"Basic and translational oncology"
Italian-French Erasmus Intensive Course in Oncology organized in collaboration with
European Master of Genetics - University Paris7-Paris5

Translational research in breast cancer

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Outline

- Introduction to the clinical classification
- Molecular characterization and intertumor heterogeneity

  → Examples of clinical applications
- The challenge of intratumor heterogeneity
- The role of the tumor immune microenvironment
- Novel translational research tools and future perspectives

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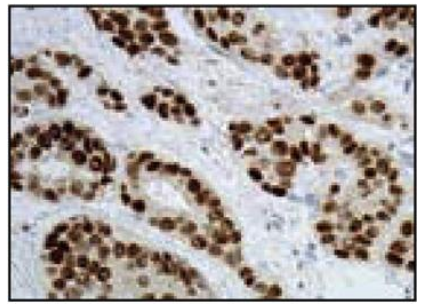
Clinical classification of breast cancer (BC) – **markers**

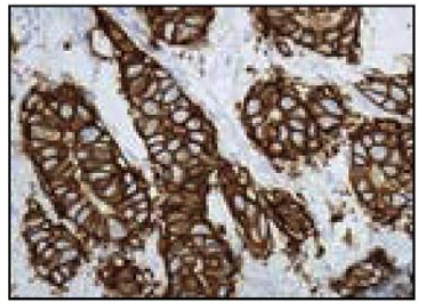
Biomarker	Method and threshold	Use	LOE
ER	IHC; positive if ≥1%	• Essential for the characterization of the IHC luminal group • Poor prognostic marker if negative • Predictive marker for endocrine treatment • Mandatory for endocrine treatment prescription	I
PR	IHC; positive if ≥1%	• If negative, tumour classified as IHC luminal B • Strong poor prognostic marker if negative • Predictive marker for endocrine treatment	I
HER2	• IHC; positive if >10% complete membrane staining (3+) • Single-probe ISH; positive if HER2 ≥6 copies • Dual-probe ISH; positive if HER2 and CEP17 ≥2 and HER2 ≥4 copies, or HER2 and CEP17 <2 and HER2 ≥6 copies	• Essential to characterize HER2-enriched (ER-negative) disease and luminal B, HER2-positive • Prognostic marker • Predictive marker for anti-HER2 treatment • Mandatory for anti-HER2 therapy	I (IHC) and I (ISH)
Ki67	IHC; no final consensus on cut-off value but values <10% are considered low and >30% are considered high[a]	Absence of international consensus for scoring and threshold	I
		Prognostic value in ER-positive, HER2-negative tumours (primary tumours and post-neoadjuvant tumour residues)	I
		Absence of prognostic value in HER2-positive disease or TNBC	I
		Predictive of response to neoadjuvant endocrine therapy[a]	I
		Predictive of response to neoadjuvant chemotherapy	Expert opinion
		If elevated, chemotherapy is often prescribed in ER-positive, HER2-negative tumours	Expert opinion
		Part of the IHC definition of luminal tumours whereby when Ki67 is low, luminal A tumour likely and when Ki67 high, luminal B tumour likely	Expert opinion

ER = estrogen receptor; PR = progesteron receptor; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; ISH = *in situ* hybridization; LOE = level of evidence.

A



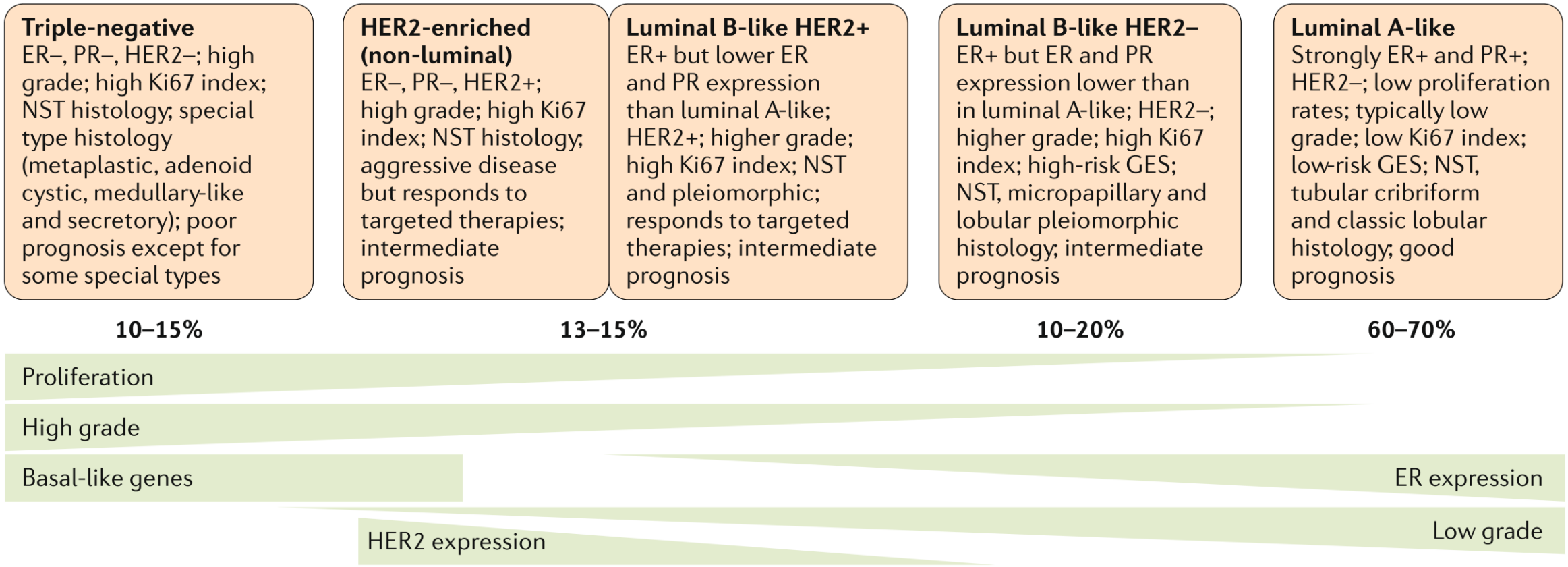
B



Expression of (**A**) ER and (**B**) HER2 assessed by **IHC**

Rivenbark AG, et al. Am J Pathol 2013
Harbeck N, et al. Nat Rev Dis Primers 2019

Clinical classification – ***subtypes*** based on immunohistochemistry



Harbeck N, et al. Nat Rev Dis Primers 2019

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Gene expression-based "intrinsic" subtypes – *PAM50*

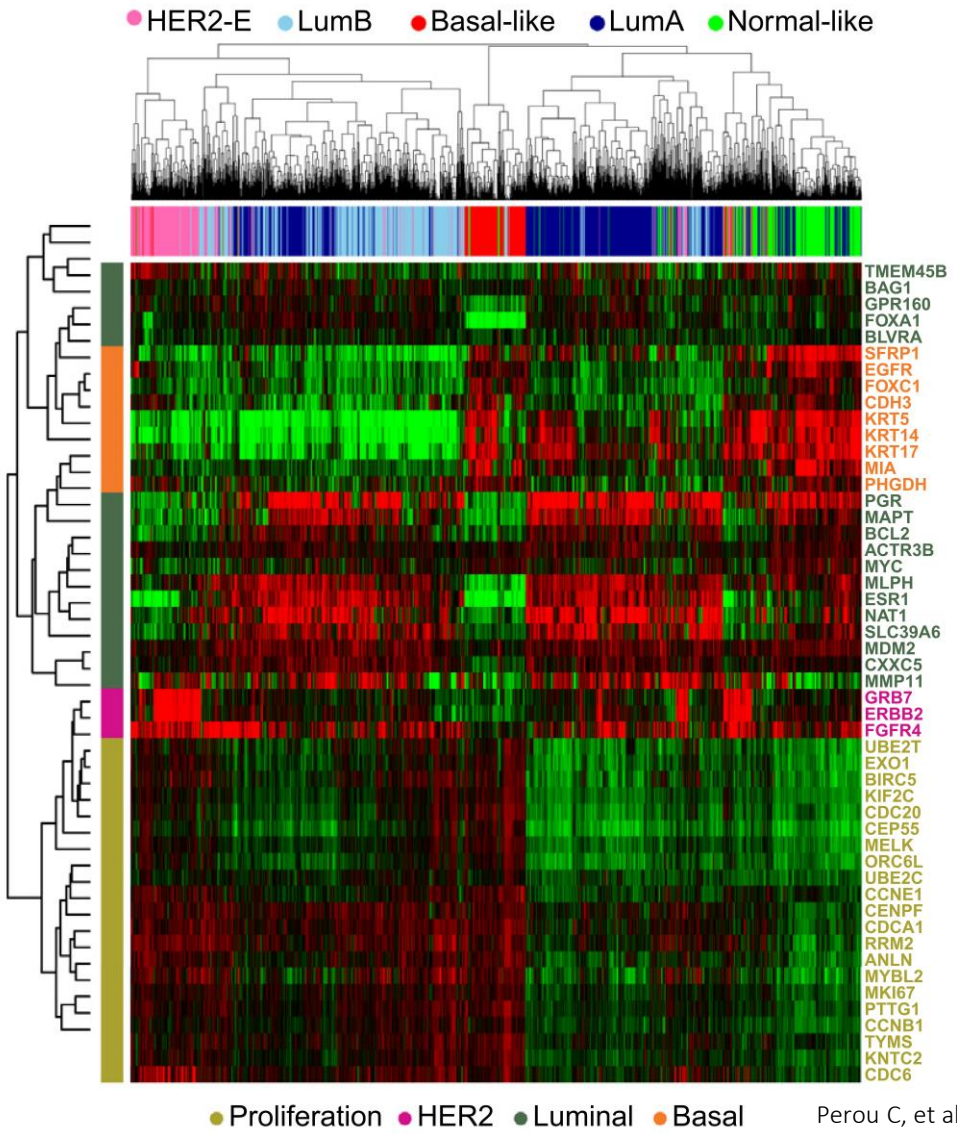
- 2000: **Gene expression studies** initially identified 4 "intrinsic" subtypes

- Subsequent studies **refined** the classification and demonstrated **prognostic significance**

- Prediction Analysis of Microarray (**PAM**) 50 subtypes (based on **50 genes**):

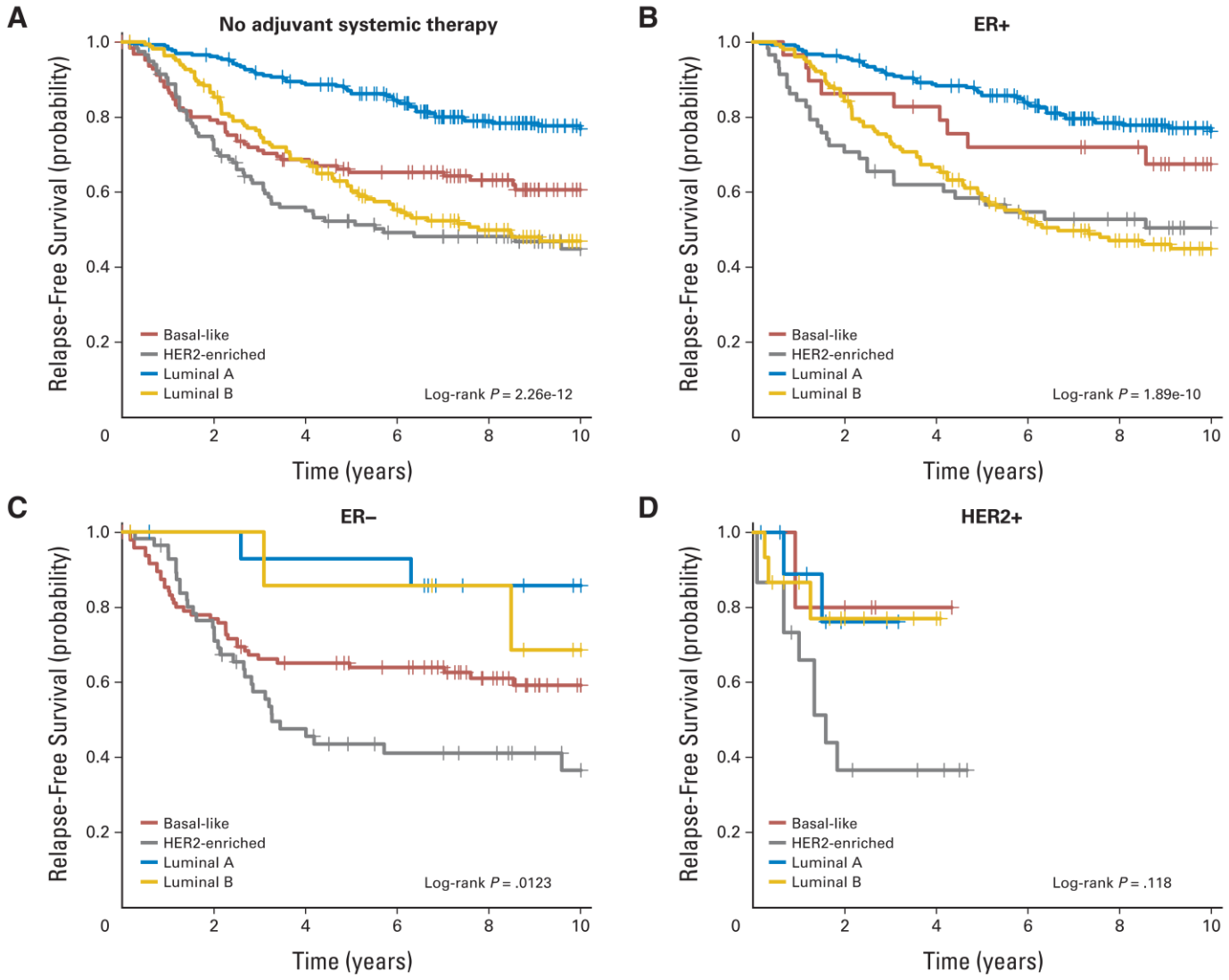
- **Luminal A**
- **Luminal B**
- **HER2-enriched**
- **Basal-like**
- **Normal-like** (may represent non-cancer cells "contaminating" bulk tissue samples)

→ **Differences in biological processes**



Perou C, et al. Nature 2000
Parker JS, et al. J Clin Oncol 2009
Prat A, et al. Breast 2015

PAM50 subtypes and ***prognosis***



Parker JS, et al. J Clin Oncol 2009

PAM50 *vs* "clinical" classification

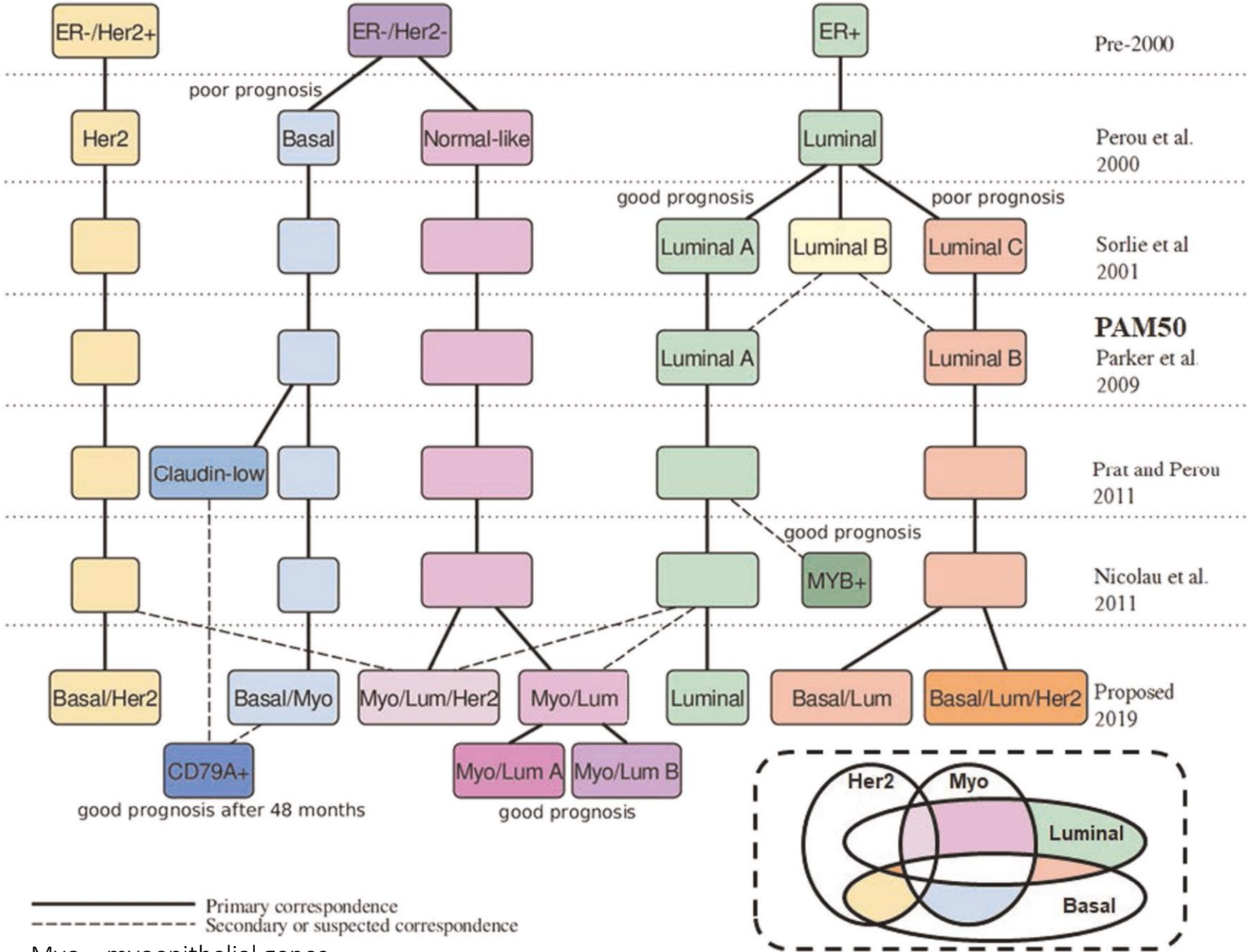
Distribution of the PAM50 intrinsic subtypes within the pathology-based groups.[a]

IHC-based group	References	N	PAM50 intrinsic subtype distribution			
			Luminal A	Luminal B	HER2-enriched	Basal-like
HR+/HER2−	[10,14,16–22]	4295	60.3%	31.9%	6.6%	1.2%
Luminal A	[10,14,17,21]	637	62.2%	27.0%	10.2%	0.6%
Luminal B	[10,14,17,21]	317	34.1%	51.1%	11.0%	3.8%
HER2+	[6,23–26]	831	17.6%	26.8%	44.6%	11.0%
HER2+/HR+	[25,26]	182	33.0%	46.2%	18.7%	2.2%
HER2+/HR−	[25,26]	168	19.0%	4.2%	66.1%	10.7%
TNBC	[12–15]	868	1.6%	3.2%	9.1%	86.1%

[a] The data has been obtained from the different publications. Several studies have performed a standardized version of the PAM50 assay (RT-qPCR-based or nCounter-based) from formalin-fixed paraffin-embedded tumour tissues [10,14,17,19–22], while others have performed the microarray-based version of the PAM50 assay [6,16,18,23–26].

- Combined the data from studies for a total of 5994 independent samples
- **Overall discordance** of **~30%**
- The **two methods** to identify intrinsic biology should **not be considered the same**
- 3 or 4 biomarkers do not fully recapitulate the intrinsic subtypes of breast cancer

Prat A, et al. Breast 2015



Myo = myoepithelial genes

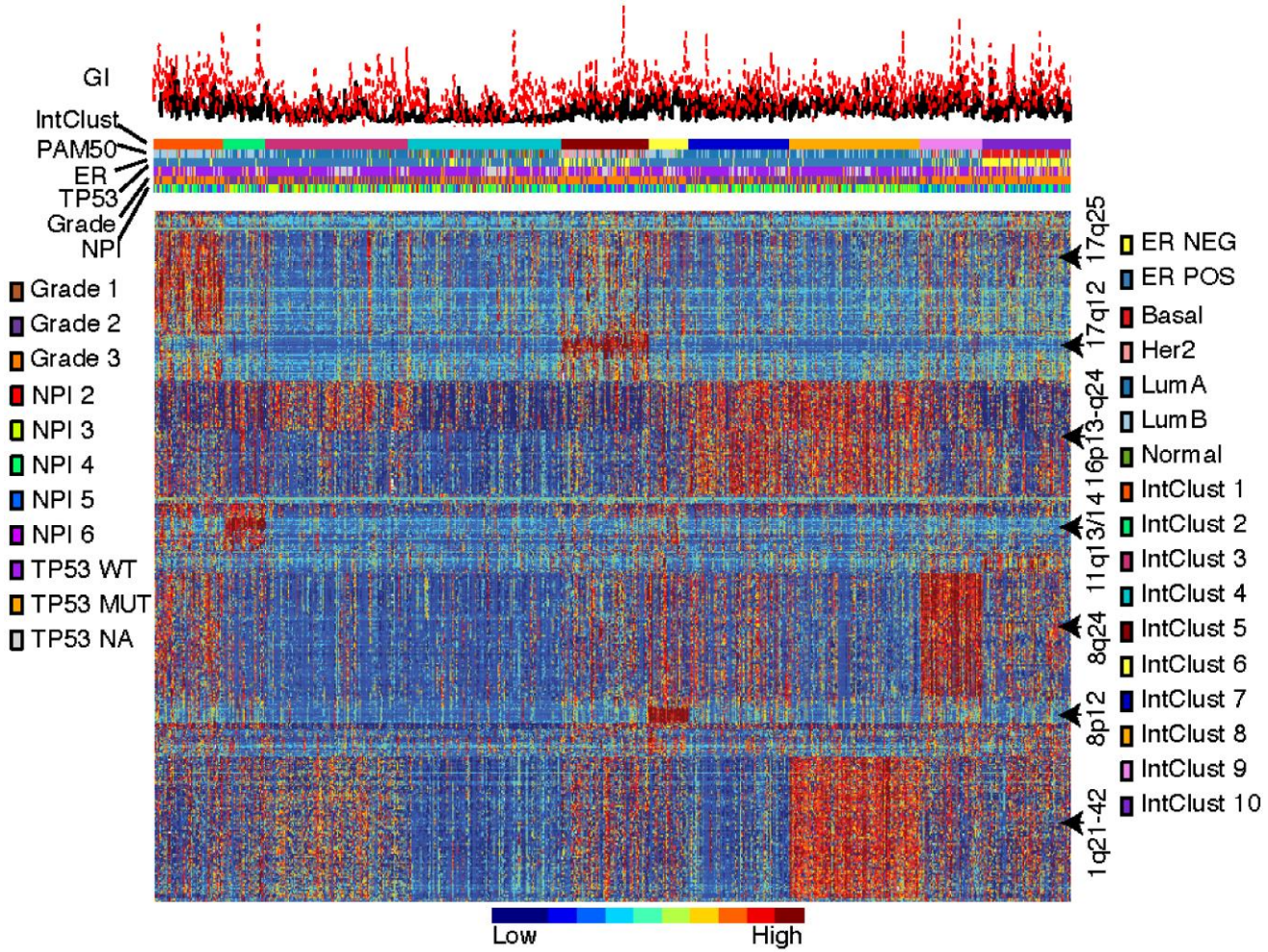
Mathews JC, et al. NPJ Breast Cancer 2019

The METABRIC* study

Somatically acquired copy number aberrations (**CNAs**) are the **dominant feature** of BC

- Collection of ~2000 primary BC samples
- **None** of the HER2-positive patients **received trastuzumab** (!)
- **Integrated analysis** of ***copy number*** and ***gene expression***

→ CNAs influencing gene expression in *cis* likely to be enriched in driver genes



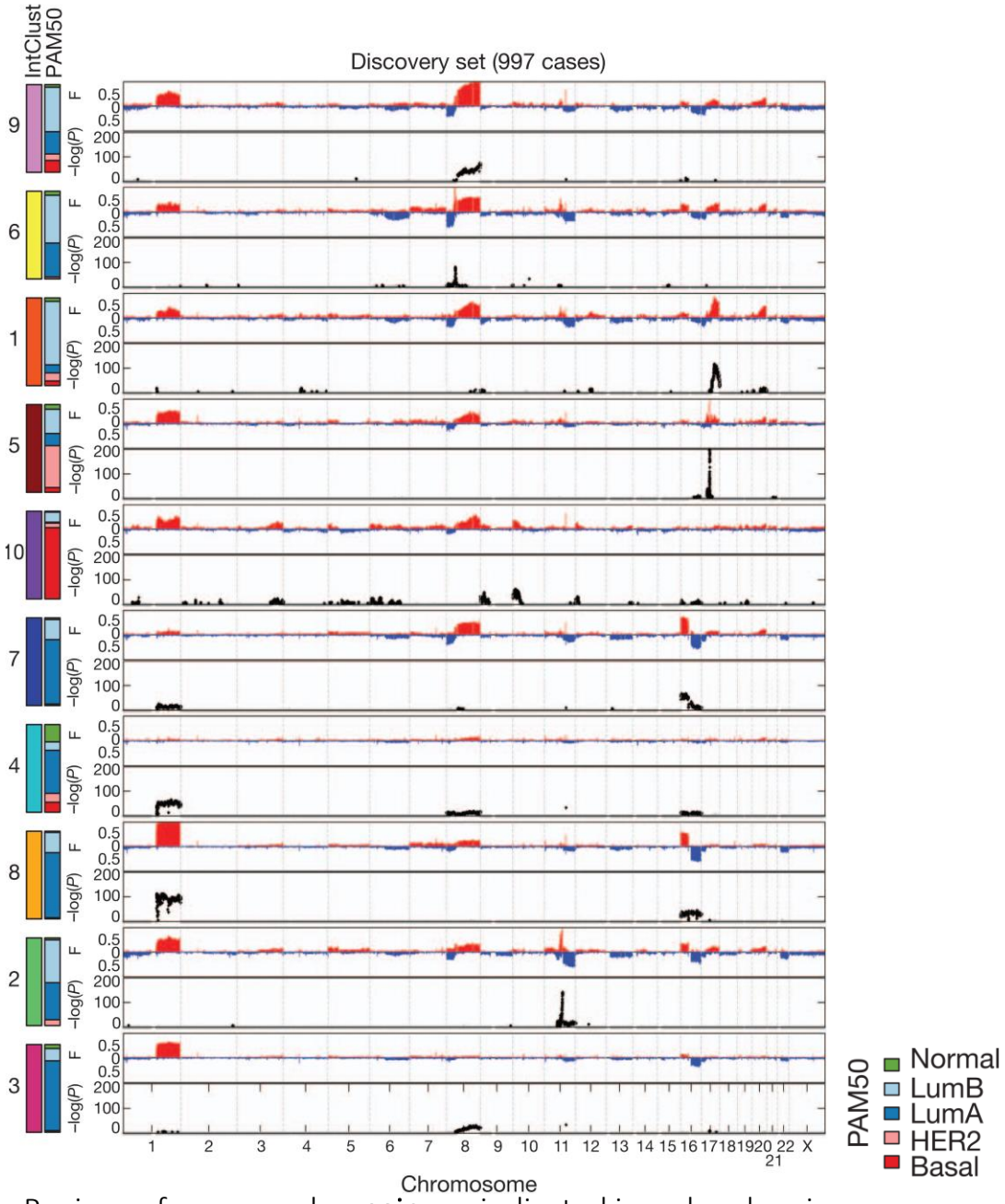
Unsupervised analysis of paired DNA–RNA profiles revealed **10 novel subgroups (Integrative Clusters - IntClusts)**

GI = genomic instability; NPI = Nottingham prognostic index

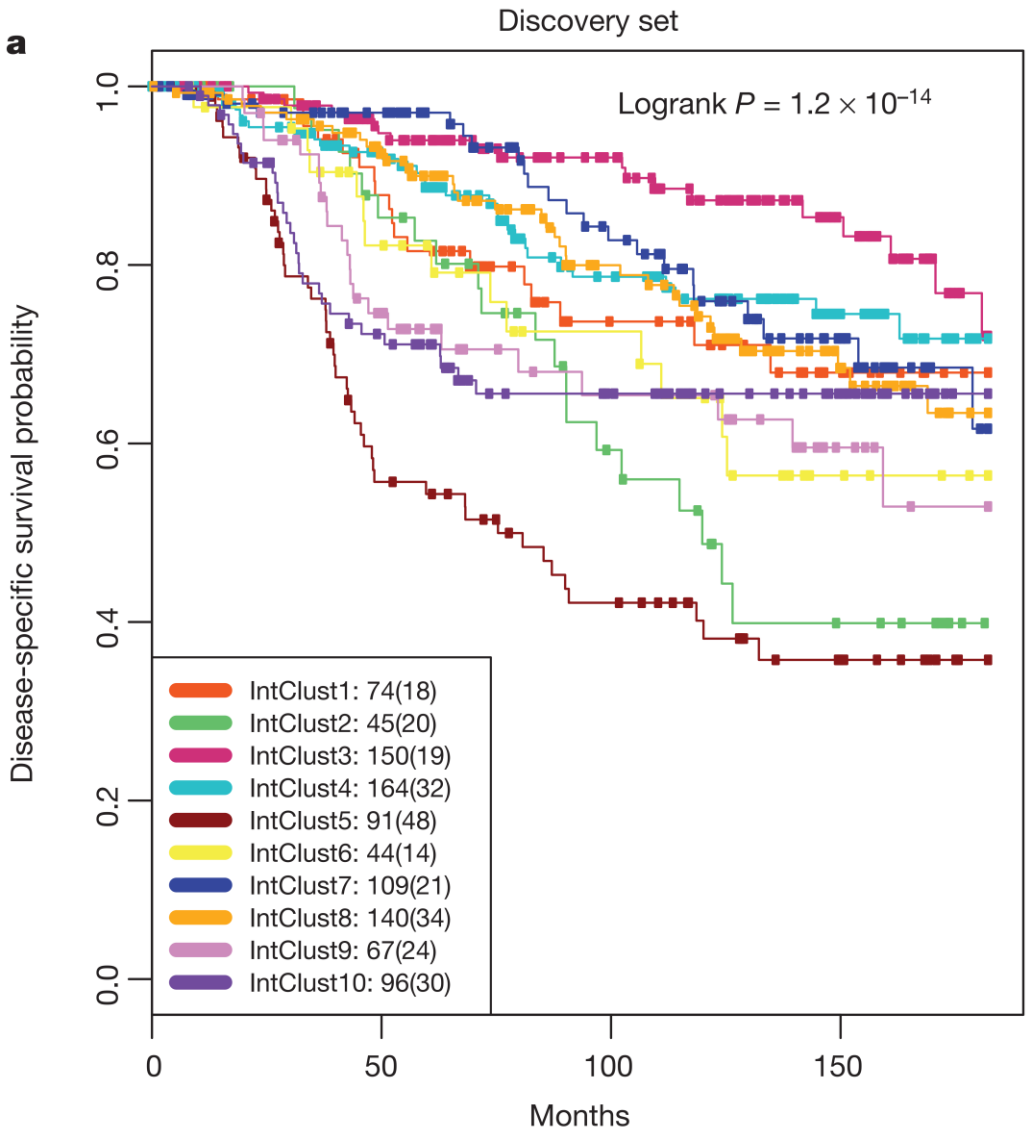
* Molecular Taxonomy of Breast Cancer International Consortium

Cis = Variant at a locus has an impact on its own expression ≠ *trans* when it is associated with genes in other sites in the genome

Curtis C, et al. Nature 2012

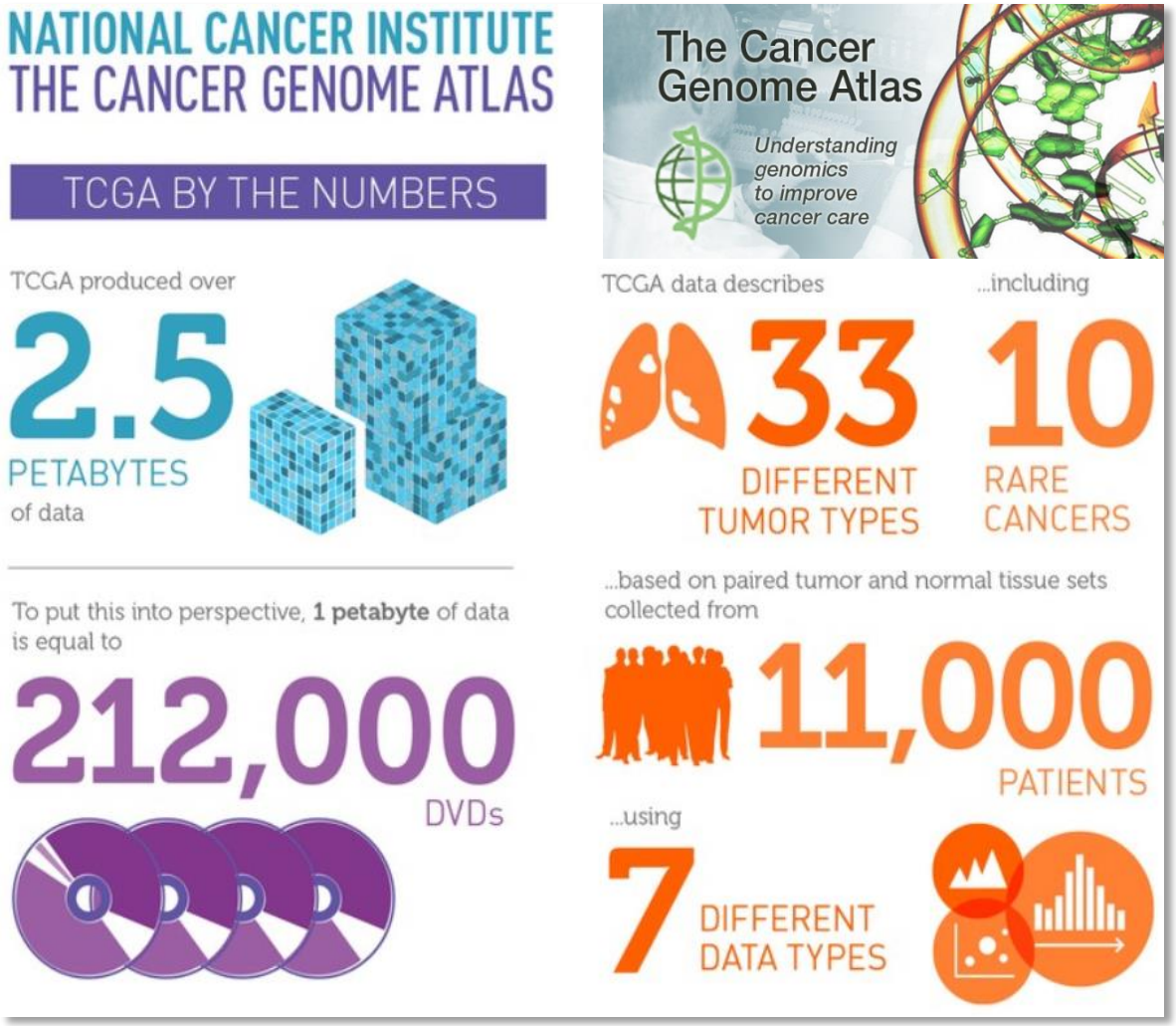


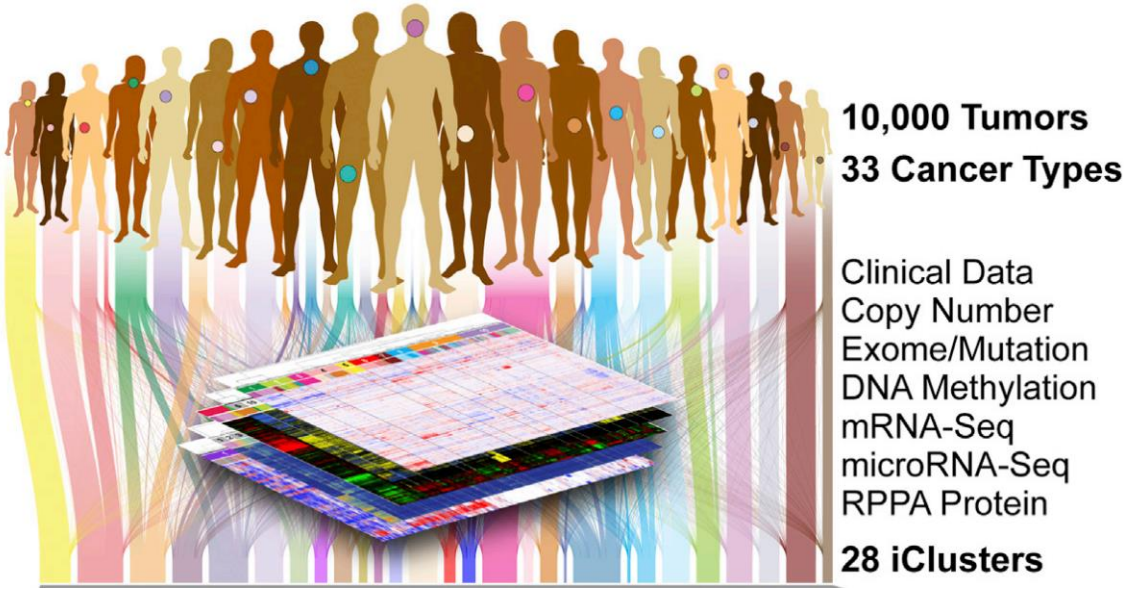
Regions of copy number **gain** are indicated in red and regions of **loss** in blue in the frequency plot



Curtis C, et al. Nature 2012

The Cancer Genome Atlas (TCGA)

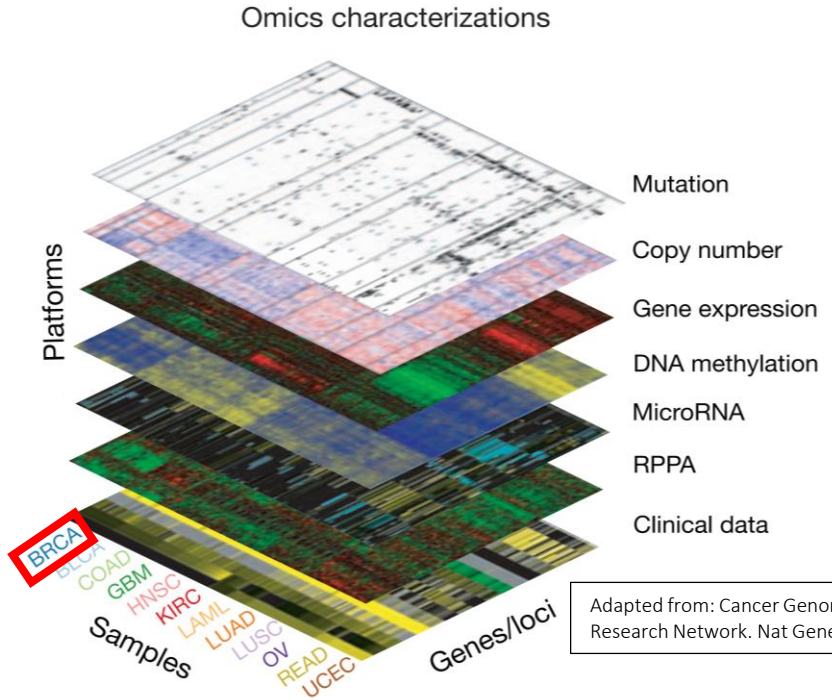




Adapted from: Hoadley KA, et al. Cell 2019

The Cancer Genome Atlas

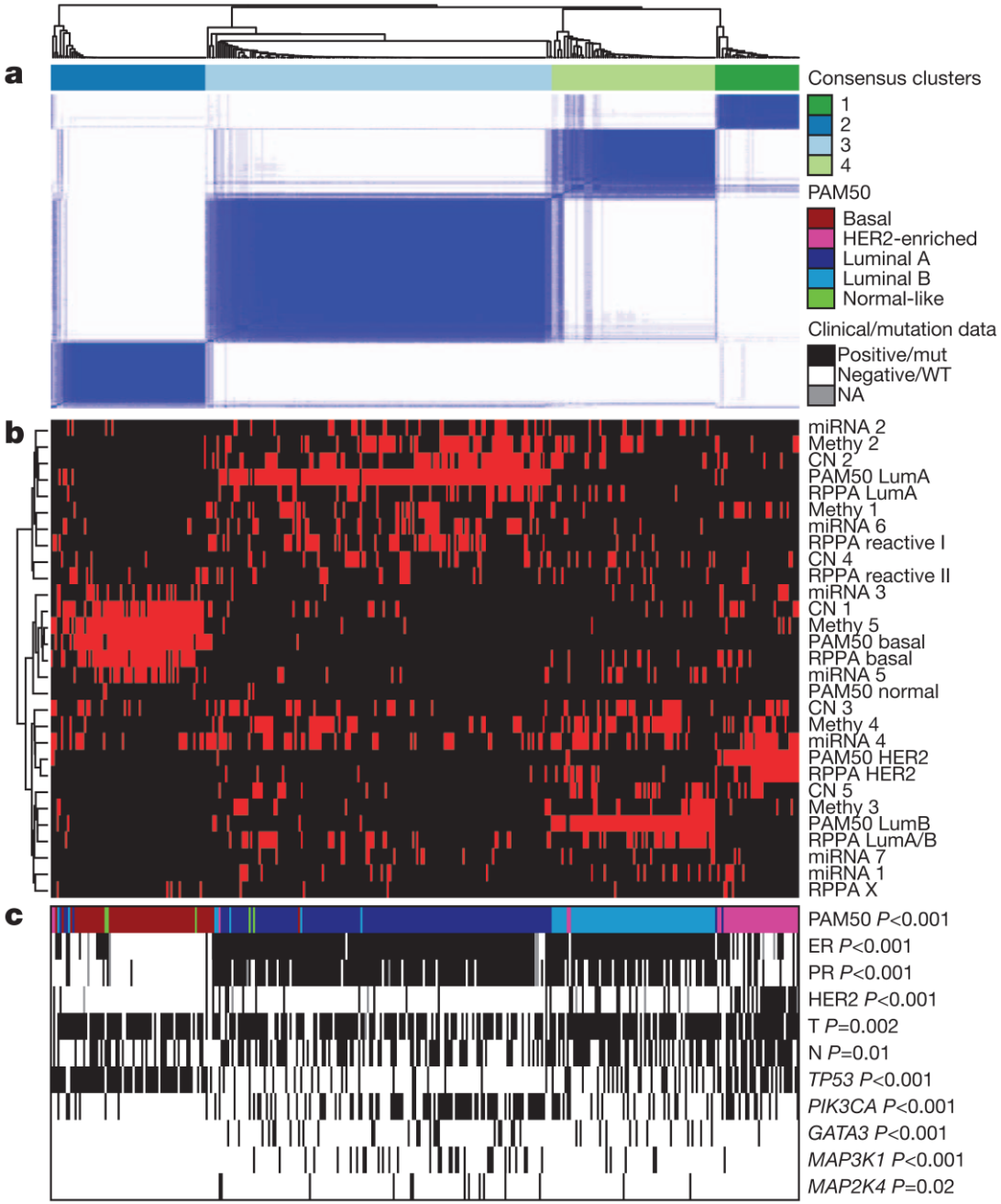
- Primary breast cancers from 825 patients



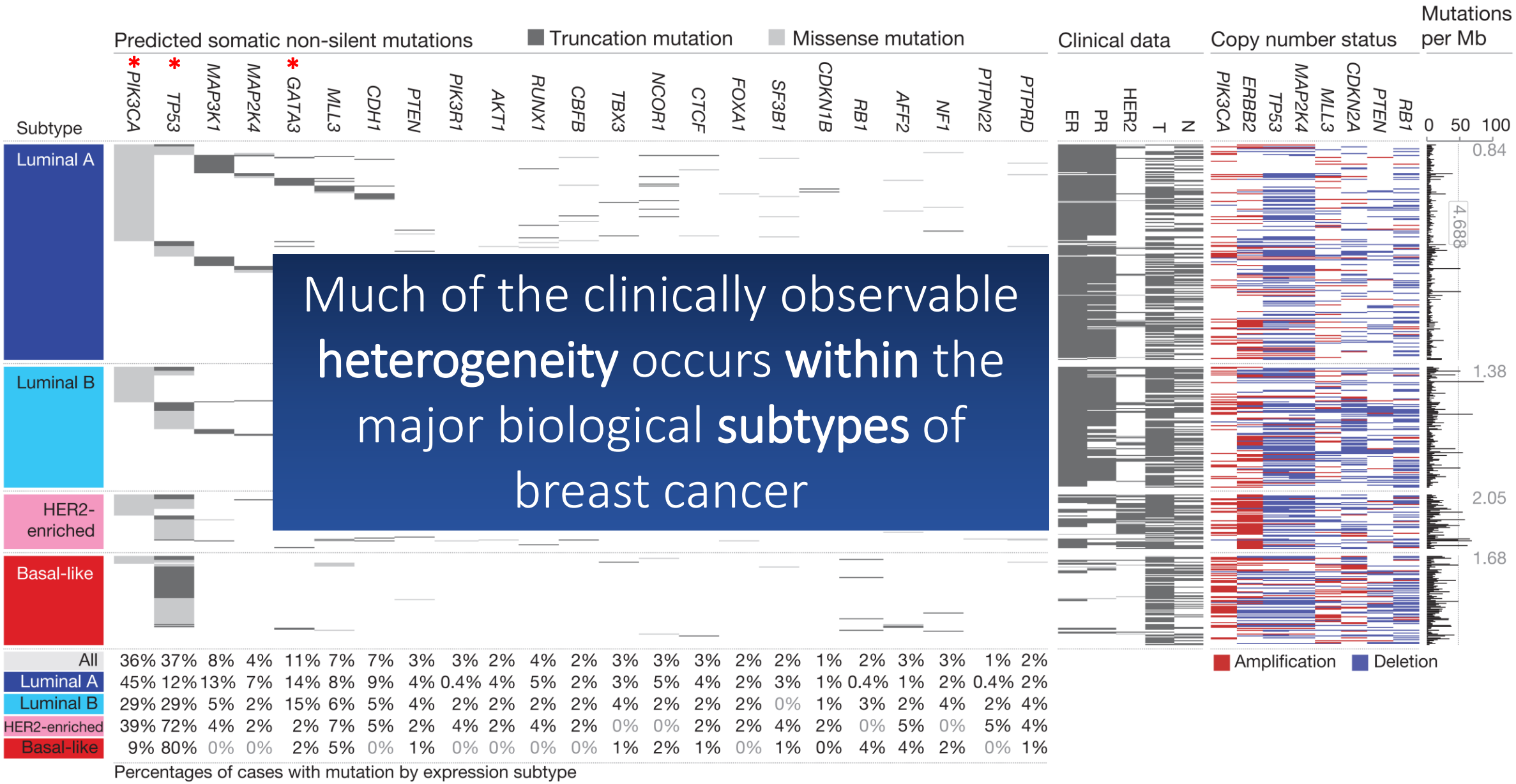
Adapted from: Cancer Genome Atlas Research Network. Nat Genet 2013

- **Unsupervised clustering** on data from five molecular platforms (N=348, not including WES) and ***integration*** of results

→ 4 main BC consensus clusters



Cancer Genome Atlas Research Network. Nature 2012



Much of the clinically observable **heterogeneity** occurs **within** the major biological **subtypes** of breast cancer

* >10% incidence across all BCs

Cancer Genome Atlas Research Network. Nature 2012

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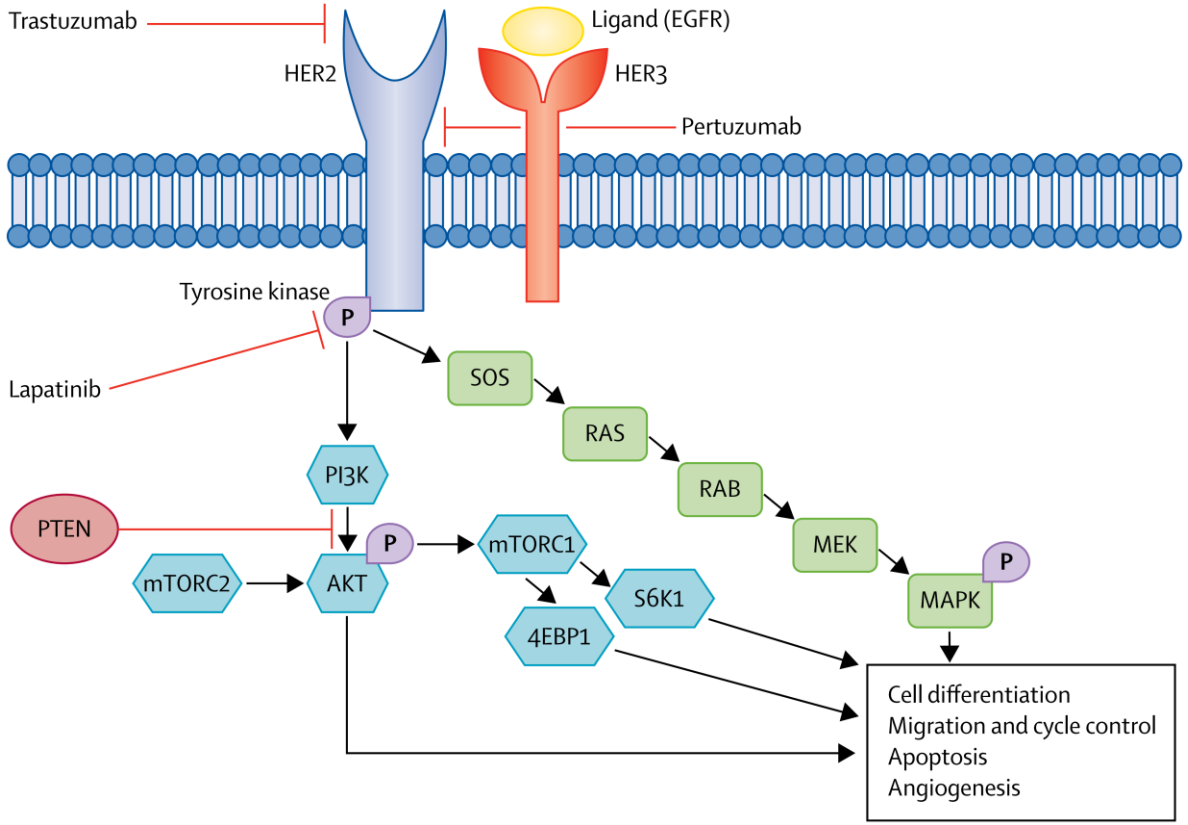
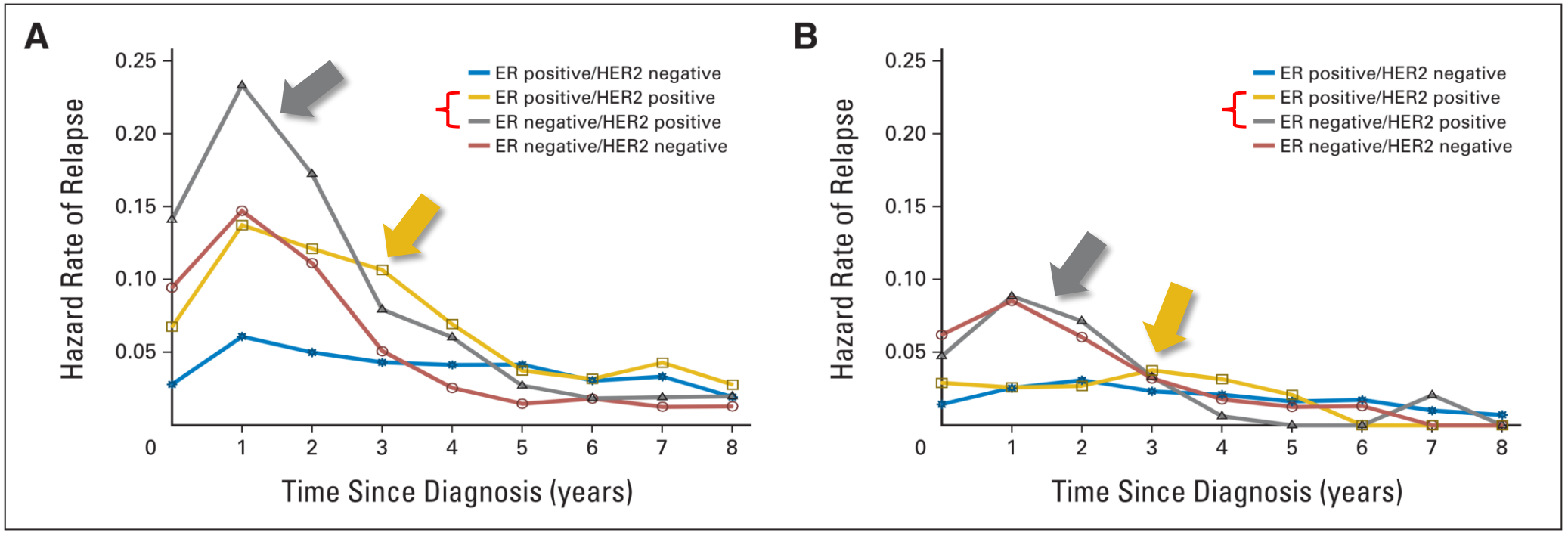
HER2-positive breast cancer

- 1980s: **discovery** of the oncogene Human Epidermal growth factor Receptor 2 (HER2)
- 1987: **amplification** of HER2 in BC is associated with *poor prognosis*
- Development of the anti-HER2 monoclonal antibody **trastuzumab**
- 1998: FDA approved trastuzumab for **metastatic** HER2+ BC
- 2005: Results of **adjuvant** trastuzumab trials
- 2013: FDA approved trastuzumab + pertuzumab + docetaxel as **neoadjuvant** treatment



Slamon DJ, et al. Science, 1987
Loibl S & Gianni L. Lancet 2017

Prognosis ***before*** and ***after*** trastuzumab



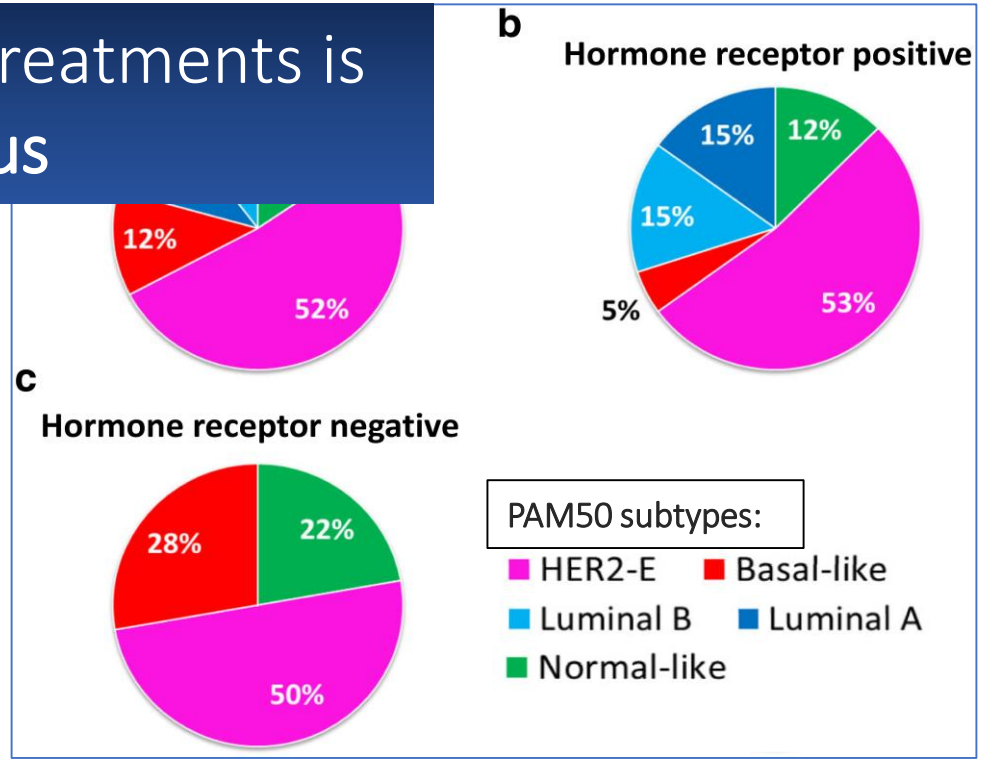
Hazard rate of relapse according to tumor subtype in **(A)** cohort 1 (1986-1992) and **(B)** cohort 2 (2004-2008)

Cossetti RJD, et al. J Clin Oncol 2015

HER2+ BC *heterogeneity*

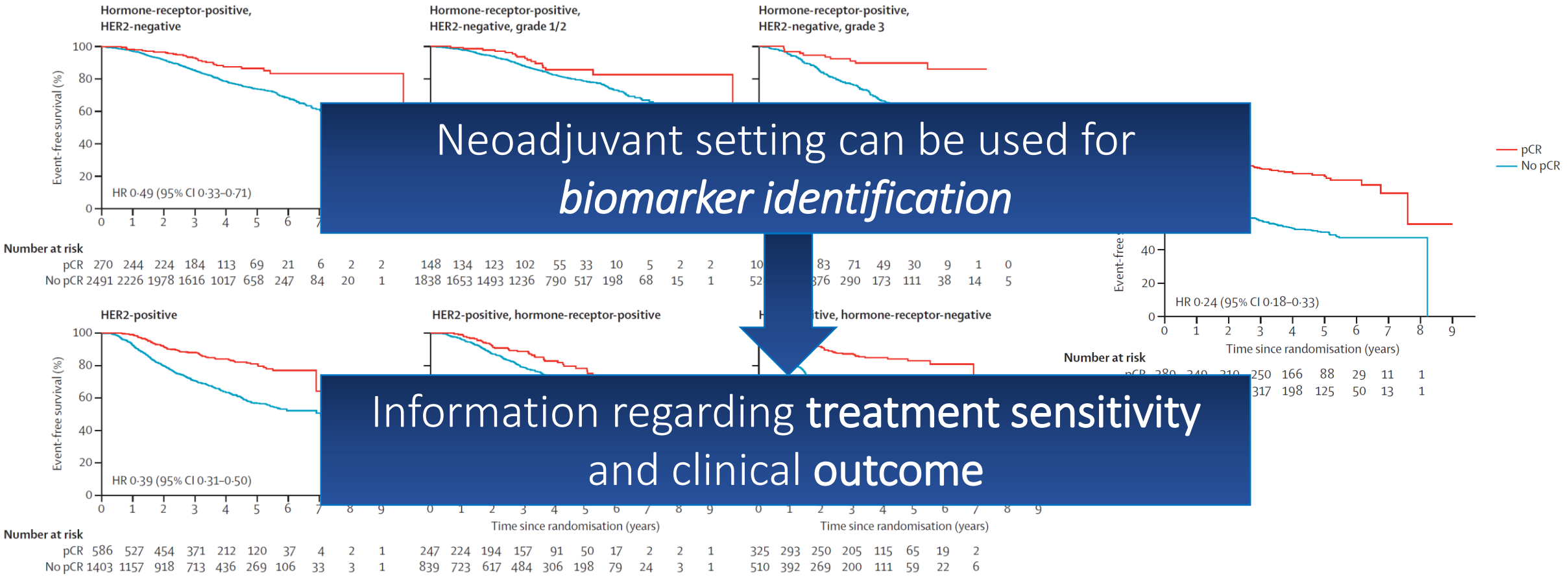
- **Histopathology** (ductal *vs* lobular)
- **HER2 positivity** (3+ *vs* 2+ with FISH amplified) and HER2 **IHC expression levels**
- **Hormone receptor** st
- **PAM50 intrinsic subtypes** [1]
- **Mutational/copy number/epigenetic** profiles [2,3]
- **Tumor-Infiltrating Lymphocytes (TIL) levels** [4]
- …

Response to anti-HER2 treatments is **heterogeneous**



[1] Gavilà J, et al. BMC Med 2019
[2] Cancer Genome atlas network, Nature 2012
[3] Pereira B, et al. Nat Commun 2016
[4] Solinas C, et al. Breast 2017

Neoadjuvant therapy – association between ***pCR*** and ***long-term outcome***



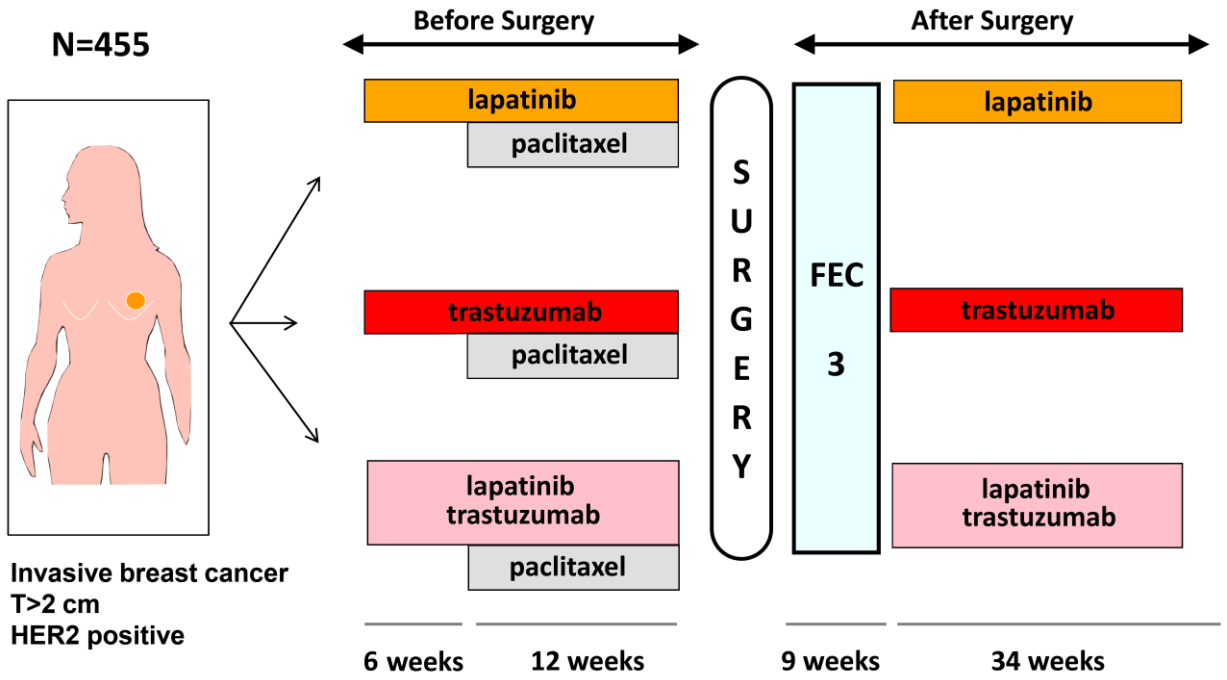
Neoadjuvant setting can be used for *biomarker identification*

Information regarding **treatment sensitivity** and clinical **outcome**

pCR = pathological complete response

Cortazar P, et al. Lancet 2014

The NeoALTTO trial



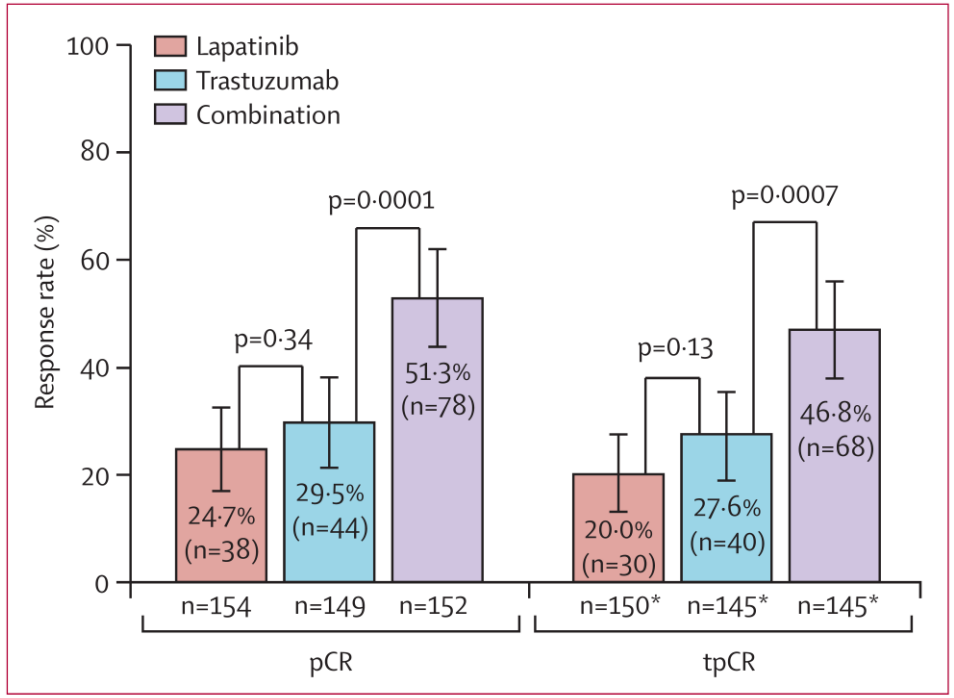


Figure 2: **Rates of pCR and of locoregional total pCR in the three treatment groups**

Baselga J, et al. Lancet 2012
De Azambuja E, et al. Lancet Oncol 2014

RNA-sequencing to predict *pCR* in the NeoALTTO trial

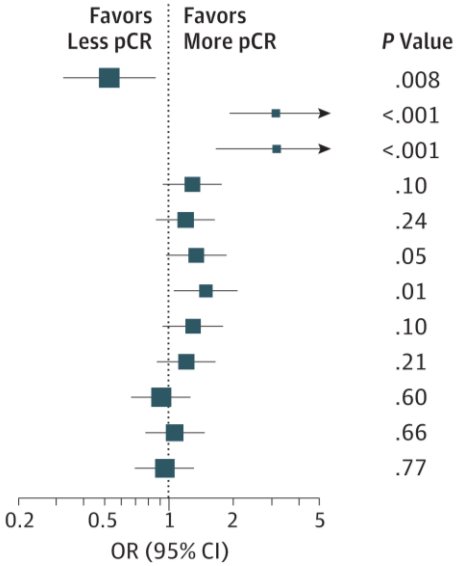
- RNA-sequencing to determine **gene expression levels**, **PAM50 subtypes**, **gene signatures** (GSs)*

- In **all treatment arms**:
  - High expression levels of **ERBB2/HER2: ↑ pCR**
  - Low levels of **ESR1: ↑ pCR**
  - **HER2-enriched** PAM50 subtype**: ↑ pCR**

- In the **combination arm:**
  - High expression of **immune** GSs: **↑ pCR**
  - High expression of **stroma** GSs: **↓ pCR**

B Adjusted for clinicopathological parameters and treatment arm

Parameter	OR (95% CI)	FDR	*P* Value
ESR1	0.53 (0.33-0.86)	0.016	.008
ERBB2/HER2	3.1 (1.9-5.1)	2.1×10^{-7}	<.001
HER2 enriched (PAM50)	3.2 (1.7-6.0)	9.9×10^{-4}	<.001
Immune1	1.3 (0.95-1.8)	0.085	.10
Immune2	1.2 (0.89-1.6)	0.16	.24
Immune3	1.3 (0.99-1.8)	0.065	.05
Genomic Grade Index	1.5 (1.1-2.1)	0.021	.01
Aurka	1.3 (0.95-1.8)	0.085	.10
AKT/mTOR	1.2 (0.89-1.6)	0.16	.21
Stroma1	0.92 (0.68-1.2)	0.36	.60
Stroma2	1.1 (0.79-1.5)	0.36	.66
AR	0.96 (0.70-1.3)	0.39	.77

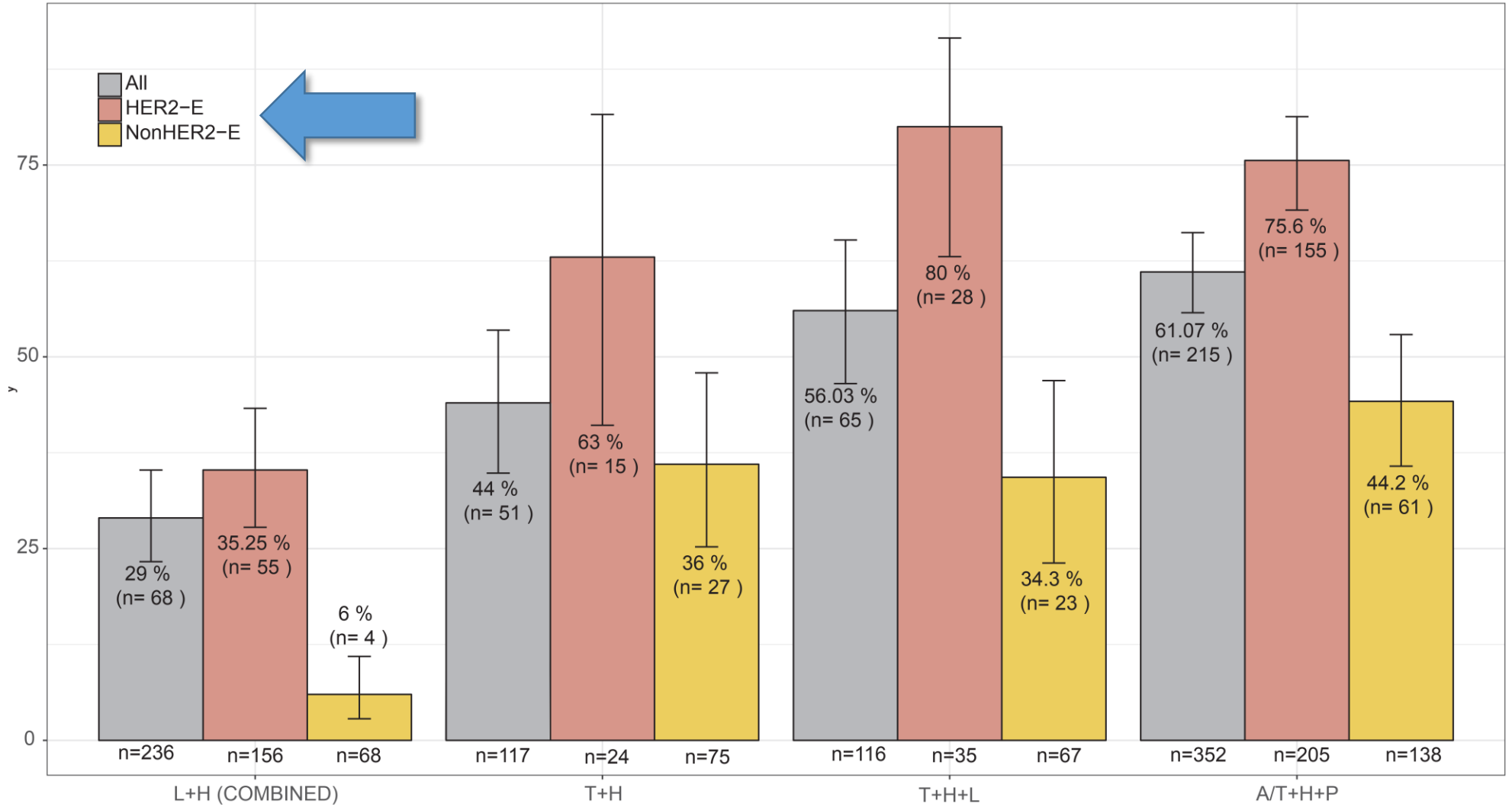


Effect of single genes and gene expression signatures on pCR adjusting for clinicopathological parameters and treatment arms.

* **Gene expression signature**: group of genes with expression pattern characterizing biological processes
→ pathway activation, prognostic/predictive biomarkers, gene sets associated to specific function, disease subgroups

Fumagalli D, et al. JAMA Oncol 2017

pCR rates according to *HER2-E PAM50 subtype*

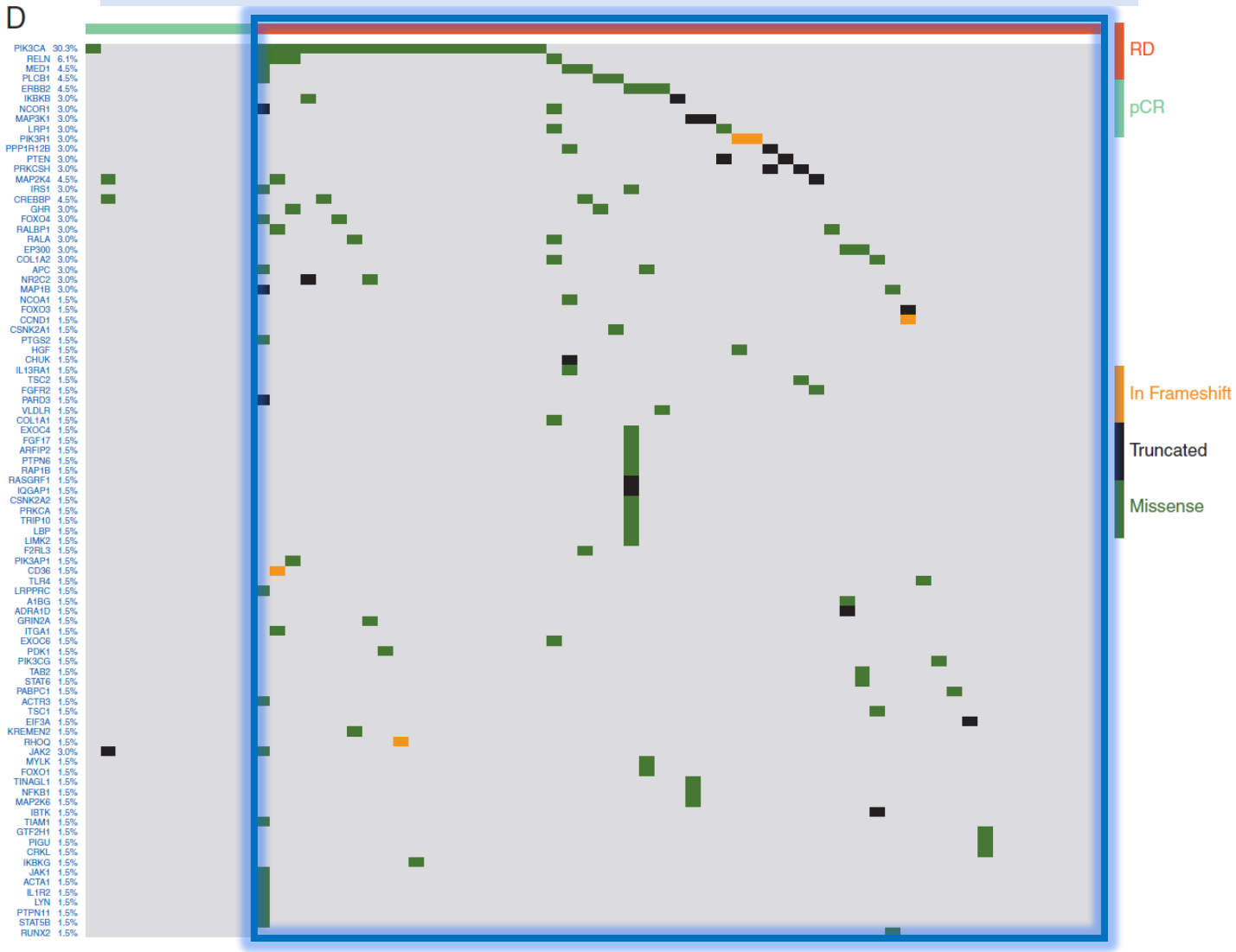


Rates of pCR according to the type of chemotherapy and anti-HER2 therapy using data from 8 neoadjuvant clinical trials in HER2+ breast cancer
H = trastuzumab; L = lapatinib; P = pertuzumab; T = taxane; A = anthracycline

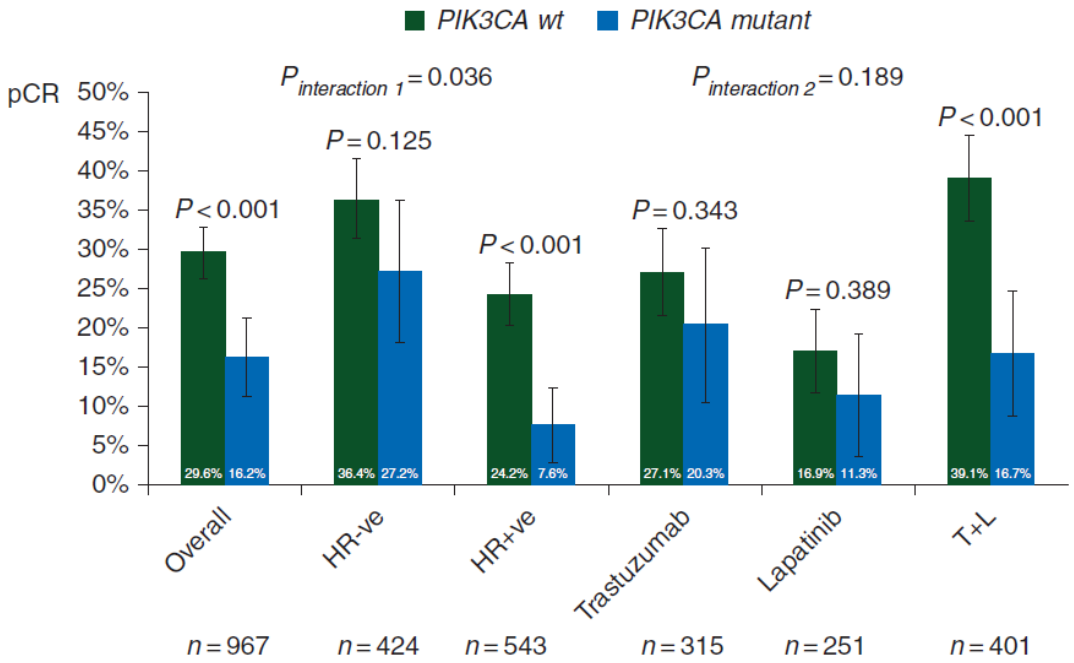
Cejalvo JM, et al. Cancer Treat Rev 2018

PIK3CA mutations to predict *pCR*

NeoALTTO trial → mutations in PIK3CA network genes



Pooled analysis from 5 prospective trials



Shi W, et al. Ann Oncol 2017
Loibl S, et al. Ann Oncol 2016

Triple-negative breast cancer

BL1: Basal-like 1

BL2: Basal-like 2

IM: Immunomodulatory

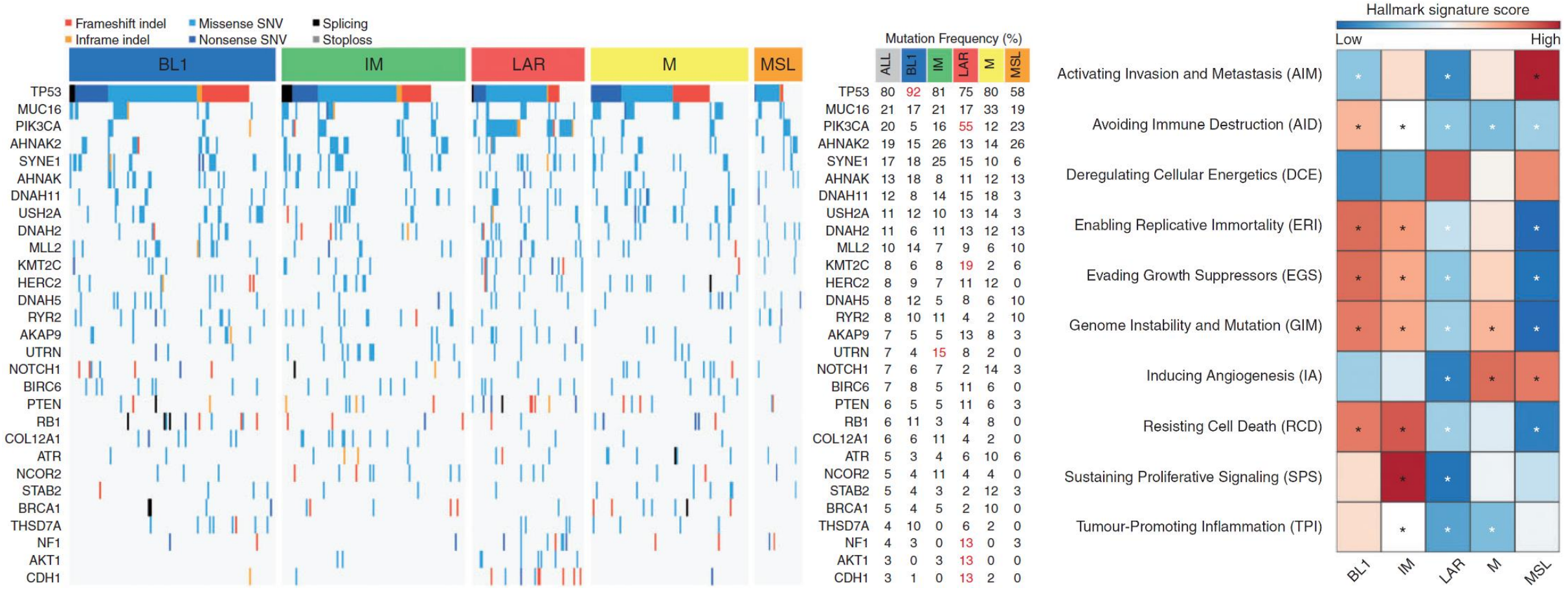
M: Mesenchymal

MSL: Mesenchymal Stem-like

LAR: Luminal Androgen receptor

Lehmann BD, et al. JCI 2011

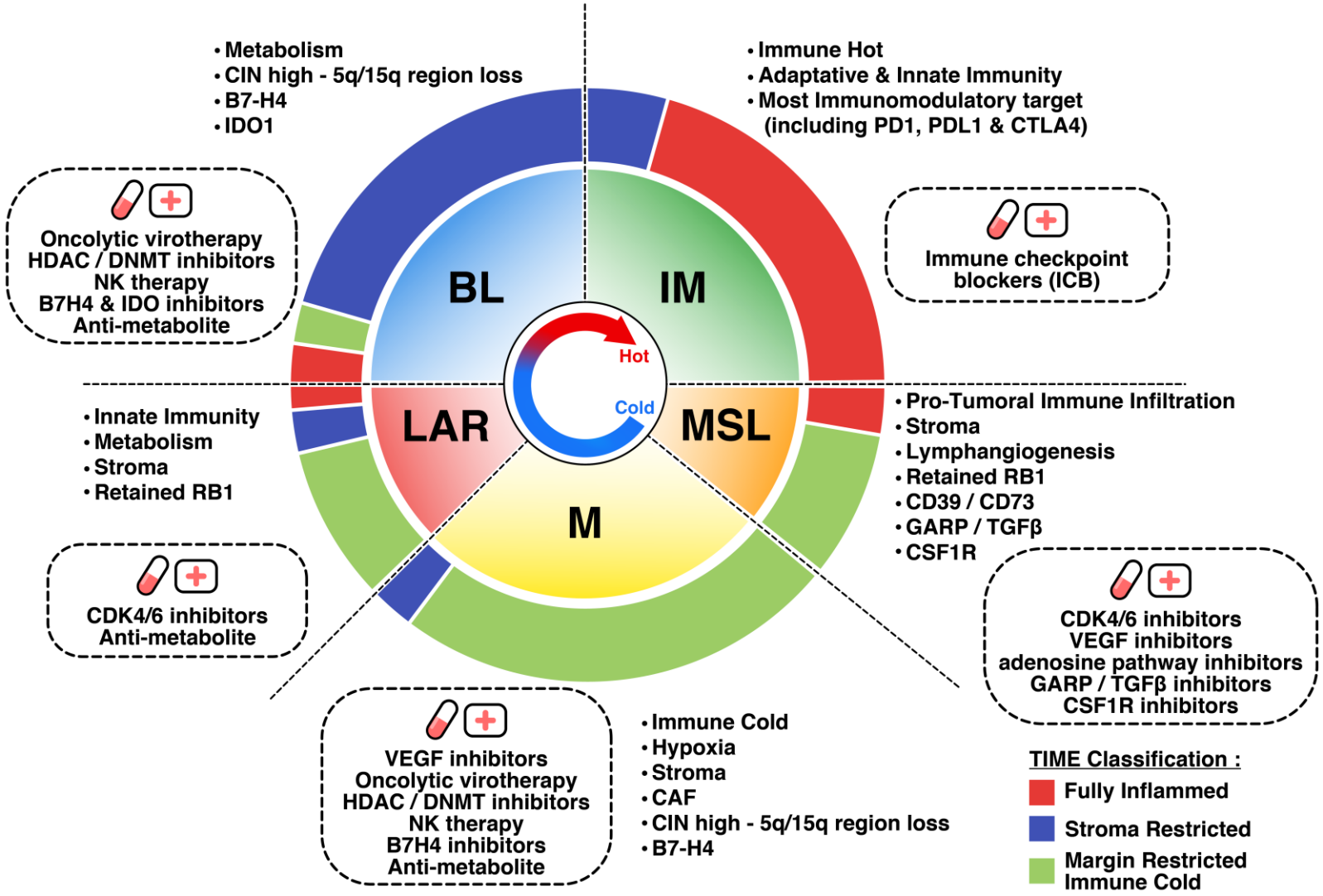
TNBC subtypes – multi-omic analysis



Significant **up-regulation** displayed in black, **down-regulation** in white

Bareche Y, et al. Ann Oncol 2018

Heterogeneity of TNBC – opportunities for personalized treatment

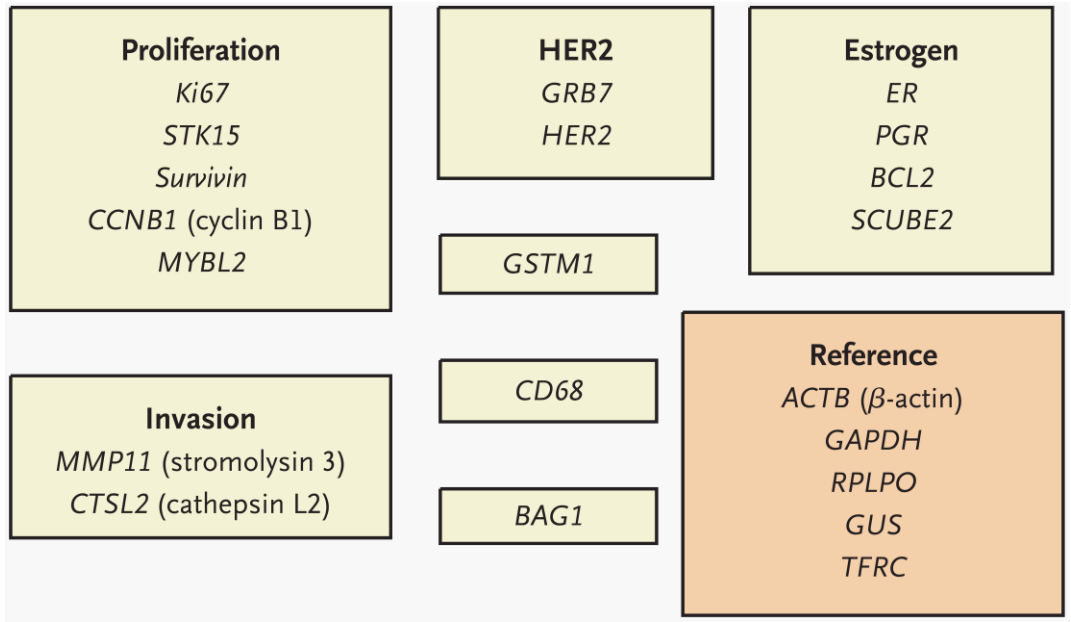


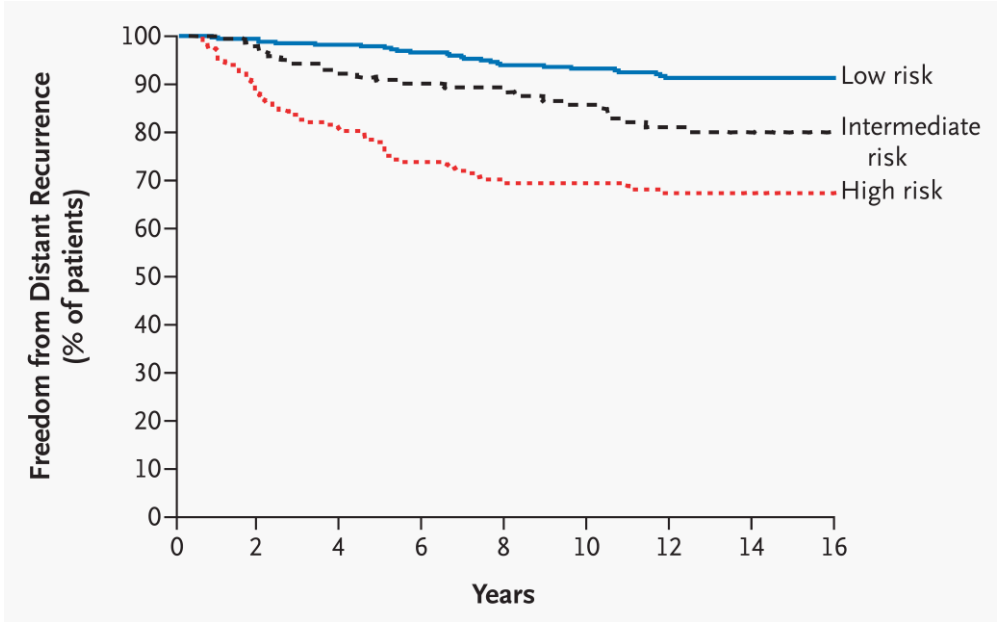
Bareche Y, et al. J Natl Cancer Inst 2019

Luminal breast cancer

Oncotype DX assay: 21 genes selected after **three independent preliminary studies** involving 447 patients and 250 candidate genes

Range of **recurrence scores** from 0 to 100 based on ***gene expression levels***

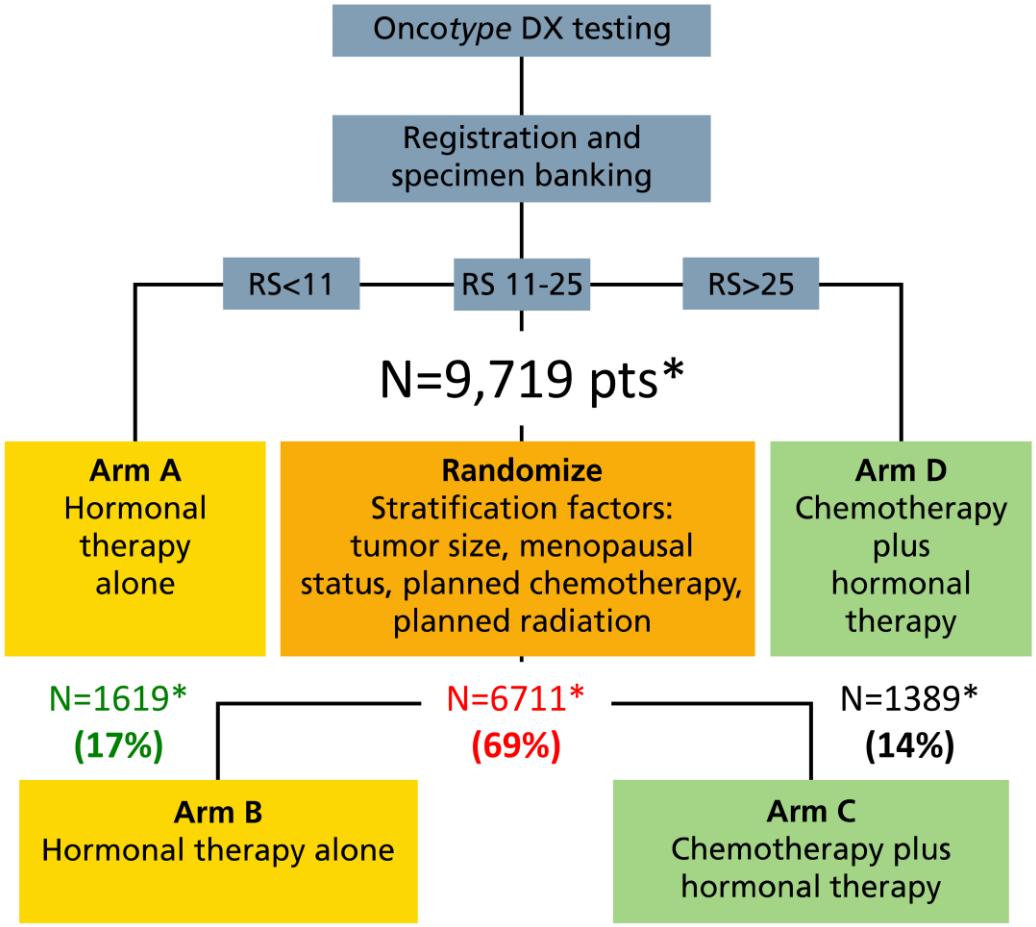




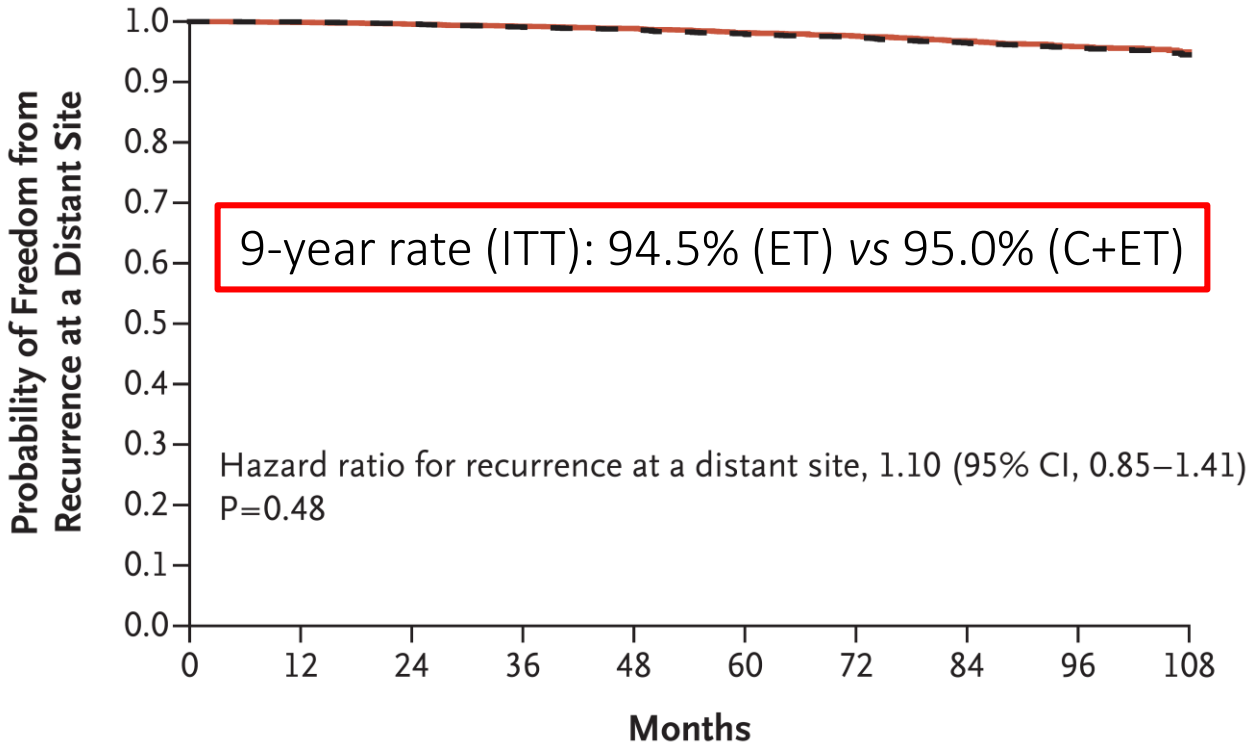
Likelihood of distant recurrence, according to Recurrence-Score Categories

Paik S, et al. NEJM 2004

The TAILORx trial – adjuvant setting



* Included in the main analysis (eligible patients with FU information)



Some **benefit** of **chemotherapy** found in women **50 years of age or younger** with a recurrence **score of 16 to 25**

Sparano JA, et al. NEJM 2018

Table 1 | **Summary of studies used in testing of different gene signatures**

Gene-signature test	Training set	Initial validation set	Proportion (%) of patients assigned to the 'low-risk' category	Clinical application
Oncotype DX[25]	447 ER+/– tumour samples from patients with LN+/– disease enrolled in three separate clinical trials, including from the tamoxifen only arm of NSABP B-20	668 ER+ and tumour samples from patients with LN– disease in the tamoxifen only arm of NSABP B-14 (including samples from the training set)	51.0	Prediction of 10-year recurrence risk in patients with ER+ and LN– disease
Prosigna[37,41]	189 ER+/– tumour samples from patients with LN+/– disease and 29 nonmalignant breast tissue biopsy samples	786 ER+/– tumour samples from patients with and LN+/– disease	28.2	Determining the prognosis of postmenopausal women with ER+ and LN+/– disease of stages 1 or 2
MammaPrint[43,44]	78 ER+/– tumour samples with a diameter <5 cm from patients <55 years of age with LN-negative disease	295 ER+/– tumour samples <5 cm in diameter from patients <53 years of age with and LN+/– disease (including samples from the training set)	40.0	Determining the prognosis of women with ER+/– and LN– disease of stages 1 or 2
Breast cancer index[59,60]	60 ER+ tumour samples from patients previously treated with tamoxifen	588 ER+ tumour samples from patients with LN– disease enrolled in the Stockholm trial	53.0	Determining the prognosis of women with ER+ and LN– disease, prediction of benefit from extended endocrine therapy
EndoPredict[65]	964 ER+ tumour samples from patients with LN+/– disease treated with tamoxifen	378 ER+ tumour samples from patients with LN+/– disease from the ABCSG-6 trial (tamoxifen-only arm) and 1,324 patients from the ABCSG-8 trial	62.6	Determining the prognosis of women with ER+ and LN+/– disease
Genomic Grade Index[74]	64 ER+ tumour samples of histological grades 1–3	• 125 ER+/– • Tumour samples of histological grades 1–3 from patients with and LN– disease	59.7	Prognosis and risk stratification based on histological grade

ER: oestrogen receptor; LN: lymph node

Kwa M, et al. Nat Rev Clin Oncol 2017

Outline

- Introduction to the clinical classification
- Molecular characterization and intertumor heterogeneity

  → Examples of clinical applications

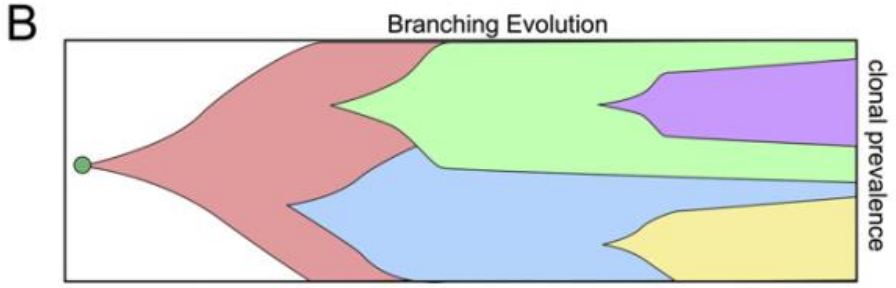
- The challenge of ***intratumor heterogeneity***
- The role of the tumor immune microenvironment
- Novel translational research tools and future perspectives

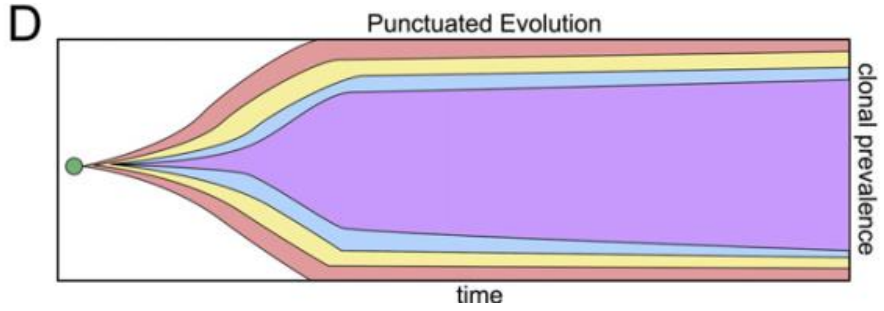
Tumor *evolution* and *heterogeneity*

Experimental data in breast cancer → *Mixed model*

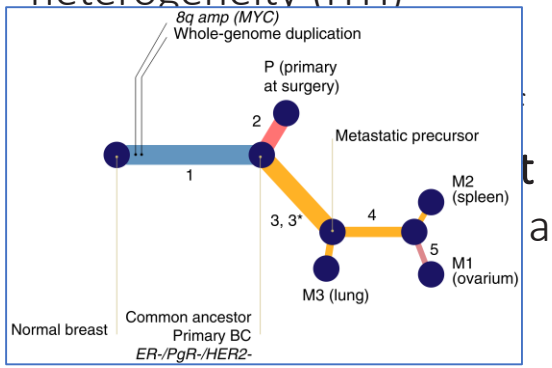
- **CNAs** and chromosomal **structural variants** follow a *PE* model (**D**)
  → They occur in ***early*** punctuated bursts of evolution, and stably expand

- Point **mutations** follow a *BE* model (**B**)
  → ***Gradual*** evolution over the lifetime of the tumor, leading to clonal expansions

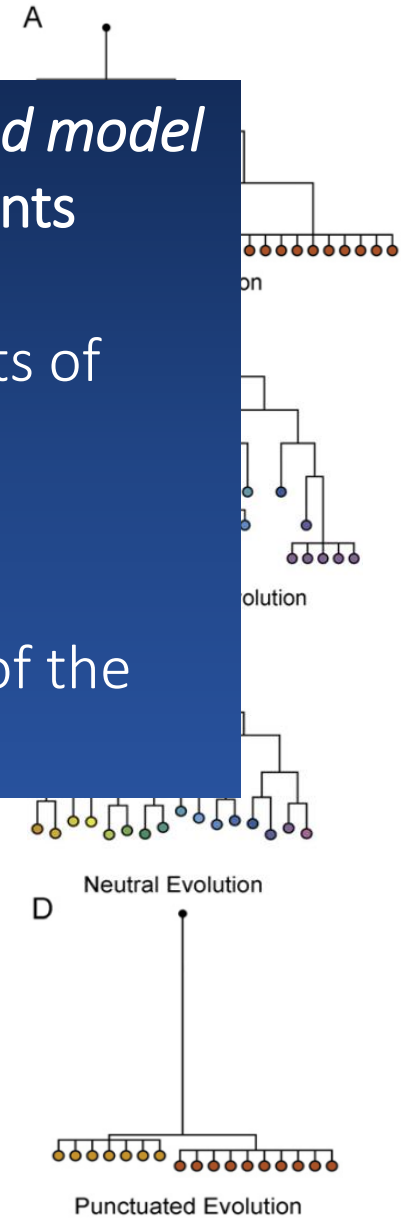




leading to genetic drift and extensive intratumor heterogeneity (ITH)



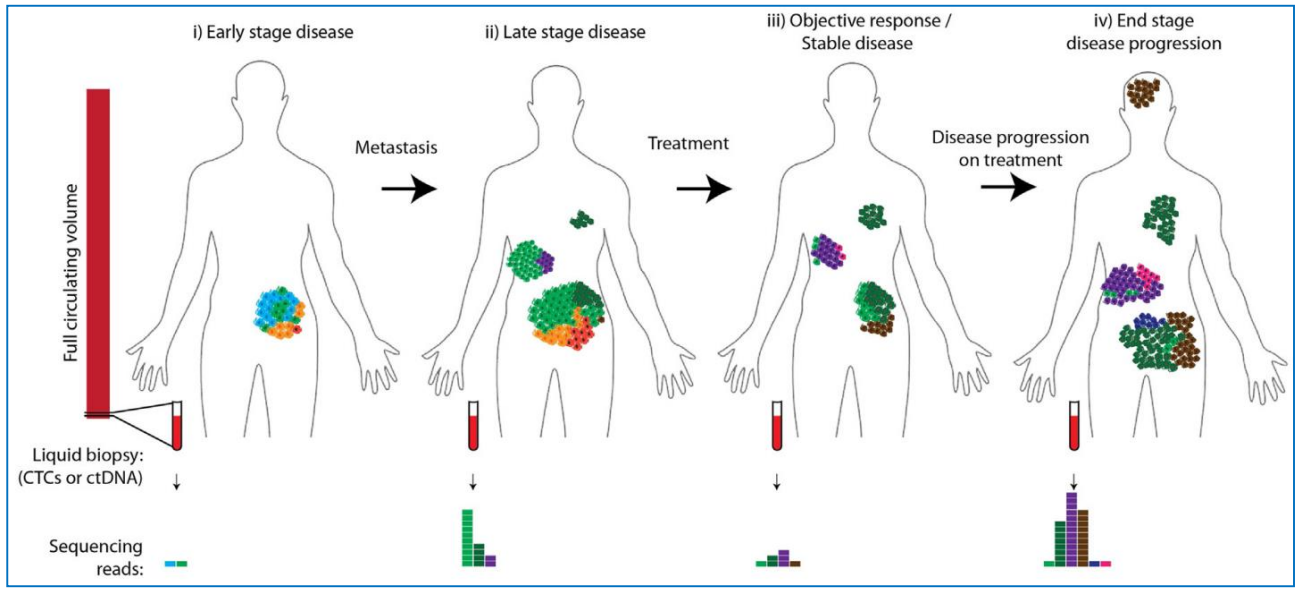
genomic **aberrations**
t **stages** of tumor
Phylogenetic analysis performed using **somatic mutations** and **copy number aberrations**
a few dominant clones

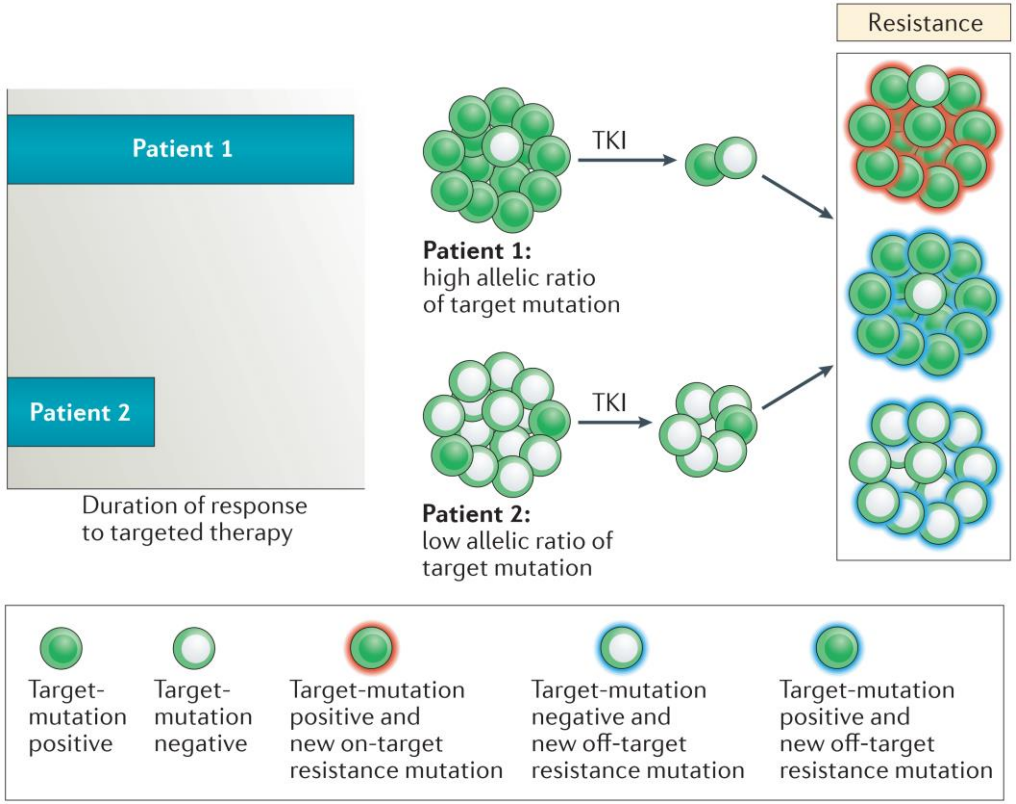


Brown D, et al. Nat Comm 2017

Davis A et al. BBA 2017

Tumor heterogeneity ***increases*** over time and is correlated to ***treatment resistance***





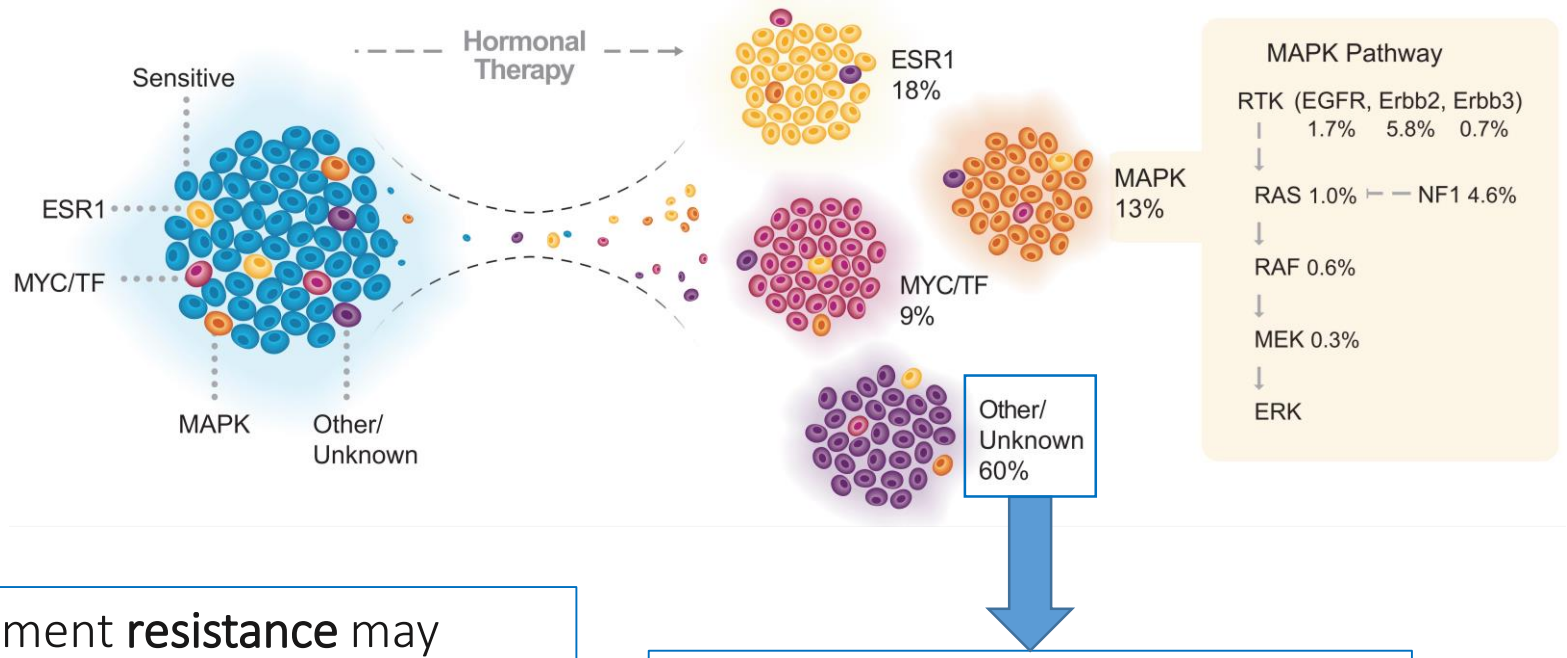
Burrel RA & Swanton C. Mol oncol 2014
Dagogo-Jack I & Shaw AT. Nat Rev Clin Oncol 2018

Taxonomy of the mechanisms of ***resistance*** to ***endocrine therapy***

Highlights

- We performed prospective sequencing of 1,501 HR$^+$ breast cancers in the clinical setting
- MAPK and TF alterations were present in 22% of 692 HR$^+$ post-endocrine therapy tumors
- MAPK and TF alterations were mutually exclusive with *ESR1* mutations
- MAPK and TF alterations were associated with shorter response to endocrine therapies

TF = transcription factor



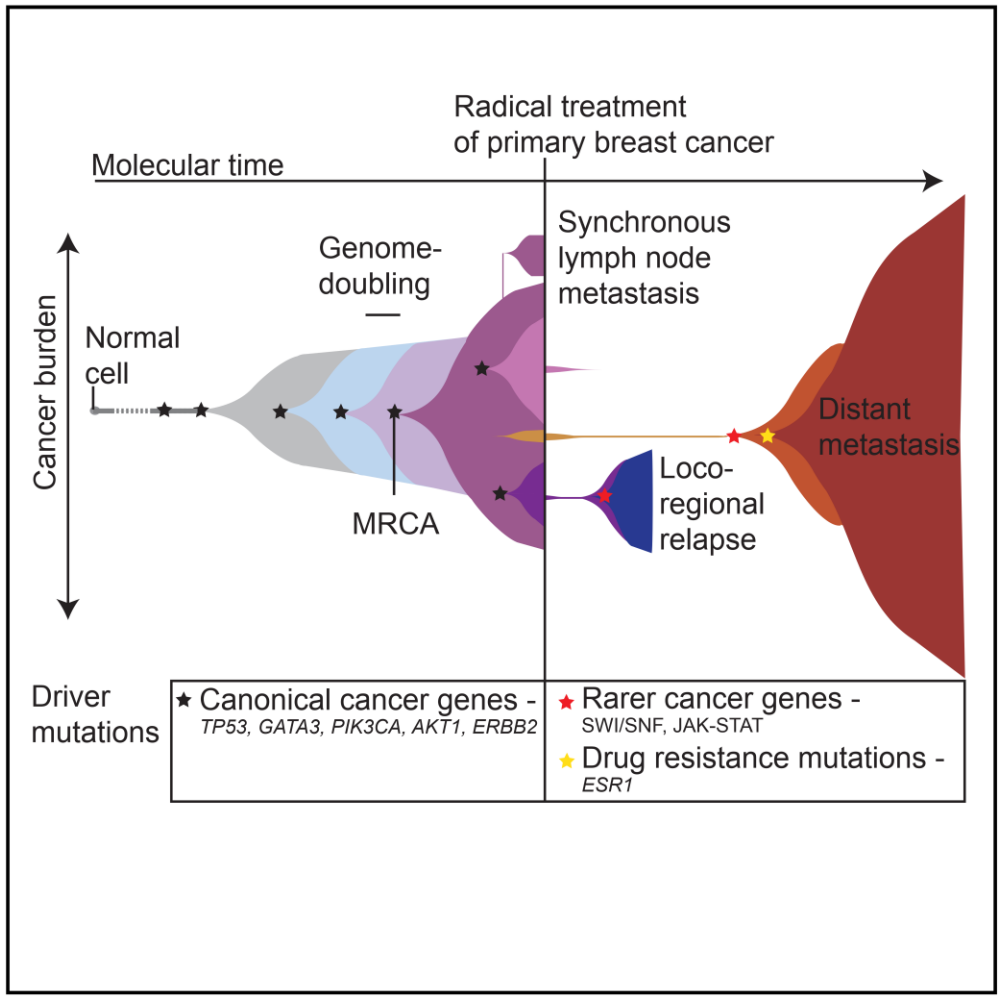
Genomics alterations associated to treatment **resistance** may

- ***pre-exist*** in the pre-treatment tumors and expand
- be ***acquired*** under the **selective pressure** of endocrine therapy

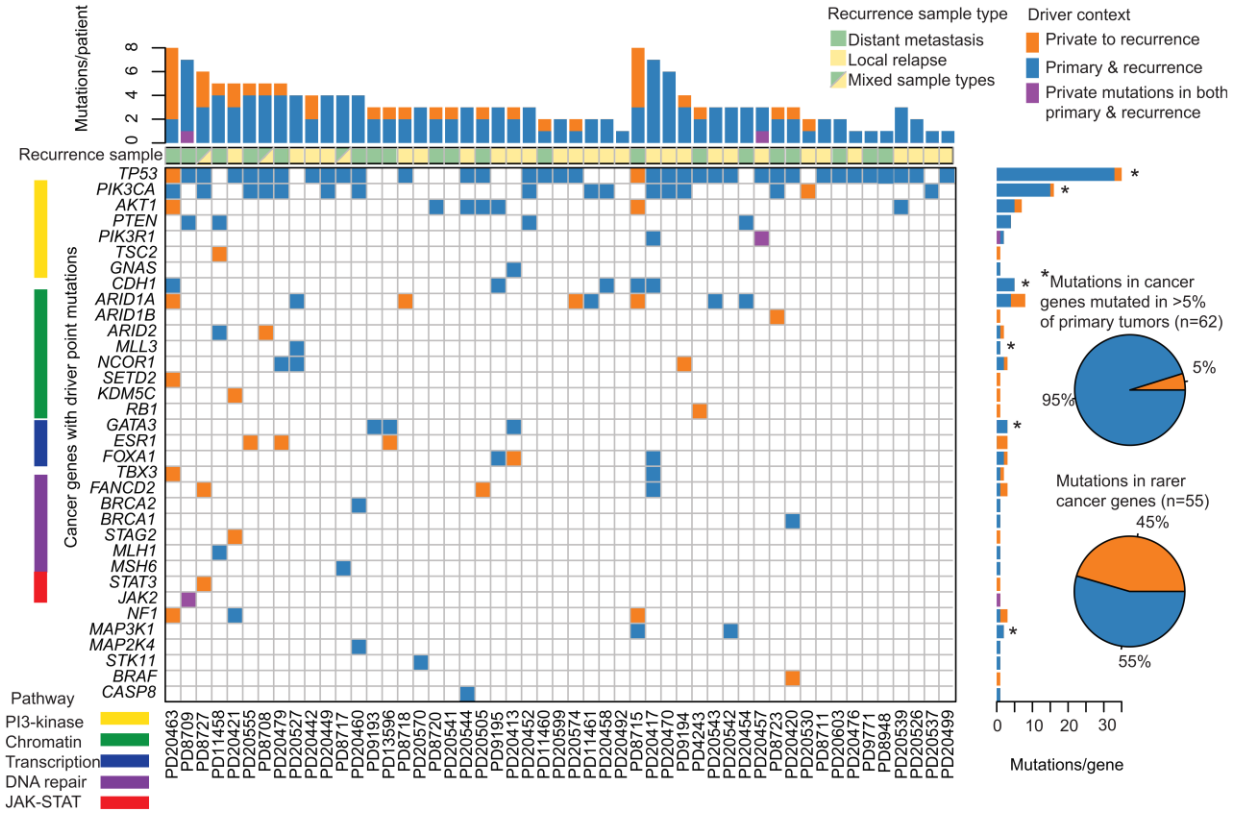
Role of transcriptional reprogramming, epigenetics, tumor microenvironment…?
→ **Go beyond "single gene" vision**

Razavi P, et al. Cancer Cell 2018

Progression of breast cancer – **when?**

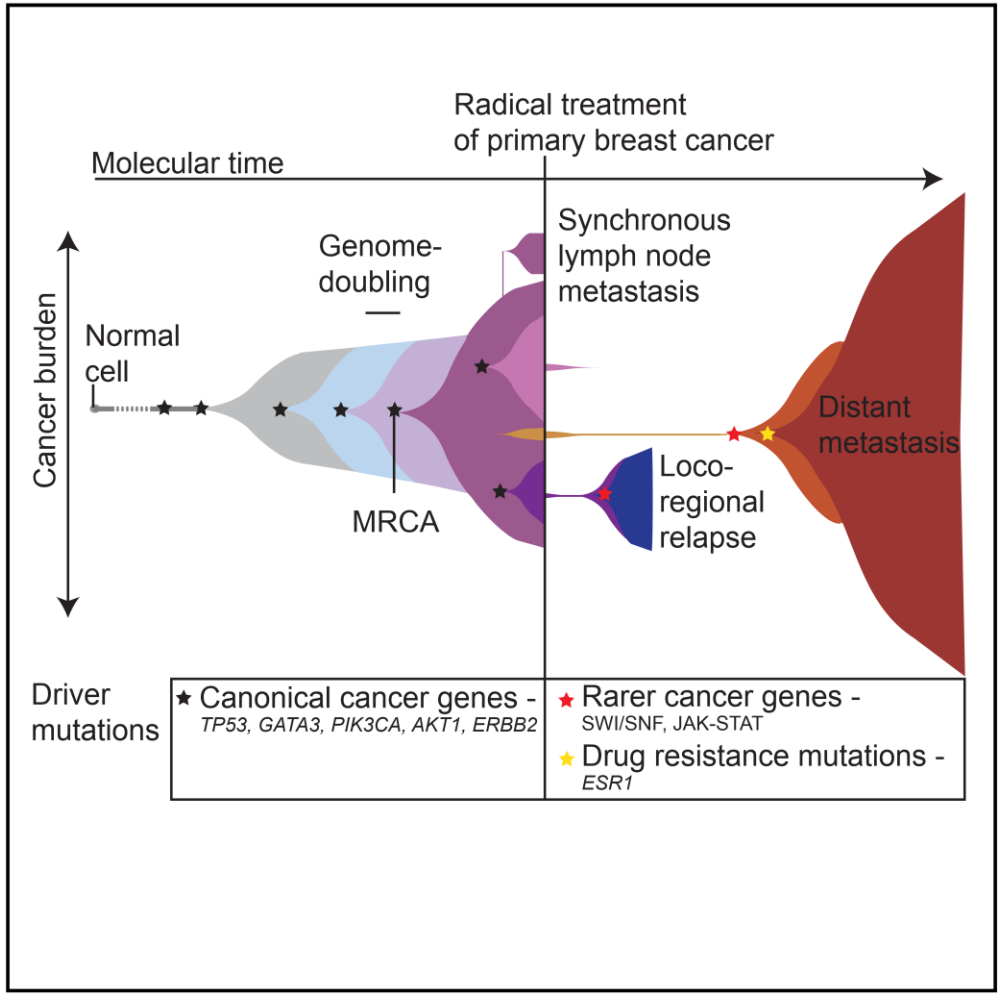


Metastases mostly ***disseminate late*** from primary breast tumors, keeping most drivers, but **continue to acquire mutations**



Yates LR, et al. Cancer Cell 2017

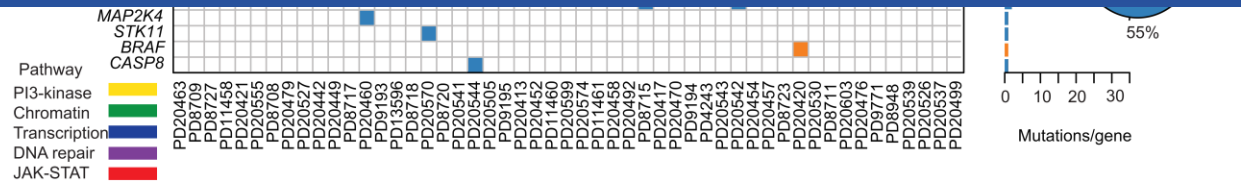
Progression of breast cancer – **when?**



Metastases mostly ***disseminate late*** from primary breast tumors, keeping most drivers, but **continue to acquire mutations**

- The genome of the **primary** tumor represents a good ***proxy*** for that of the cells that ultimately seeded the **relapse**
  → Important for **adjuvant treatments**

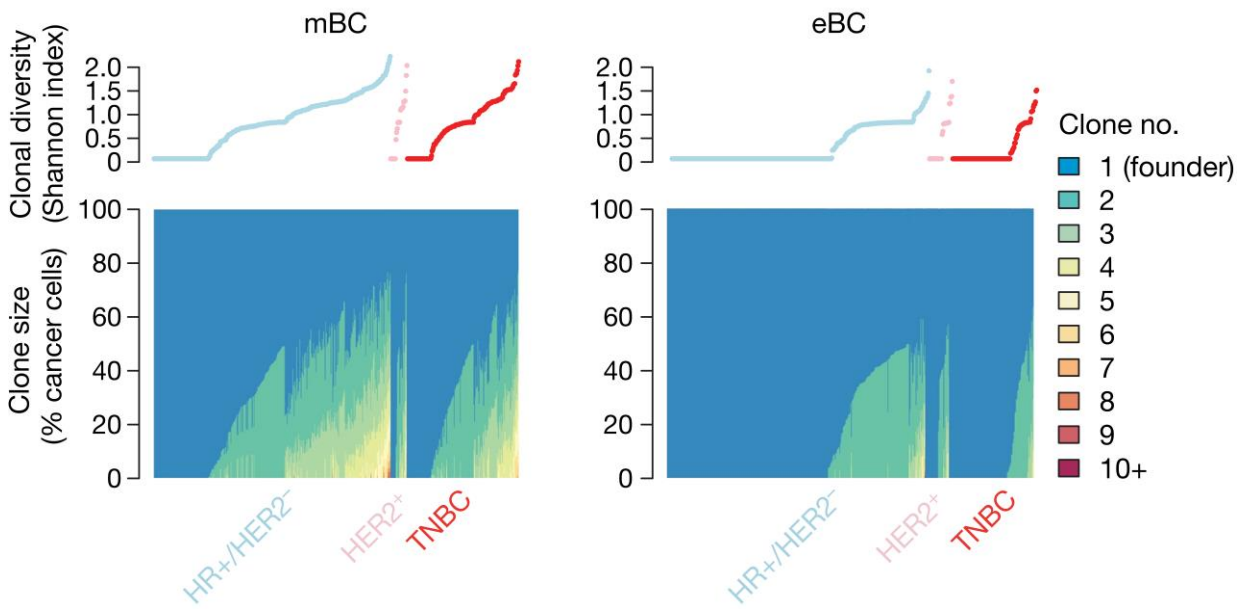
- The genome of a **metastatic clone** undergoes extended **changes** by the time it has expanded to be **clinically detectable**



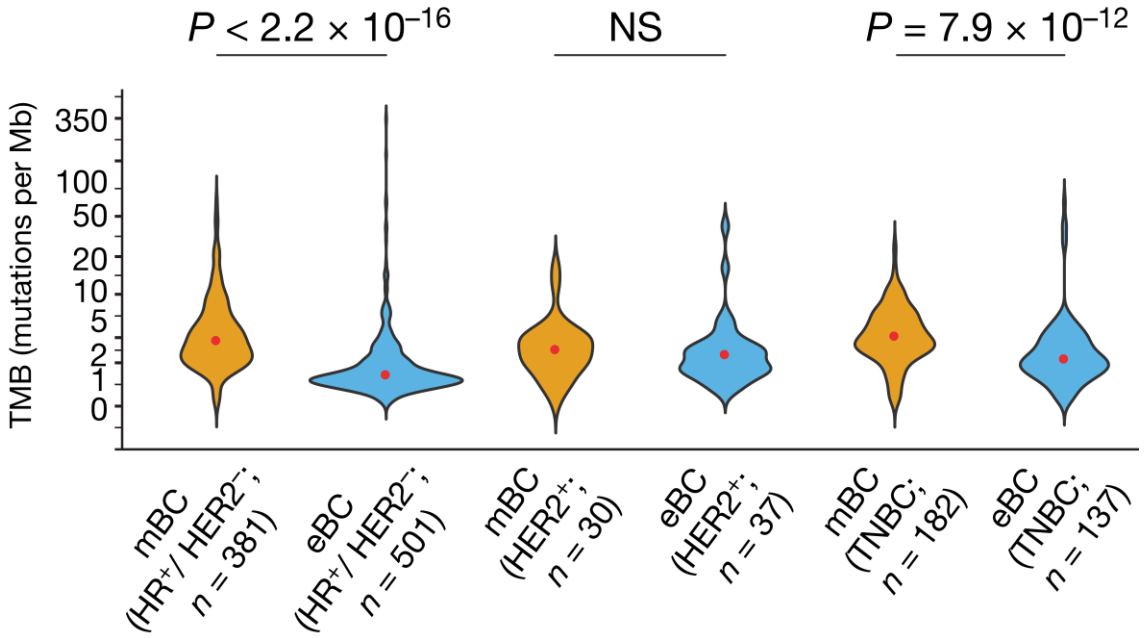
Yates LR, et al. Cancer Cell 2017

Advanced-stage BCs are ***more complex*** than early-stage BCs

Increase in the ***clonal diversity*** in mBC



Increase of ***mutational load*** in mBC



eBC = early-stage breast cancer
mBC = metastatic breast cancer
TMB = tumor mutational burder

Bertucci F, et al. Nature 2019

Outline

- Introduction to the clinical classification
- Molecular characterization and intertumor heterogeneity

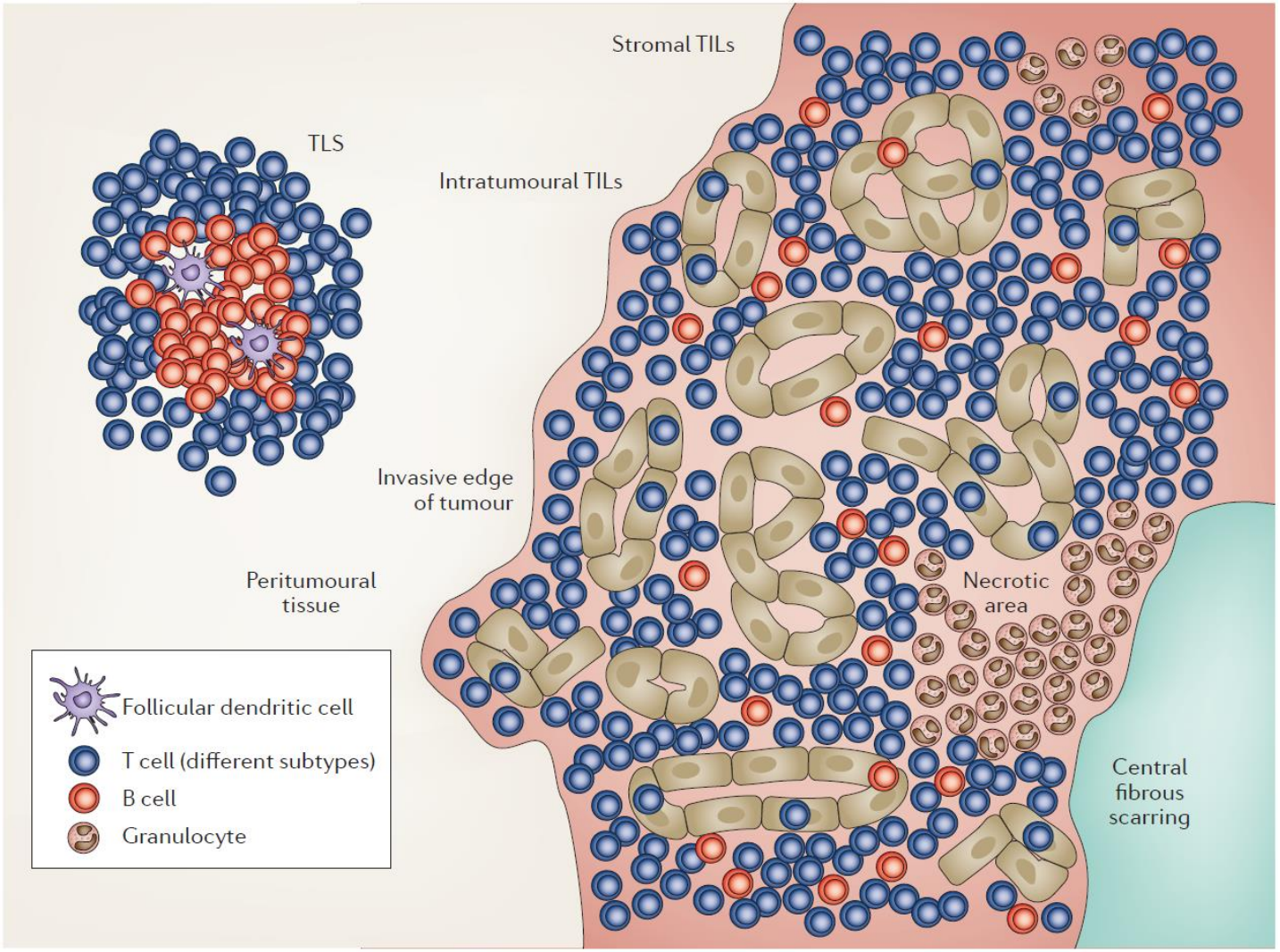
  → Examples of clinical applications

- The challenge of intratumor heterogeneity
- The role of the ***tumor immune microenvironment***
- Novel translational research tools and future perspectives

The ***immune*** microenvironment

Characterization of the tumor immune microenvironment can be performed at different levels

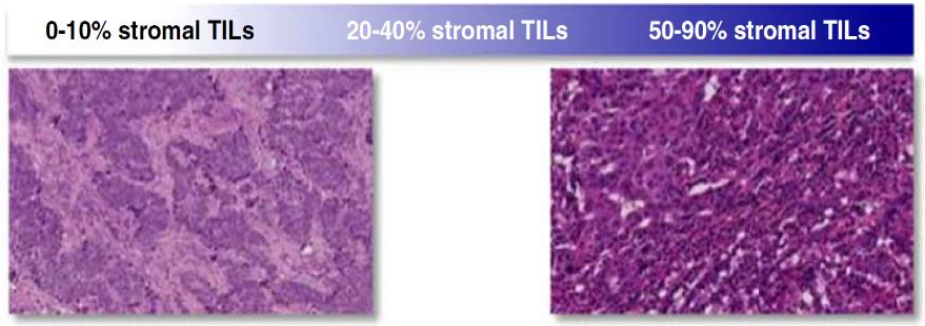
- ***Quantification*** of tumor-infiltrating lymphocytes (TILs, e.g. H&E staining)
- Characterization of TIL ***subpopulations*** (e.g. IHC, IF, flow cytometry)
- Description of the TIL ***geographic distribution***



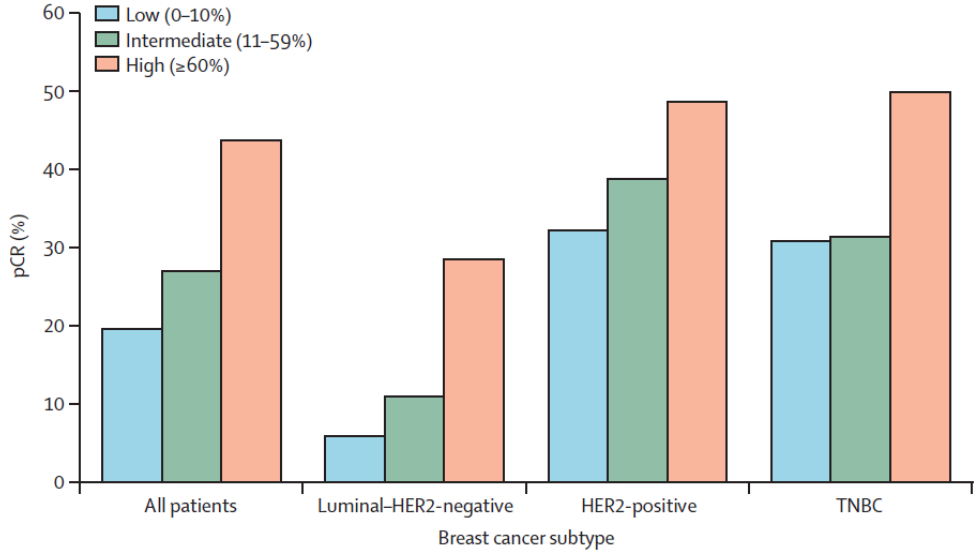
Savas P, et al. Nat Rev Clin Oncol 2016

TIL levels as a biomarker

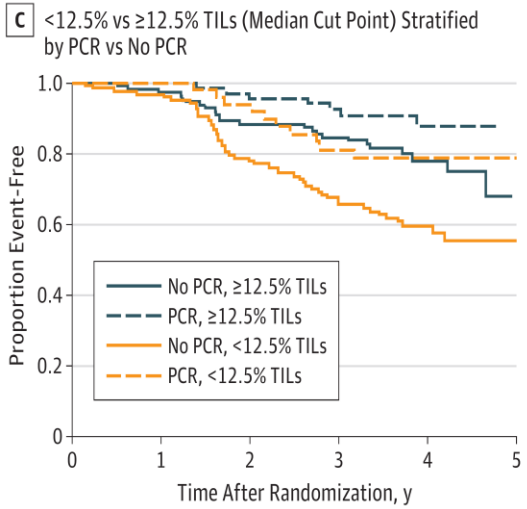
Current clinical data establish the clinical validity of higher **TIL levels** as a ***predictive*** and ***prognostic biomarker***

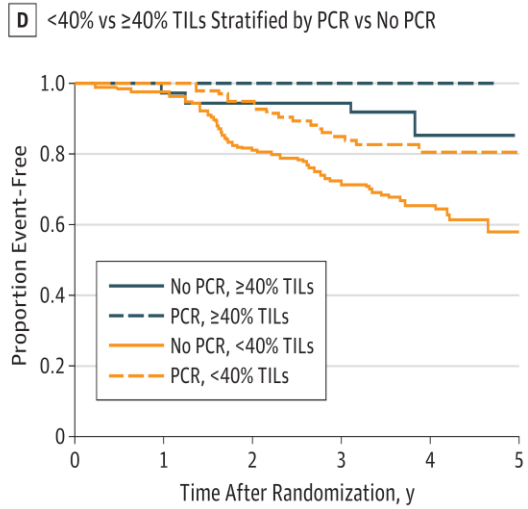


Pooled analysis of 3771 BC patients treated with **neoadjuvant** therapy



Higher TIL levels → better **Event-Free Survival** independently of pCR in NeoALTTO



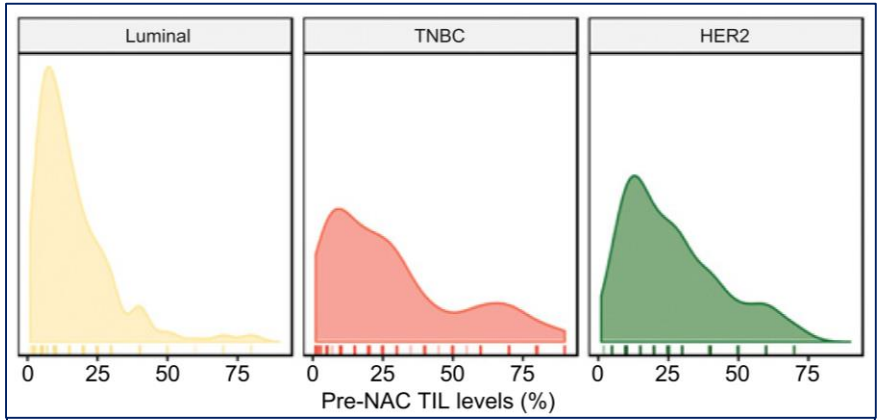


Solinas C, et al. Breast 2017
Denkert C, et al. Lancet Oncol 2018
Salgado R, et al. JAMA Oncol 2015

TIL levels in breast cancer **subtypes**

Median ***levels of stromal TILs*** (scored following international guidelines [1], usually higher than intratumoral TILs):

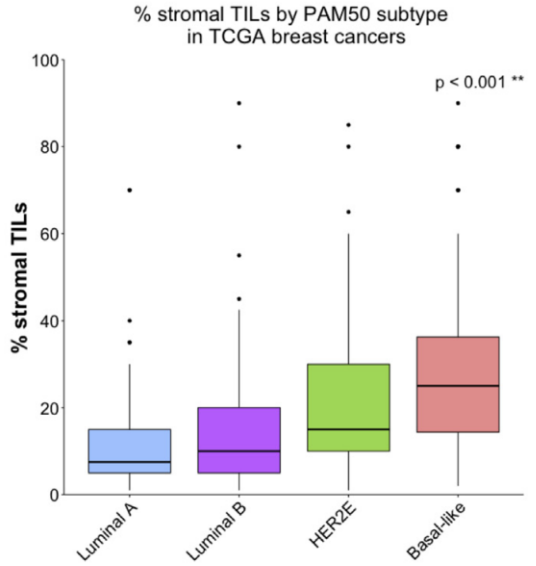
- **Luminal** BC → 7-10%
- **HER2-positive** BC → 15-20%
- **TNBC** → 15-20%



Distribution of pre-neoadjuvant chemotherapy (NAC) TIL levels, by BC subtype (kernel density plot)

HER2-positive and **triple-negative** BCs are considered more ***immunogenic*** than **luminal** BC

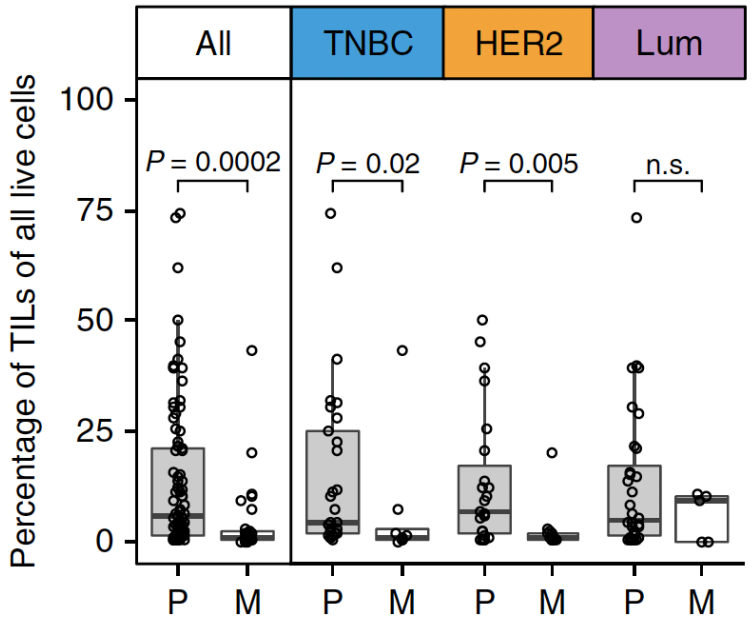
- Mutational load
- Neoantigen load
- Antigen presentation
- Immunosuppressive environment
- …

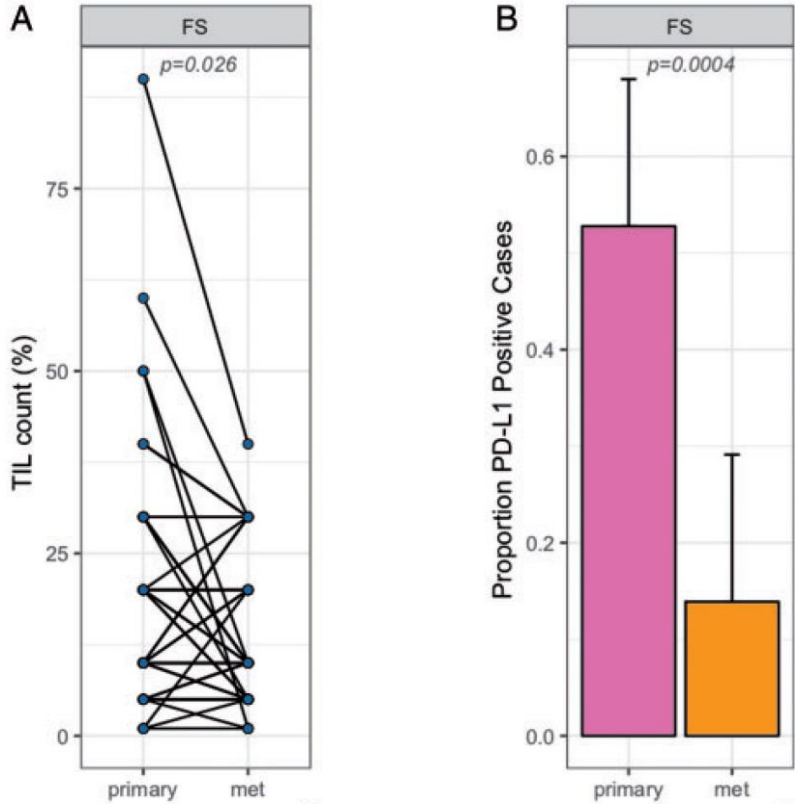


[1] Salgado R, et al. Ann Oncol 2015
Luen S, et al. Breast 2016
Solinas C, et al. Breast 2017
Hamy AS, et al. Clin Cancer Res 2019

TIL levels in **primary** *vs* **metastatic** breast cancer

Levels of TILs (and PD-L1) are ***lower*** in ***metastatic*** lesions compared to the ***primary*** tumor
→ ***immune escape***





TIL and PD-L1 protein expression in paired primary and metastatic cancers assessed on full sections (FSs)
(A) TIL count (%)
(B) PD-L1 positivity rates, defined as ≥1% of stromal or tumor cells showing IHC staining

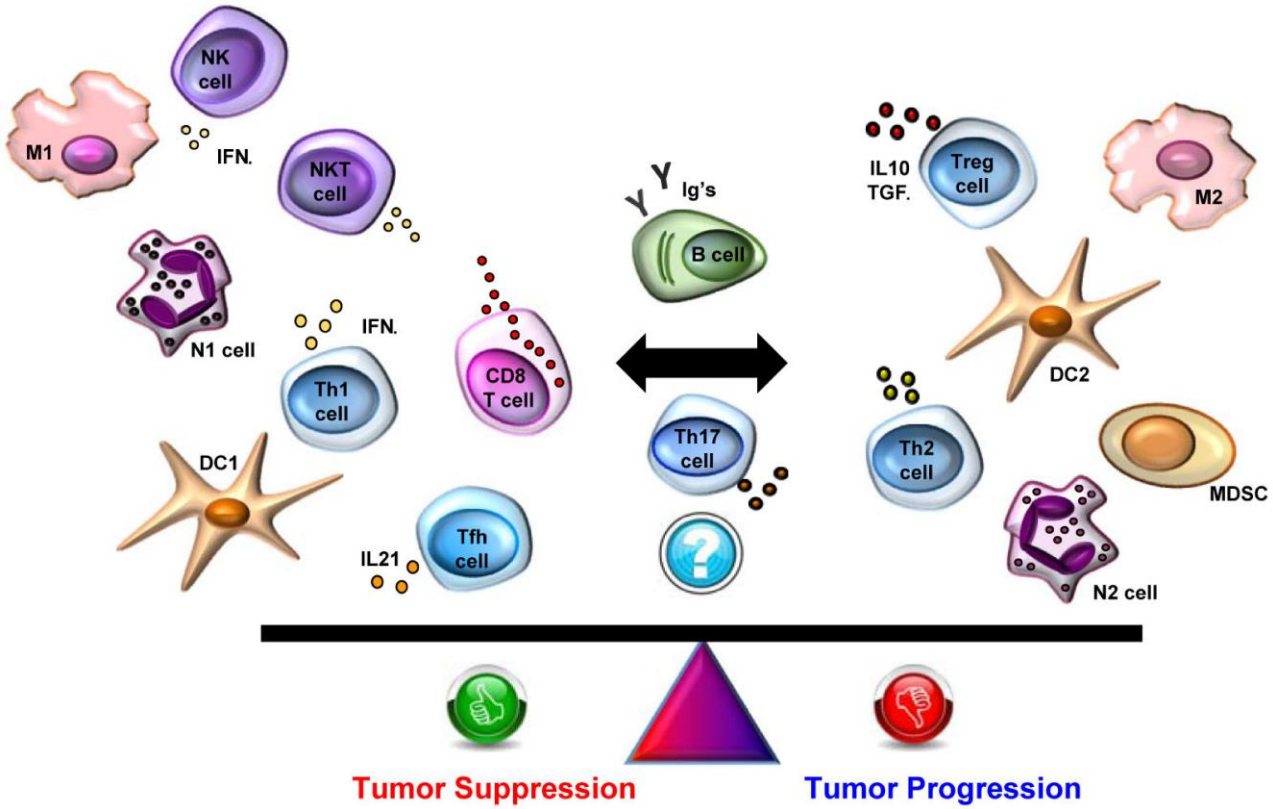
Szekely B, et al. Ann Oncol 2018
Savas P, et al. Nat Med 2018

Characterization of *TIL subpopulations*

The cellular constituents of the host immune response to tumors can:

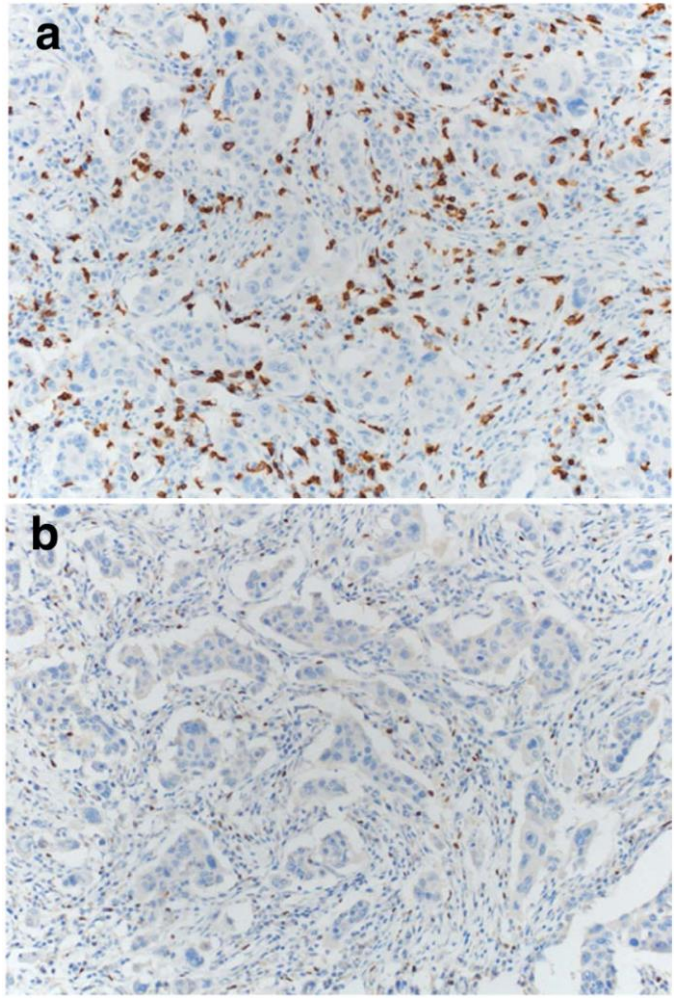
- ***control*** tumor growth
- contribute to an immunosuppressive environment that ***promotes*** tumor progression

TIL levels alone may not be enough when searching for robust biomarkers

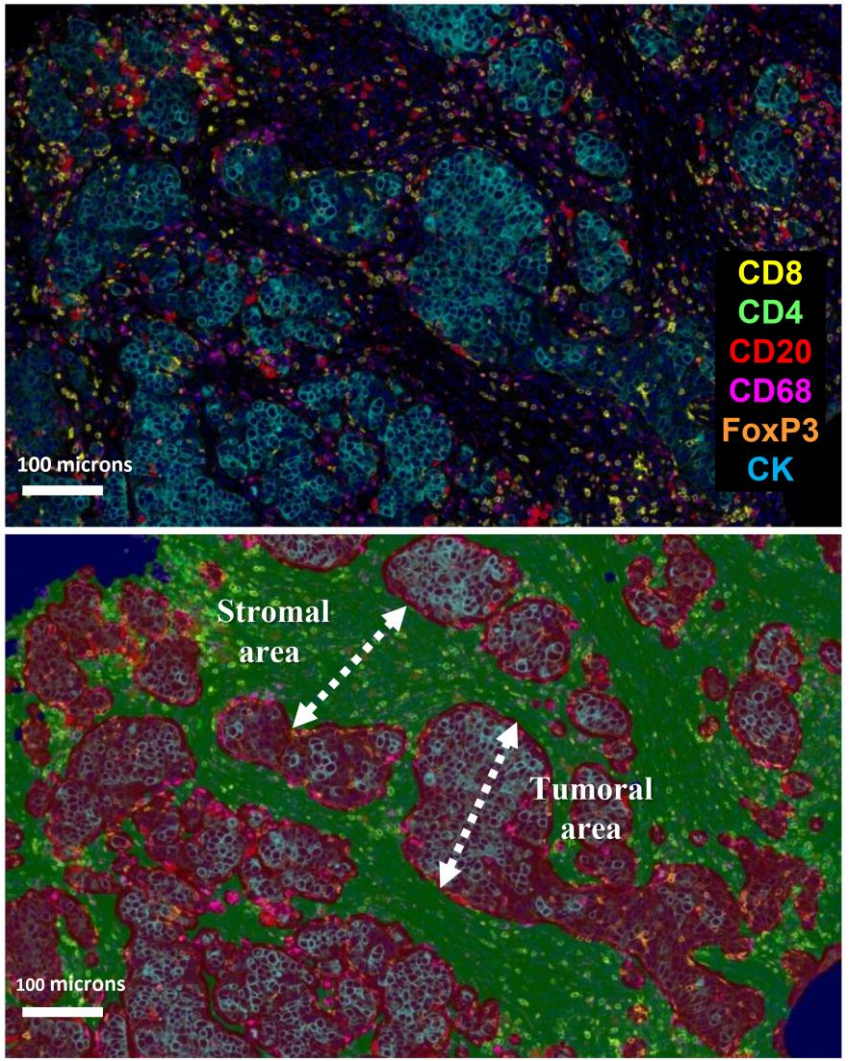


Hendry S, et al. Adv Anat Pathol 2017

Characterization of *TIL subpopulations – Methods*



Tumor CD8+ (**a**) and FOXP3+ (**b**) expression as assessed with immunohistochemistry



Multiplexed immunofluorescence of tumor infiltrates

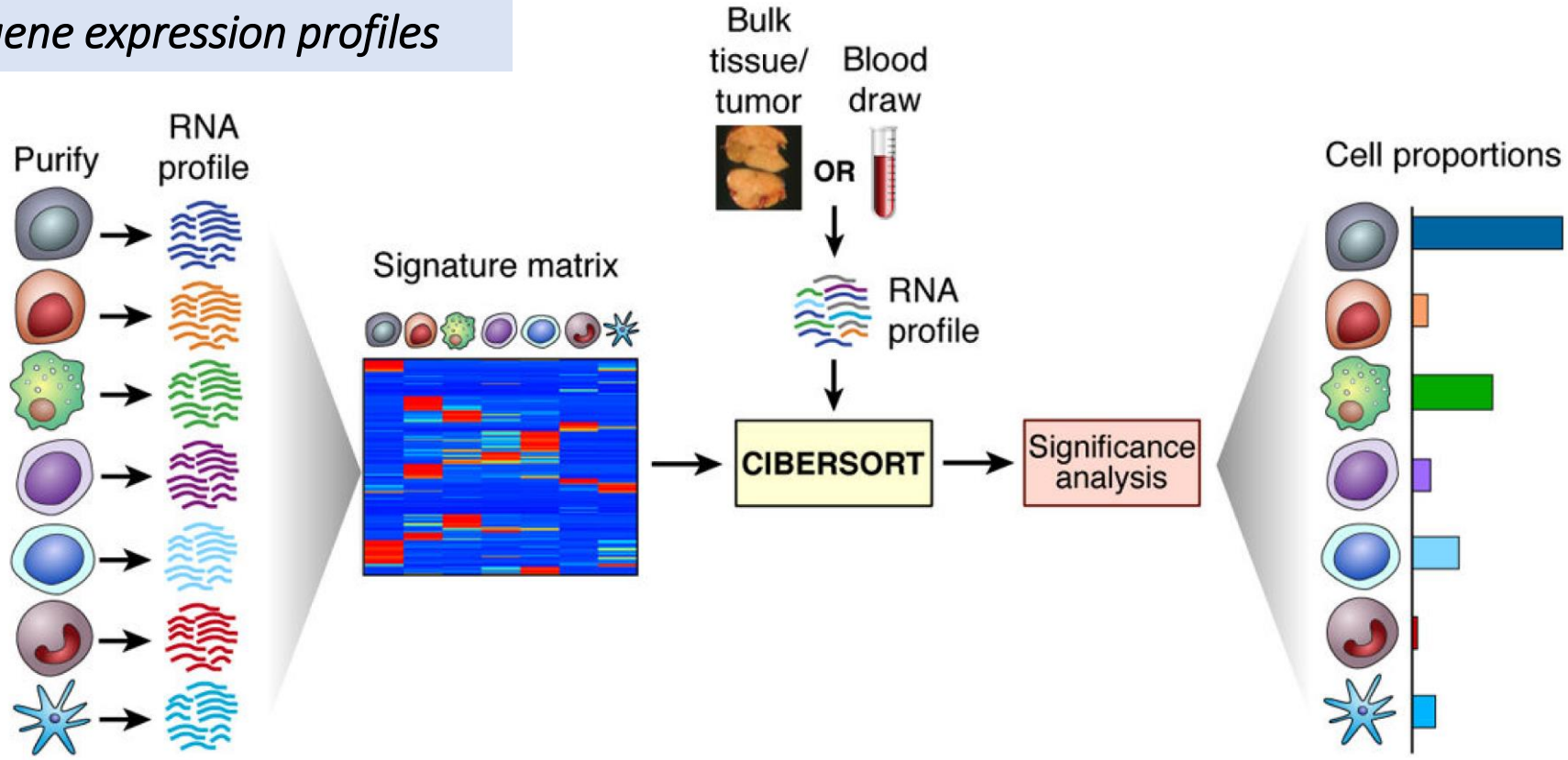
CD8+ → cytotoxic T cells
CD4+ → helper T cells
CD20+ → B cells
CD68+ → Macrophages
FoxP3+ → regulatory T cells
CK → Cytokeratin (epithelial cells)

Cluster characterized by high **CD4**, **CD8**, **CD20 stromal-TILs** and **CD20 intratumoral-TILs** associated with **higher pCR rates** after **lapatinib + trastuzumab**

Lee KH, et al. BMC Cancer 2018
De Angelis C, et al. Clin Cancer Res 2019

Characterization of *TIL subpopulations – Bioinformatics tools*

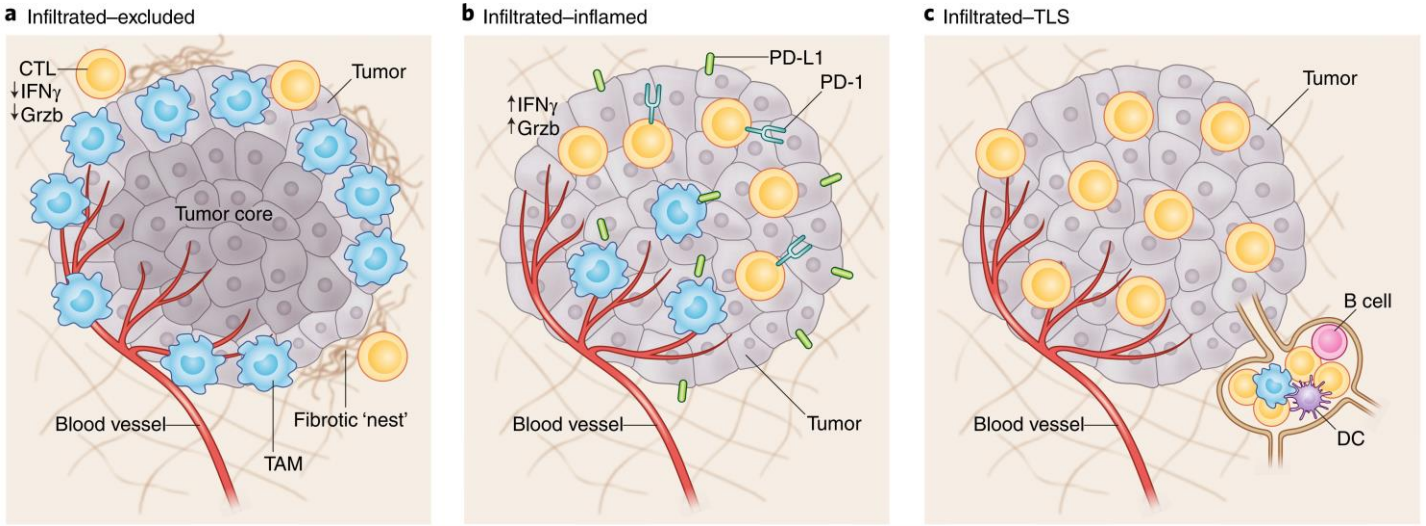
Computational methods can be used to estimate TIL **levels** and/or **subpopulations** from ***bulk tissue gene expression profiles***



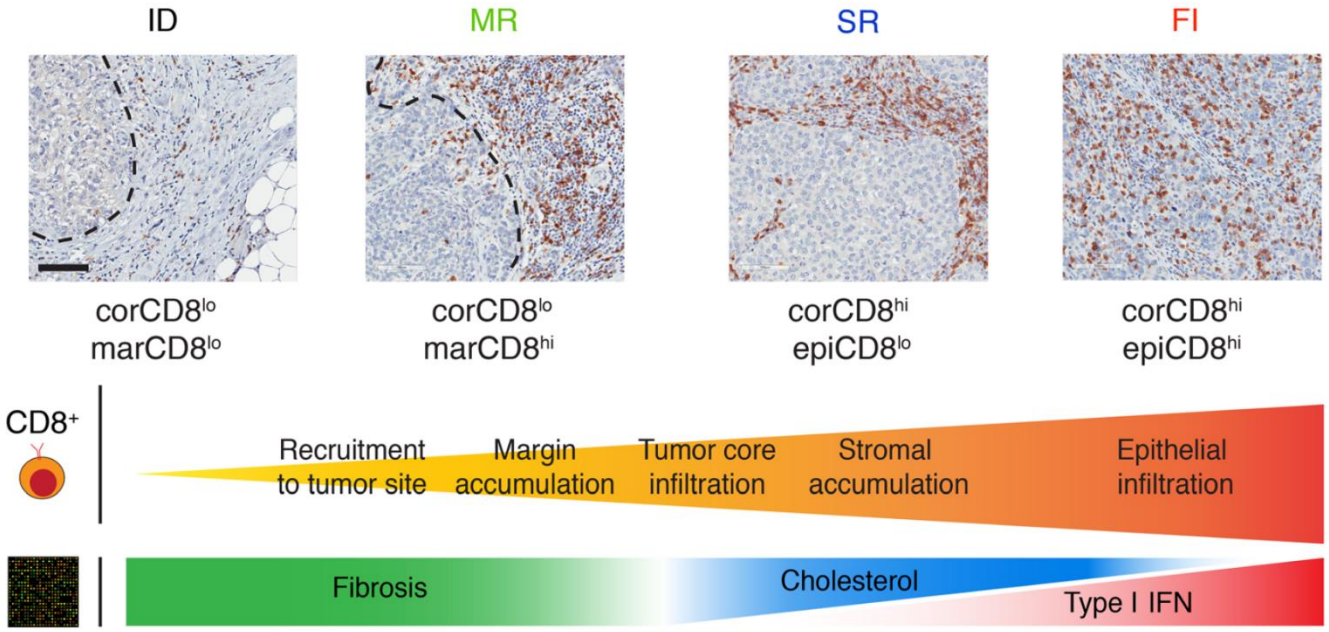
Overview of CIBERSORT

Chen B, et al. Methods Mol Biol 2018

Immune phenotyping – *geographic distribution*



TLS = tertiary lymphoid structures
CTL = cytotoxic lymphocyte
TAM = tumor-associated macrophages
DC = dendritic cell



Tumor Immune-Microenvironment (*TIME*) **classification** in TNBC

ID: Immune Desert

MR: Margin-Restricted

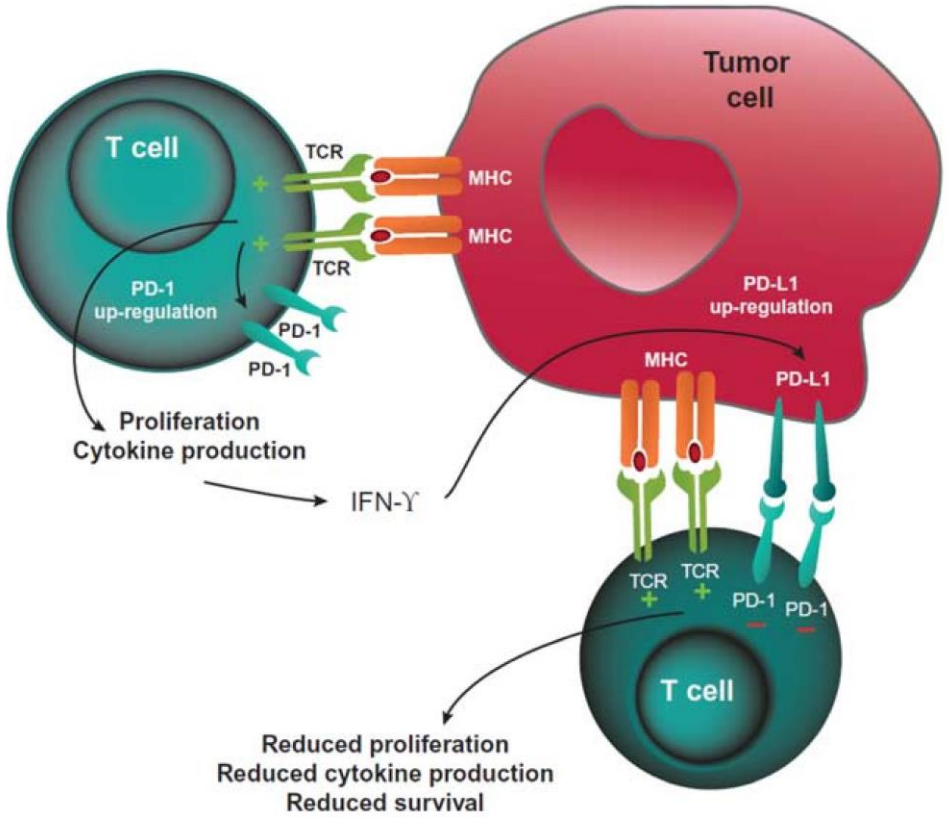
SR: Stroma-Restricted

FI: Fully Inflamed

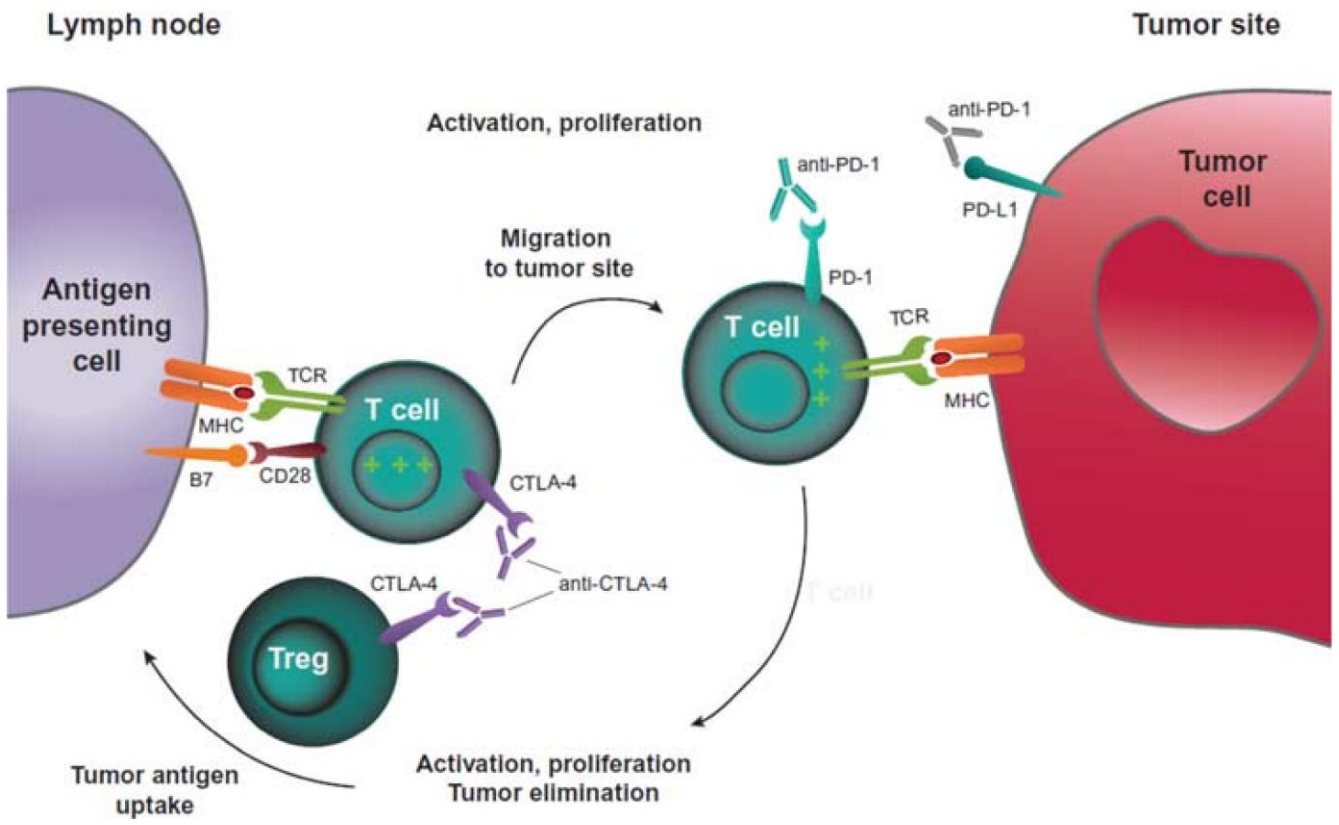
Binnewies M, et al. Nat Med 2018
Gruosso T, et al. J clin Invest 2019

TILs and *PD1/PD-L1* axis

PD-1-mediated inhibition of T cells

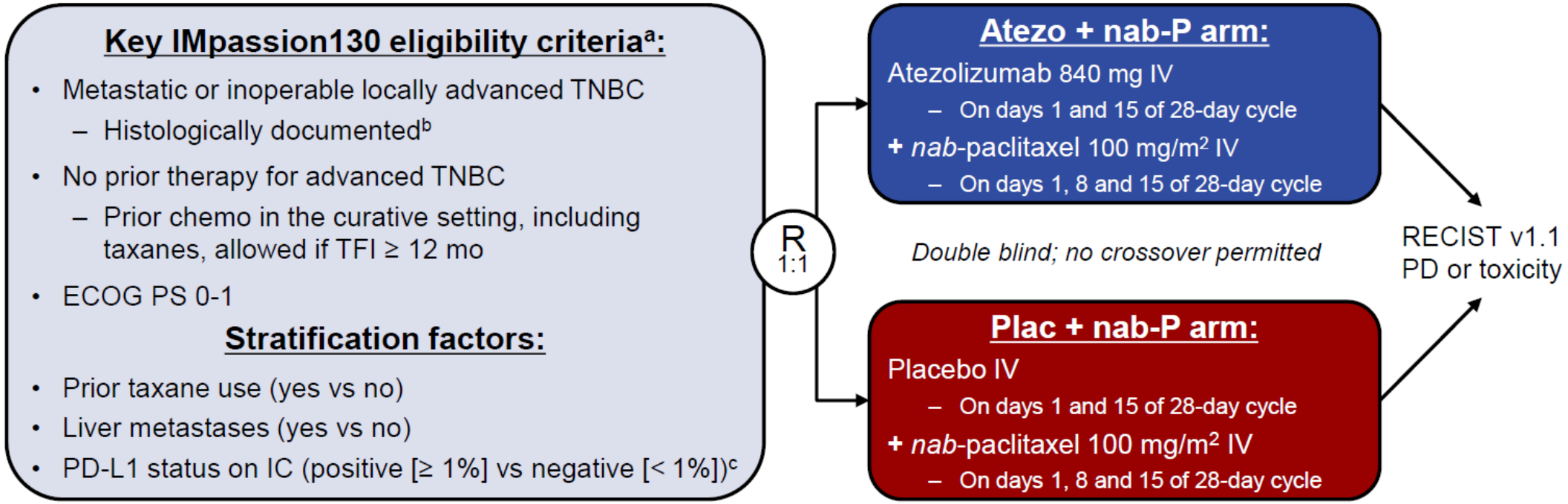


CTLA-4 and PD-1 pathway blockade



Buchbinder EI & Desai A. Am J Clin Oncol 2016

IMpassion130 – study design



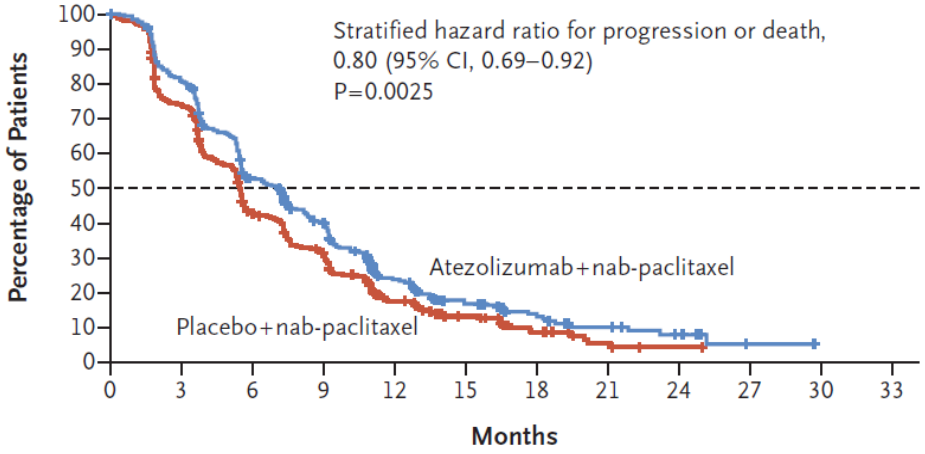
- Co-primary endpoints were PFS and OS in the ITT and PD-L1+ populations[d]
  - Key secondary efficacy endpoints (ORR and DOR) and safety were also evaluated

Schmid P, et al. NEJM 2018

IMpassion130 – results – **PFS**

A Progression-free Survival in the Intention-to-Treat Population

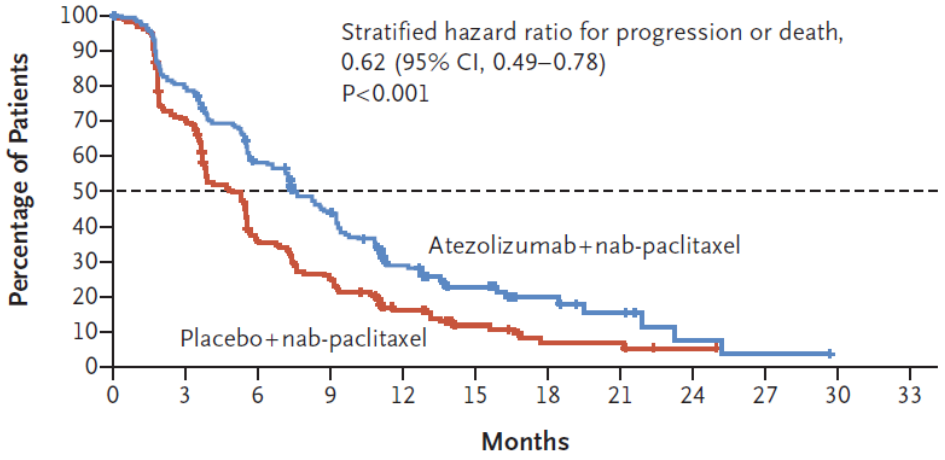
	No. of Events/ No. of Patients	Median Progression-free Survival (95% CI)	1-Yr Rate of Progression-free Survival (95% CI)
		mo	%
Atezolizumab+Nab-Paclitaxel	358/451	7.2 (5.6–7.5)	23.7 (19.6–27.9)
Placebo+Nab-Paclitaxel	378/451	5.5 (5.3–5.6)	17.7 (14.0–21.4)



No. at Risk												
Atezolizumab+ nab-paclitaxel	451	360	226	164	77	34	20	11	6	1	NE	NE
Placebo+ nab-paclitaxel	451	327	183	130	57	29	13	5	1	NE	NE	NE

B Progression-free Survival in the PD-L1–Positive Subgroup

	No. of Events/ No. of Patients	Median Progression-free Survival (95% CI)	1-Yr Rate of Progression-free Survival (95% CI)
		mo	%
Atezolizumab+Nab-Paclitaxel	138/185	7.5 (6.7–9.2)	29.1 (22.2–36.1)
Placebo+Nab-Paclitaxel	157/184	5.0 (3.8–5.6)	16.4 (10.8–22.0)



No. at Risk												
Atezolizumab+ nab-paclitaxel	185	146	104	75	38	19	10	6	2	1	NE	NE
Placebo+ nab-paclitaxel	184	127	62	44	22	11	5	5	1	NE	NE	NE

Schmid P, et al. NEJM 2018

Table 1 | **Factors that predict response to immune checkpoint inhibitor therapy**

Factor	Association with favourable clinical outcome	Validated in phase III clinical trial?	Predictive versus prognostic[a]	Cancer type	Tissue type for biomarker assessment[b]	Possible assay type for biomarker assessment
Tumour mutation burden	Positive	Yes	Predictive	Multiple cancer types	Blood or tumour tissue	NGS WES or targeted gene panel sequencing
PDL1 expression	Positive	Yes	Predictive	Multiple cancer types	Tumour tissue	Immunohistochemistry
Copy number variation	Negative	TBD	Prognostic, predictive or both	Multiple cancer types	Tumour tissue	NGS WES or targeted gene panel sequencing
HLA class I diversity	Positive	TBD	Predictive	Melanoma and NSCLC	Blood	NGS WES or PCR-based typing
LOH at HLA class I alleles	Negative	TBD	Predictive	Melanoma	Tumour tissue	TBD
T cell repertoire clonality change	Positive	TBD	Predictive	Melanoma	Tumour tissue or blood	TBD
T cell-inflamed microenvironment	Positive	TBD	Prognostic, predictive or both	Multiple cancer types	Tumour tissue	NGS RNA-seq or immunostaining
SERPINB3 or *SERPINB4* mutations	Positive	TBD	Predictive	Melanoma	Tumour tissue	NGS WES
Gut microbial diversity	Positive	TBD	Predictive	Melanoma	Oral or gut	PCR or NGS
Specific gut microbial species	Positive or negative	TBD	Predictive	Melanoma	Oral or gut	PCR or NGS
TGFβ expression	Negative	TBD	Predictive	Colon cancer and urothelial cancer	Tumour tissue	NGS RNA-seq or expression panel
Mutations in the β-catenin pathway	Negative	TBD	Predictive	Melanoma	Tumour tissue or blood	NGS WES, targeted gene panel sequencing or RNA-seq
JAK2 mutations (rare)[c]	Negative	TBD	Predictive	Melanoma	Tumour tissue or blood	NGS WES or targeted gene panel sequencing
B2M mutations (rare)[c]	Negative	TBD	Predictive	Melanoma	Tumour tissue or blood	NGS WES or targeted gene panel sequencing
STK11 mutations (common)	Negative	TBD	Predictive	NSCLC	Tumour tissue or blood	NGS WES or targeted gene panel sequencing

HLA, human leukocyte antigen; LOH, loss of heterozygosity; NSCLC, non-small-cell lung cancer; NGS, next-generation sequencing; PDL1, programmed cell death 1 ligand 1; RNA-seq, RNA sequencing; TBD, to be determined; TGFβ, transforming growth factor-β; WES, whole-exome sequencing. [a]Predictive refers to a given biomarker that has an effect dependent on the immune checkpoint inhibitor therapy, and prognostic refers to a biomarker that has a specific effect independent of the therapy. [b]Blood detection of mutations refers to cell-free DNA analysis. [c]*JAK2* and *B2M* mutations are controversial. Responses have been seen in patients with these mutations. Intratumoural heterogeneity likely needs to be assessed along with these mutations.

Havel J, et al. Nat Rev Cancer 2019

Outline

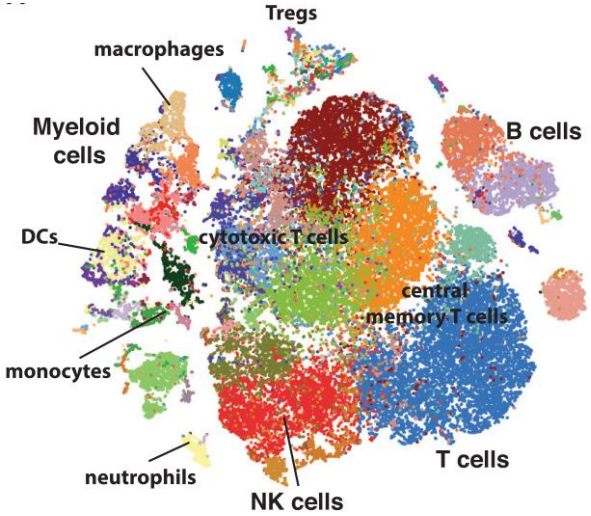
- Introduction to the clinical classification
- Molecular characterization and intertumor heterogeneity

  → Examples of clinical applications

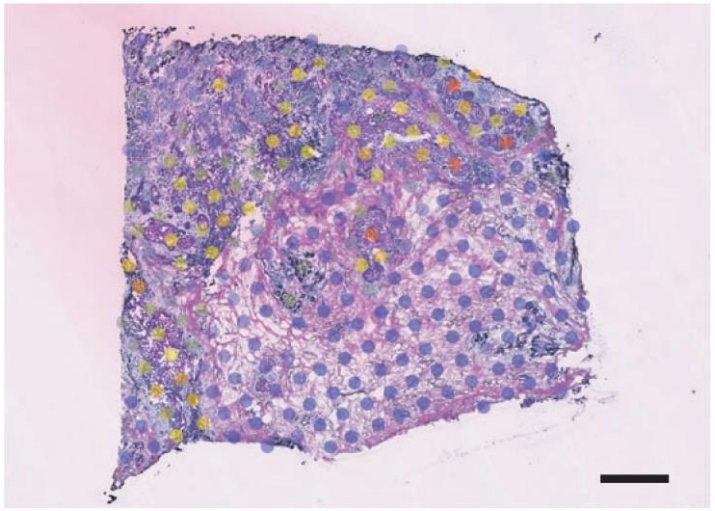
- The challenge of intratumor heterogeneity
- The role of the tumor immune microenvironment
- ***Novel translational research tools*** and ***future perspectives***

Technological advances

Single-cell analysis



Spatial transcriptomics



Azizi E, et al. Cell 2018
Salmén F, et al. Nat Protoc 2018

Single-cell analysis

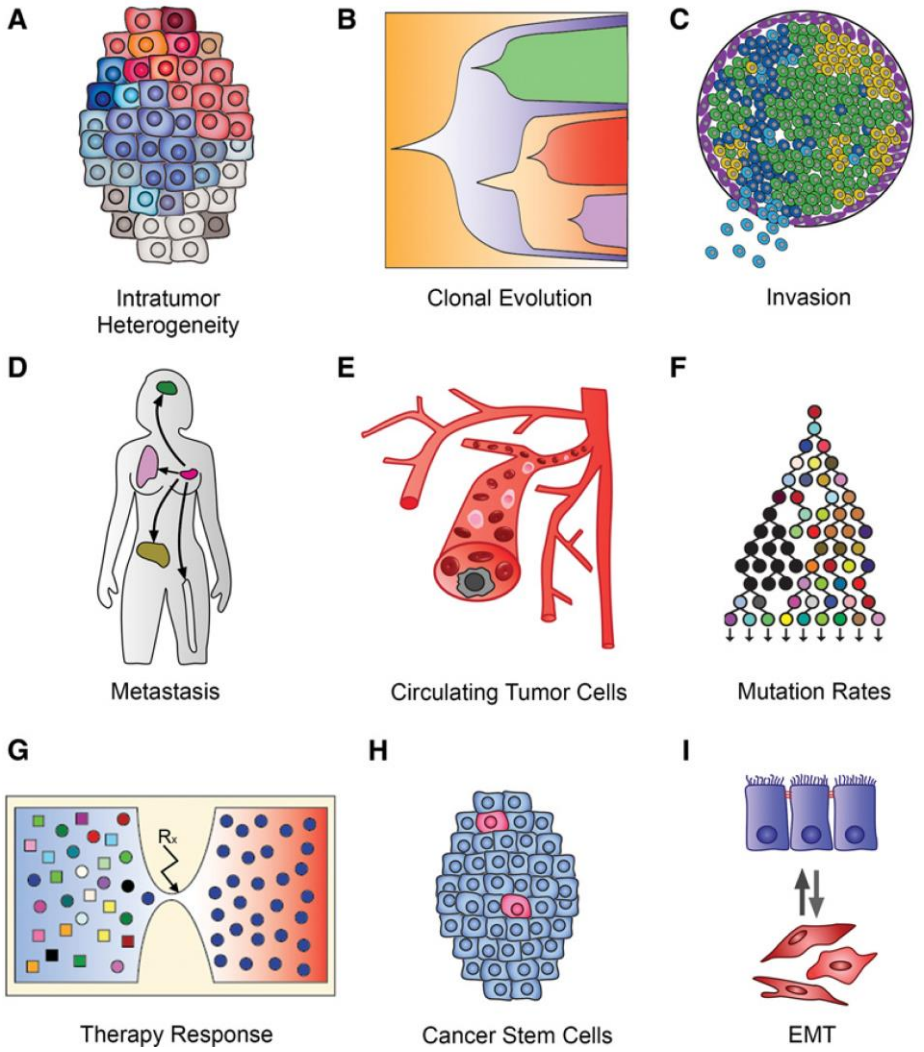
Bulk tissue → mixture of **different types of cells** (tumor cell subpopulations, immune cells, stroma, …)

→ **Transcriptomics/genomics studies** use RNA/DNA sequencing of **homogenized samples**

→ Averaged transcriptome and mixture of mutational/CNAs data from **different cell types**

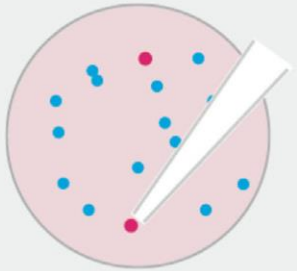
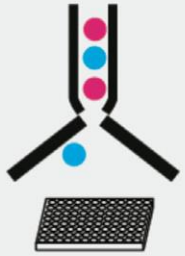
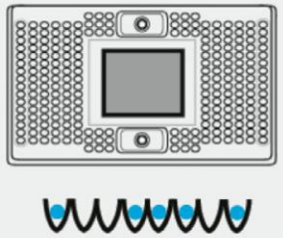
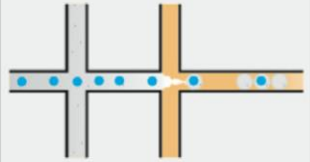
- Examples of single-cell "omics" techniques:
- **RNA-sequencing**
- **DNA-sequencing** (e.g. for CNV)
- ATAC-sequencing (for single-cell **epigenomics**)
- **Immune profiling** (e.g. cell surface proteins, antigen specificity)

Applications of single-cell sequencing in cancer research



Navin NE. Genome Res 2015

Single cell ***isolation methods*** for RNA-seq

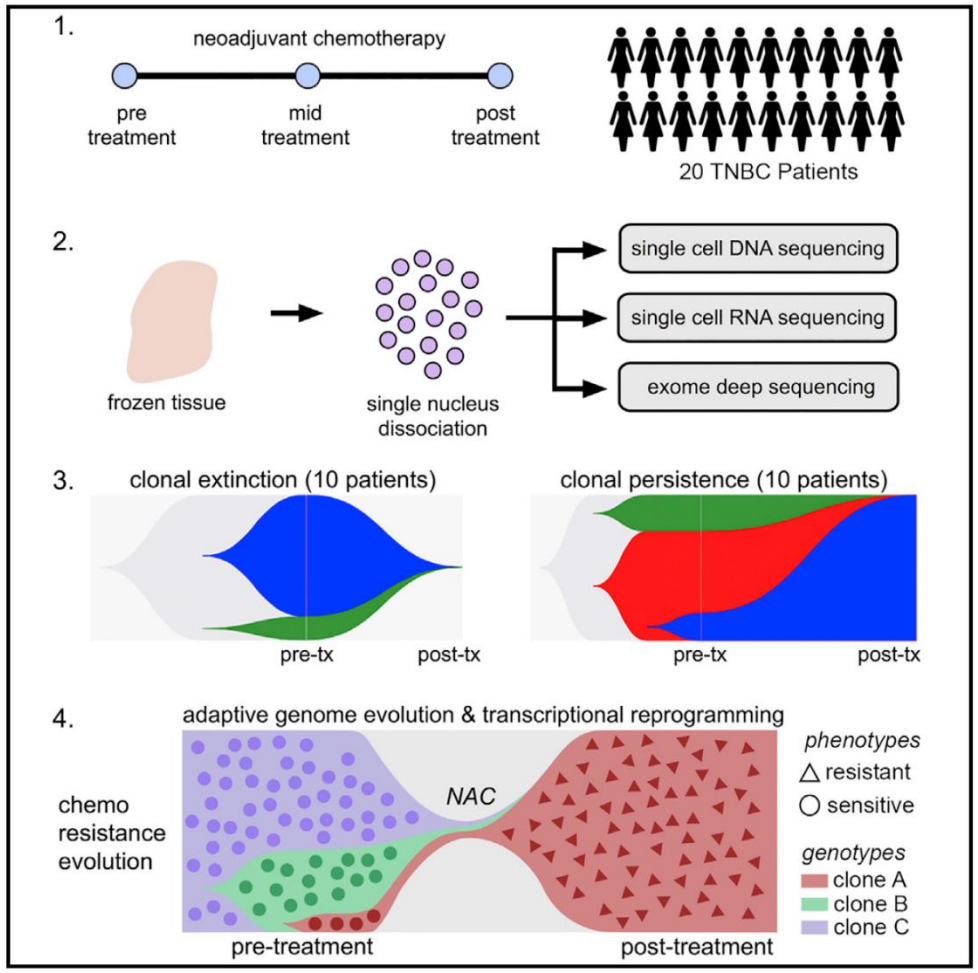
	Micro-manipulation / Automated Pipetting	FACS	Microwell encapsulation	Droplet encapsulation
				
Cell Stress	Low	Moderate	Moderate	Moderate
Selection	Yes	Yes	No* / Yes++	No*
Doublet	Low	Low	Low-High	Moderate
Throughput	Low	Moderate	Moderate	High
Capture efficency	Low	Moderate	Moderate	Low-Moderate
Academic / Commerical scRNA workflow	- CellenONE (Cellenion)‡ - Smart-Seq2 (42)	- MARS-Seq (39) - Smart-Seq2 (42)	- C1 (Fluidigm) - ddSeq (Biorad / Illumina) - ICell8 (Clontech)++ - Rhapsody (BD)	- InDrop (1CellBio) - DropSeq (Dolomite-bio) - 10X (Chromium)
Example of use	Fragile rare cells	Rare cells based on phenotype or marking	Large cell numbers	Large cell numbers

‡Automated pipetting system
*Preselection or enrichment can be performed prior
++Only reagents added to wells containing singlets, determined by system
FWP: Fluidigm white paper
PB: Product brochure / manual

Nguyen A, et al. Front Immunol 2018

Chemoresistance evolution in TNBC delineated by single-cell sequencing

Graphical Abstract



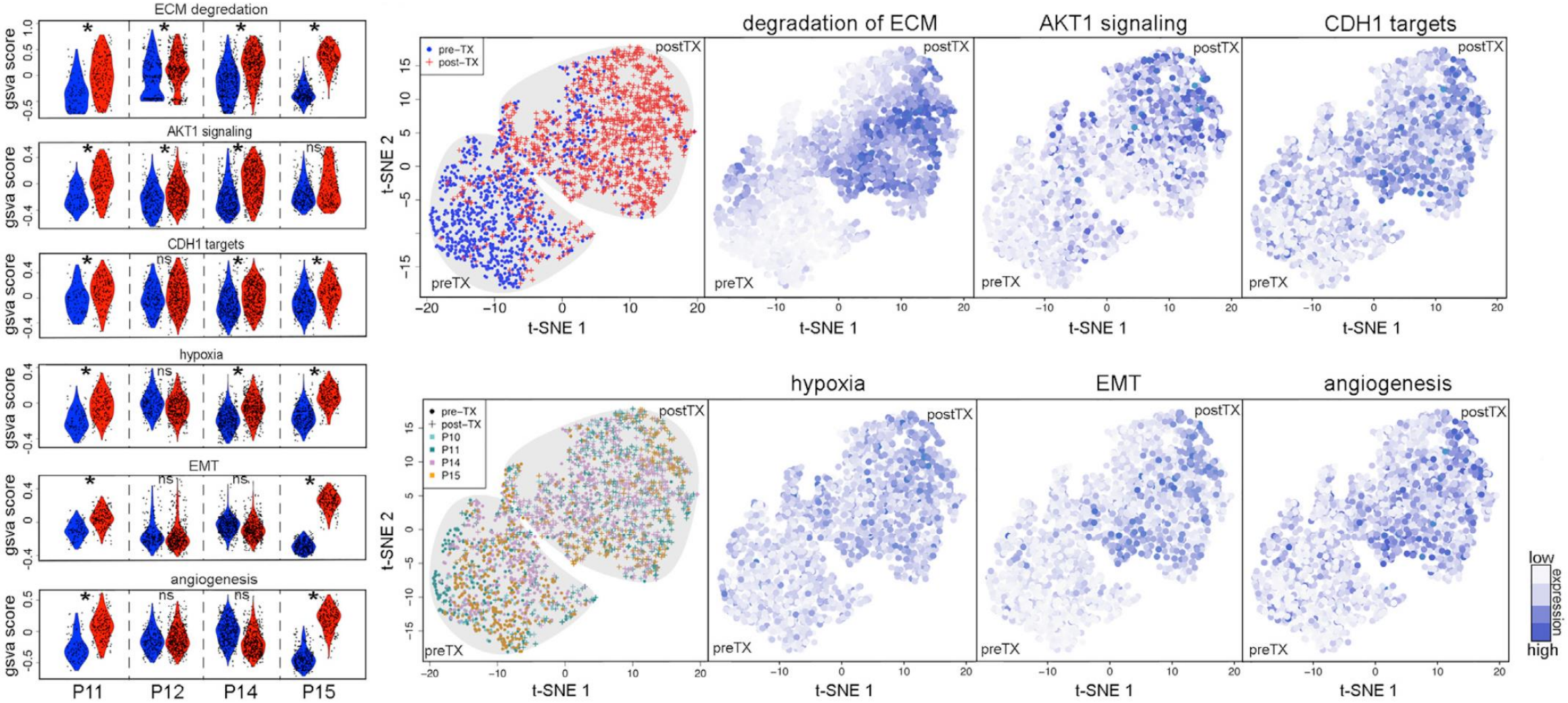
30-50% of TNBCs are **resistant** to NeoADJ chemotherapy
→ lack of genomic biomarkers

Highlights

- **Single-cell sequencing of breast cancer patients treated with chemotherapy**
- **Patients showed clonal persistence or extinction in response to therapy**
- **Resistance occurred through adaptive selection of pre-existing genomic aberrations**
- **Chemotherapy induced transcriptional reprogramming of resistant signatures**

Kim C, et al. Cell 2018

Gene signatures associated with ***chemoresistance*** in TNBC
→ **transcriptional reprogramming** and **therapeutic opportunities** to overcome resistance



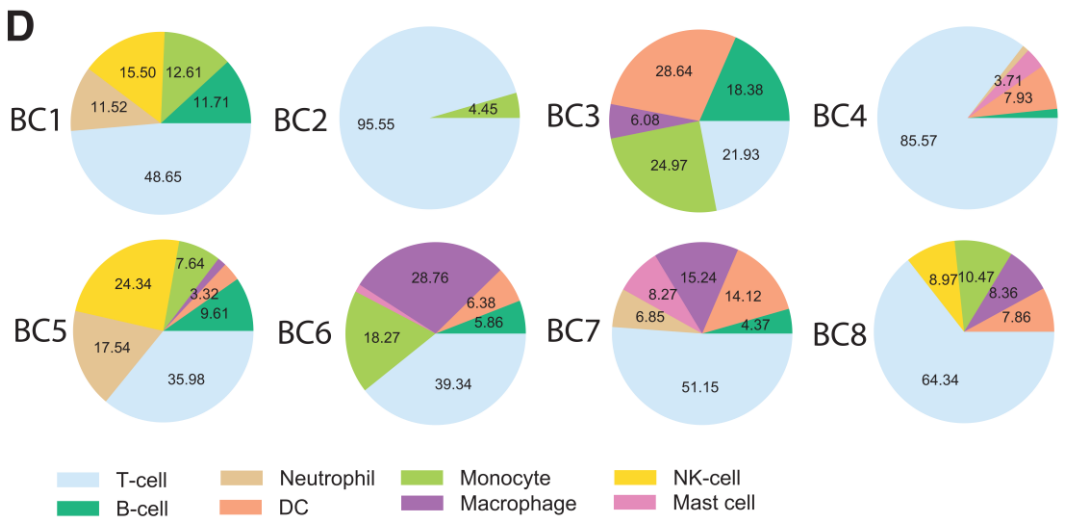
Combined single-cell data from four clonal persistence patients

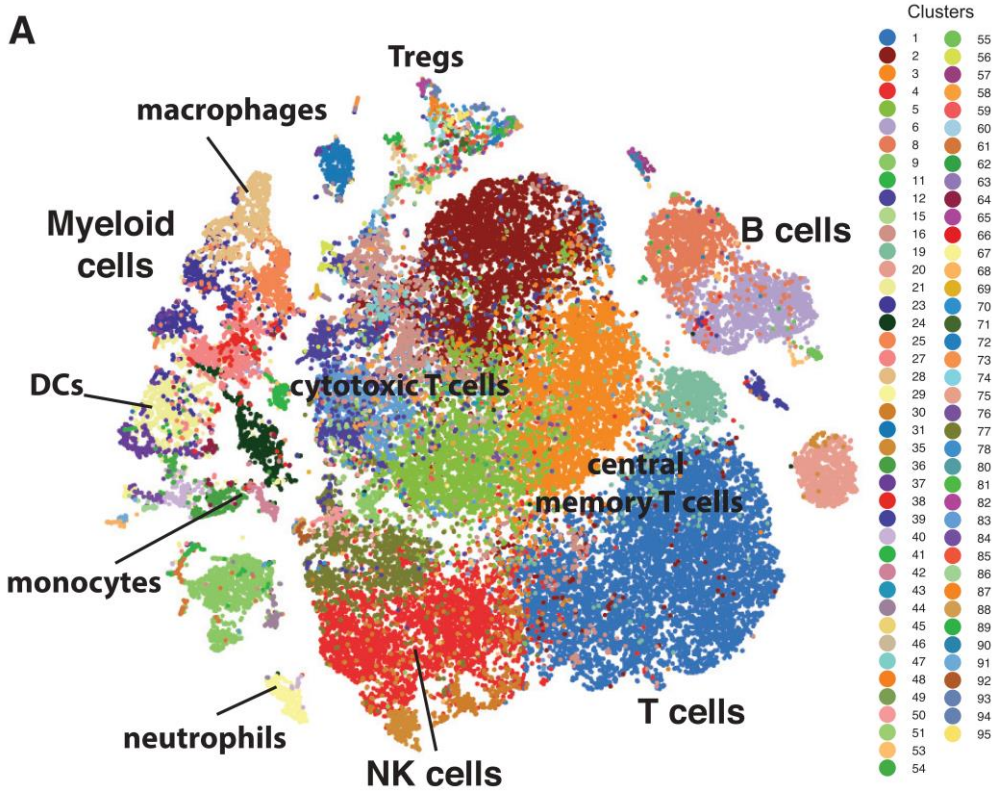
Kim C, et al. Cell 2018

Characterization of the tumor *immune microenvironment*

- **Single-cell RNA-sequencing** of breast tumor immune microenvironment to build ***immune atlas*** in breast carcinoma

- This atlas revealed **vast diversity** in immune cells of both the adaptive and innate immune systems





Breast immune cell atlas constructed from combining all patient samples (BC1-8) and tissues projected with t-SNE. Each dot represents a cell, colored by cluster.
t-SNE = t-distributed stochastic neighbor embedding

Azizi E, et al. Cell 2018

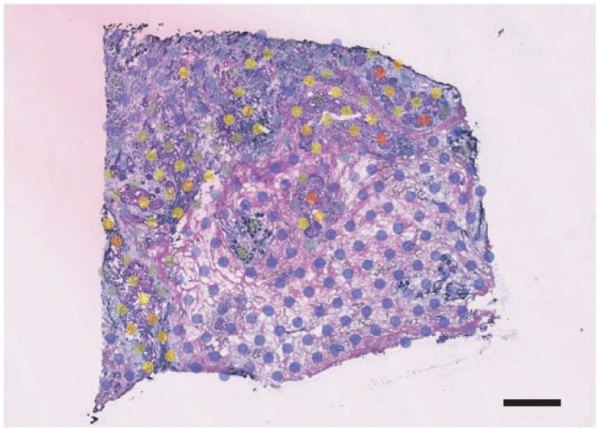
Spatially resolved transcriptomics

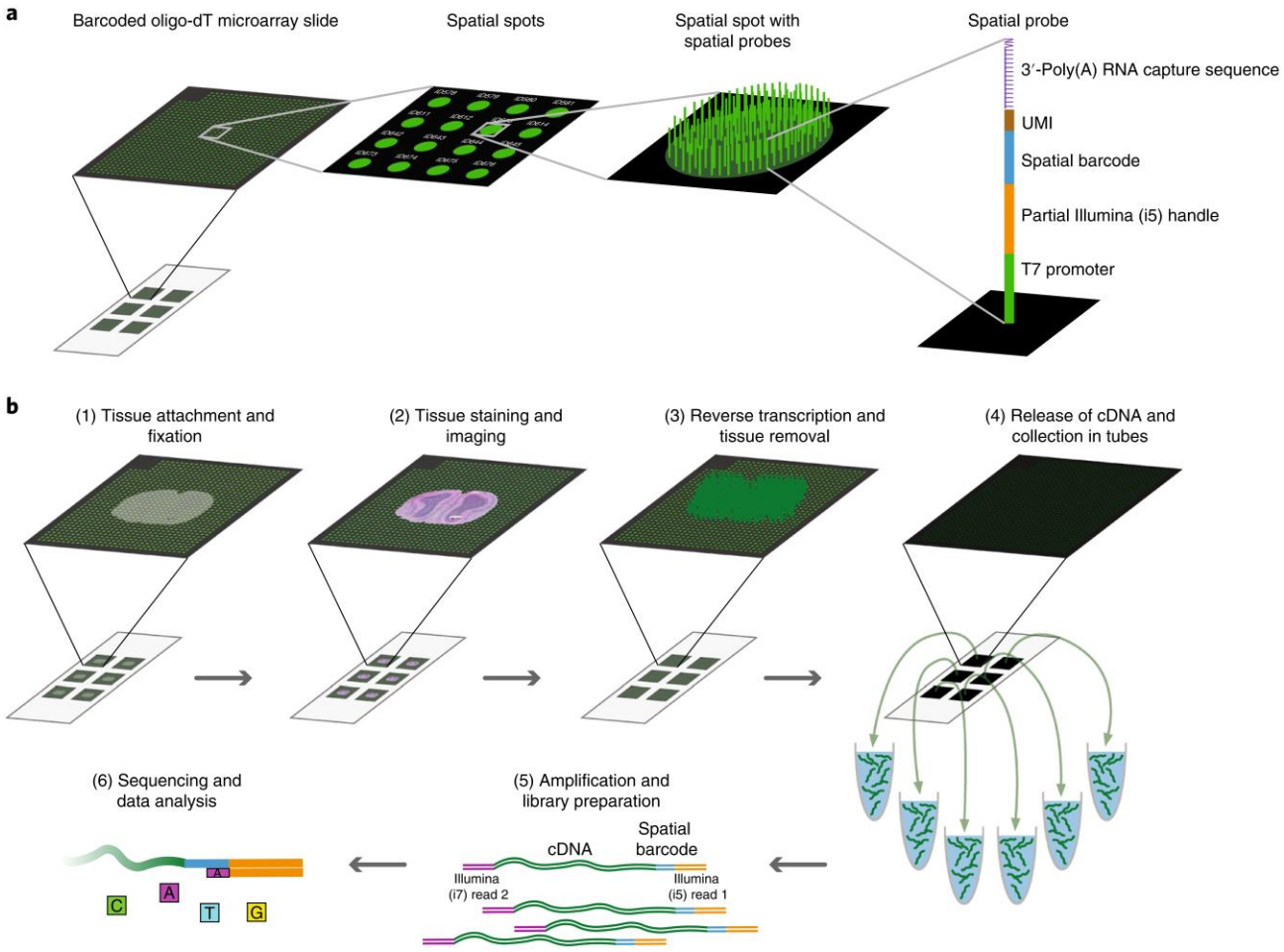
- **Single-cells** are collected from suspensions of dissociated tissue

→ *loss of spatial information*

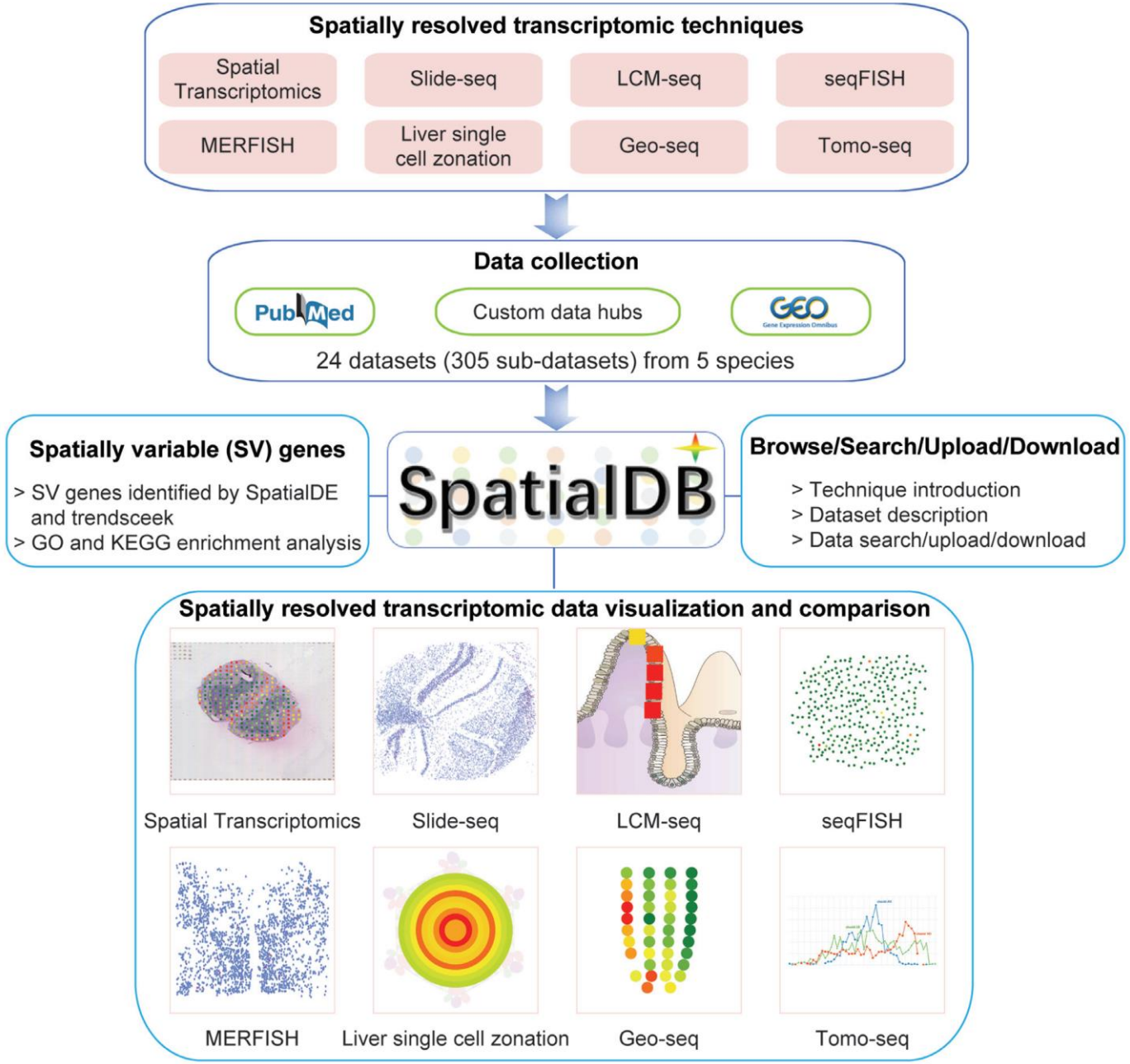
- Spatial transcriptomics allows to **retain** the **positional context** of *gene expression levels* and to combine those data with *morphological information*

- Resolution down to **1-10 cells**





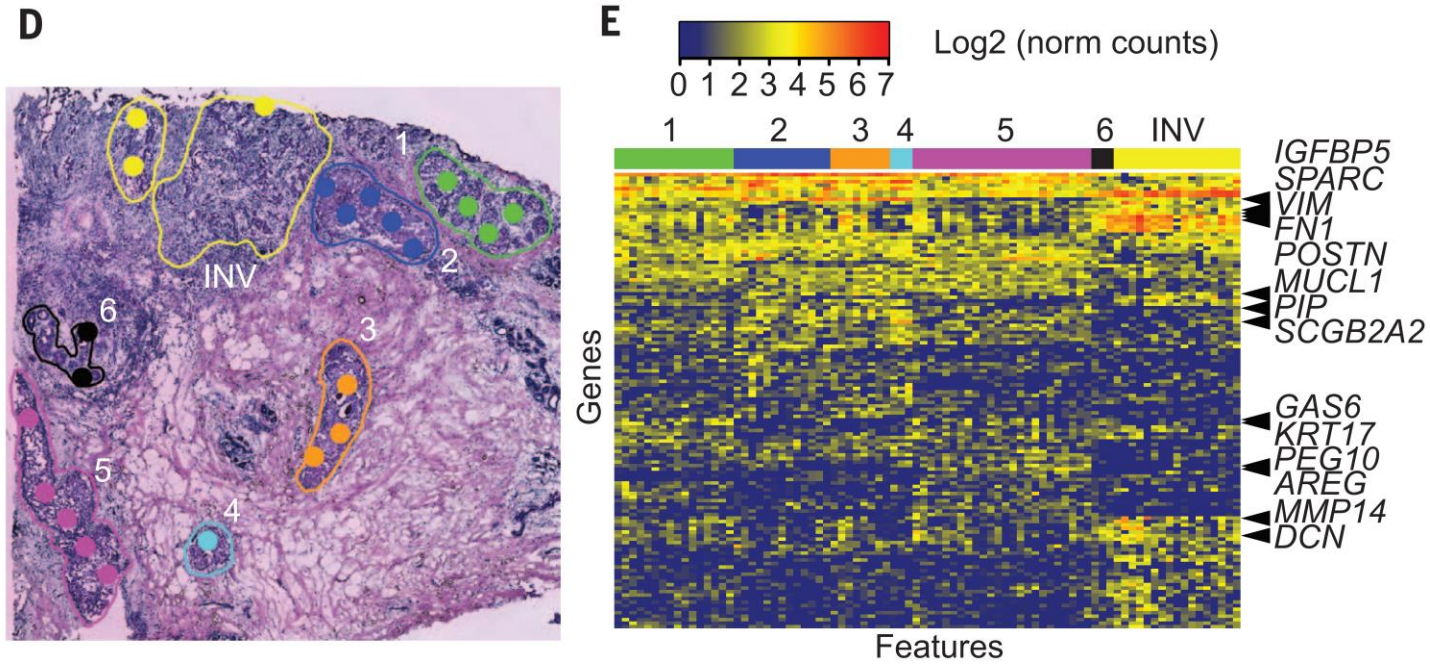
Ståhl PL, et al. Science 2016
Salmén F, et al. Nat Protoc 2018



Fan Z, et al. Nucleic Acids Res 2019

Spatial transcriptomics and ***tumor heterogeneity***

- Different tumor areas (e.g. ductal carcinoma *in situ*) can present high degree of **heterogeneity** in gene expression
- Unexpected level of heterogeneity within a biopsy, which would **not** be **possible to detect** with regular **bulk transcriptome analysis**

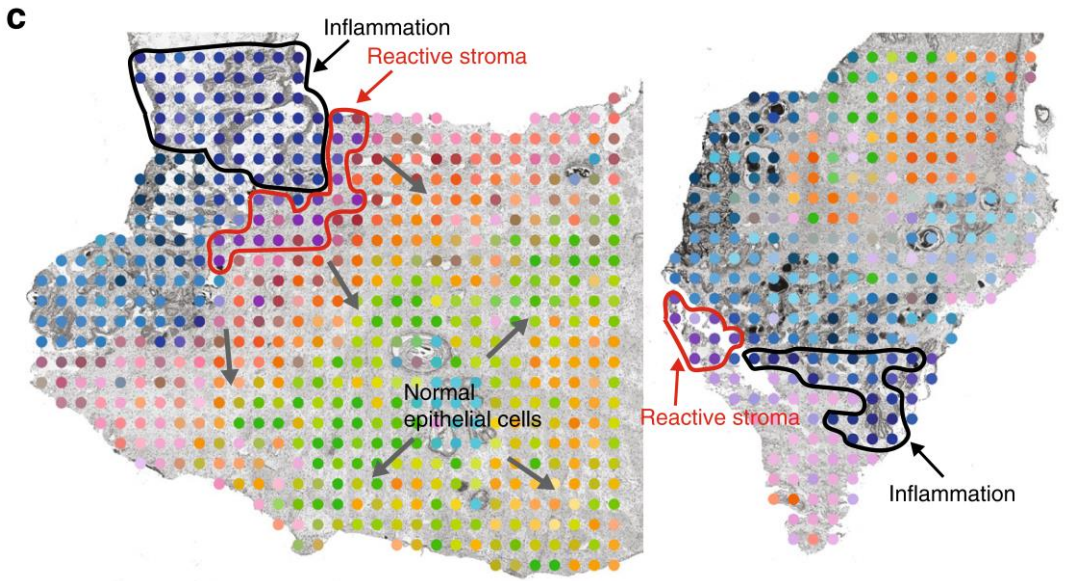


(**D**) Histological section of a breast cancer biopsy containing invasive ductal cancer (INV) and six separate areas of ductal cancer in situ (1 to 6),with analyzed spatial transcriptomics features.
(**E**) Gene expression heat map over the different areas in four adjacent sections (D)

Ståhl PL, et al. Science 2016
Salmén F, et al. Nat Protoc 2018

Spatial transcriptomics – *potential applications*

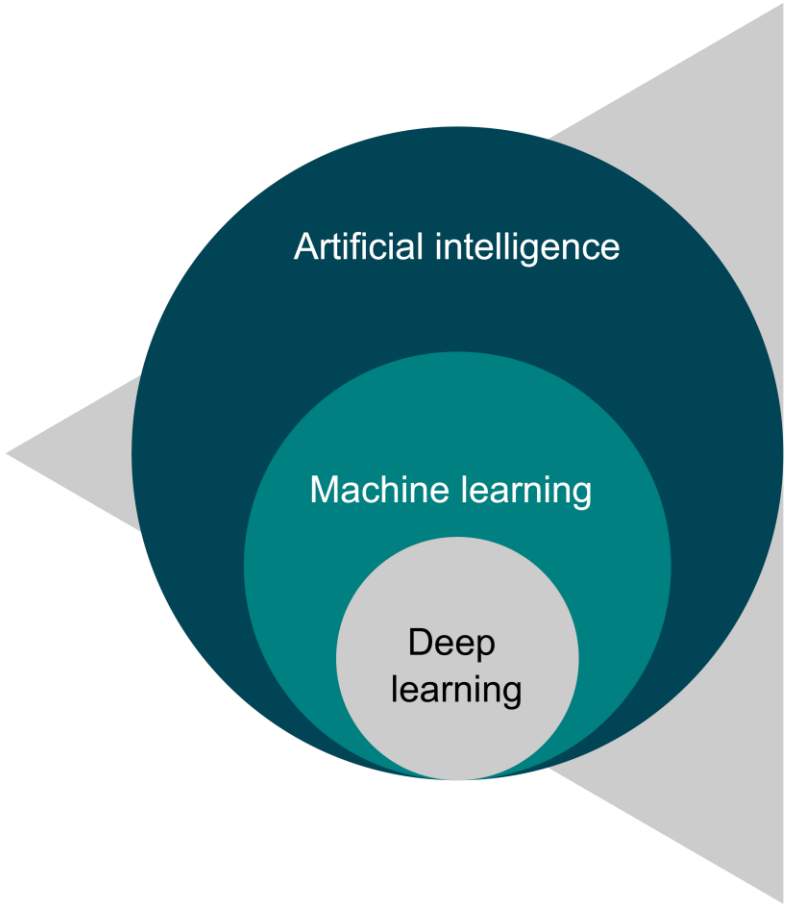
- **Measure gene activity** and **map biological processes** (e.g. EMT, CSC, immune response)
- Correlation between **gene expression** and **morphological** intratumor **heterogeneity**
- Characterization of heterogeneous tumor cell **subpopulations**
- Characterization of **cell-cell interactions** (e.g. tumor cells and microenvironment, including immune and stroma cells)



Stromal heterogeneity and reactive stroma in the microenvironment of inflammation in prostate cancer

Ståhl PL, et al. Science 2016
Berglund E, et al. Nat Commun 2018

Artificial Intelligence



Artificial intelligence

Artificial Intelligence is the science of making machines do things requiring human intelligence. It is human intelligence in machine format where computer programs develop data-based decisions and perform tasks normally performed by humans.
Artificial intelligence is any computer program that does something smart.

Machine learning

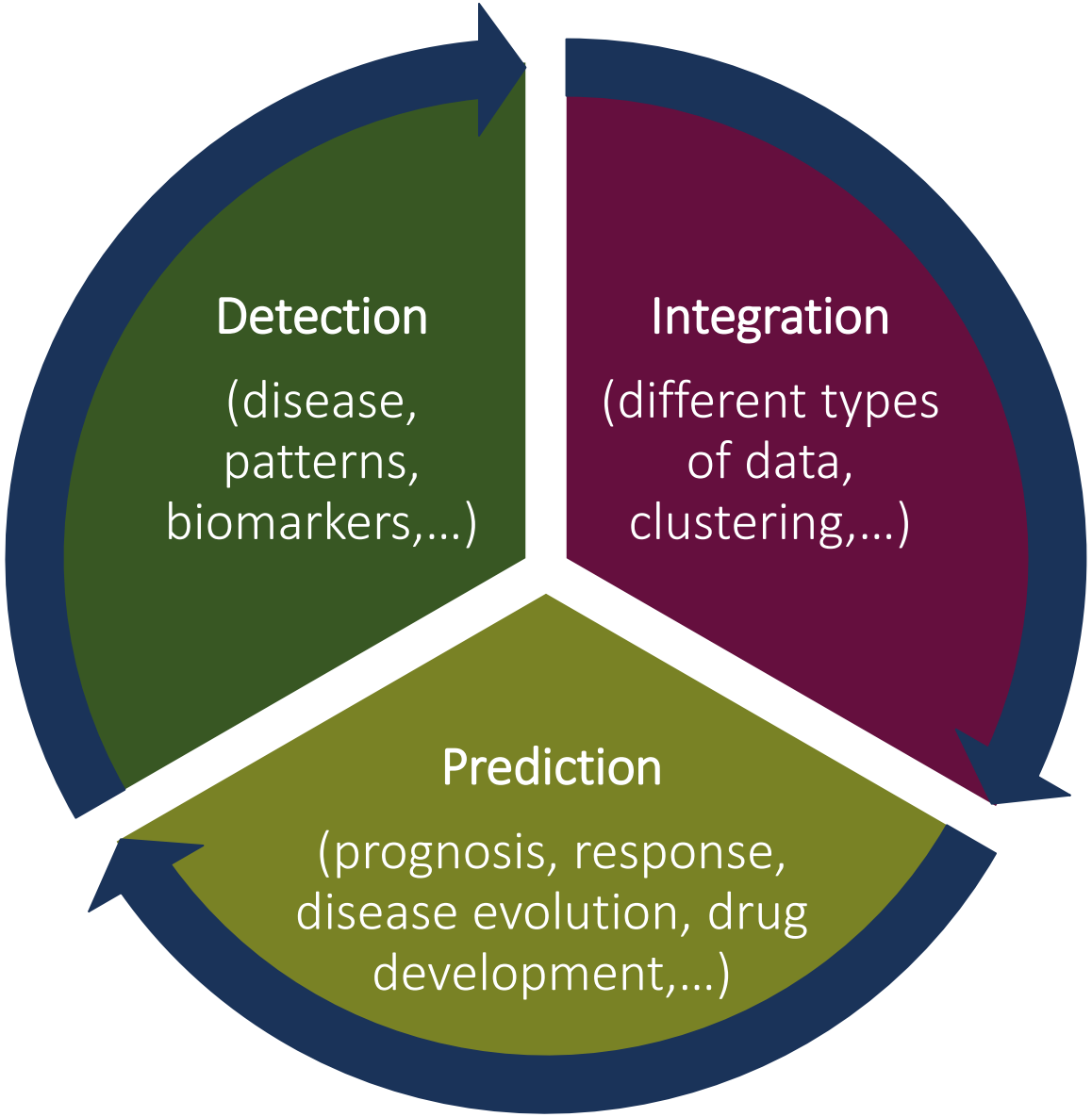
Machine learning takes artificial intelligence a step further in the way that algorithms are programmed to learn and improve without the need for human data input and reprogramming.

Deep learning

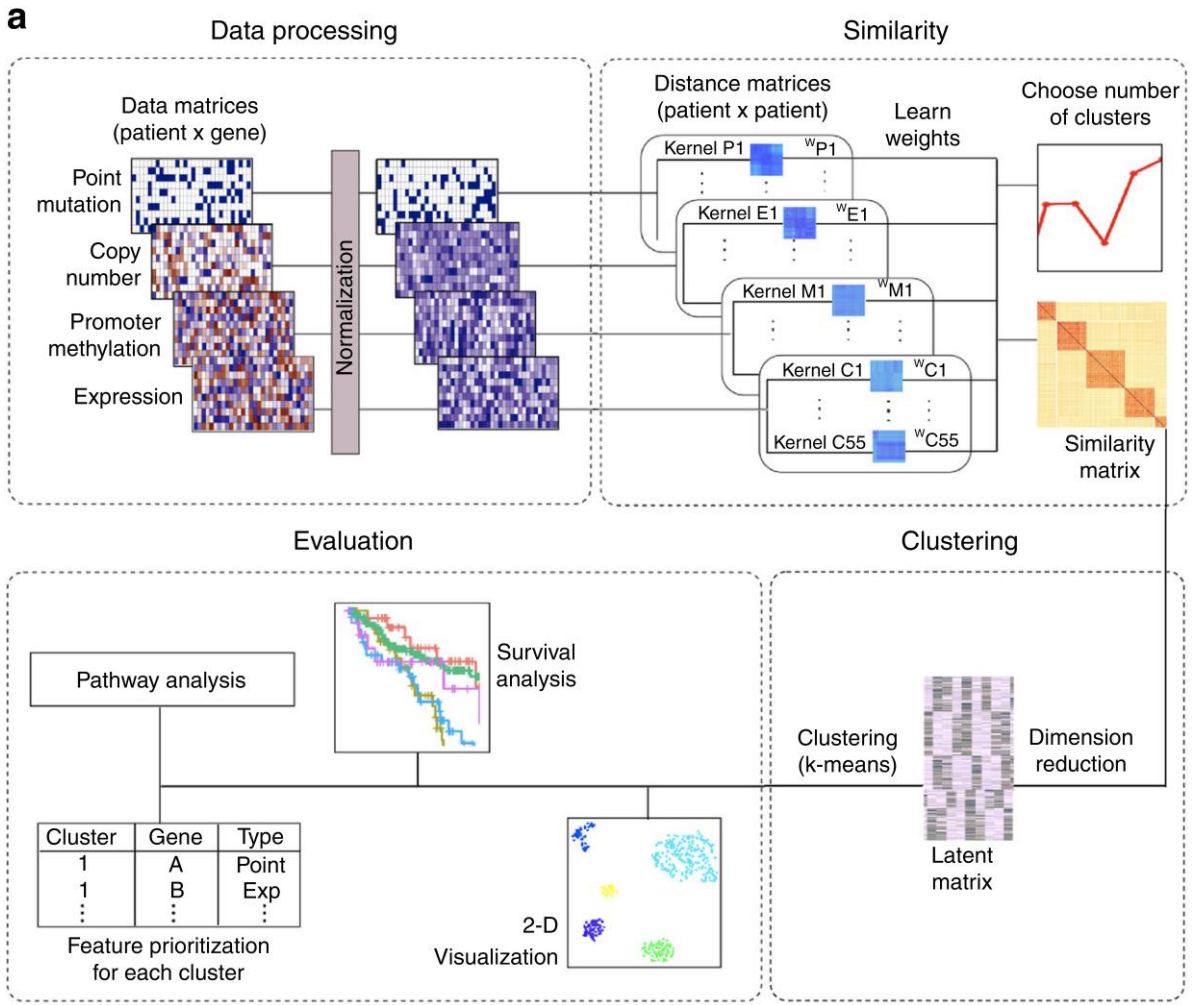
Deep learning is the next generation of machine learning that introduces multiple layers of learning from massive datasets. Deep learning decisions and data classifications are refined at each layer to produce accurate insights.

Chan HCS, et al. Trends Pharmacol Sci 2019

Artificial intelligence in oncology



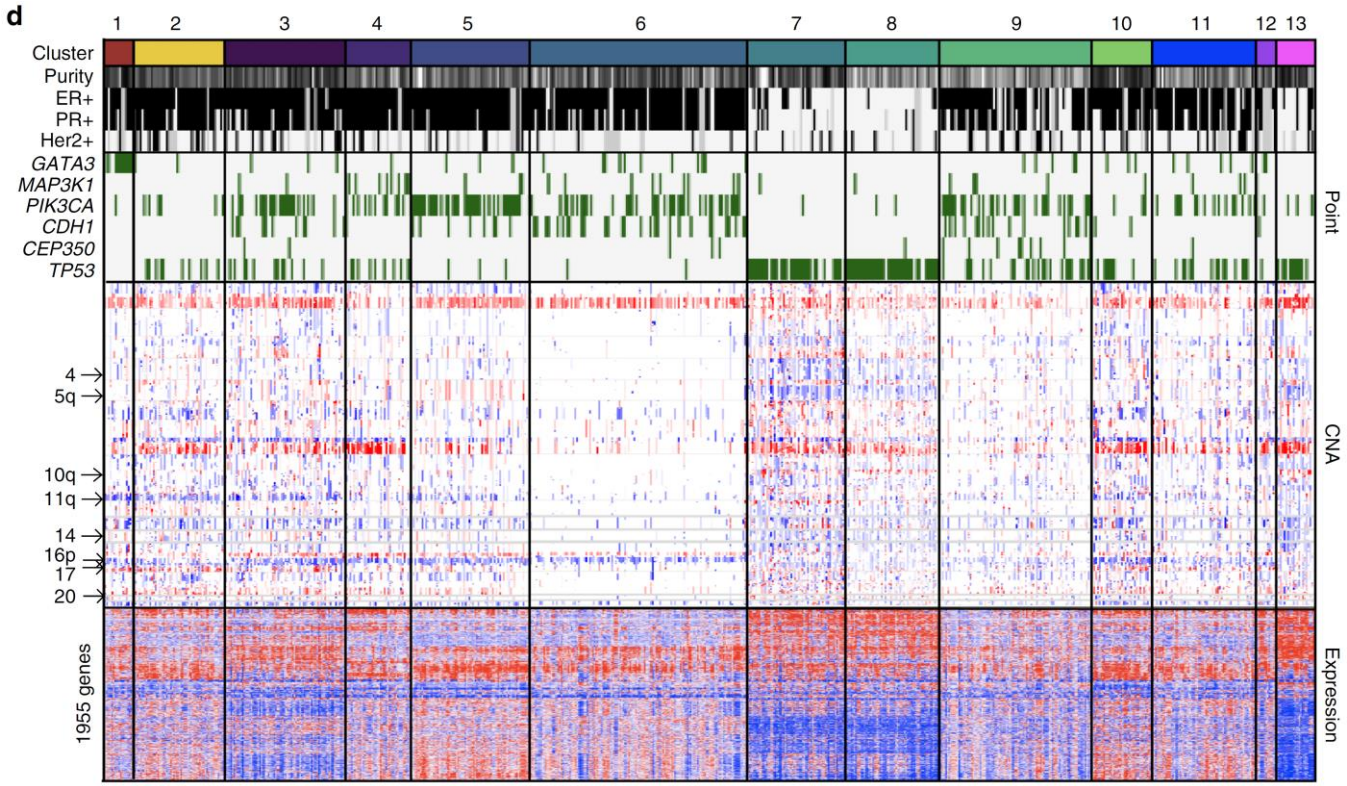
Integration of *multi-omic data* to predict prognosis



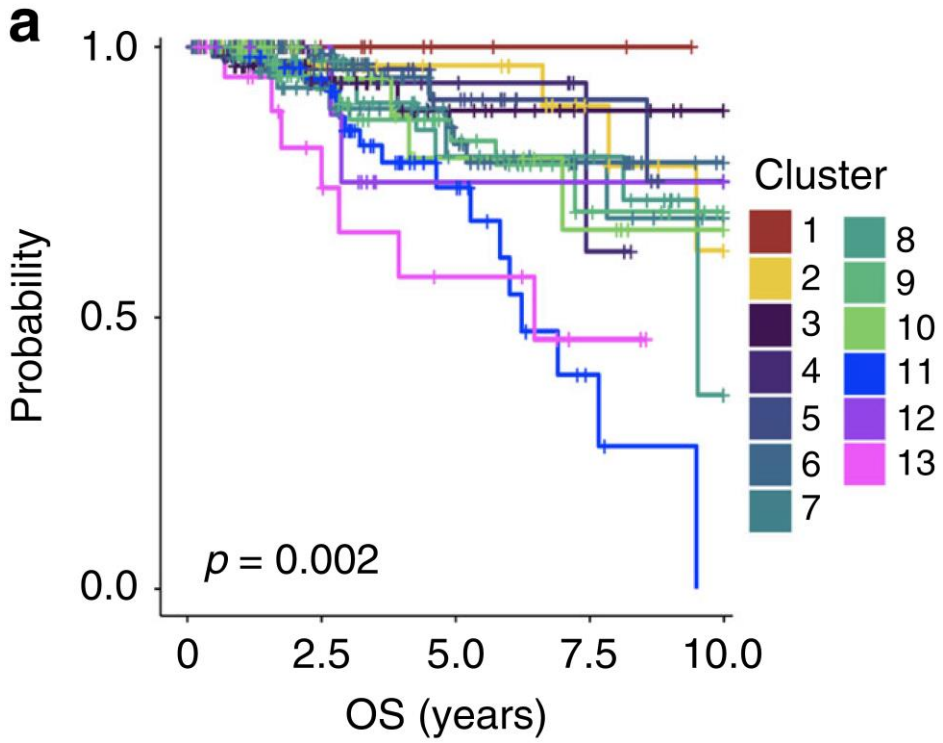
- Cancer Integration via Multikernel Learning (CIMLR) method applied to *multi-omic data* from **36 cancer types** from **TCGA** to reveal **molecular subtypes**
- Discovered ***subtypes*** exhibit significant differences in patient **survival** for 27 cancer types
- This method ***outperformed*** current state-of-the-art tools in **speed**, **accuracy**, and **prediction** of patient survival

Ramazzotti D, et al. Nat Commun 2018

CIMLR in breast cancer



In breast cancer, CIMLR separates 663 tumors into **13 clusters** with different overall and disease-specific survival



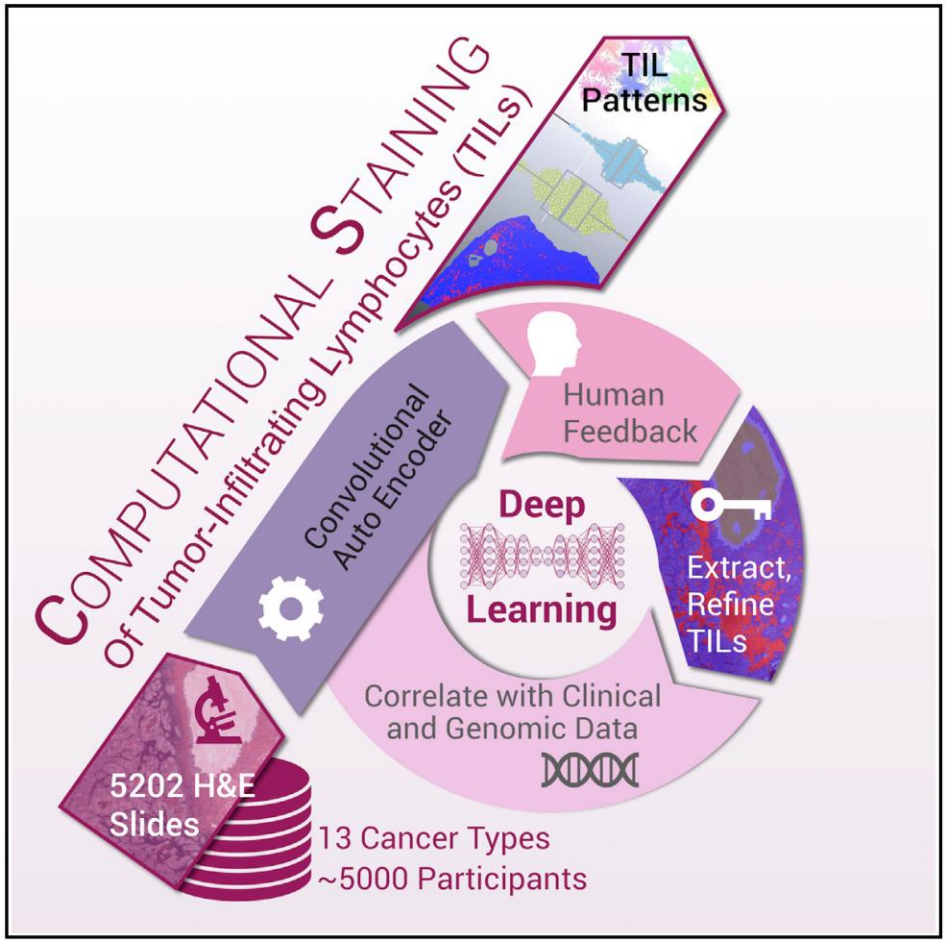
Kaplan–Meier curves showing **overall survival** for the 13 clusters of **breast cancer**

Ramazzotti D, et al. Nat Commun 2018

Digital pathology and *machine learning* to score Tumor Infiltrating Lymphocytes

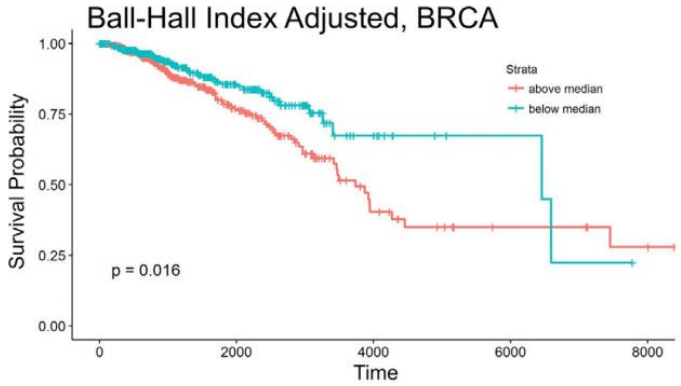
Highlights

- Deep learning based computational stain for staining tumor-infiltrating lymphocytes (TILs)
- TIL patterns generated from 4,759 TCGA subjects (5,202 H&E slides), 13 cancer types
- Computationally stained TILs correlate with pathologist eye and molecular estimates
- TIL patterns linked to tumor and immune molecular features, cancer type, and outcome

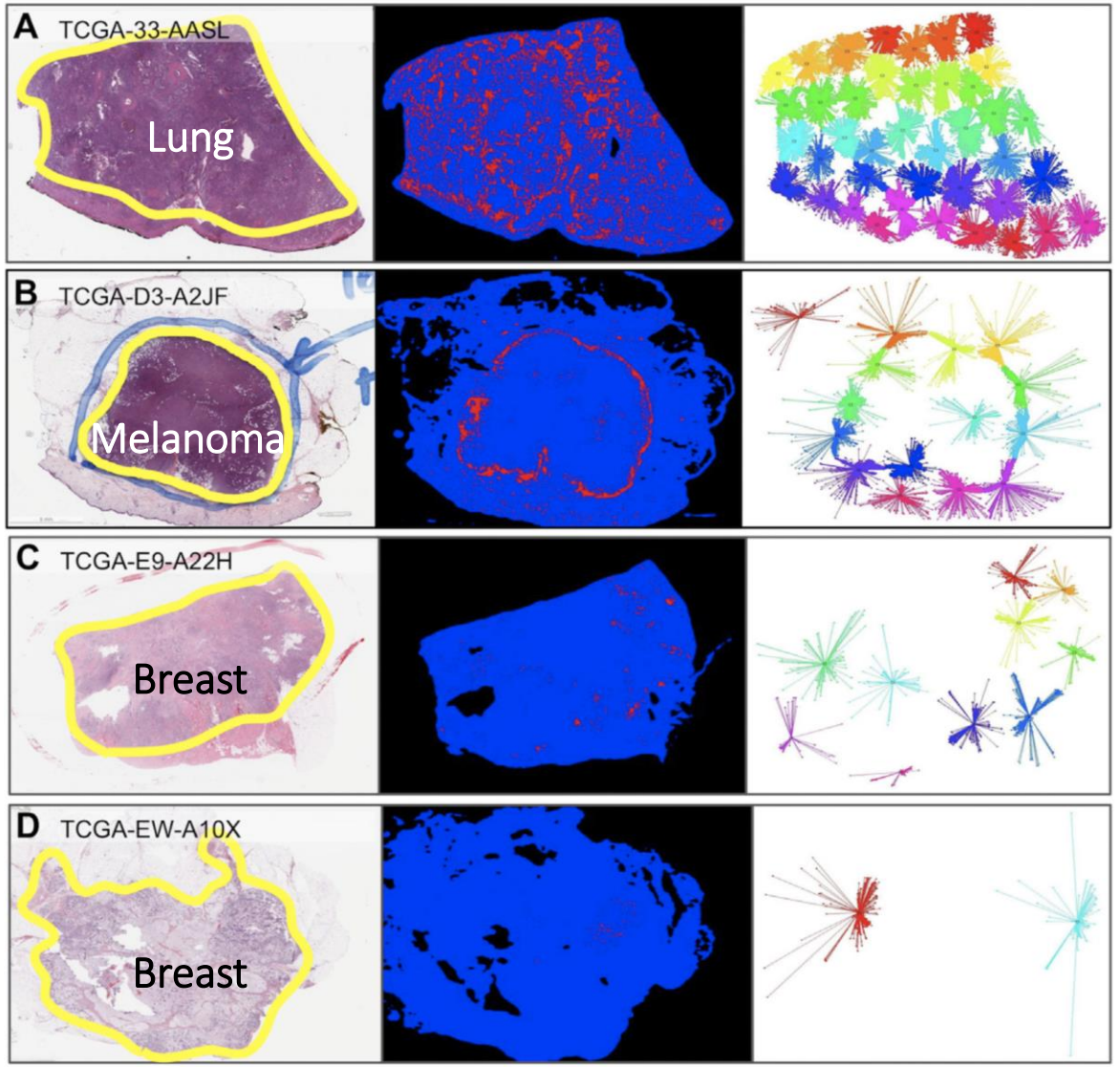


Saltz J, et al. Cell Rep 2018

Automated assessment of **local structures in the TIL infiltrate** and association with molecular and clinical readouts



Association of TIL Local Spatial Structure with survival in breast cancer (BRCA)

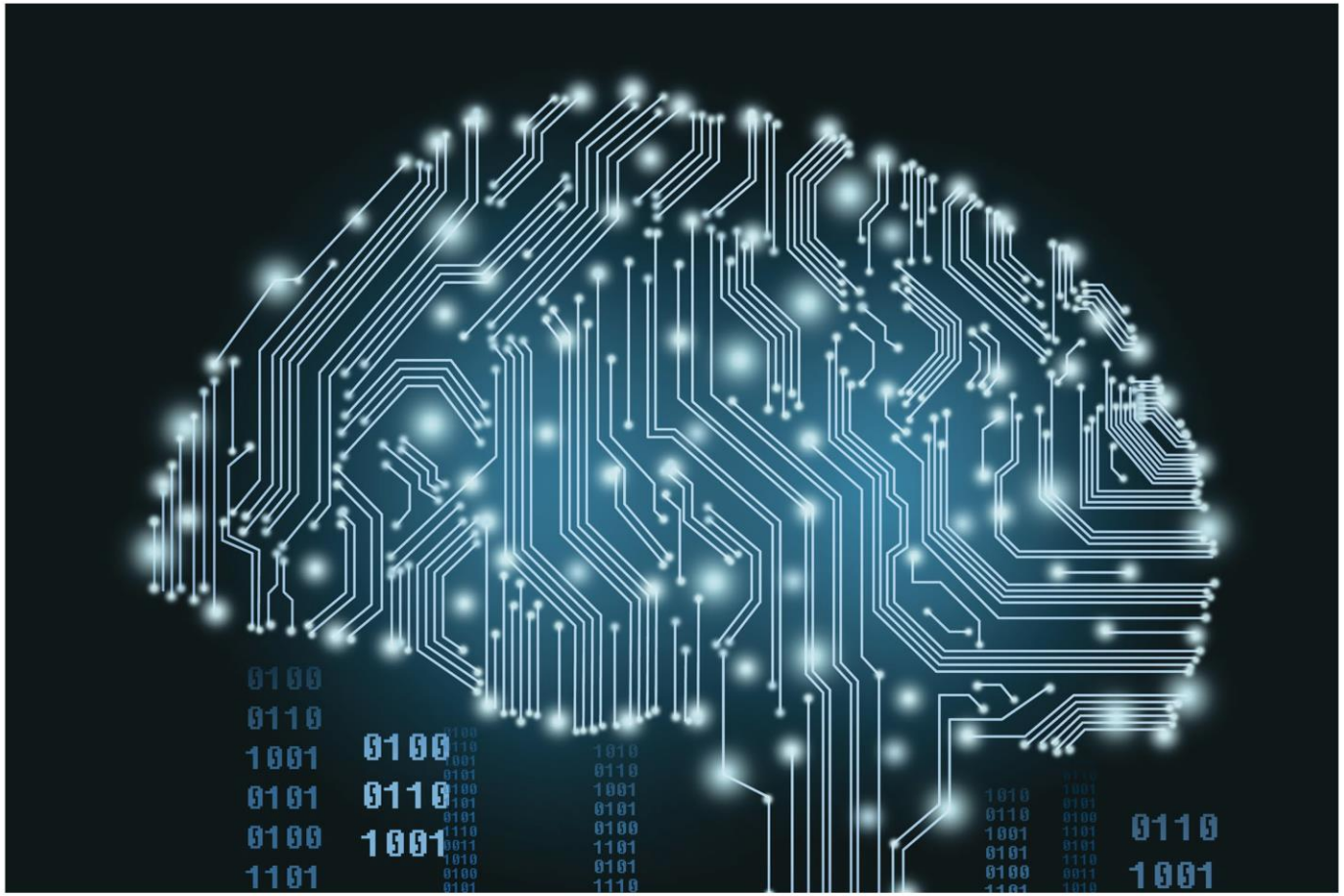


E

Participant Barcode	Study	Number of TIL Patches	TIL fraction	Number of TIL Clusters	Cluster Size Mean	Within-Cluster Dispersion Mean	Cluster Extent Mean	Ball Hall Index	Banfield Raftery Index	C Index	Determinant Ratio Index	Global Pattern
TCGA-33-AASL	LUSC	26245	20.6	40	656.1	293456	41.0	447	159518	0.015	2065.4	Brisk Diffuse
TCGA-D3-A2JF	SKCM	6832	4.9	18	379.6	238600	82.1	771	43456	0.022	790.0	Brisk Band-like
TCGA-E9-A22H	BRCA	1000	1.5	10	100.0	54876	51.9	560	6174	0.025	343.0	Non-Brisk Multifocal
TCGA-EW-A1OX	BRCA	285	0.1	2	142.5	430332	223.0	3093	2283	0.000	29.6	Non-Brisk Focal

(A–D) Four cases representing different degrees of lymphocyte infiltration.
Left: H&E diagnostic image at low magnification with tumor regions circled in yellow.
Middle: TIL map; red represents a positive TIL patch, blue represents a tissue region with no TIL patch, while black represents no tissue.
Right: diagrams of clusters of TIL patches derived from the affinity propagation clustering of the TIL patches.

Saltz J, et al. Cell Rep 2018



Credit: Dong Wenjie / Moment / Getty

AI added to the curriculum for doctors-to-be

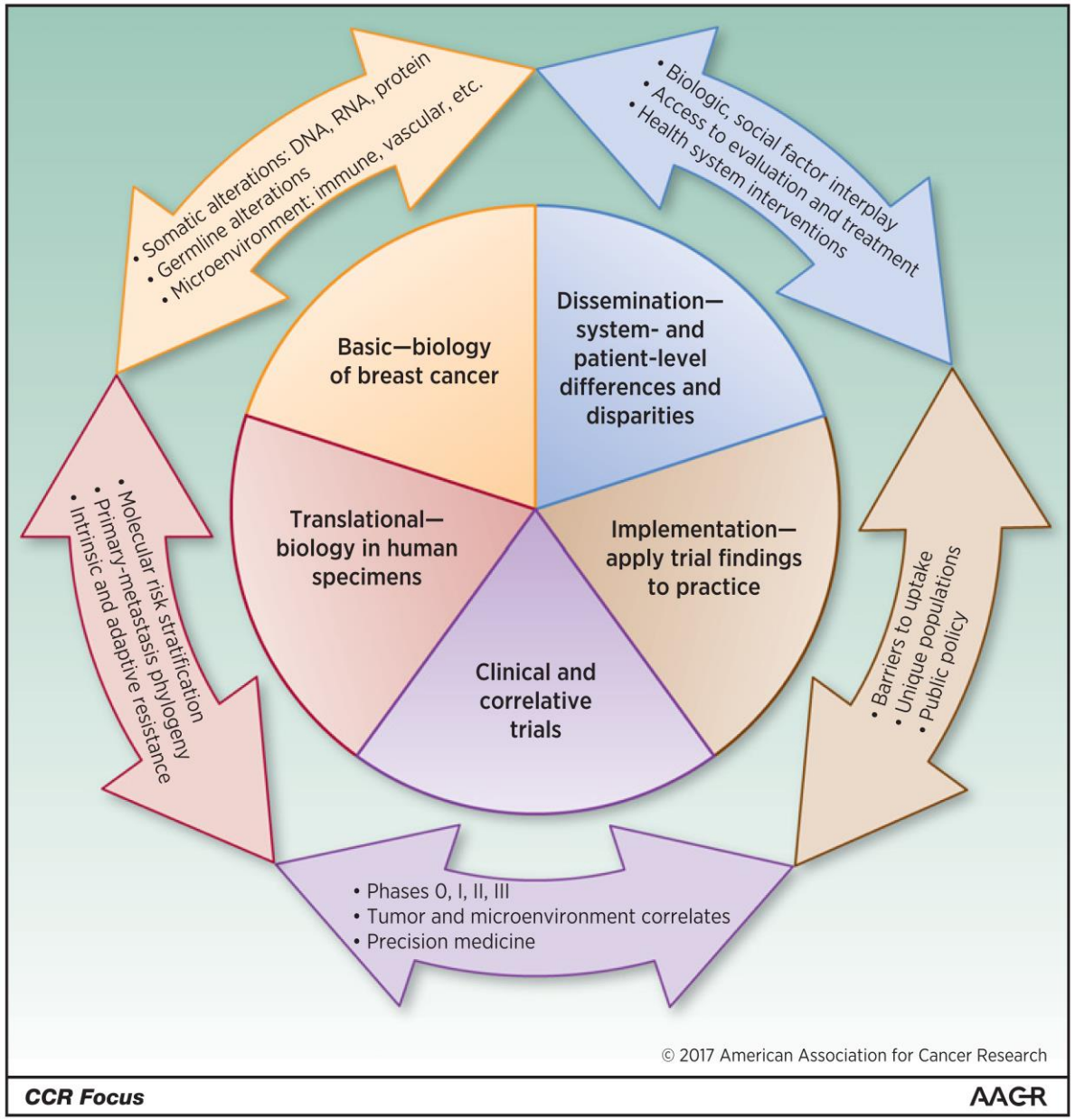
Medical schools and graduate research programs embrace artificial intelligence.

With the technological revolution of AI, may come an educational one: ***medical researchers*** will have to **understand** the basics of **artificial intelligence**, and, conversely, ***computer scientists*** will have to be **trained** to **understand medicine**

Brouillette M. Nat Med 2019

Conclusions

- Breast cancer is a **heterogeneous** disease
- **Inter-** and **intra- tumor heterogeneity**
  → Therapeutic and clinical implications
- **Translational research** allows a better understanding of breast cancer **biology** and of the mechanisms of **treatment resistance/sensitivity**
- **Biomarker** identification
  → **Integration** of multiple "omics" data
- These findings can be applied to **refine patient prognosis** (risk of relapse/progression) and to allow **treatment personalization** at a patient-level



Partridge AH & Carey LA. Clin Cancer Res 2017

Thank you

Merci

Grazie

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