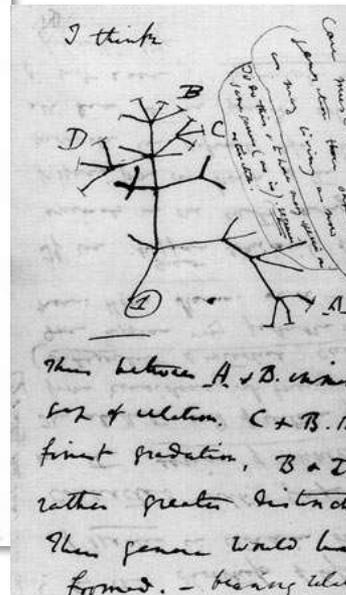


# Brystkreft

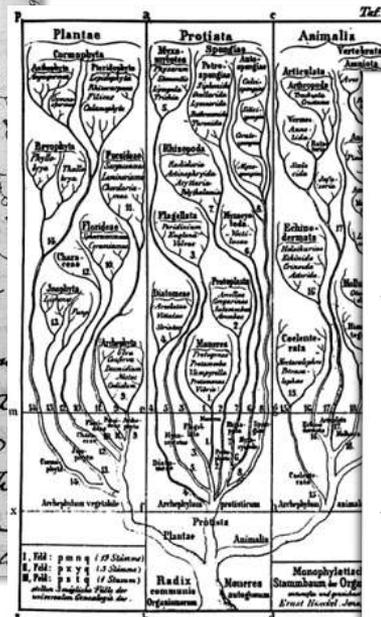
– en sykdom med ulike ansikt



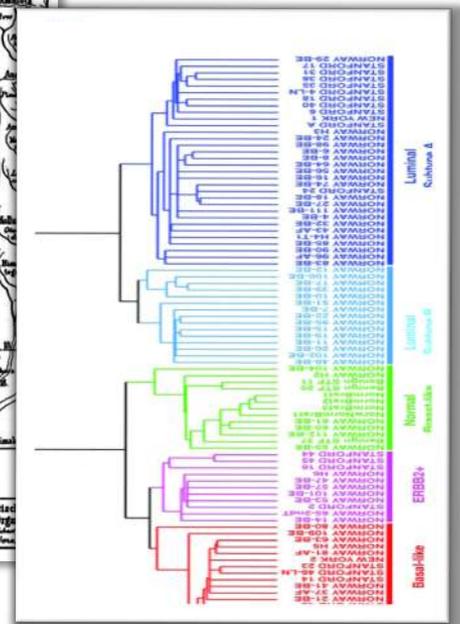
Linnaeus



Darwin



Haeckel

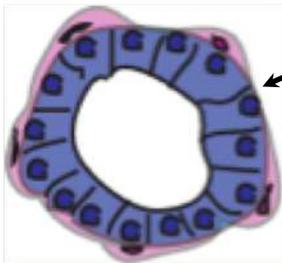
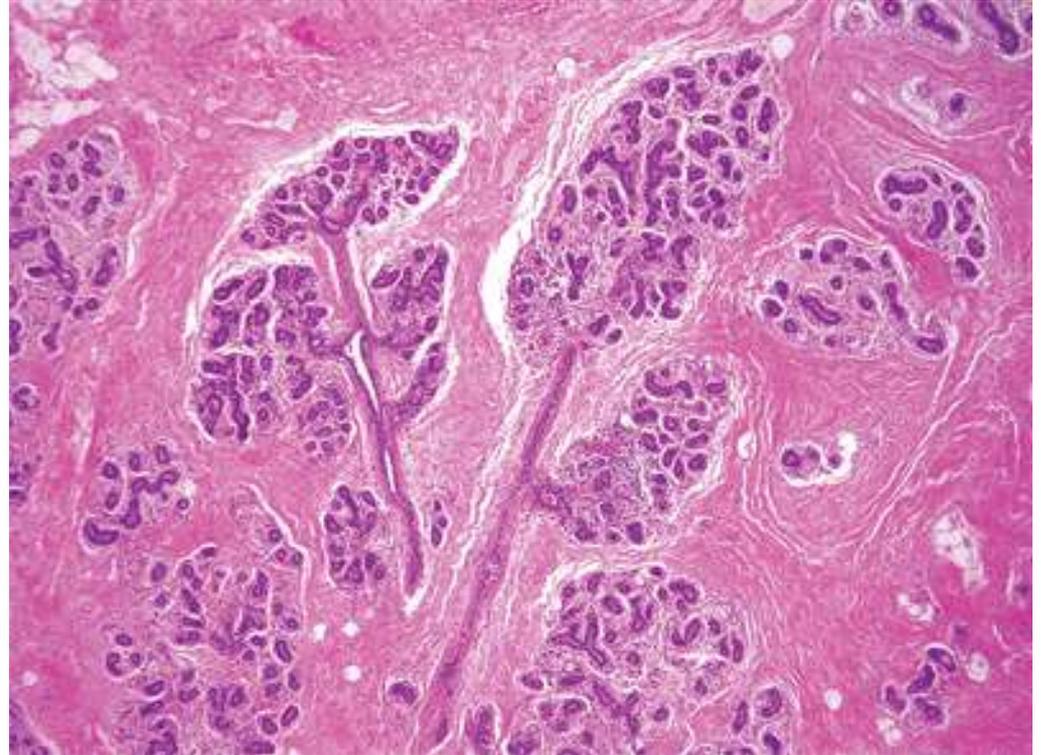
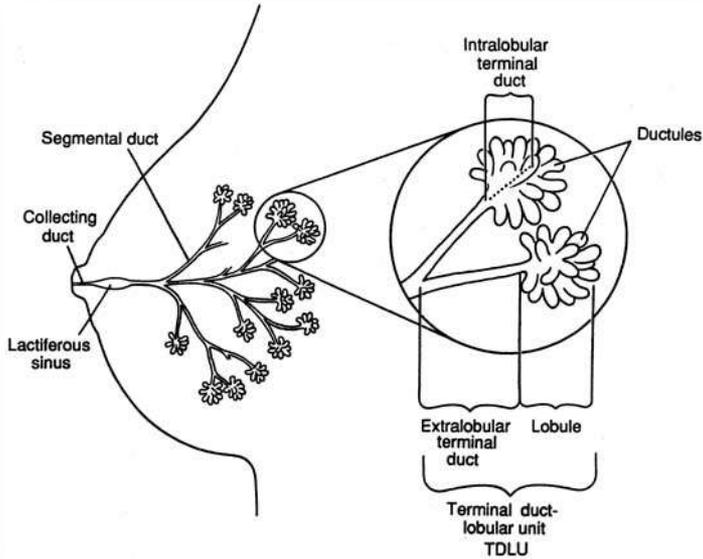


Perou

## 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

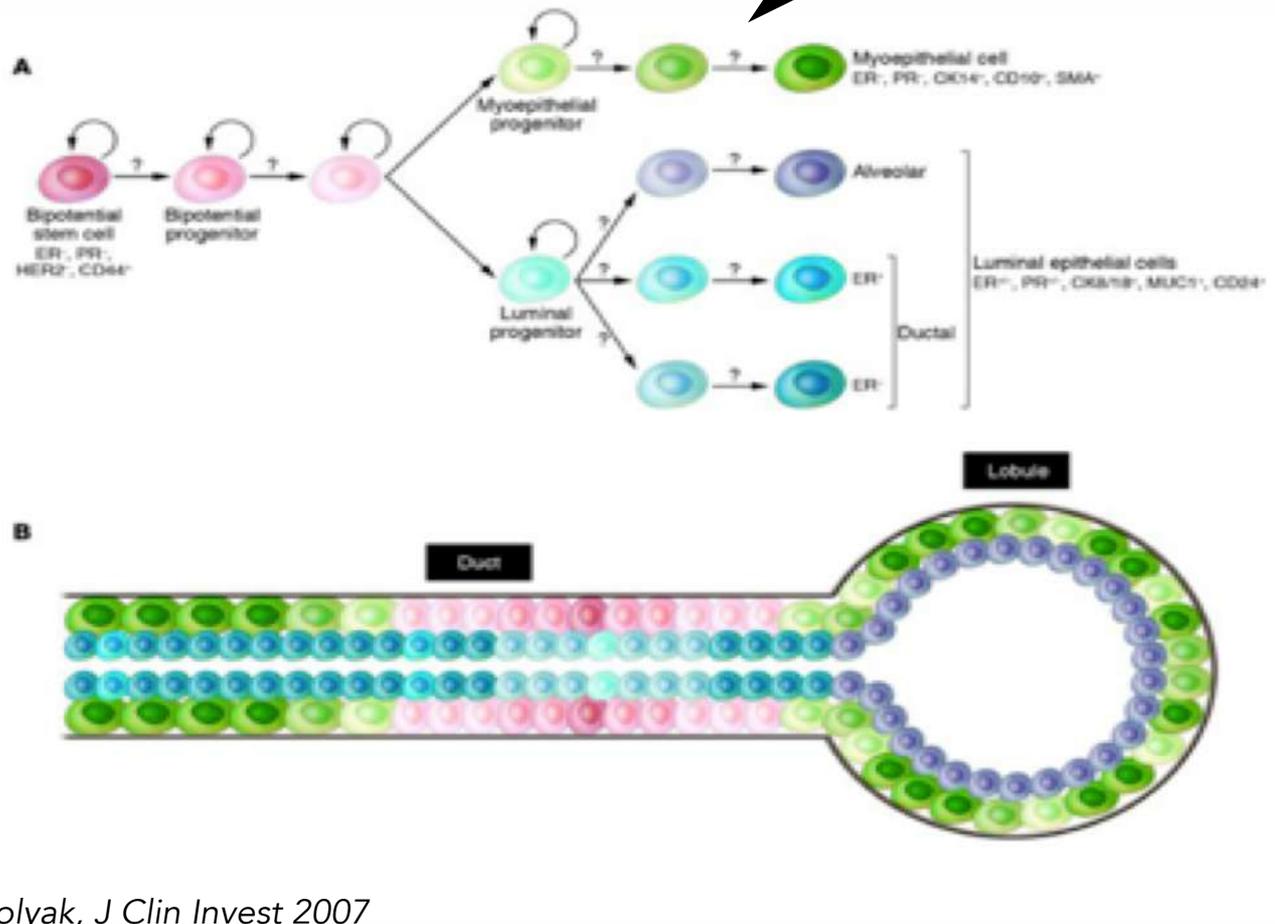


Luminal  
epithelial cells

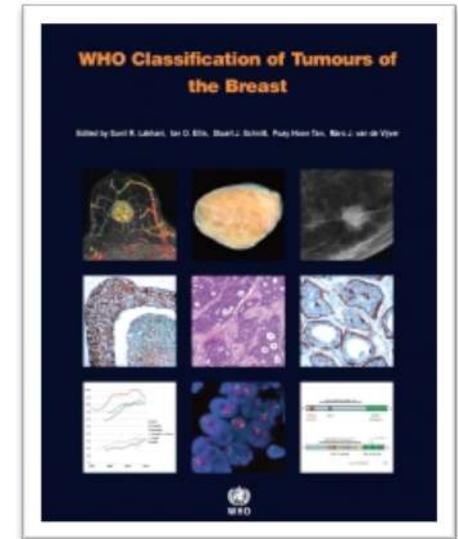
Myoepithelial/basal cells

# An assumed hierarchical relationship between the cell types

Differentiation

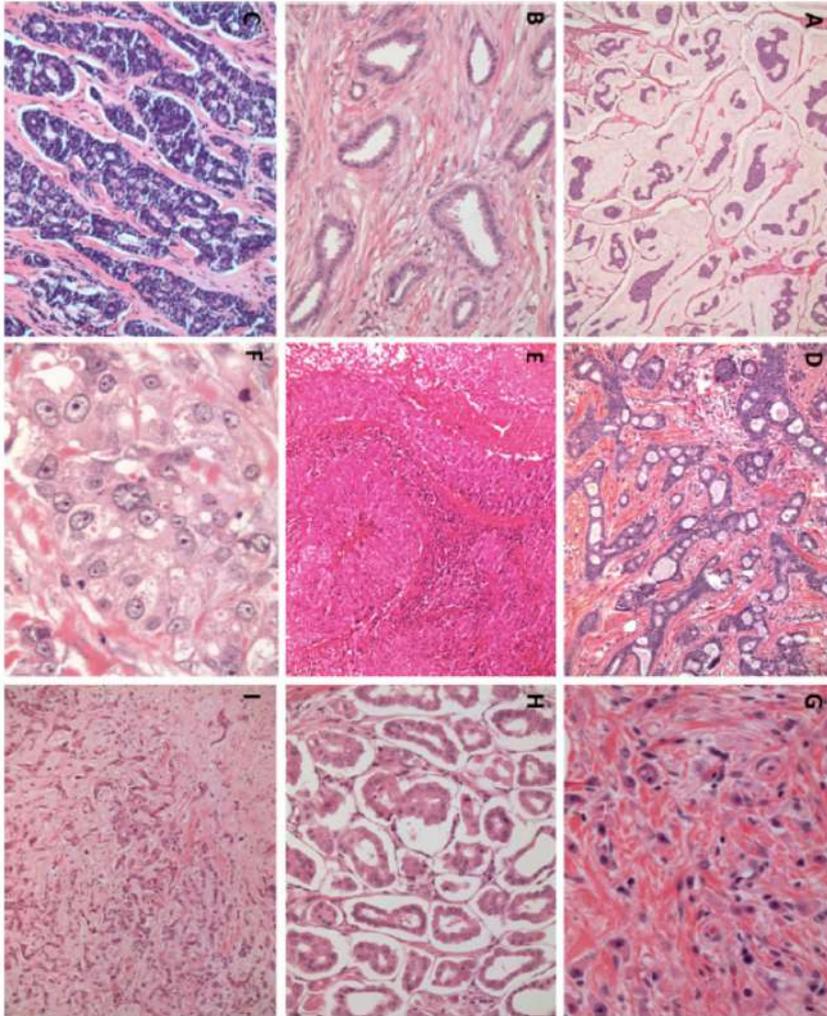


# Morphology based classification

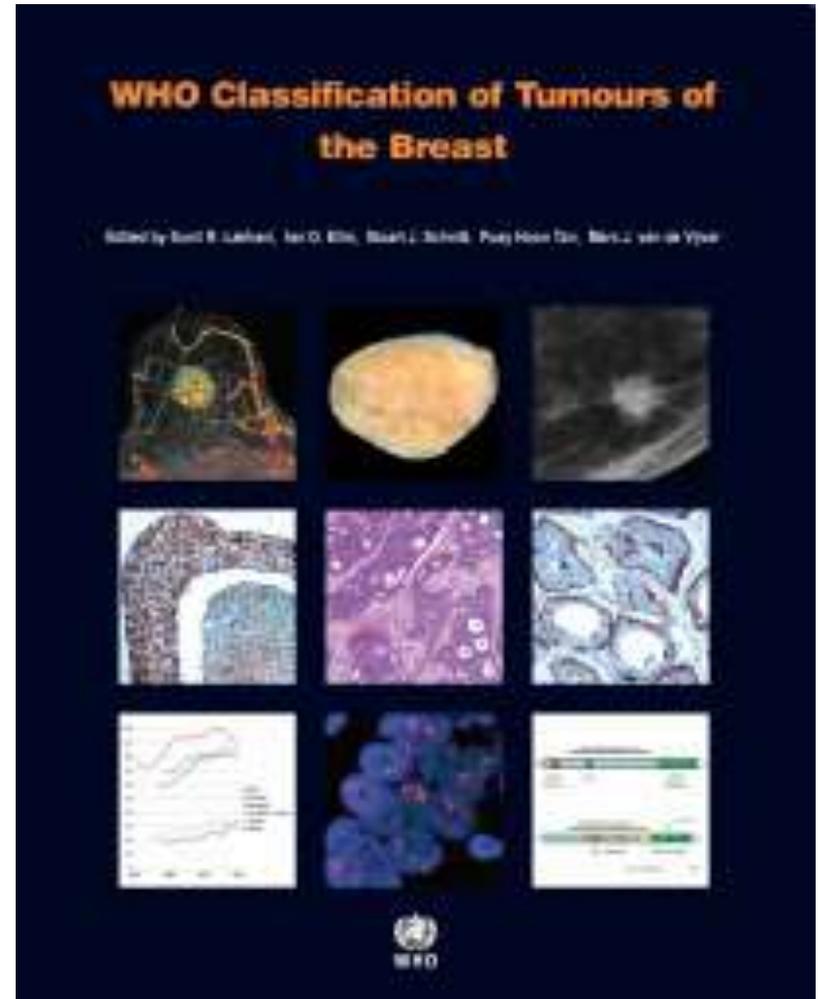


- Invasive carcinomas of no special type, NST (previously known as “ductal”) – a wide specter
- Special type carcinomas
- Mixed carcinomas

# Classification by morphology



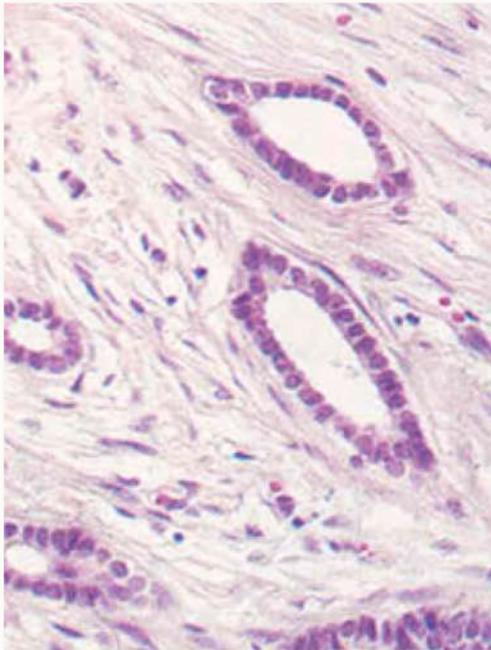
Dieci, The Oncologist 2014



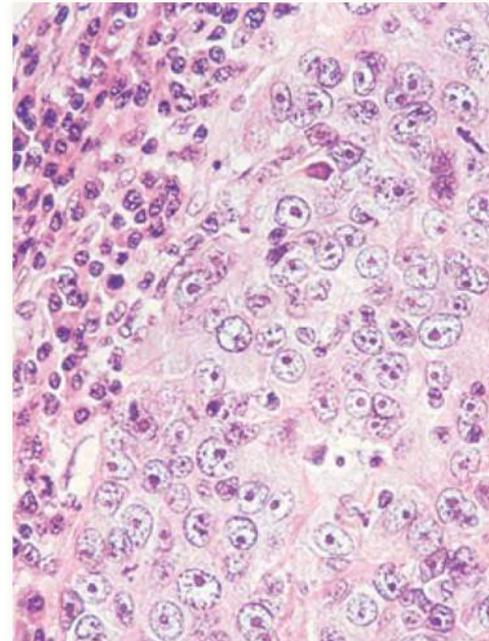
# Morphology = phenotype!

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

Indolent behavior:



Aggressive behavior:

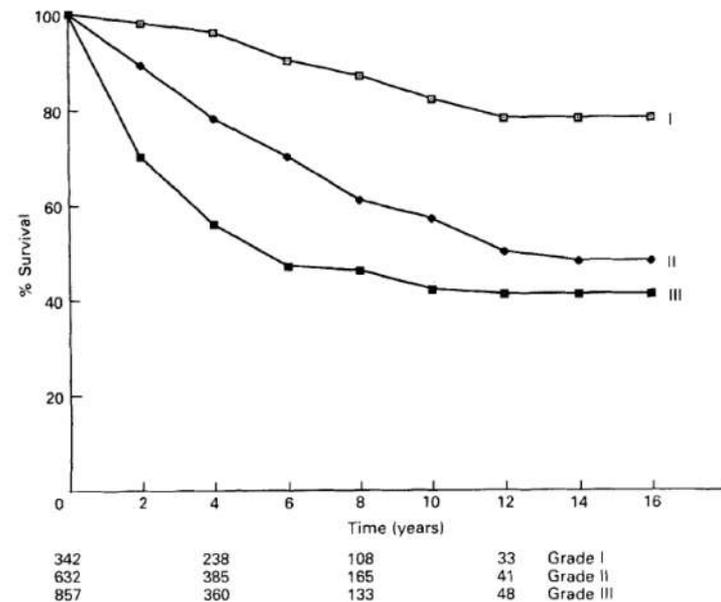


# Histological grade

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

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

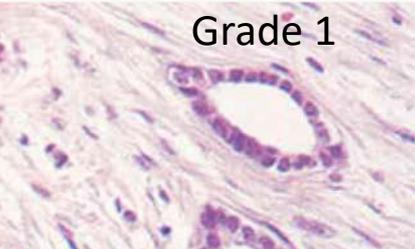
3–5 points: grade I —well-differentiated  
 6–7 points: grade II —moderately differentiated  
 8–9 points: grade III—poorly differentiated



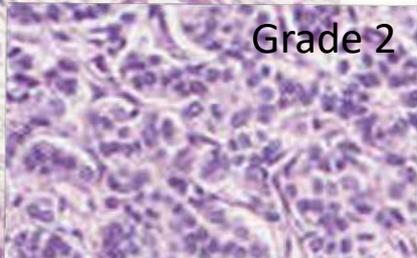
*Elston and Ellis, Histopathology, 1991*

# Grouping of breast cancer - 2020

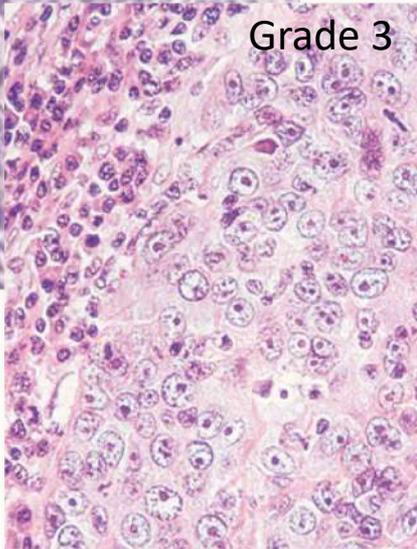
Histological grade  
Grade 1



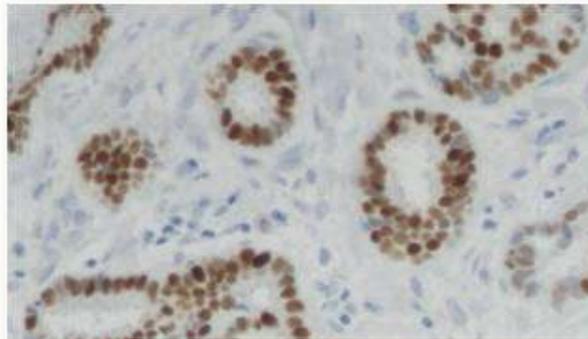
Grade 2



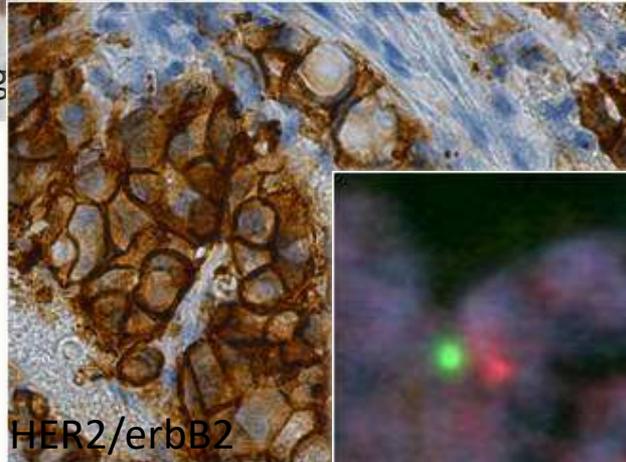
Grade 3



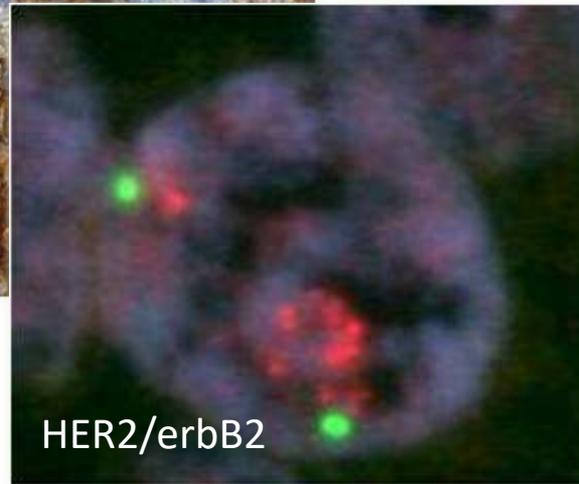
ER/PgR/Ki67/HER2



Estrog



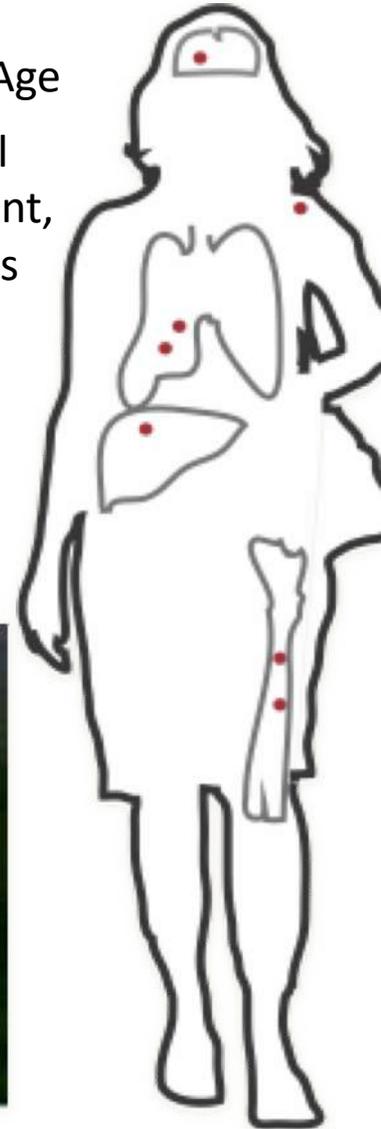
HER2/erbB2



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

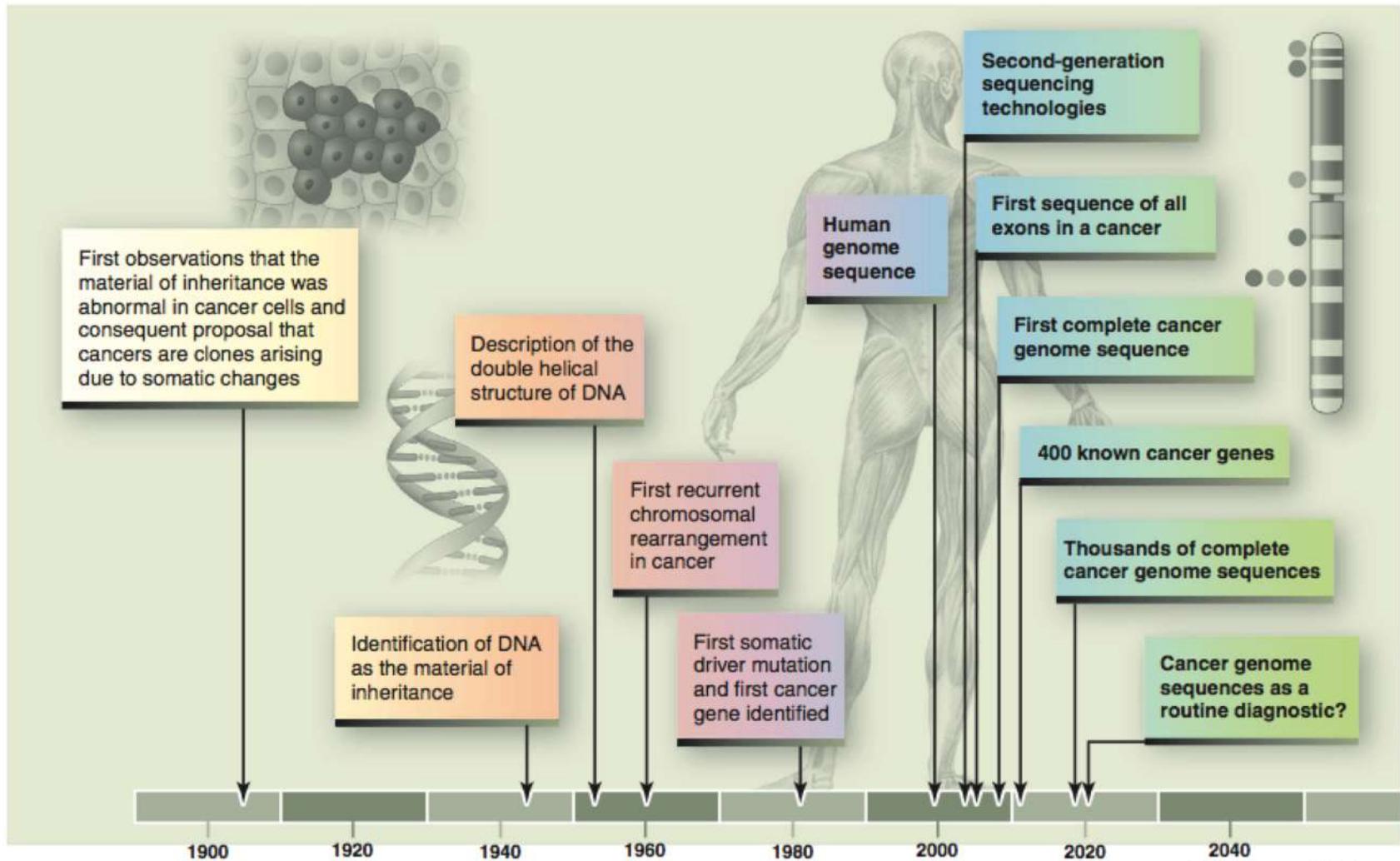


Fig. 1. Time line showing key events in the investigation of the cancer genome.

# 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

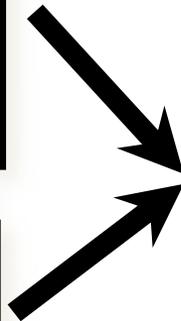
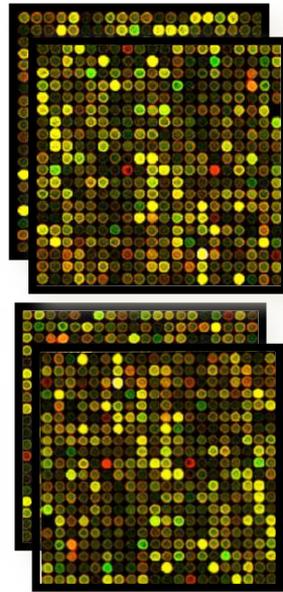
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- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- And are the designated class the same throughout the entire evolution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

# Identification of biomarker/signatures for treatment prediction

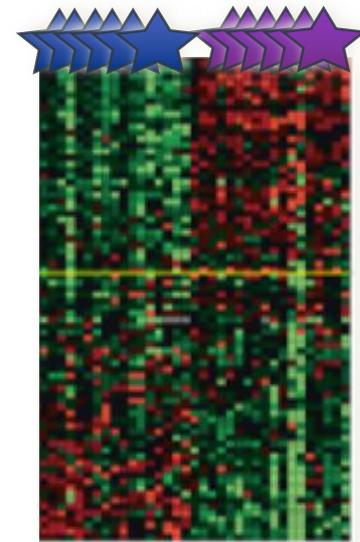
Patient samples with **same treatment**, comparison of findings in responders vs. non-responders

Supervised analyses

Selected tumor samples



Molecular markers with predictive or prognostic potential

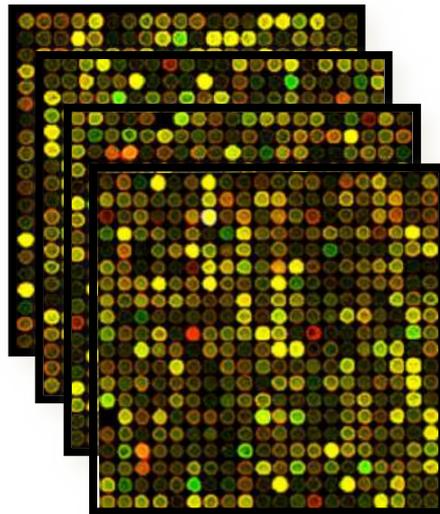
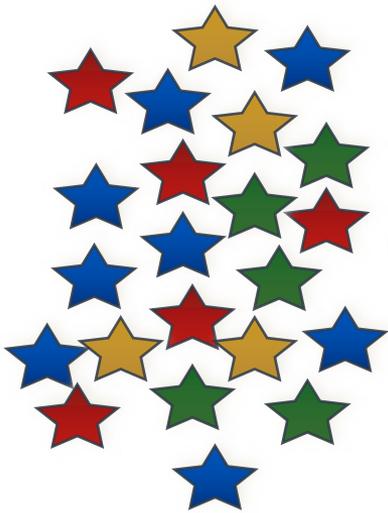


# Identification of biomarker/signatures recognizing biological distinct traits

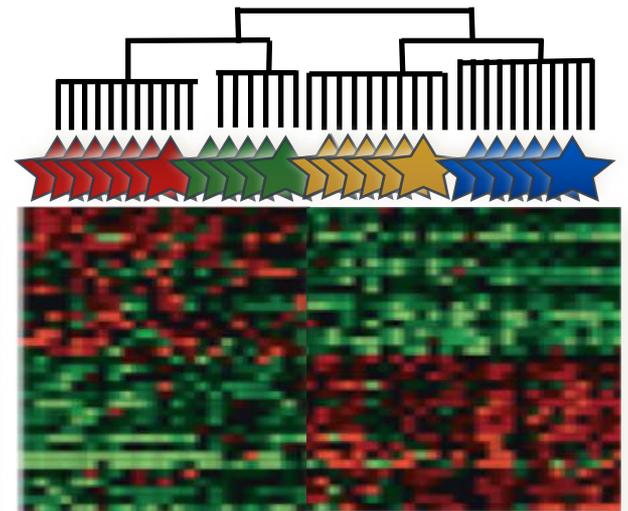
Patient samples **regardless of treatment type**, comparison of findings across all individual samples

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 VIJVER, M.D., Ph.D., YUDONG D. HE, Ph.D., LAURA J. VAN 'T VEER, AUGUSTIJN A.M. HART, M.Sc., DOREN W. VOSKUIJL, 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 SJOUERD RODENHUIS, M.D., Ph.D., EMIEL T. RUTGERS, M.D., STEPHEN H. ANDRÉ, Ph.D., 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-

**A**DJUVANT improves disease outcome in both premenopausal and postmenopausal women up to age 70.<sup>1,2</sup> It is generally prognostic for breast cancer.<sup>3,4</sup> The main prognostic factors are age, tumor size, histologic type of the tumor, and hormone-receptor status. Several factors have been investigated to predict the outcome of distant recurrence, but only limited predictive value has been demonstrated.

Using complementary DNA (cDNA) microarrays to analyze breast-cancer tumors with distinct patterns of gene expression, we recently identified a 70-gene prognosis profile in patients with lymph-node-negative disease. In addition, microarray analysis distinguished cancers associated with either a poor prognosis<sup>5,9</sup> and to distant recurrence<sup>6,10</sup> and lymph-node-positive disease.<sup>11</sup> Using inkjet-synthesis technology, we recently identified a 70-gene prognosis profile in patients with lymph-node-negative disease.

From the Divisions of Diagnostic and Therapeutic Oncology (M.J.V., A.A.H., G.J.S., D.W.V., M.P., D.A.G., L.J.V., R.B.), Biometrics (T.V.), and Biostatistics (R.B.), Netherlands Cancer Institute, Theoretical Biomedical Genetics, Amsterdam (M.J.V., D.H., H.D.), and the Departments of Biometrics (D.A.G.), Biostatistics (R.B.), and Biostatistics (R.B.), Netherlands Cancer Institute, Theoretical Biomedical Genetics, Amsterdam (D.H., H.D.), and the Department of Biometrics (D.A.G.), Netherlands Cancer Institute, Theoretical Biomedical Genetics, Amsterdam (D.H., H.D.), and the Department of Biometrics (D.A.G.), Netherlands Cancer Institute, Theoretical Biomedical Genetics, Amsterdam (D.H., H.D.).

N Engl J Med, Vol. 347, No. 25 • December 19, 2002

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"70-gene profile"

"Recurrence score"

THE NEW ENGLAND JOURNAL OF MEDICINE

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. Taesung Park, Ph.D., William Hillier, 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 histopathologic measures.

METHODS

We tested whether the results of a reverse-transcriptase-polymerase chain reaction (RT-PCR) assay of 21 prospectively selected genes in paraffin-embedded tumor tissue 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 expression of 21 genes and 5 reference genes were used in a prospectively defined algorithm to 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. The recurrence scores of 668 patients categorized as having a low, intermediate, or high risk by the algorithm were 51, 22, and 27 percent, respectively. The Kaplan-Meier estimate of distant recurrence-free survival was 96.6 percent for patients in the low-risk group, 92.6 percent for patients in the intermediate-risk group, and 85.4 percent for patients in the high-risk group.

The recurrence score could be used as a continuous function to predict distant recurrence-free survival. The recurrence score was also predictive of distant recurrence-free survival in patients with node-negative, estrogen-receptor-positive breast cancer.

CONCLUSIONS

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

N Engl J Med 347:127-137, 2002

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

Howard Y. Chang<sup>1,2,3</sup>, Dmitry S. A. Nuyten<sup>4,5,6</sup>, Julie B. Sneddon<sup>7</sup>, Trevor Hastie<sup>8</sup>, Robert Tibshirani<sup>9</sup>, Therese Sorlie<sup>10</sup>, Hongyue Dai<sup>11</sup>, Yudong D. He<sup>12</sup>, Laura J. van't Veer<sup>13</sup>, Harry Bartelink<sup>14</sup>, Matt van de Rijl<sup>15</sup>, Patrick O. Brown<sup>16</sup>, and Marc J. van de Vijver<sup>17</sup>

<sup>1</sup>Program in Epithelial Biology, Department of Biodynamics, <sup>2</sup>Health Research and Policy, and <sup>3</sup>Pathology, and <sup>4</sup>Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305; <sup>5</sup>Department of Diagnostic Oncology and <sup>6</sup>Radiation Oncology, the Netherlands Cancer Institute, Amsterdam 121, <sup>7</sup>Genetic Association, the Netherlands Cancer Institute, Seattle, WA 98108 and <sup>8</sup>Biostatistics Center, Harvard Medical School, Boston, MA 02115, <sup>9</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>10</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>11</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>12</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>13</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>14</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>15</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>16</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115, <sup>17</sup>Department of Biostatistics, Harvard School of Public Health, Boston, MA 02115

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 supervised predictors of metastasis ("70-gene prognosis signature").

microarray | prognosis | wound healing | metastasis | treatment decision

A 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 applied to normal tissues

(CSR) genes and their canonical expression patterns in fibroblasts activated with serum, the soluble fraction of clotted 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 in 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 is to test 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

Microarray analysis of gene expression patterns in 295 breast cancer patients from the Netherlands Cancer Institute. The expression of each gene was measured in all samples to 25,000 A, and measurement of expression was based on national standard clinical trials at the Netherlands Cancer Institute.

"Wound Response"

Discussion

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

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

Therese Sørlie<sup>1,2,3</sup>, Charles M. Perou<sup>4,5</sup>, Robert Tibshirani<sup>6</sup>, Turid Aas<sup>1</sup>, Stephanie Geisler<sup>9</sup>, Hilde Johnsen<sup>8</sup>, Trevor Hastie<sup>6</sup>, Michael B. Eisen<sup>7</sup>, Matt van de Rijjn<sup>1</sup>, Stefanie S. Jeffrey<sup>1</sup>, Thor Thorsen<sup>8</sup>, Hanne Quist<sup>1</sup>, John C. Matese<sup>6</sup>, Patrick O. Brown<sup>7</sup>, David Botstein<sup>6</sup>, Per Eystein Lønning<sup>9</sup>, and Anne-Lise Borresen-Dale<sup>6,8</sup>

Departments of <sup>1</sup>Genetics and <sup>2</sup>Surgery, The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway; <sup>3</sup>Department of Genetics and Lindeberg Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599; Departments of <sup>4</sup>Health Research and Policy and Statistics, <sup>5</sup>Genetics, Pathology, <sup>6</sup>Surgery, and <sup>7</sup>Biochemistry and Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305; Departments of <sup>8</sup>Medicine (Section of Oncology), <sup>9</sup>Surgery, and <sup>10</sup>Biochemical Endocrinology, Haukeland University Hospital, N-5021 Bergen, Norway; and <sup>11</sup>Life Sciences Division, Lawrence Berkeley National Laboratory, 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 microarrays to correlate tumor characteristics to clinical outcome. In 85 cDNA microarray experiments representing 78 breast carcinomas, and four normal breast tissues were analyzed. Hierarchical clustering, as reported previously, showed that breast 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 N<sub>2</sub> 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 (T<sub>3</sub>/T<sub>4</sub> and/or N<sub>2</sub> 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](http://www.pnas.org).

**RNA Analysis.** Total RNA was isolated by phenol-chloroform extraction (Trizol, GIBCO/BRL), and mRNA was purified by either magnetic separation using Dynabeads (Dynal) or 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: [at@labmed.uio.no](mailto:at@labmed.uio.no).

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559 clones representing 494 unique genes

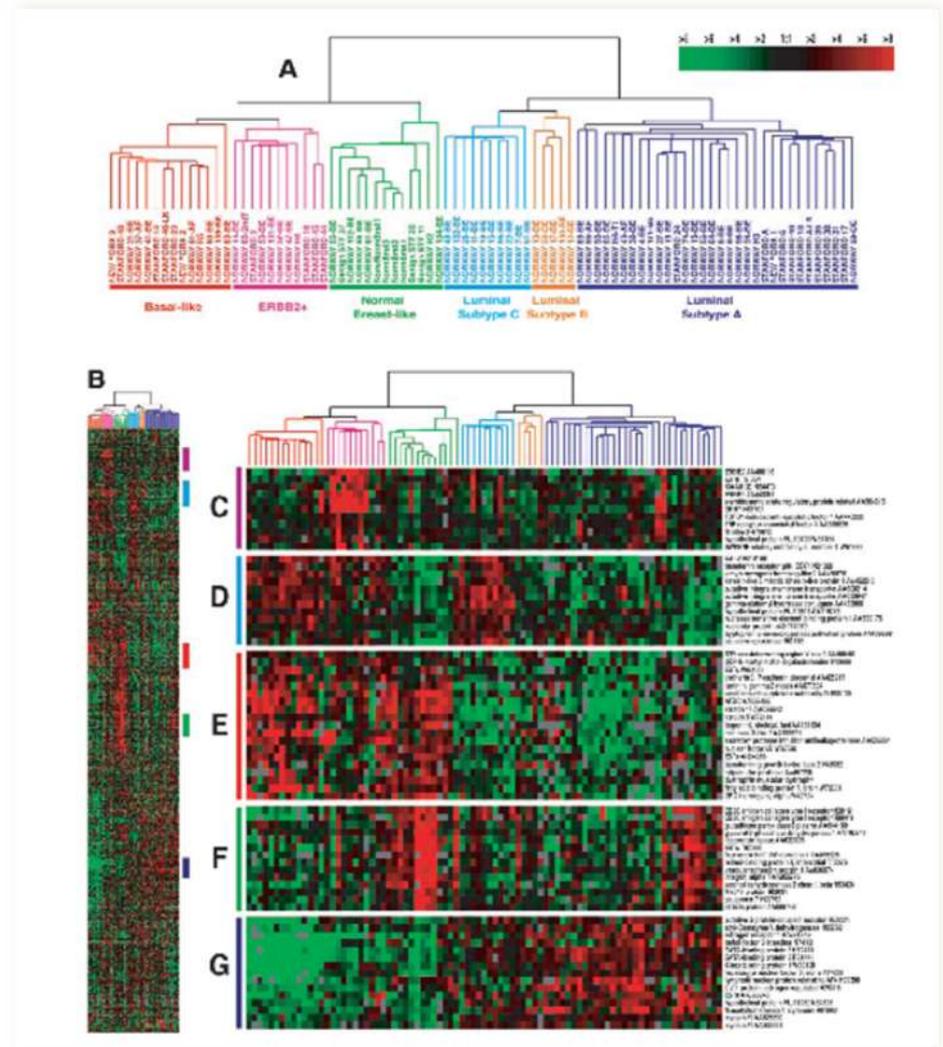
of phenotypic variation, might provide the basis for an improved taxonomy of cancer (11–14).

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

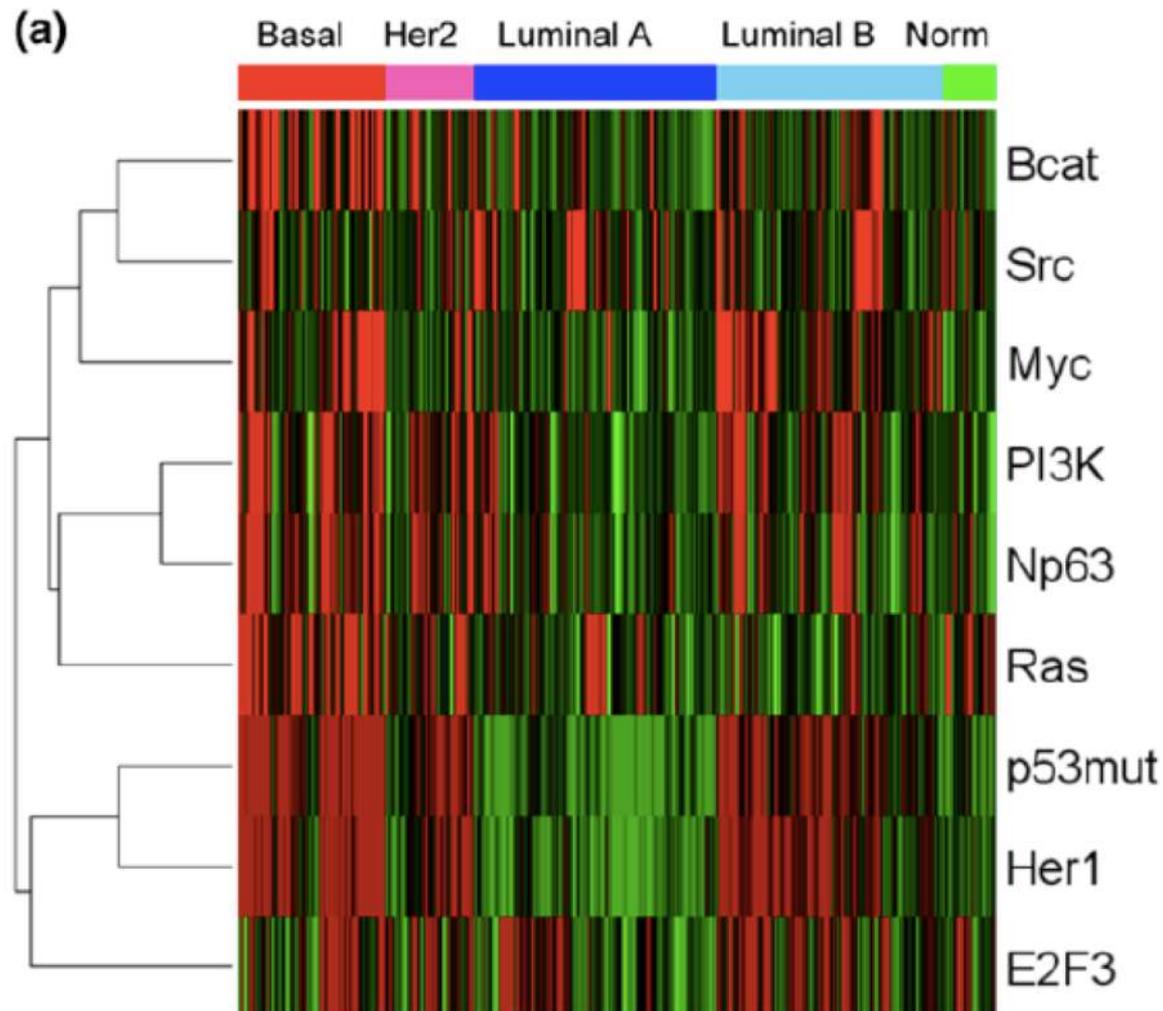
- 561 genes selected as most “intrinsic” for individual tumors before and after treatment
- Clustering of other sets of tumors by the expression of these genes group them into five main groups

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

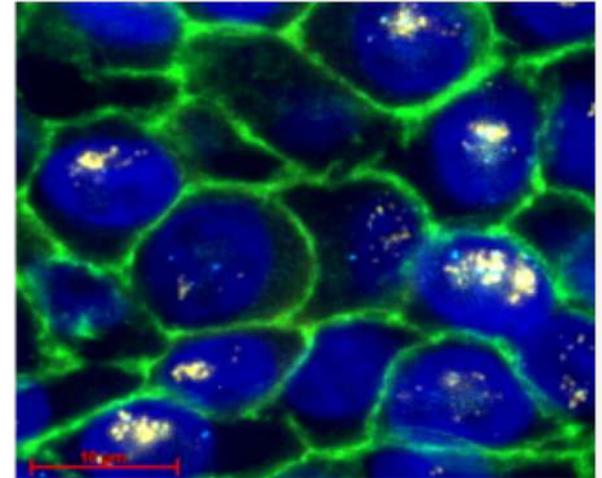
# HER2-enriched and basal-like breast cancer

## 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 $\geq 1\%$ <sup>a</sup>
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 <sup>b</sup> . Low or absent nodal involvement (N 0–3), smaller T size (T1 T2).
Intermediate	Multiparameter molecular marker 'intermediate' if available <sup>c</sup> . 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 <sup>b</sup> . More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).

<sup>a</sup>ER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.

<sup>b</sup>Ki-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.

<sup>c</sup>Not all multiparameter molecular marker tests report an intermediate score.

# 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	"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 $\geq 1\%$ <sup>a</sup> 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 <sup>b</sup> . 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

<sup>a</sup>ER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.  
<sup>b</sup>Ki-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.  
<sup>c</sup>Not 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....





*Non metter sempre piedi in a tua casa,  
ma solo perché lei ti sembra comoda.  
Cesar. Non cambiate opinioni.*





...but different genotype!

# DNA Translocations and copy number changes

Few alterations...

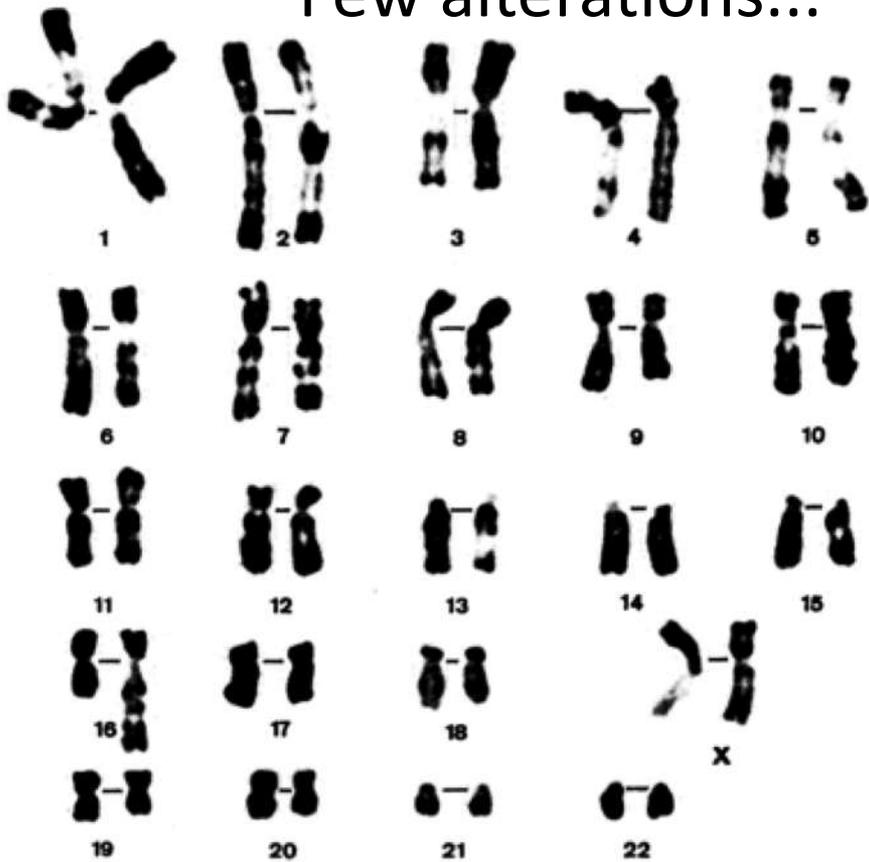
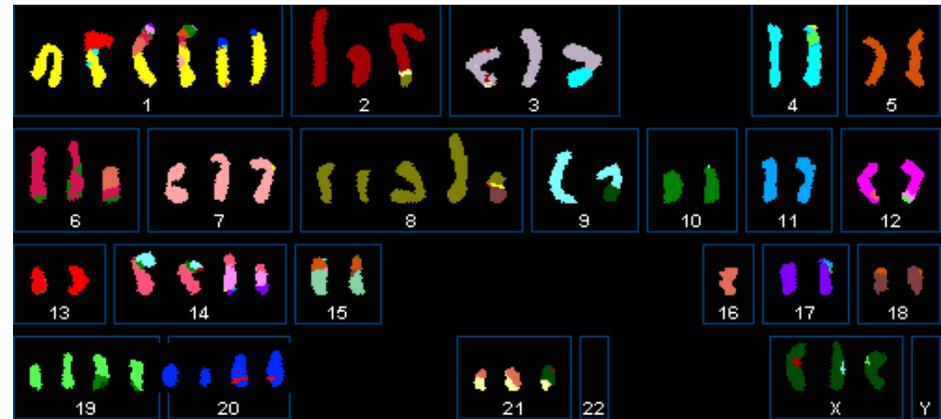
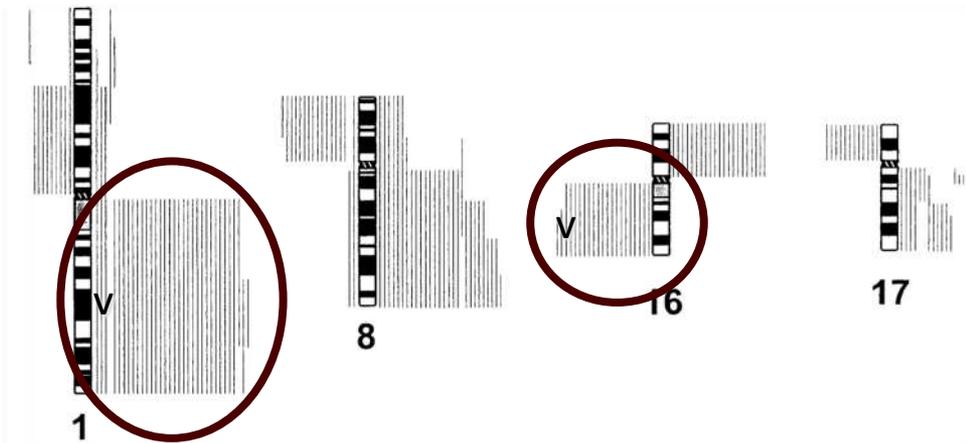


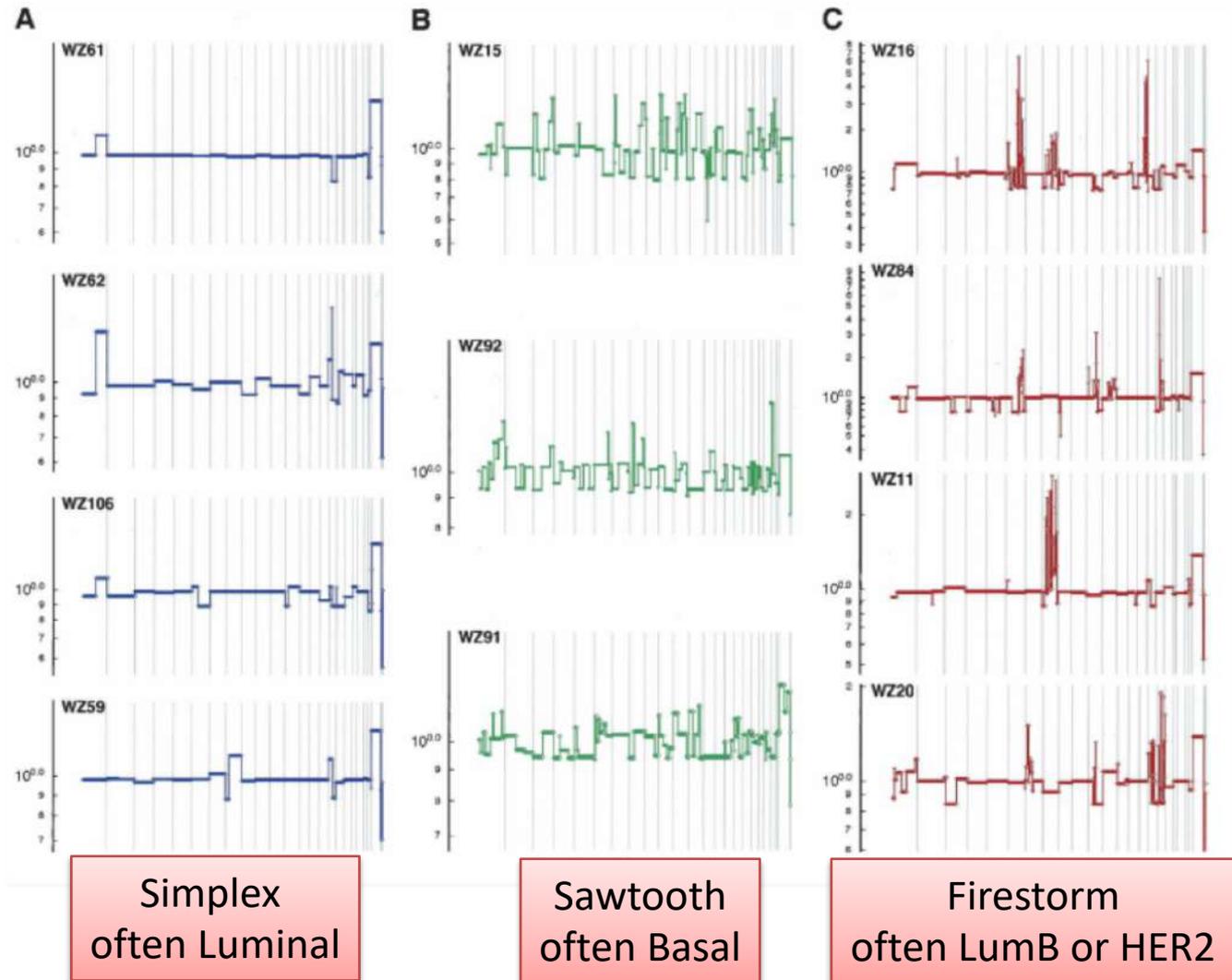
Figure 1 Karyotype from case 27, exhibiting der(1q16p) as the sole anomaly.



...many alterations

# Patterns of genomic rearrangements

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

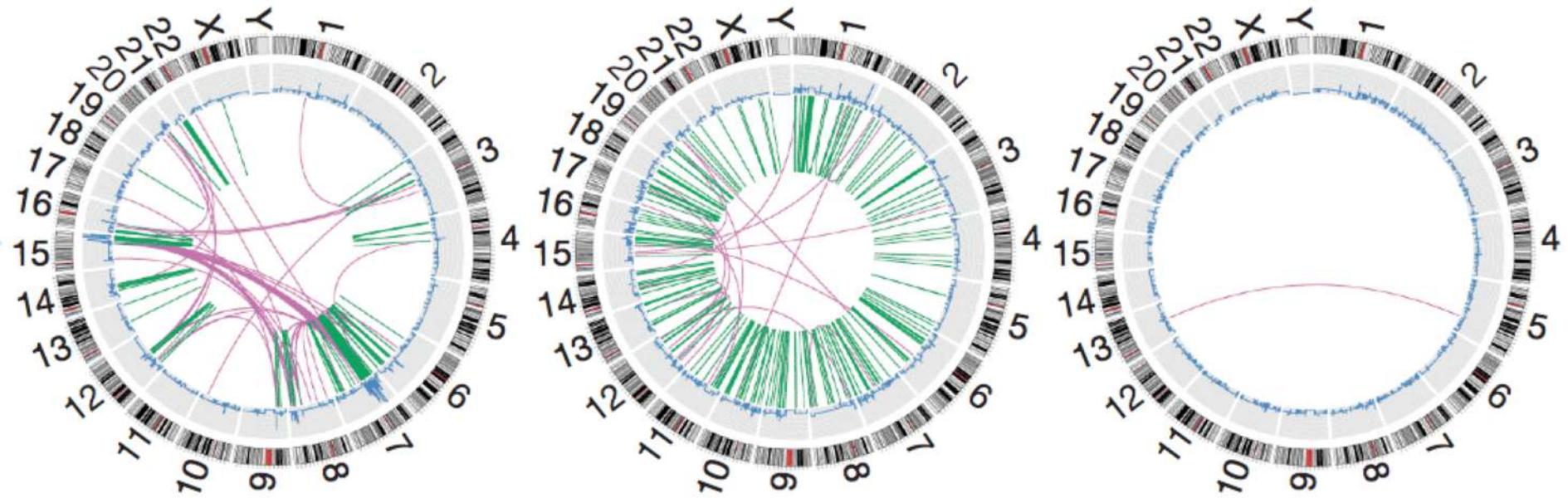


# Sequenced breast cancer genomes - structural rearrangements

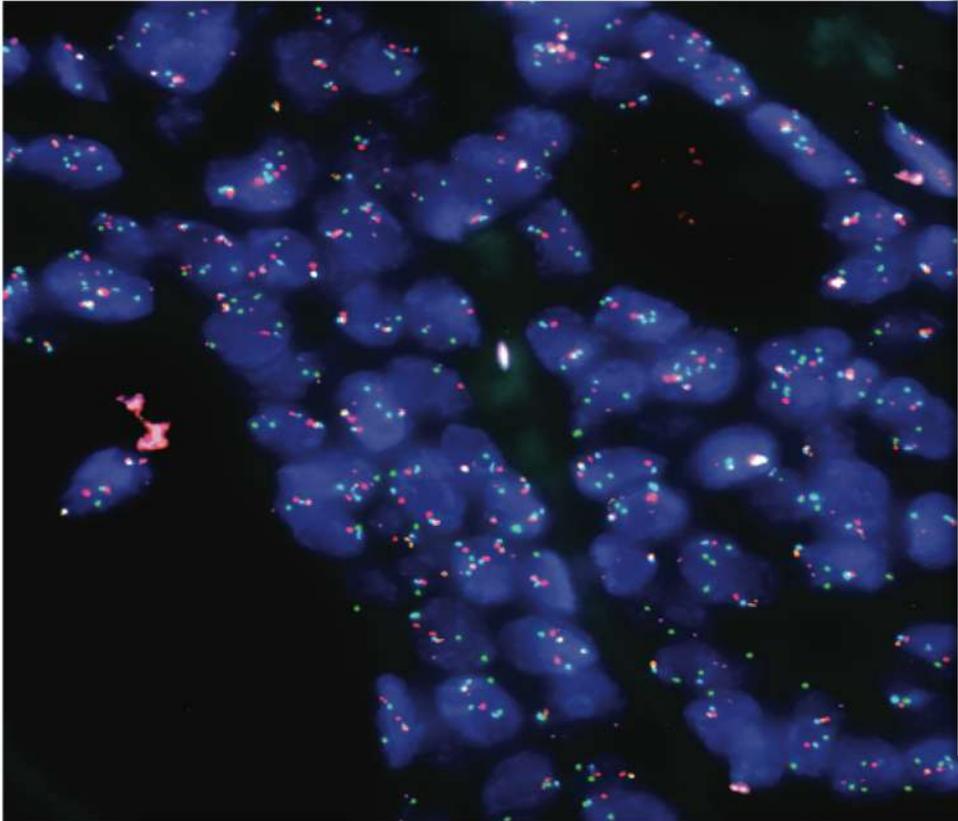
PD3668a  
ER<sup>+</sup> PR<sup>+</sup> ERBB2<sup>-</sup>

PD3664a  
ER<sup>-</sup> PR<sup>-</sup> ERBB2<sup>-</sup>

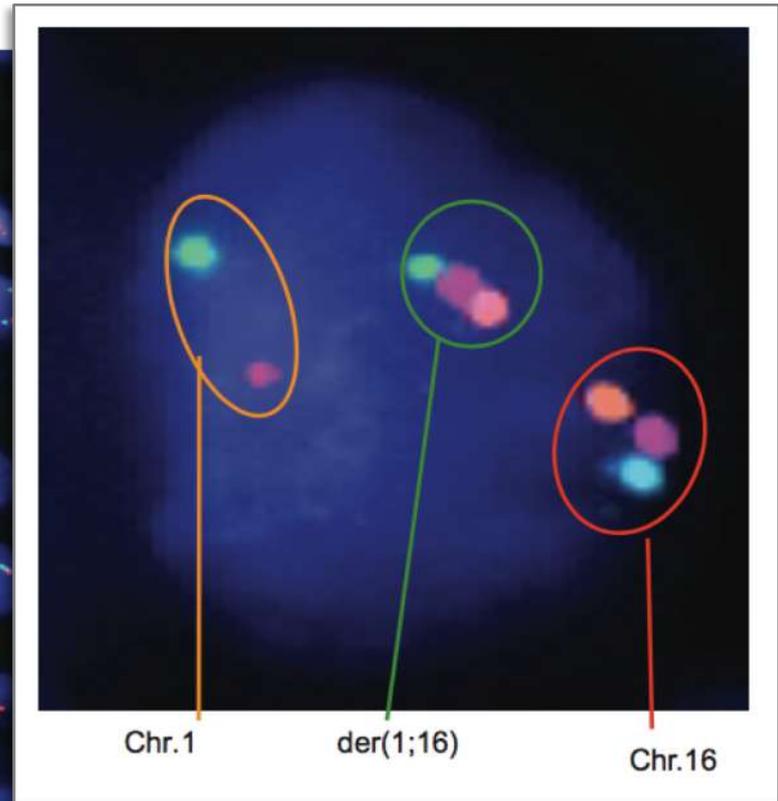
PD3667a  
ER<sup>+</sup> PR<sup>+</sup> ERBB2<sup>-</sup>



# 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

Clust  
M50

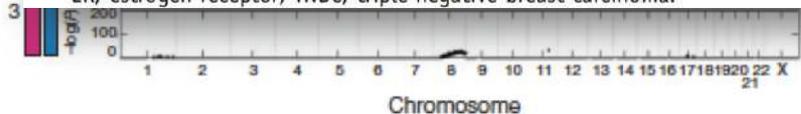
9  
6  
1  
5  
10  
7  
4  
8  
2  
3

**Table 1** Overview of the Integrative Cluster Subtypes and the Dominating Properties with Regard to Copy Number Driving Events, Biomarkers, Type of DNA Architecture, <sup>46</sup> 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 <sup>+</sup> (HER2 <sup>+</sup> )	Simplex/firestorm (chromosome 17q)	Luminal B	Intermediate
2	Chromosome 11	ER <sup>+</sup>	Firestorm (chromosome 11q)	Luminal A and B	Poor
3	Very few	ER <sup>+</sup>	Simplex/flat	Luminal A	Good
4	Very few	ER <sup>+</sup> /ER <sup>-</sup>	Sawtooth/flat	Luminal A (mixed)	Good (immune cells)
5	Chromosome 17 ( <i>HER2</i> gene)	ER <sup>-</sup> (ER <sup>+</sup> )/HER2 <sup>+</sup>	Firestorm (chromosome 17q)	Luminal B and HER2	Extremely poor (in pre- Herceptin cohorts)
6	8p deletion	ER <sup>+</sup>	Simplex/firestorm (chromosome 8p/ chromosome 11q)	Luminal B	Intermediate
7	Chromosome 16	ER <sup>+</sup>	Simplex (chromosome 8q/chromosome 16q)	Luminal A	Good
8	Chromosome 1, Chromosome 16	ER <sup>+</sup>	Simplex (chromosome 1q/chromosome 16q)	Luminal A	Good
9	Chromosome 8/ Chromosome 20	ER <sup>+</sup> (ER <sup>-</sup> )	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

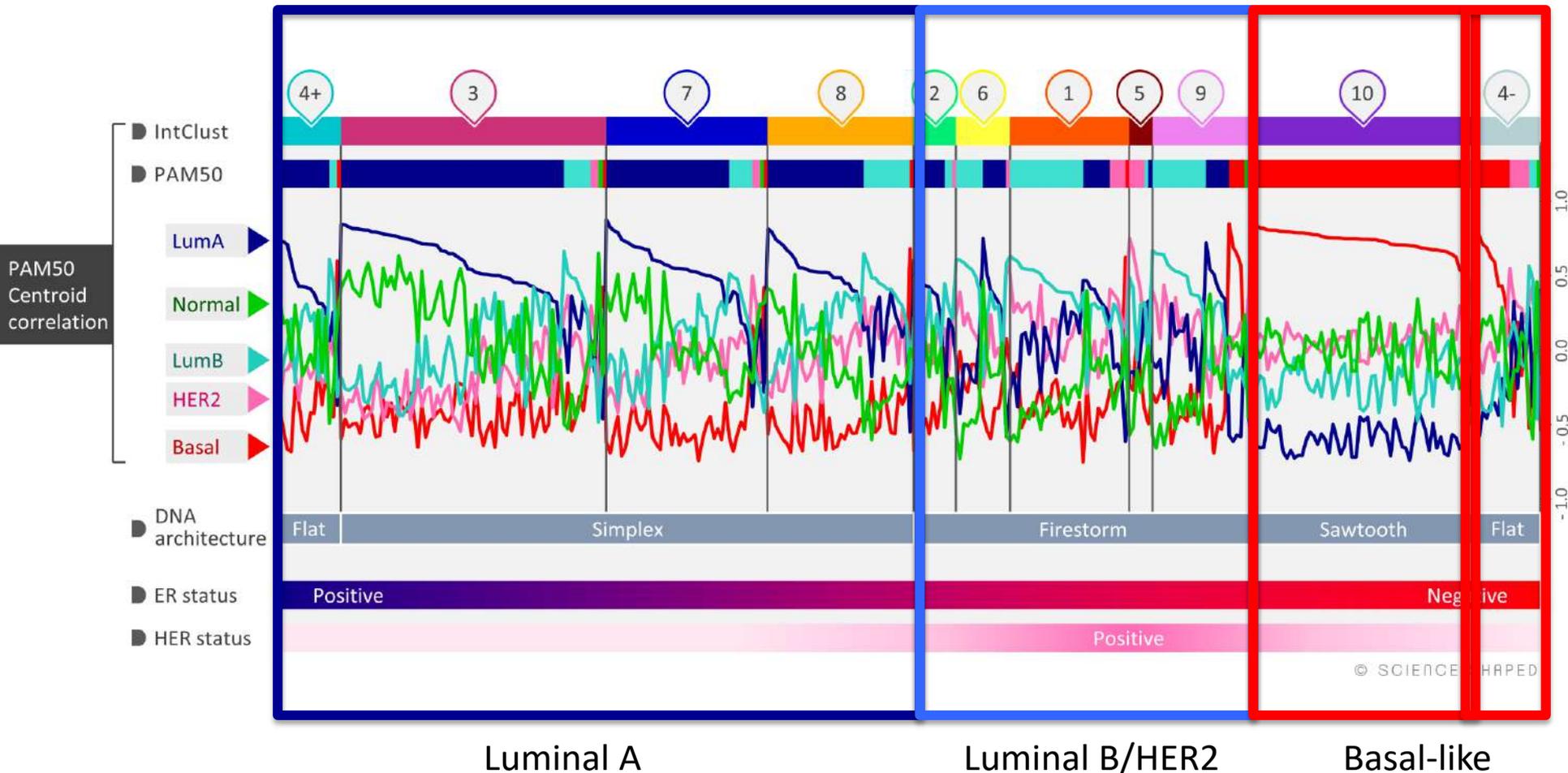
ER, estrogen receptor; TNBC, triple-negative breast carcinoma.

Russnes et al. *Am J Pathology*, 2017



Curtis et al. *Nature*, 2012

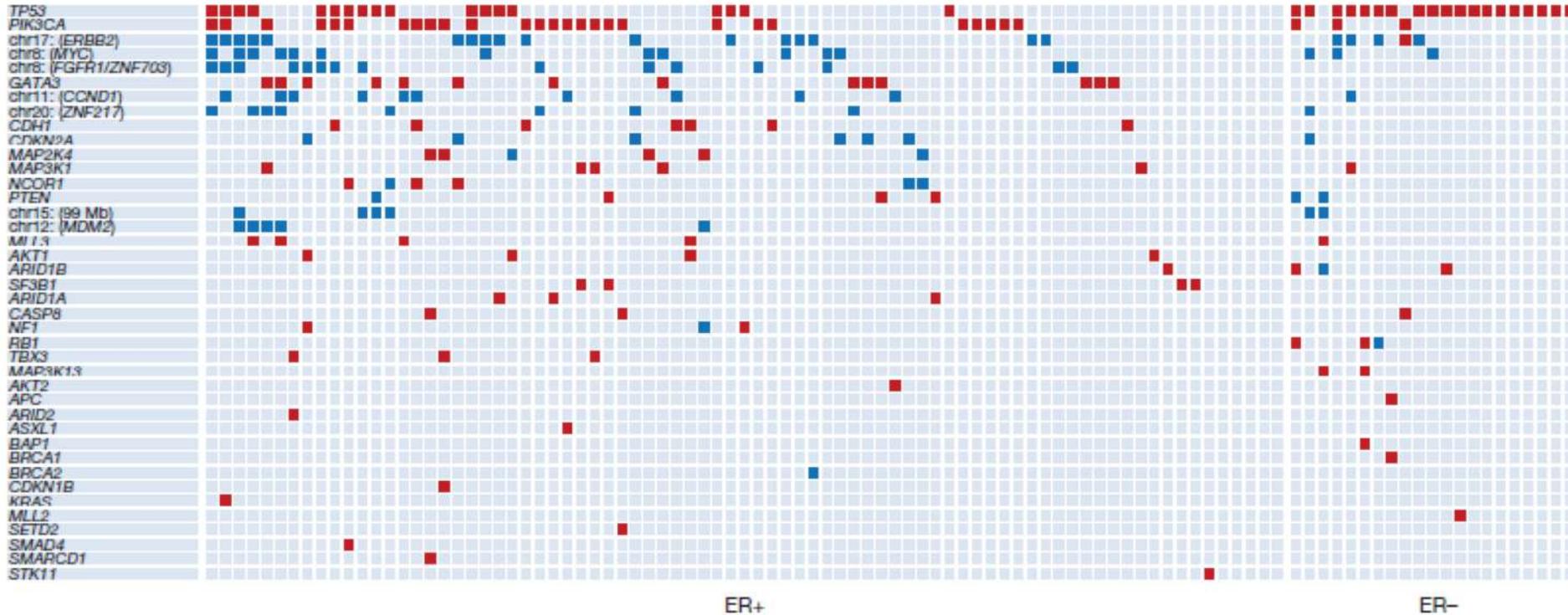
# PAM50 – IntClust – DNA architecture



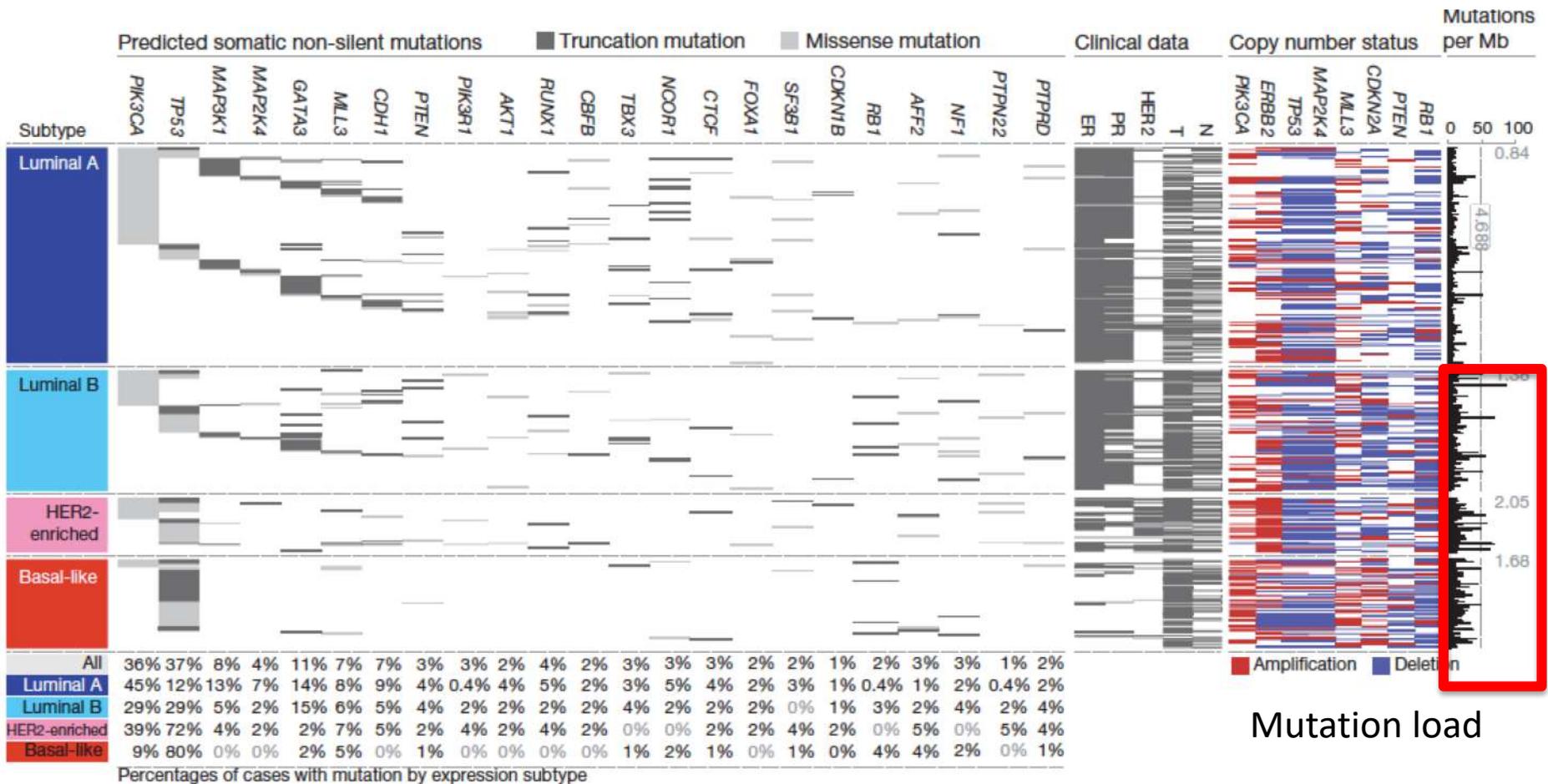
NB: centroid classification has five dimensions!

# Mutations - “Personal” profiles?

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



# 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

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Intermediate	Multiparameter molecular marker 'intermediate' if available <sup>c</sup> . 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 clearly high Ki-67 <sup>b</sup> . More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).

**"LumA-like"**

**"intermediate group"**

**"LumB-like"**

**Multiparameter molecular marker needed**

<sup>a</sup>ER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.

<sup>b</sup>Ki-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.

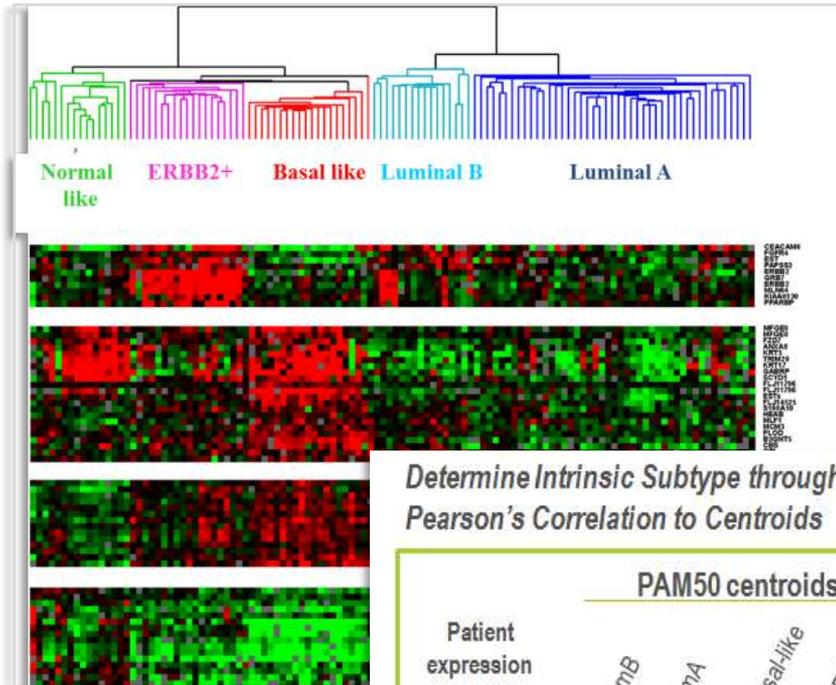
<sup>c</sup>Not all multi

# Luminal disease is defined as a spectrum

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

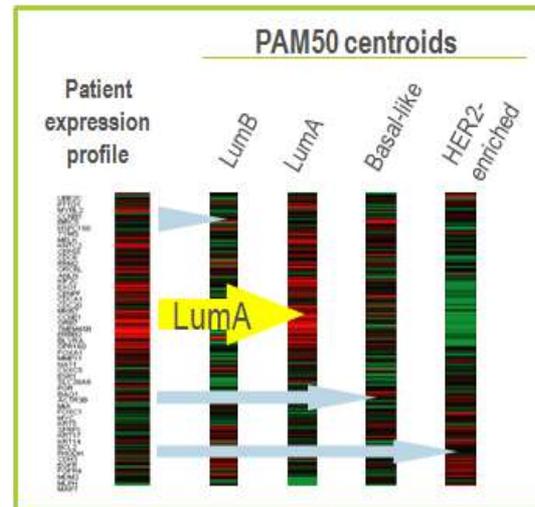
- Oncotype Dx
- Mammaprint
- BCI
- IHC4
- Rotterdam signature
- Prosigna (PAM50 ROR)
- Endopredict
- Mammostrat
- MammaTyper
  
- ...but Ki-67 is easy and cheaper

# From intrinsic subtypes to PAM50 to Prosigna



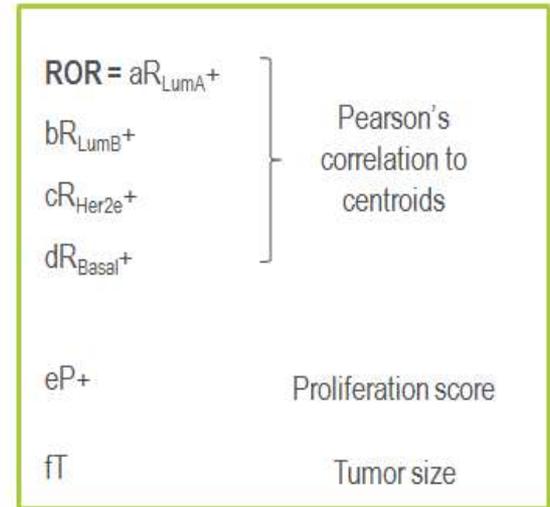
- PAM50: **5 subtypes** (Parker et al. JCO 2009)
- Prosigna™ Breast Cancer Prognostic Gene Signature Assay on the nCounter® Dx Analysis System (Nanostring)  
**4 subtypes and ROR score**
- Assignment of subclass by **centroide correlation**

Determine Intrinsic Subtype through Pearson's Correlation to Centroids

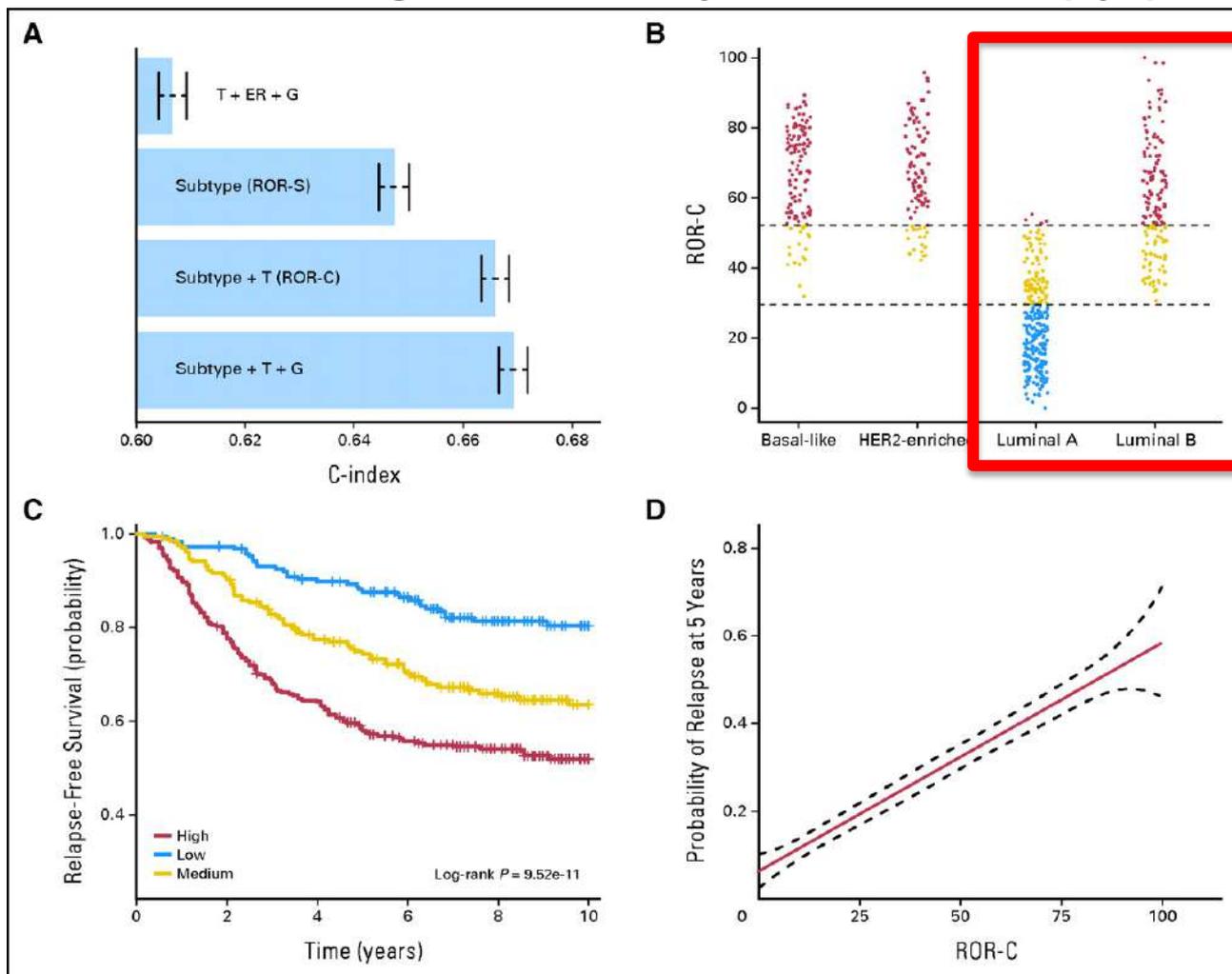


Sørli, Perou et al PNAS, 2006

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

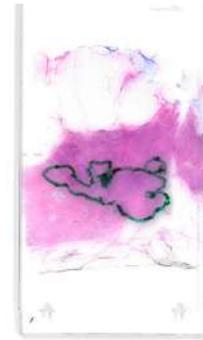
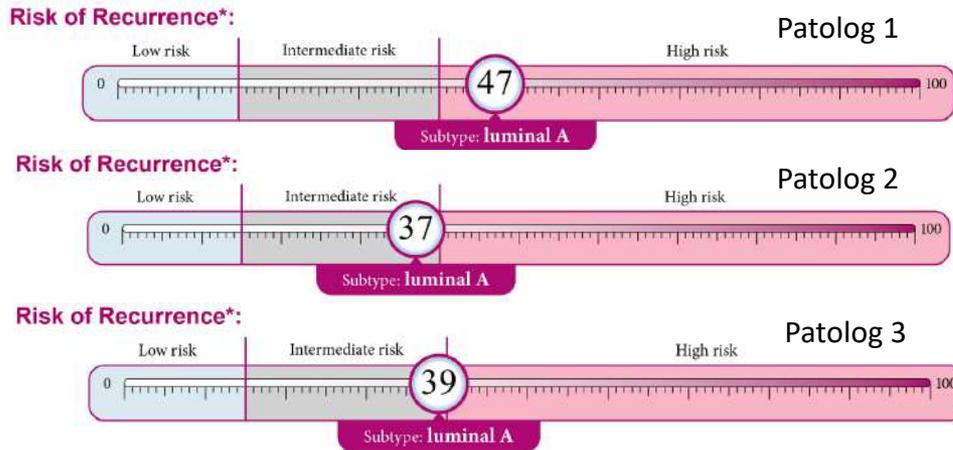
- Not PCR based – suited for RNA from FFPE
- Can be run as both a research instrument and a diagnostic instrument (black box)
- Up to 800 genes (can do DNA and protein as well)



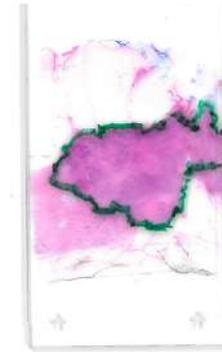
# Challenge: regional intra tumor heterogeneity

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

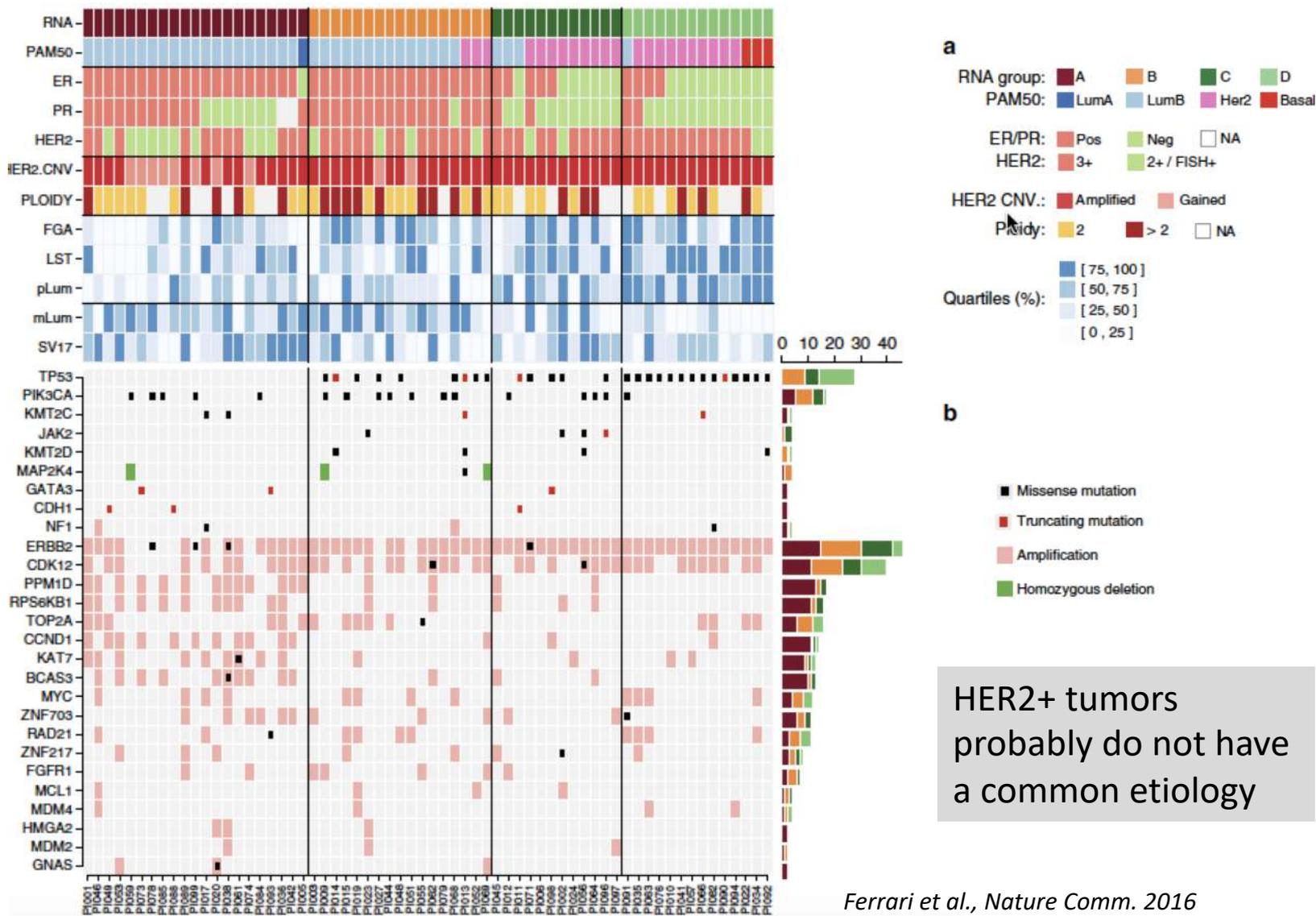
## Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer

Matthew D. Burstein<sup>1</sup>, Anna Tsimelzon<sup>2</sup>, Graham M. Poage<sup>3</sup>, Kyle R. Covington<sup>2</sup>, Alejandro Contreras<sup>2,4</sup>, Suzanne A.W. Fuqua<sup>2</sup>, Michelle I Savage<sup>3</sup>, C. Kent Osborne<sup>2</sup>, Susan G. Hilsenbeck<sup>2</sup>, Jenny C. Chang<sup>5</sup>, Gordon B. Mills<sup>6</sup>, Ching C. Lau<sup>7</sup>, and Powel H. Brown<sup>3</sup>

# Molecular pathways enriched in the four groups

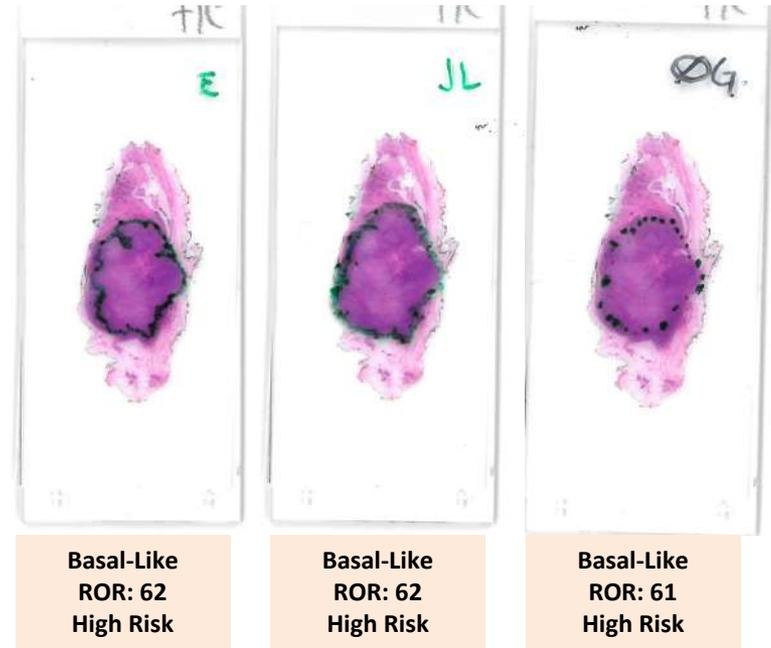
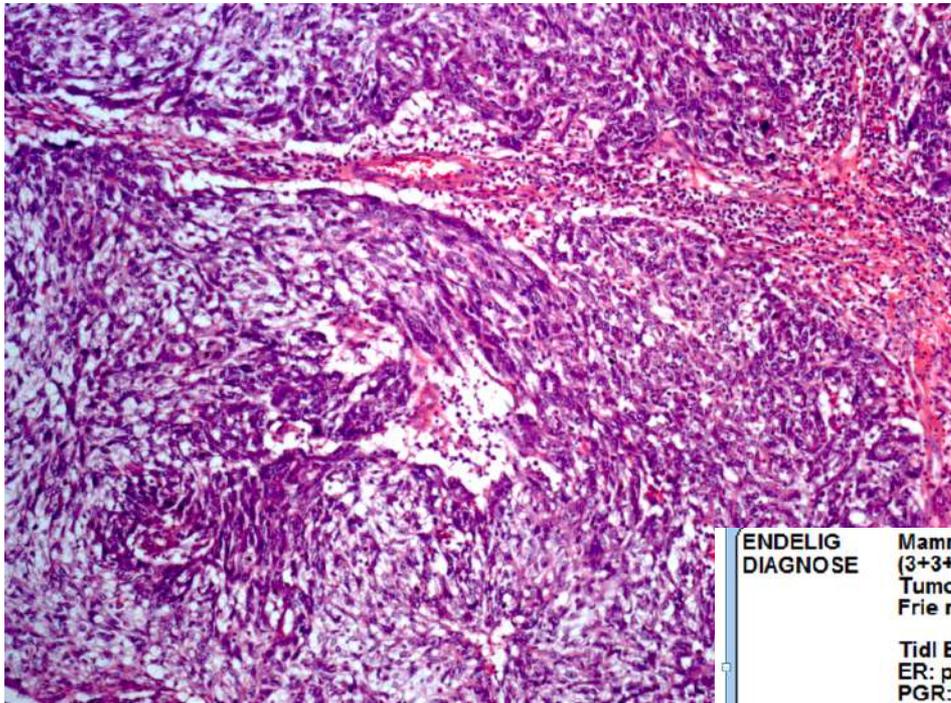
	Subtype 1 Luminal AR (LAR)			Subtype 2 Mesenchymal (MES)			Subtype 3 Basal-like immune suppressed (BLIS)			Subtype 4 Basal-like immune activated (BLIA)		
	Disc	Val	Ext	Disc	Val	Ext	Disc	Val	Ext	Disc	Val	Ext
Ingenuity canonical pathways												
Prolactin signaling	Blue	Blue	Blue	White	White	White	White	White	White	Red	Red	Red
Aryl hydrocarbon receptor signaling	Red	Red	Red	White	White	White	White	White	White	White	White	White
ErbB4 signaling	Blue	Blue	Blue	White	White	White	White	White	White	White	White	White
Estrogen-mediated S-phase entry	Blue	Blue	Blue	White	White	White	White	White	White	White	White	White
Xenobiotic metabolism signaling	Red	Red	Red	White	White	White	White	White	White	White	White	White
Cell cycle: G <sub>2</sub> -M DNA damage checkpoint regulation	Blue	Blue	Blue	Blue	Blue	Blue	White	White	White	Red	Red	Red
Coagulation system	White	White	White	Red	Red	Red	White	White	White	White	White	White
ATM signaling	White	White	White	Blue	Blue	Blue	White	White	White	Red	Red	Red
Hereditary breast cancer signaling	White	White	White	Blue	Blue	Blue	White	White	White	White	White	White
Mitotic roles of Polo-like kinase	White	White	White	Blue	Blue	Blue	White	White	White	White	White	White
Cell-cycle control of chromosomal replication	White	Blue	Blue	Blue	Blue	Blue	White	White	White	White	White	White
Mismatch repair in eukaryotes	White	White	White	Blue	Blue	Blue	White	White	White	White	White	White
DNA damage-induced 14-3-3 sigma signaling	White	White	White	Blue	Blue	Blue	White	White	White	White	White	White
Complement system	White	White	White	Red	Red	Red	White	White	White	White	White	White
Extrinsic prothrombin activation pathway	White	White	White	Red	Red	Red	White	White	White	White	White	White
Hepatic fibrosis / hepatic stellate cell activation	White	White	White	Red	Red	Red	White	White	White	Red	Red	Red
Natural killer cell signaling	White	White	White	White	White	White	Blue	Blue	Blue	Red	Red	Red
Calcium-induced T lymphocyte apoptosis	White	White	White	White	White	White	White	White	White	Red	Red	Red
Type I diabetes mellitus signaling	White	White	White	White	White	White	Blue	Blue	Blue	Red	Red	Red
Cytotoxic T lymphocyte-mediated apoptosis of target cells	White	White	White	White	White	White	Blue	Blue	Blue	Red	Red	Red
B-cell development	White	White	White	White	White	White	Blue	Blue	Blue	White	White	White
Tumoricidal function of hepatic natural killer cells	White	White	White	White	White	White	Blue	Blue	Blue	Red	Red	Red
Antigen presentation pathway	White	White	White	White	White	White	Blue	Blue	Blue	Red	Red	Red
Leukocyte extravasation signaling	White	White	White	White	White	White	Blue	Blue	Blue	Red	Red	Red
Cdc42 signaling	White	White	White	White	White	White	White	White	White	Blue	Blue	Blue
Allograft rejection signaling	White	White	White	White	White	White	White	White	White	Red	Red	Red
Altered T-cell and B-cell signaling in rheumatoid arthritis	White	White	White	White	White	White	White	White	White	Red	Red	Red
Autoimmune thyroid disease signaling	White	White	White	White	White	White	White	White	White	Red	Red	Red
T-Helper cell differentiation	White	White	White	White	White	White	White	White	White	Blue	Blue	Blue
Graft-versus-host disease signaling	White	White	White	White	White	White	White	White	White	Red	Red	Red
Nur77 signaling in T lymphocytes	White	White	White	White	White	White	White	White	White	Red	Red	Red
OX40 signaling pathway	White	White	White	White	White	White	White	White	White	Blue	Blue	Blue
ICOS-ICOSL signaling in T helper cells	White	White	White	White	White	White	White	White	White	Red	Red	Red
NF-kB activation by viruses	White	White	White	White	White	White	White	White	White	Red	Red	Red
Apoptosis signaling	White	White	White	White	White	White	White	White	White	Red	Red	Red
Tec kinase signaling	White	White	White	White	White	White	White	White	White	Blue	Blue	Blue
CCR5 signaling in macrophages	White	White	White	White	White	White	White	White	White	Red	Red	Red
Production of nitric oxide and reactive oxygen species in macrophages	White	White	White	White	White	White	White	White	White	Red	Red	Red
IL15 signaling	White	White	White	White	White	White	White	White	White	Red	Red	Red
Role of pattern recognition receptors in recognition of bacteria and viruses	White	White	White	White	White	White	White	White	White	Red	Red	Red

# 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 dukalt karsinom, histologisk grad 3 (3+3+3p)  
Tumordiameter 23 mm  
Frie reseksjonsreider, 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

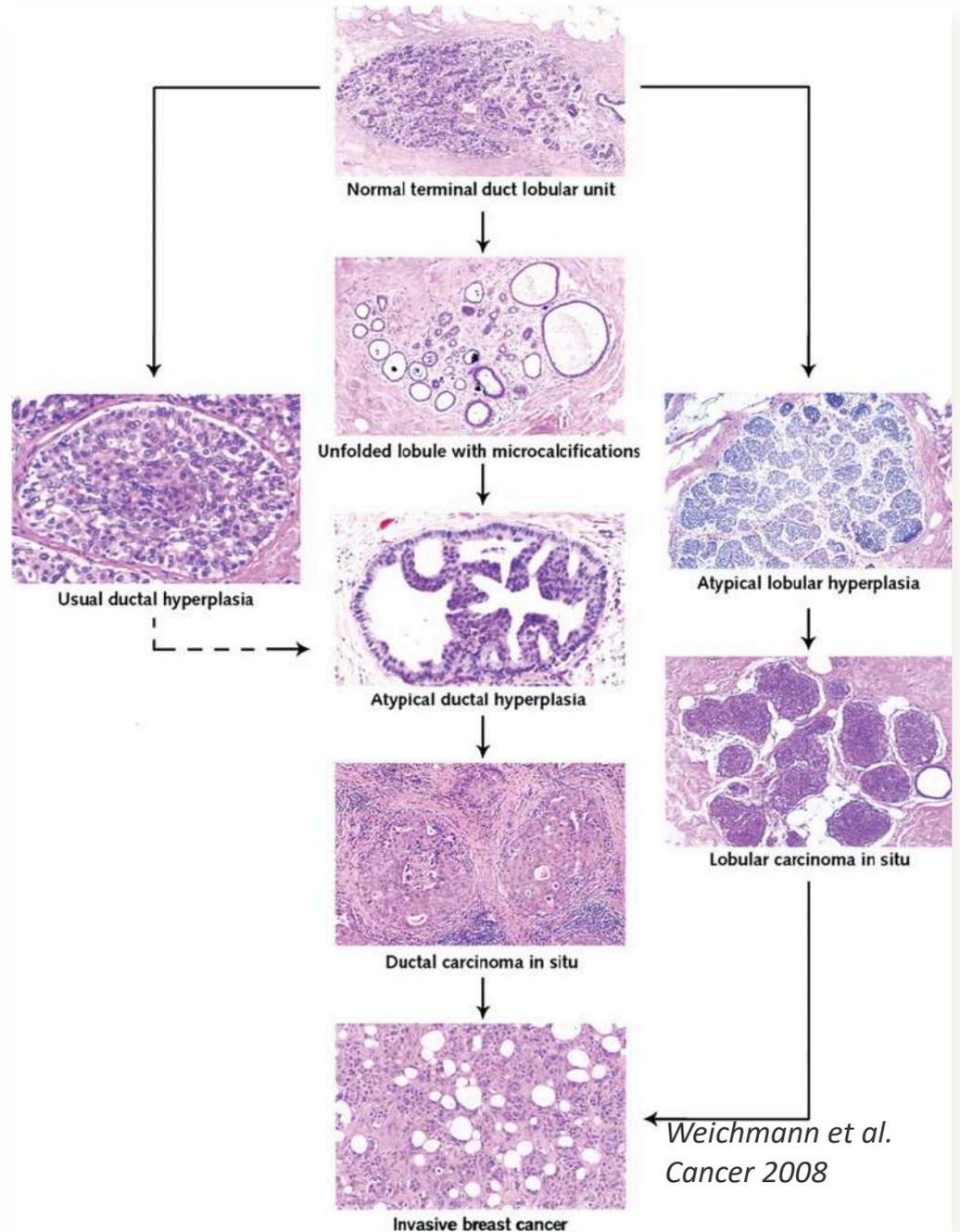
BU15 28335:  
En sentinel lymfeknute uten påviste patologiske forandringer

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

# Molecular based classification

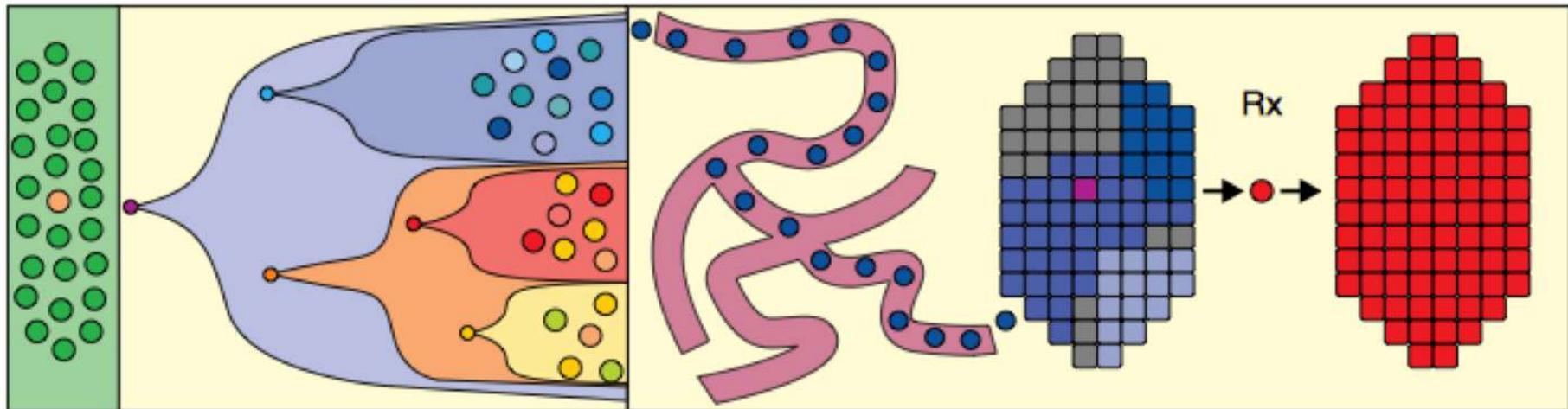
- Biomarkers/signatures for treatment prediction?
- Biomarkers/signatures recognizing biological distinct traits?
- Genomic – transcriptomic – metabolomic – proteomic features?
- Integrated approaches?
- Are they recapitulating already established classes...?
- What is the clinical implication?
- **And 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



Transformation

Clonal Evolution

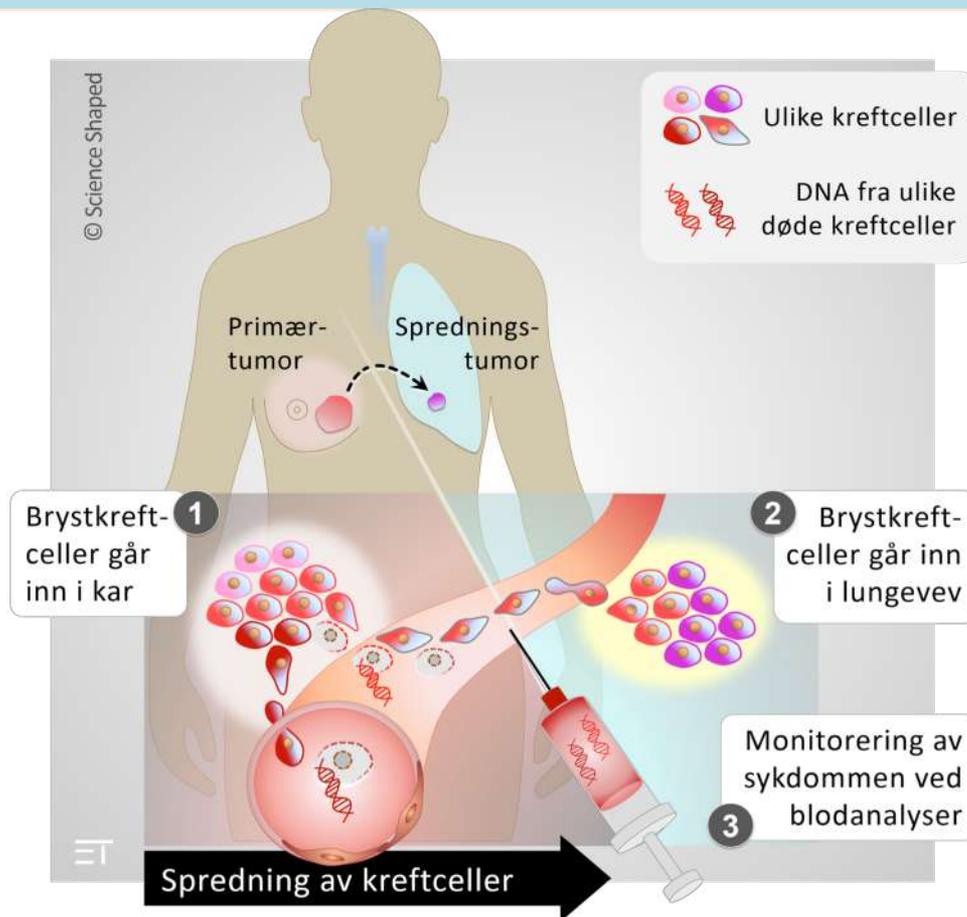
Metastasis

Chemoresistance

At what time point can metastatic potential be revealed?  
Prediction of therapy response?

# 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
<b>Assay</b>	Germline DNA test	Tumor RNA-expression assay	Tumor (or circulating tumor or cell-free) DNA for genomic profiling
<b>Number of Genes Measured</b>	Varies by assay; typically 2 to 40.	Varies by assay; typically 10-100	Varies by assay; typically > 400
<b>Assay Readout</b>	Mutations in germline DNA	Patterns of gene expression often weighted with proprietary score	Mutations, deletions, amplifications in tumor DNA
<b>Clinical Role</b>	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
<b>Recommend for:</b>	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 <u>based on</u> PIK3CA or ESR1 mutations; experimental for other precision medicine purposes

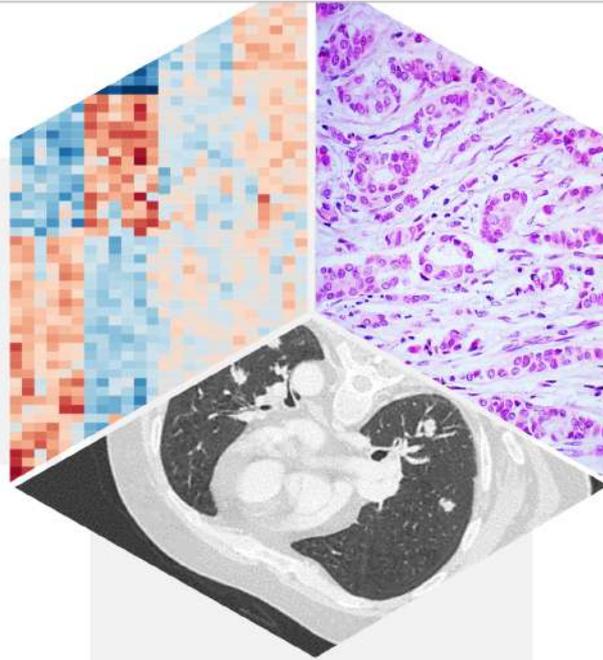
# MULTIDISCIPLINARY DIAGNOSTICS



## MOLECULAR CLASSIFICATION

- PAM50 RNA
- IntClust RNA/DNA
- Others?

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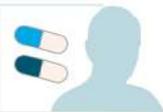
## HISTOPATHOLOGY

- 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!

