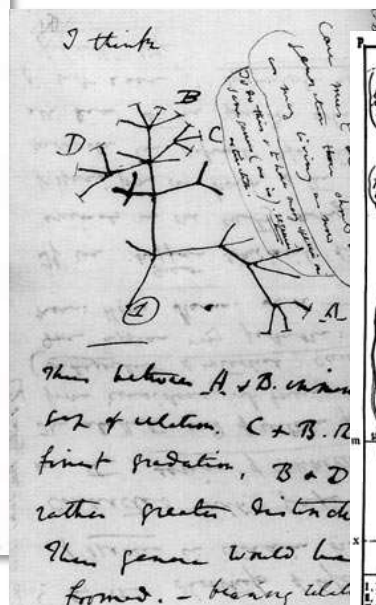


Brystkreft

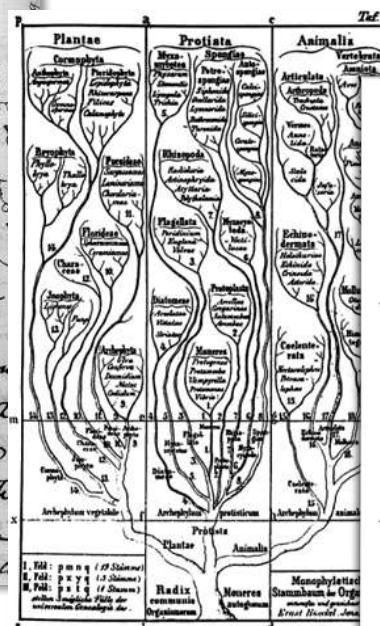
– en sykdom med ulike ansikt



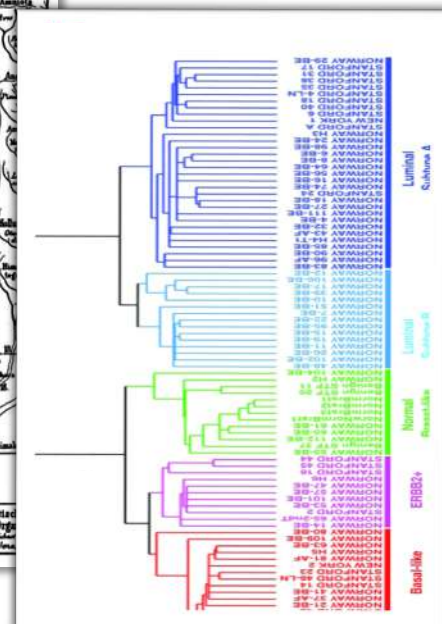
Linnaeus



Darwin



Haeckel

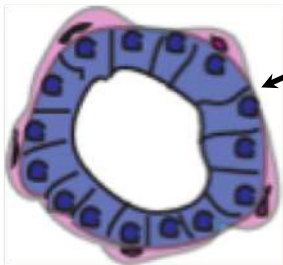
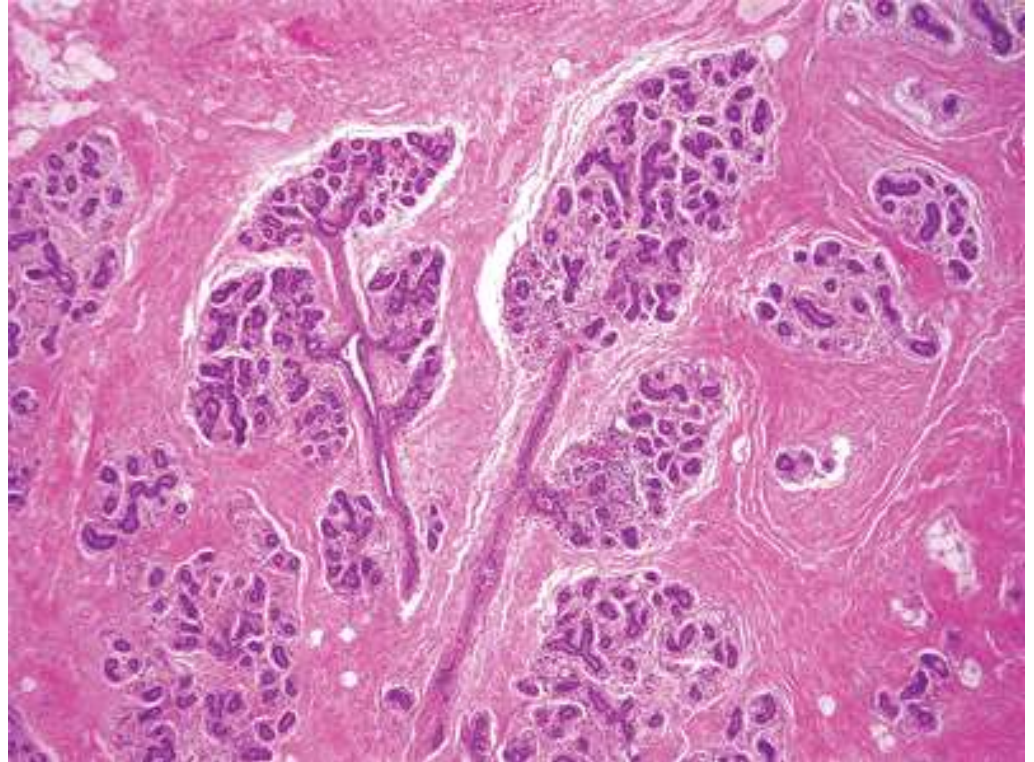
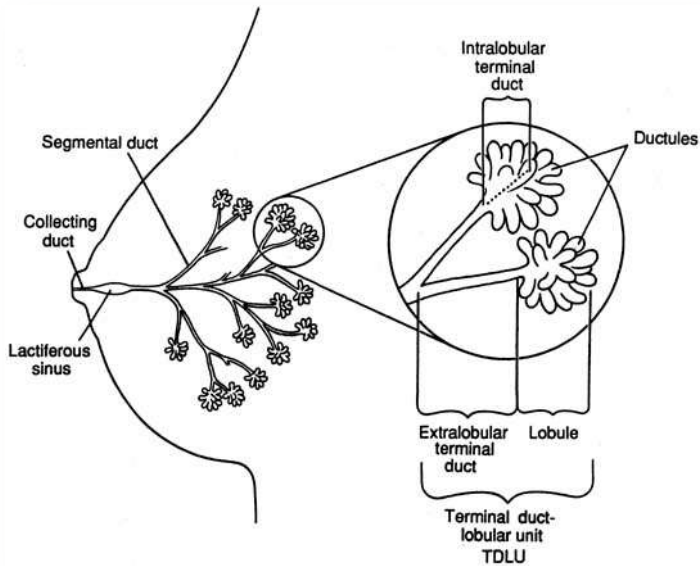


Perou

OnkoLiS 2020

Hege E. G. Russnes, MD, PhD
Dept. of Pathology and
Dept of Cancer Genetics, Institute for Cancer Research
Oslo University Hospital

The breast

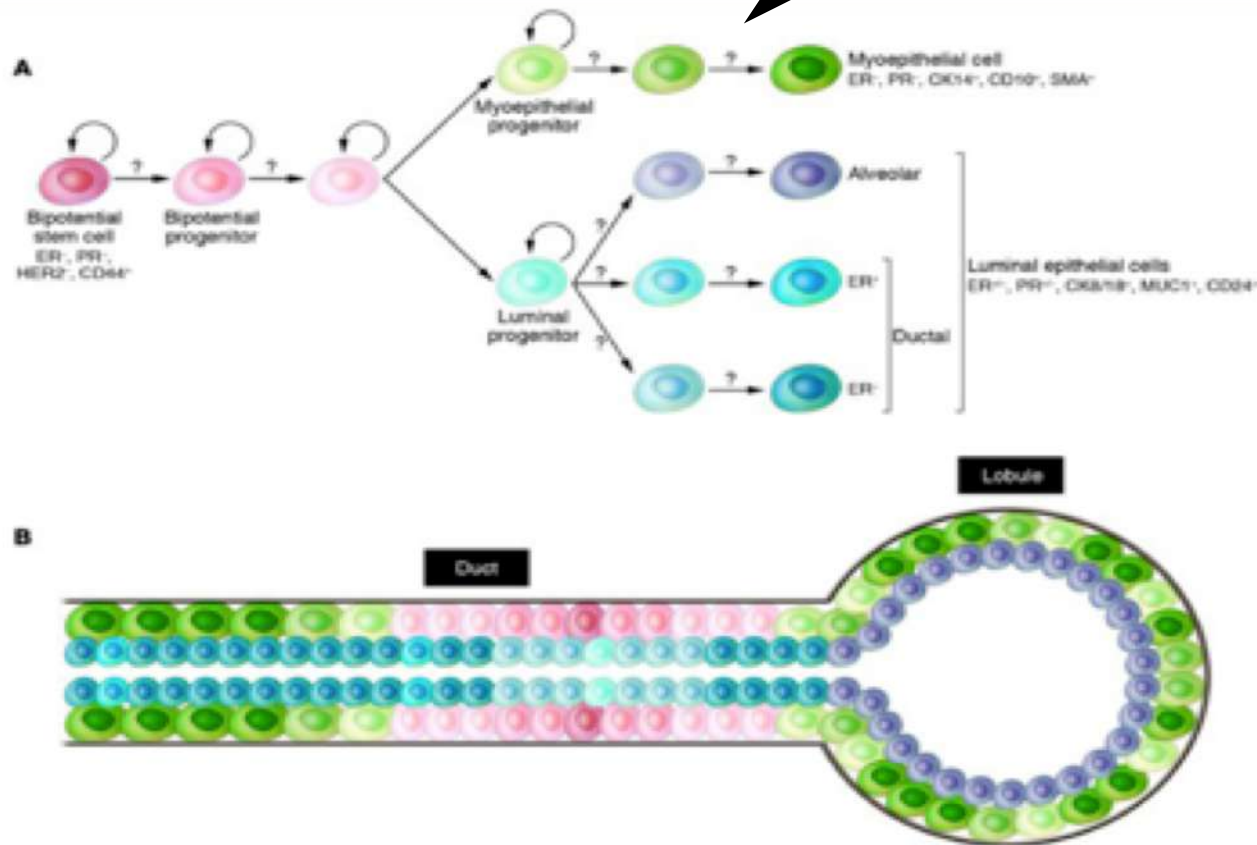


→ Luminal
epithelial cells

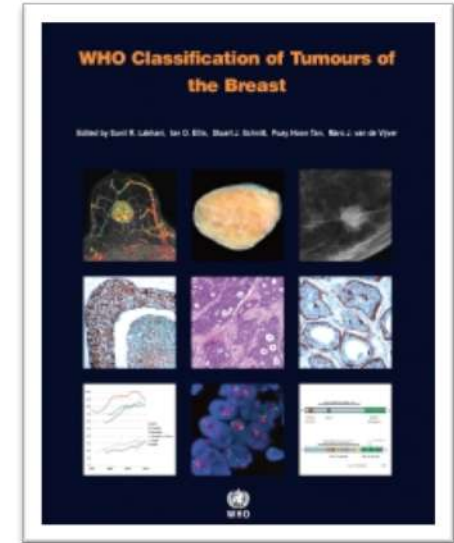
→ Myoepithelial/basal cells

An assumed hierarchical relationship between the cell types

Differentiation

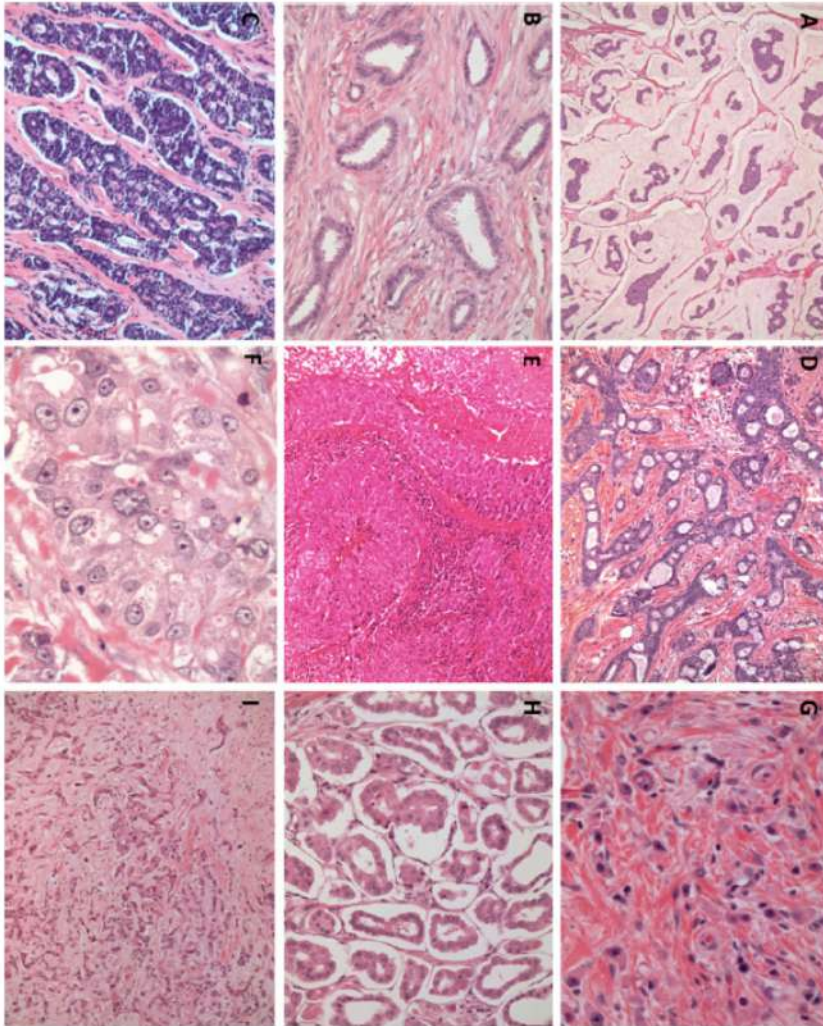


Morphology based classification

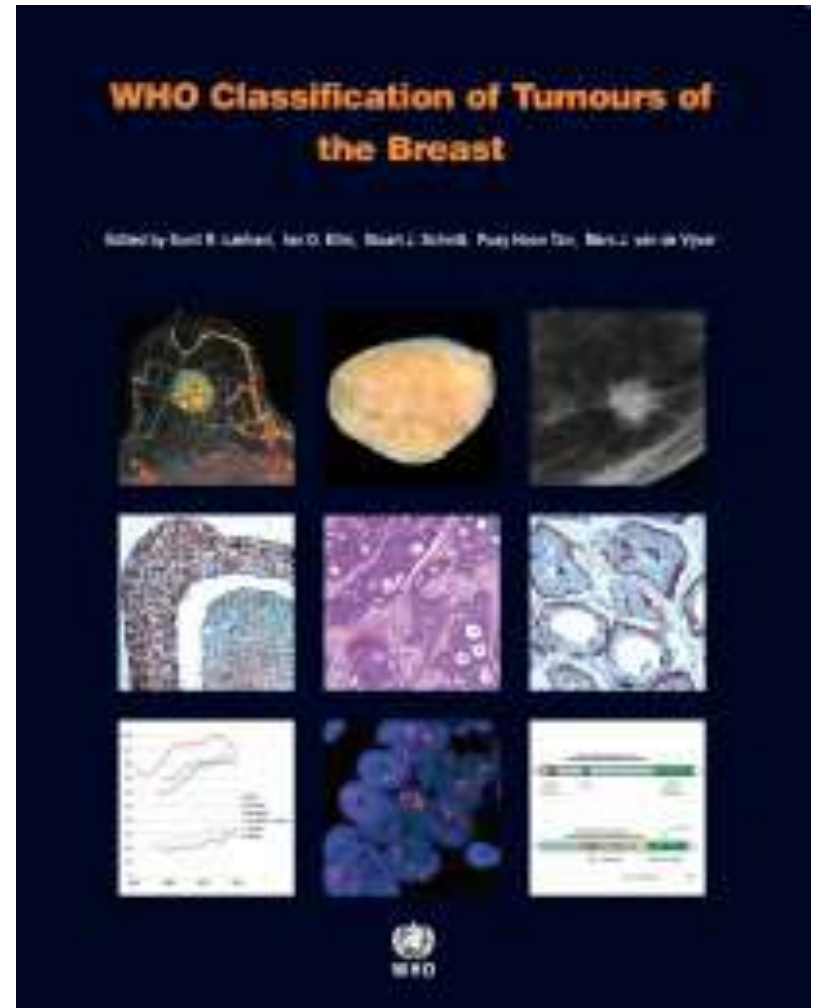


- Invasive carcinomas of no special type, NST (previously known as “ductal”) – a wide specter
- Special type carcinomas
- Mixed carcinomas

Classification by morphology



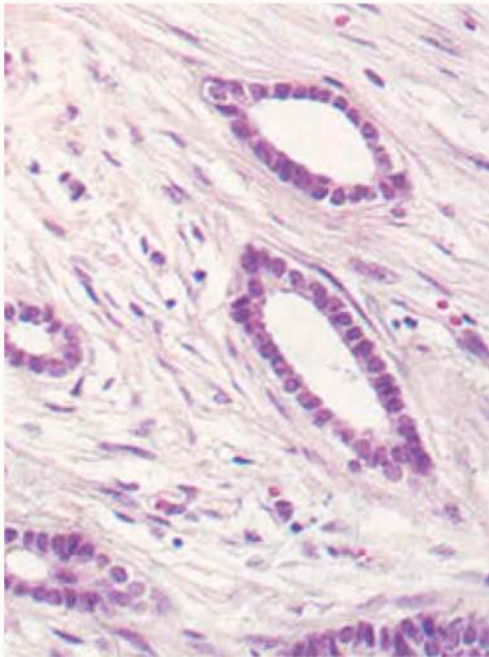
Dieci, The Oncologist 2014



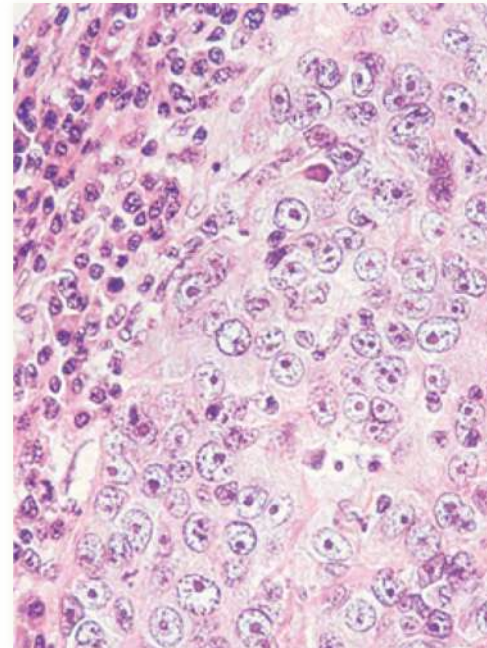
Morphology = phenotype!

“A phenotype is the ensemble of observable characteristics displayed by an organism”

Indolent behavior:



Aggressive behavior:

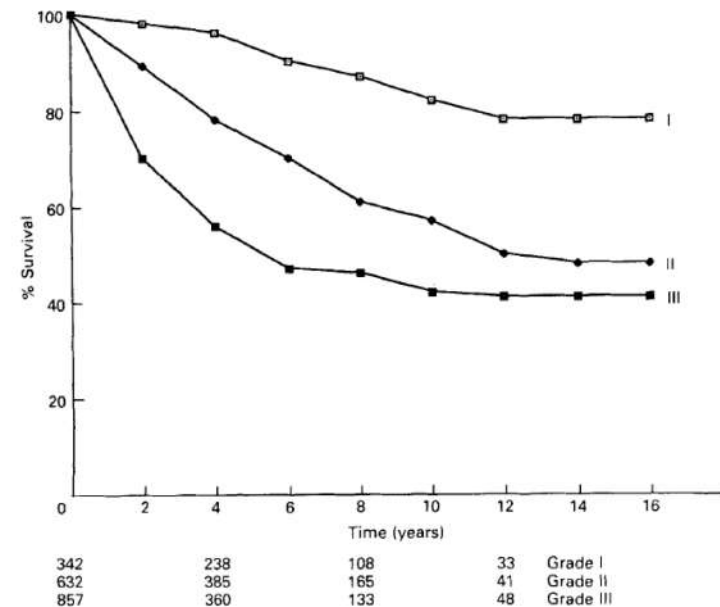


Histological grade

Table 1. Summary of semiquantitative method for assessing histological grade in breast carcinoma

Feature	Score
Tubule formation	
Majority of tumour (>75%)	1
Moderate degree (10–75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic counts	
Dependent on microscope field area (see Table 2)	1–3

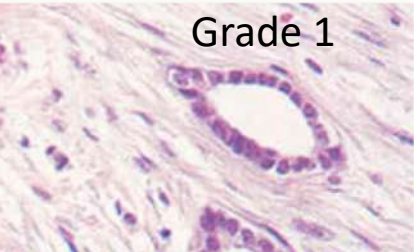
3–5 points: grade I —well-differentiated
 6–7 points: grade II —moderately differentiated
 8–9 points: grade III—poorly differentiated



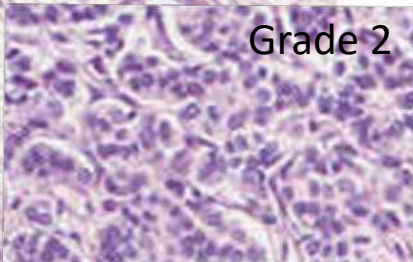
Elston and Ellis, Histopathology, 1991

Grouping of breast cancer - 2020

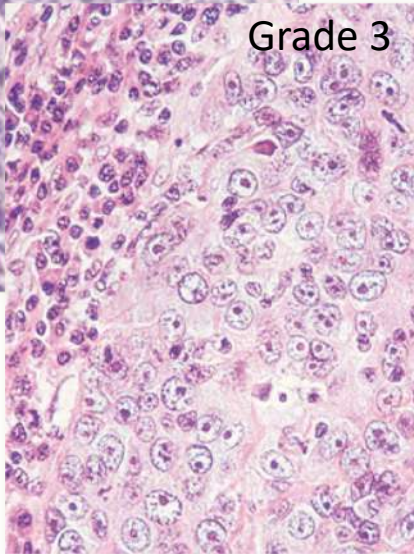
Histological grade
Grade 1



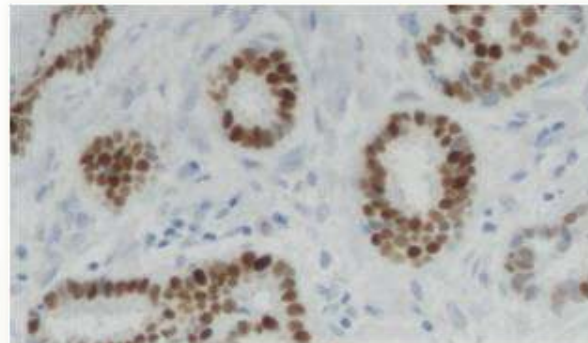
Grade 2



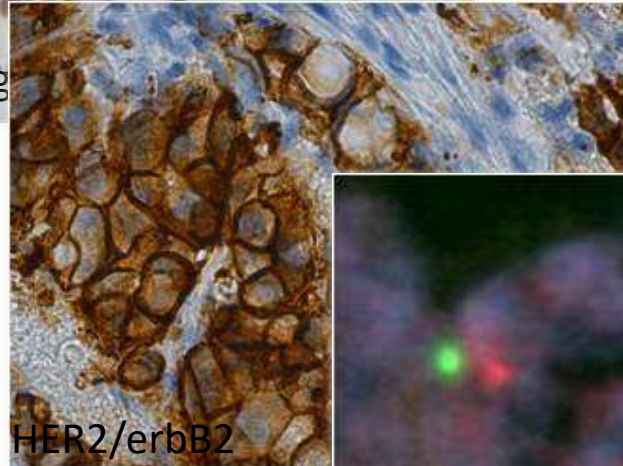
Grade 3



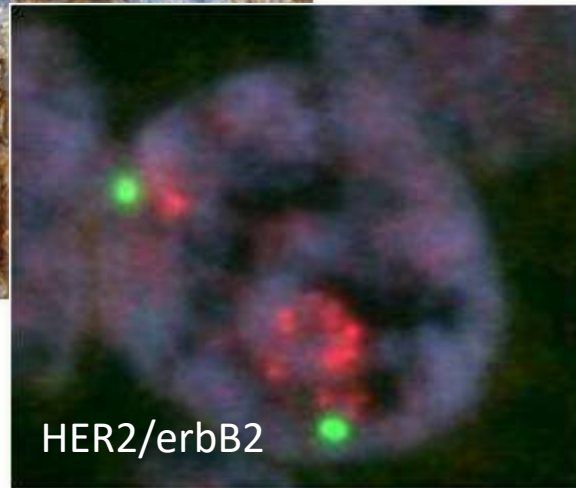
ER/PgR/Ki67/HER2



Estrog



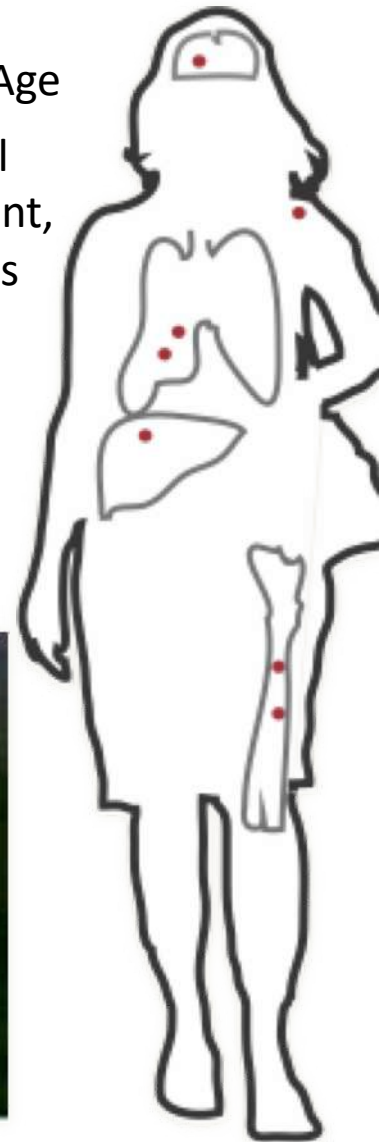
HER2/erbB2



HER2/erbB2

Age

Size, nodal
involvement,
metastases
TNM



St. Gallen consensus meeting 2015

Table 2. Treatment-oriented classification of subgroups

Clinical grouping

Triple-negative

Hormone receptor-negative and HER2-positive

Hormone receptor-positive and HER2-positive

Hormone receptor-positive and HER2-negative
luminal disease as a spectrum:

High receptor, low proliferation, low tumor
burden (luminal A-like)

Intermediate

Low receptor, high proliferation, high tumor
burden (luminal B-like)

- TNBC
- ER-/HER2+
- ER+/HER2+
- ER+/HER2-
 - Low proliferation
 - Intermediate proliferation
 - High proliferation

Revolution in technology reveals unknown biology

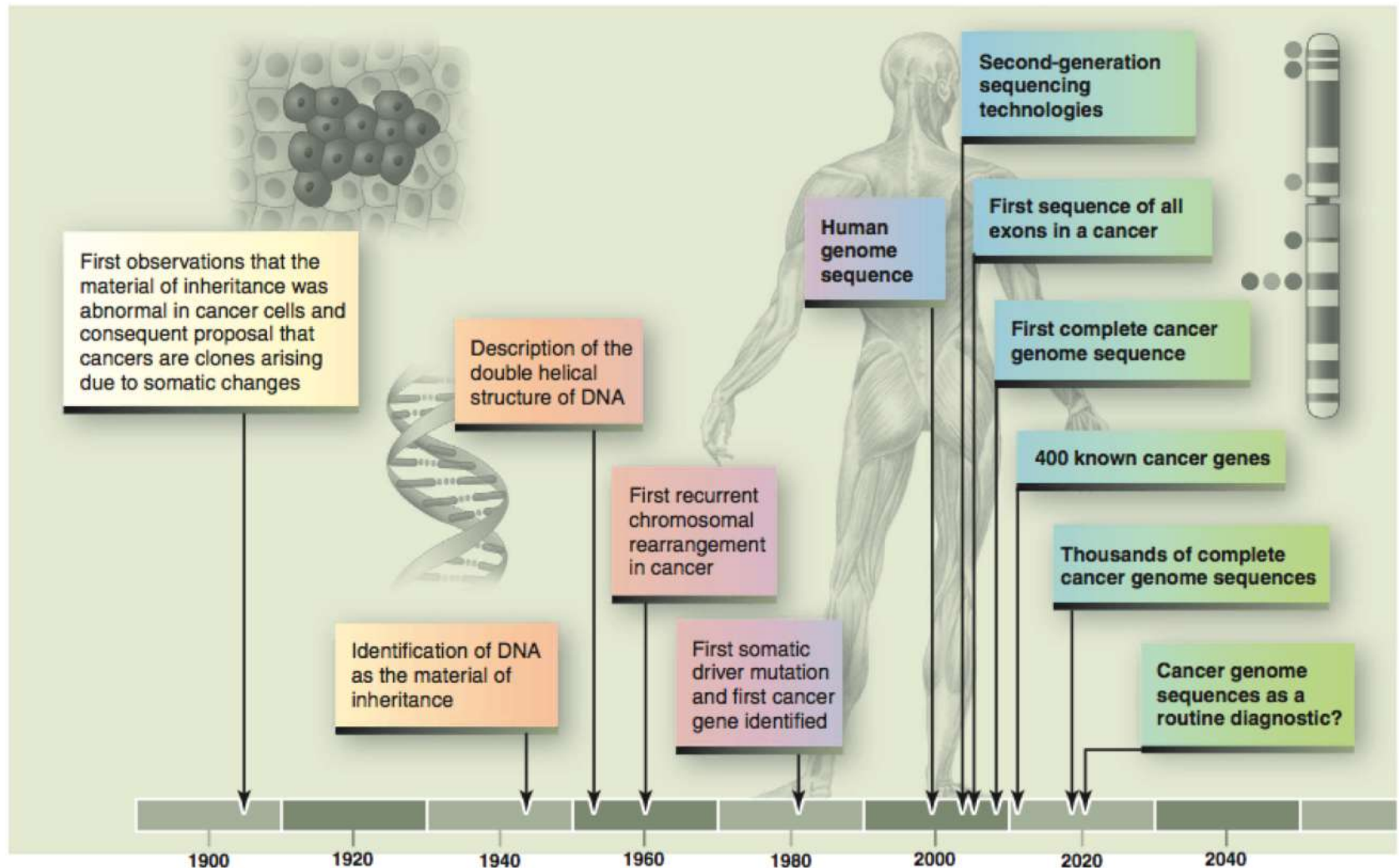


Fig. 1. Time line showing key events in the investigation of the cancer genome.

Molecular based classification

- Biomarkers/signatures for treatment prediction?
- Biomarkers/signatures recognizing biological distinct traits?
- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- And are the designated class the same throughout the entire evolution of given cancer?

Molecular based classification

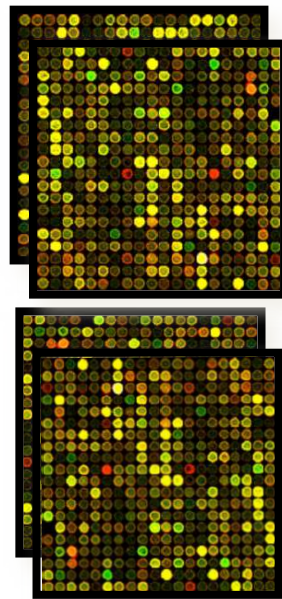
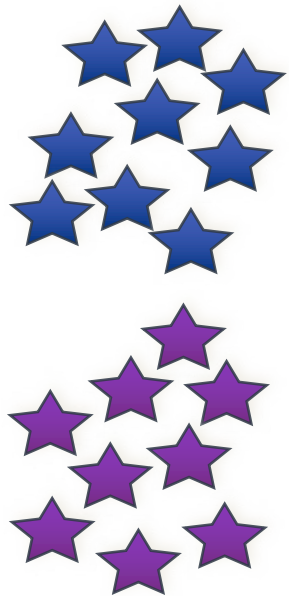
- Biomarkers/signatures for treatment prediction?
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- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- And are the designated class the same throughout the entire evolution of a given tumor?

Identification of biomarker/signatures for treatment prediction

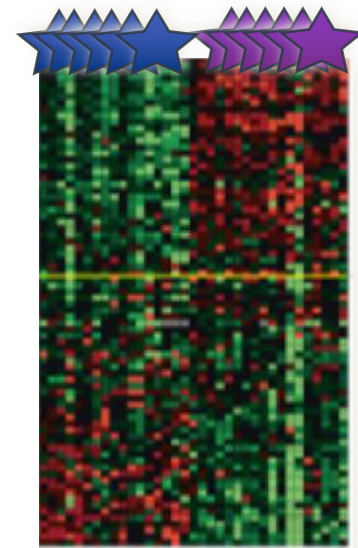
Patient samples with **same treatment**, comparison of findings in responders vs. non-responders

Supervised analyses

Selected tumor samples



Molecular markers with predictive or prognostic potential

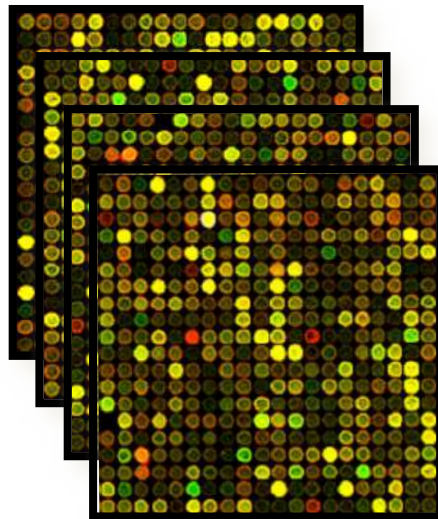
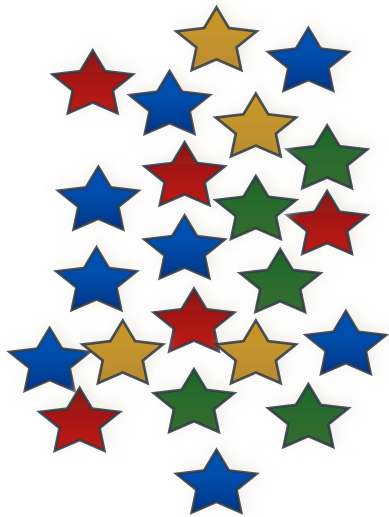


Identification of biomarker/signatures recognizing biological distinct traits

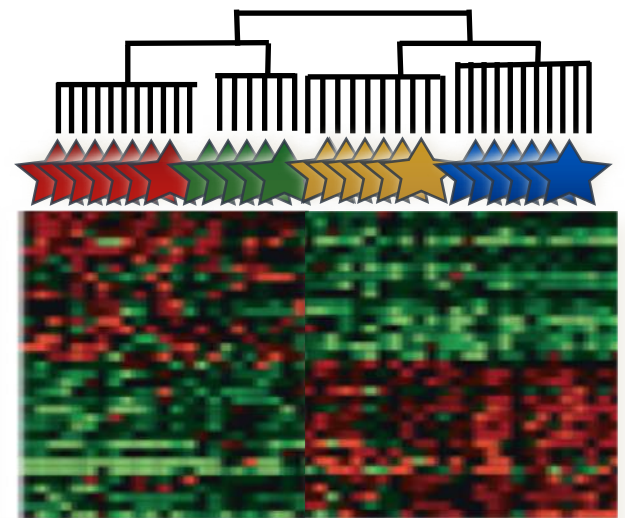
Patient samples **regardless of treatment type**, comparison of findings across all individual samples

Unsupervised analyses

Tumor samples



Molecular markers for tumor classes



Treatment prediction

Needs validation in clinical prospective trial

Important for treatment stratification

Often restricted to a specific technology and specific algorithms

Only valid for a given treatment regimen and a selected patient group

Limited usefulness for identification of novel treatment regimens

Class identification

Needs validation in clinical prospective trial

Important for treatment stratification

Often restricted to a specific technology and specific algorithms

Independent of treatment regimen, **but needs to enter** into “treatment prediction” trials

Aims at identification of novel treatment regimens



A GENE-EXPRESSION SIGNATURE AS A PREDICTOR
IN BREAST CANCER

MARC J. VAN DE VLIET, M.D., Ph.D., YUDONG D. HE, Ph.D., LAURA J. VAN 'T VEER,
AUGUSTINUS A.M. HART, M.Sc., DOREN W. VOSKUI, Ph.D., GEORGE J. SCHREIBER, M.S.,
CHRIS ROBERTS, Ph.D., MATTHEW J. MARTON, Ph.D., MARK PARRISH, DOUWE A.
ANNUSKA GLAS, Ph.D., LEONIE DELAHAYE, TONY VAN DER VELDE, HARRY BAR
SJOERD RODENHUIS, M.D., Ph.D., EMIEL T. RUTGERS, M.D., STEPHEN H.
AND RENE BERNARDS, Ph.D.

ABSTRACT

Background. A more accurate means of prognostication in breast cancer will improve the selection of patients for adjuvant systemic therapy.

Methods. Using microarray analysis to evaluate our previously established 70-gene prognosis profile, we classified a series of 295 consecutive patients with primary breast carcinomas as having a gene-expression signature associated with either a poor prognosis or a good prognosis. All patients had stage I or II breast cancer and were younger than 53 years old; 151 had lymph-node-negative disease, and 144 had lymph-node-positive disease. We evaluated the predictive power of the prognosis profile using univariable and multivariable statistical analyses.

Results. Among the 295 patients, 180 had a poor-prognosis signature and 115 had a good-prognosis signature, and the mean (\pm SE) overall 10-year survival rates were 54.6 \pm 4.4 percent and 94.5 \pm 2.6 percent, re-

ADJUVANT improves disease both premenopausal women up to lymph-node-negative cancer.^{1,2} It is generally prognostic features therapy.^{3,4} The main prognostic features are age, tumor size, histologic type of the tumor, and hormone-receptor status. Factors have been investigated the outcome of disease limited predictive.

Using complementary to analyze breast-cancer tumors with distinct patterns they termed "basal type" subgroups differ with increase in patients with low addition, microarray distinguish cancers associated mutations^{5,9} and to detect^{6,9,10} and lymph-node.

Using inkjet-synthesis rays, we recently identified

From the Divisions of Diagnostic
D.A. A.W., A.G., L.D., R.
(S.R.), Biometrics (T.V.),
Genetics (R.B.), Center
for Biomedical Genetics, and
Land, Wash. (T.D.H., H.D.).
gene regions to Dr. Irene
Netherlands; Cancer Institute
Netherlands; or at Erasmus
Drs. van de Vijver, He,
and

N Engl J Med, Vol. 347, No. 25 • December

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ORIGINAL ARTICLE

A Multigene Assay to Predict Recurrence of
Tamoxifen-Treated, Node-Negative Breast Cancer

Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang,
Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin,
Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew W.
Tasung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher,
D. Lawrence Wickerham, M.D., John Bryant, Ph.D.,
and Norman Wolmark, M.D.

ABSTRACT

BACKGROUND

The likelihood of distant recurrence in patients with breast cancer who have lymph nodes and estrogen-receptor-positive tumors is poorly defined by histopathological measures.

METHODS

We tested whether the results of a reverse-transcriptase-polymerase (RT-PCR) assay of 21 prospectively selected genes in paraffin-embedded would correlate with the likelihood of distant recurrence in patients with tamoxifen-treated breast cancer who were enrolled in the National Breast and Bowel Project clinical trial B-14. The levels of expressed related genes and 5 reference genes were used in a prospectively defined calculate a recurrence score and to determine a risk group (low, intermediate, or high) for each patient.

RESULTS

Adequate RT-PCR profiles were obtained in 668 of 675 tumor blocks. Of patients categorized as having a low, intermediate, or high risk by were 51, 22, and 27 percent, respectively. The Kaplan-Meier estimate

"Recurrence score"

could be used as a continuous function to predict distant recurrence rates.

CONCLUSIONS

The recurrence score has been validated as quantifying the likelihood of recurrence in tamoxifen-treated patients with node-negative, estrogen-breast cancer.

N Engl J Med 347:1272-1283, December 30, 2002

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Robustness, scalability, and integration of a
wound-response gene expression signature
in predicting breast cancer survival

Howard Y. Chang^{1,2,3,4}, Dmitry S. A. Nuyten^{1,2,3,4}, Julie B. Sneddon¹, Trevor Hastie¹, Robert Tibshirani¹, Therese Sorlie^{1,2,3},
Hongyue Dai¹, Yudong D. He^{1,2}, Laura J. van't Veer^{1,2}, Harry Bartelink¹, Matt van de Rijdt¹, Patrick O. Brown^{1,2,3},
and Marc J. van de Vijver^{1,2,3,4}

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Contributed by Patrick O. Brown, January 5, 2003

Based on the hypothesis that features of the molecular program of normal wound healing might play an important role in cancer metastasis, we previously identified consistent features in the transcriptional response of normal fibroblasts to serum, and used this "wound-response signature" to reveal links between wound healing and cancer progression in a variety of common epithelial tumors. Here, in a consecutive series of 295 early breast cancer patients, we show that both overall survival and distant metastasis-free survival are markedly diminished in patients whose tumors expressed this wound-response signature compared to tumors that did not express this signature. A gene expression centroid of the wound-response signature provides a basis for prospectively assigning a prognostic score that can be scaled to suit different clinical purposes. The wound-response signature improves risk stratification independently of known clinical-pathologic risk factors and previously established prognostic signatures based on unsupervised hierarchical clustering ("molecular subtypes") or expression predictors of metastasis ("70-gene prognosis signature").

microarray | prognosis | wound healing | metastasis | treatment decision

In a recent survey, microarray analysis of gene expression patterns has provided a way to improve the diagnosis and risk stratification of many cancers (1–5). Unsupervised analysis of global gene expression patterns has identified molecularly distinct subtypes of cancer, distinguished by extensive differences in gene expression, in diseases that were considered homogeneous based on classical diagnostic methods (1, 3, 4, 7). The molecular subtypes are often associated with different clinical outcomes. Global gene expression analysis can also be employed for treatment

response ("CSR") genes and their canonical expression pattern in fibroblasts activated with serum, the soluble fraction of clotting blood and an important initiator of wound healing *in vivo*. The CSR genes were chosen to minimize overlap with cell cycle genes, but instead appeared to represent other important processes in wound healing, such as matrix remodeling, cell motility, and angiogenesis, processes that are likely also to contribute to cancer invasion and metastasis. In several common epithelial tumors such as breast, lung, and gastric cancers, expression of the wound-response signature predicted poor overall survival and increased risk of metastasis (10). These initial findings demonstrate the promise of using hypothesis-driven gene expression signatures to provide insights from existing gene expression profiles of cancers. However, as is other methodologies, reproducibility and scales for interpretation need to be evaluated before this strategy can be generally adopted for biologic discovery and clinical use.

The best validation of a gene signature's prognostic value lies in its ability to predict outcome in large independent data sets. Here we examine a database of 295 breast cancer patients from the Netherlands Cancer Institute that had previously been used to identify and validate a prognostic gene expression profile, defined by a set of 70 genes (5, 9). We used this data set to test the reproducibility of the association between the wound-response signature and breast cancer progression, and to investigate how the information from diverse gene expression signatures identified by various means might be integrated both biologically and for clinical use.

Materials and Methods

Gene expression data were obtained from the microarray analysis of 295 breast cancer patients from the Netherlands Cancer Institute. Each patient sample was analyzed on a 10,000 A, and measurement of expression was based on national protein clinical trials at the patients who had lymph

"Wound Response"

metastasis.

Gene expression patterns provide a common language among biologic phenomena and allow an alternative approach to infer physiologic and molecular mechanisms from complex human disease states (1, 10, 11, 12). Starting with the gene expression profile of cells manipulated *in vitro* to simulate a biologic process, the expression profile can then be used to interpret the gene expression data of human cancers and test specific hypotheses. To understand the similarities between wound healing and cancer, Chang *et al.* (10) identified a set of "core serum

expression signature" (CSR) genes and their canonical expression pattern in fibroblasts activated with serum, the soluble fraction of clotting blood and an important initiator of wound healing *in vivo*. The CSR genes were chosen to minimize overlap with cell cycle genes, but instead appeared to represent other important processes in wound healing, such as matrix remodeling, cell motility, and angiogenesis, processes that are likely also to contribute to cancer invasion and metastasis. In several common epithelial tumors such as breast, lung, and gastric cancers, expression of the wound-response signature predicted poor overall survival and increased risk of metastasis (10). These initial findings demonstrate the promise of using hypothesis-driven gene expression signatures to provide insights from existing gene expression profiles of cancers. However, as is other methodologies, reproducibility and scales for interpretation need to be evaluated before this strategy can be generally adopted for biologic discovery and clinical use.

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Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications

Therese Sørlie^{a,b,c}, Charles M. Perou^{a,d}, Robert Tibshirani^e, Turid Aas^f, Stephanie Geisler^g, Hilde Johnsen^b, Trevor Hastie^h, Michael B. Eisen^b, Matt van de Rijnⁱ, Stefanie S. Jeffrey^j, Thor Thorsen^k, Hanne Quist^l, John C. Matese^l, Patrick O. Brown^m, David Botsteinⁿ, Per Eystein Lønning^g, and Anne-Lise Borresen-Dale^{b,n}

Departments of ^aGenetics and ^bSurgery, The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway; ^cDepartment of Genetics and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599; Departments of ^dHealth Research and Policy and Statistics, ^eGenetics, Pathology, Surgery, and ^fBiochemistry and Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305; Departments of ^gMedicine (Section of Oncology), ^hSurgery, and ⁱBiochemical Endocrinology, Haukeland University Hospital, N-5021 Bergen, Norway; and ^jLife Sciences Division, Lawrence Berkeley National Laboratories, and Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720

Contributed by David Botstein, July 17, 2001

"Intrinsic classification"

This study was to classify breast carcinomas based on gene expression patterns derived from cDNA microarray experiments representing 78 breast carcinomas, and four normal breast tissues were used as controls. Hierarchical clustering of the gene expression data revealed that the cancers could be classified into a basal epithelial-like group, an *ERBB2*-overexpressing group and a normal breast-like group based on variations in gene expression. A novel finding was that the previously characterized luminal epithelial/estrogen receptor-positive group could be divided into at least two subgroups, each with a distinctive expression profile. These subtypes proved to be reasonably robust by clustering using two different gene sets: first, a set of 456 cDNA clones previously selected to reflect intrinsic properties of the tumors and, second, a gene set that highly correlated with patient outcome. Survival analyses on a subcohort of patients with locally advanced breast cancer uniformly treated in a prospective study showed significantly different outcomes for the patients belonging to the various groups, including a poor prognosis for the basal-like subtype and a significant difference in outcome for the two estrogen receptor-positive groups.

The biology of breast cancer remains poorly understood. Although lymph node metastases (1), histologic grade (2), expression of steroid and growth factor receptors (3, 4), estrogen-inducible genes like cathepsin D (5), protooncogenes like *ERBB2* (6), and mutations in the *TP53* gene (7, 8) all have been correlated to prognosis, knowledge about individual prognostic factors provides limited information about the biology of the disease. Thus, because of their internal correlations in multivariate analysis, the prognostic value of many of these parameters fades away (9, 10).

The cellular and molecular heterogeneity of breast tumors and the large number of genes potentially involved in controlling cell

correlations between gene expression patterns and clinically relevant parameters. We found that classification of tumors based on gene expression patterns can be used as a prognostic marker with respect to overall and relapse-free survival in a subset of patients that had received uniform therapy. One finding was the separation of estrogen receptor (ER)-positive tumors into at least two distinctive groups with characteristic gene expression profiles and different prognosis.

Materials and Methods

Patients and Tumor Specimens. A total of 78 breast carcinomas (71 ductal, five lobular, and two ductal carcinomas *in situ* obtained from 77 different individuals; two independent tumors from one individual diagnosed at different times) and three fibroadenomas were analyzed in this study. These include 40 tumors that were previously analyzed and described (14). Four normal breast tissue samples from different individuals also were included, three of which were pooled normal breast samples from multiple individuals (CLONTECH). In summary, 85 tissue samples representing 84 individuals were analyzed. Tissue samples were snap-frozen in liquid N₂ and stored at -170°C or -80°C. All tumor specimens analyzed contained more than 50% tumor cells. Fifty-one of the patients were part of a prospective study on locally advanced breast cancer (T₃/T₄ and/or N₂ tumors) treated with doxorubicin monotherapy before surgery followed by adjuvant tamoxifen in the case of positive ER and/or progesterone receptor (PgR) status (15). All but three patients were treated with tamoxifen. ER and PgR status was determined by using ligand-binding assays, and mutation analysis of the *TP53* gene was performed as described (15). All common polymorphisms were recorded, but are considered wild type in this study. A detailed list of all samples and clinical data for the patients is included in Table 1, which is published as supporting information on the PNAS web site, www.pnas.org.

RNA Analysis. Total RNA was isolated by phenol-guanidine extraction (Trizol, GIBCO/BRL), and mRNA was purified by either magnetic separation using Dynabeads (Dynal) or by using the Invitrogen FastTrack 2.0 Kit. All experiments and the production of microarrays were performed as described (14), with detailed protocols available at <http://cmgm>.

Abbreviations: ER, estrogen receptor; SAM, significance analysis of microarrays.

*T.S. and C.M.P. contributed equally to this work.

†To whom reprint requests should be addressed. E-mail: atb@labmed.uio.no.

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559 clones representing 494 unique genes

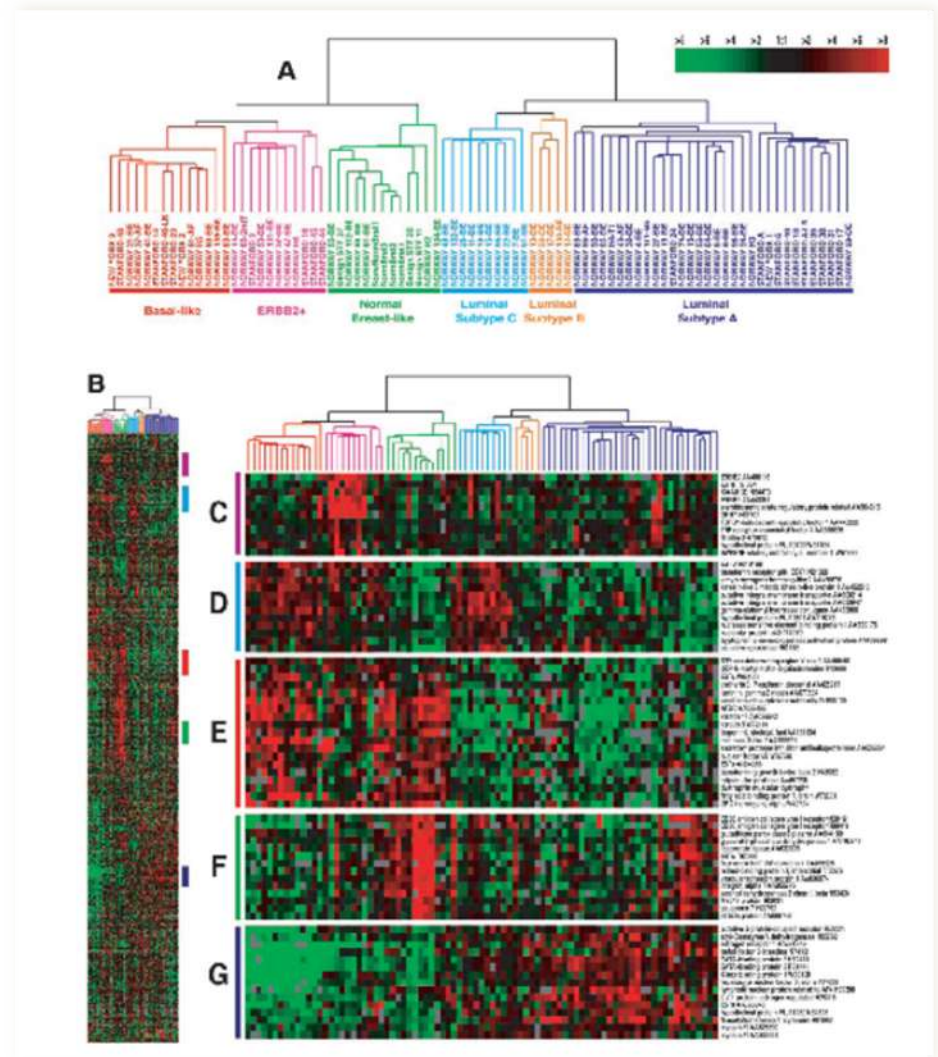
and the presence of phenotypic variation, might provide the basis for an improved taxonomy of cancer (11–14).

Recently, we reported that variations in gene expression patterns in 40 grossly dissected human breast tumors analyzed by cDNA microarrays and hierarchical clustering provided a distinctive "molecular portrait" of each tumor, and that the tumors could be classified into subtypes based solely on differences in these patterns (14). The present work refines our previous classifications by analyzing a larger number of tumors and explores the clinical value of the subtypes by searching for

Molecular subtypes by gene expression

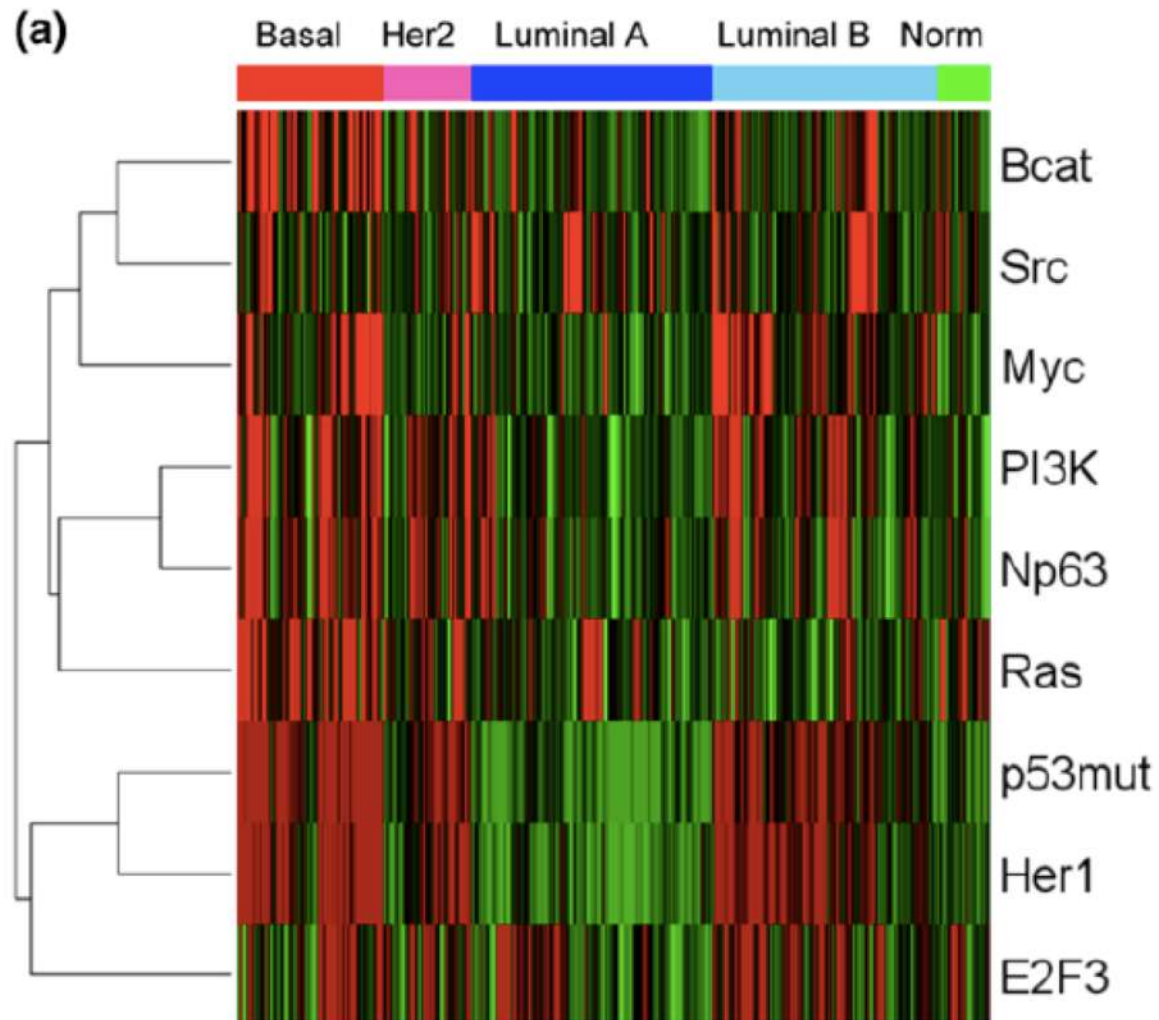
- 561 genes selected as most “intrinsic” for individual tumors before and after treatment
- Clustering of other sets of tumors by the expression of these genes group them into five main groups

Luminal A, Luminal B, HER2-enriched, Basal-like and Normal-like.



Pathway differences - phenotypes

- Some pathways vary between subtypes
- Some pathways vary within subtypes



Luminal breast cancer

Luminal A characteristics:

- ~60% of breast cancers
- ER and PR positive
- Tend to have low proliferation level
- Do not overexpress HER2
- Includes ER positive special type cancers (tubular, mucinous)
- High expression of hormone receptors and associated genes
- Respond to endocrine therapy
- Good prognosis, a large subset are cured by surgery alone (of post menopausal patients)

Luminal B characteristics:

- ~10% of breast cancers
- ER positive but can be PgR low
- High proliferation level
- Respond to endocrine therapy and chemotherapy
- Adverse prognosis if not treated appropriately

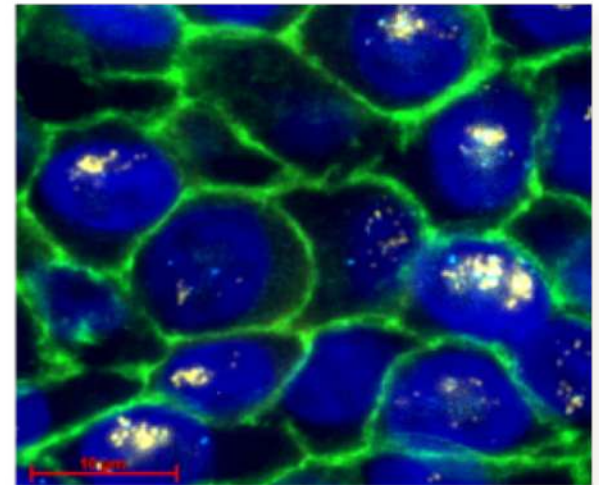
HER2-enriched and basal-like breast cancer

HER2- enriched

- Can be either ER+ or ER-
- HER2 pathway active
- Can have gain of HER2 (low level or high level)
- High proliferating
- Can have extensive immune cell infiltration
- Can respond to chemotherapy
- Very slim prognosis until HER2 target therapy was introduced (Trastuzumab)
- Dual-blockage is promising (to avoid resistance, i.e. relapse)
- NB: a HER2 enriched tumor can be clinically HER2 negative...

Basal-like:

- ER-/PgR-/HER2-
- Frequently grade 3
- Solid growth
- High proliferation
- Can have extensive immune cell infiltration
- Can be positive for CK5/6, EGFR
- Can respond to chemotherapy



Immunofluorescence:

Blue: DAPI (nuclear)

Green: HER2 protein

Yellow: HER2 gene probe

Light blue: Cent 17 probe

St. Gallen consensus meeting 2015

Table 2. Treatment-oriented classification of subgroups of breast cancer

Clinical grouping	Notes
Triple-negative	Negative ER, PgR, and HER2
Hormone receptor-negative and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-negative luminal disease as a spectrum:	ER and/or PgR positive $\geq 1\%$ ^a
High receptor, low proliferation, low tumor burden (luminal A-like)	Multiparameter molecular marker 'favorable prognosis' if available. High ER/PgR and clearly low Ki-67 ^b . Low or absent nodal involvement (N 0–3), smaller T size (T1 T2).
Intermediate	Multiparameter molecular marker 'intermediate' if available ^c . Uncertainty persists about degree of risk and responsiveness to endocrine and cytotoxic therapies.
Low receptor, high proliferation, high tumor burden (luminal B-like)	Multiparameter molecular marker 'unfavorable prognosis' if available. Lower ER/PgR with clearly high Ki-67 ^b . More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).

^aER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.

^bKi-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low.

^cNot all multiparameter molecular marker tests report an intermediate score.

St. Gallen consensus meeting 2015

Clinical grouping	Notes	
Triple-negative	Negative ER, PgR, and HER2	"Basal-like"
Hormone receptor-negative and HER2-positive	ASCO/CAP guidelines	"HER2-enriched"
Hormone receptor-positive and HER2-positive	ASCO/CAP guidelines	"Luminal B/HER2-like"
Hormone receptor-positive and HER2-negative luminal disease as a spectrum: High receptor, low proliferation, low tumor burden (luminal A-like)	ER and/or PgR positive $\geq 1\%^a$ Multiparameter molecular marker 'favorable prognosis' if available. High ER/PgR and	"Luminal A-like"
Intermediate		
Low receptor, high proliferation, high tumor burden (luminal B-like)	Multiparameter molecular marker 'unfavorable prognosis' if available. Lower ER/PgR with clearly high Ki-67 ^b . More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).	"Luminal B-like"

Still IHC phenotypes for diagnosis – but luminal disease in need of more

^aER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.
^bKi-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low.
^cNot all multiparameter molecular marker tests report an intermediate score.

Molecular based classification

- Biomarkers/signatures for treatment prediction?
- Biomarkers/signatures recognizing biological distinct traits?
- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- And are the designated class the same throughout the entire evolution of a given tumor?

Similarities in phenotype....







...but different genotype!

DNA Translocations and copy number changes

Few alterations...

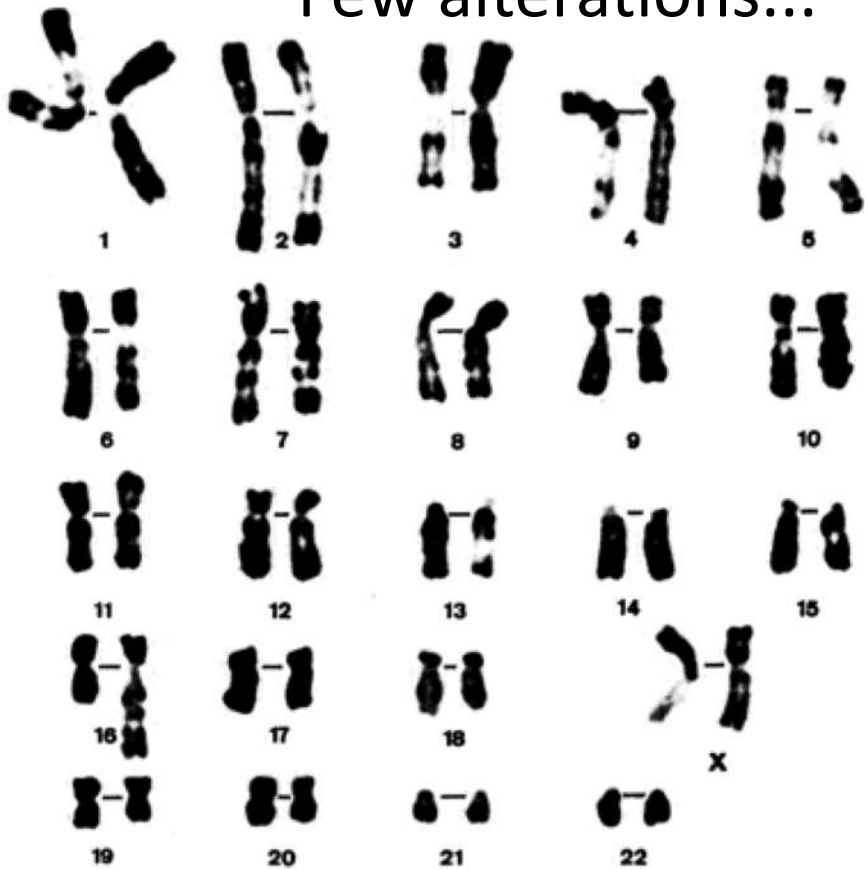
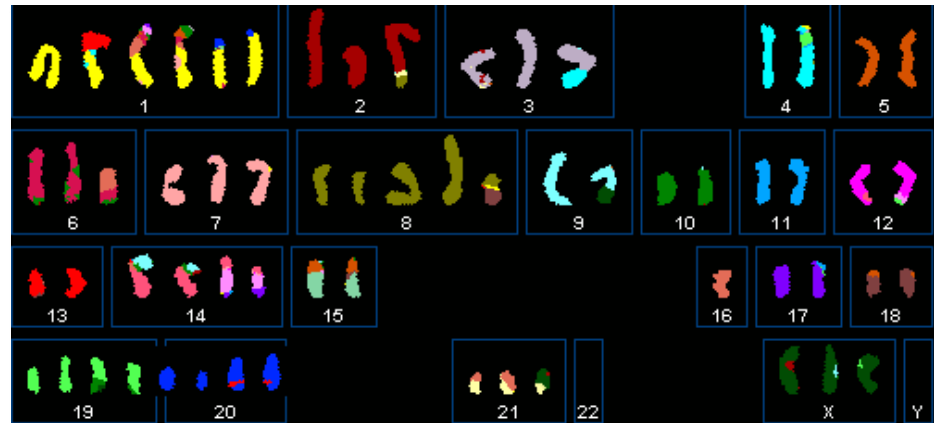
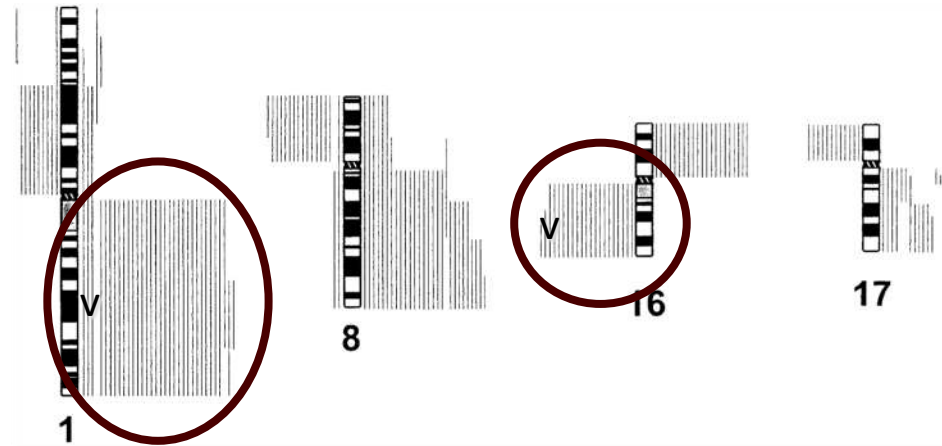


Figure 1 Karyotype from case 27, exhibiting der(1q16p) as the sole anomaly.



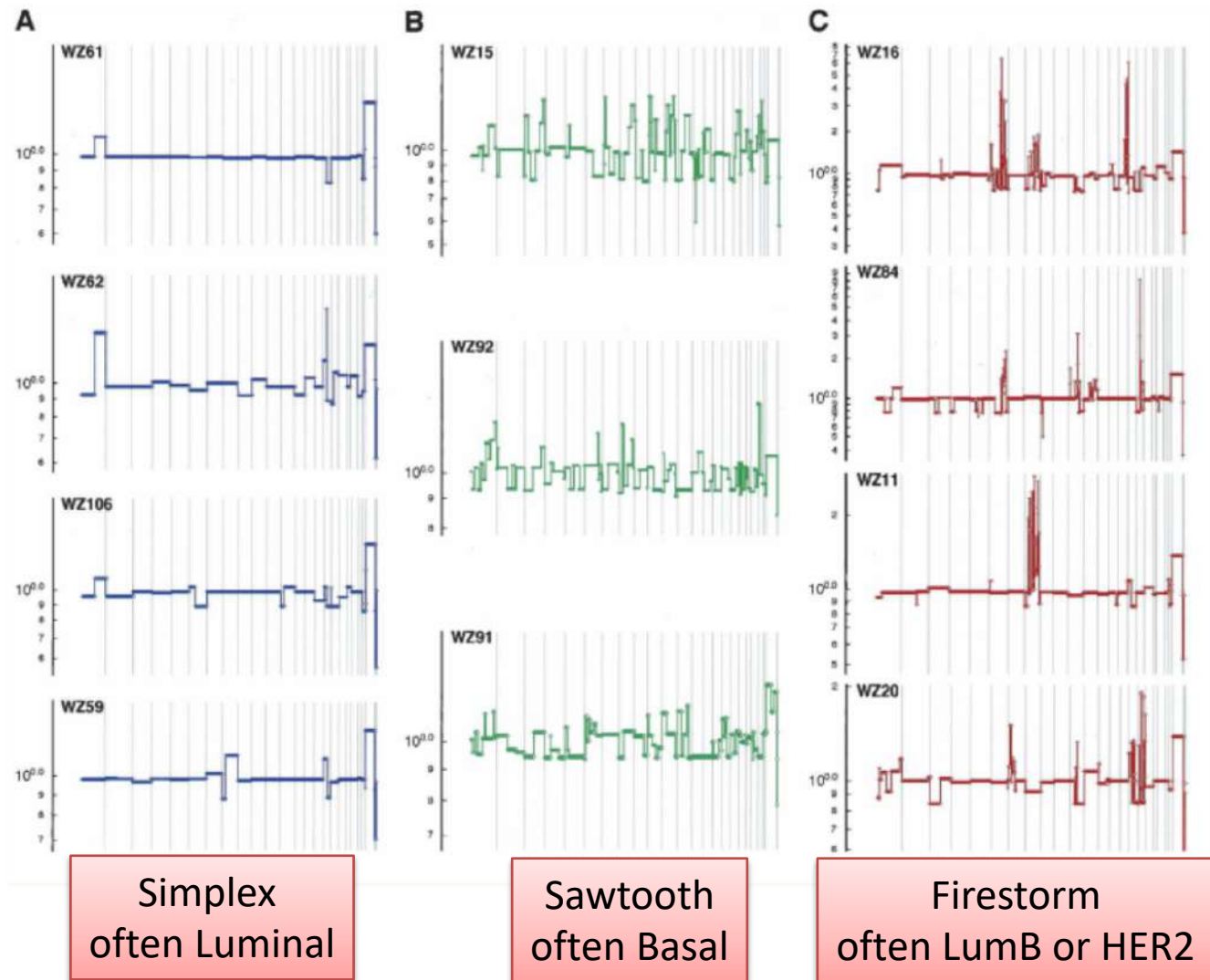
Dutrillaux et al. Cancer Genet Cytogenet 1990

MDA-MB-157, From <http://www.path.cam.ac.uk>

...many alterations

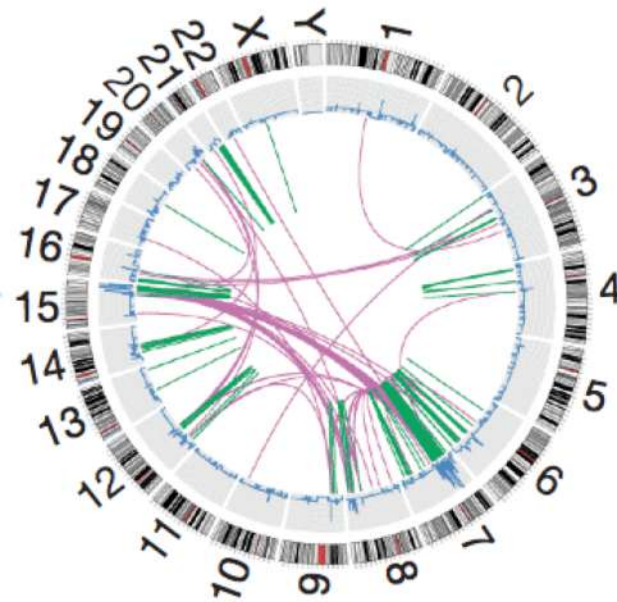
Patterns of genomic rearrangements

- Breast cancer genomes show three main patterns of alterations
 - simplex
 - complex/sawtooth
 - complex/fires form

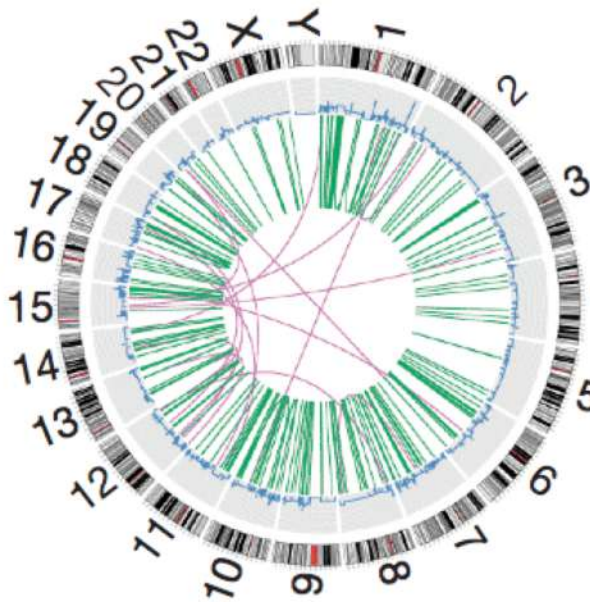


Sequenced breast cancer genomes - structural rearrangements

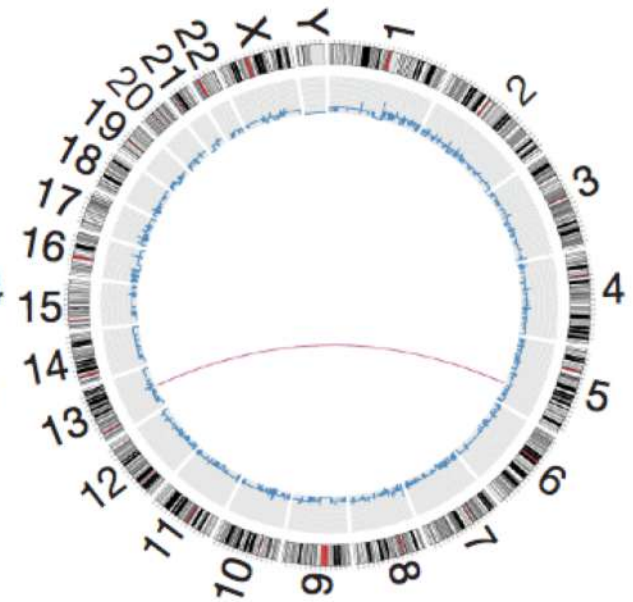
PD3668a
ER⁺ PR⁺ ERBB2⁻



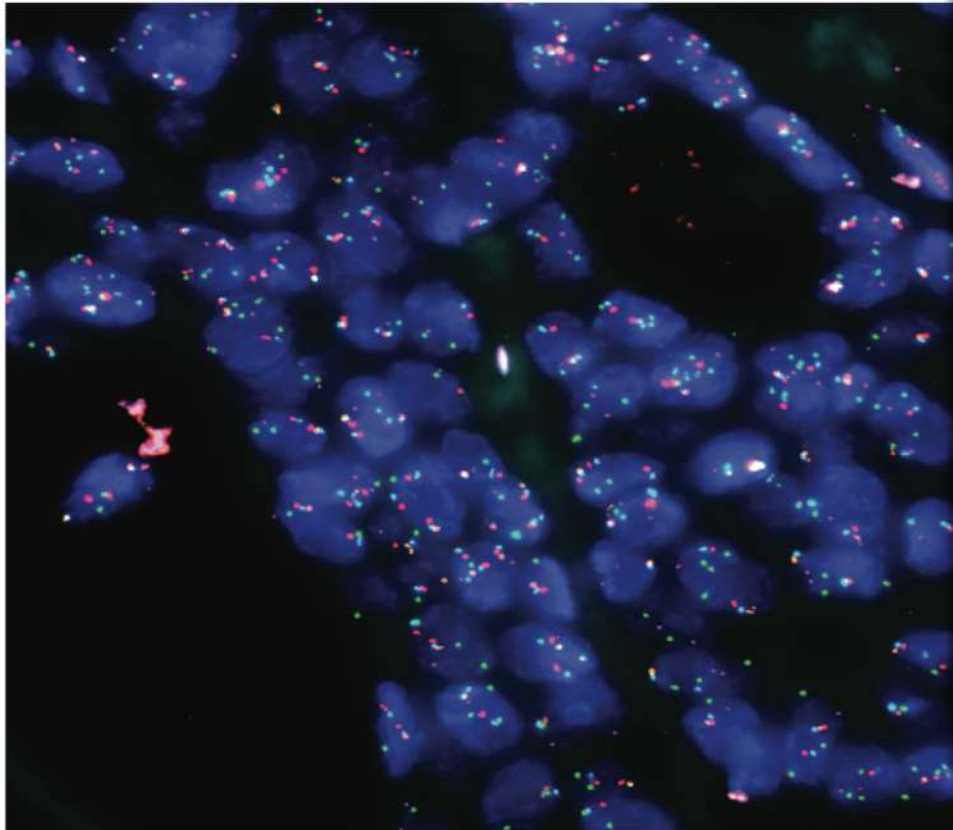
PD3664a
ER⁻ PR⁻ ERBB2⁻



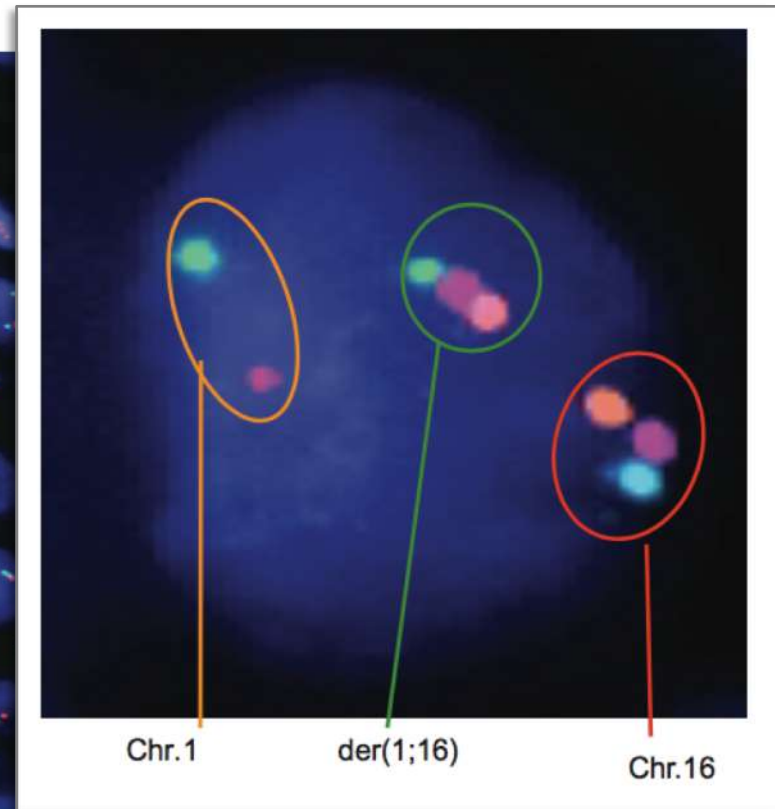
PD3667a
ER⁺ PR⁺ ERBB2⁻



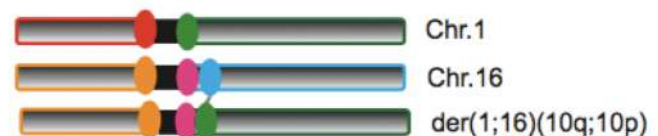
Centromere close translocations; gain and losses of whole chromosome arms



Coll. With A. Zetterberg, CCK, KI, Stockholm



Probe combinations in tumor cells



Rye et al, Genes Chrom Cancer 2015

Class discovery by integrating DNA alterations and gene expression data

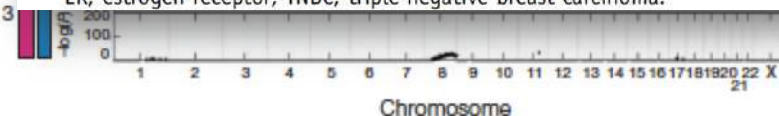
Different genomic drivers across ER+ breast cancer

Table 1 Overview of the Integrative Cluster Subtypes and the Dominating Properties with Regard to Copy Number Driving Events, Biomarkers, Type of DNA Architecture,⁴⁶ Dominant PAM50 Subtype, and Clinical Outcome

Integrative cluster group	Copy number driver	Pathology biomarker class	DNA architecture	Dominant PAM50	Clinical characteristics (survival)
1	Chromosome 17/ chromosome 20	ER ⁺ (HER2 ⁺)	Simplex/firestorm (chromosome 17q)	Luminal B	Intermediate
2	Chromosome 11	ER ⁺	Firestorm (chromosome 11q)	Luminal A and B	Poor
3	Very few	ER ⁺	Simplex/flat	Luminal A	Good
4	Very few	ER ⁺ /ER ⁻	Sawtooth/flat	Luminal A (mixed)	Good (immune cells)
5	Chromosome 17 (HER2 gene)	ER ⁻ (ER ⁺)/HER2 ⁺	Firestorm (chromosome 17q)	Luminal B and HER2	Extremely poor (in pre- Herceptin cohorts)
6	8p deletion	ER ⁺	Simplex/firestorm (chromosome 8p/ chromosome 11q)	Luminal B	Intermediate
7	Chromosome 16	ER ⁺	Simplex (chromosome 8q/chromosome 16q)	Luminal A	Good
8	Chromosome 1, Chromosome 16	ER ⁺	Simplex (chromosome 1q/chromosome 16q)	Luminal A	Good
9	Chromosome 8/ Chromosome 20	ER ⁺ (ER ⁻)	Simplex/firestorm (chromosome 8q/ chromosome 20q)	Luminal B (mixed)	Intermediate
10	Chromosome 5, Chromosome 8, Chromosome 10, Chromosome 12	TNBC	Complex/sawtooth	Basal-like	Poor 5-year, good long-term if survival

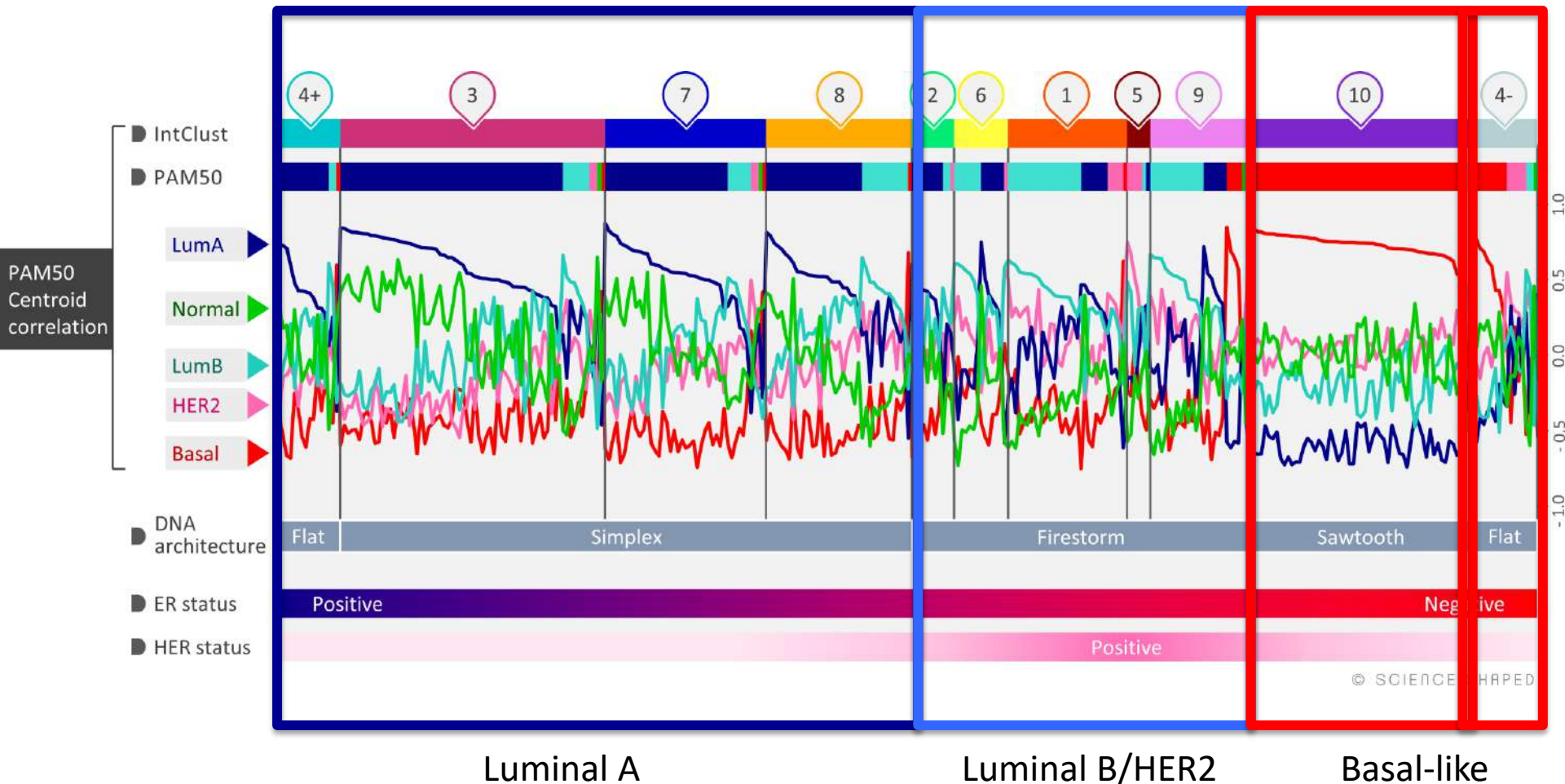
ER, estrogen receptor; TNBC, triple-negative breast carcinoma.

Russnes et al. Am J Pathology, 2017



Curtis et al. Nature, 2012

PAM50 – IntClust – DNA architecture

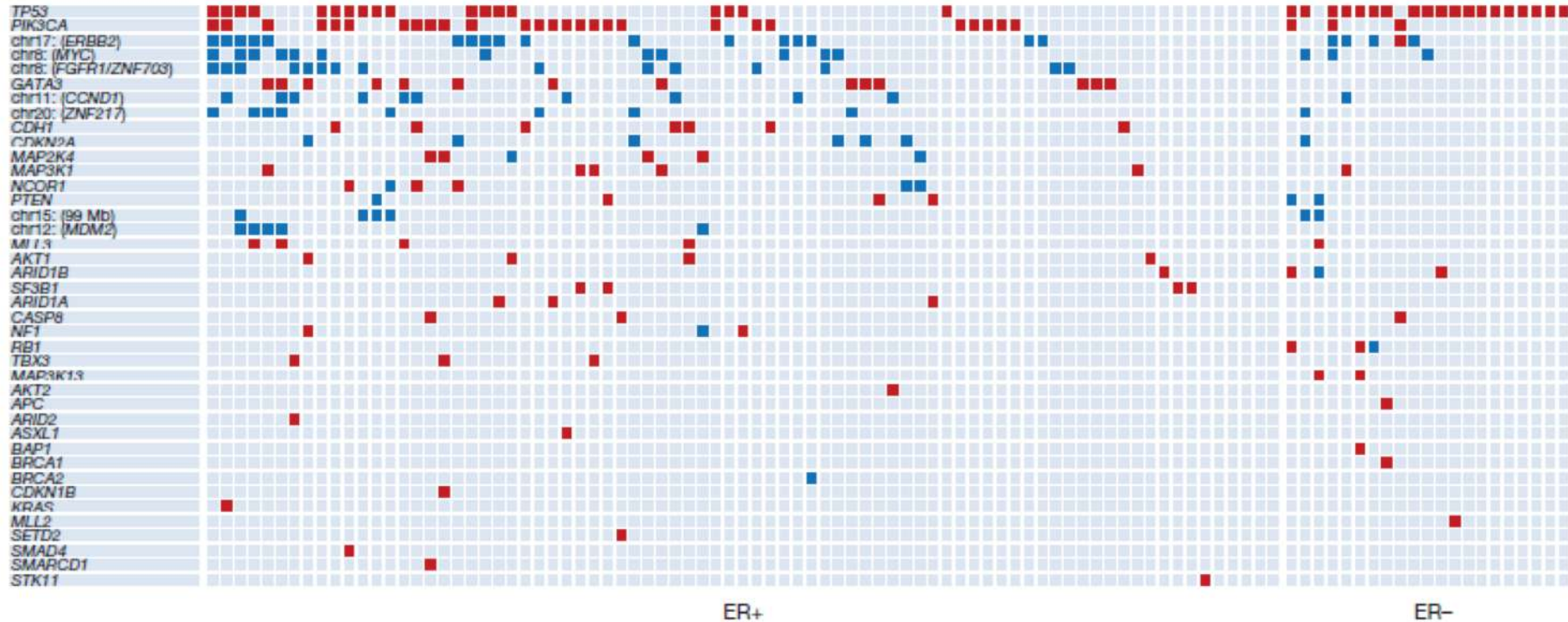


NB: centroid classification has five dimensions!

Russnes et al., Am J of Pathology, 2017

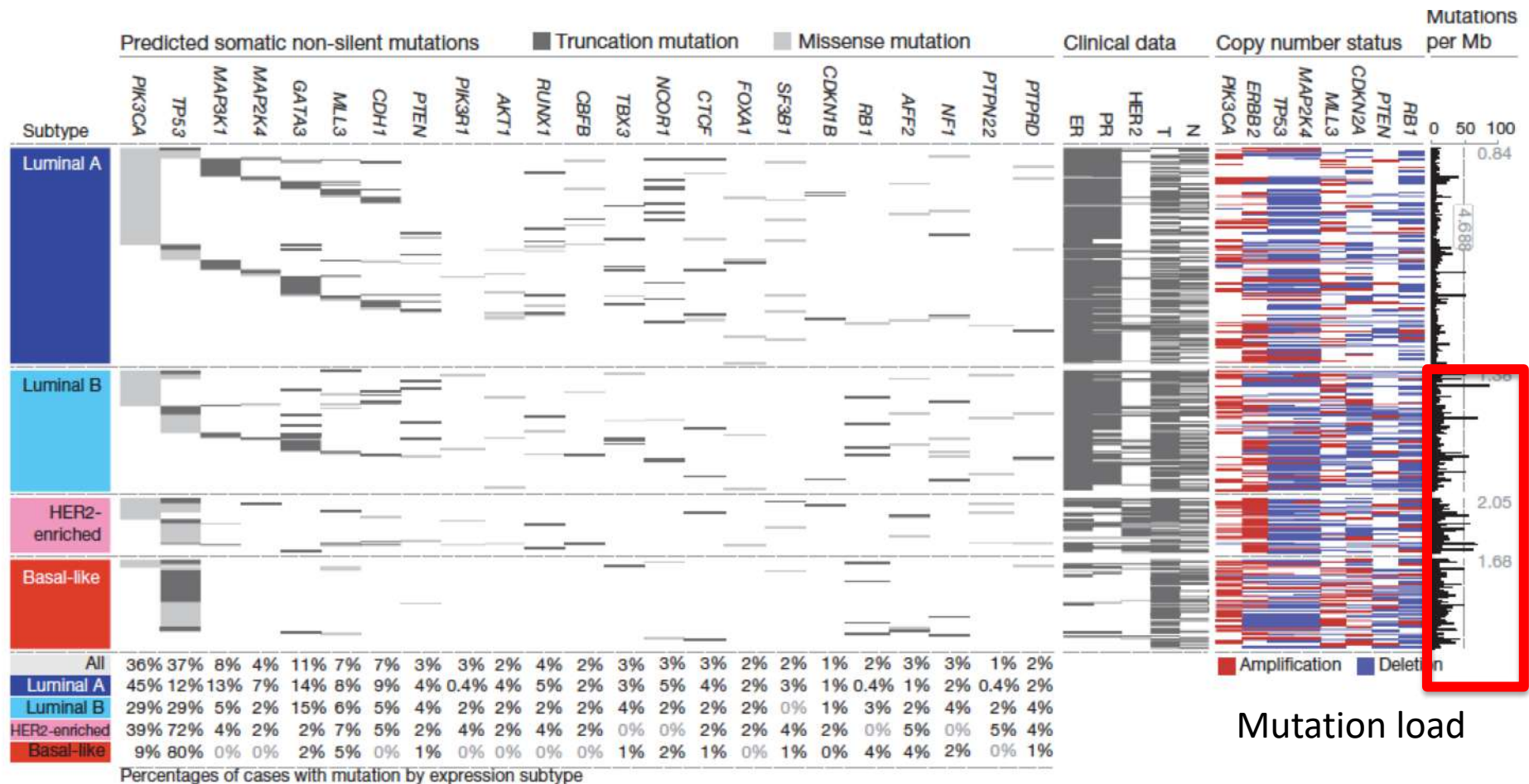
Mutations - “Personal” profiles?

100 breast cancer samples, 40 genes -> a total of 73 different combinations of mutated genes!



Stephens et al. Nature 2012

A specter of DNA mutations



Molecular based classification

- Biomarkers/signatures for treatment prediction?
- Biomarkers/signatures recognizing biological distinct traits?
- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- And are the designated class the same throughout the entire evolution of a given tumor?

Yes – but clearly adding more!

Luminal breast cancer

Table 2. Treatment-oriented classification of subgroups of breast cancer

Clinical grouping	Notes
Triple-negative	Negative ER, PgR, and HER2
Hormone receptor-negative and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-negative luminal disease as a spectrum:	ER and/or PgR positive $\geq 1\%$ ^a
High receptor, low proliferation, low tumor burden (luminal A-like)	Multiparameter molecular marker 'favorable prognosis' if available. High ER/PgR and clearly low Ki-67 ^b . Low or absent nodal involvement (N 0–3), smaller T size (T1 T2).
Intermediate	"intermediate group" Multiparameter molecular marker 'intermediate' if available ^c . Uncertainty persists about degree of risk and responsiveness to endocrine and cytotoxic therapies.
Low receptor, high proliferation, high tumor burden (luminal B-like)	"LumB-like" Multiparameter molecular marker 'unfavorable prognosis' if available. Lower ER/PgR clearly high Ki-67 ^b . More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).

^aER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.

^bKi-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low.

^cNot all multi

Multiparameter molecular marker needed

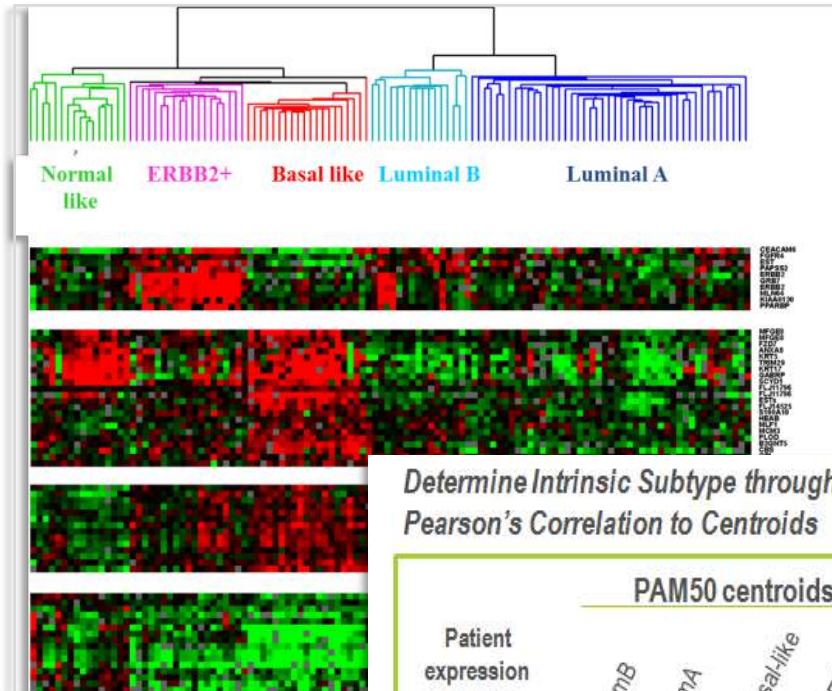
Luminal disease is defined as a spectrum

Several validated molecular multimarker tests predict prognosis and/or therapy response:

- Oncotype Dx
- Mammaprint
- BCI
- IHC4
- Rotterdam signature
- Prosigna (PAM50 ROR)
- Endopredict
- Mammostrat
- MammaTyper

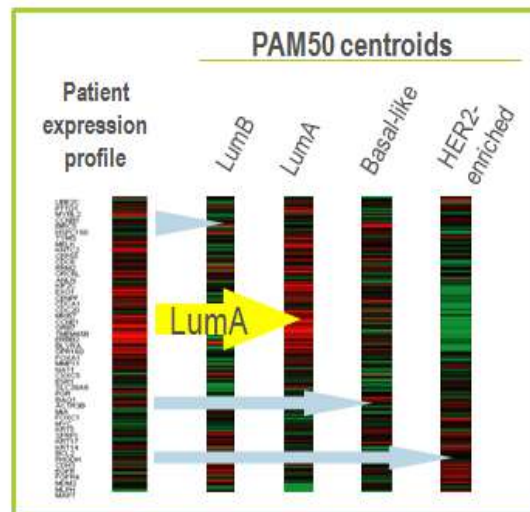
- ...but Ki-67 is easy and cheaper

From intrinsic subtypes to PAM50 to Prosigna



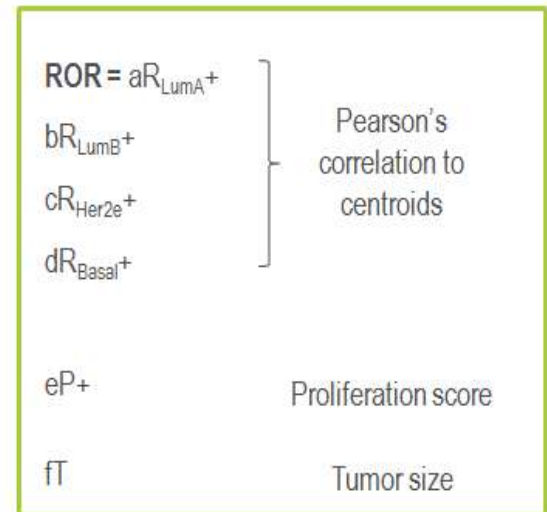
Sørli, Perou et al PNAS, 2006

Determine Intrinsic Subtype through Pearson's Correlation to Centroids

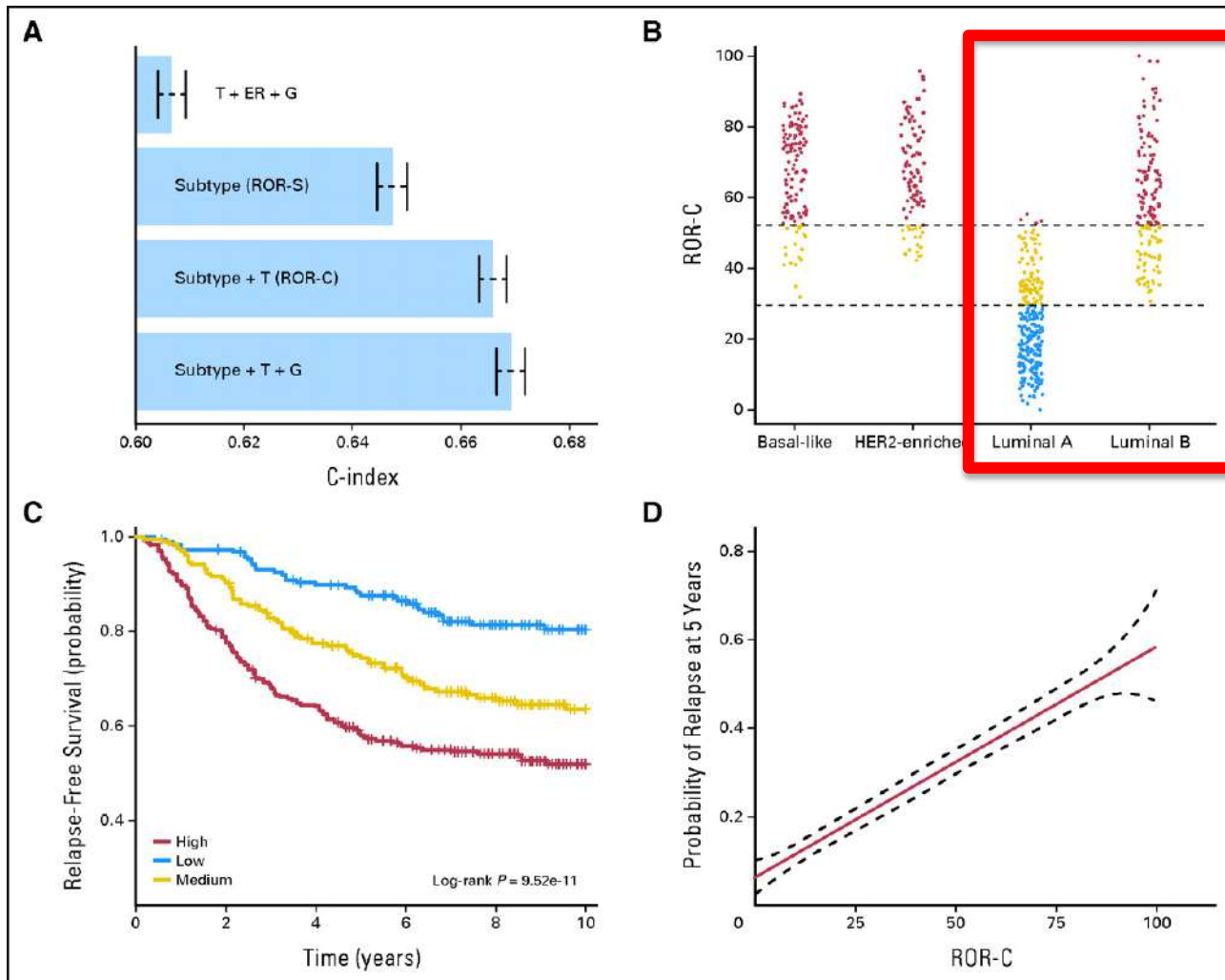


- PAM50: **5 subtypes** (Parker et al. JCO 2009)
- Prosigna™ Breast Cancer Prognostic Gene Signature Assay on the nCounter® Dx Analysis System (Nanostring)
4 subtypes and ROR score
- Assignment of subclass by **centroide correlation**

Calculate Risk of Recurrence (ROR) Score



PAM50/Prosigna: Risk of relapse (ROR) predictions using a test set of node-negative, no systemic therapy patients.



Joel S. Parker et al. JCO 2009;27:1160-1167

NanoString nCounter Analysis System

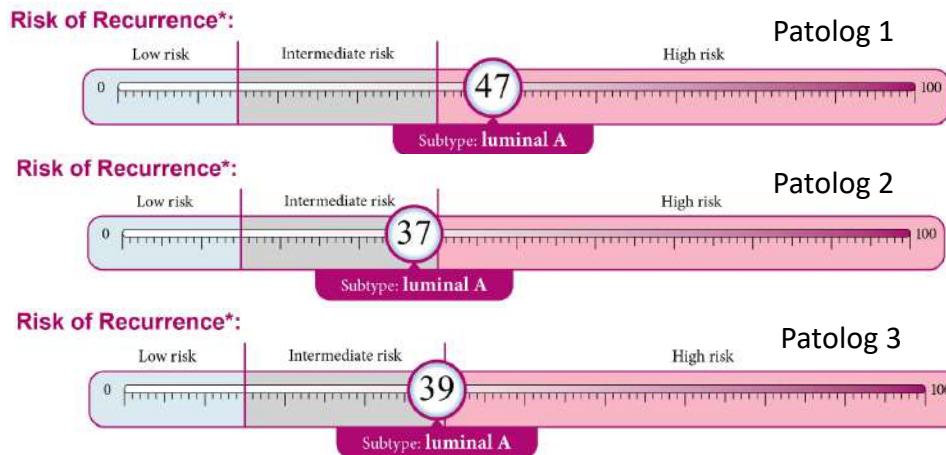
- Not PCR based – suited for RNA from FFPE
- Can be run as both a research instrument and a diagnostic instrument (black box)
- Up to 800 genes (can do DNA and protein as well)



Challenge: regional intra tumor heterogeneity

Tumor area selection by three pathologists:

Prosigna score:



Luminal A
ROR: 47
High risk



Luminal A
ROR: 37
Intermed.
risk



Luminal A
ROR: 39
Intermed.
risk

Morphology is of importance: the selection of area can determine use of adjuvant chemotherapy or not!

The diversity of Basal-like tumors

Biology of Human Tumors

Clinical
Cancer
Research

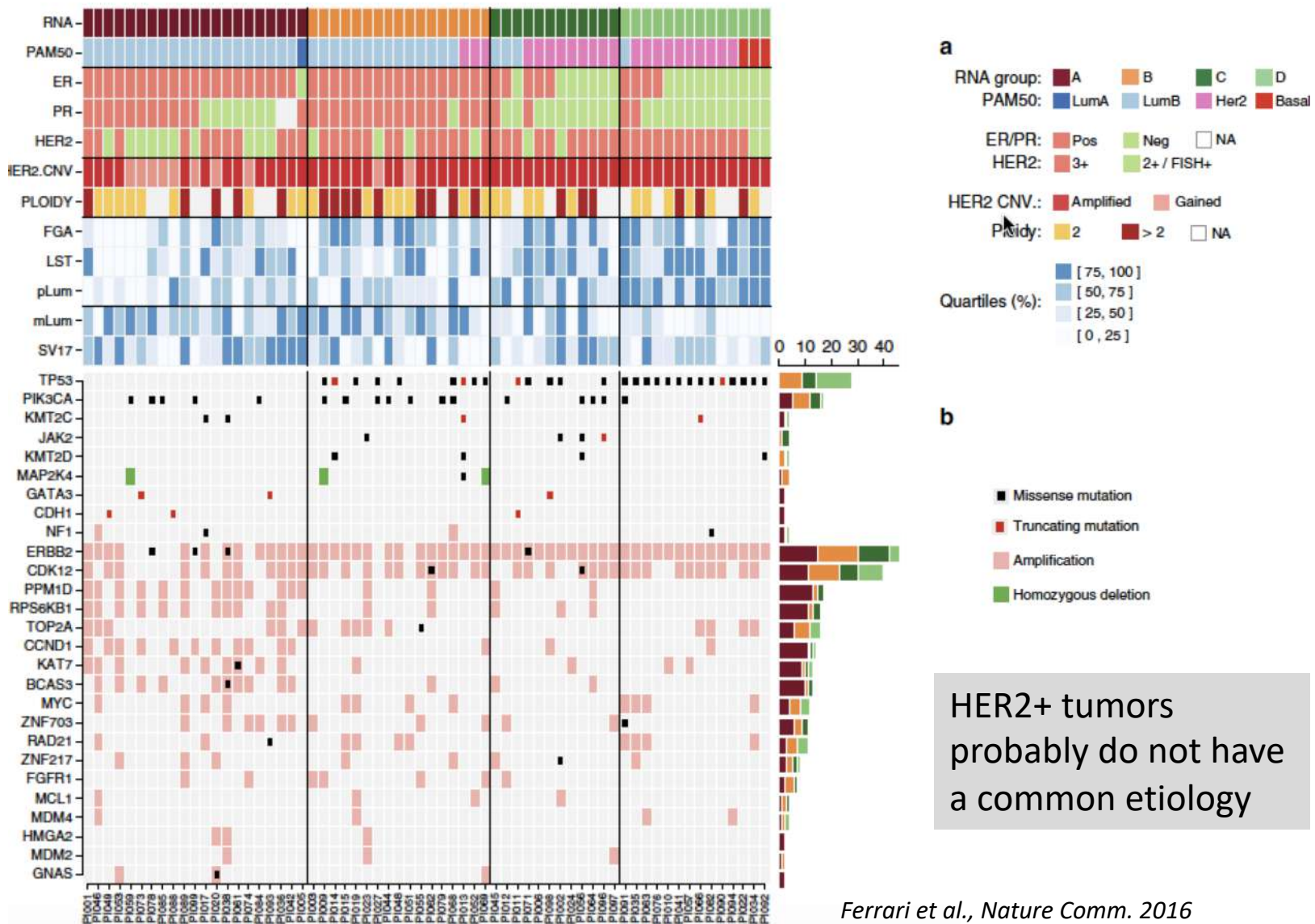
Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer

Matthew D. Burstein¹, Anna Tsimelzon², Graham M. Poage³, Kyle R. Covington², Alejandro Contreras^{2,4}, Suzanne A.W. Fuqua², Michelle I. Savage³, C. Kent Osborne², Susan G. Hilsenbeck², Jenny C. Chang⁵, Gordon B. Mills⁶, Ching C. Lau⁷, and Powel H. Brown³

Molecular pathways enriched in the four groups

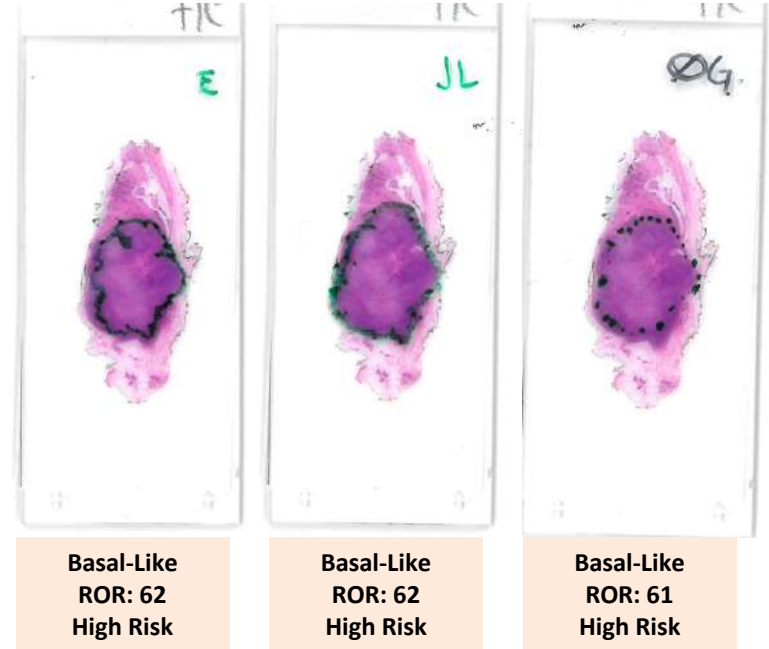
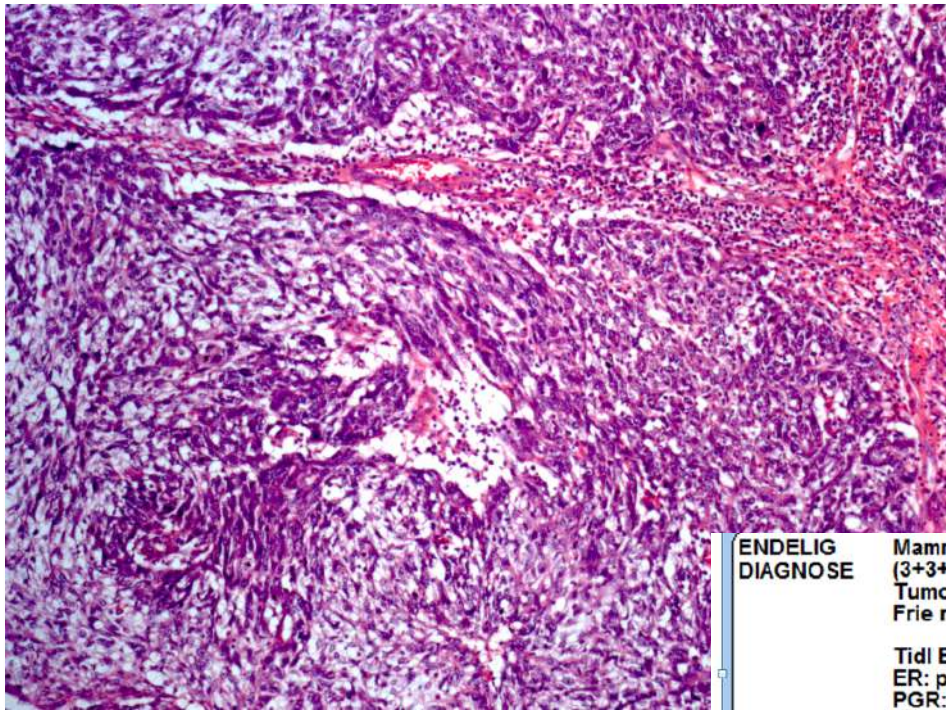
[illegible]

The diversity of HER2+ tumors



Diagnostic challenge: ER status by molecular multimarker test

Tumor area selection by three pathologists:



NB: Not ER+ by gene PAM50, and medullary BC is most frequently ER-

**ENDELIG
DIAGNOSE** Mammaresektat (ve. side) med infiltrerende duktalt karsinom, histologisk grad 3 (3+3+3p)
Tumordiameter 23 mm
Frie reseksjonsrender, knapp ventralt (under 0,5 mm)

Tidl BM15 13094:
ER: positiv (ca. 20%)
PGR: negativ (0%)
Her2: score=0 ved immunhistokjemi (klinisk negativ)
Ki-67 score: >75% gjennom hele tumor

BU15 28335:
En sentinel lymfeknute uten påviste patologiske forandringer

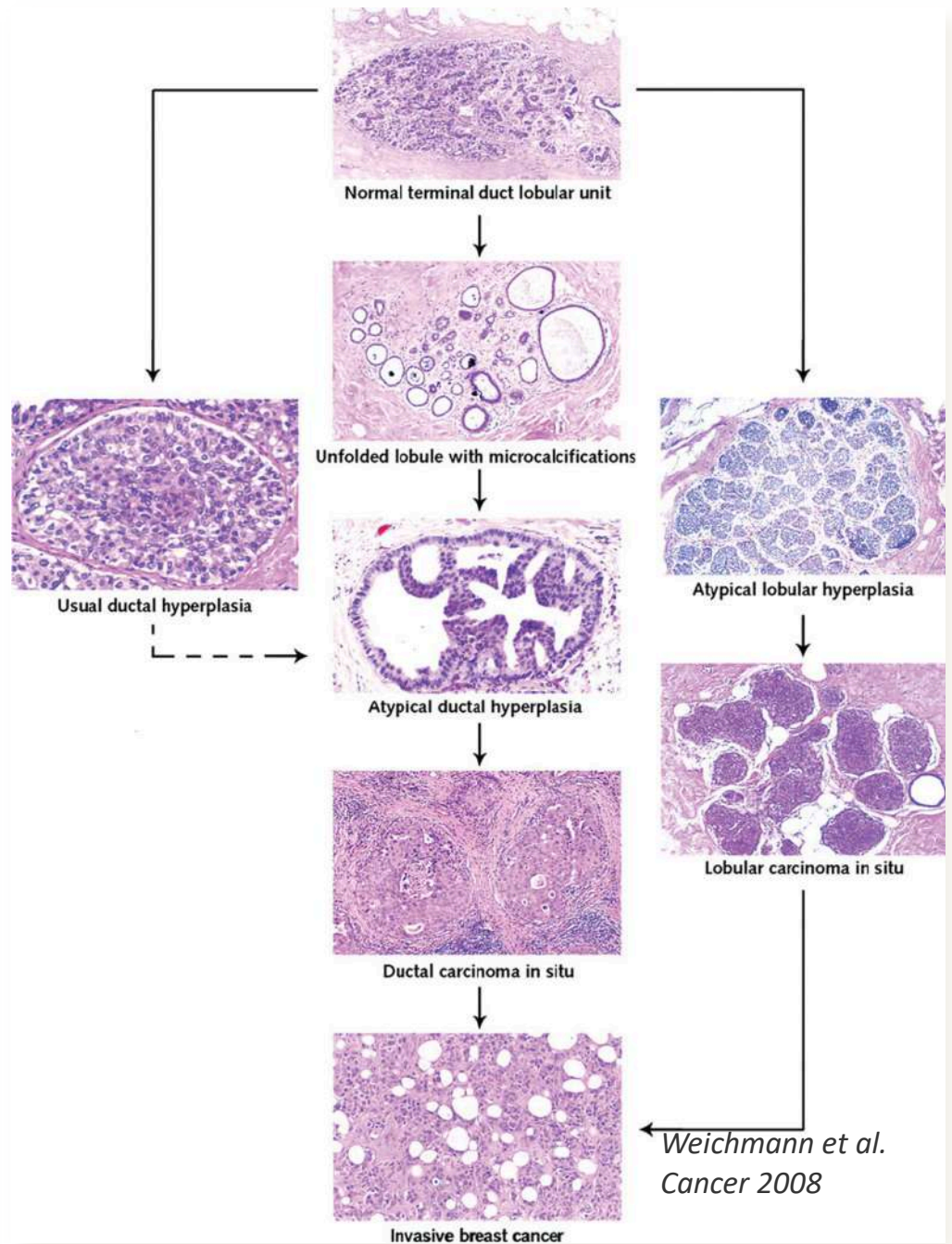
SNOMED
Vurdering

T 04030 M 85003 pT2 pN0 pMx G3 F 12391 F 12645 E her2N P ki763 P 11010
Reseptorstatus, HER-2 og Ki-67 er gjentatt i operasjonspreparatet med samme resultat som i grovnalsbiopsi.
Tumor har morfologisk visse medullære trekk.

Molecular based classification

- Biomarkers/signatures for treatment prediction?
- Biomarkers/signatures recognizing biological distinct traits?
- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- And are the designated class the same throughout the entire evolution of a given tumor?

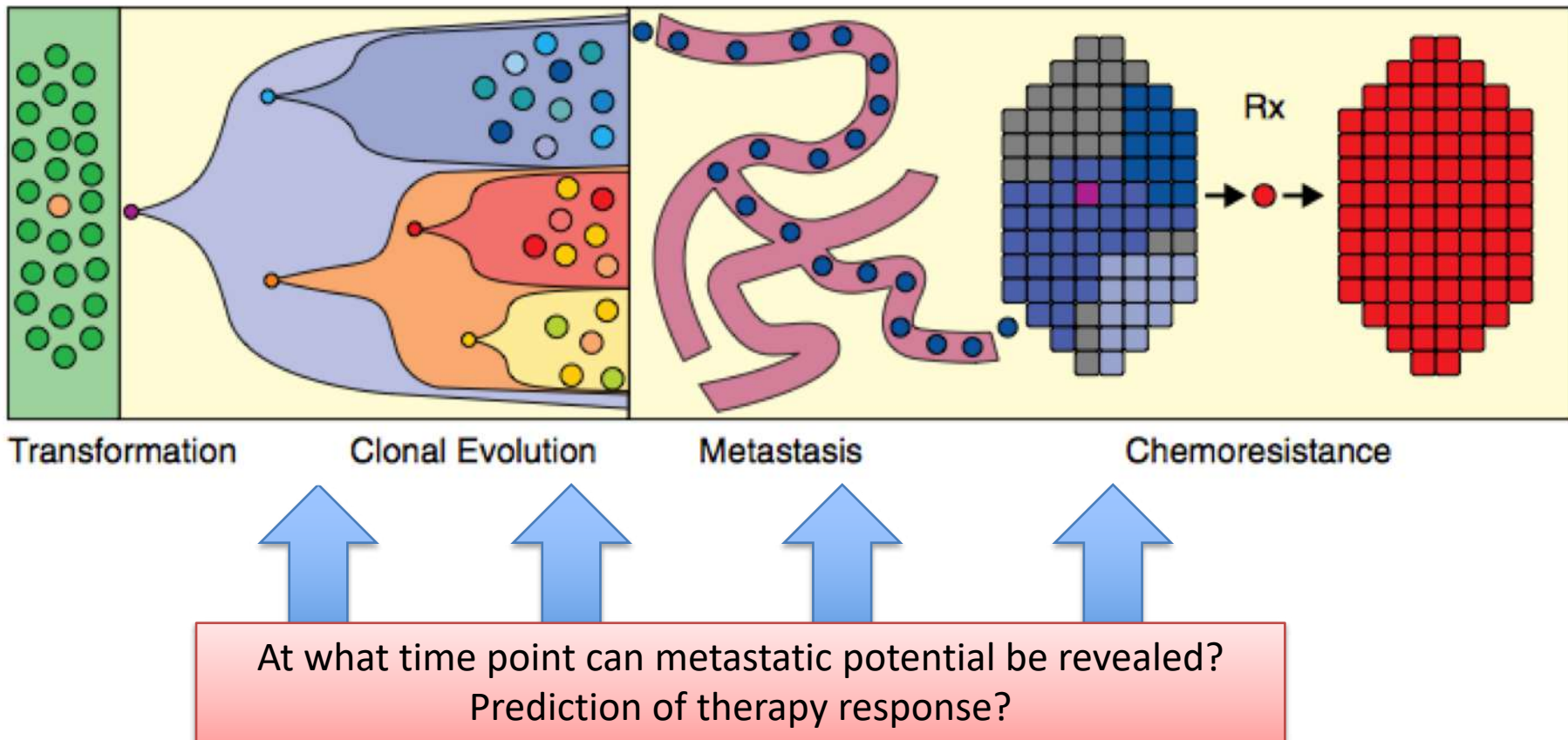
Pre-invasive disease:
Many different
histological appearances
with uncertain
relationship...



Heterogeneity and evolution

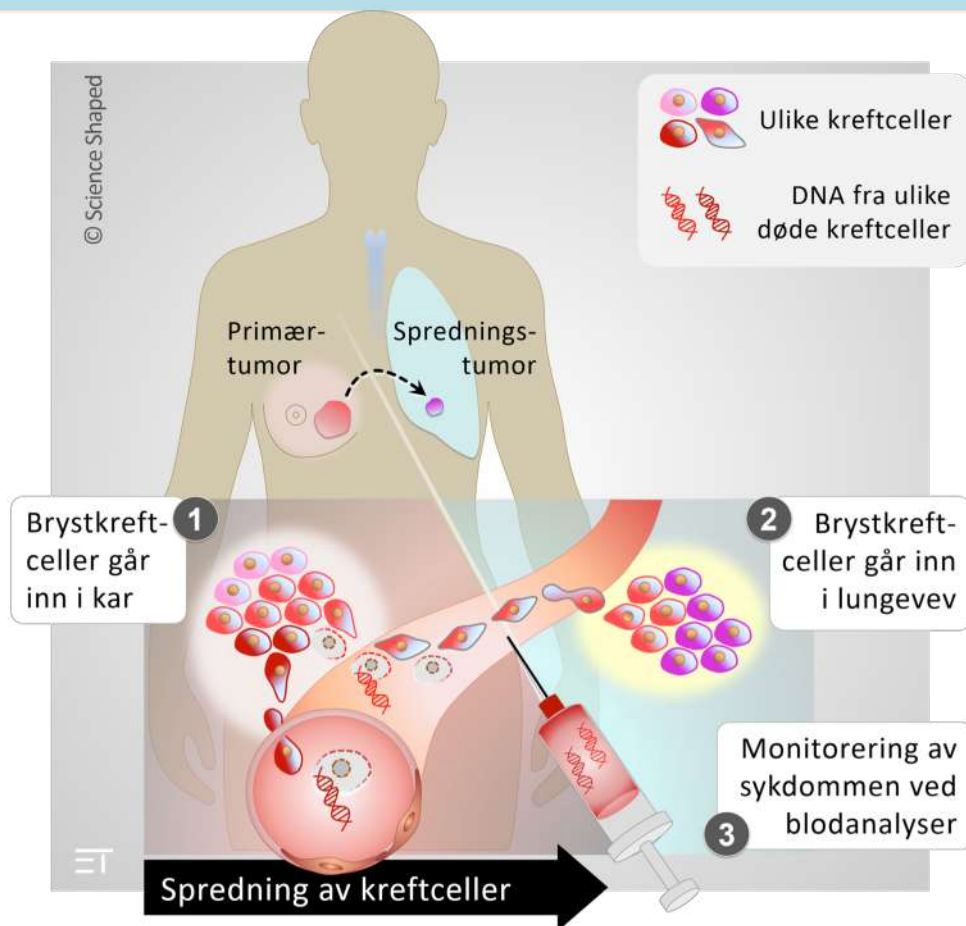
- disease progression

Navin, Genome Biol 2014



Liquid biopsies

Development and standardization of protocols and assays for cell-free tumor DNA detection in peripheral blood



- Promising for monitoring neo-adjuvant treatment
- Promising in metastatic setting
- But need many markers or NGS based tests
- NB: value of circulating cells (CTC) or disseminated cells (DTC) needs to be considered

Molecular classification of breast cancer

2019, ASCO Educational book:

	Hereditary Cancer Risk	Tumor Gene-Expression Signatures	Tumor Genomic Mutations
Assay	Germline DNA test	Tumor RNA-expression assay	Tumor (or circulating tumor or cell-free) DNA for genomic profiling
Number of Genes Measured	Varies by assay; typically 2 to 40.	Varies by assay; typically 10-100	Varies by assay; typically > 400
Assay Readout	Mutations in germline DNA	Patterns of gene expression often weighted with proprietary score	Mutations, deletions, amplifications in tumor DNA
Clinical Role	Defining hereditary cancer syndromes (e.g., BRCA1/2); identifying patients for selected therapy in metastatic breast cancer with PARP inhibitors or platinum analogs	Prognostic markers for outcome in ER+ breast cancer; predicting benefit from adjuvant chemotherapy in ER+ breast cancer	Identifying mutations for targeted therapy in metastatic breast cancer, including dynamic evolution of mutations associated with treatment resistance; potential surrogate for cancer burden in setting of metastatic disease
Recommend for:	All patients with <u>metastatic breast cancer</u> ; patients with <u>early-stage breast cancer</u> with family history or other clinical features associated with hereditary cancer syndromes	Women with <u>early-stage ER+</u> breast cancer, typically stage 1 or 2, for deciding whether to recommend adjuvant chemotherapy in addition to endocrine therapy	Selection of endocrine/targeted treatments in <u>advanced ER+</u> breast cancer based on PIK3CA or ESR1 mutations; experimental for other precision medicine purposes

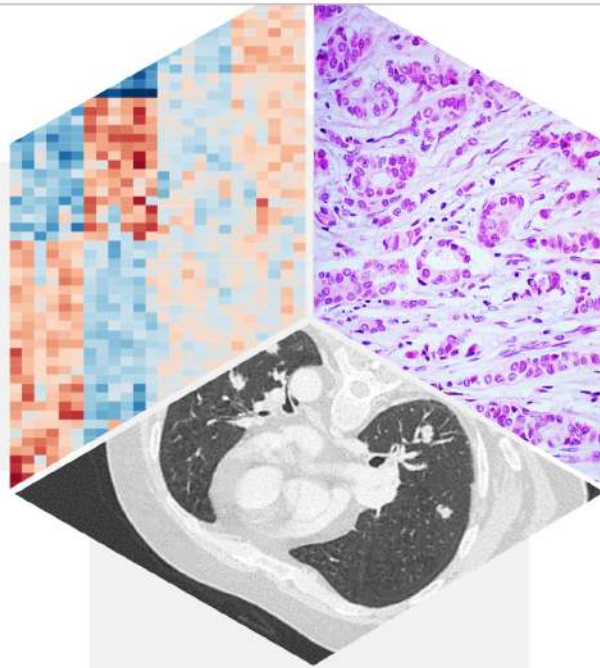
MULTIDISCIPLINARY DIAGNOSTICS



MOLECULAR CLASSIFICATION

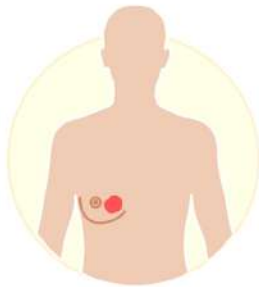
- PAM50 RNA
- IntClust RNA/DNA
- Others?

© SCIENCE SHAPED



HISTOPATHOLOGY

- Histological type, grade
- Size
- ER, PgR, HER2, Ki67



CLINICAL INFORMATION

- Age, heredity
- Clinical examination
- Imaging



Deciding standard treatment



Selection for clinical trials



Plan for follow-up



Focused translational research

Tusen takk!

