Genome-Wide Profiling of Breast Cancer: History and Lessons from Studies before NGS Era

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Conventional factors that Tx decision was based on

- Tumor size
- Lymph node metastasis
- Histological grade
- ER, PR, HER-2

Tumor size

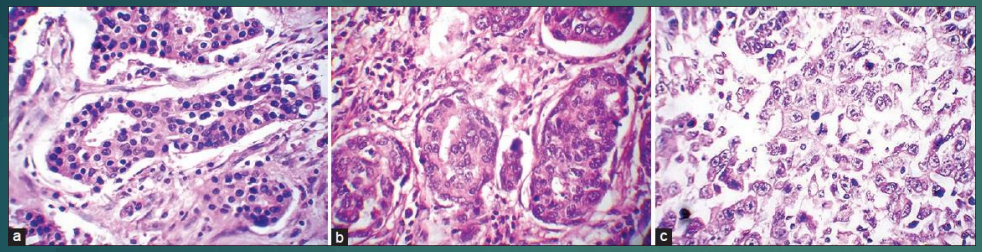
- How old is the tumor?

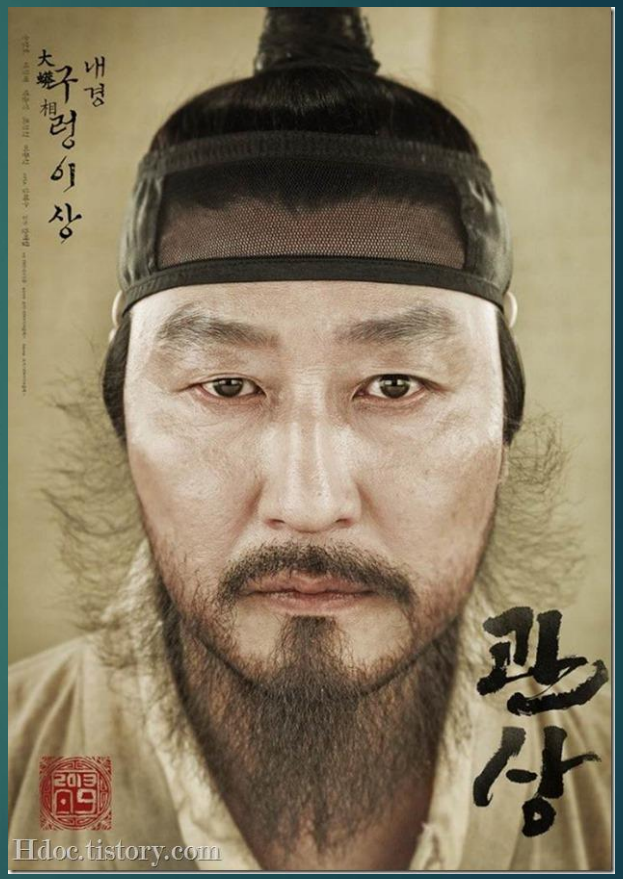
- How fast the tumor grows (before detection)

Lymph node involvement

- Better predictor of prognosis than the tumor size
- It can happen by chance (it also means how old the tumor is) not by the ability of the tumor to metastasize
- Anyway it means tumor can be separated from his family and move further and live alone for at least limited time

Histologic grade

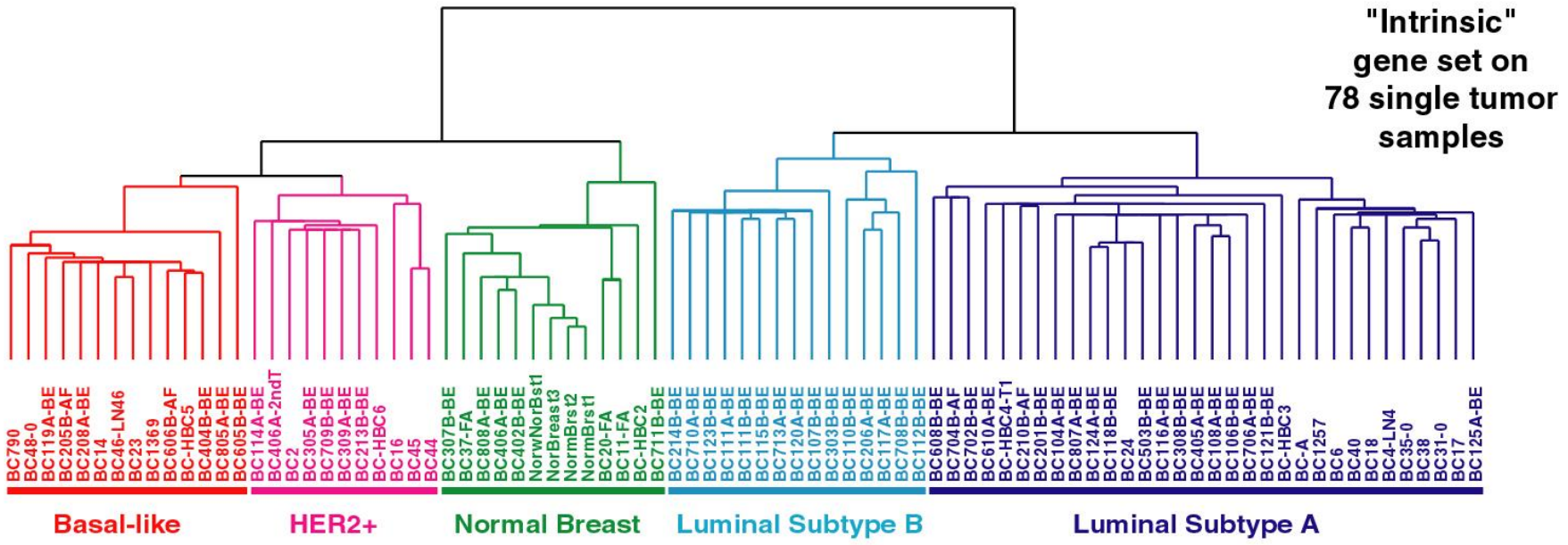


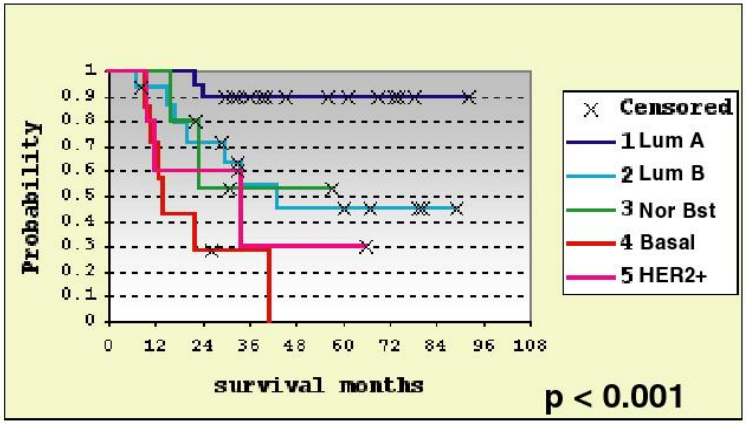


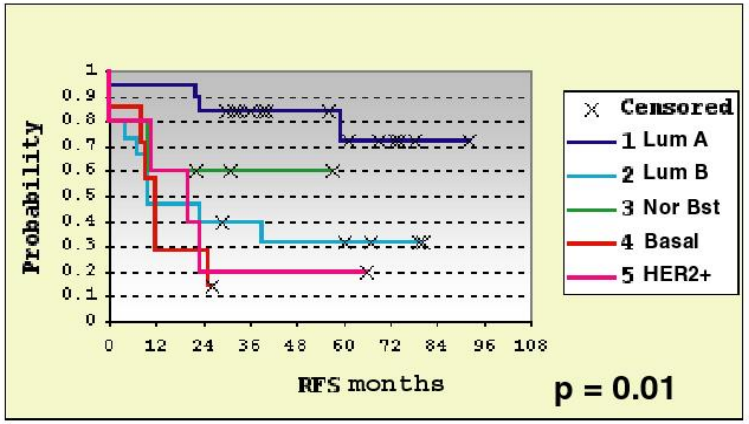
physiognomy

Molecular profiling

Molecular portraits of breast cancer
(source: Perou & Sorlie et al, Nature 2000)







Intrinsic molecular classification (Perou and Sorlie)

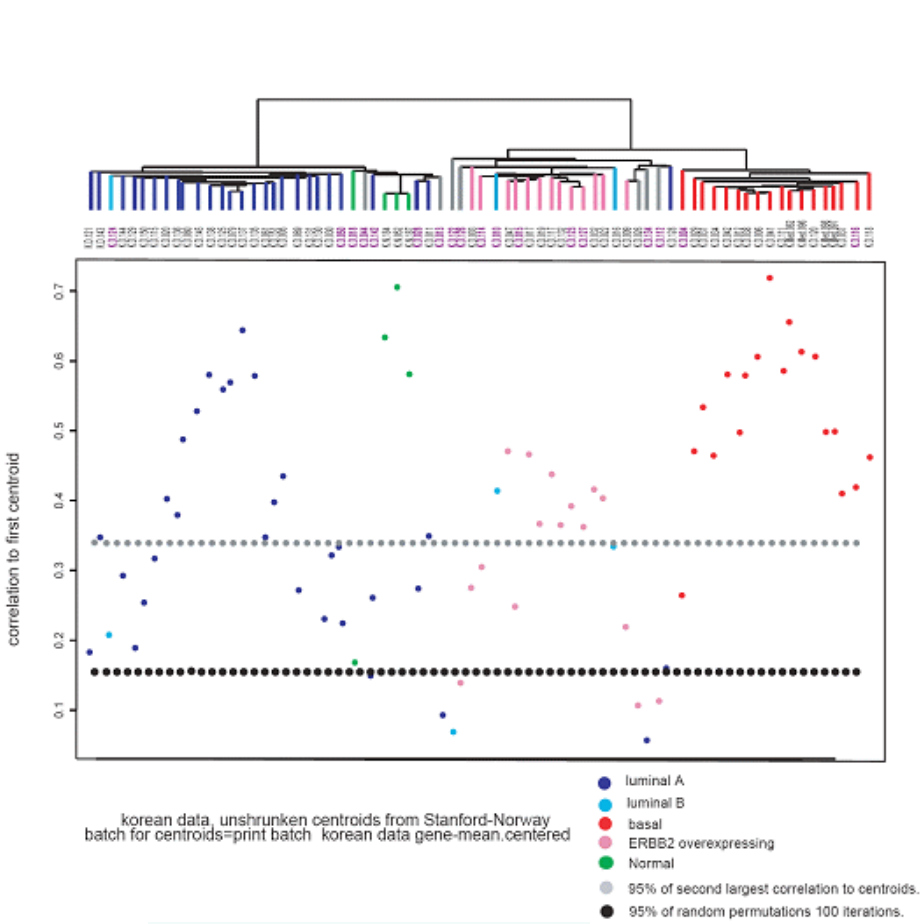
- Intrinsic genes: Genes whose expression vary more between tumors than between repeated samples of the same tumors
- Hierarchical clustering (Unsupervised analysis)
- ER+ and ER-negative tumors are fundamentally distinct
- At least four molecular subtypes of breast ca
- 3 important gene groups: ER and ER related genes, proliferation genes, and HER-2 amplicon genes in chr17
- The most stable and reproducible separation was basal-like tumors

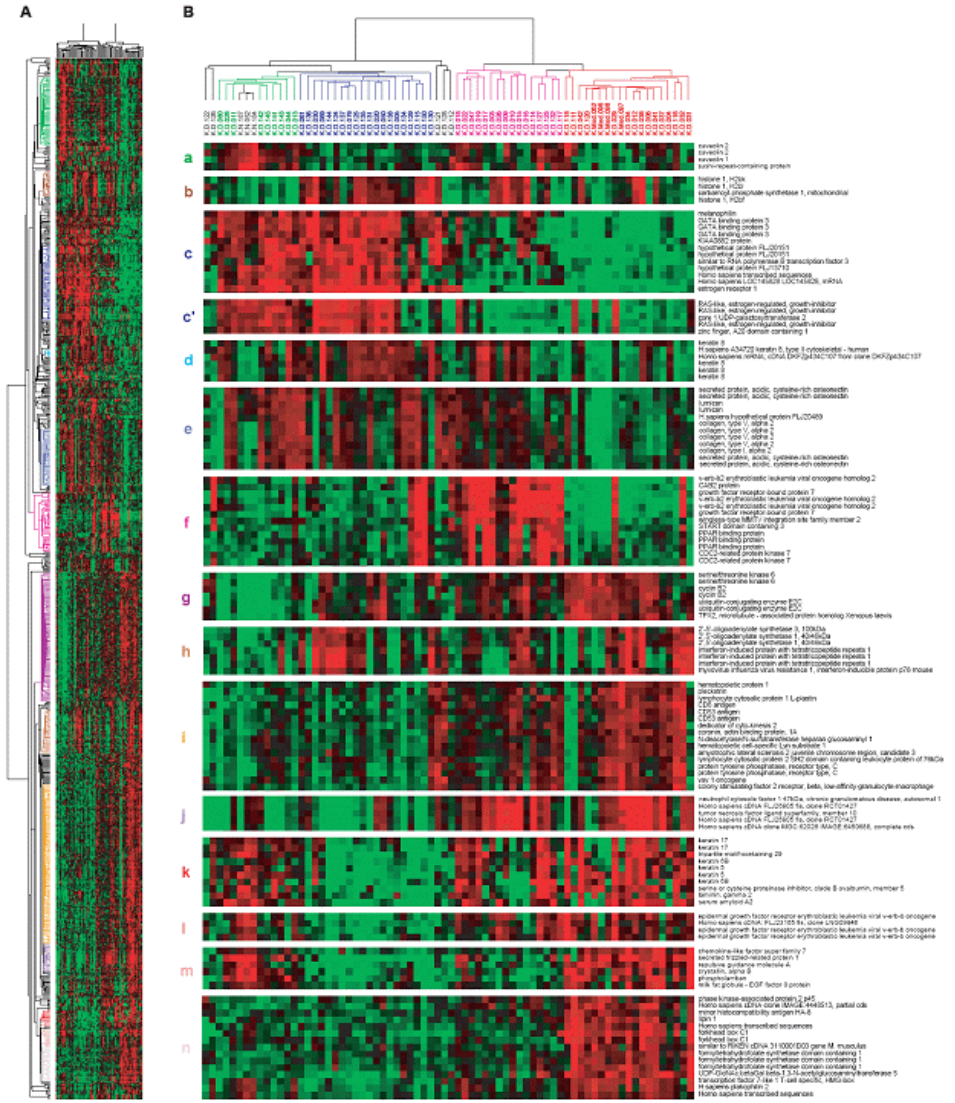
Intrinsic molecular classification: limitations

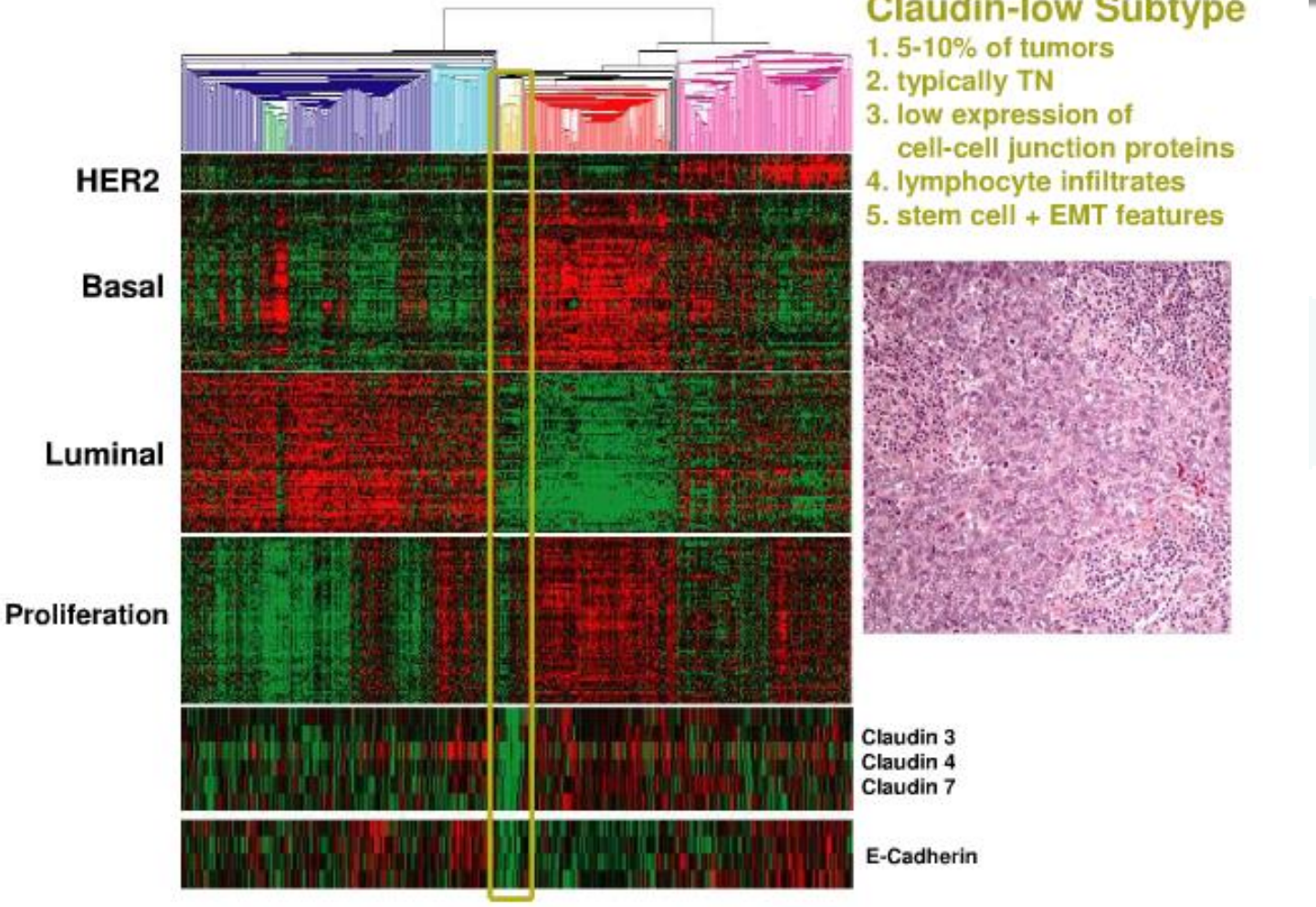
- Intrinsic gene is real?
- How to classify a newcomer
- How many subtypes exist?
- Is it clinically useful? (prognostic and predictive value) beyond just ER, PR, HER-2, Ki67?

Characterization of molecular subtypes of Korean breast cancer: An ethnically and clinically distinct population

WONSHIK HAN[1], MONICA NICOLAU[2], DONG-YOUNG NOH[1] and STEFANIE S. JEFFREY[3]



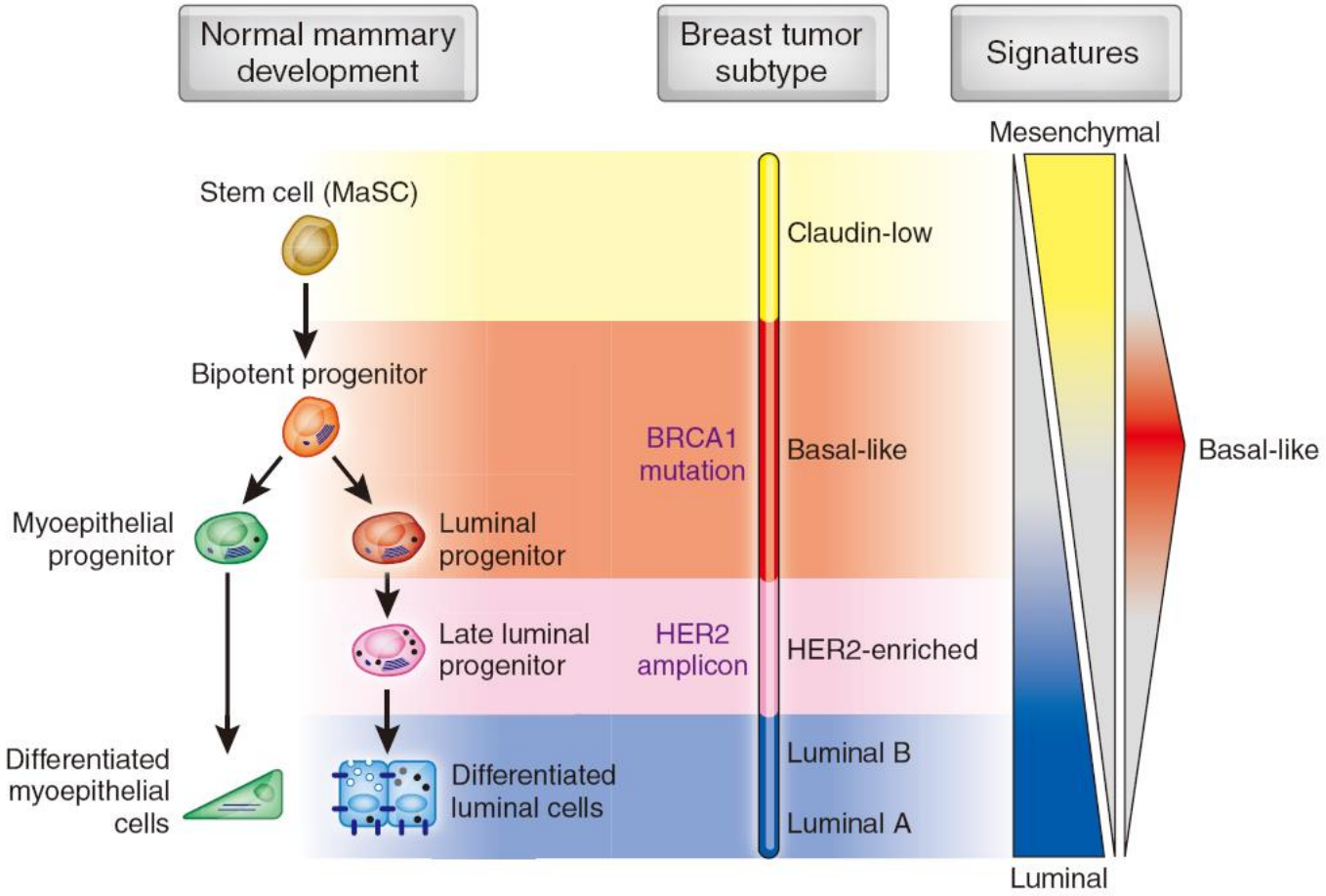




Perou CM, SABCS 2009

Mammary development meets cancer genomics

Aleix Prat & Charles M Perou

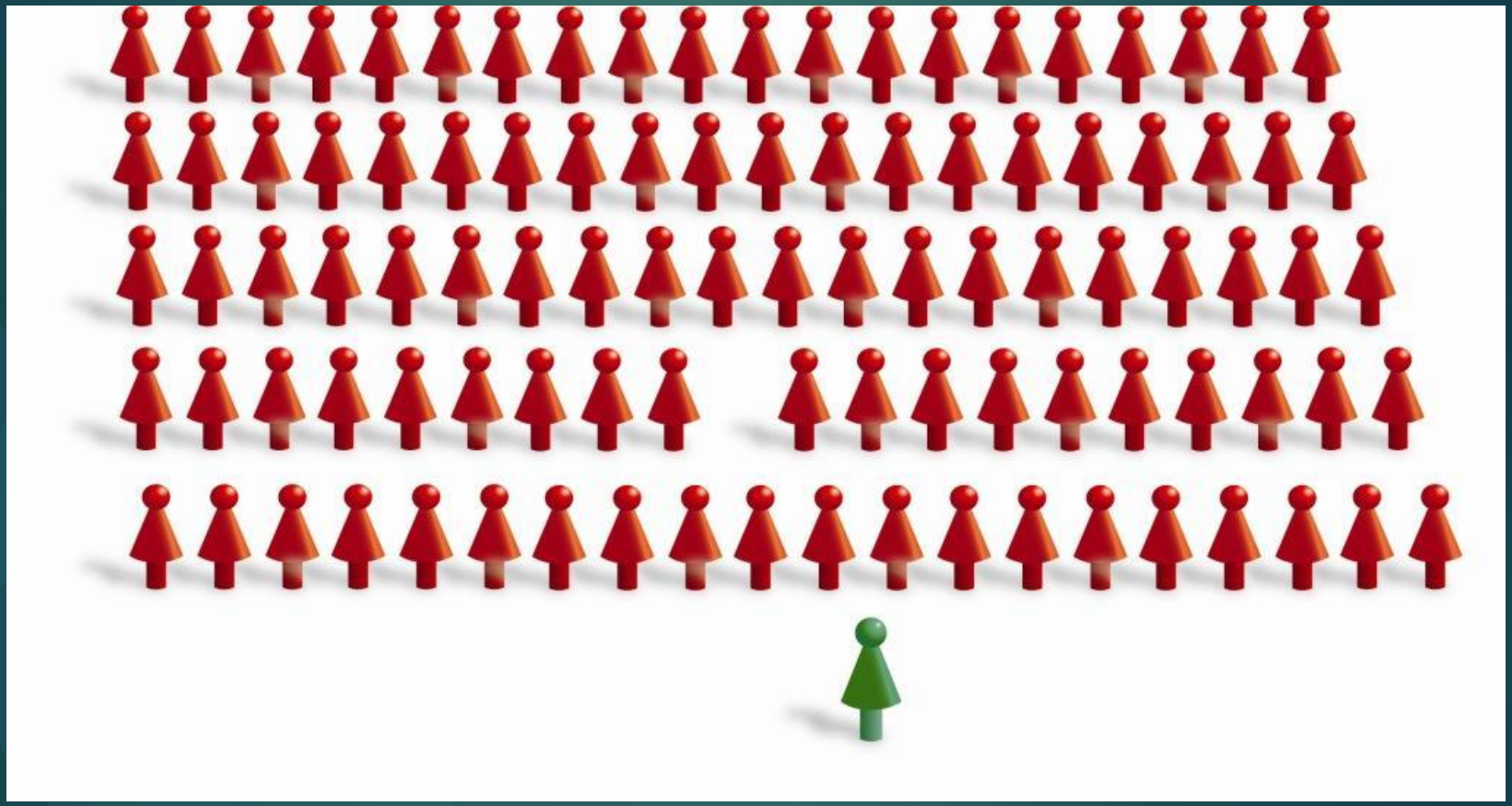


Nature Medicine 2009

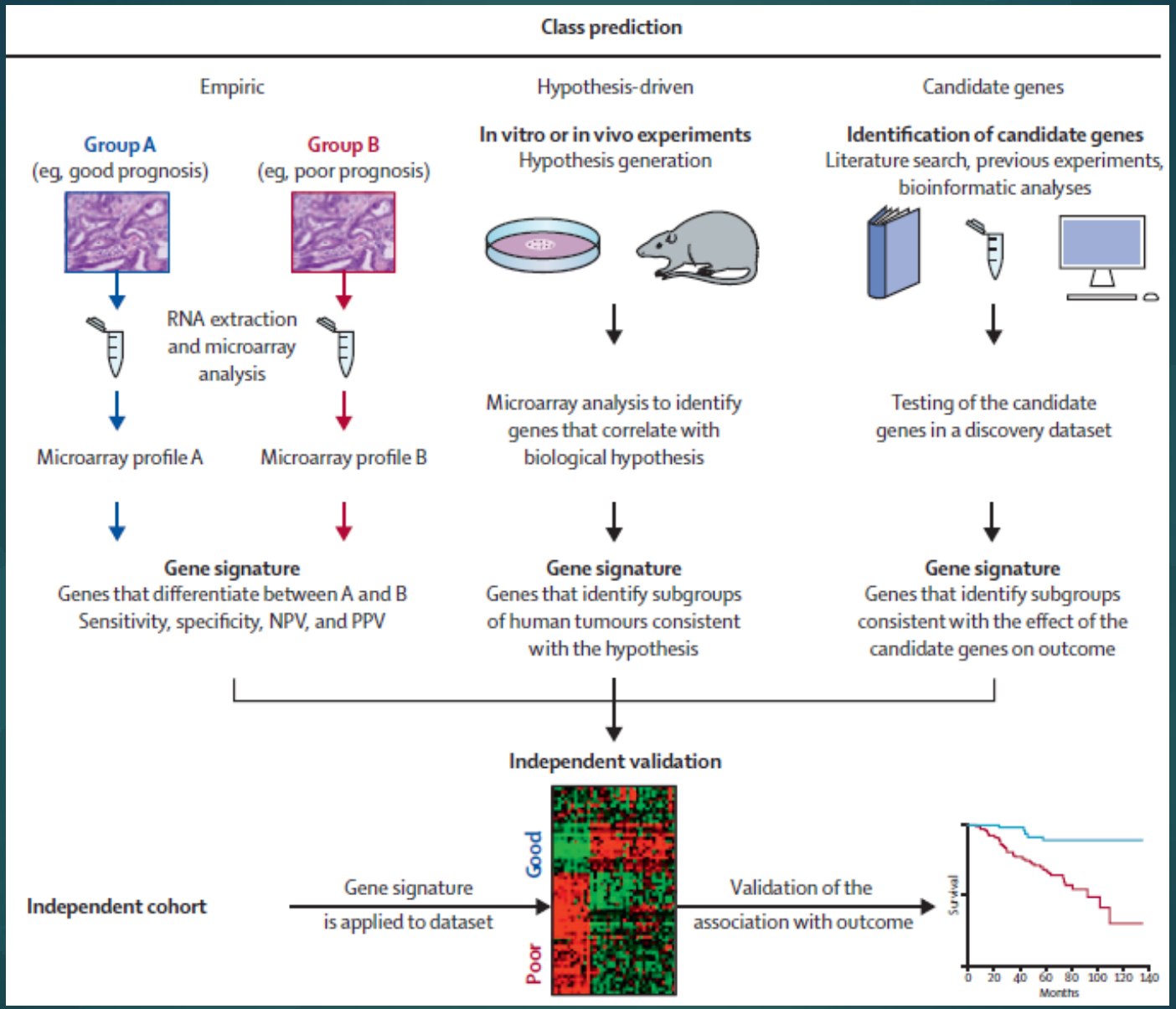
Prognostic Signatures

AIMING TO IDENTIFY PATIENTS WITH DISEASE OF SUFFICIENTLY GOOD PROGNOSIS TO ALLOW THE SAFE OMISSION OF ADJUVANT CHEMOTHERAPY

Only one out of 100 women will benefit from adjuvant chemo-therapy



Most common breast cancer today: T1, N0, ER+, Grade 2.



Reis-Filho and Pusztai, Lancet 2011

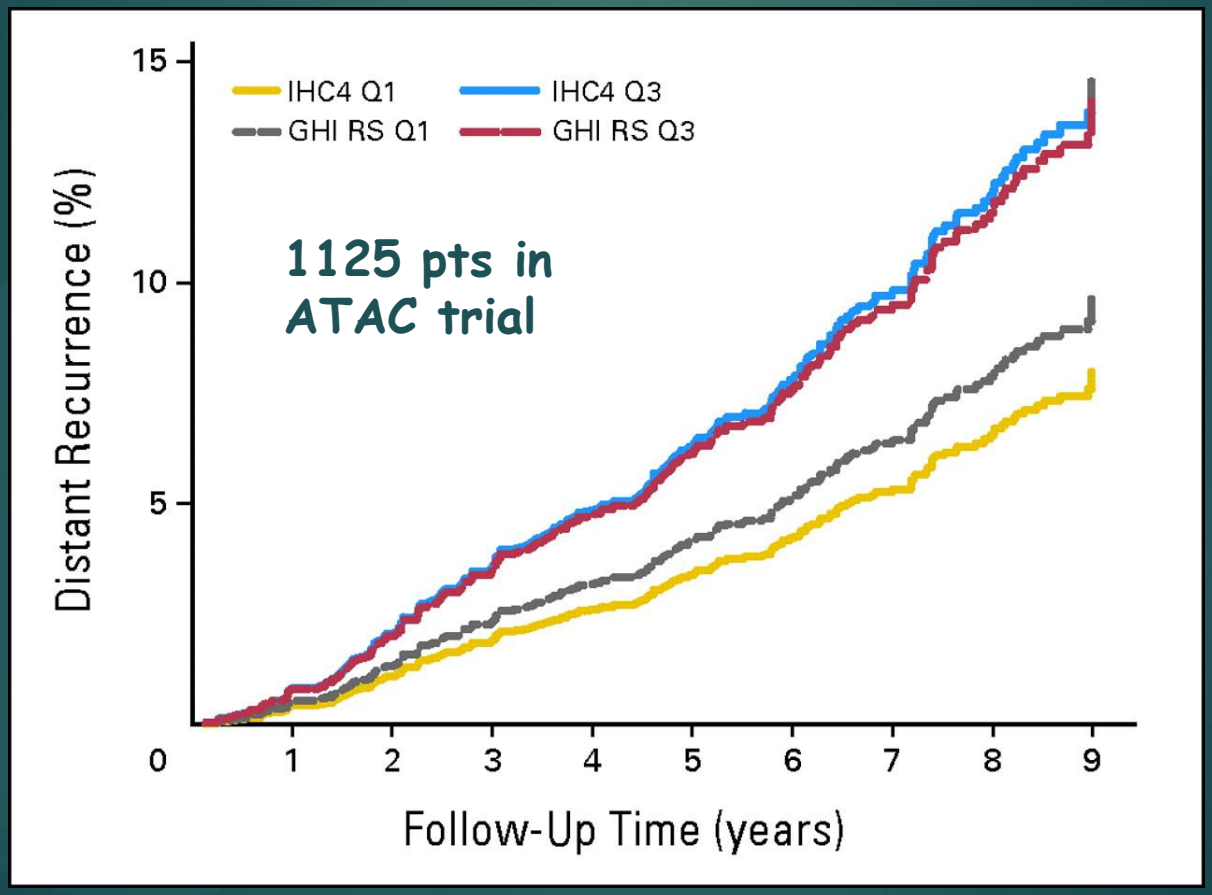
Commercially available multi-gene expression signatures in breast cancer

- MammaPrint (70-gene signature)
- Veridex 76-gene
- Oncotype DX (21-gene singnature)
- Breast Cancer Index (HoxB13:IL17BR, Theros™)
- Genomic grade index (MapQuant Dx)
- PAM50 (Prosigna™)

Commercially available prognostic multigene signatures for breast cancer

- Despite differences in the genes that compose each of the signatures, they largely identify the same group of patients as having poor prognosis disease (Fan et al. NEJM 2006)
- The unifying characteristic is the high expression of proliferation-related genes
- Almost invariably classify ER-negative cancers as of poor prognosis
- Tumor size and lymph-node status provide prognostic information that is independent of that offered by prognostic signatures

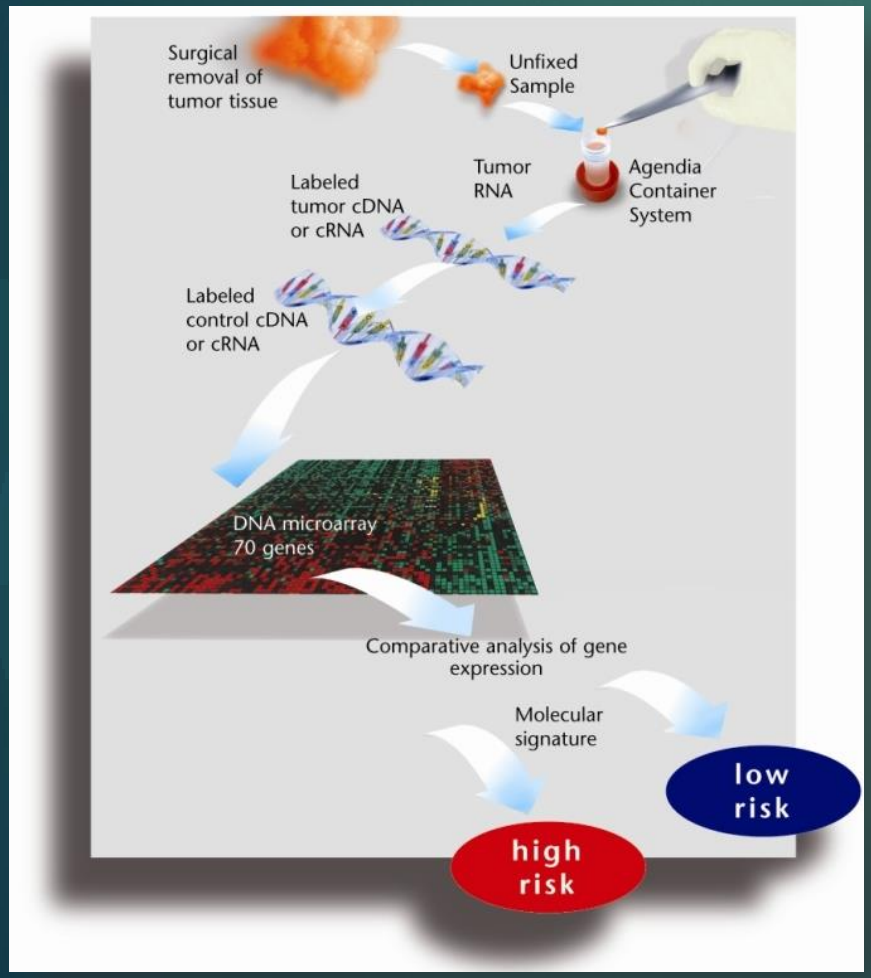
Information beyond ER, PR, HER-2, and Ki67 might be limited

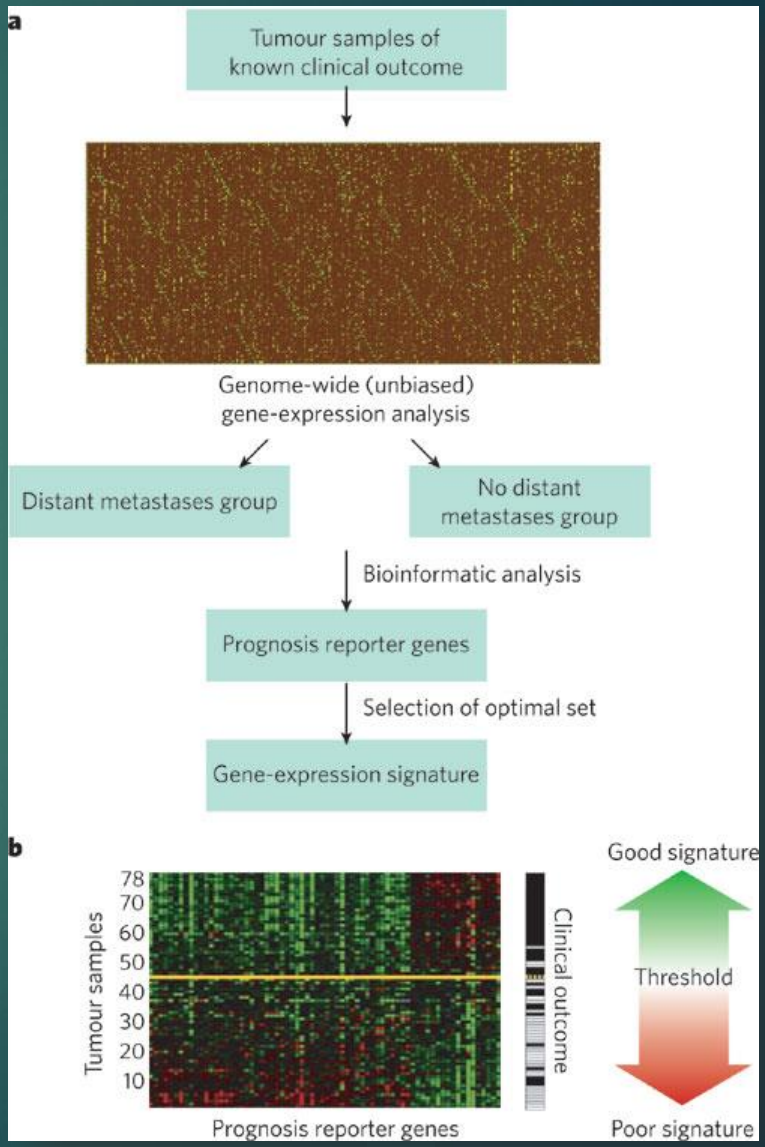


Cuzick et al. JCO 2011

mammaprint™

decoding breast cancer.

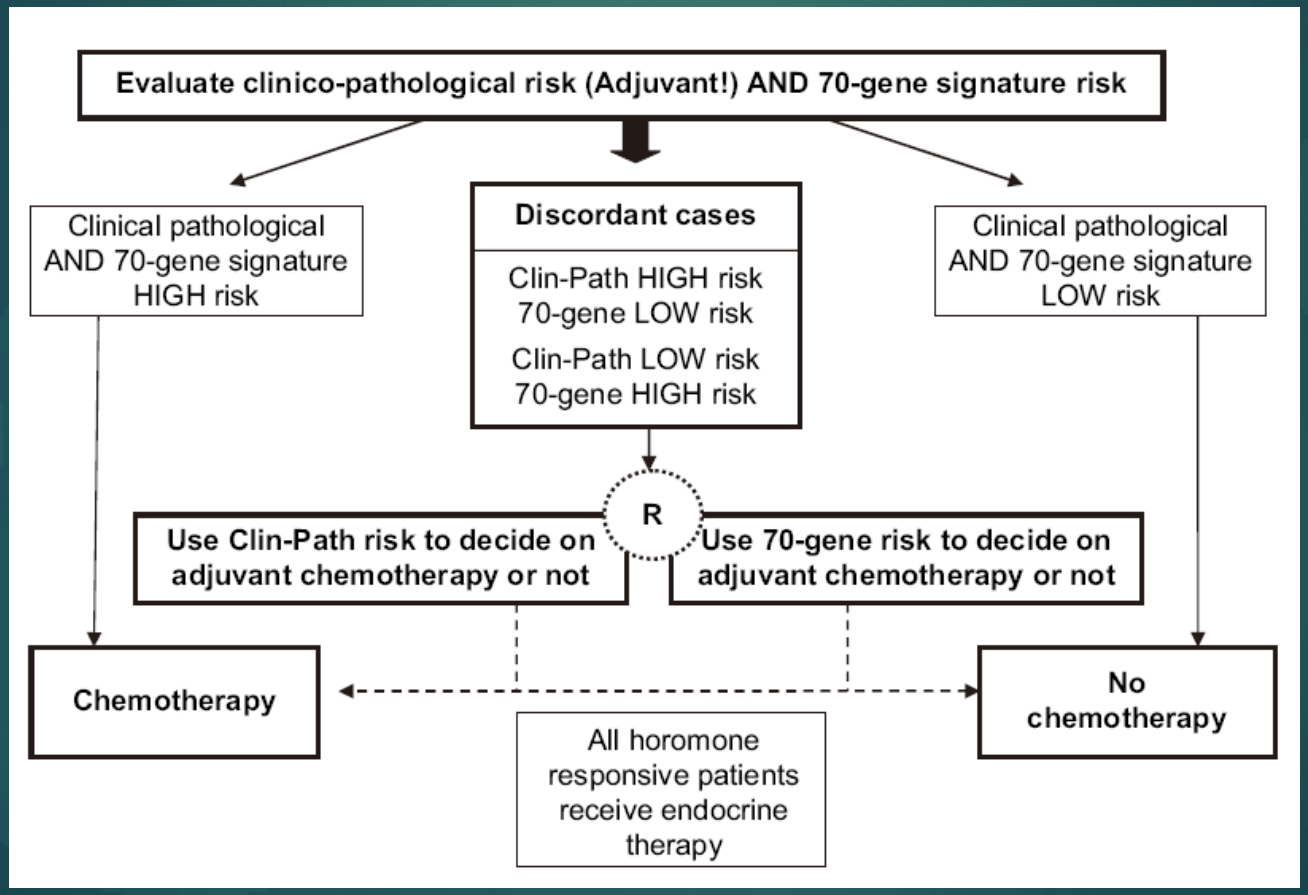




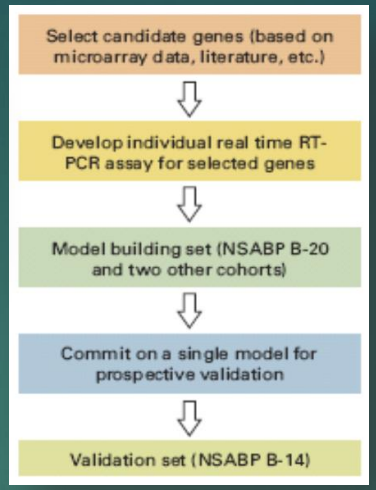
70-gene signature (MammaPrint)

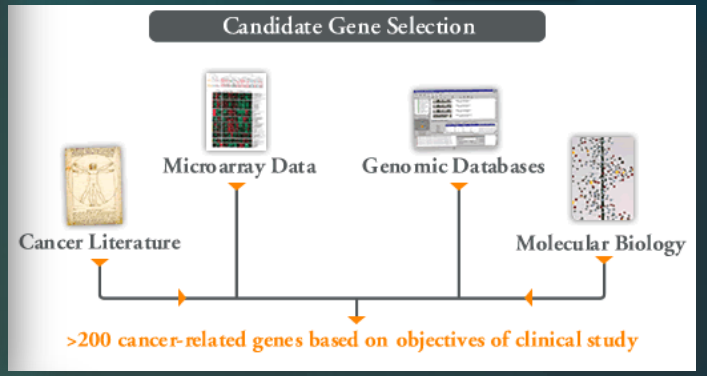
- FDA approved
- Could reduce high risk group from 85% to 60%
- Level II of evidence from retrospective studies
- Require fresh or frozen samples
- Discriminatory power for ER-negative disease is very small (only 0–5% of patients with ER-negative disease are classified as having good prognosis)

EORTC-BIG MIDACT (MIcroarray for Node negative Disease may Avoid ChemoTherapy) Trial Design
6,000 Node-negative breast cancer



oncotype DX®
Breast Cancer Assay





Proliferation
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

Estrogen
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

Invasion
Stromelysin 3
Cathepsin L2

CD68

HER-2
GRB7
HER-2

Reference
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS = + 0.47 × HER-2 group score
- 0.34 × ER group score
+ 1.04 × proliferation group
+ 0.10 × invasion group score
+ 0.05 × CD68
- 0.08 × GSTM1
- 0.07 × BAG1

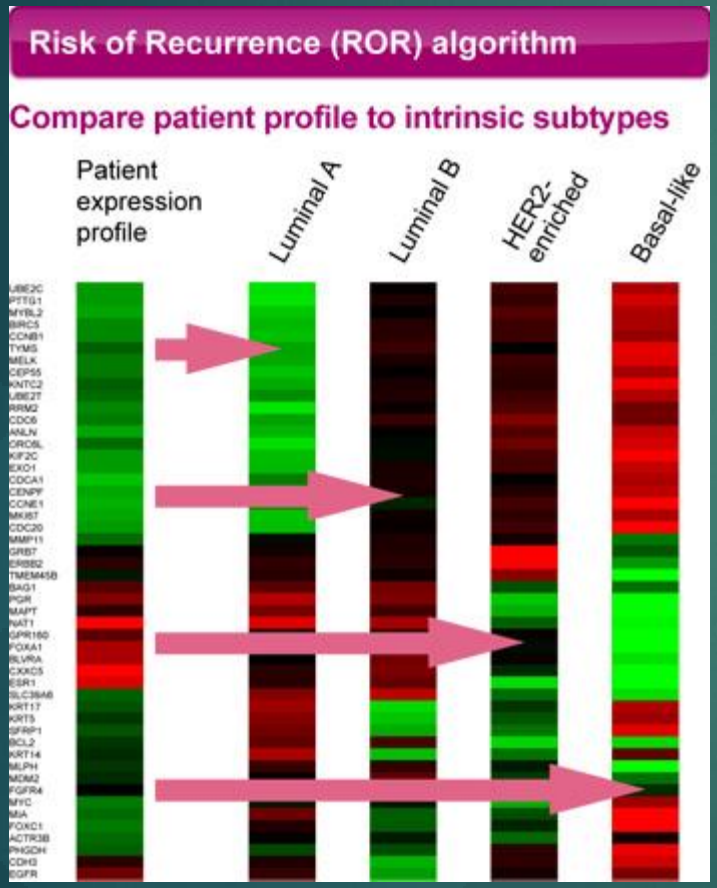
Category	RS (0 – 100)
Low risk	RS < 18
Intermediate risk	RS ≥ 18 and < 31
High risk	RS ≥ 31

Panel of 21 genes for the Recurrence-Score Algorithm
Real time-PCR of 21 genes from FFPE

21-gene score (Oncotype DX)

- Can use FFPE tissue sections
- Level I evidence
- Incorporated in NCCN guideline
- Recommended in ASCO guideline
- Expanded evidence in patients treated AI and patients have up to 3+ LNs (RxPONDER trial)
- Uncertain "intermediate" risk group (TAILORx trial)
- A model combining RS with traditional anatomical pathological factors could be more prognostic than RS alone

PAM50 (Prosigna™)



ROR is derived from a algorithm based on the PAM50 gene signature, intrinsic subtype, tumor size, and proliferation score

Better differentiation of intermediate- and higher-risk groups of Oncotype DX

Dowsett M et al. JCO 2013

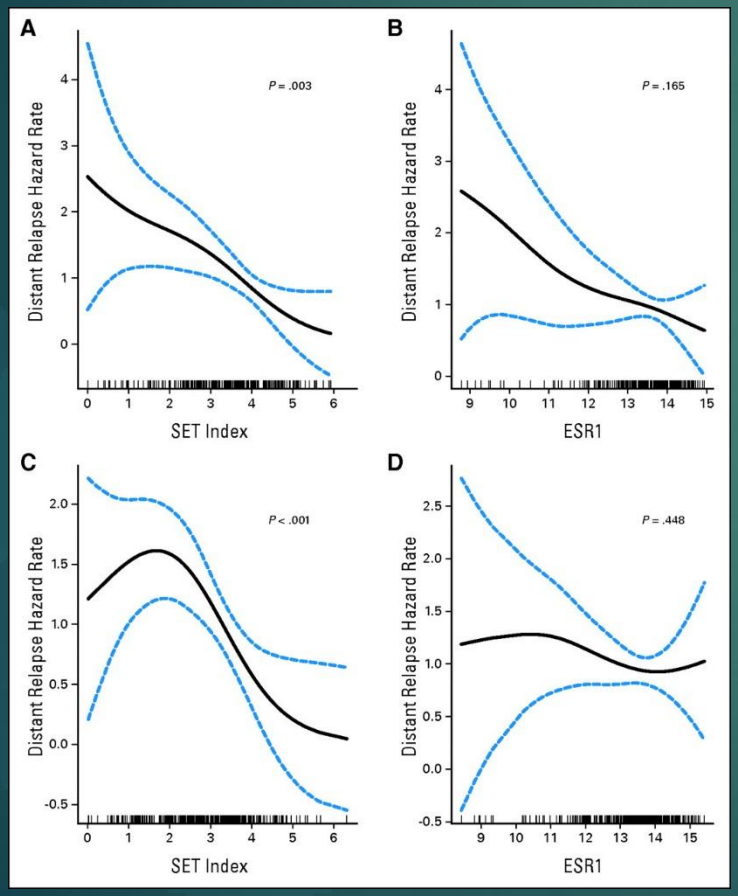
Multigene predictors of response to chemotherapy

Authors	Number of cases[a]	Regimen	Chemotherapy	Chemosensitivity evaluation	Technology	Method	Signature	NPV	PPV	Accuracy
Chang *et al.* [116]	24 discovery 6 validation	Neoadjuvant	Docetaxel	Clinical response	cDNA microarray	Supervised	92 genes	83%	92%	88%
Ayers *et al.* [90]	24 discovery 12 validation	Neoadjuvant	T/FAC	pCR	cDNA microarray	Supervised	74 genes	73%	100% (3/3)	78%
Iwao-Koizumi *et al.* [91]	44 discovery 26 validation	Neoadjuvant	Docetaxel	Clinical response	High-throughput RT-PCR	Supervised	85 genes	90.9%	73.3%	80.7%
Gianni *et al.* [70]	89 discovery 92 validation	Neoadjuvant	TA	pCR	qRT-PCR/ DNA microarray	Supervised	86 genes	-	-	-
Hess *et al.* [92]	82 discovery 51 validation	Neoadjuvant	T/FAC	pCR	cDNA microarray	Supervised	30 genes	96%	52%	76%
Thuerigen *et al.* [93]	52 discovery 48 validation	Neoadjuvant	G-ET	pCR	cDNA microarray	Supervised	512 genes	95%	64%	88%
Farmer *et al.* [103]	63	Neoadjuvant	FEC	pCR	cDNA microarray	Metagene approach	Stromal metagene	81%	57%	65%

There is no validated and commercially available gene signature to predict response to a specific therapeutic agent

Colombo et al. Breast Cancer Research 2011

Sensitivity to endocrine therapy (SET) index



- Defined from 165 genes coexpressed with ESR1 in 437 microarray profiles
- It predicted survival benefit from adjuvant endocrine therapy, not inherent prognosis

Symmans et al. JCO 2010

The lessons from Genome wide profiling studies

- Gene expression analysis has changed the way breast cancer is perceived, and it is no longer regarded as a single disease
- ER+ and ER-negative cancers represent molecularly and clinically distinct diseases
- Proliferation genes and expressions are important as a prognostic factor for ER+ cancers
- Multi-gene prognostic signatures provide information that is complementary to that provided by anatomical prognostic variables
- However, they have limited clinical value for patients with ER-negative disease
- The knowledge acquired from microarray-based gene expression profiling studies will help in the development of the next generation of genomic predictors