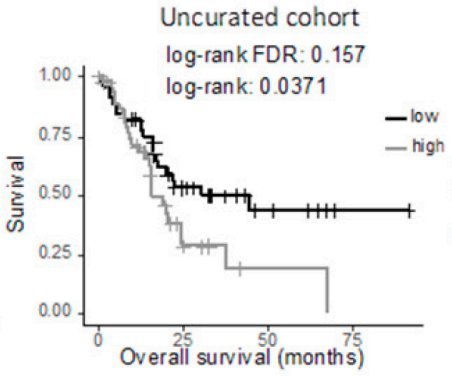
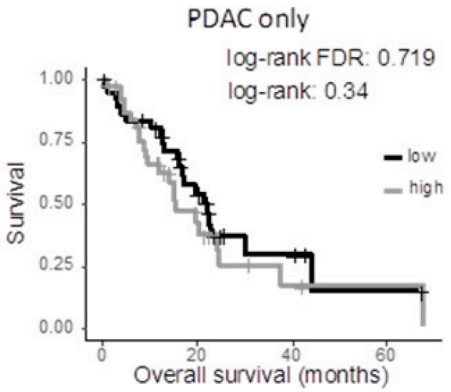




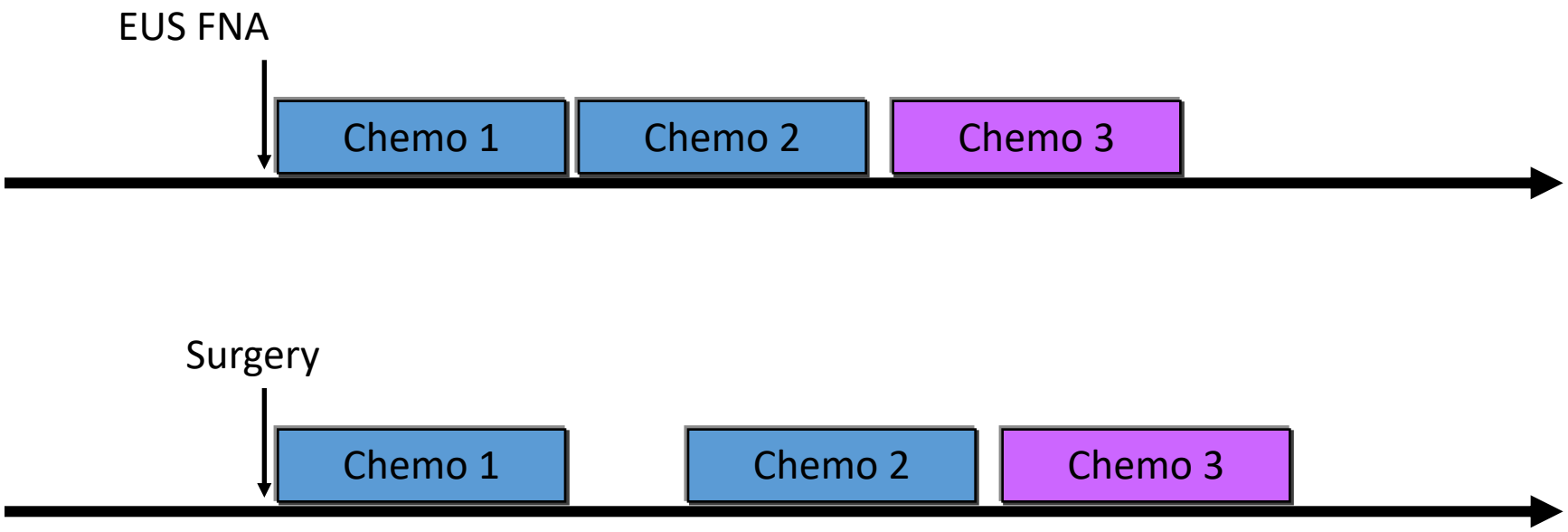






Nicolle *et al. Cancers* 2019

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



- Clonal selection induced by multiple therapies

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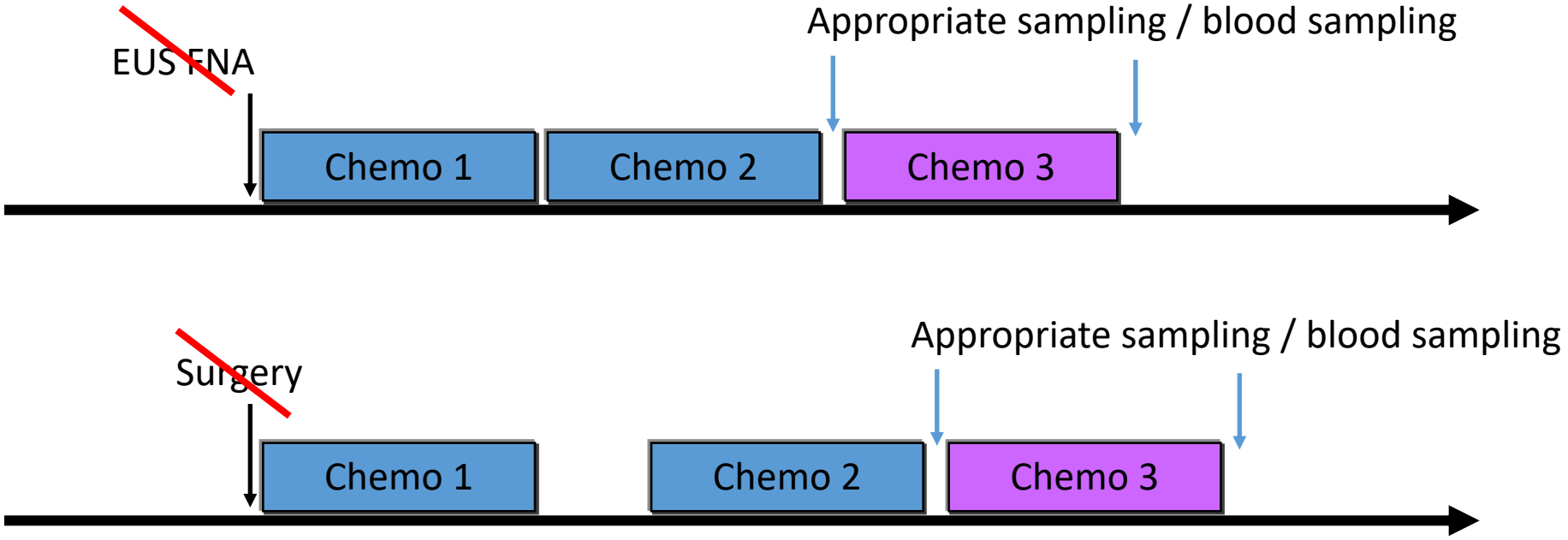
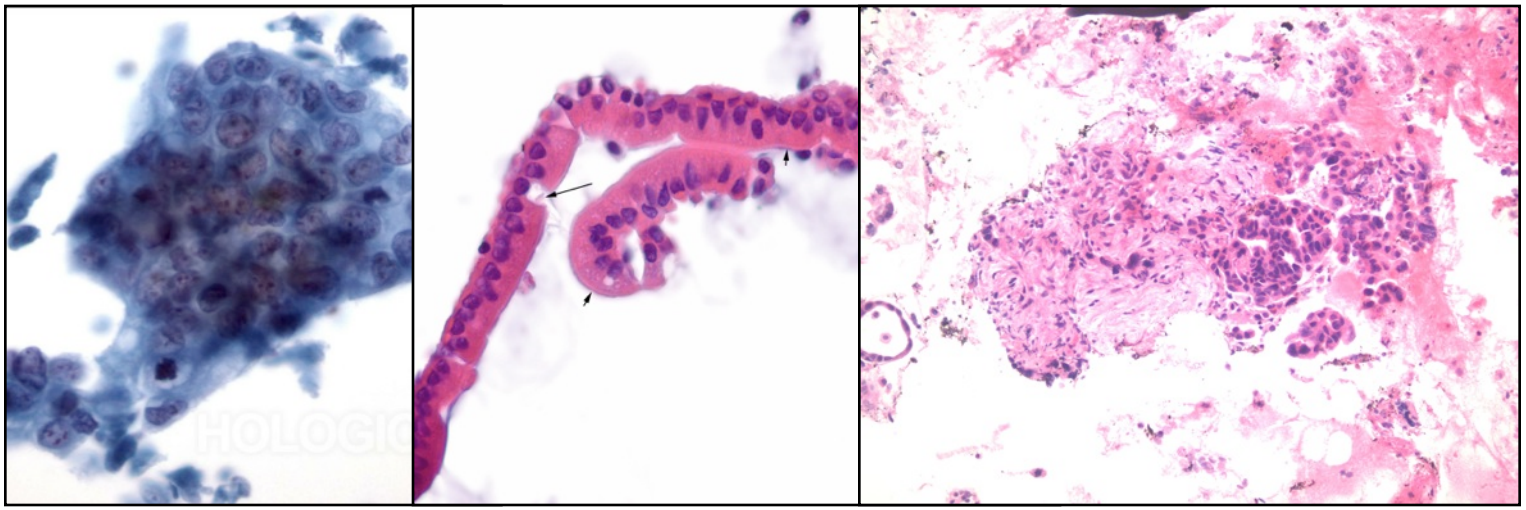


- Clonal selection induced by multiple therapies

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

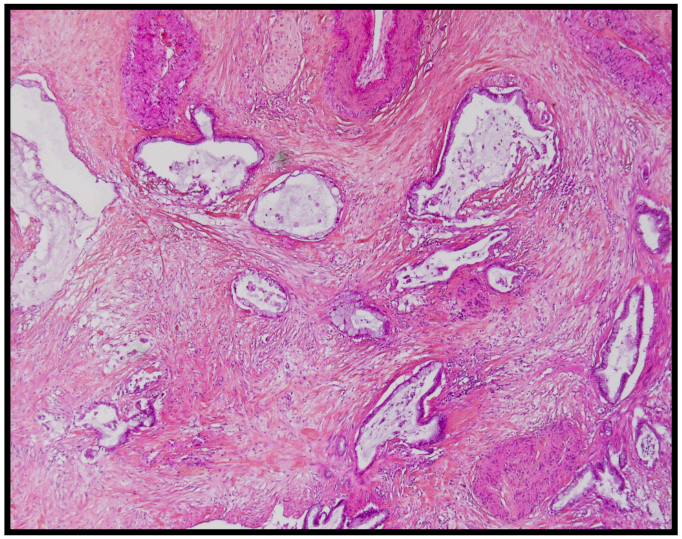


Protein on Tum cells	OK	OK	OK
Mutation on Tum cell	+/- OK	++/- OK	OK
Exp (mi)ARN	+/- OK (richness)	+/- OK (richness)	+/- OK (richness)
Protein in stroma	NON	NON	+/- OK

Liver biopsy+++, true cut +++

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

Surgical specimen



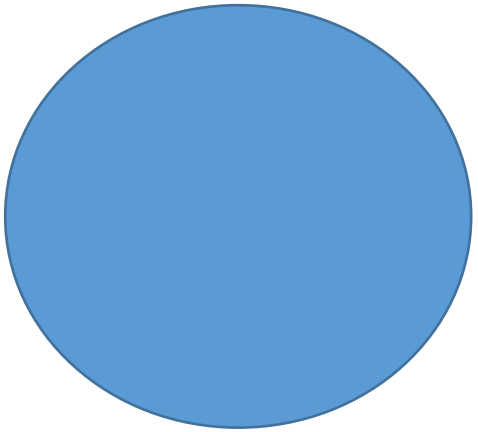
Protein on Tum cells	OK
Mutation on Tum cell	OK
Exp (mi)ARN	OK
Protein in stroma	OK

Tumor
+/- sphere
3cm diameter

14cm^3

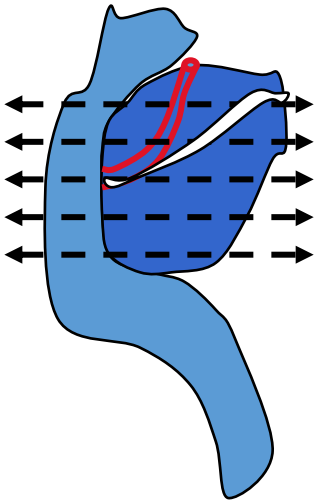
Frozen carrot
0.6cm wide
0.4 cm thick

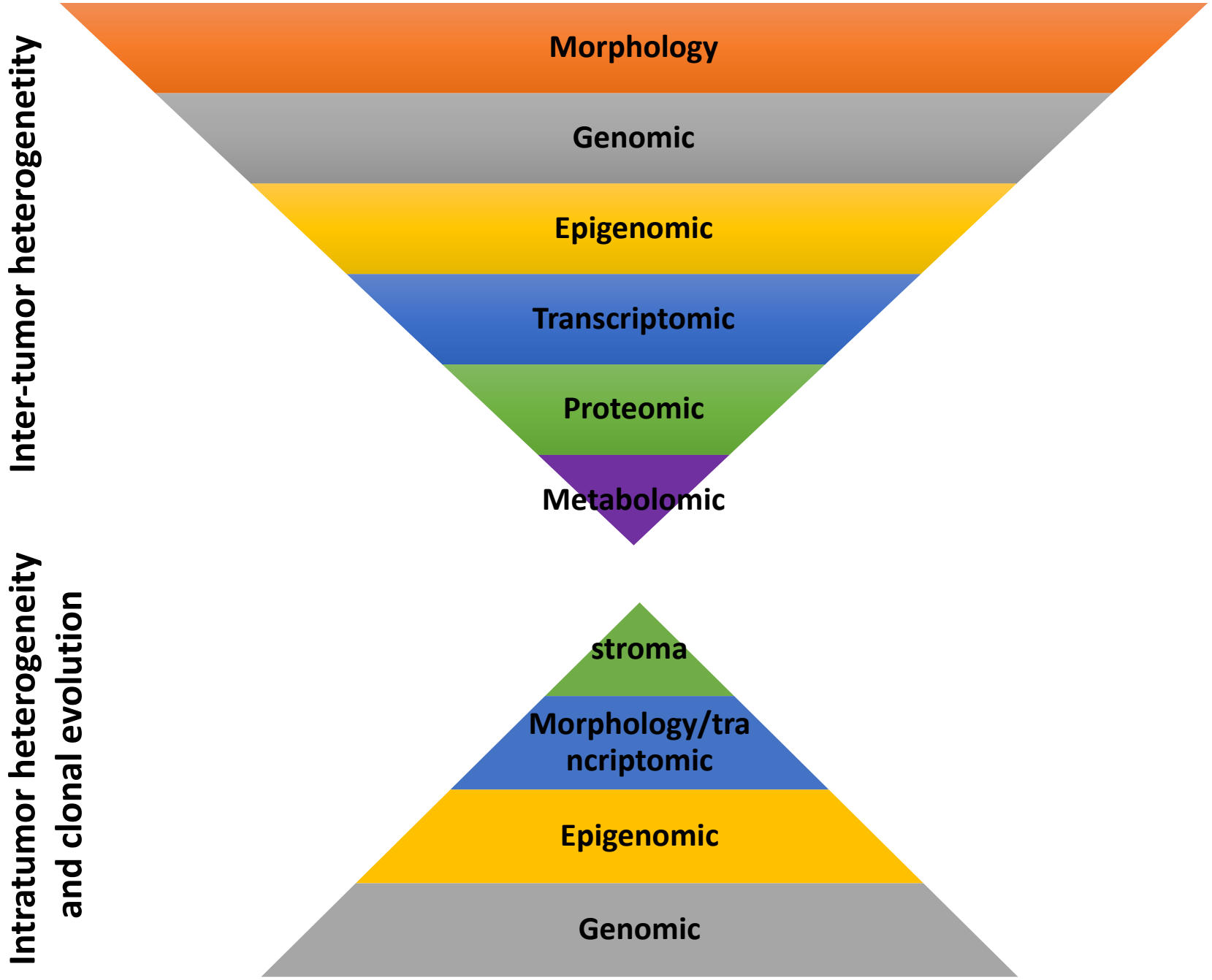
0.11cm^3

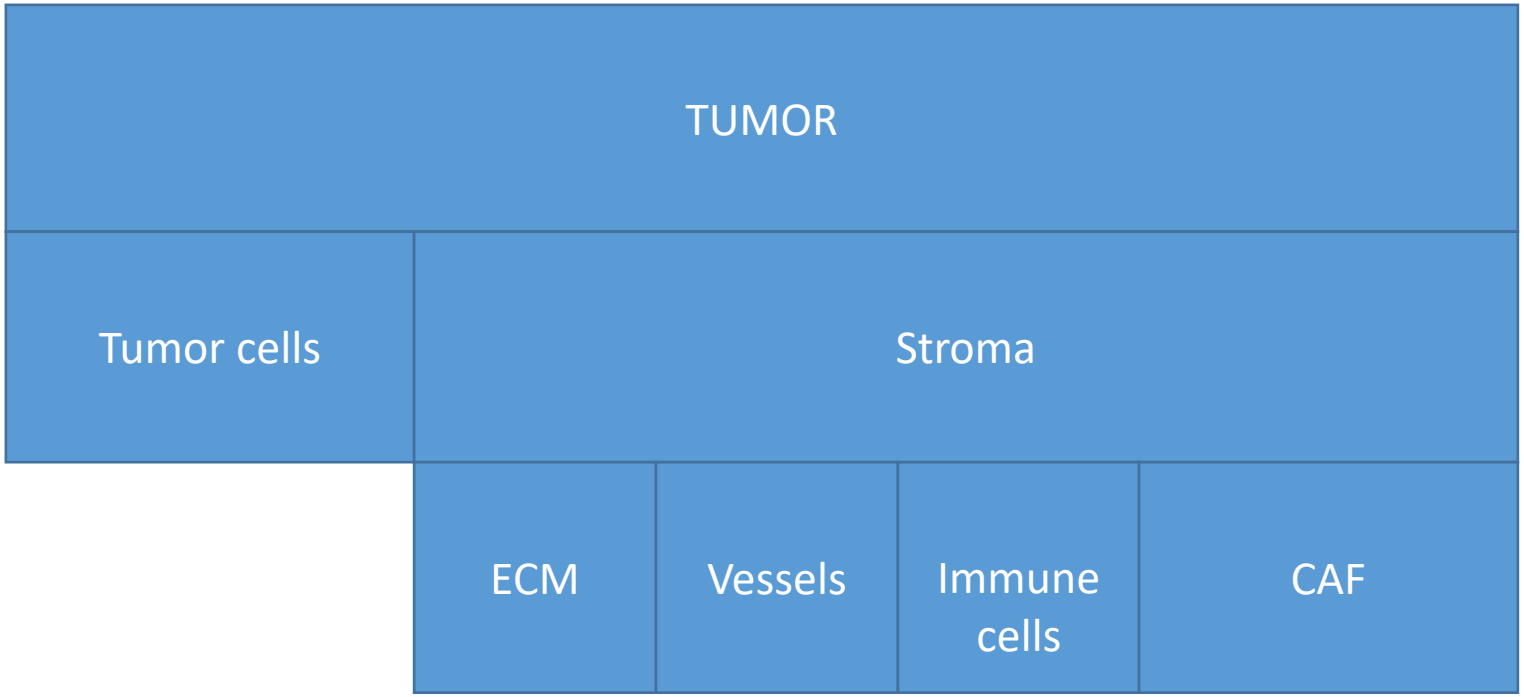


Spatialized sampling…

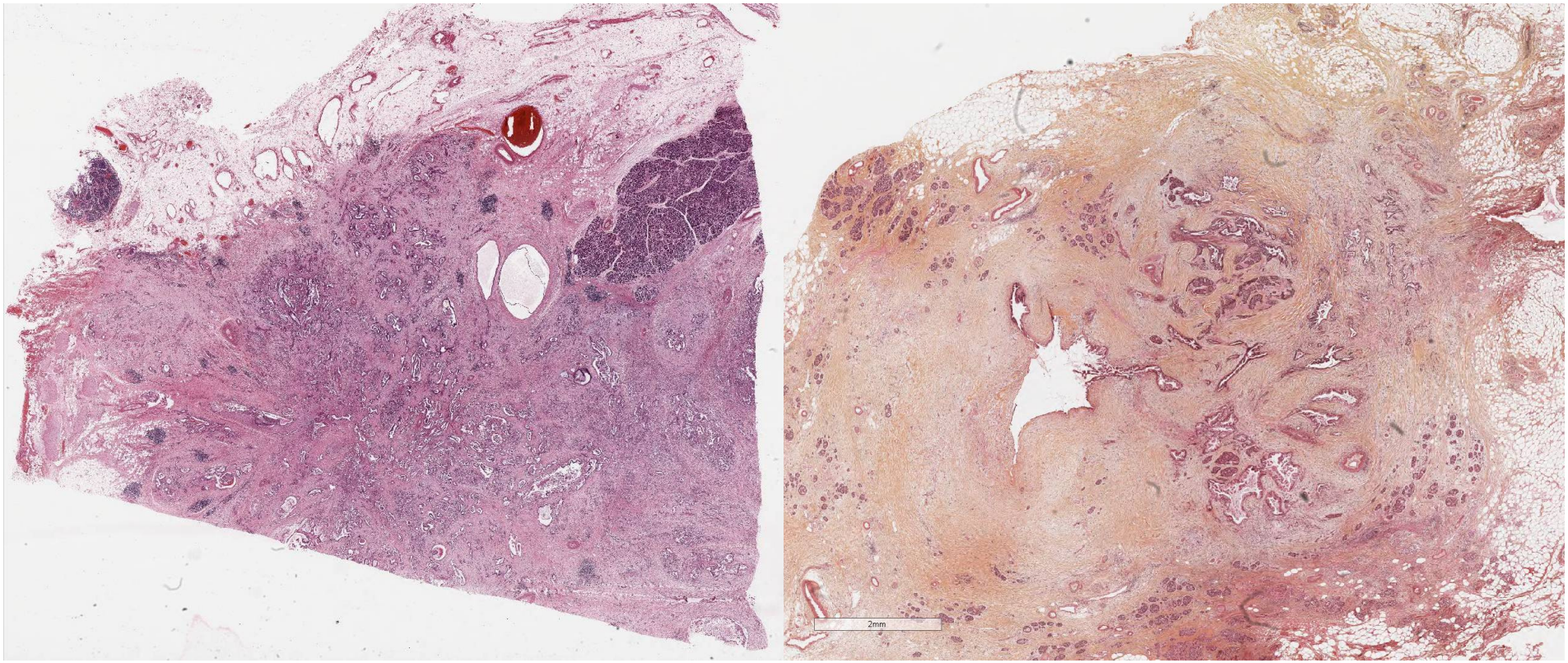
…yes but usually with formalin fixed paraffin embeded samples…




Inter-tumor heterogenetity
Morphology
Genomic
Epigenomic
Transcriptomic
Proteomic
Metabolomic
Intratumor heterogeneity and clonal evolution
stroma
Morphology/tra ncriptomic
Epigenomic
Genomic

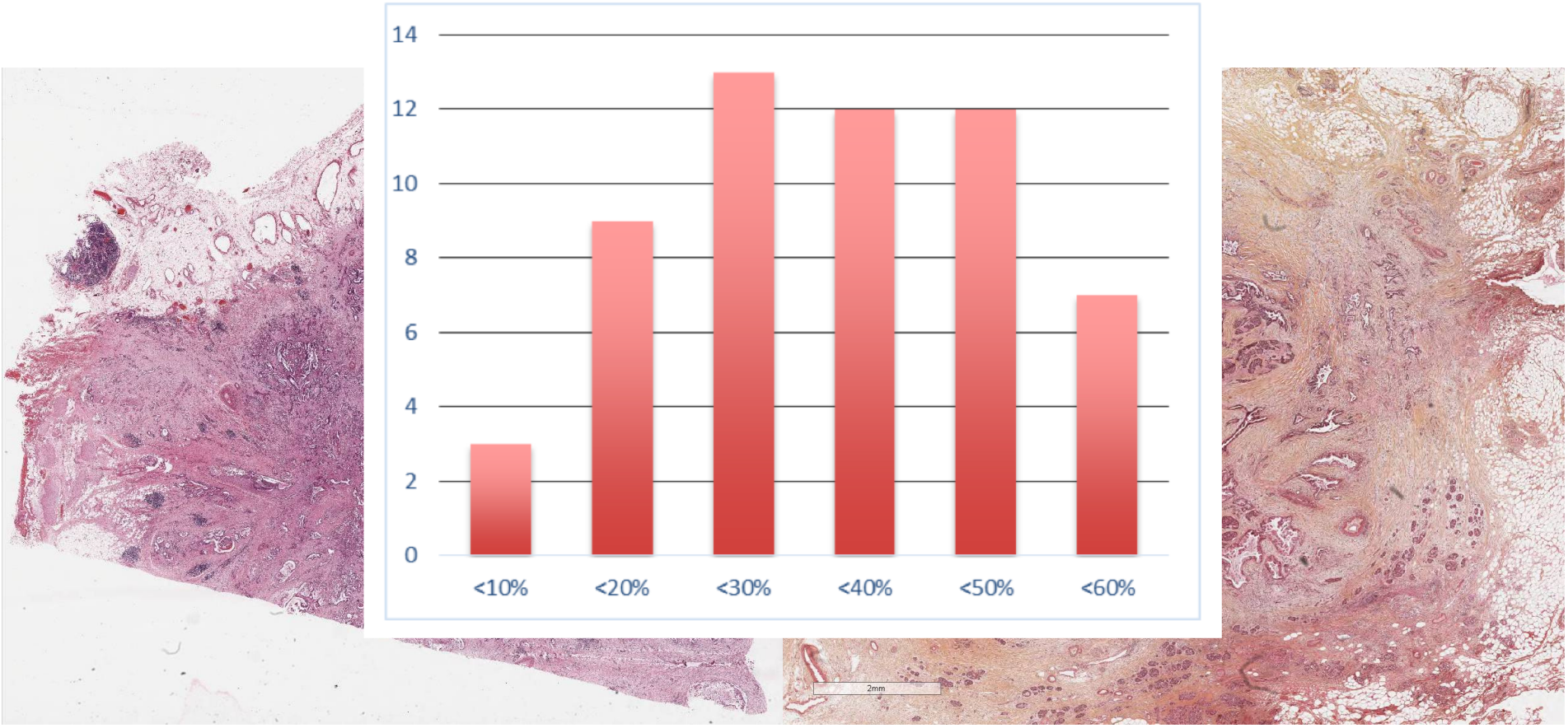

TUMOR
Tumor cells
Stroma
ECM
Vessels
Immune cells
CAF

Inter-tumor morphological heterogenetity



Heterogeneity of the tumor cell – stroma ratio

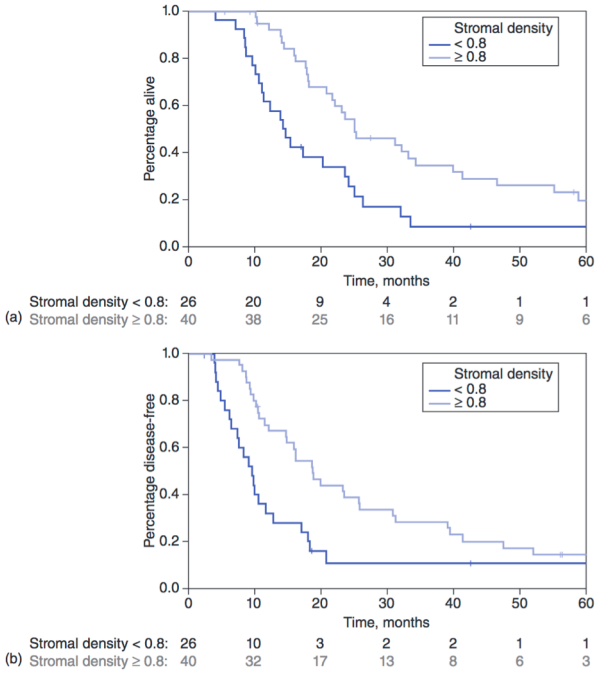
Inter-tumor morphological heterogenetity



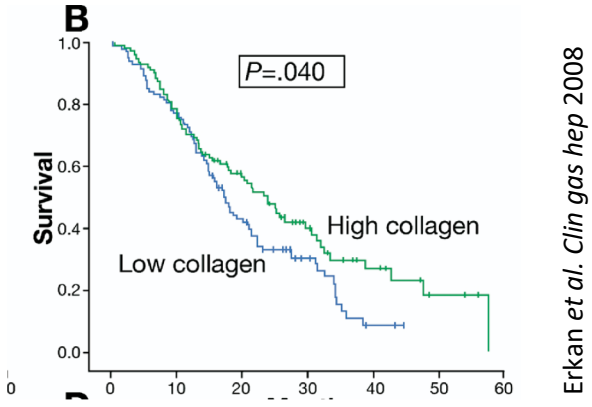
Heterogeneity of the tumor cells – stroma ratio

Inter-tumor morphological heterogenetity

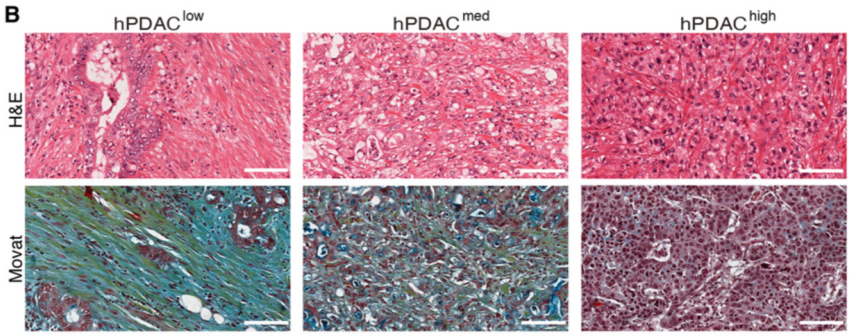
Stroma abundance– pronostic role?

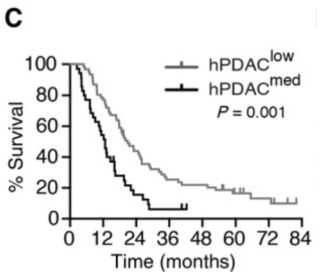


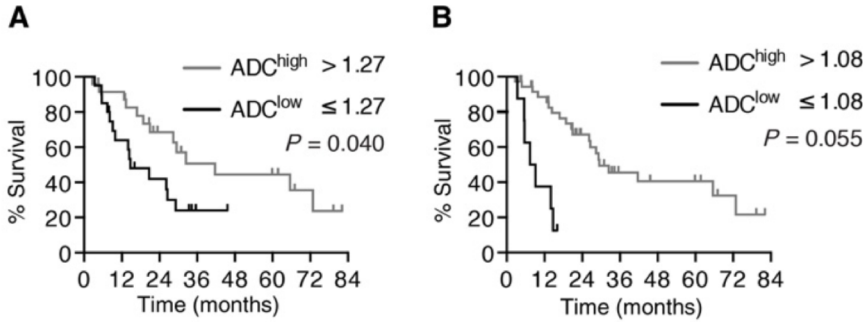
Bever *et al. HPB* 2015, 292

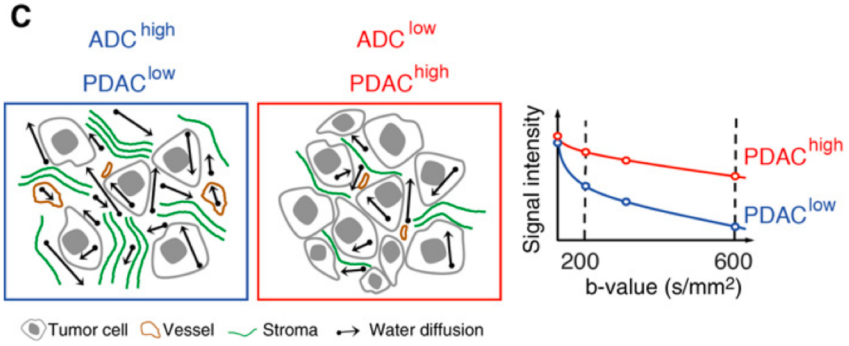


Erkan *et al. Clin gas hep* 2008





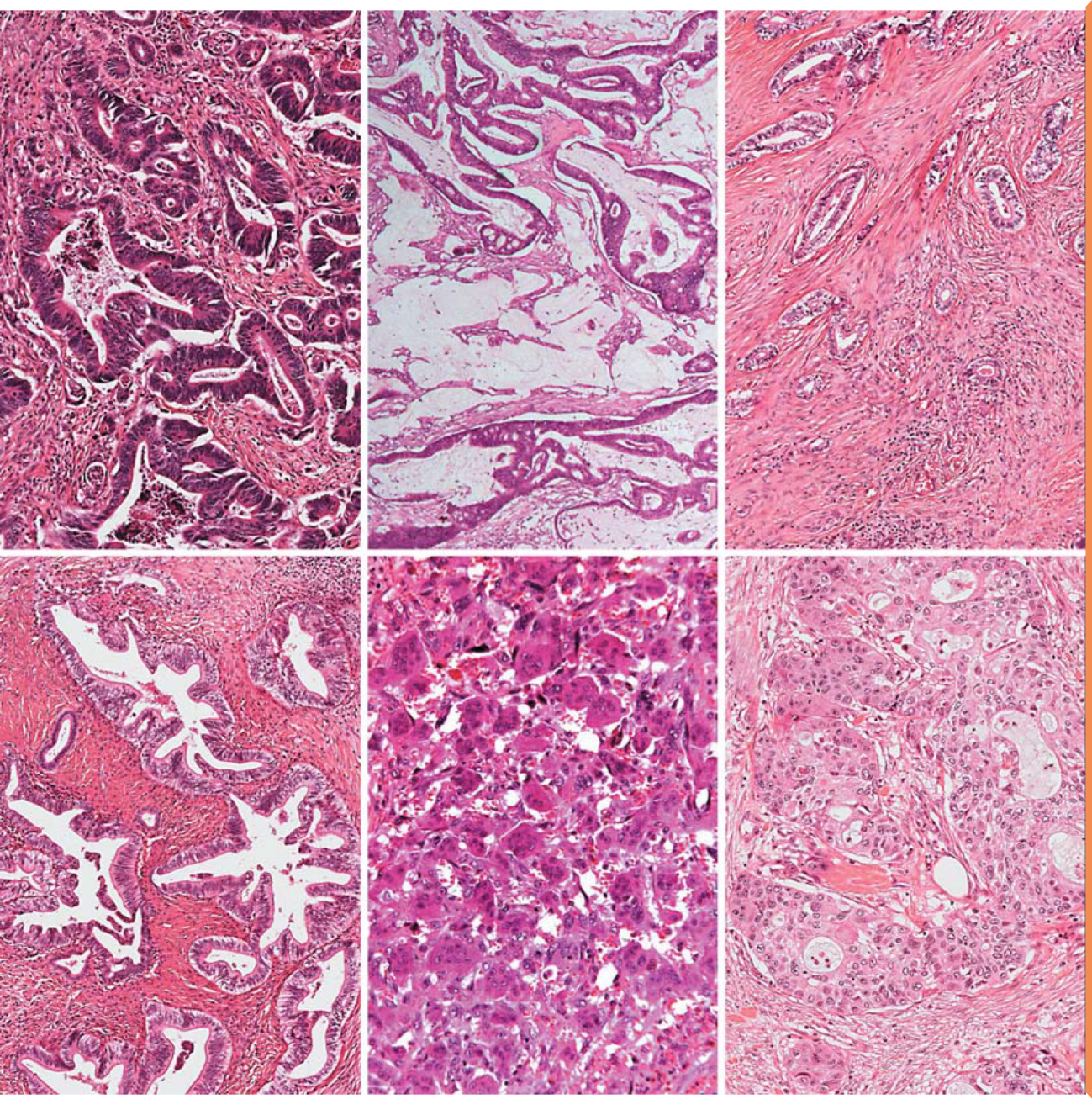




Heid *et al. Clin can res* 2017, 1461

Small sample (<100 pts)

Inter-tumor morphological heterogenetity



Inter-tumor morphological heterogenetity

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*

Schlitter *et al*. Sci reports 2017

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More frequent *MYC amplification*?

NGS/IHC *KRAS/CDKN2A/SMAD4/TP53*
No major difference

Schlitter *et al*. Sci reports 2017

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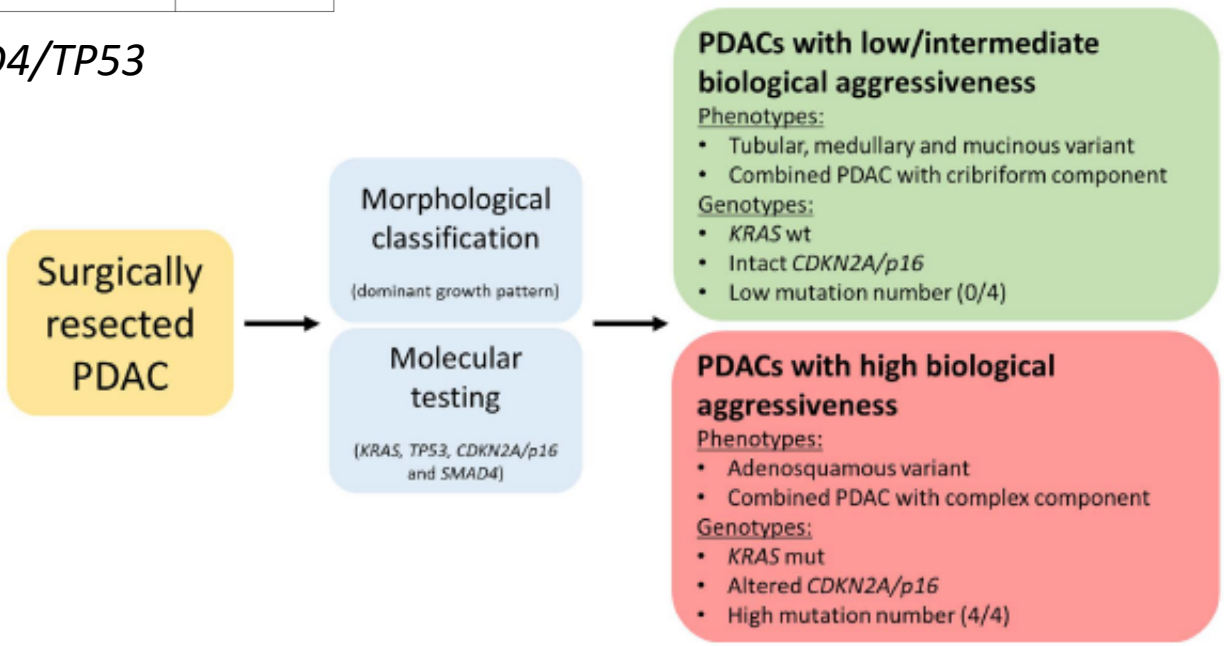
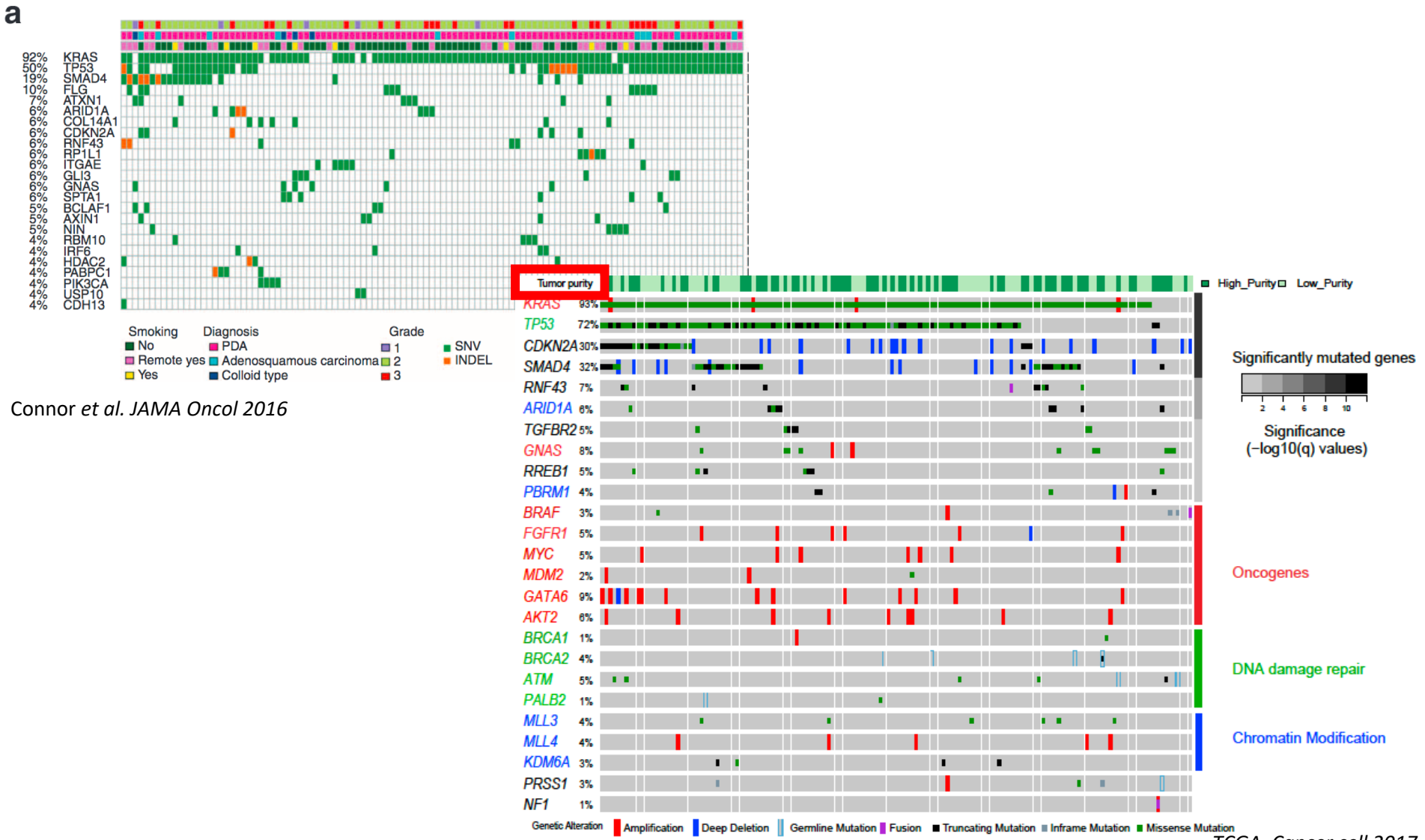


Schlitter *et al*. Sci reports 2017

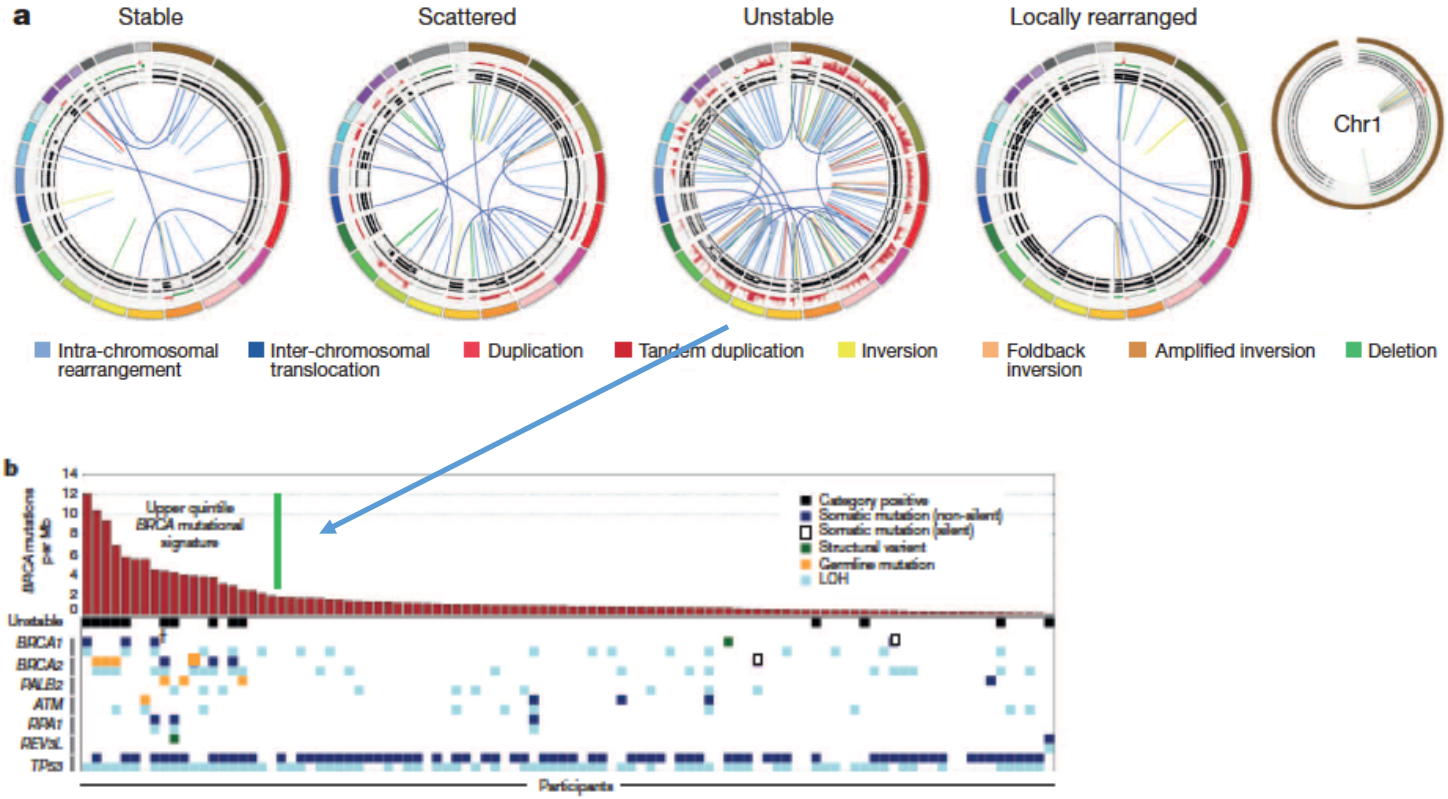
3 large datasets (ICGC (456 pts), TCGA (150 pts), Connor *et al.* (148 pts) – similar results



Connor *et al. JAMA Oncol 2016*

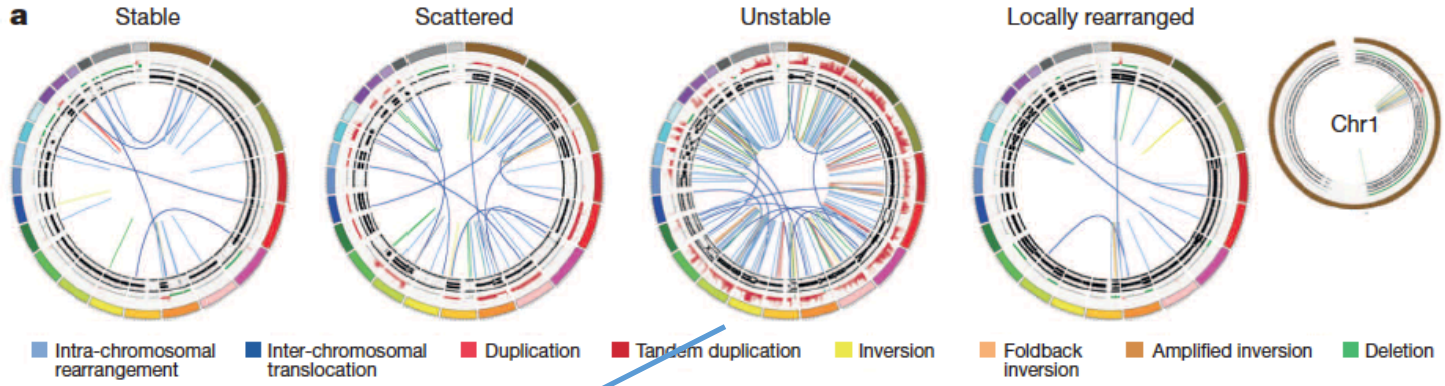
TCGA, Cancer cell 2017

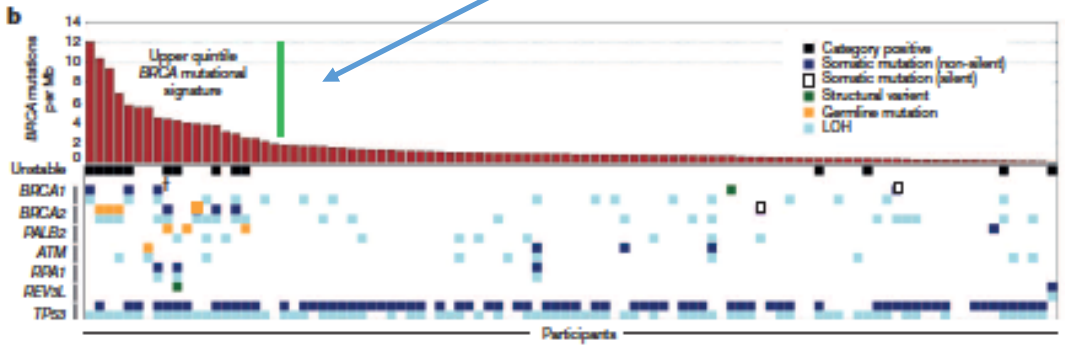
Genomic



Waddell et a. Nature 2015

Genomic





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

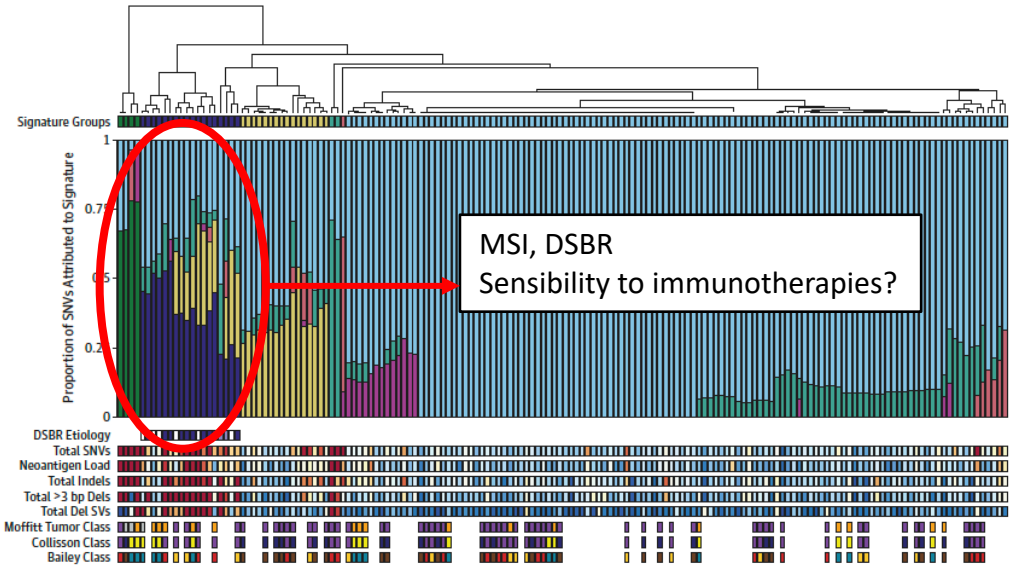
* there are 119 tumours from 115 cases in the Age Related discovery group
** there are 18 tumours from 17 cases in the DSBR discovery group
*** there are 2 tumours from 1 case in the APOBEC discovery group

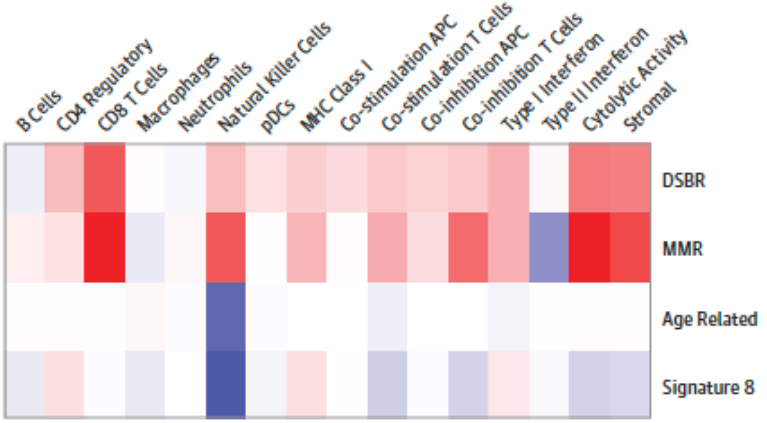
Benefit from PARPi in maintenance therapy (POLO trial)

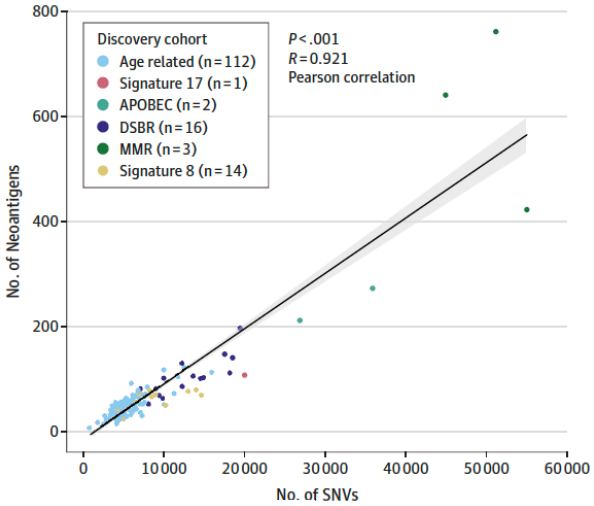
Waddell et a. Nature 2015

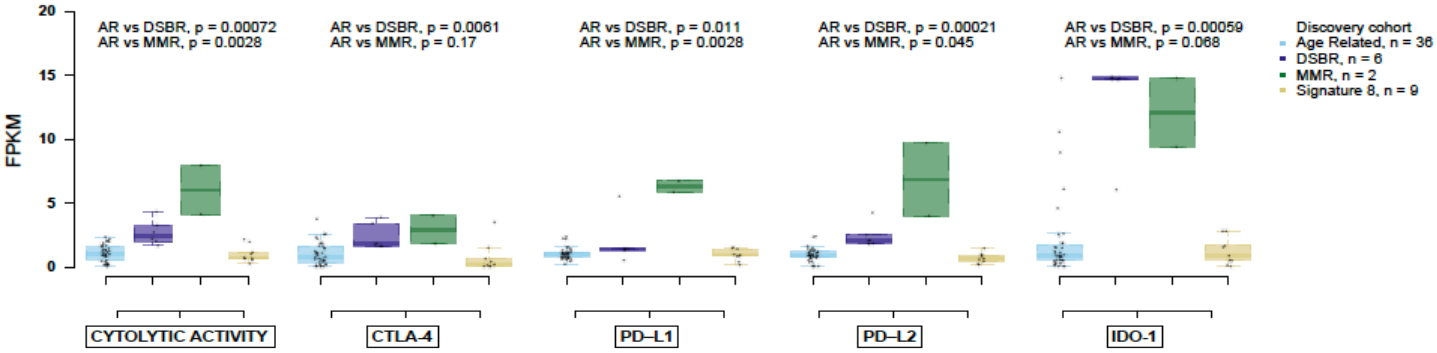
Connor *et al. JAMA Oncol 2016*

Genomic





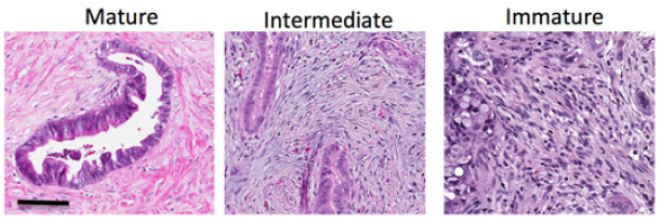




Connor *et al. JAMA Oncol 2016*

Genomic

Type of stroma



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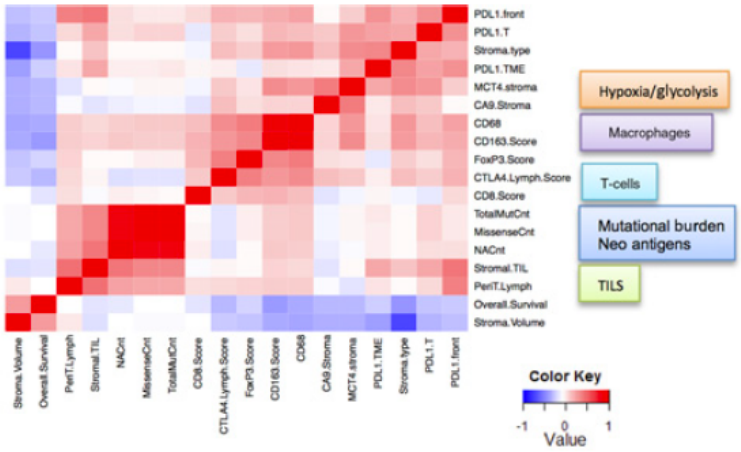
Type of immune infiltrate



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Mutationnal profile (WES)

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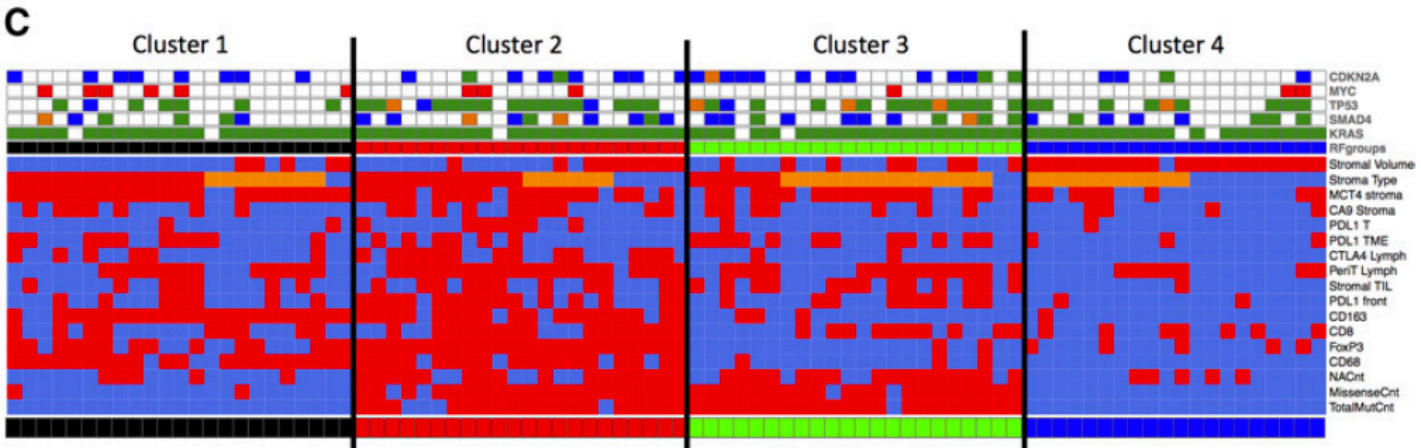


Mutationally cold

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

Hot

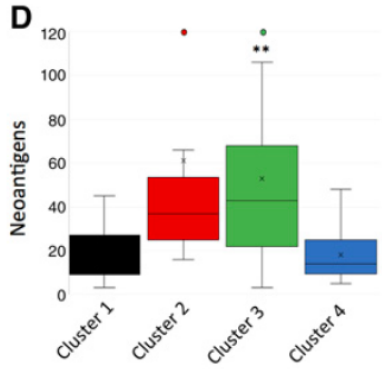
Intermediate stroma
High neoAg
Pleomorphic immune infiltrate

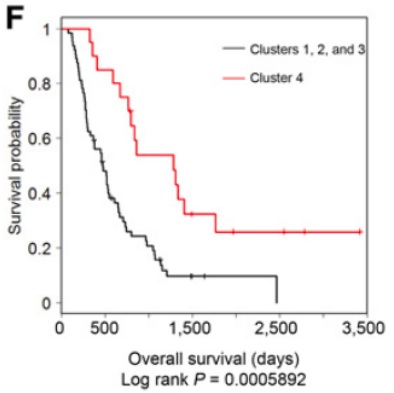
Mutationally active

Intermediate stroma
High neoAg
TIL
Low macrophage

Cold

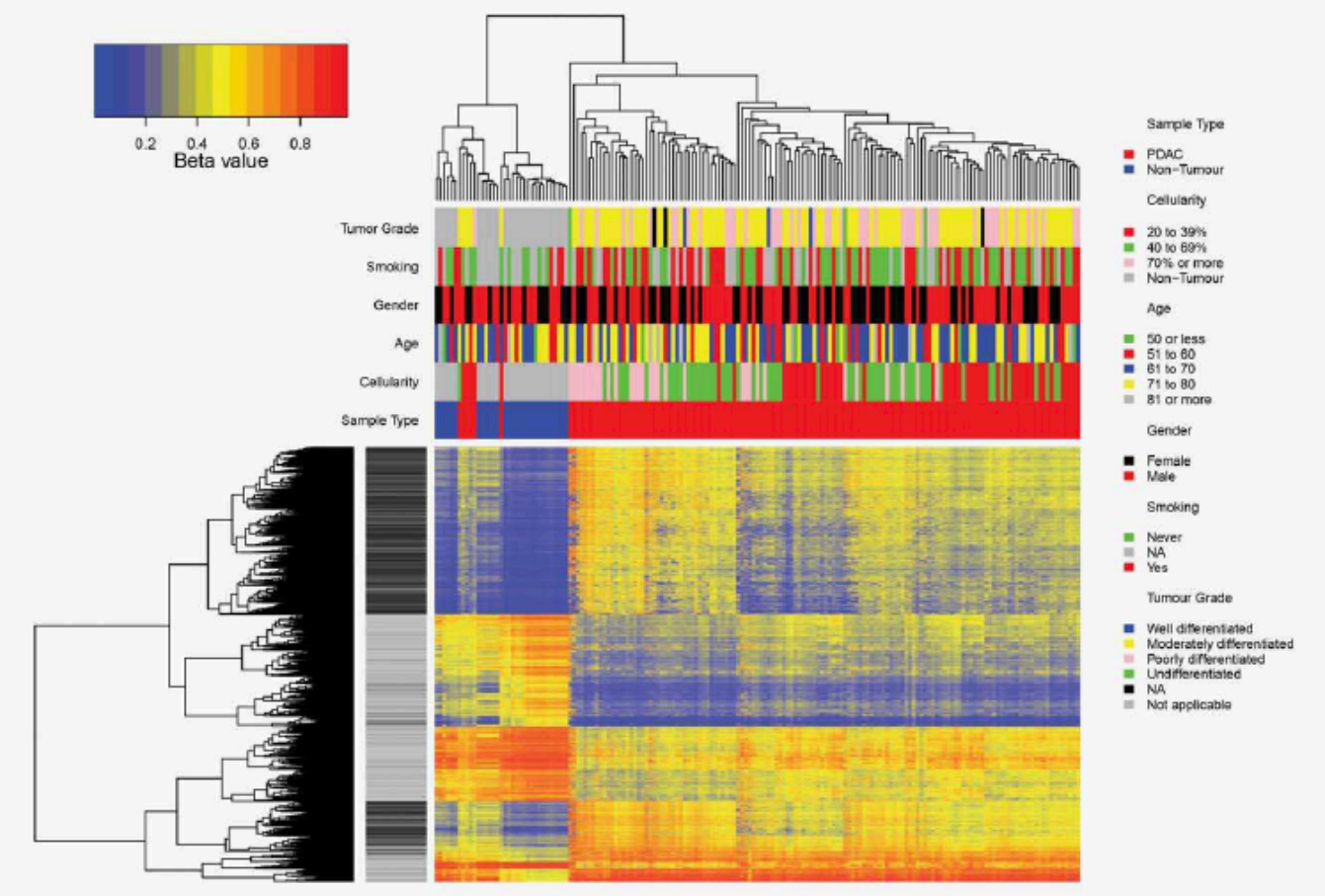
Abundant stroma
Mature stroma mature
Low neoAg
Low mutation in driver genes
Low immune infiltrate





Knudsen *et al. Clin can res* 2017

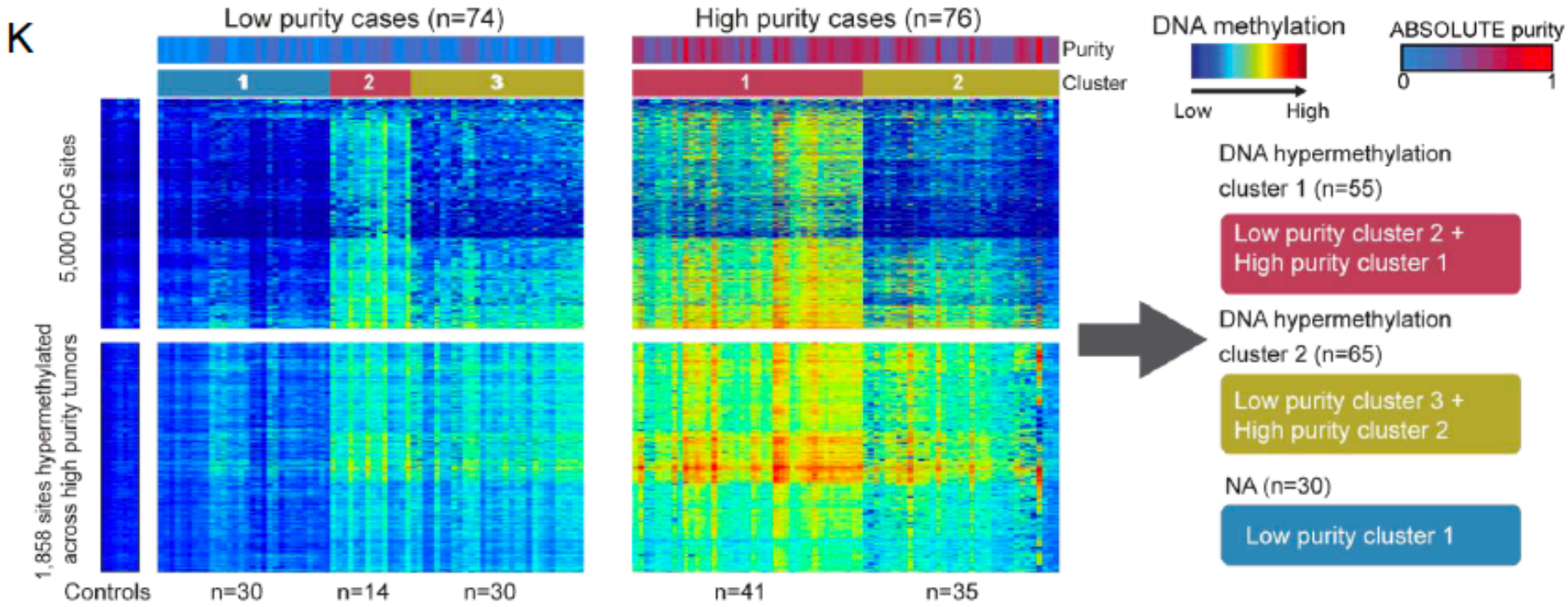
More studies are needed+++, possible interest in diagnostic (circulating DNA)



Nones *et al. IJC* 2014

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

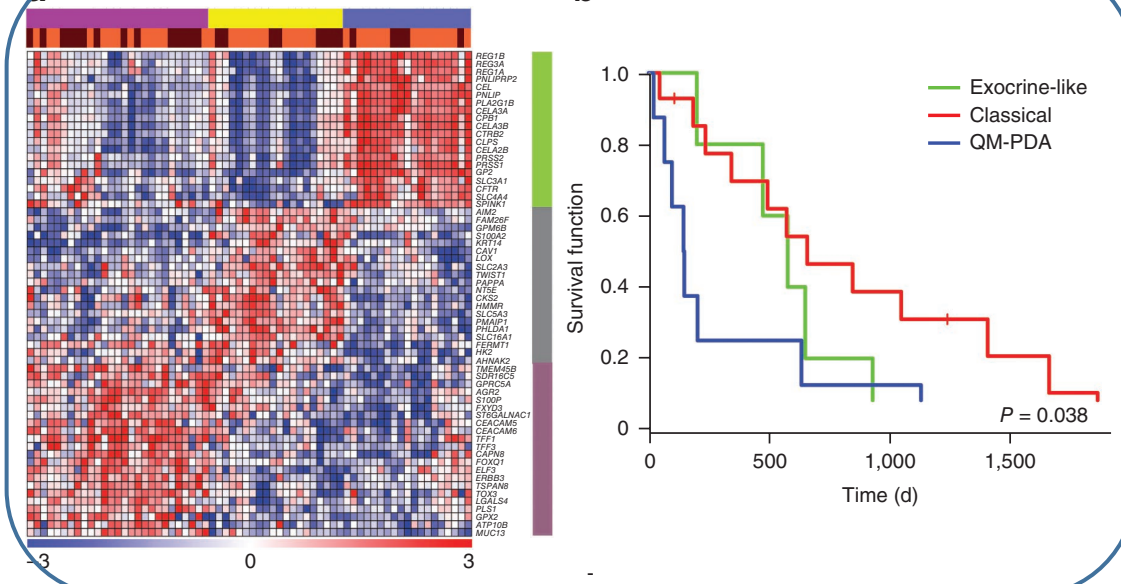
⬆ MET

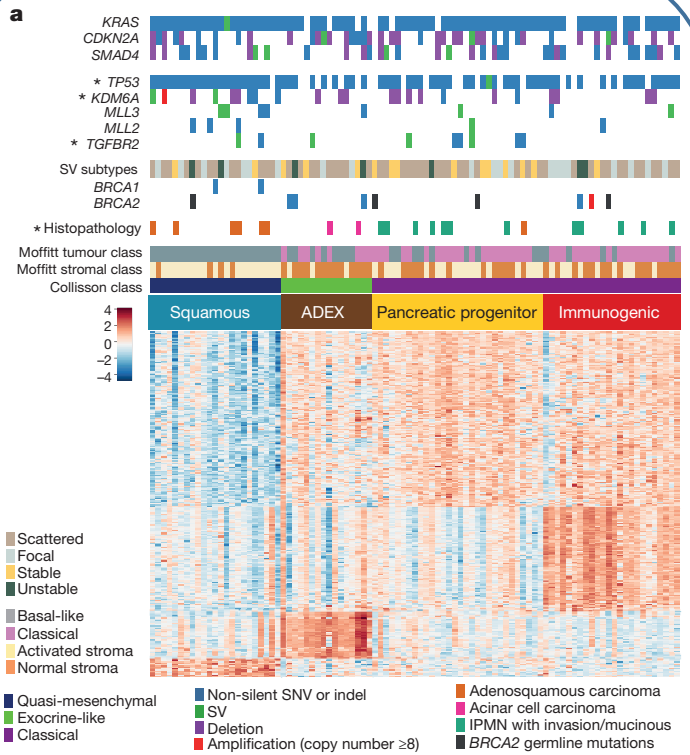


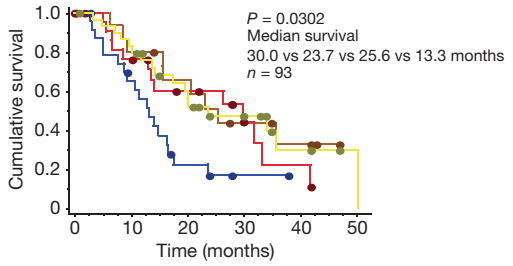
See also Lomberk *et al. Nat Com* 2018

TCGA, Cancer cell 2017

Transcriptomic

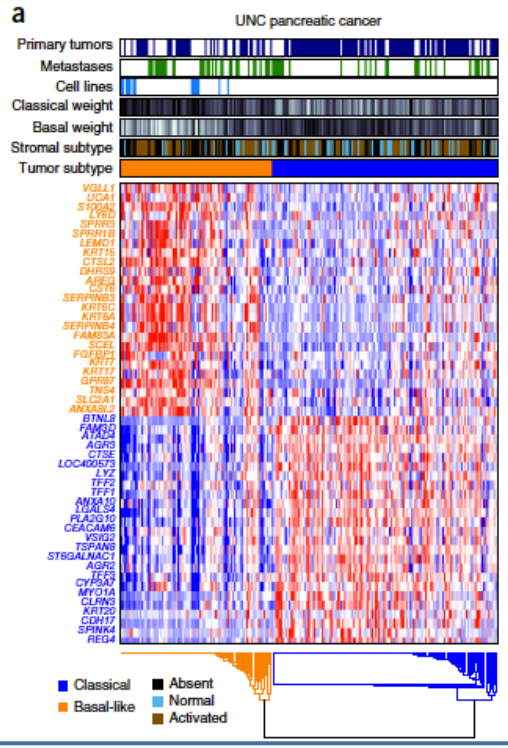


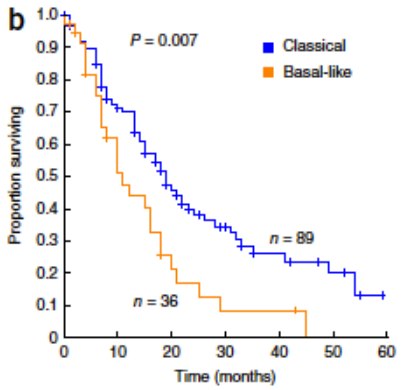




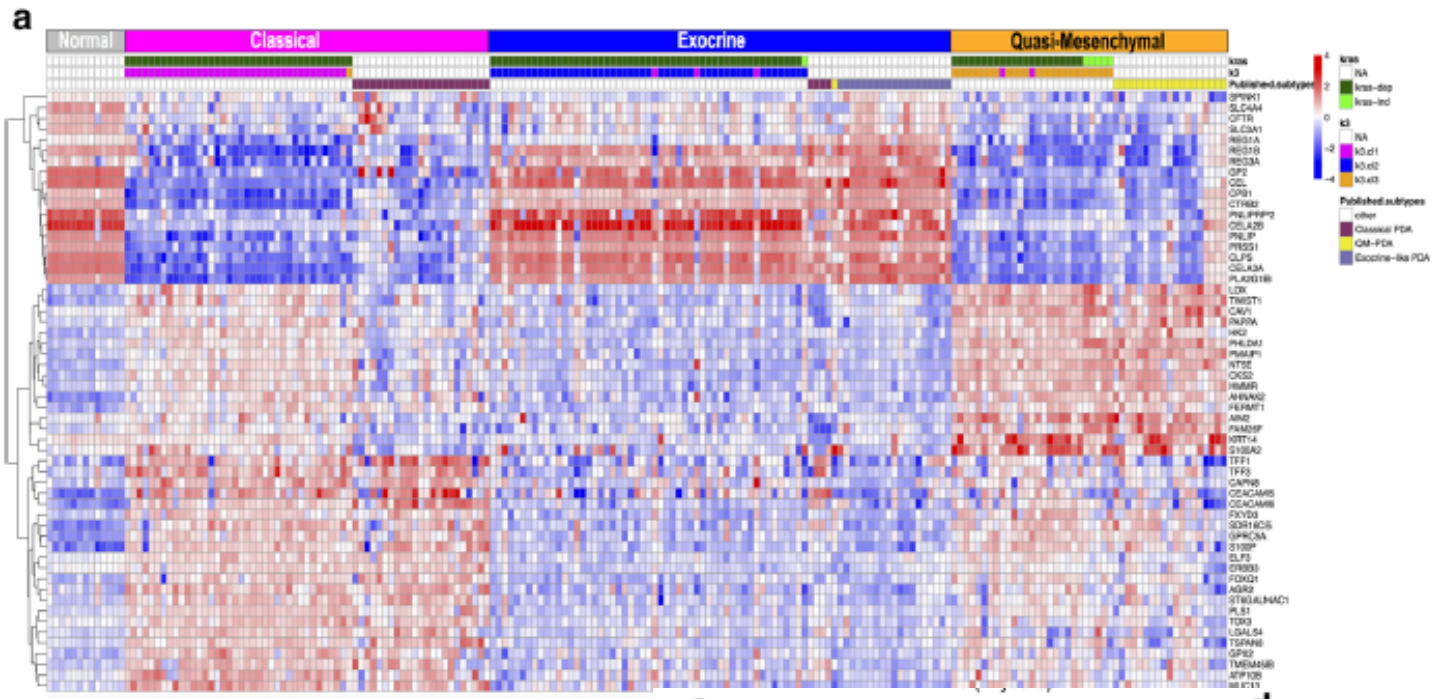
Bailey et al. Nature 2016

Moffit et al. Nat Gen 2015





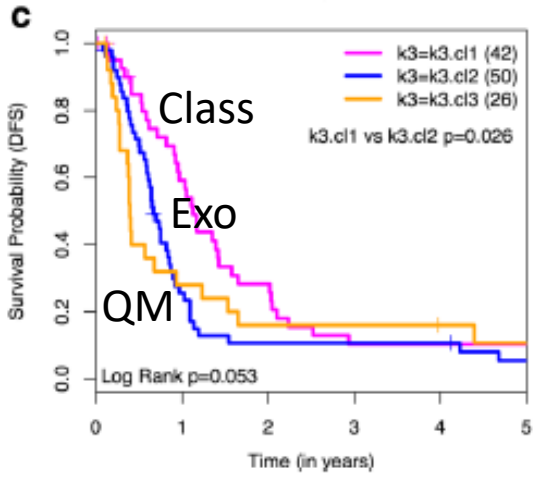
PDAC transcriptomic subtypes, how many? Do they all exist?

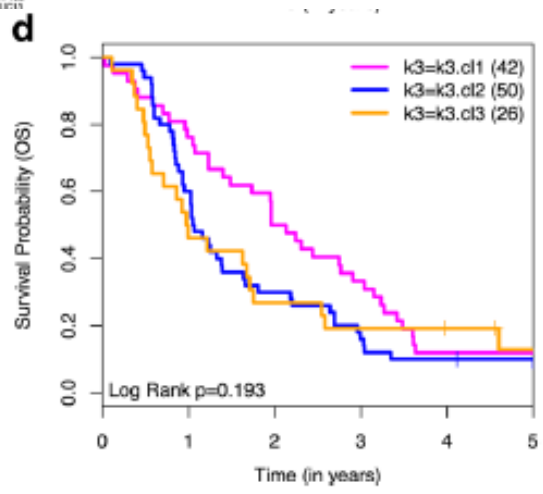


b

	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

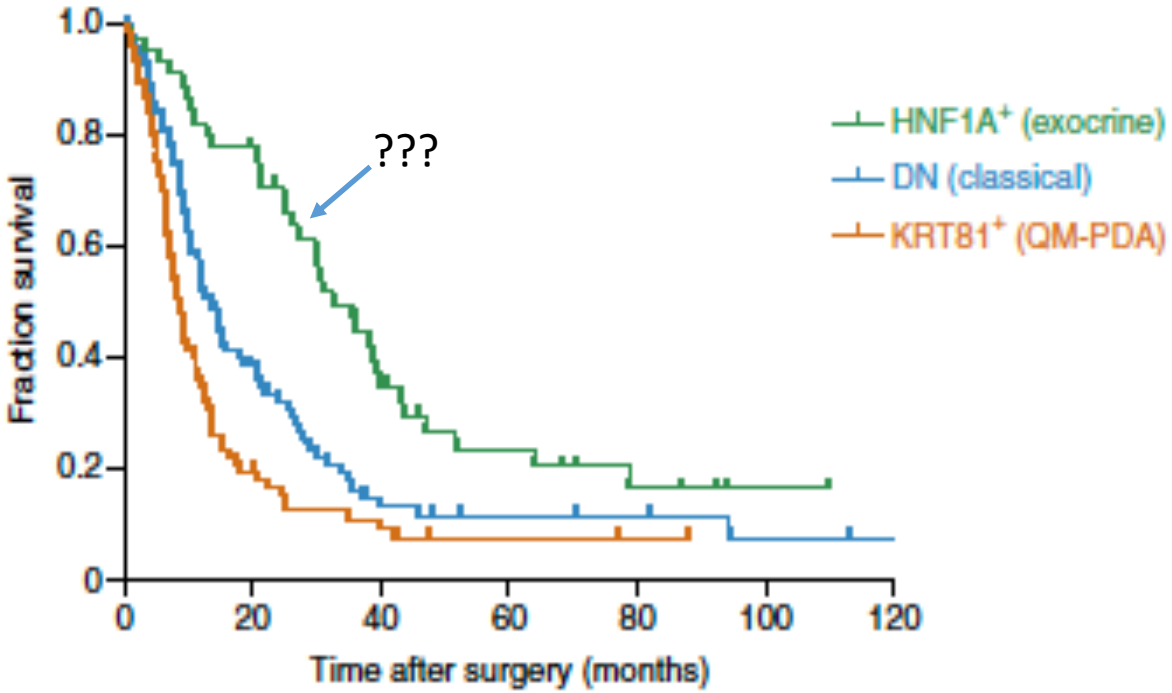




Janky *et al. BMC Cancer* 2016

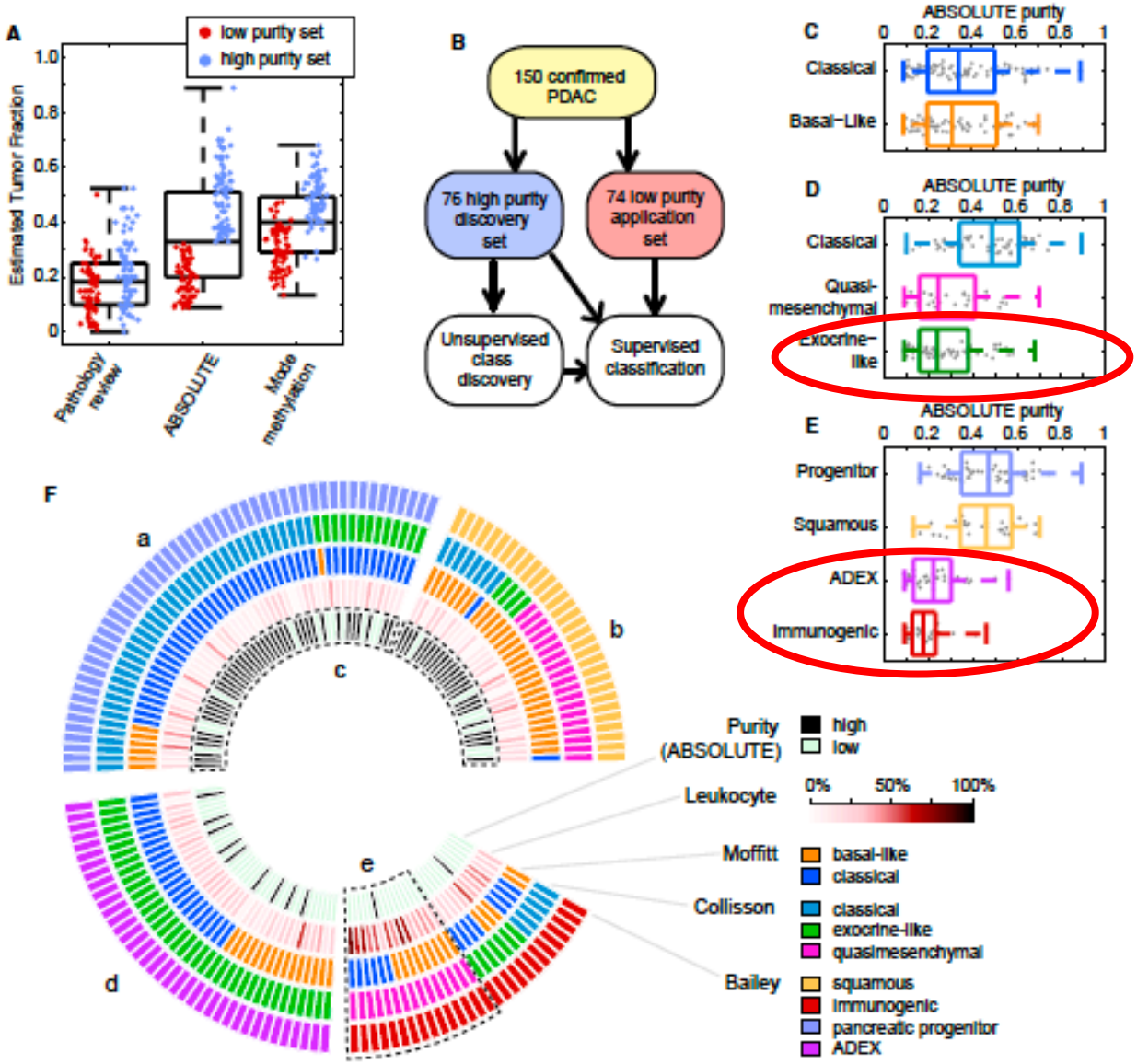
PDAC transcriptomic subtypes, how many? Do they all exist?

231patients



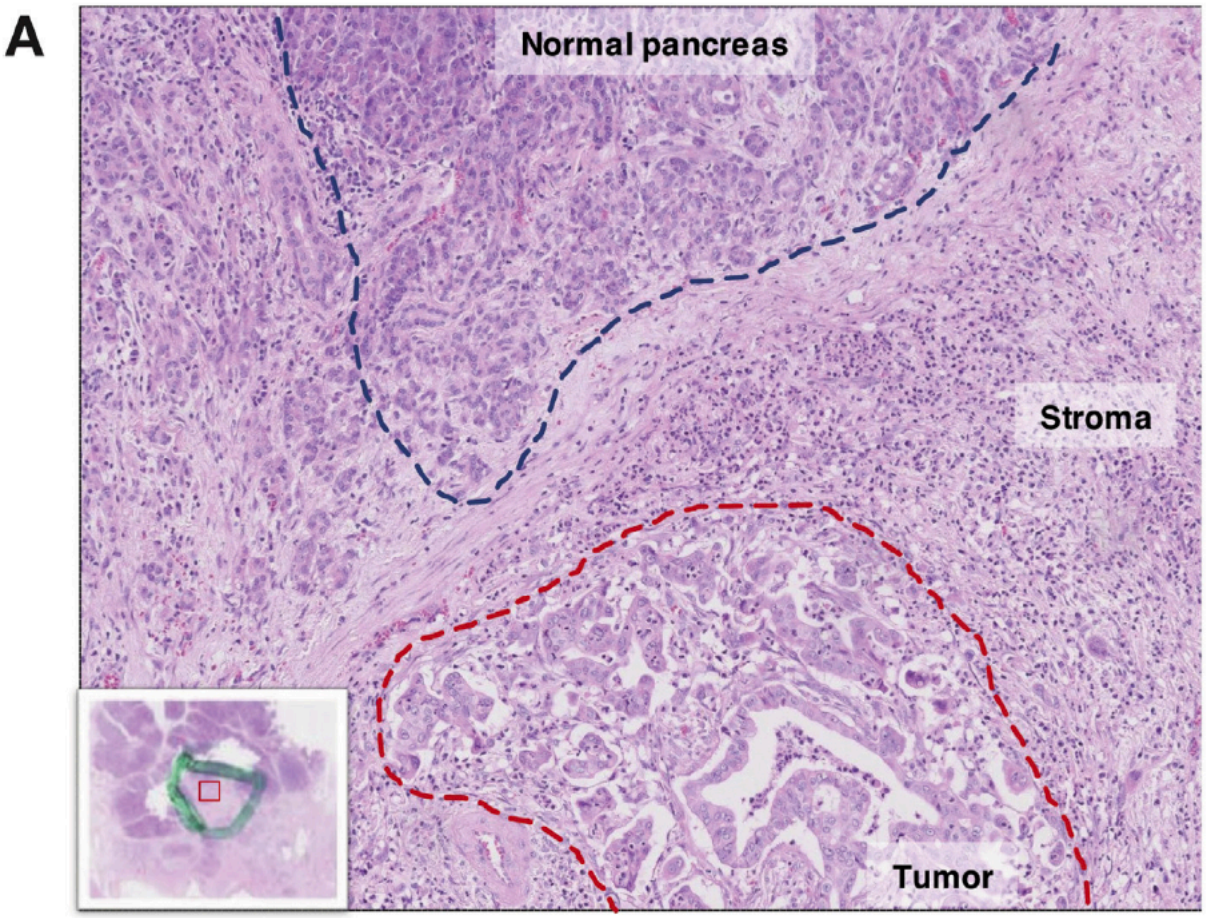
Noll *et al. Nat Med* 2015

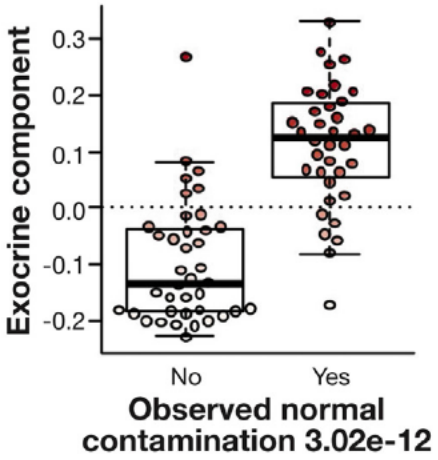
PDAC transcriptomic subtypes, how many? Do they all exist?

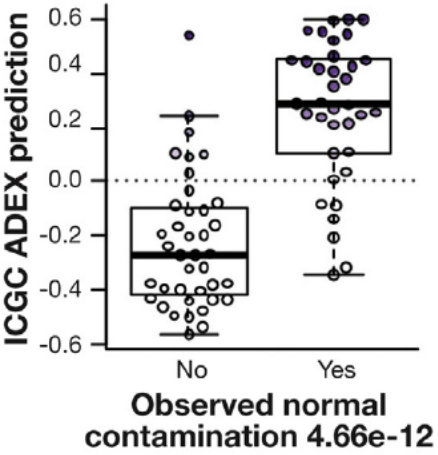


TCGA, Cancer cell 2017

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



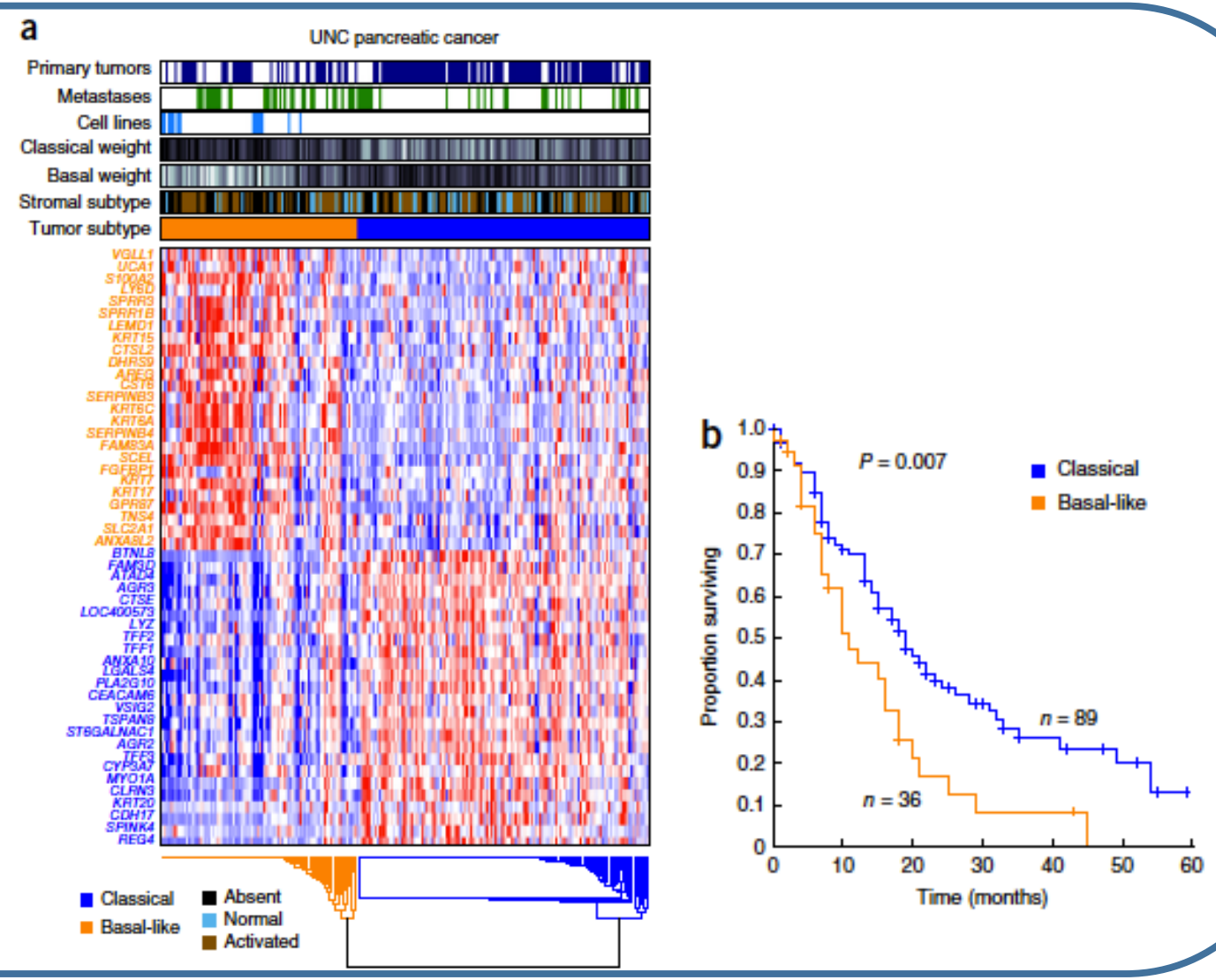




Puleo *et al, Gastroenterology* 2018

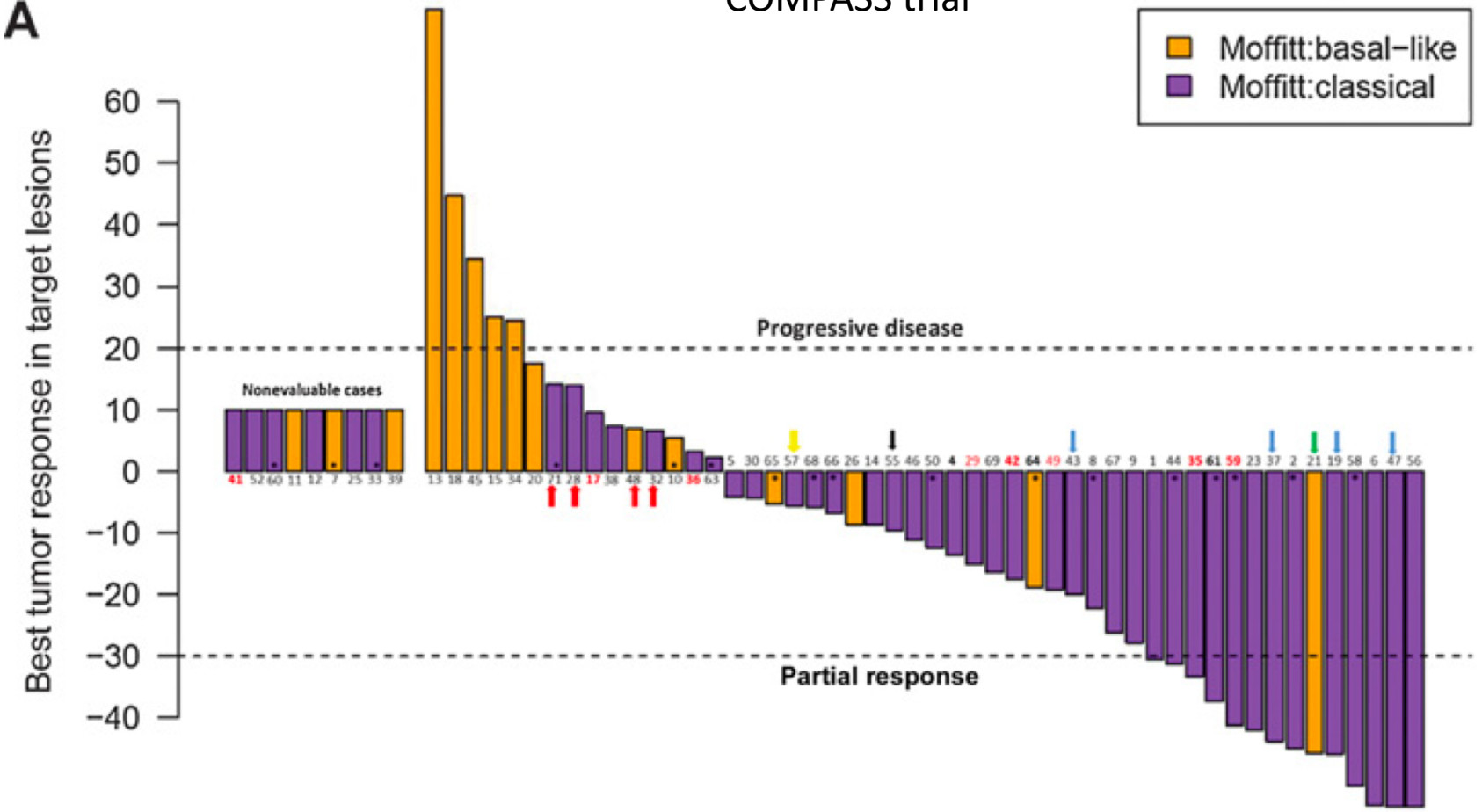
2 main transcriptomic tumor subtypes with different prognosis

Moffit *et al. Nat Gen* 2015



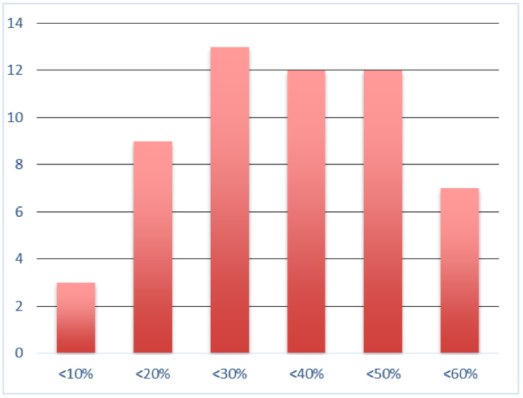
Molecular subtypes may have an important clinical utility++++

COMPASS trial

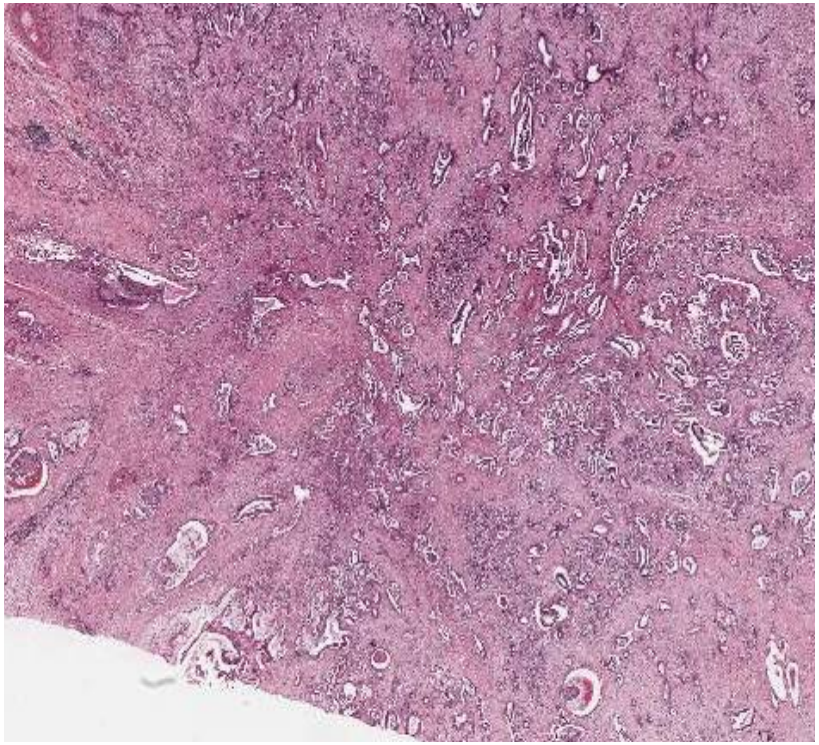


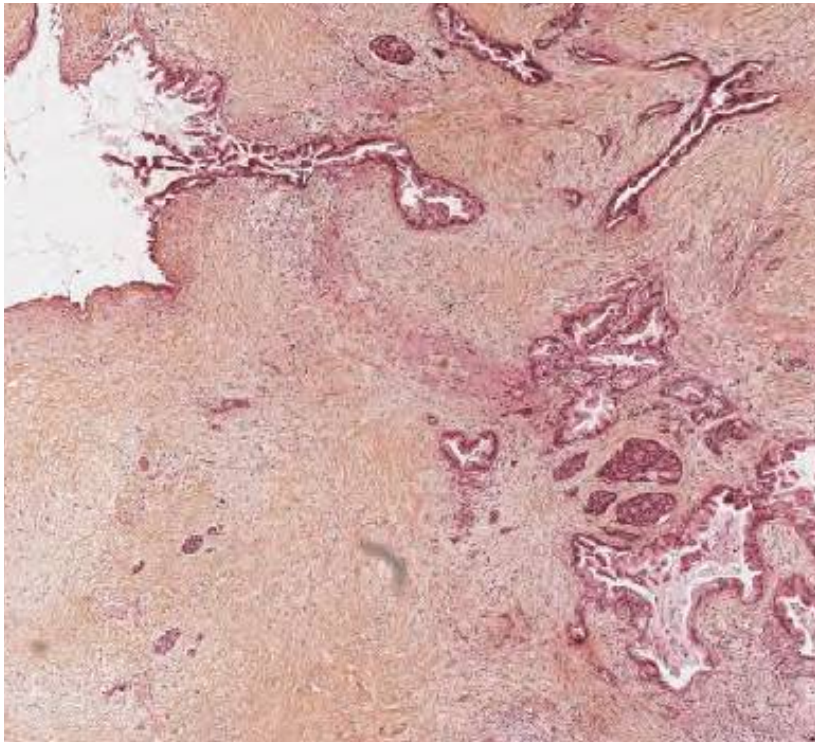
Aung *et al. Clin can res* 2018

Transcriptomic



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





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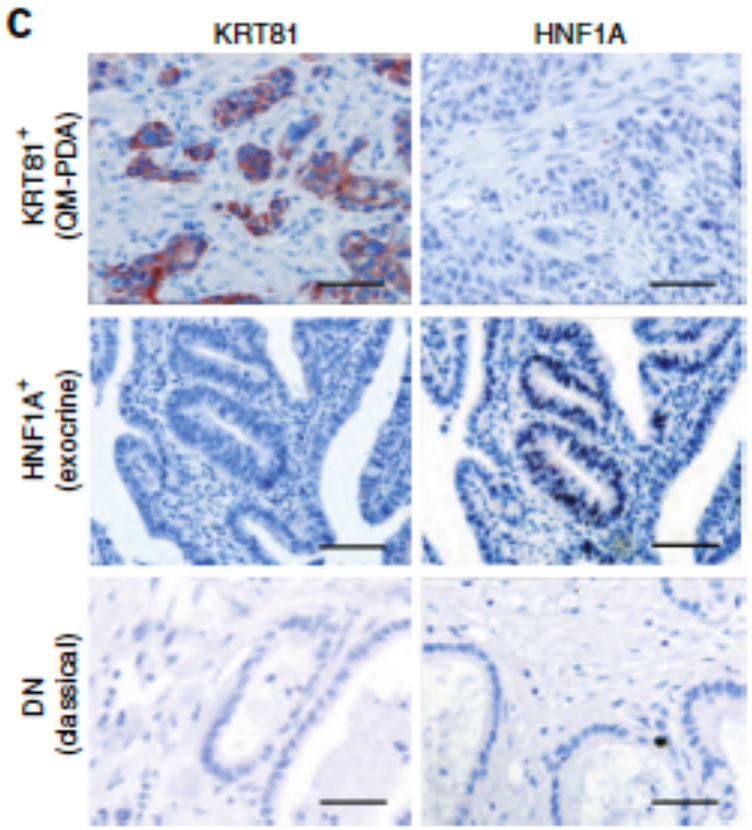
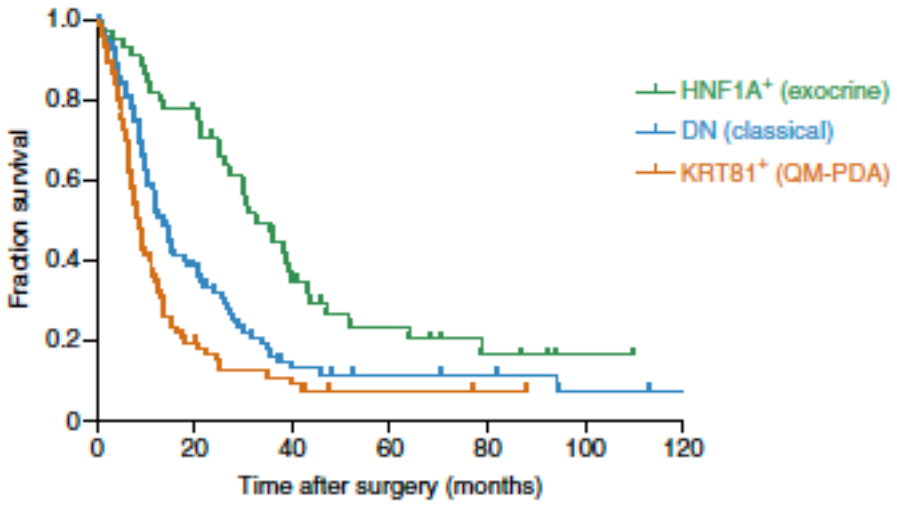
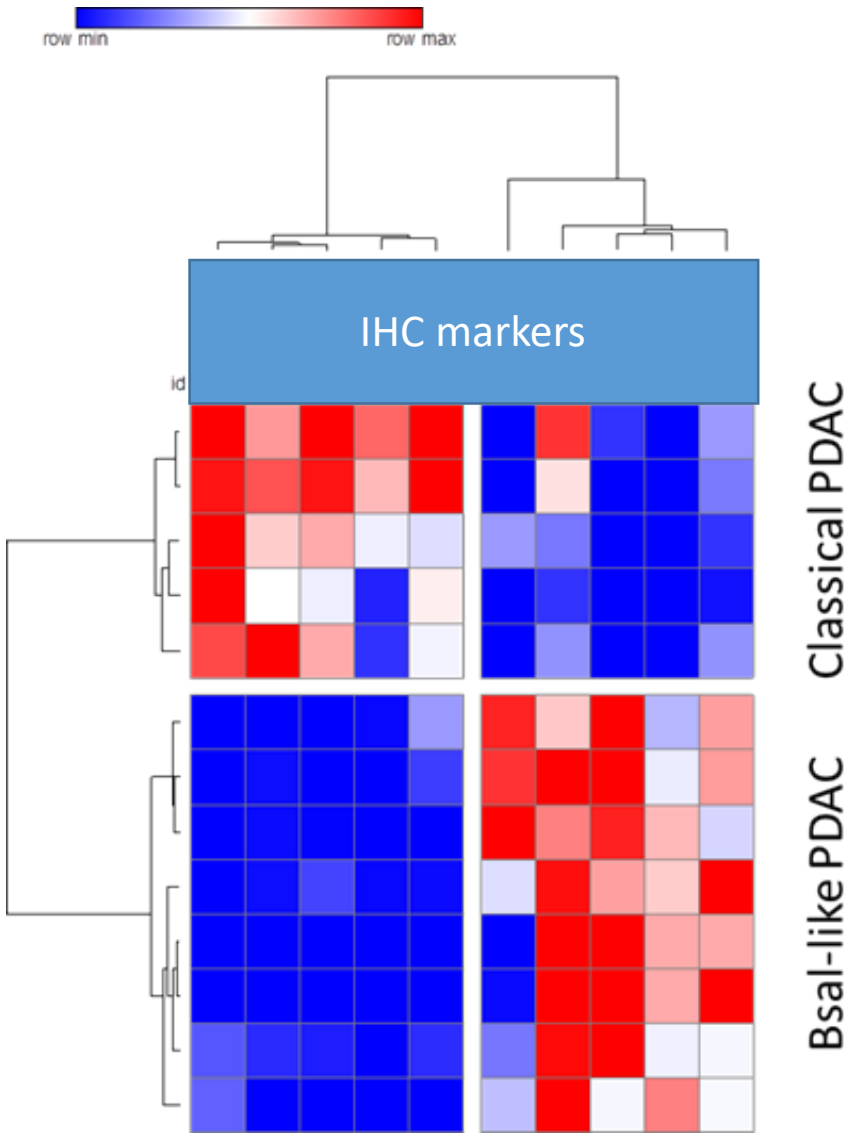


What do we do with double +?

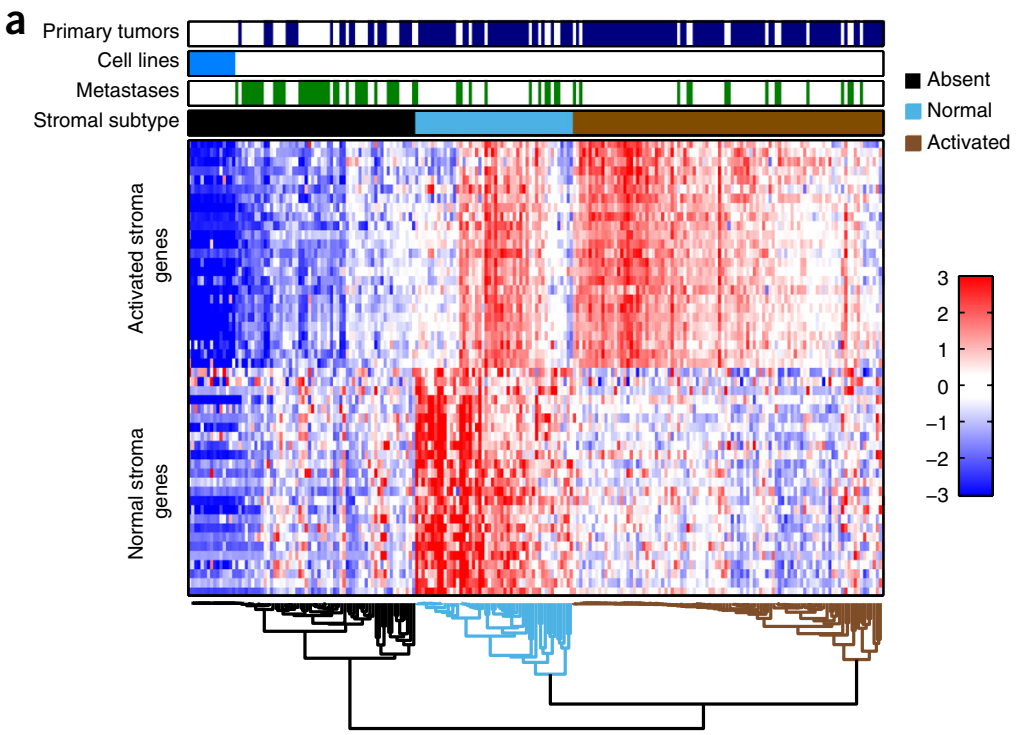


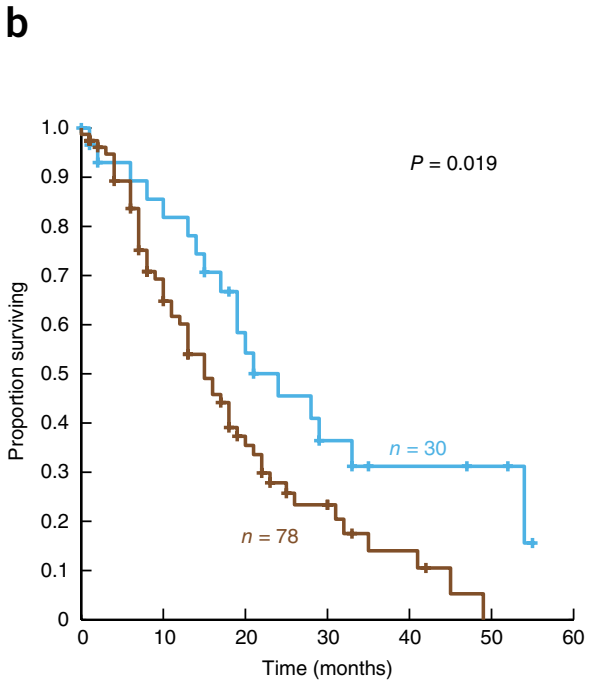
Noll *et al. Nat Med* 2015

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



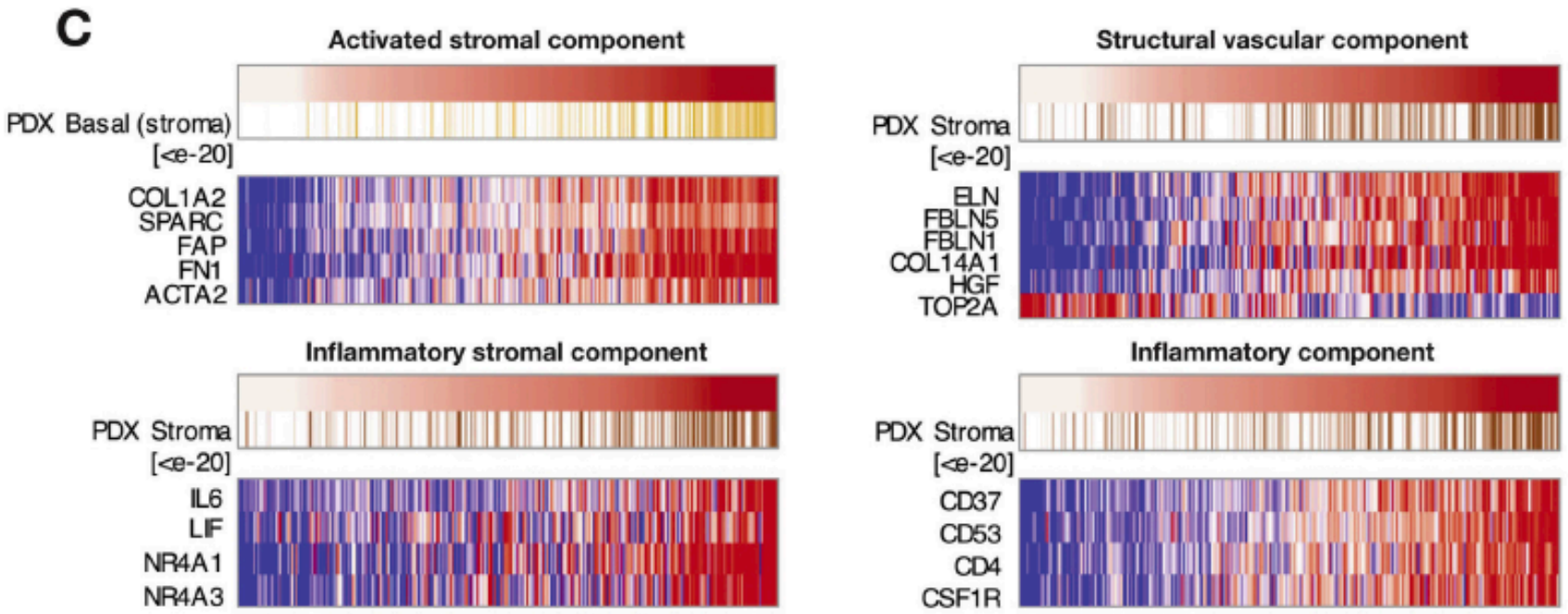
And the stroma is also heterogeneous…





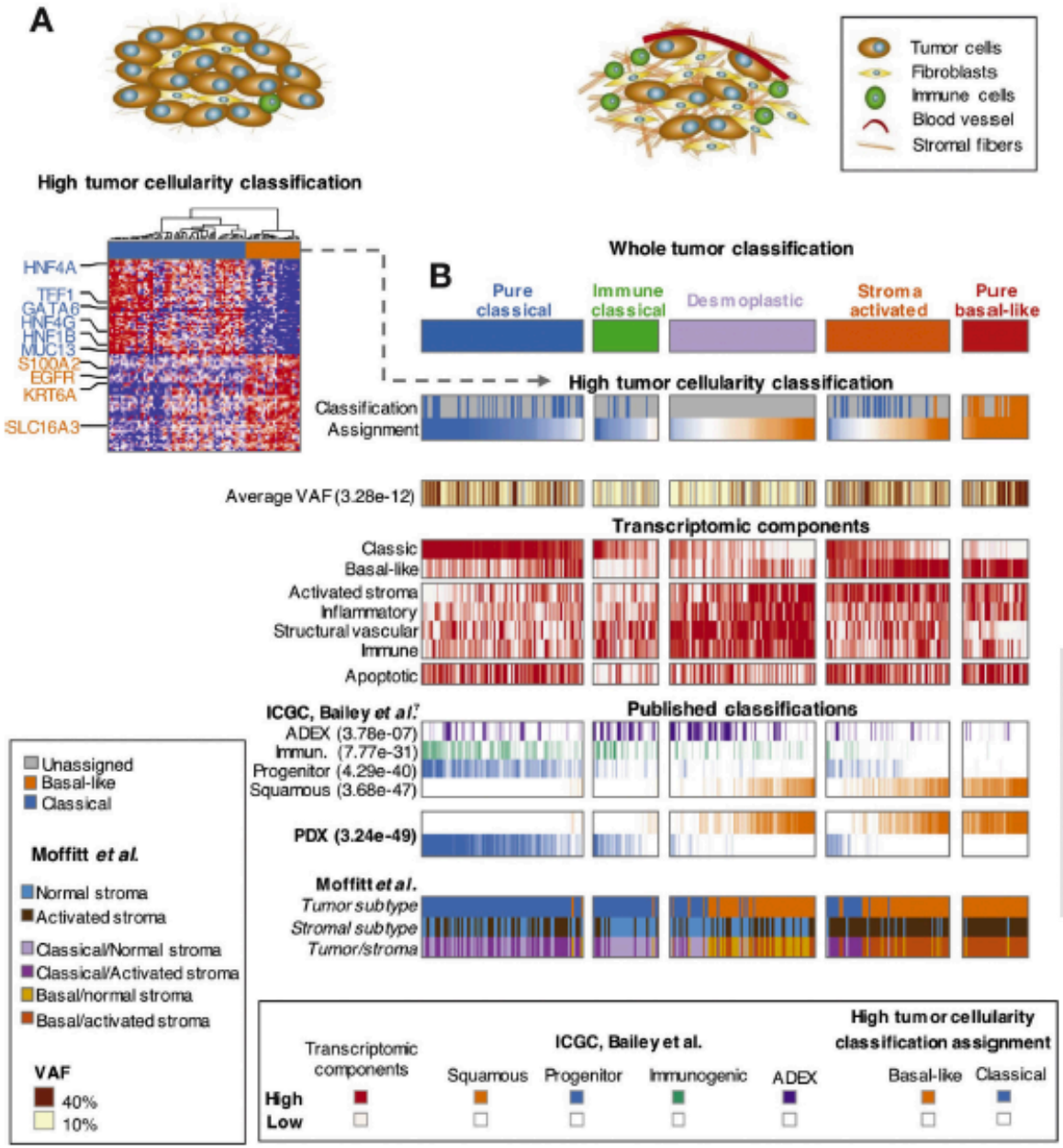
Moffitt et al. Nature genetics 2015

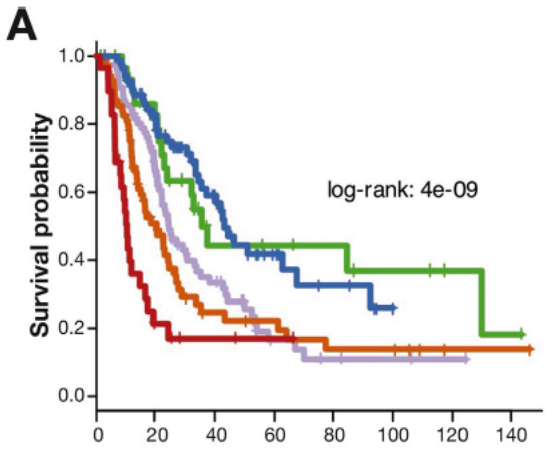
Transcriptomic

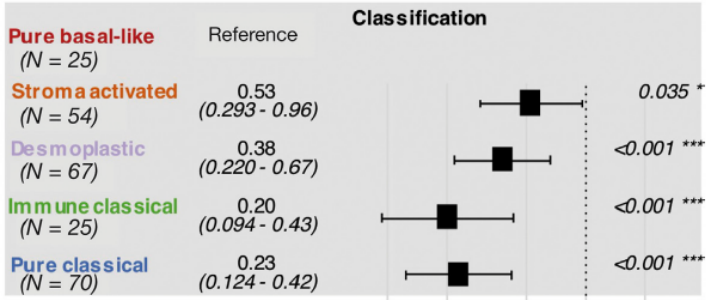


Puleo *et al, Gastroenterology* 2018

Transcriptomic

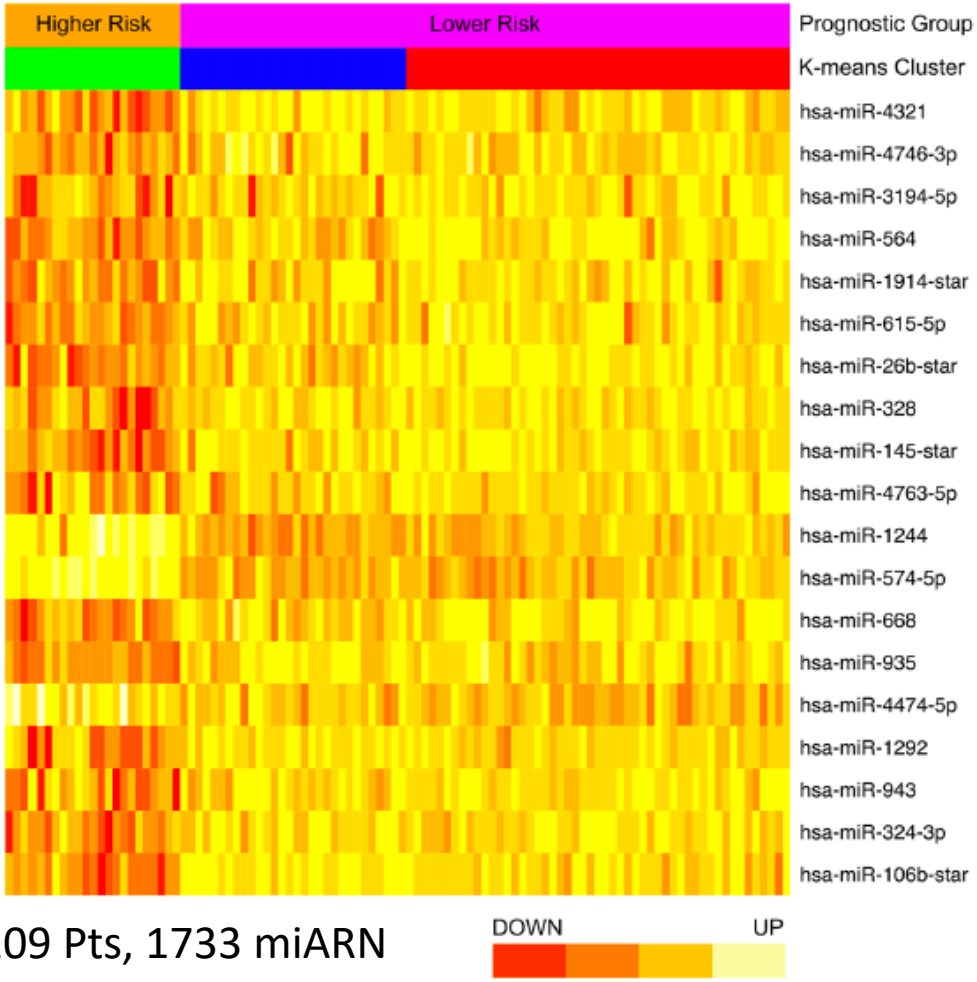




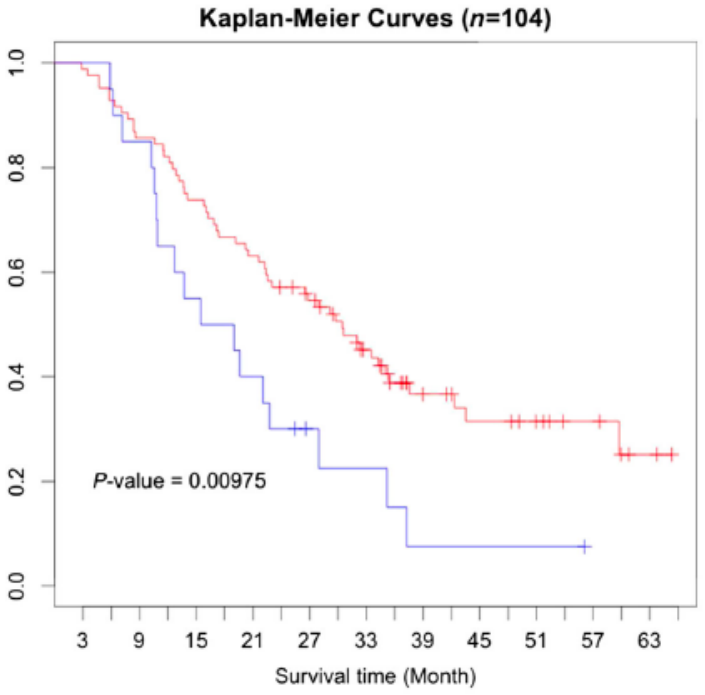


Puleo *et al, Gastroenterology* 2018

There is also heterogeneity in non coding RNA…

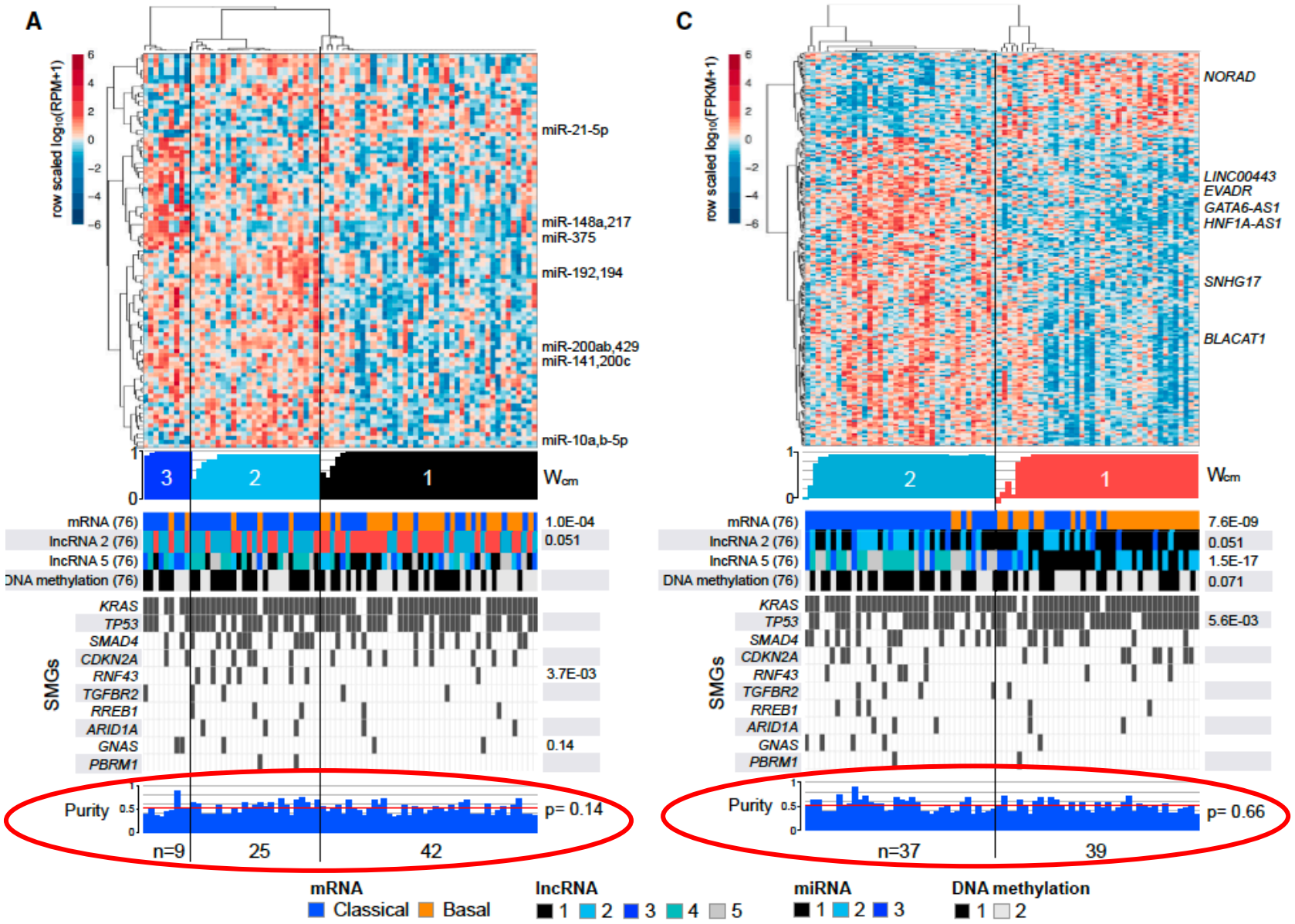


109 Pts, 1733 miARN



Namkung *et al. J Gastroenterol Hepatol*. 2016

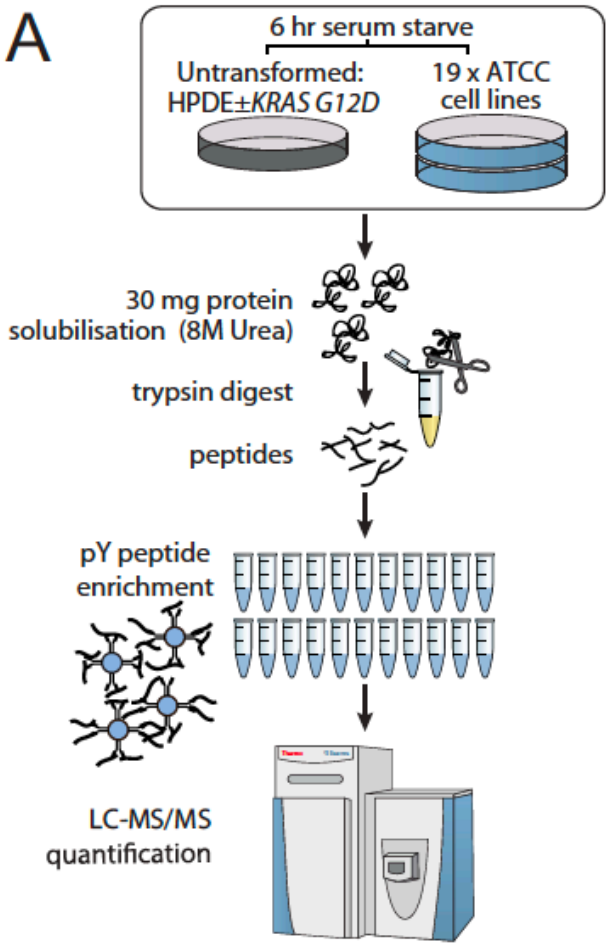
There is also heterogeneity in long non coding RNA…

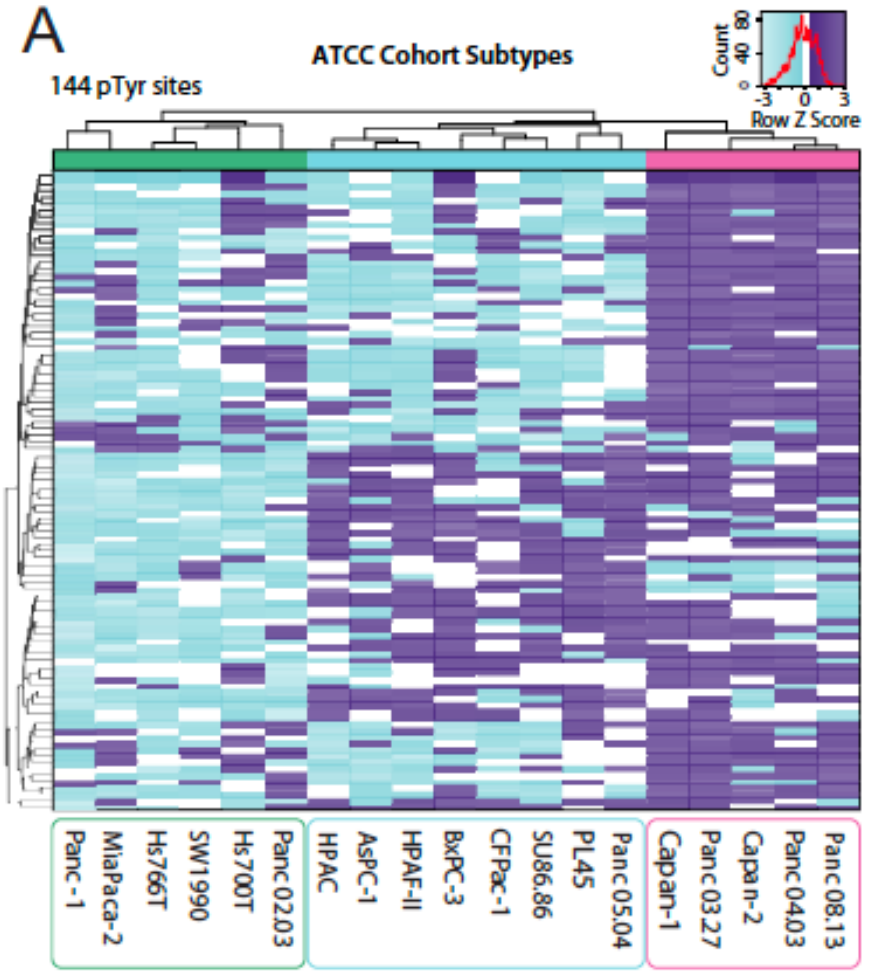


TCGA, Cancer cell 2017

Very few studies!

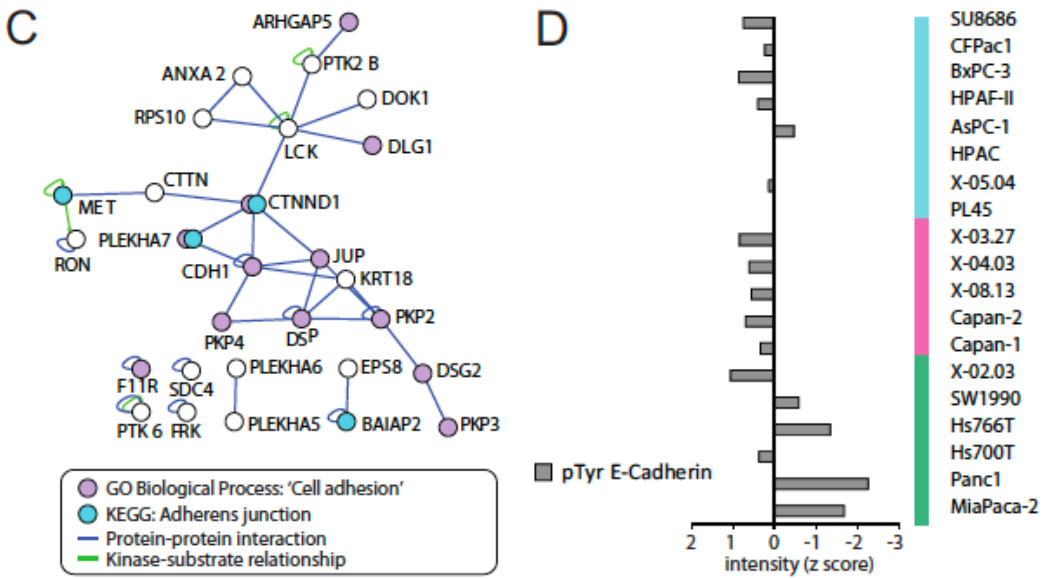
Phosphoproteome heterogeneity….(cell lines)…..



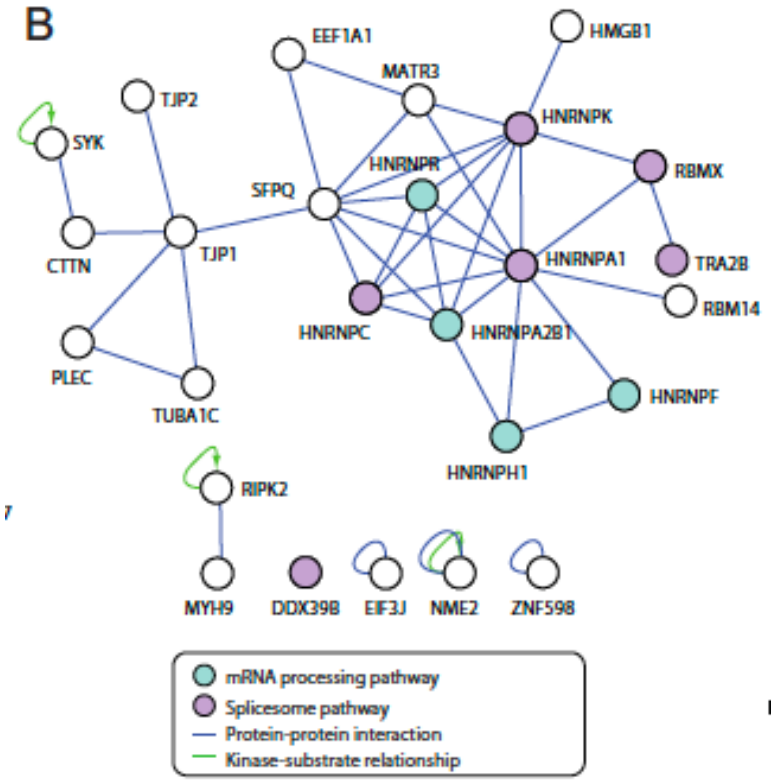


Humphrey *et al. Moll cell proteomics* 2016

Phosphoproteome heterogeneity….(cell lines)…..

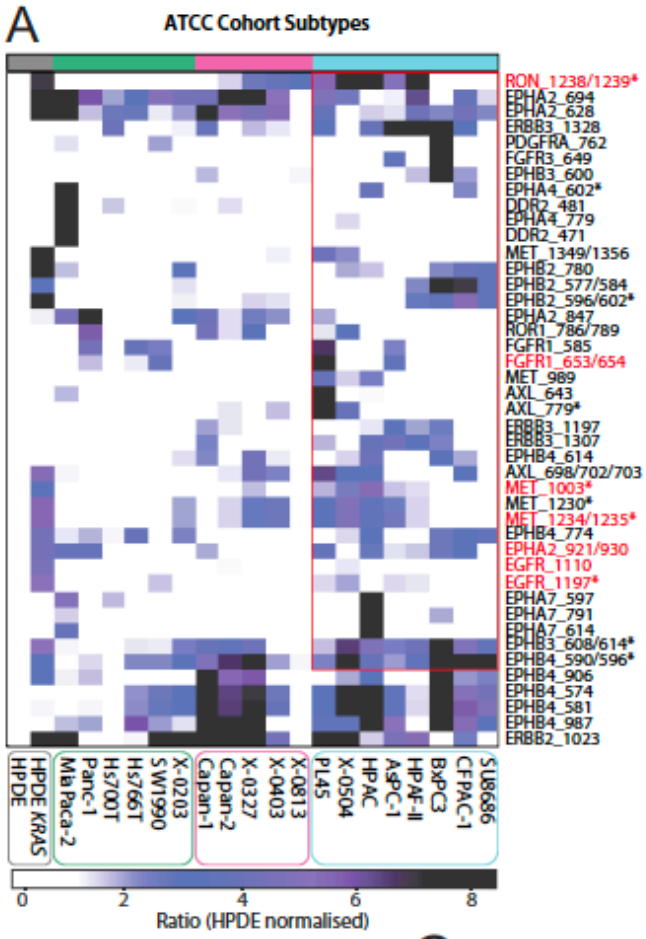


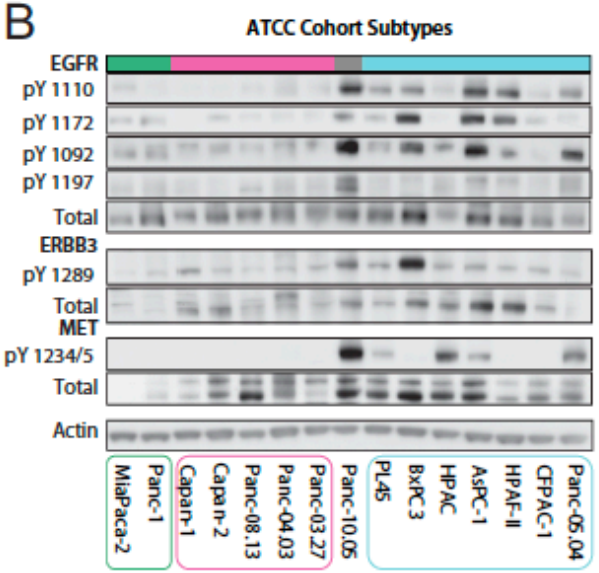
Type 1: EMT, low global phosphorylation



Type 2: Gene processing

Humphrey *et al. Moll cell proteomics* 2016





Type 3: Strong tyrosine kinase activity

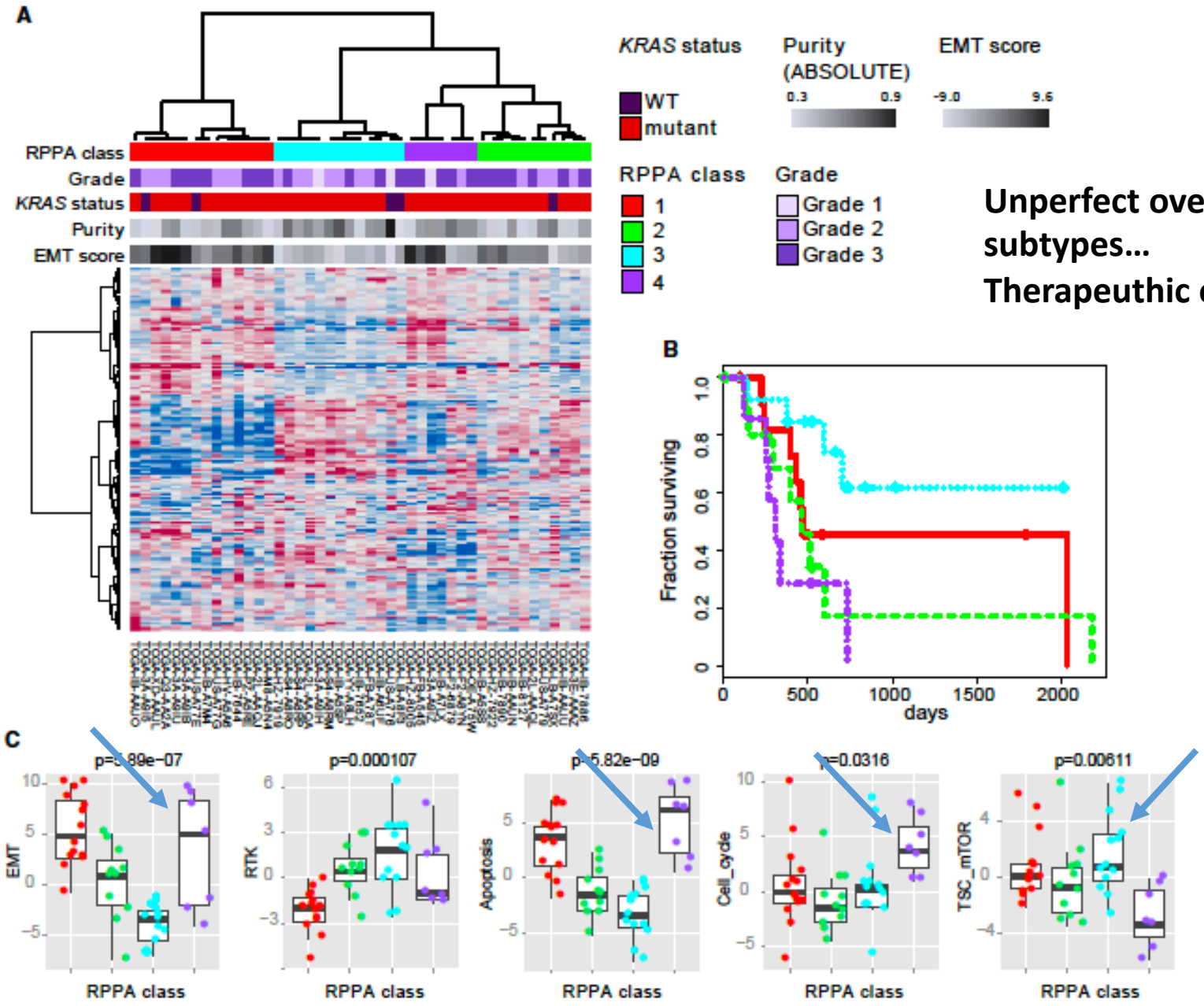
Sensitivity to TKi?

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

pTyr Abundance
- High relative pTyr
- Medium relative pTyr
- Low relative pTyr

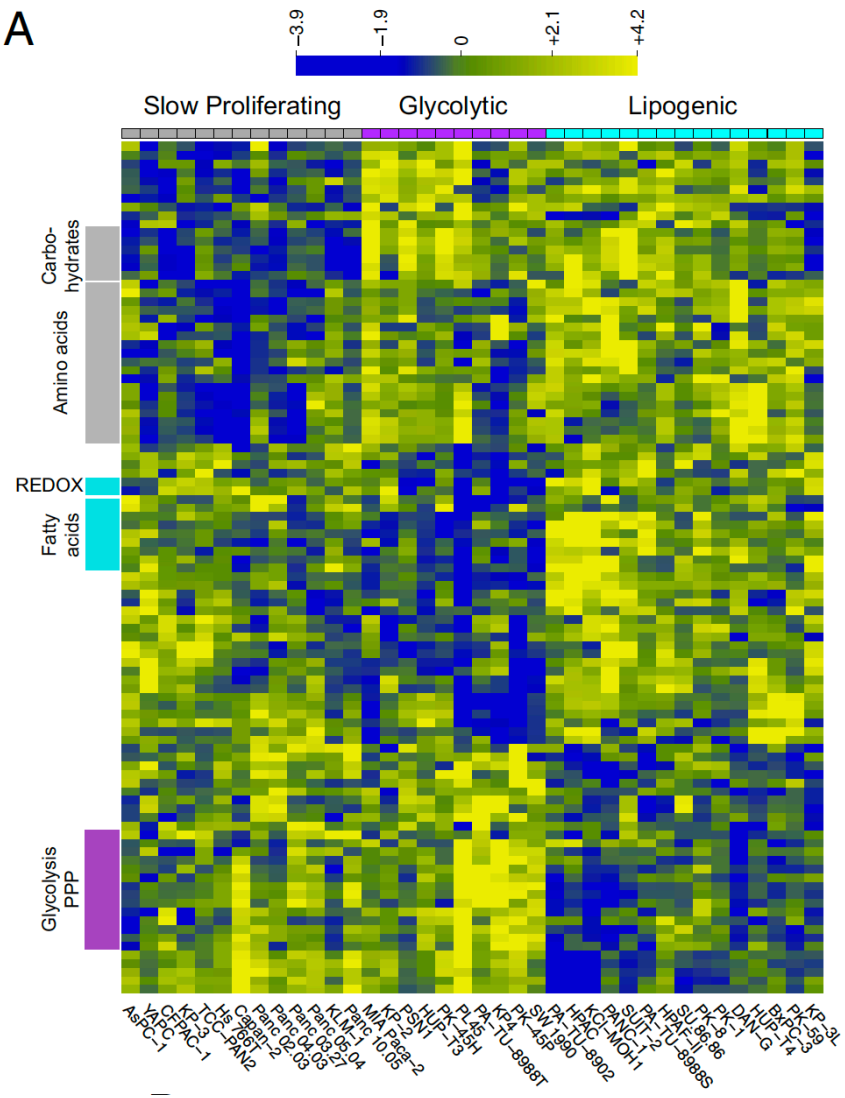
Humphrey *et al. Moll cell proteomics* 2016

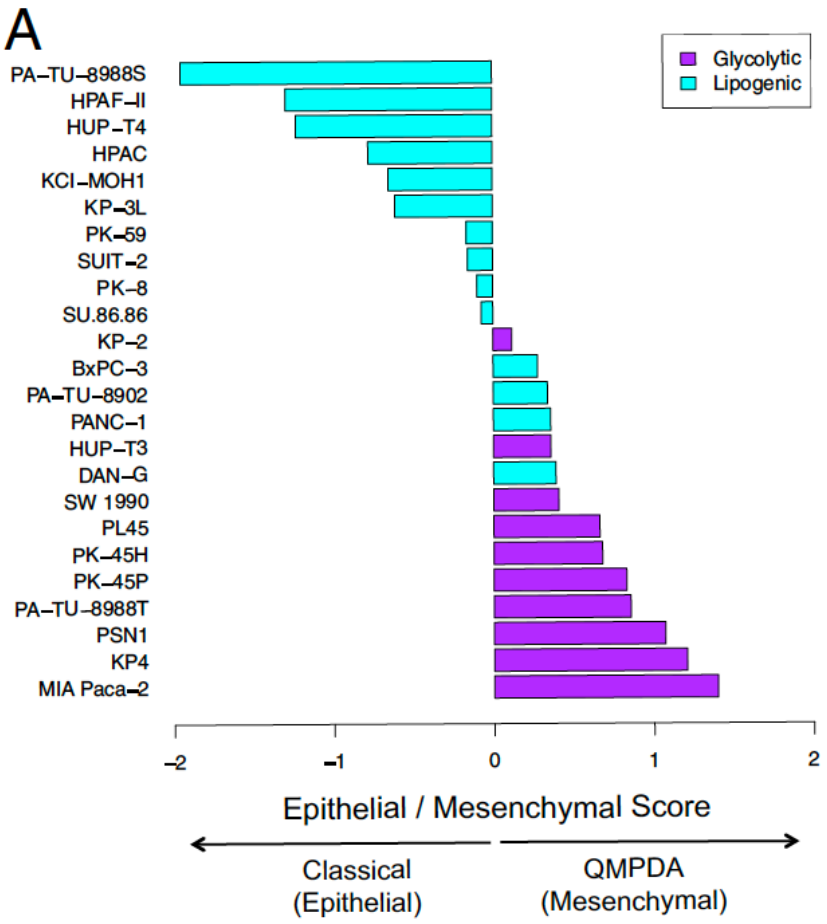


Unperfect overlap with the subtypes…
Therapeuthic opportunities?

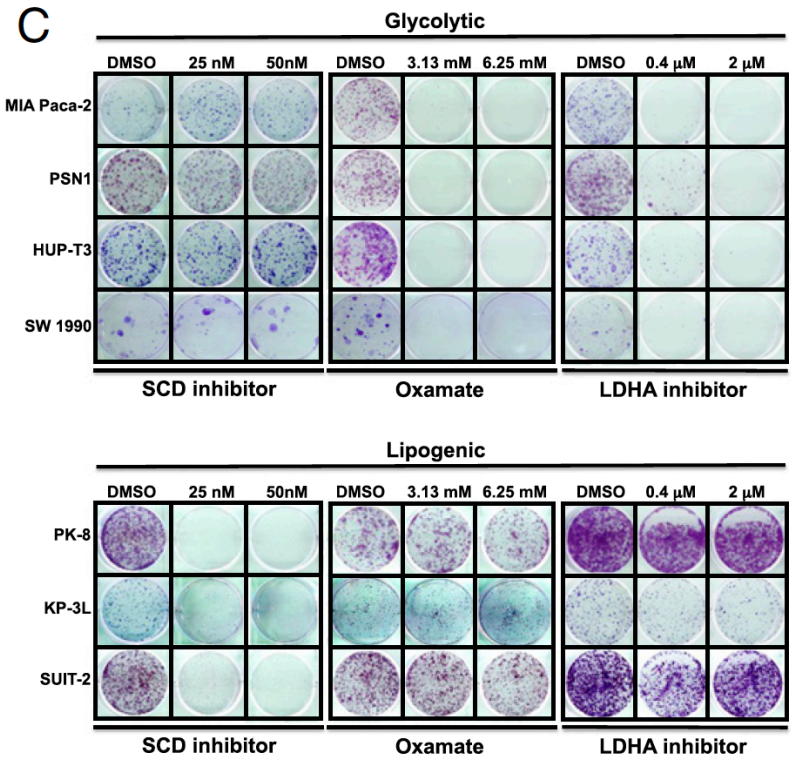
TCGA, Cancer cell 2017

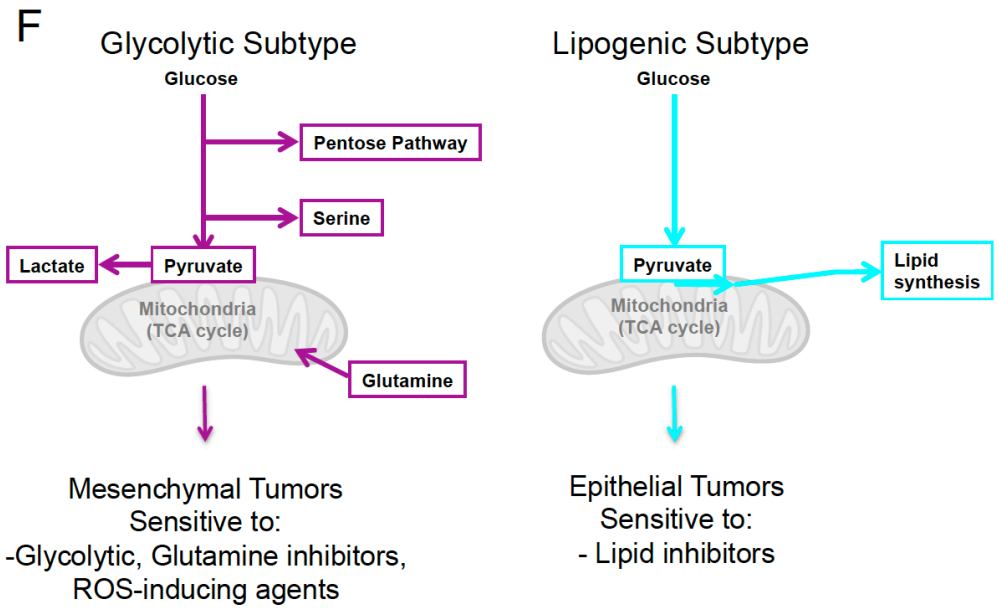
Critical in PDAC growth. Numerous publications on individual mechanisms++++





Daemen *et al. PNAS* 2015



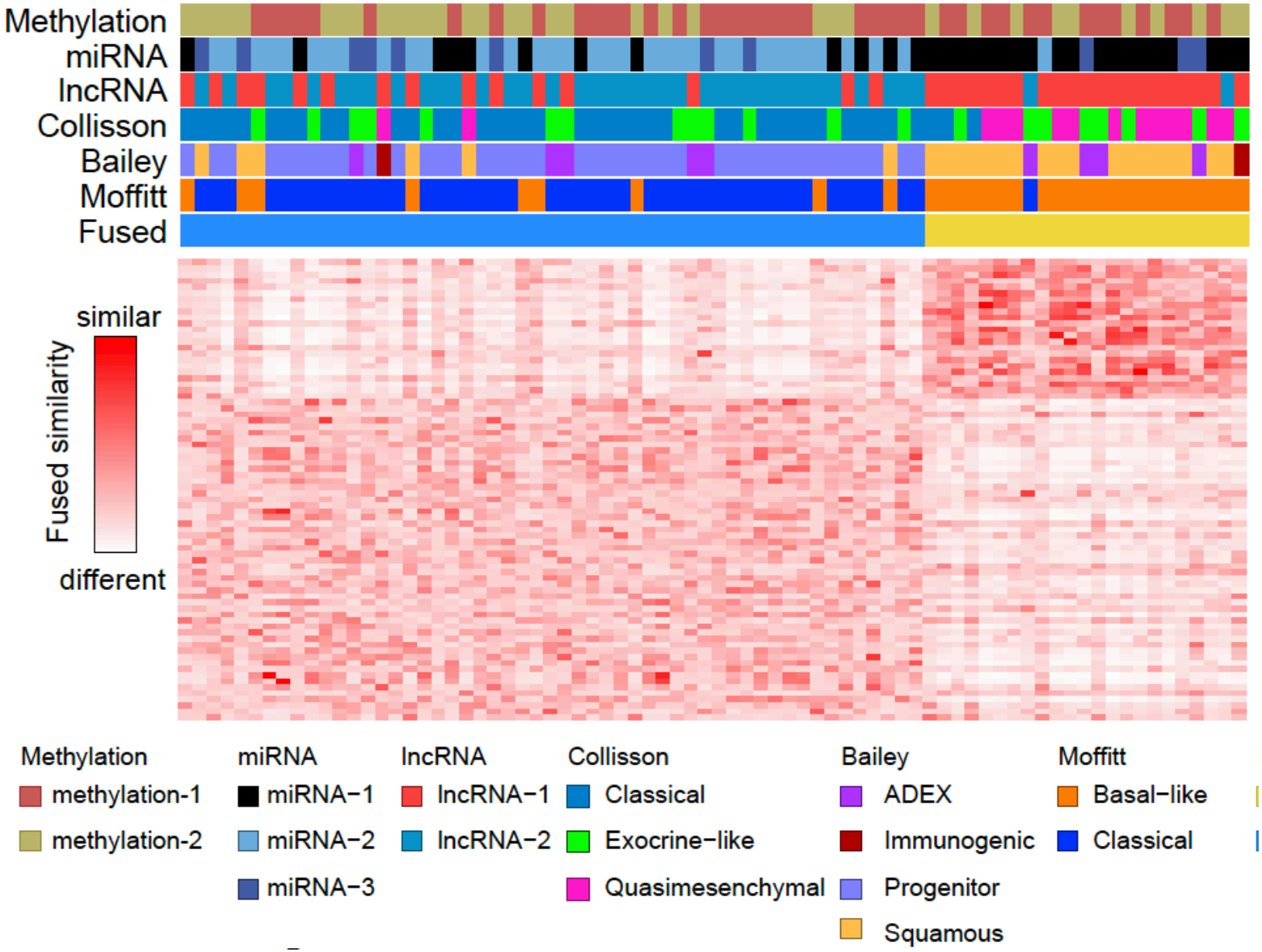


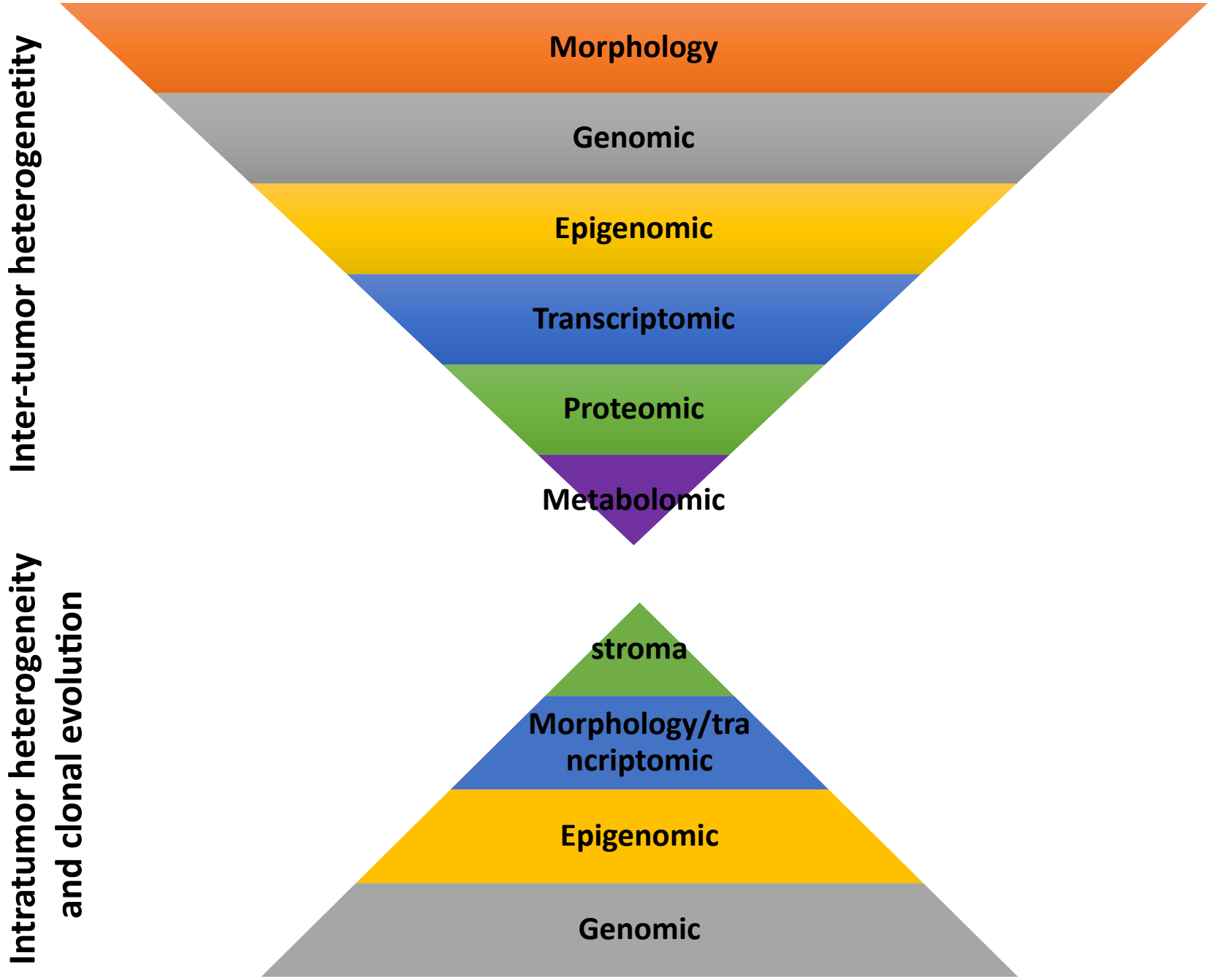
Human tumors ?

Daemen *et al. PNAS* 2015

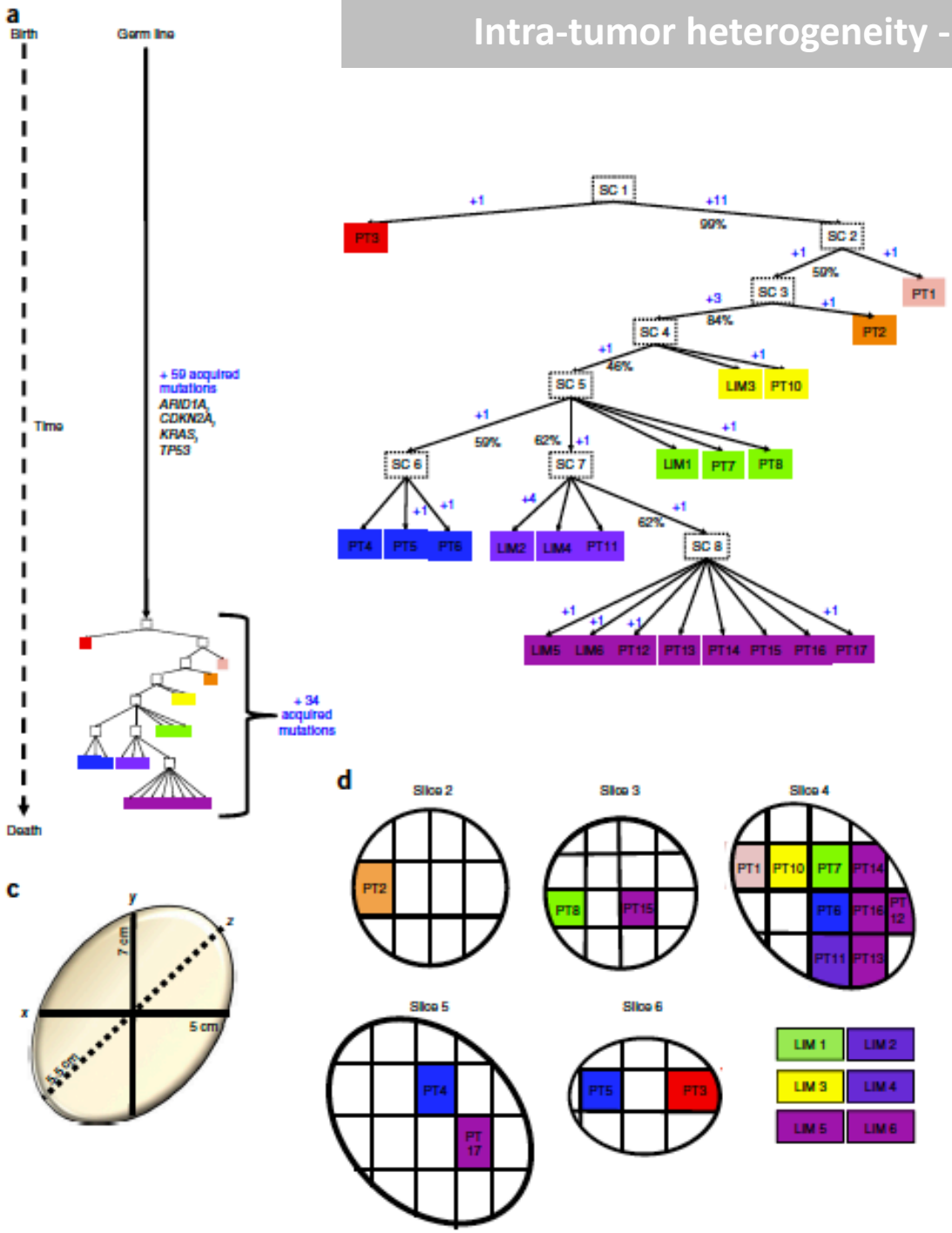
Inter-tumor heterogeneity - conclusion

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




Inter-tumor heterogenetity
Morphology
Genomic
Epigenomic
Transcriptomic
Proteomic
Metabolomic
Intratumor heterogeneity and clonal evolution
stroma
Morphology/tra ncriptomic
Epigenomic
Genomic

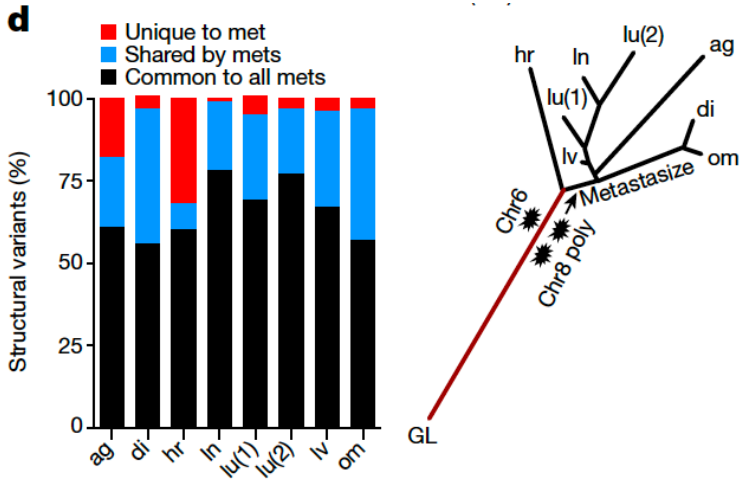
Intra-tumor heterogeneity - genomic



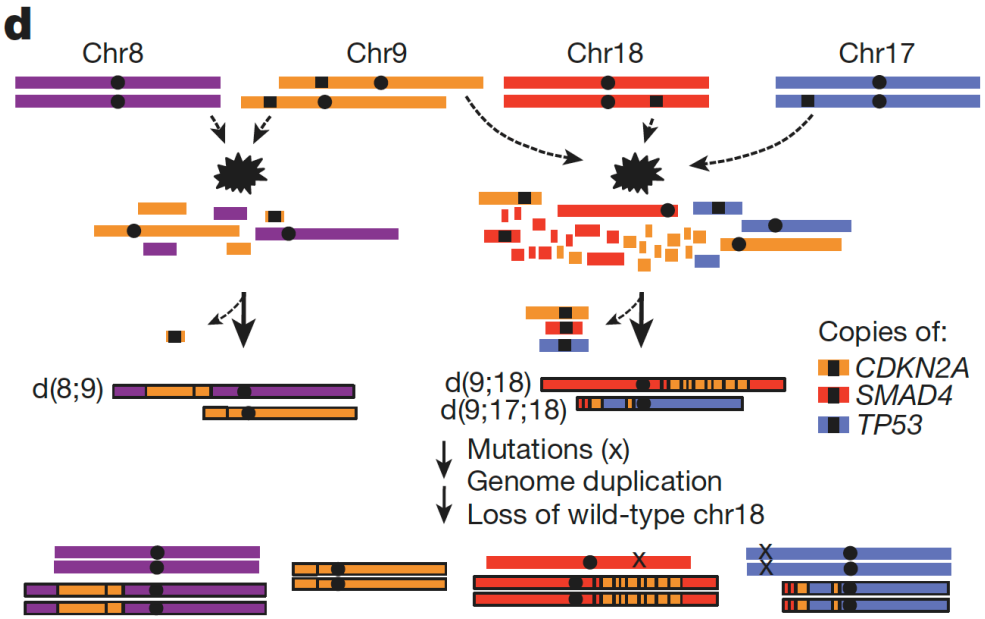
- **Low heterogeneity of classical driver genes**
- **Most genomic events happen early**
- **No « mestastasis » gene**
- **Physical and genomic spatialisation are different+++**

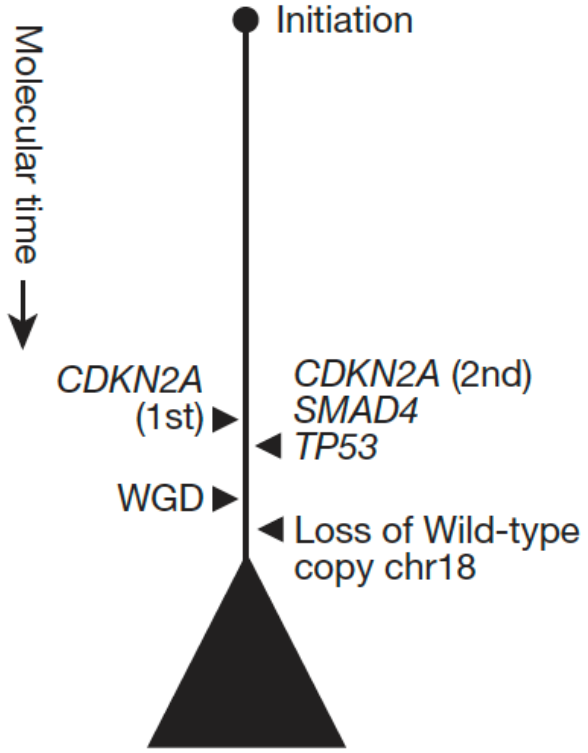
Makohon-Moore *et al. Nat Gen* 2017
Yachida *et al. Nature* 2010

Intra-tumor heterogeneity - genomic



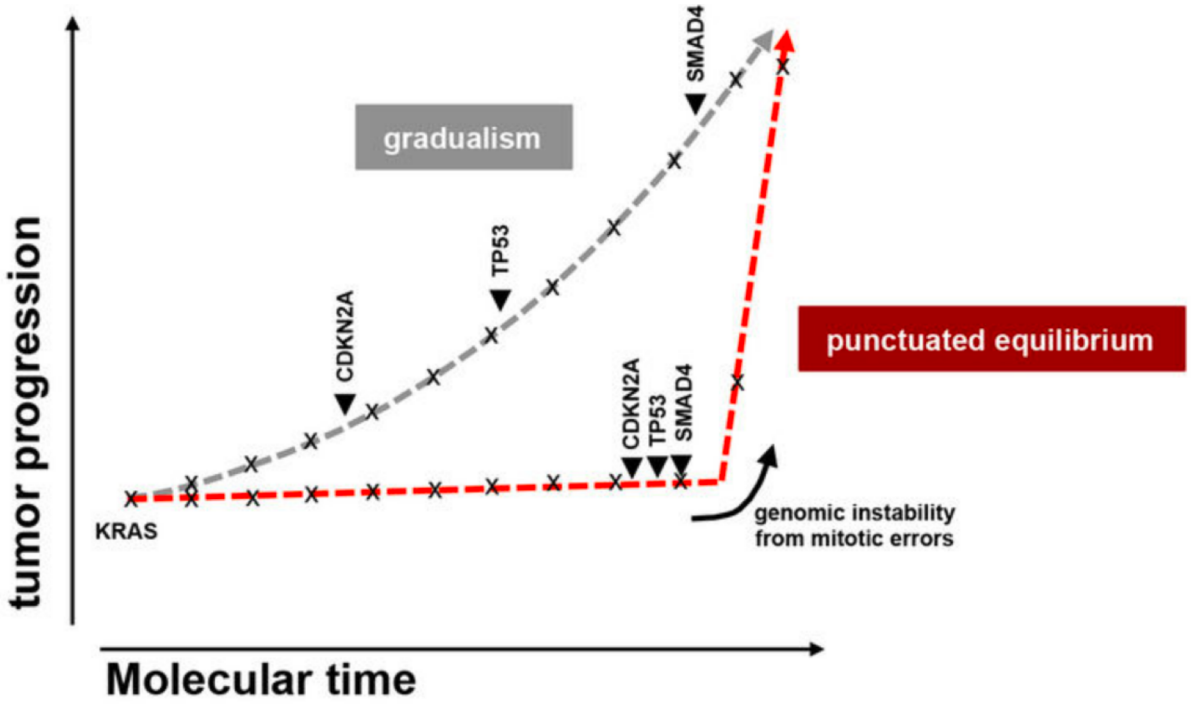
- Most genomic events happen before the first metastase
- 50% of tumeurs are not diploïd (T ou H)
- Multiples simultaneous genomic alterations

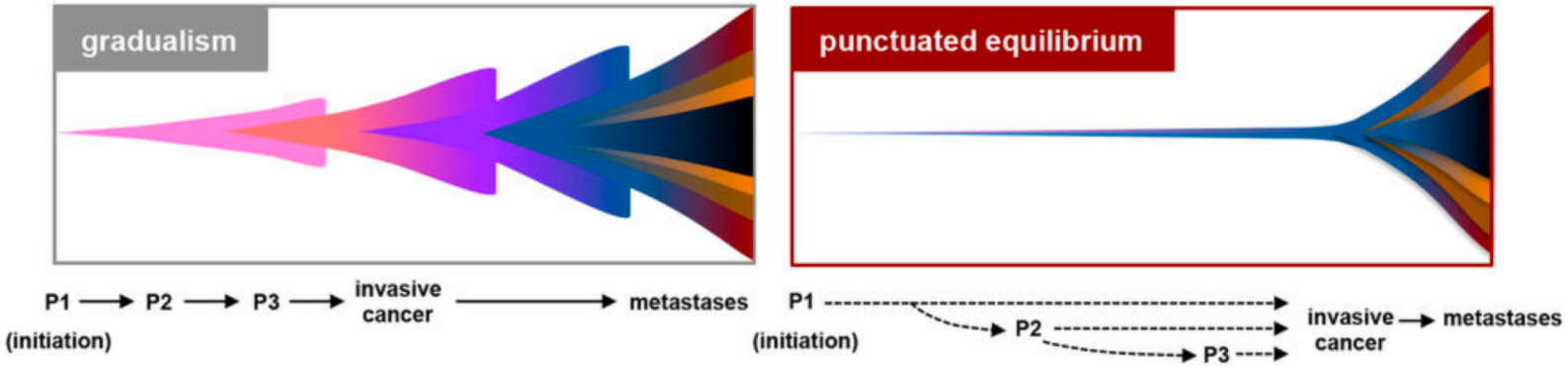




Notta *et al. Nature* 2016

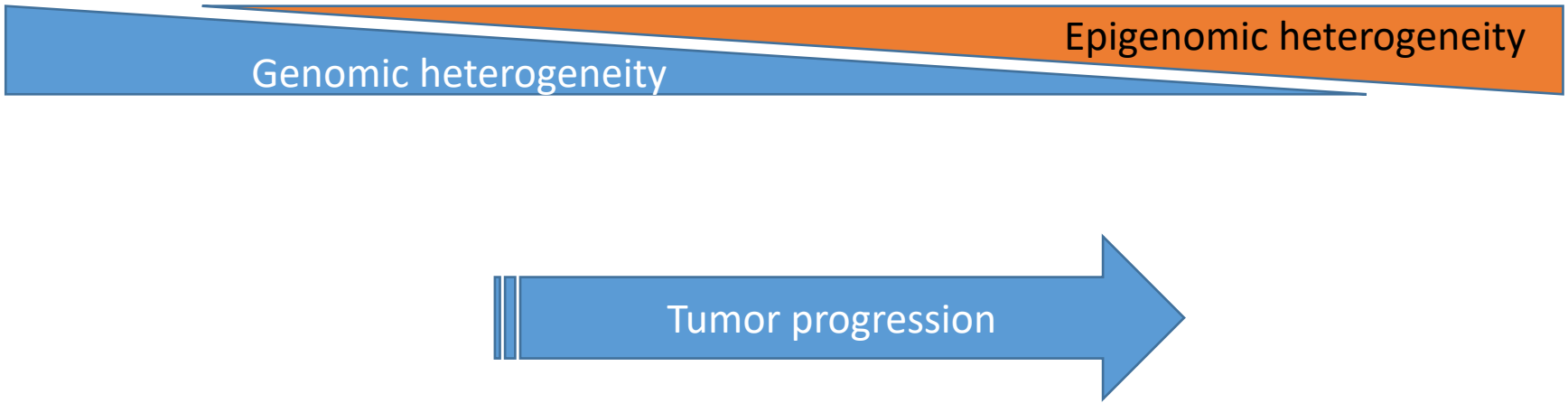
How to follow high risk patients???





Notta *et al. Nature* 2016

Intra-tumor heterogeneity - epigenomic



Intra-tumor heterogeneity - epigenomic

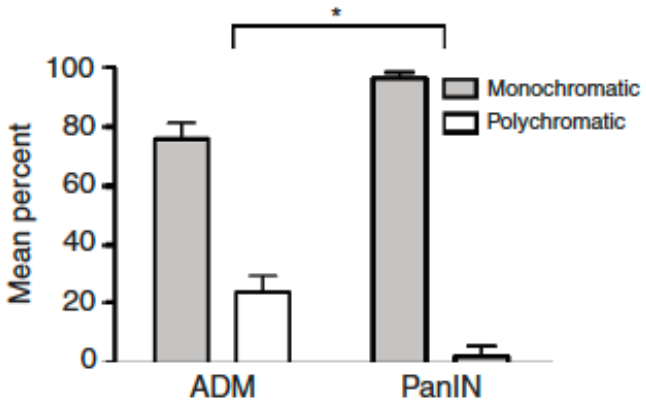
Epigenomic heterogeneity

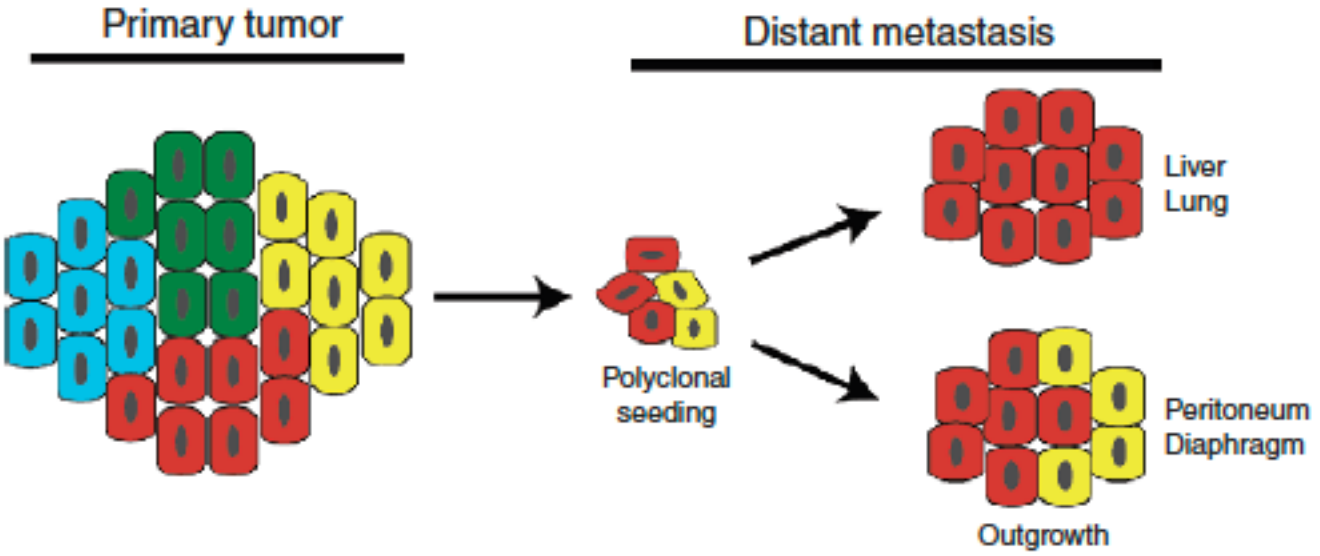
Genomic heterogeneity

C

ADM and PanIN counts

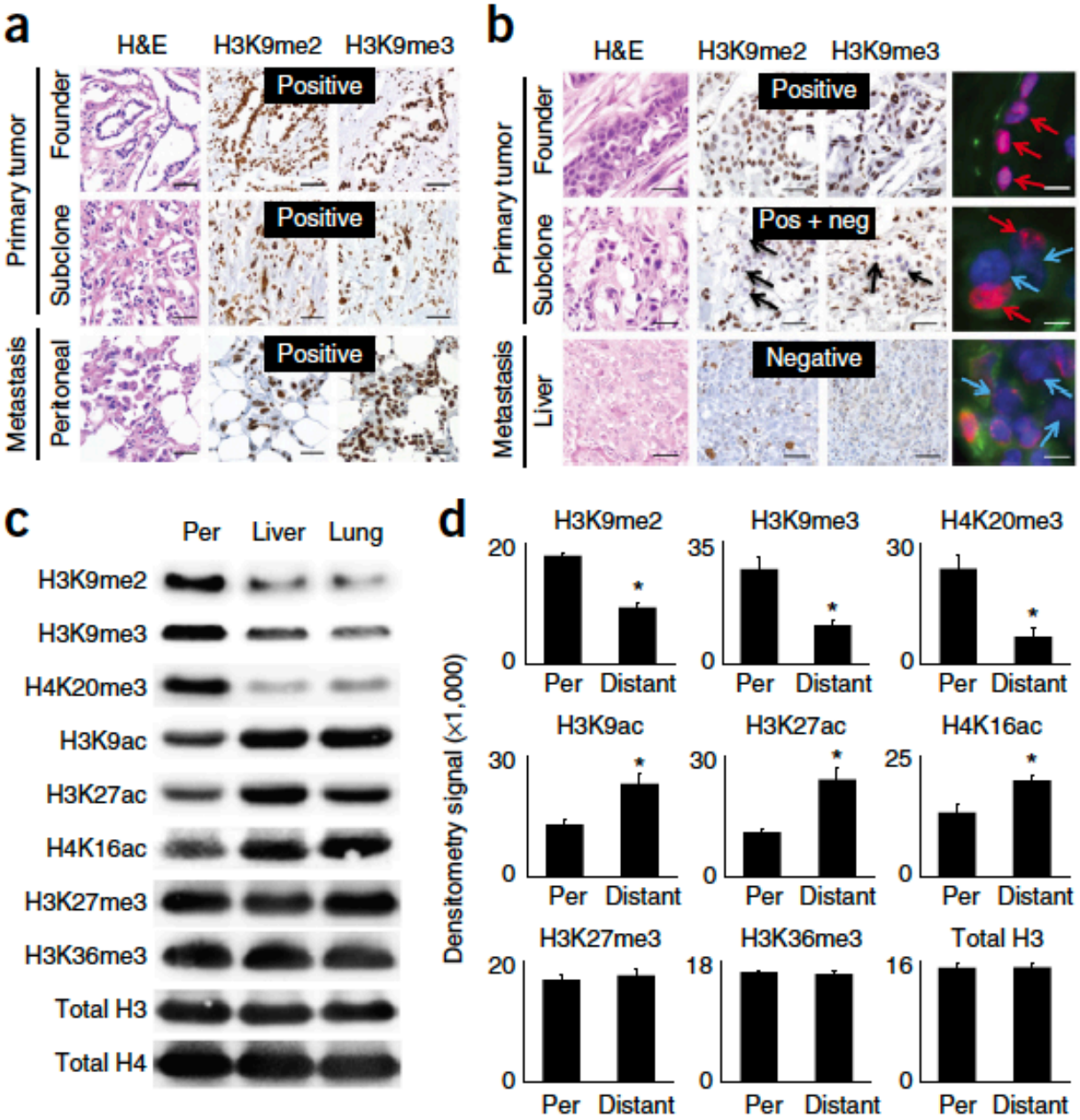
Lesion	Monochromatic	Polychromatic	Total
ADM	149 (76%)	46 (24%)	195
PanIN	141 (97%)	4 (3%)	145





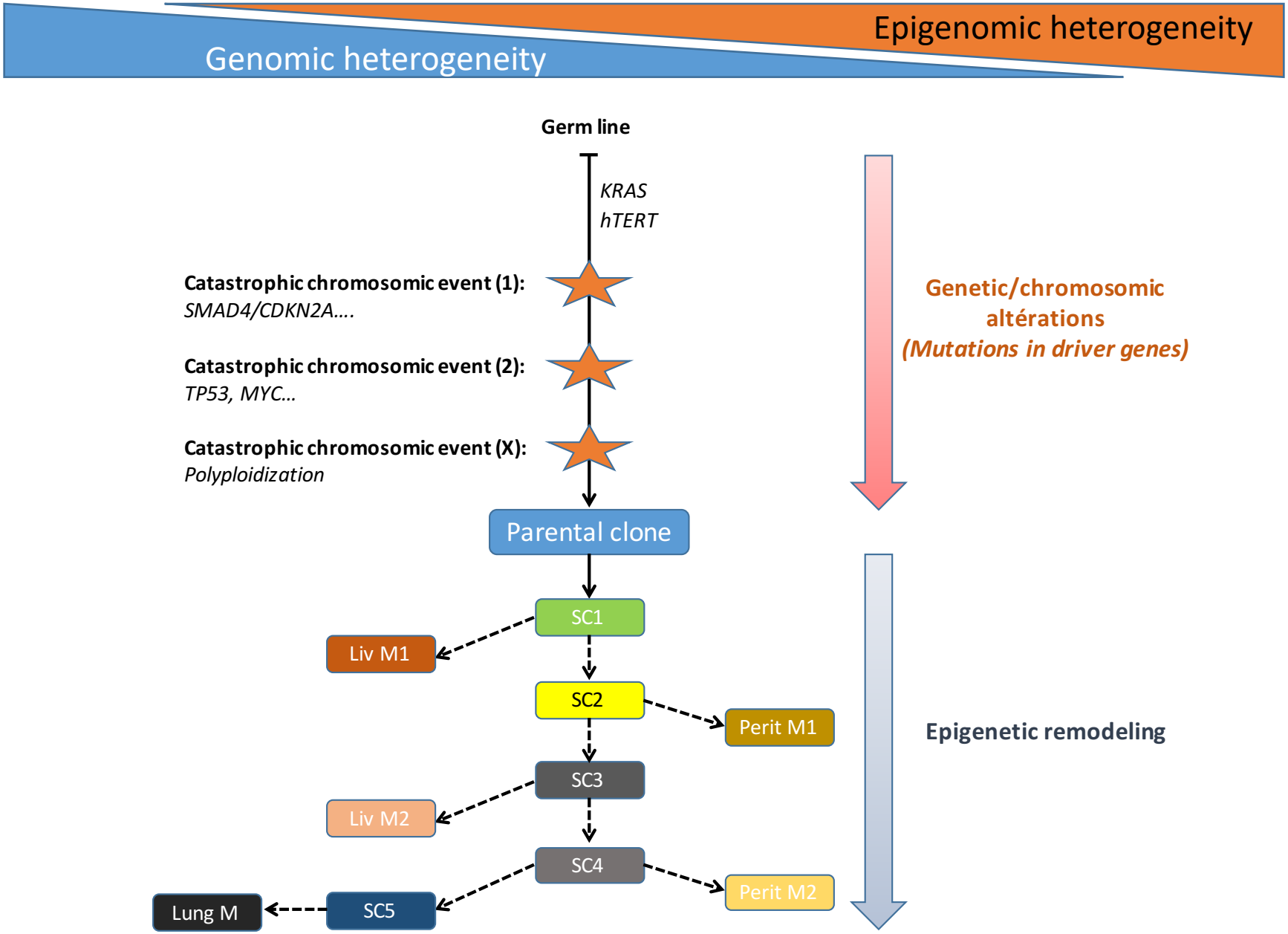
Maddipati *et al*. *Cancer discovery* 2015

Intra-tumor heterogeneity - epigenomic



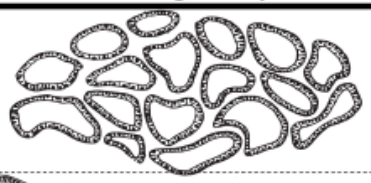
Mc Donals *Nat Gen* 2016

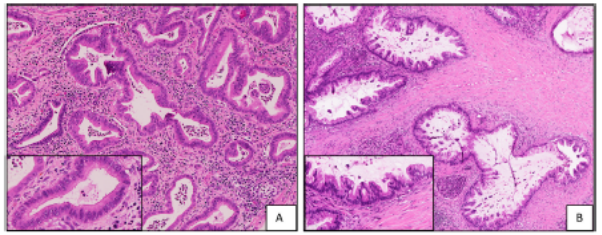
Intra-tumor heterogeneity - epigenomic

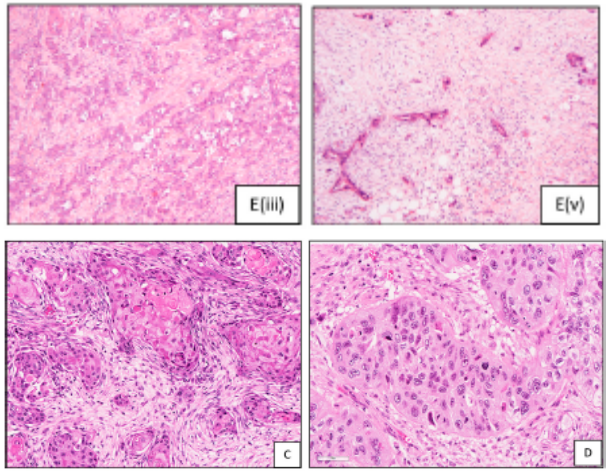


If tumor were pure, that would be too easy…..

A

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

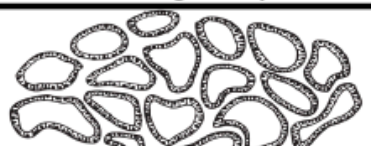




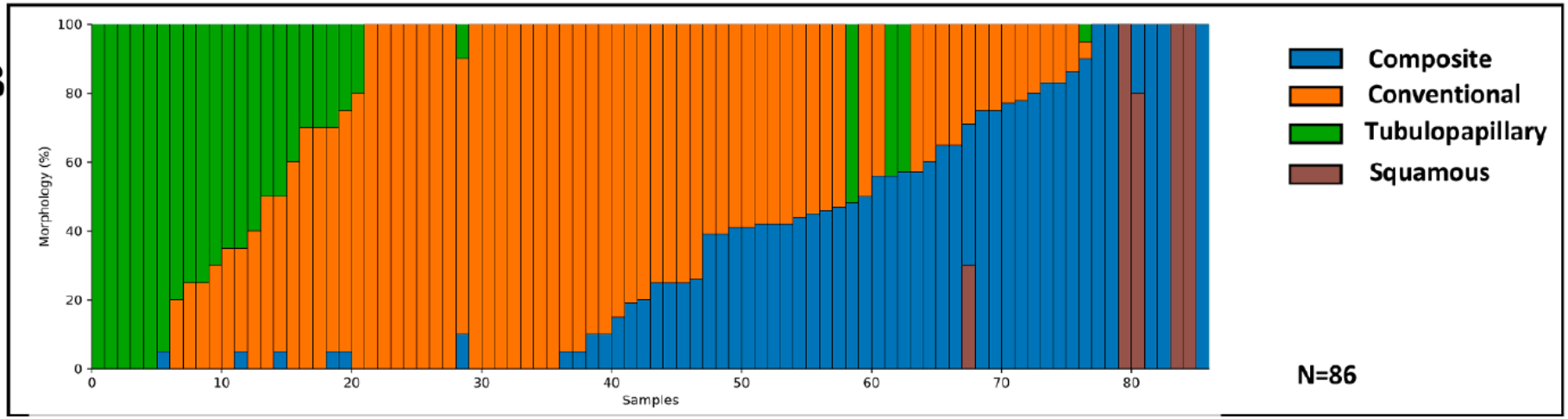
N Kalimuthu S, et al. Gut 2019

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

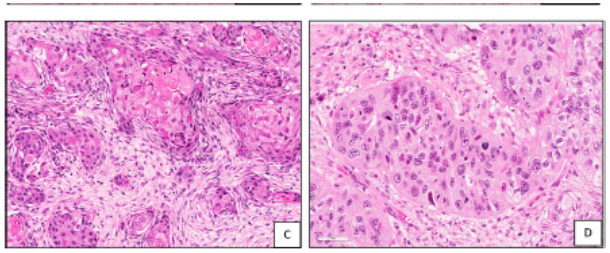
A

"Gland forming" component	Patterns
	Conventional

B

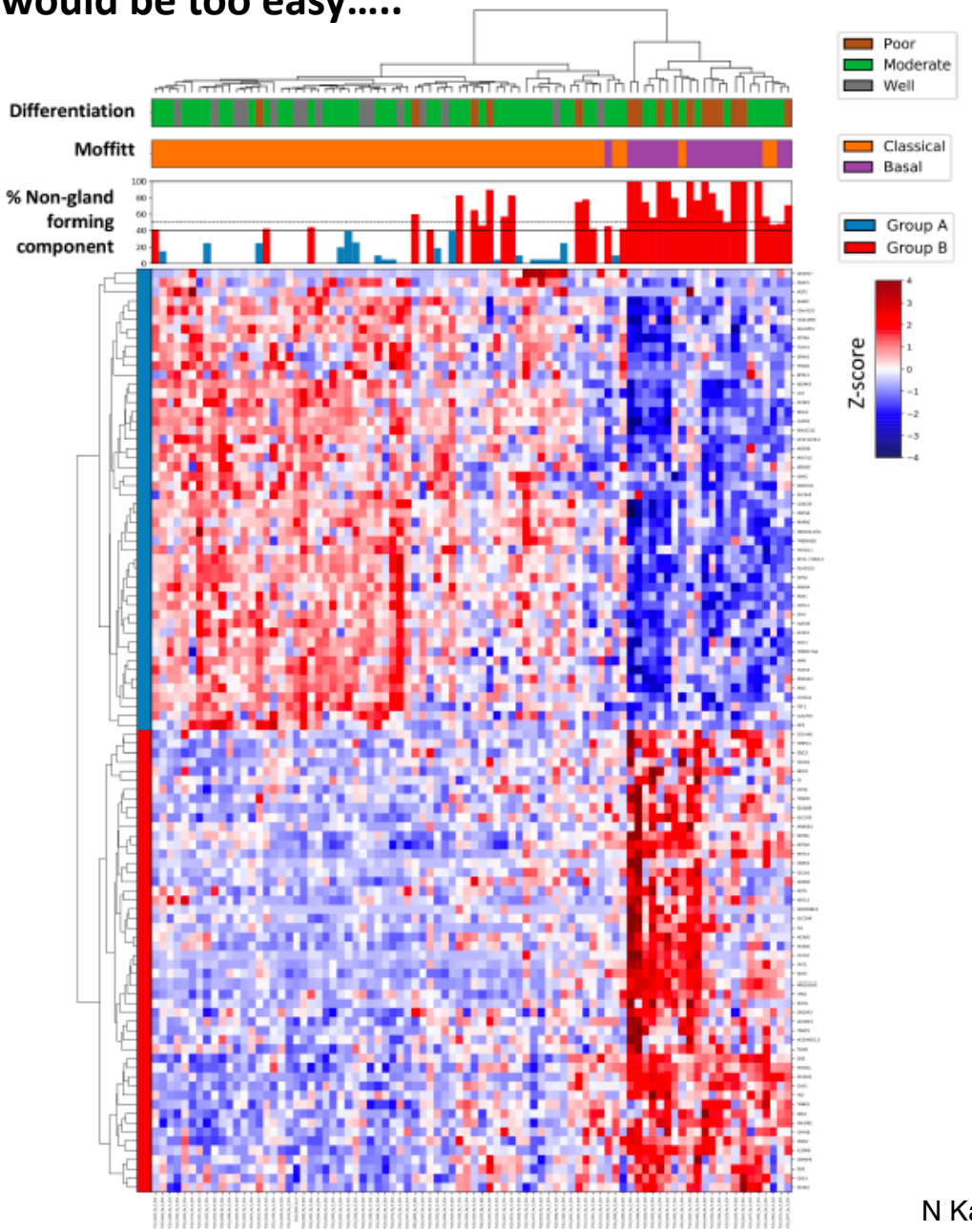


	Squamous



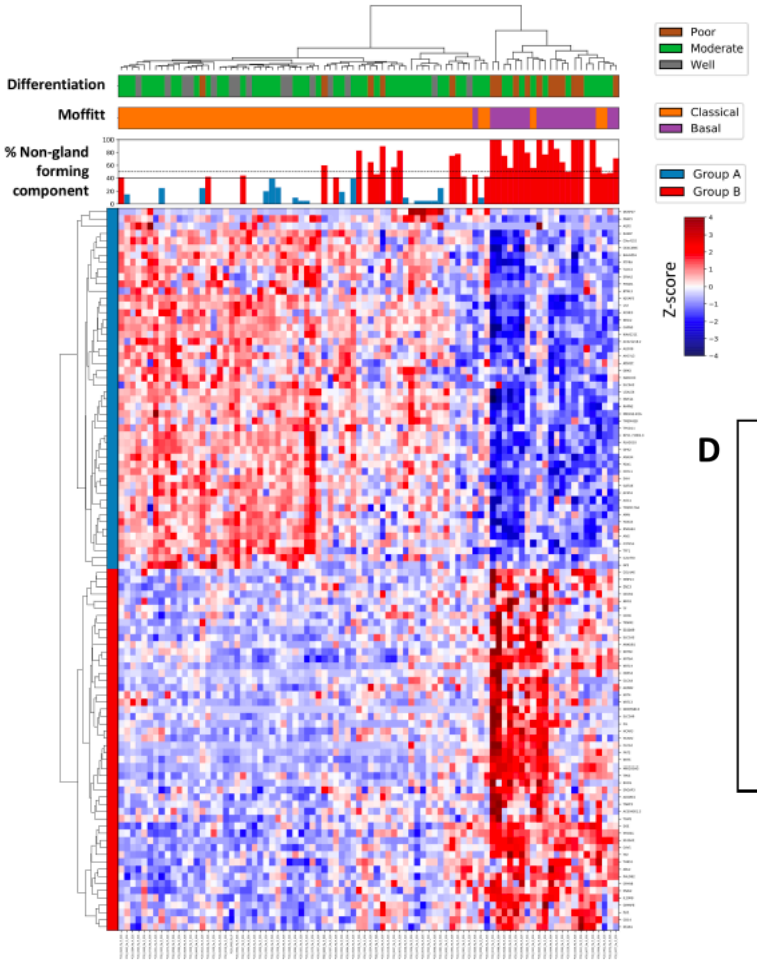
N Kalimuthu S, et al. Gut 2019

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

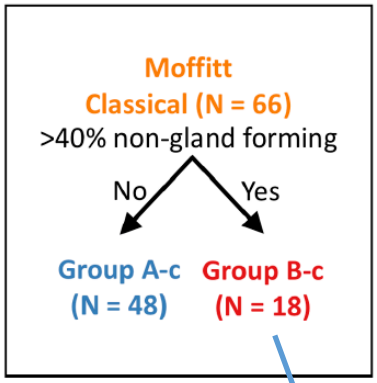


N Kalimuthu S, et al. Gut 2019

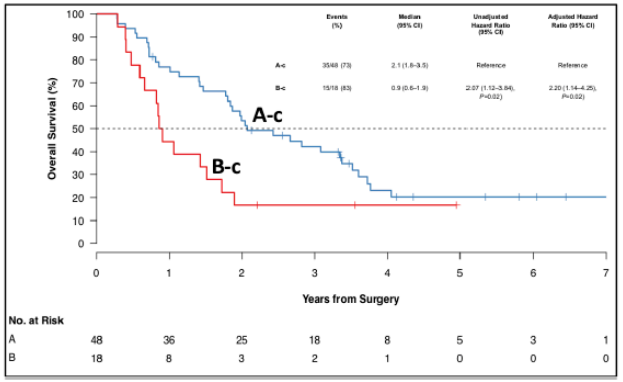
If tumor were pure, that would be too easy…..



D

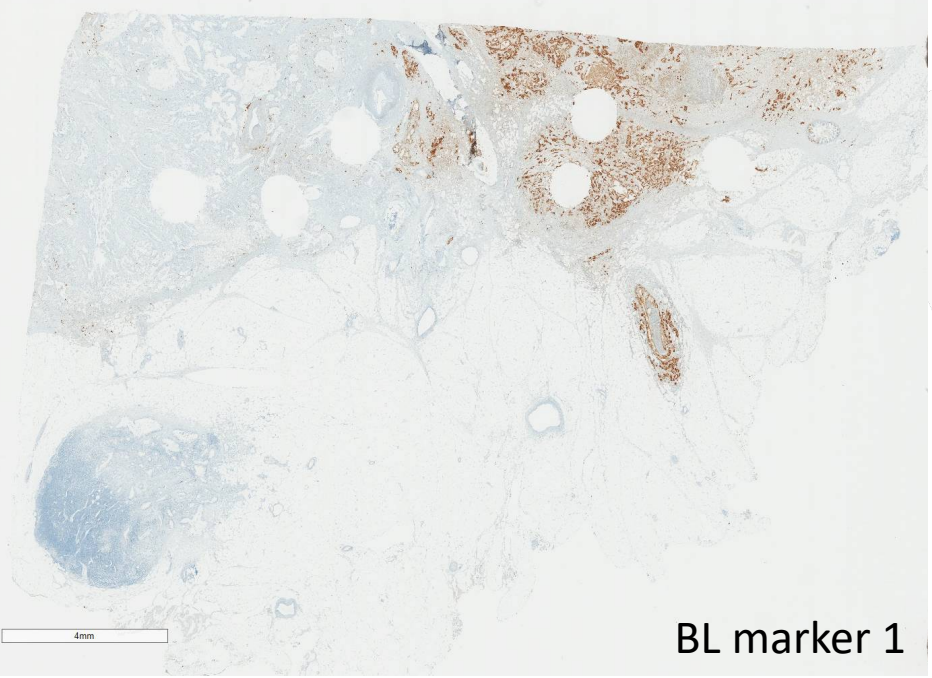


E



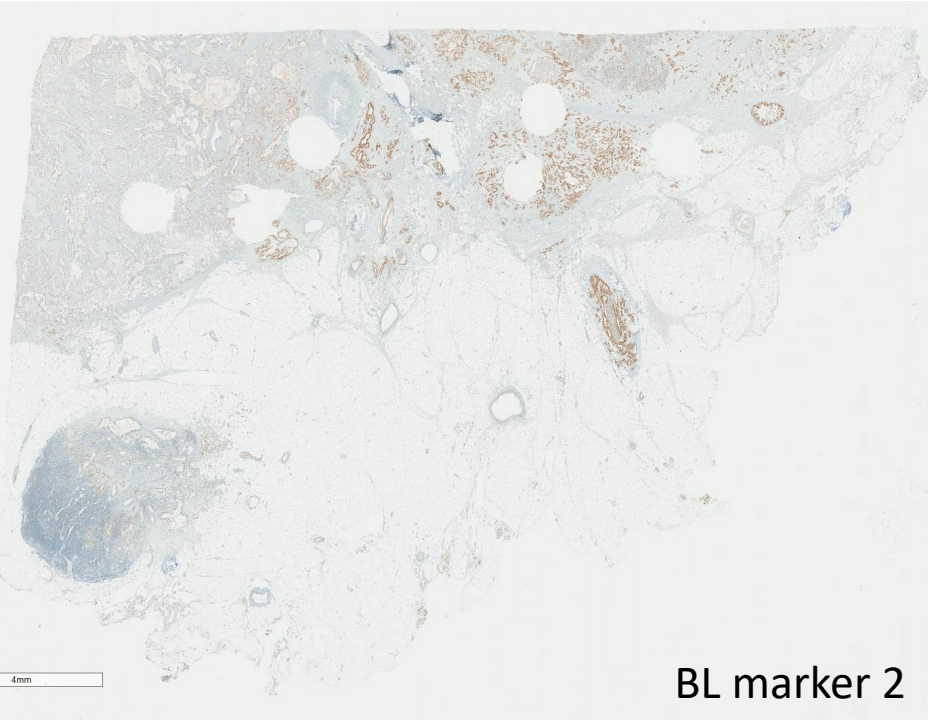
« classical » tumors with a basal like subpopulation?

N Kalimuthu S, et al. Gut 2019

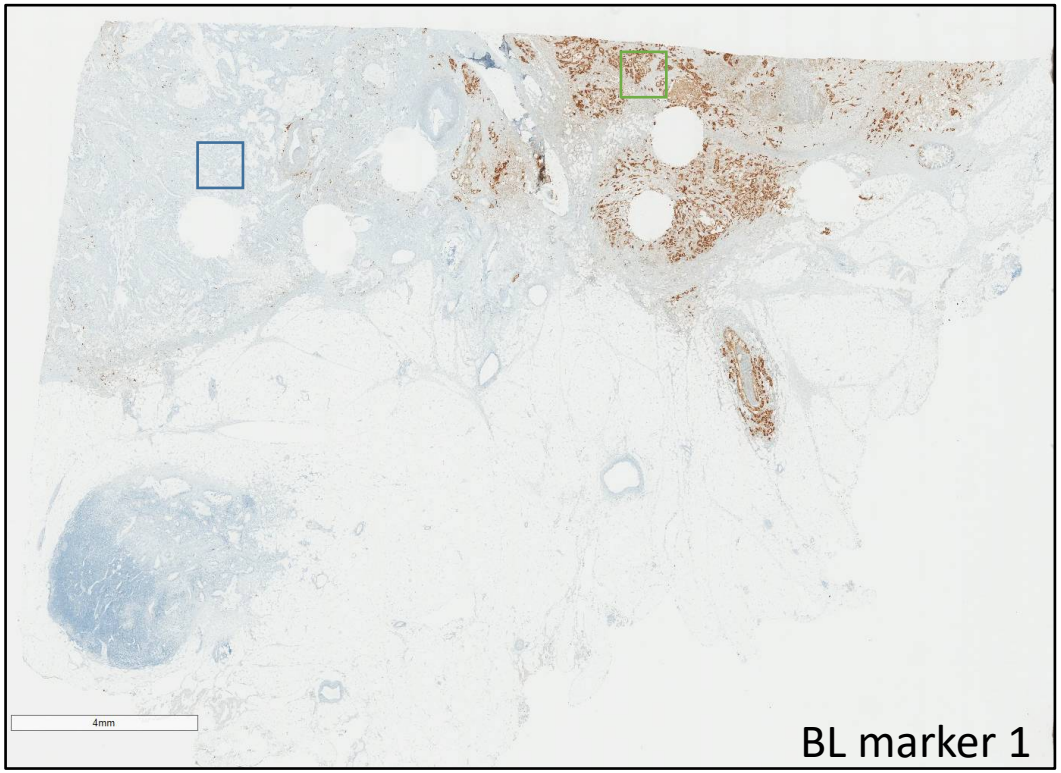


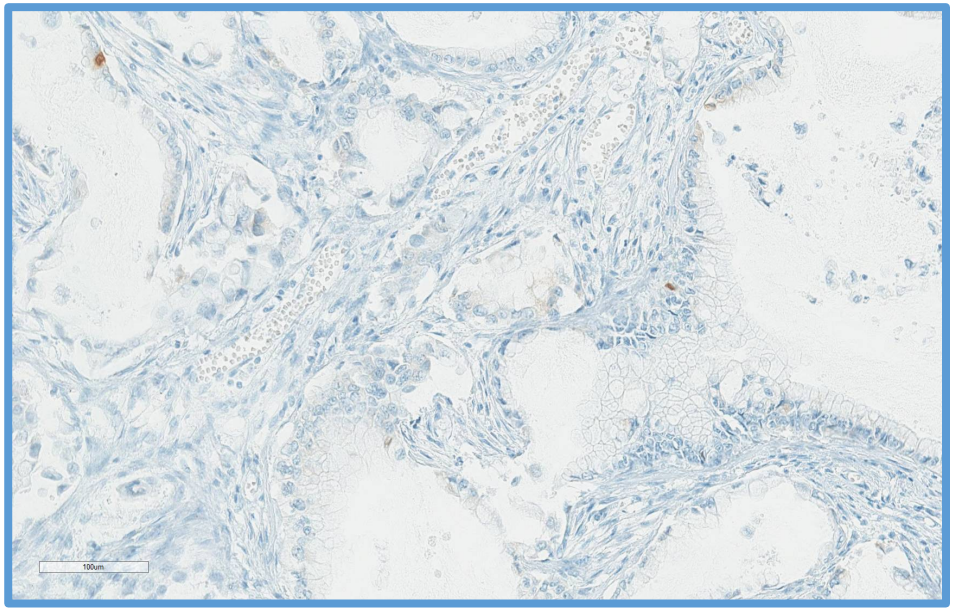
BL marker 1

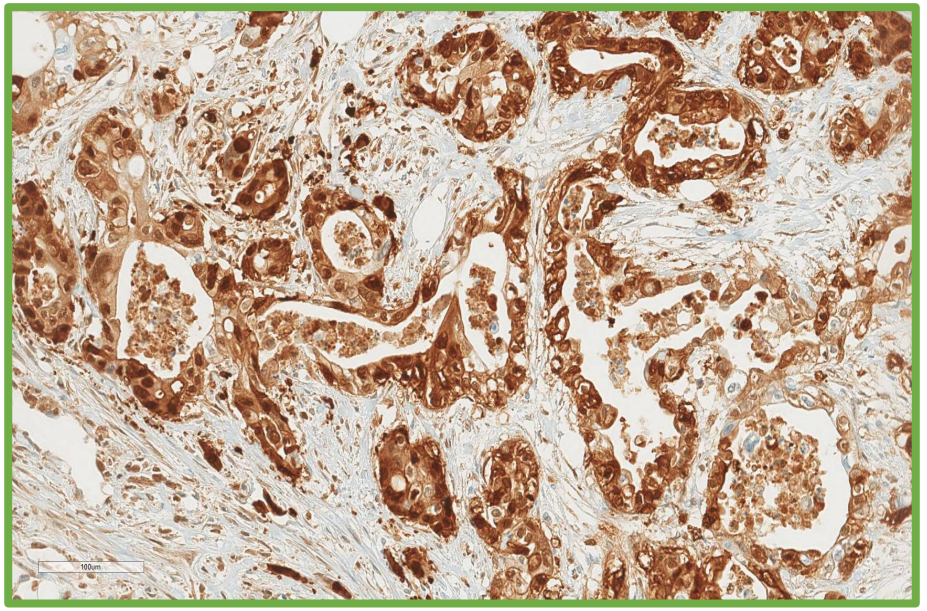
Class marker 1



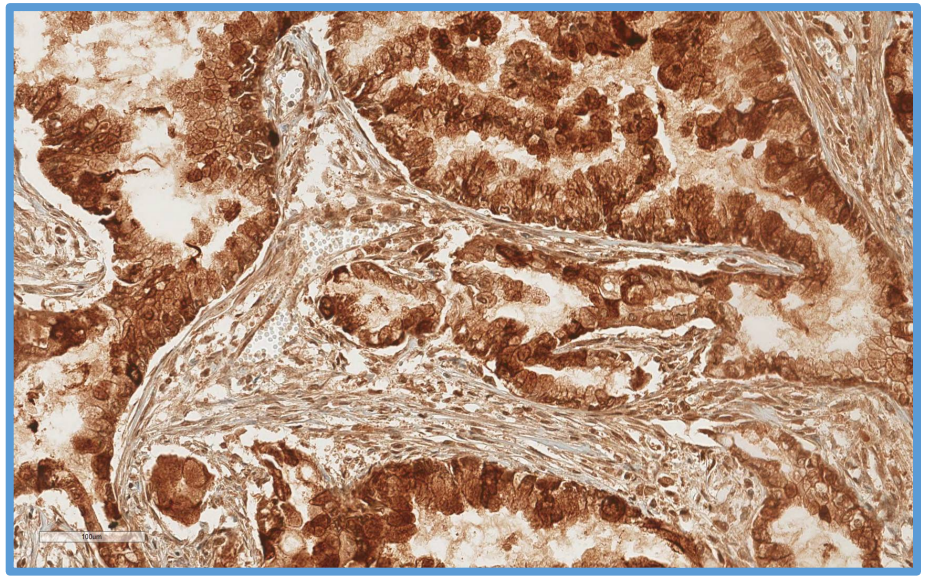
BL marker 2

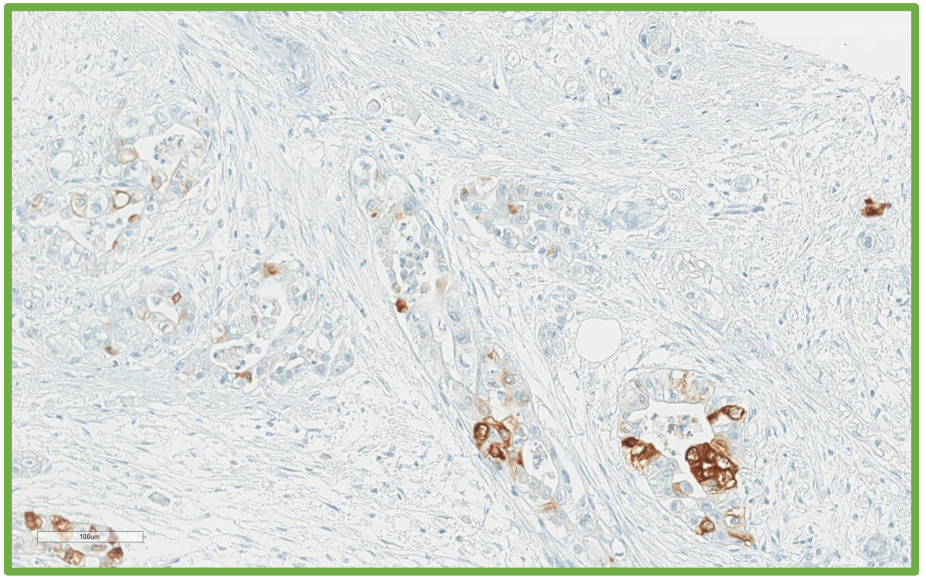

4mm
BL marker 1


100um

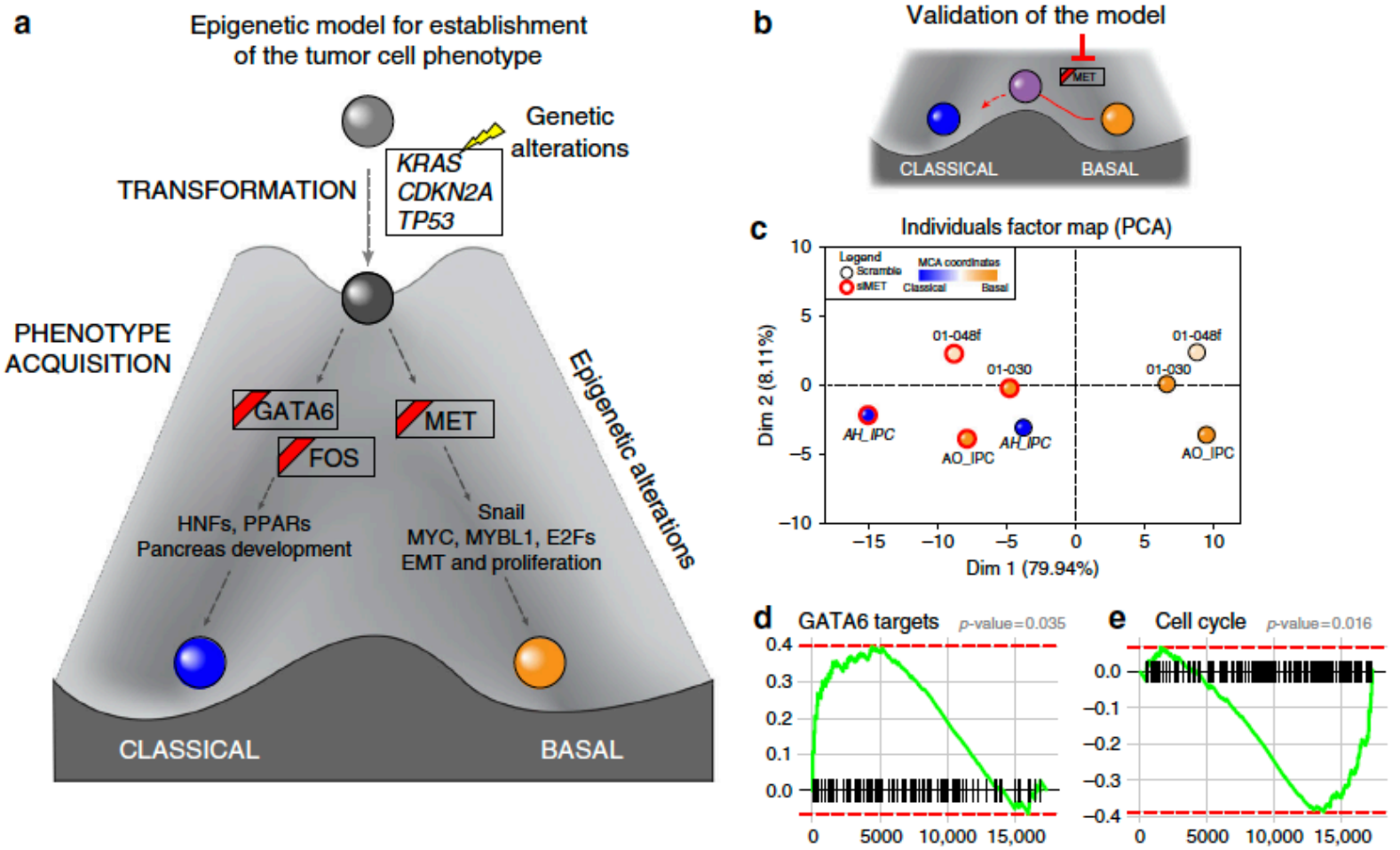

100um

Classical marker 1



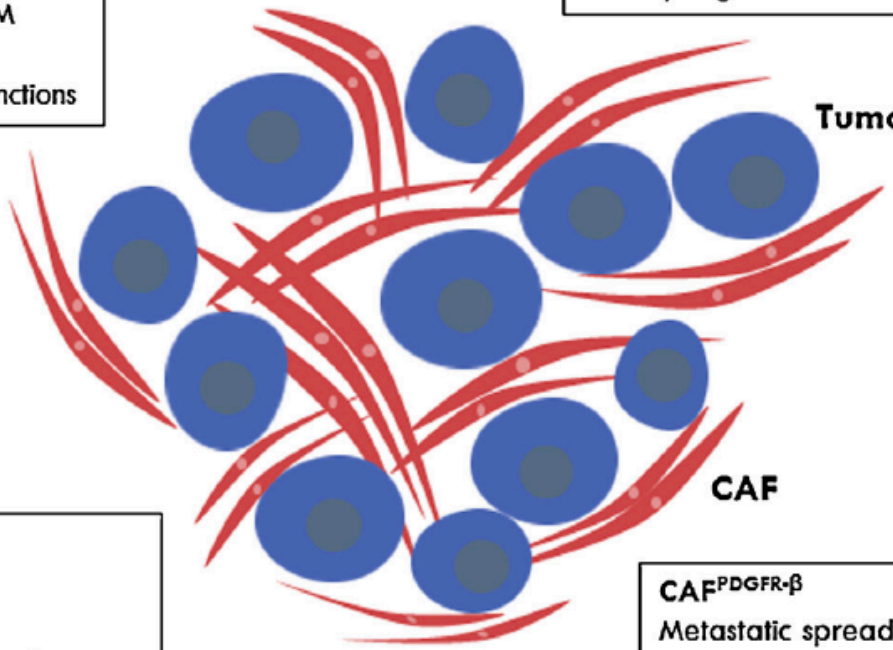


Is there an epigenetic-driven plasticity between subtypes?



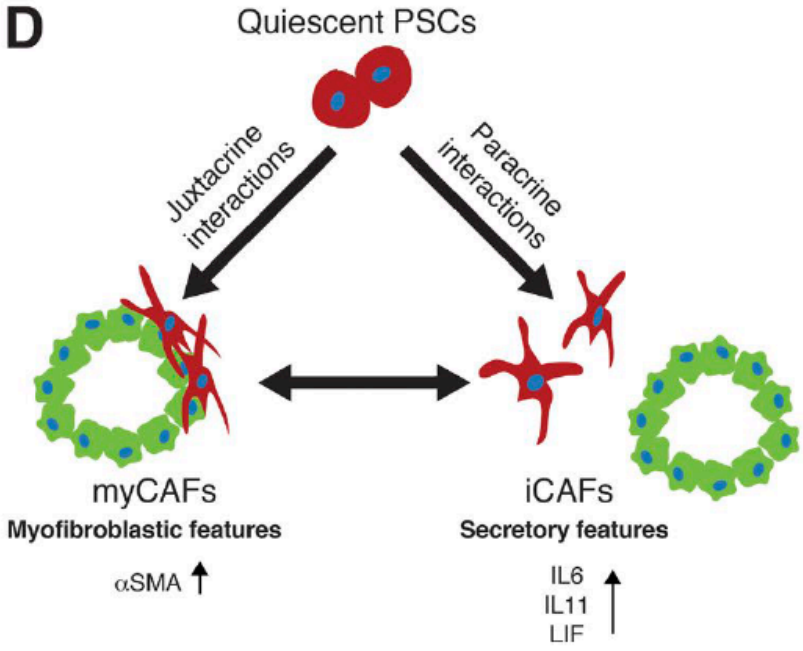
Lomberk *et al. Nat Com* 2018

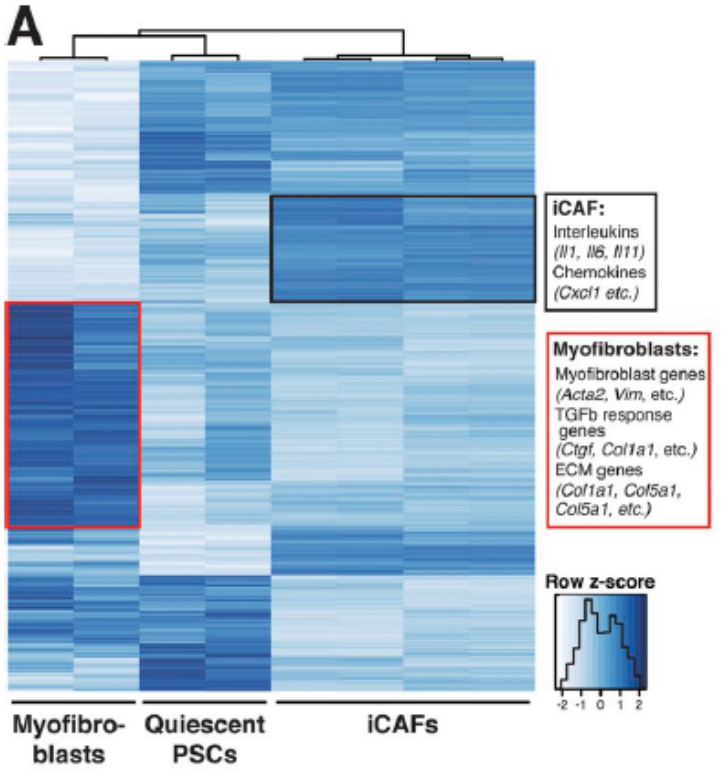
Intra-tumor heterogeneity - stroma

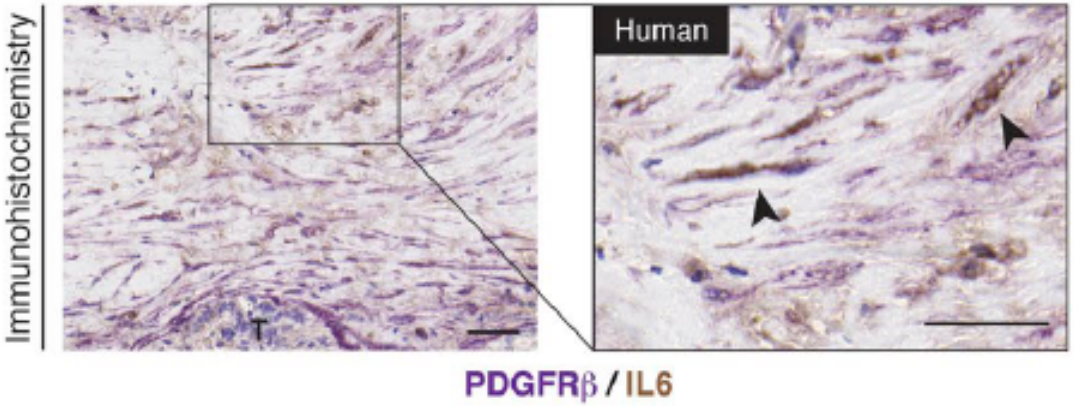


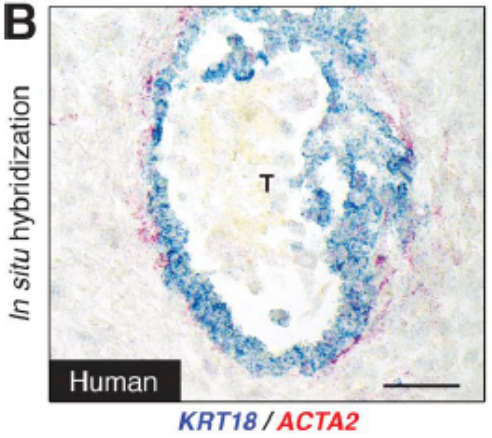
E. Cortez et al. / Seminars in Cancer Biology 25 (2014) 3–9

Intra-tumor heterogeneity - stroma



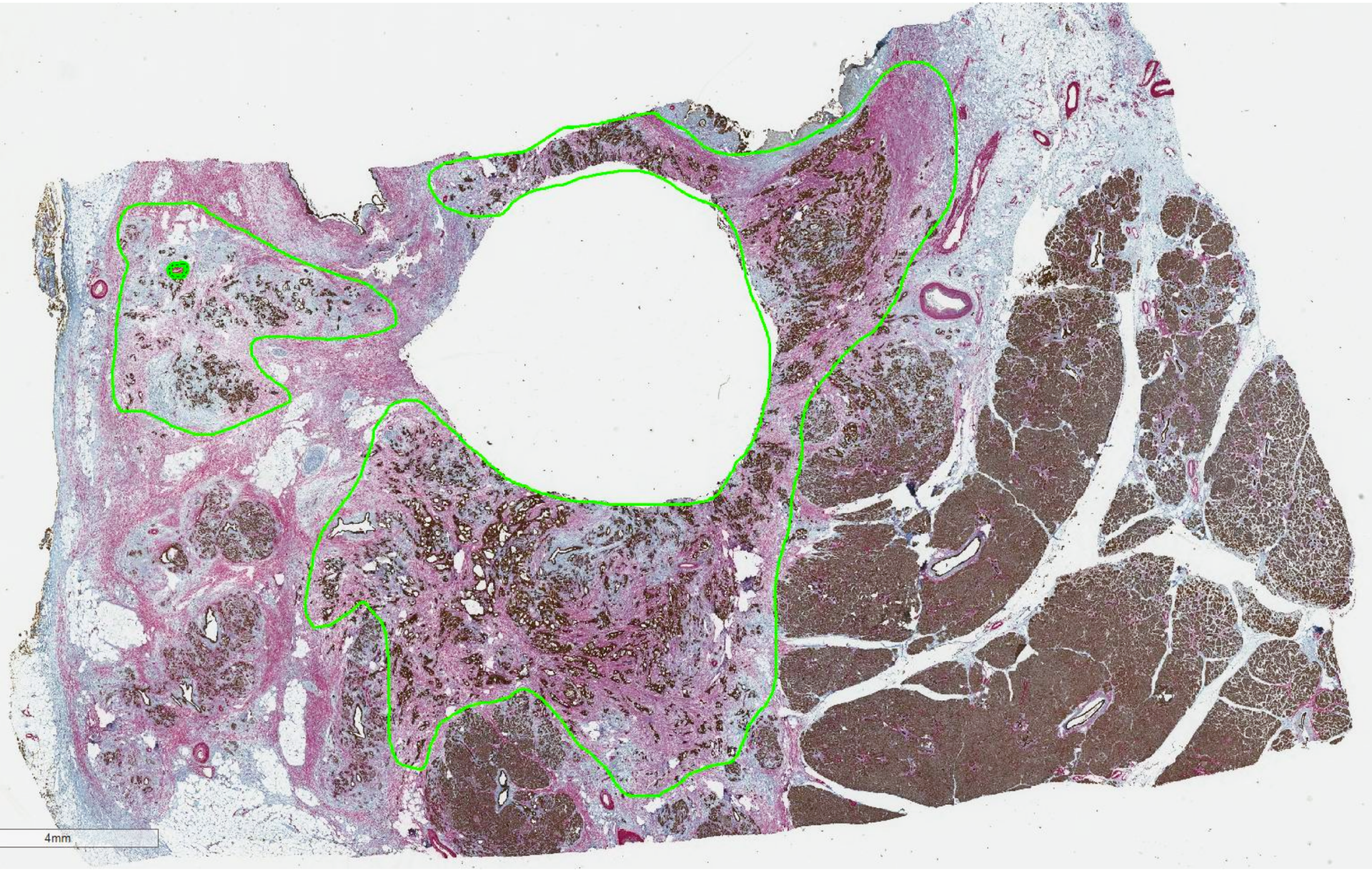




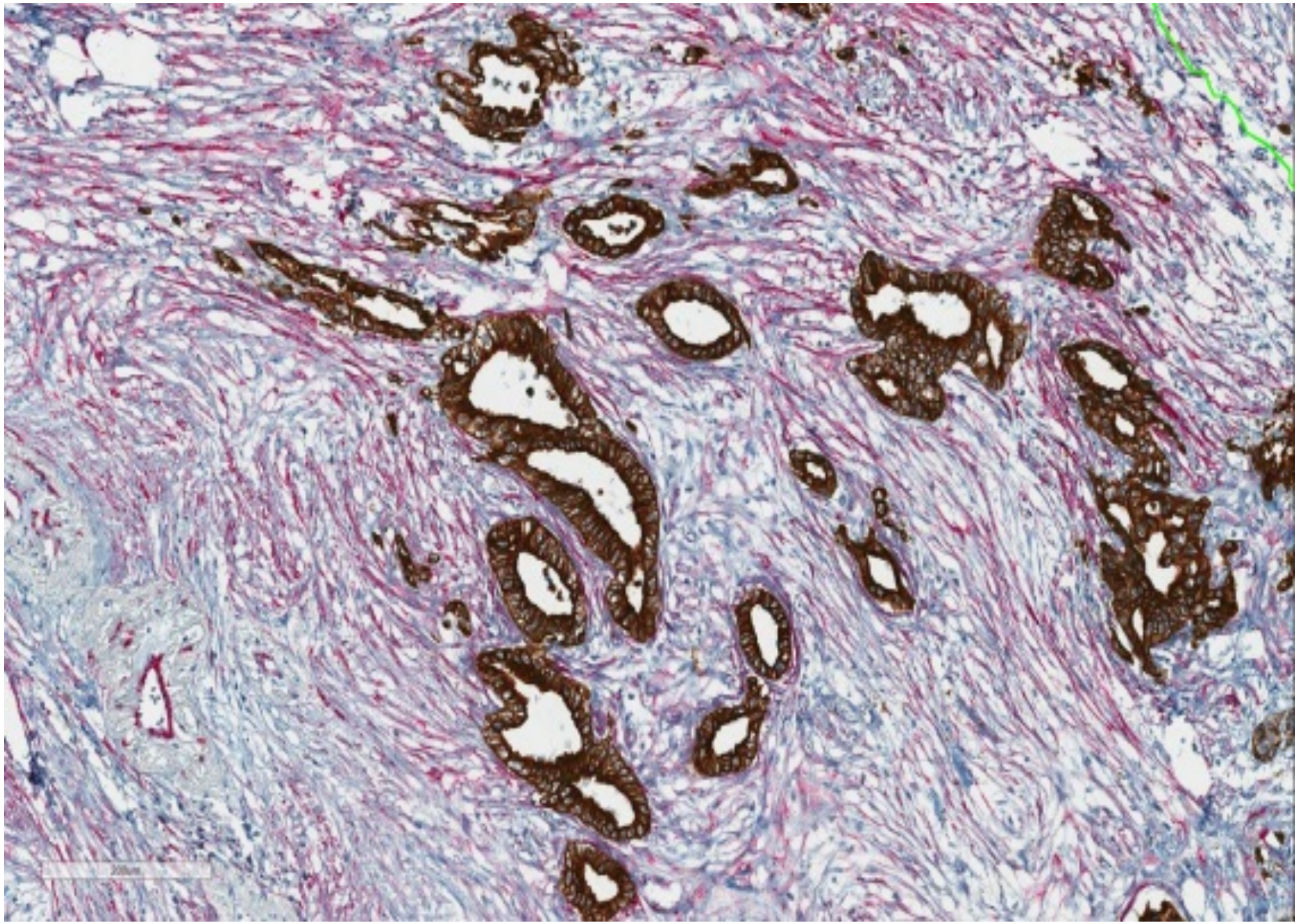


Ohlund *et al., J Exp Med* 2017

Intra-tumor heterogeneity - stroma

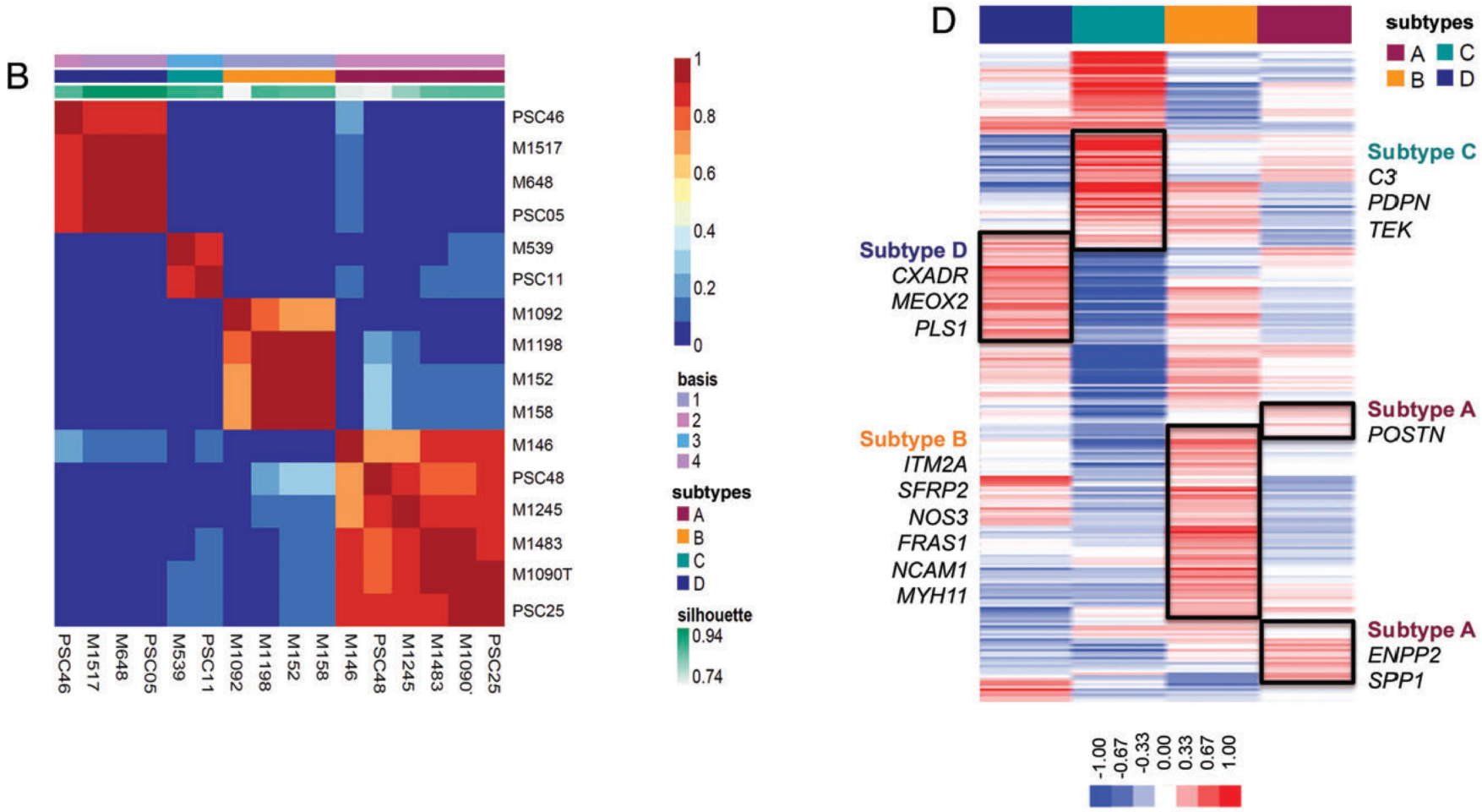


Intra-tumor heterogeneity - stroma



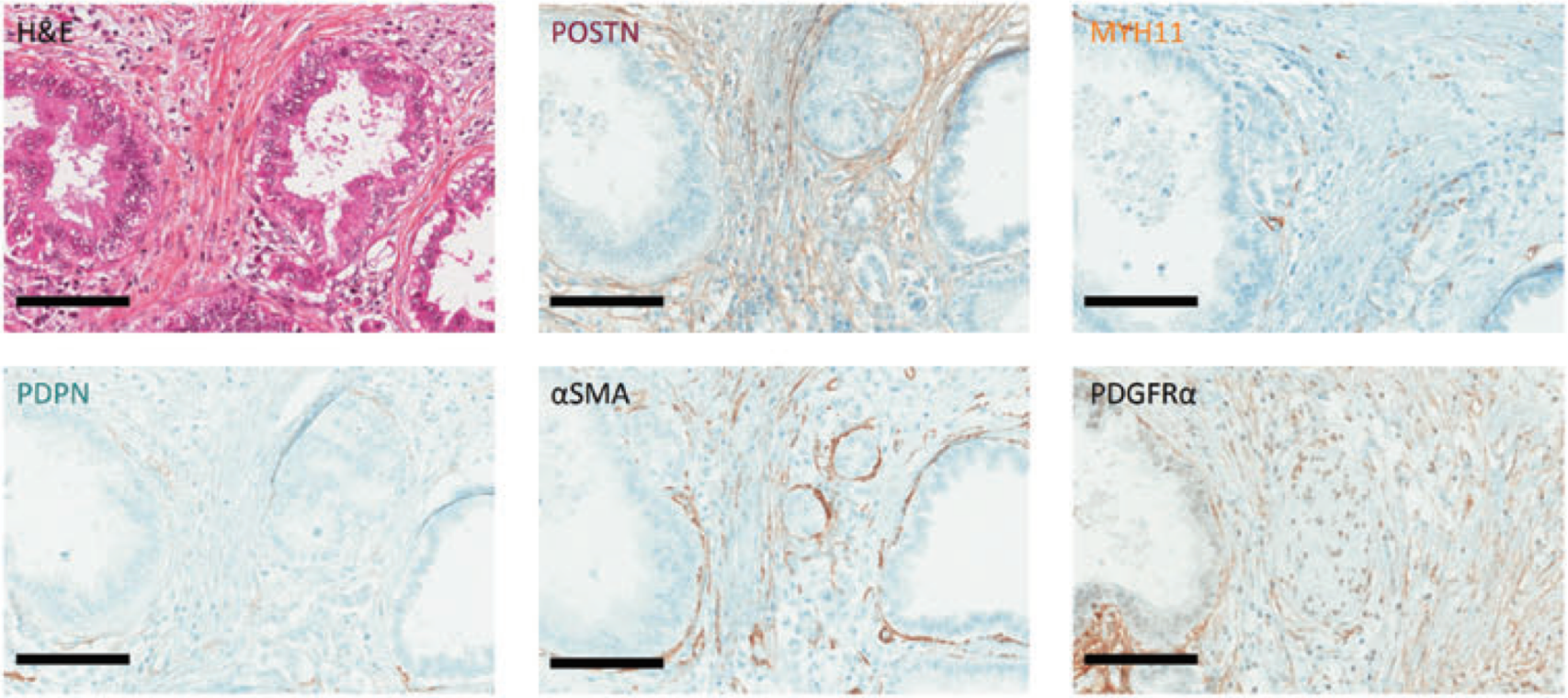
In humans, it may be a but more complicated….

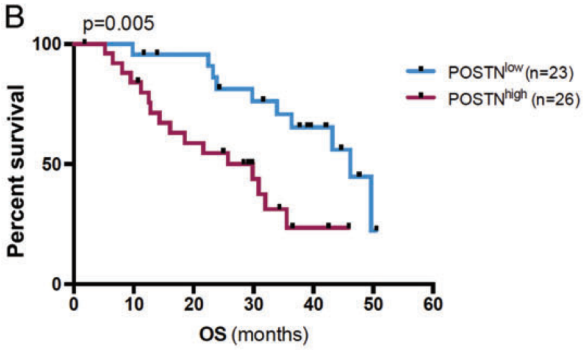
Patient-derived CAF



Neuzillet *et al. J Pathol* 2019

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





Neuzillet *et al. J Pathol* 2019

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

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