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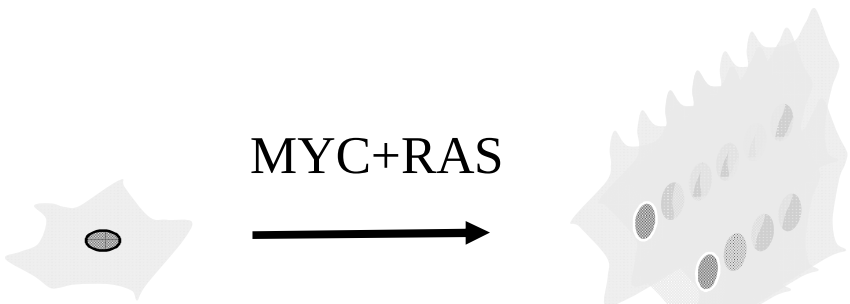
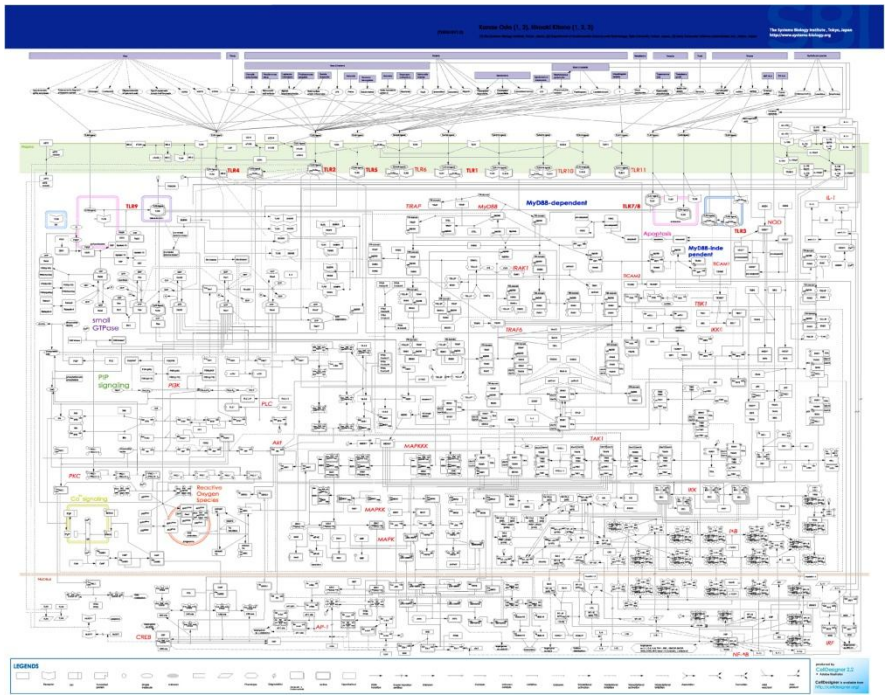
Nuevas tecnologías para el análisis genético de factores pronósticos y predictivos en cáncer de mama

25 years of progress in cancer research



Normal cell

Cancer

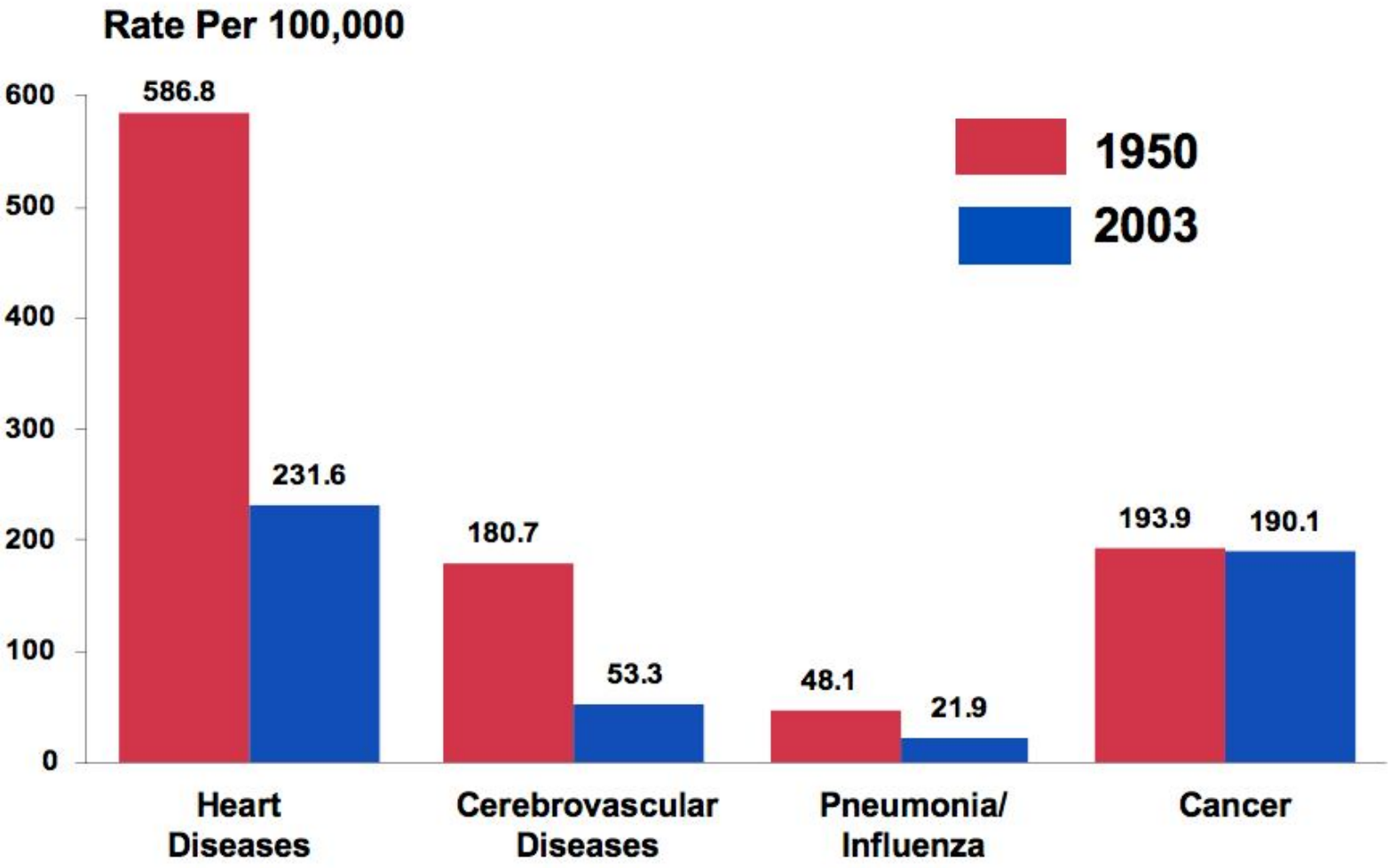


1980s

Today

…with embarrassingly little patient benefit!

1950 - 2003



* Age-adjusted to 2000 US standard population.
Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.
2003 Mortality Data: US Mortality Public Use Data Tape, 2003, NCHS, Centers for Disease Control and Prevention, 2006

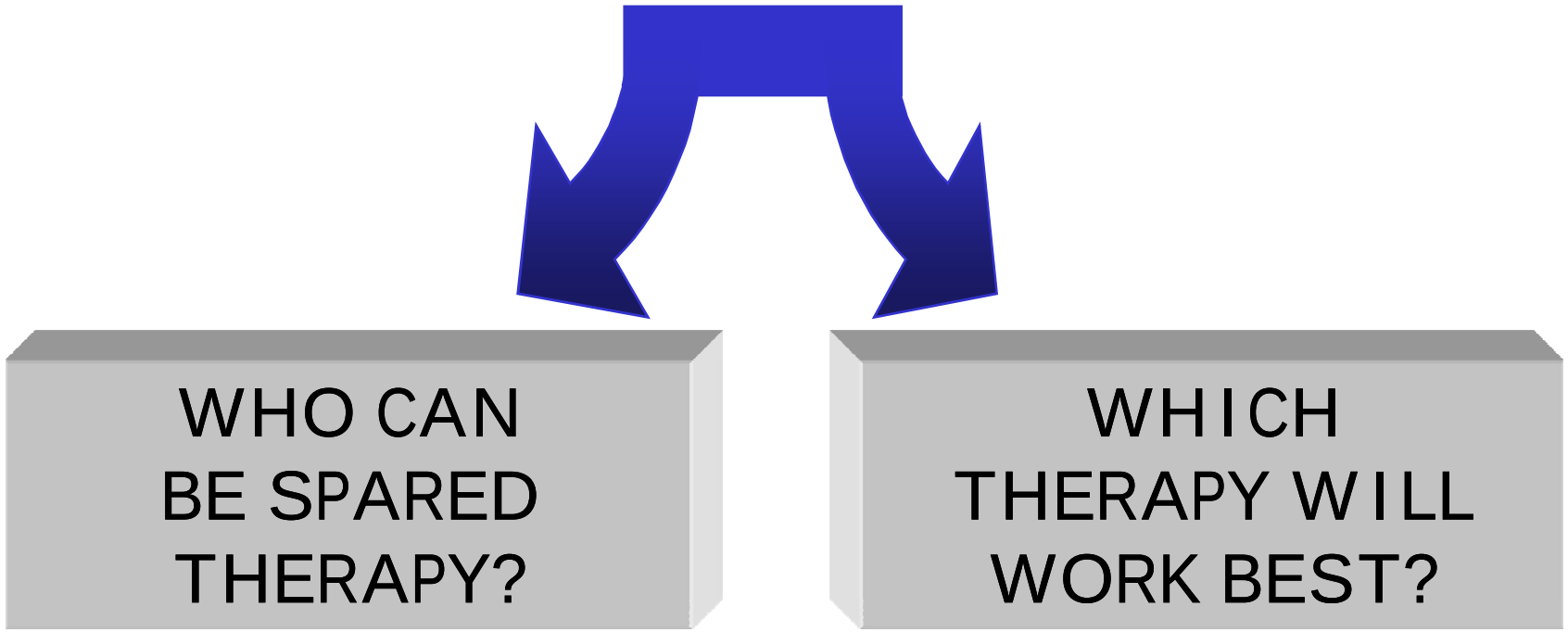
In the post genomic era:

- We still use the light microscope for cancer diagnosis
- We still use many broadly-acting cytotoxic drugs to treat cancer

Genomic technologies can help up to:

- Develop better cancer diagnostics
- Develop more specific cancer therapeutics

Therapy decision making for early stage breast cancer:



Prognostic factors needed

Predictive factors needed

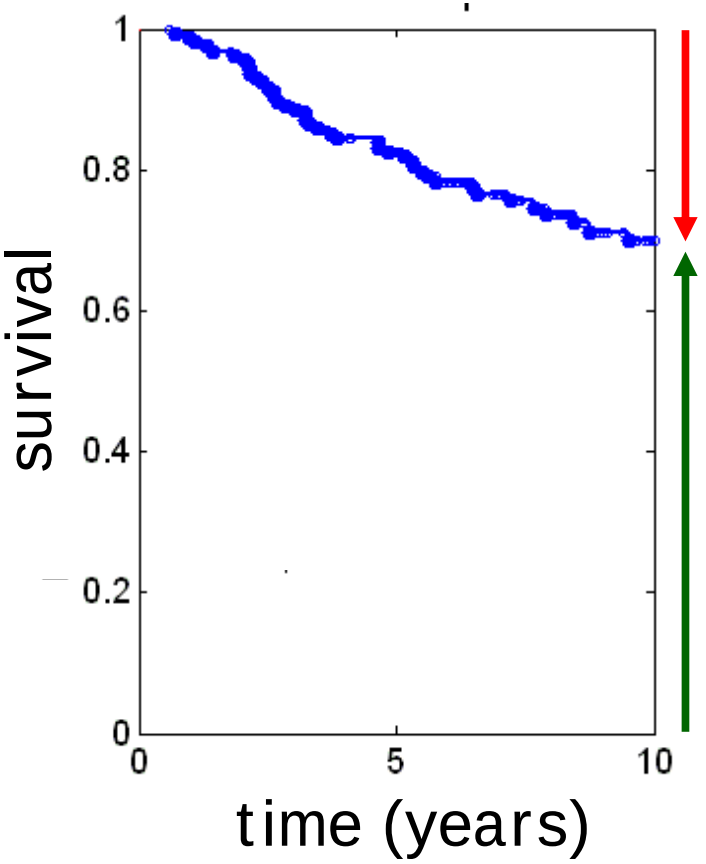
Treatment Decisions in Breast Cancer…

- **Classical pathological indexes:**
  - Nottingham Prognostic Index (NPI)
  - Adjuvant! Online (AO)
  - St. Gallen criteria
  - NCCN
- These guidelines result in **overtreatment** of many patients with chemotherapy.
- A more precise stratification of **poor versus good-prognostic patients** and into **responders versus non-responders** to therapeutic agents is needed.

Breast Cancer - Survival

premenopausal patients, lymph node negative

Kaplan-Meier Survival Curves



~30% die of breast cancer

~70% survive breast cancer

Who to treat ?

How to treat ?

Current Clinical Management

lymph node negative breast cancer

adjuvant treatment selection criteria

- NIH (US) consensus criteria: > 95%
- St Gallen (EU) consensus criteria: > 80%
  receive adjuvant chemo- and/or hormonal therapy

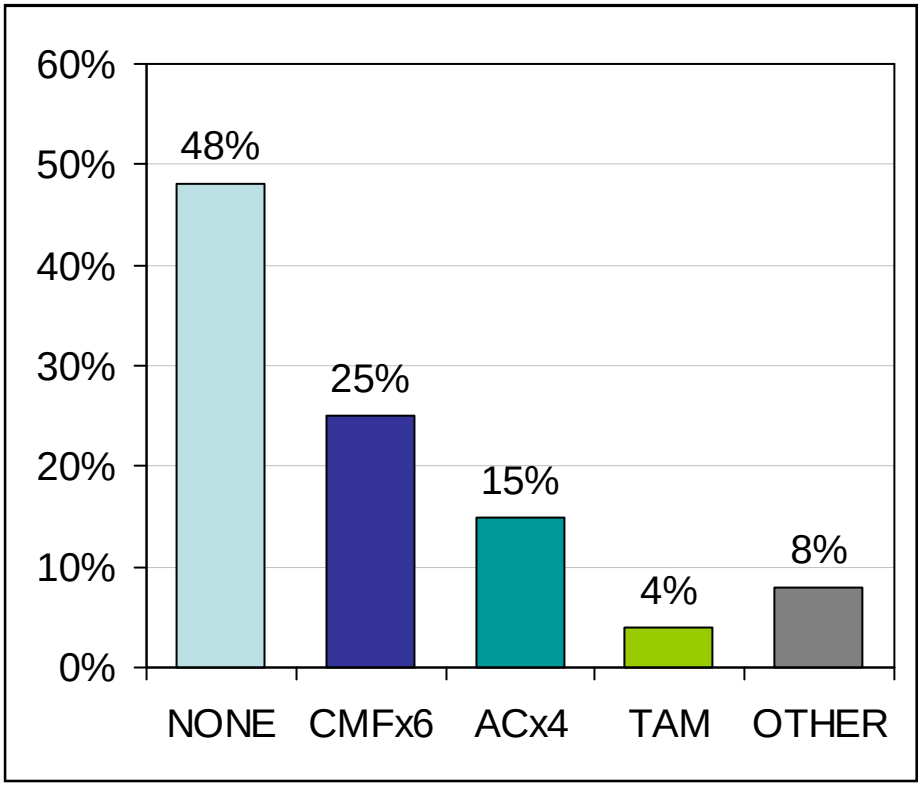
As only 30-40% of these patients develop distant metastases, some 40-60% of patients are over-treated with adjuvant (chemo)therapy

Breast Cancer: The Treatment Dilemma

61 y-old, fit, postmenopausal

Node negative
pT = 0.9 cm
ductal cancer
ER and PR negative
HER2 negative
Grade 2

Choices of 40 experts world-wide



Courtesy: Martine Piccart

The Chemotherapy dilemma: Common Presentation Of Breast Cancer T1 N0 ER+ Grade 2

Need To Treat 100 Women

Costly, many suffer unnecessary side effects

And Only One Benefits!

Genomic tests can help!

Courtesy: Peter Ravdin

Few genes, little information

Tumor cell behavior is determined by the activity of many genes

- The activity of one or a few genes cannot predict tumor cell behavior in a reliable way.
- We need tools to measure the activity of many genes in a single experiment

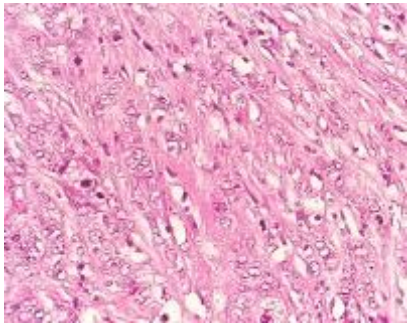
Predicting disease outcome in cancer: New tools:

DNA microarray technology

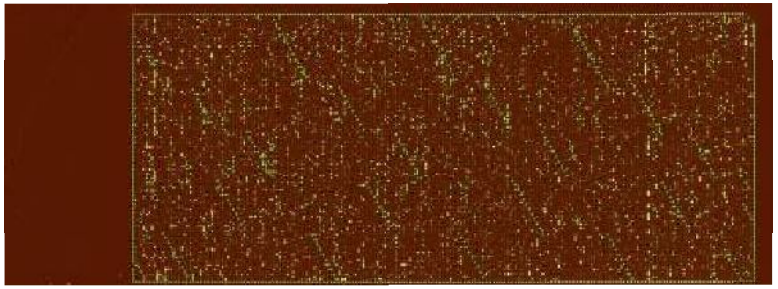
- Allows us to determine the activity of thousands of genes in a single experiment

Old and new diagnostics

Micro-scope



Micro-array

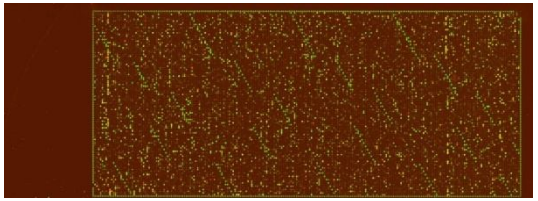


Application of Microarrays in Oncology

- Screen individual genes differentially expressed between normal and cancer tissue
- ➔ novel drug targets
- ➔ prognostic and predictive markers
- Monitor interactions among hundreds of genes *in vitro* or during therapy *in vivo*
- ➔ investigate key pathways in carcinogenesis
- **Classify cancer into various clinically relevant subgroups and refine diagnosis and prognosis**
- ➔ **identify patients with low risk of relapse**
- ➔ **identify patients likely to respond to particular therapies**

Identification of a breast cancer prognosis profile

78 breast tumors (‘83-’94)
patients < 55 years
lymph node negative (LN0)
no adjuvant therapy



Unbiased full genome
gene expression analysis

Prognosis Reporter Genes

distant metastases
< 5 years (n=34)

no distant metastases
in at least 5 years (n=44)

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Competing interests statement

The authors declare that they have no competing financial interests.

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Gene expression profiling predicts clinical outcome of breast cancer

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Breast cancer patients with the same stage of disease can have markedly different treatment responses and overall outcome. The strongest predictors for metastases (for example, lymph node status and histological grade) fail to classify accurately breast tumours according to their clinical behaviour[1–3]. Chemotherapy or hormonal therapy reduces the risk of distant metastases by approximately one-third; however, 70–80% of patients receiving this treatment would have survived without it[4,5]. None of the signatures of breast cancer gene expression reported to date[6–12] allow for patient-tailored therapy strategies. Here we used DNA microarray analysis on primary breast tumours of 117 young patients, and applied supervised classification to identify a gene expression signature strongly predictive of a short interval to distant metastases ('poor prognosis' signature) in patients without tumour cells in local lymph nodes at diagnosis (lymph node negative). In addition, we established a signature that identifies tumours of *BRCA1* carriers. The poor prognosis signature consists of genes regulating cell cycle, invasion, metastasis and angiogenesis. This gene expression profile will outperform all currently used clinical parameters in predicting disease outcome. Our findings provide a strategy to select patients who would benefit from adjuvant therapy.

We selected 98 primary breast cancers: 34 from patients who developed distant metastases within 5 years, 44 from patients who continued to be disease-free after a period of at least 5 years, 18 from patients with *BRCA1* germline mutations, and 2 from *BRCA2* carriers. All 'sporadic' patients were lymph node negative, and under 55 years of age at diagnosis. From each patient, 5 μg total RNA was isolated from snap-frozen tumour material and used to derive complementary RNA (cRNA). A reference cRNA pool was made by pooling equal amounts of cRNA from each of the sporadic carcinomas. Two hybridizations were carried out for each tumour using a fluorescent dye reversal technique on microarrays containing approximately 25,000 human genes synthesized by inkjet technology[13]. Fluorescence intensities of scanned images were quantified, normalized and corrected to yield the transcript abundance of a gene as an intensity ratio with respect to that of the signal of the reference pool[14]. Some 5,000 genes were significantly regulated across the group of samples (that is, at least a twofold difference and a *P*-value of less than 0.01 in more than five tumours).

An unsupervised, hierarchical clustering algorithm allowed us to cluster the 98 tumours on the basis of their similarities measured over these approximately 5,000 significant genes. Similarly, the ~5,000 genes were clustered on the basis of their similarities measured over the group of 98 tumours (Fig. 1a). In the dendrograms shown in Fig. 1a (left and top), the length and the subdivision of the branches displays the relatedness of the breast tumours (left) and the expression of the genes (top). Two distinct groups of tumours are the dominant feature in this two-dimensional display (top and bottom of plot, representing 62 and 36 tumours, respectively), suggesting that the tumours can be divided into two types on the basis of this set of ~5,000 significant genes. Notably, in the upper group only 34% of the sporadic patients were from the group who developed distant metastases within 5 years, whereas in the lower group 70% of the sporadic patients had progressive disease (Fig. 1b). Thus, using unsupervised clustering we can already, to some extent, distinguish between 'good prognosis' and 'poor prognosis' tumours.

To gain insight into the genes of the dominant expression signatures, we associated them with histopathological data; for example, oestrogen receptor (ER)-α expression as determined by immunohistochemical (IHC) staining (Fig. 1b). Out of 39 IHC-stained tumours negative for ER-α expression (ER negative), 34 clustered together in the bottom branch of the tumour dendrogram. In the enlargement shown in Fig. 1c, a group of downregulated genes is represented containing both the ER-α gene (*ESR1*) and genes that are apparently co-regulated with ER, some of which are known ER target genes. A second dominant gene cluster is associated with lymphocytic infiltrate and includes several genes expressed primarily by B and T cells (Fig. 1d).

Sixteen out of eighteen tumours of *BRCA1* carriers are found in the bottom branch intermingled with sporadic tumours. This is consistent with the idea that most *BRCA1* mutant tumours are ER negative and manifest a higher amount of lymphocytic infiltrate[15]. The two tumours of *BRCA2* carriers are part of the upper cluster of tumours and do not show similarity with *BRCA1* tumours. Neither high histological grade nor angioinvasion is a specific feature of either of the clusters (Fig. 1b). We conclude that unsupervised clustering detects two subgroups of breast cancers, which differ in ER status and lymphocytic infiltration. A similar conclusion has also been reported previously[7,16].

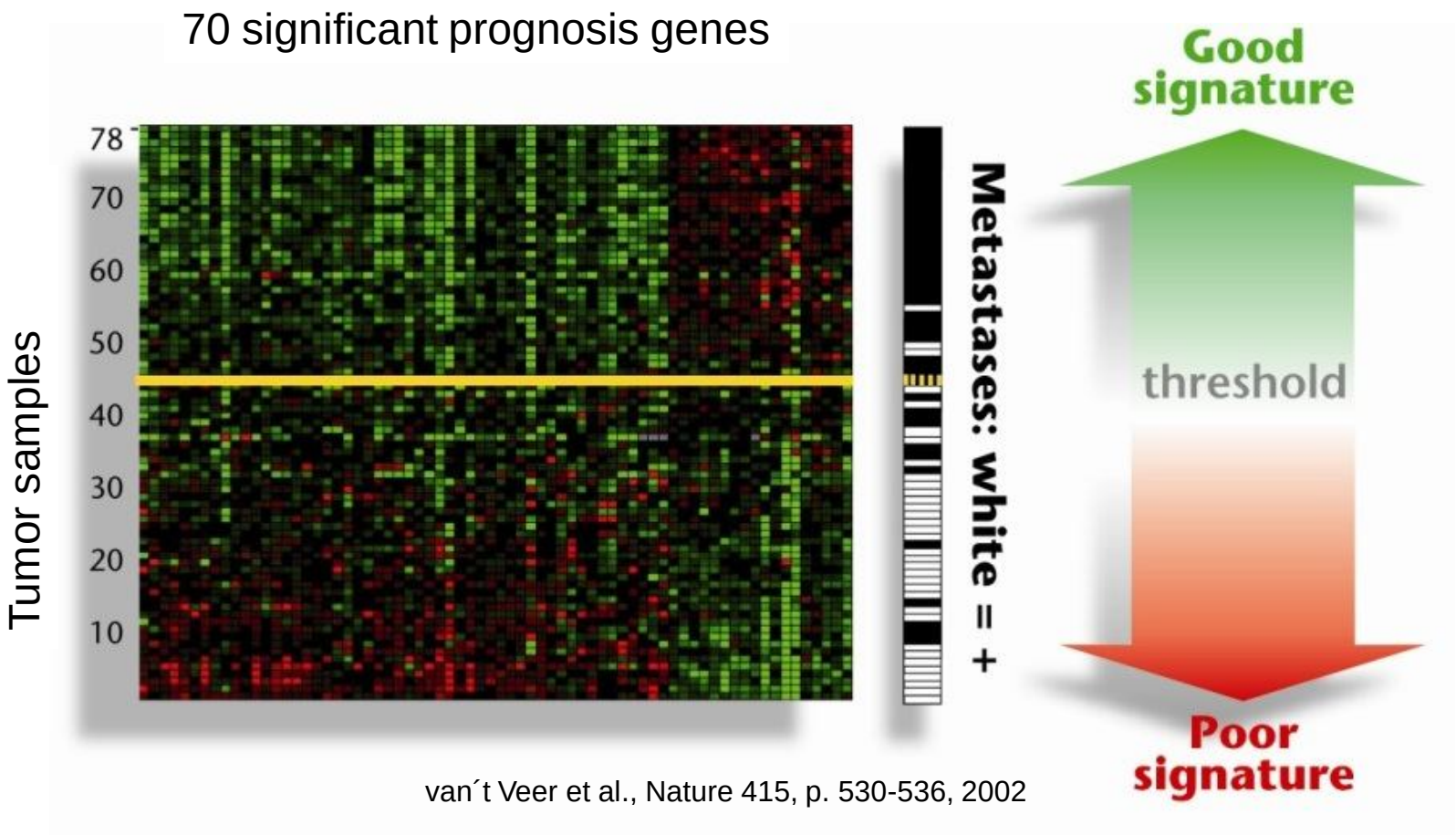
The 78 sporadic lymph-node-negative patients were selected specifically to search for a prognostic signature in their gene expression profiles. Forty-four patients remained free of disease

Discovery:

Van 't Veer et al. (2002)
Nature **415**, 530-536.

70 Gene Prognosis Profile

Supervised analysis



van´t Veer et al., Nature 415, p. 530-536, 2002

threshold set with 10% false negatives

91 % sensitivity, 73% specificity

The New England
Journal of Medicine

VOLUME 347 DECEMBER 19, 2002 NUMBER 25

A GENE-EXPRESSION SIGNATURE AS A PREDICTOR OF SURVIVAL IN BREAST CANCER

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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 (±SE) overall 10-year survival rates were 54.6±4.4 percent and 94.5±2.6 percent, respectively. At 10 years, the probability of remaining free of distant metastases was 50.6±4.5 percent in the group with a poor-prognosis signature and 85.2±4.3 percent in the group with a good-prognosis signature. The estimated hazard ratio for distant metastases in the group with a poor-prognosis signature, as compared with the group with the good-prognosis signature, was 5.1 (95 percent confidence interval, 2.9 to 9.0; P<0.001). This ratio remained significant when the groups were analyzed according to lymph-node status. Multivariable Cox regression analysis showed that the prognosis profile was a strong independent factor in predicting disease outcome.

Conclusions The gene-expression profile we studied is a more powerful predictor of the outcome of disease in young patients with breast cancer than standard systems based on clinical and histologic criteria. (N Engl J Med 2002;347:1999-2009.)

ADJUVANT systemic therapy substantially improves disease-free and overall survival in both premenopausal and postmenopausal women up to the age of 70 years with lymph-node–negative or lymph-node–positive breast cancer.[1,2] It is generally agreed that patients with poor prognostic features benefit the most from adjuvant therapy.[3,4] The main prognostic factors in breast cancer are age, tumor size, status of axillary lymph nodes, histologic type of the tumor, pathological grade, and hormone-receptor status. A large number of other factors have been investigated for their potential to predict the outcome of disease, but in general, they have only limited predictive power.[5]

Using complementary DNA (cDNA) microarrays to analyze breast-cancer tissue, Perou et al. identified tumors with distinct patterns of gene expression that they termed "basal type" and "luminal type."[6] These subgroups differ with respect to the outcome of disease in patients with locally advanced breast cancer.[7] In addition, microarray analysis has been used to distinguish cancers associated with *BRCA1* or *BRCA2* mutations[8,9] and to determine estrogen-receptor status[6,9,10] and lymph-node status.[11,12]

Using inkjet-synthesized oligonucleotide microarrays, we recently identified a gene-expression profile

From the Divisions of Diagnostic Oncology (M.J.V., L.J.V., D.W.V., H.L.P., D.A., A.W., A.G., L.D.), Radiotherapy (A.A.M.H., H.B.), Medical Oncology (S.R.), Biometrics (T.V.), Surgical Oncology (E.T.R.), and Molecular Carcinogenesis (R.B.), Netherlands Cancer Institute, Amsterdam; the Center for Biomedical Genetics, Amsterdam (R.B.); and Rosetta Inpharmatics, Kirkland, Wash. (Y.D.H., H.D., G.J.S., C.R., M.J.M., M.P., S.H.F.). Address reprint requests to Dr. Bernards at the Division of Molecular Carcinogenesis, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands, or at r.bernards@nki.nl.

Drs. van de Vijver, He, and van 't Veer contributed equally to this article.

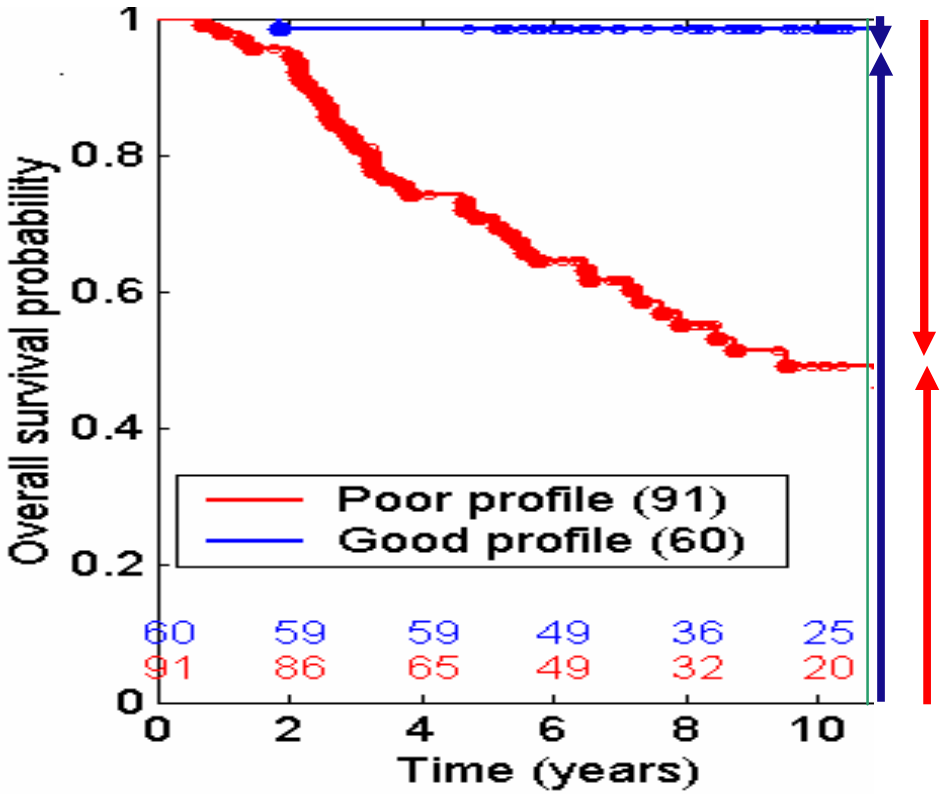
First validation:

Van de Vijver et al. (2002)

New England J. Med. **347**, 1999-2009.

295 patients

Validation of the 70 gene profile in LN0 patients



good profile:
~4% die of breast cancer
~96% survive breast cancer

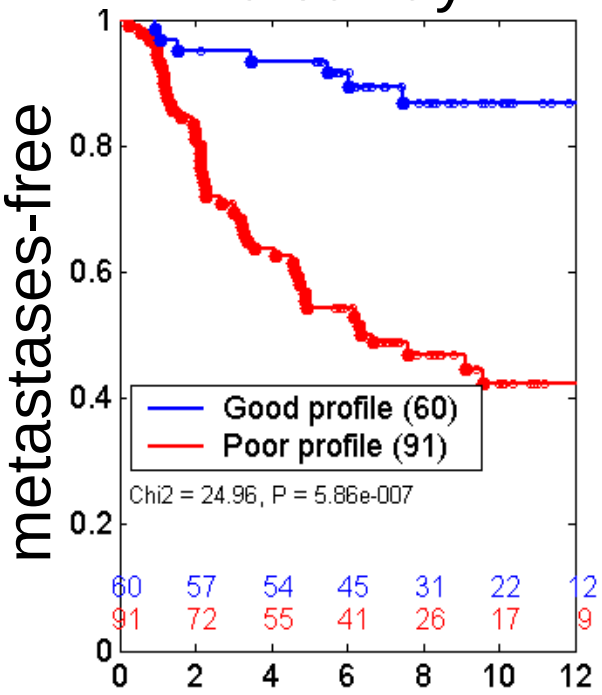
poor profile:
~50% die of breast cancer
~50% survive breast cancer

40% good profile ; 60% poor profile

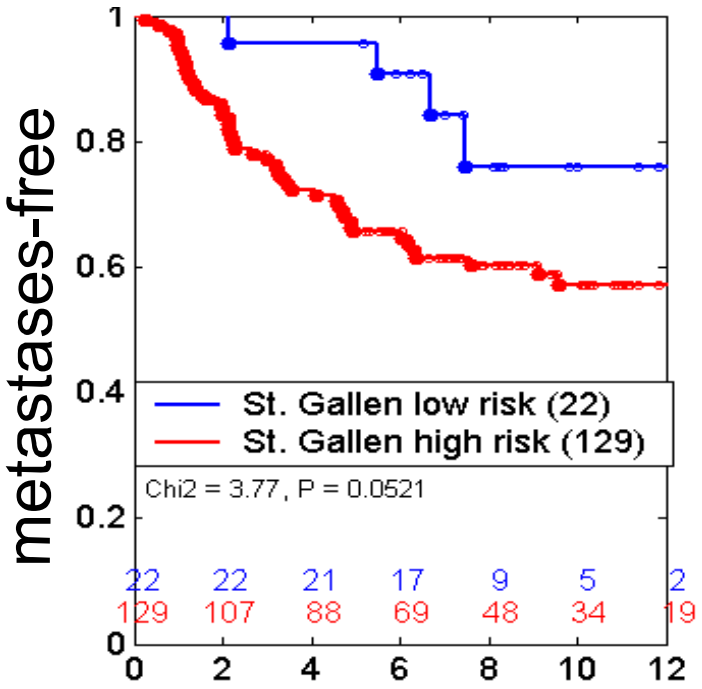
Microarray: Improved Clinical Management

Profiling vs St Gallen selection

Microarray



St Gallen



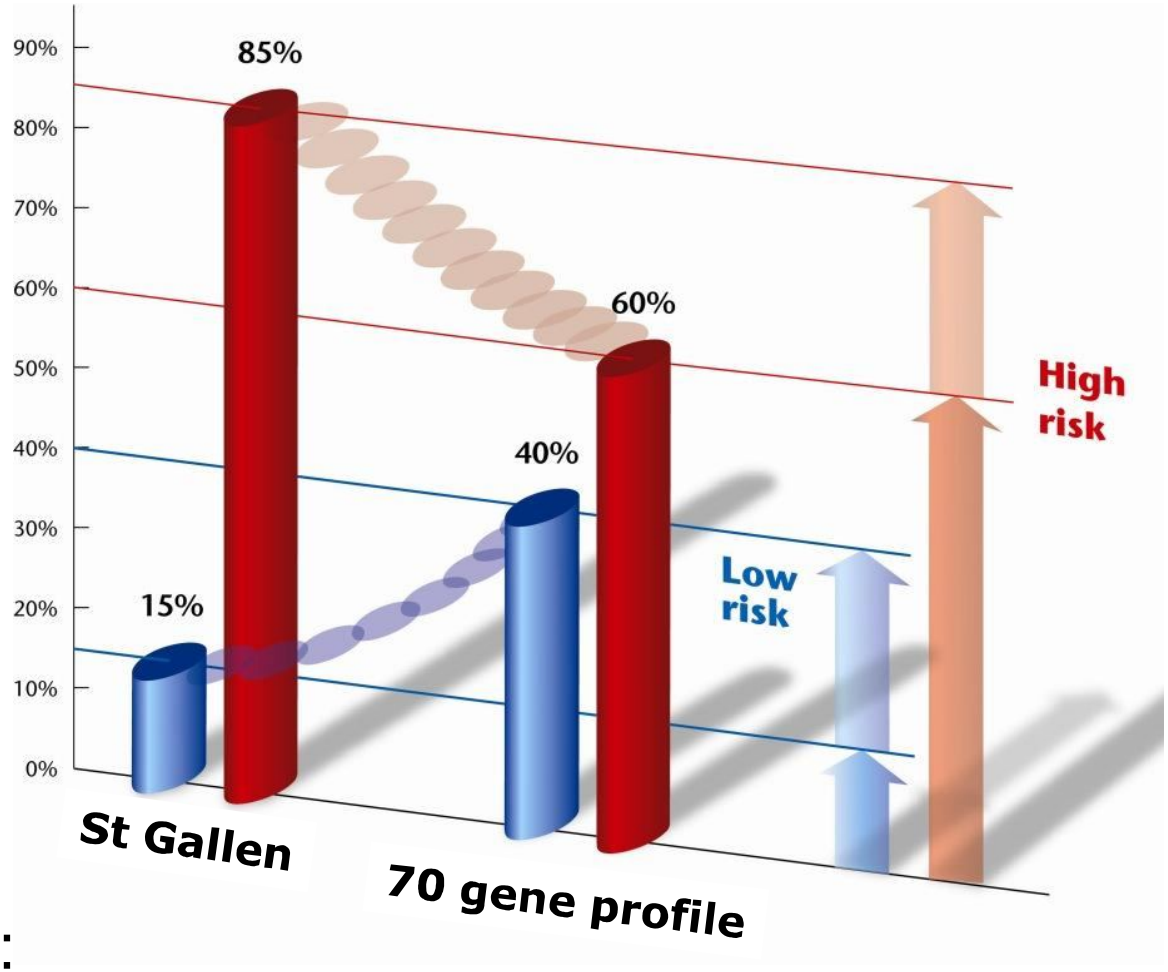
Profiling
improved prediction
and more accurate

Profiling:
40% in good profile
60 % in poor profile

St Gallen:
<15% in low risk
85% in high risk

NEJM 347, p1999-2009, 2002

70 gene profile vs St Gallen guidelines



Gene profiling:
Reduction adjuvant chemotherapy selection
Avoiding both over- and undertreatment
Improved prognosis prediction

NEJM 347, p1999-2009, 2002

Validation and Clinical Utility of a 70-Gene Prognostic Signature for Women With Node-Negative Breast Cancer

Marc Buyse, Sherene Loi, Laura van't Veer, Giuseppe Viale, Mauro Delorenzi, Annuska M. Glas, Mahasti Saghatchian d'Assignies, Jonas Bergh, Rosette Lidereau, Paul Ellis, Adrian Harris, Jan Bogaerts, Patrick Therasse, Arno Floore, Mohamed Amakrane, Fanny Piette, Emiel Rutgers, Christos Sotiriou, Fatima Cardoso, Martine J. Piccart

On behalf of the TRANSBIG Consortium

Background: A 70-gene signature was previously shown to have prognostic value in patients with node-negative breast cancer. Our goal was to validate the signature in an independent group of patients. *Methods:* Patients (n = 307, with 137 events after a median follow-up of 13.6 years) from five European centers were divided into high- and low-risk groups based on the gene signature classification and on clinical risk classifications. Patients were assigned to the gene signature low-risk group if their 5-year distant metastasis–free survival probability as estimated by the gene signature was greater than 90%. Patients were assigned to the clinicopathologic low-risk group if their 10-year survival probability, as estimated by Adjuvant! software, was greater than 88% (for estrogen receptor [ER]–positive patients) or 92% (for ER-negative patients). Hazard ratios (HRs) were estimated to compare time to distant metastases, disease-free survival, and overall survival in high- versus low-risk groups. *Results:* The 70-gene signature outperformed the clinicopathologic risk assessment in predicting all endpoints. For time to distant metastases, the gene signature yielded HR = 2.32 (95% confidence interval [CI] = 1.35 to 4.00) without adjustment for clinical risk and hazard ratios ranging from 2.13 to 2.15 after adjustment for various estimates of clinical risk; clinicopathologic risk using Adjuvant! software yielded an unadjusted HR = 1.68 (95% CI = 0.92 to 3.07). For overall survival, the gene signature yielded an unadjusted HR = 2.79 (95% CI = 1.60 to 4.87) and adjusted hazard ratios ranging from 2.63 to 2.89; clinicopathologic risk yielded an unadjusted HR = 1.67 (95% CI = 0.93 to 2.98). For patients in the gene signature high-risk group, 10-year overall survival was 0.69 for patients in both the low– and high–clinical risk groups; for patients in the gene signature low-risk group, the 10-year survival rates were 0.88 and 0.89, respectively. *Conclusions:* The 70-gene signature adds independent prognostic information to clinicopathologic risk assessment for patients with early breast cancer. [J Natl Cancer Inst 2006;98:1183–92]

Microarray technology is revolutionizing our understanding of cancer biology through the simultaneous study of the expression of tens of thousands of genes, or even of the entire human genome. Differential gene expression or molecular profiling has the potential to substantially refine cancer prognosis, well beyond what is currently possible with the clinical and pathologic indicators used thus far for this purpose. Several studies have recently used microarrays to classify breast tumors on the basis of their gene expression profiles *(1–7)*. These studies have consistently revealed considerable molecular diversity in breast cancer that often corresponds to distinct clinical phenotypes. Two major types, basal and luminal, have been identified by gene expression profiling of breast cancer, each with the potential to be subdivided into two or three subtypes. In addition, these molecular portraits seem to be remarkably stable as tumors progress from primary to metastatic disease *(8)*.

In one of the microarray studies *(5)*, investigators from the Netherlands Cancer Institute in Amsterdam (NKI) studied a narrowly defined subset of breast cancer patients, i.e., those aged 55 years or younger who were diagnosed with tumors smaller than 5 cm (T1/T2), had no nodal involvement (N0) or metastases (M0), and were treated only with local–regional therapies. The expression of 231 genes was found to be statistically significantly associated with disease outcome, as defined by the presence of distant metastasis within 5 years. This group of genes was reduced subsequently to a core set of 70 genes that together

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Correspondence to: BIG/TRANSBIG Secretariat, Institut Jules Bordet, 121 Boulevard de Waterloo, 1000 Brussels, Belgium (e-mail: TRANSBIG@bordet.be).

See "Notes" following "References."

DOI: 10.1093/jnci/djj329

Second validation:

Buyse et al. (2006)

JNCI. **98**, 1183-1192.

302 patients

Adjuvant!

Adjuvant! for Breast Cancer (Version 8.0)

Patient Information

Age: 55

Comorbidity: Perfect Health

ER Status: Positive

Tumor Grade: Grade 3

Tumor Size: 2.1 - 3.0 cm

Positive Nodes: > 9

Calculate For: Mortality

10 Year Risk: 79 Prognostic

Adjuvant Therapy Effectiveness

Horm: Aromatase Inhibitor for 5 yrs

Chemo: Anthra >4 Cycles >2 agnts.

Hormonal Therapy: 32

Chemotherapy: 33

Combined Therapy: 54

No additional therapy:

20.0 alive in 10 years.

78.0 die of cancer.

2.0 die of other causes.

With hormonal therapy: Benefit = 13.6 alive.

With chemotherapy: Benefit = 14.2 alive.

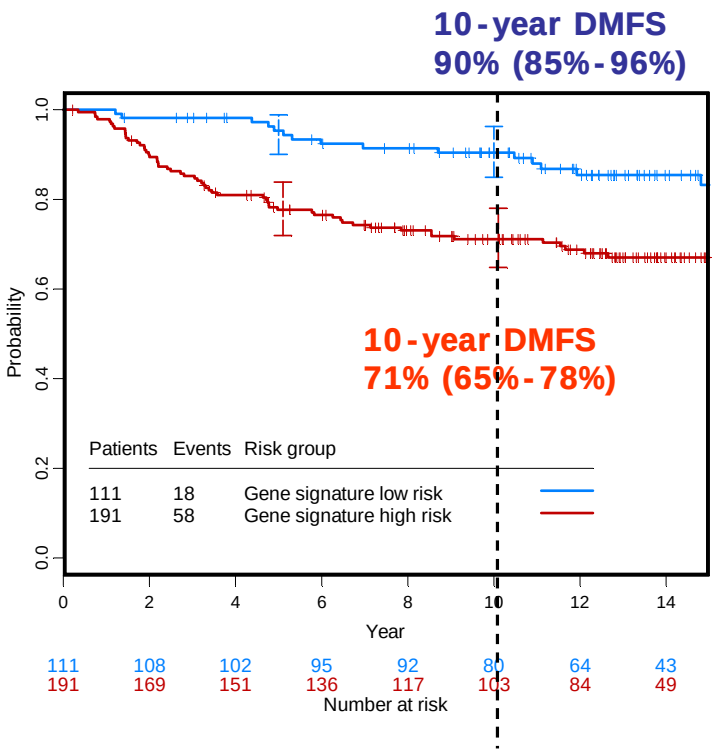
With combined therapy: Benefit = 27.6 alive.

Print Results PDF | Access Help and Clinical Evidence | Images for Consultations

Metastasis-free survival

70 genes versus Adjuvant!

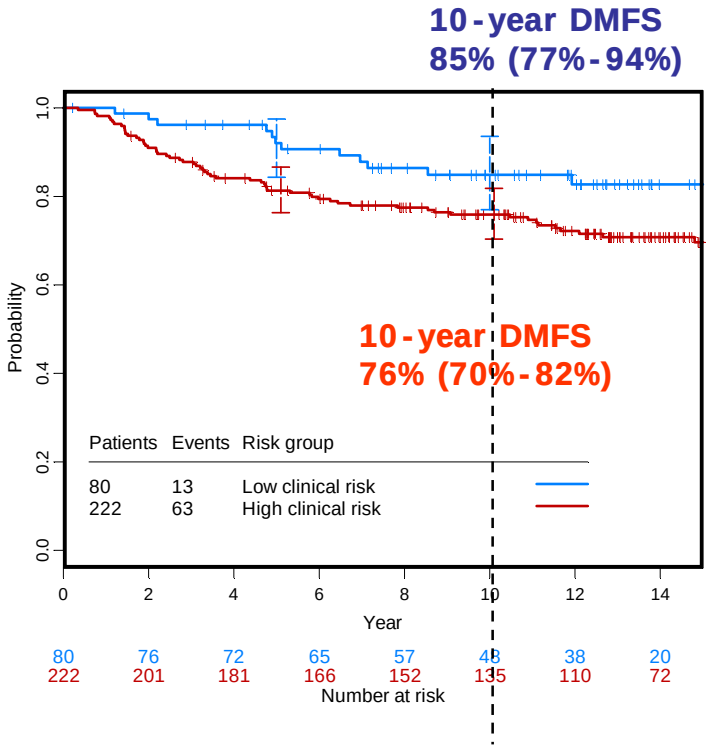
70 gene profile



Low risk profile

High risk profile

Adjuvant! online



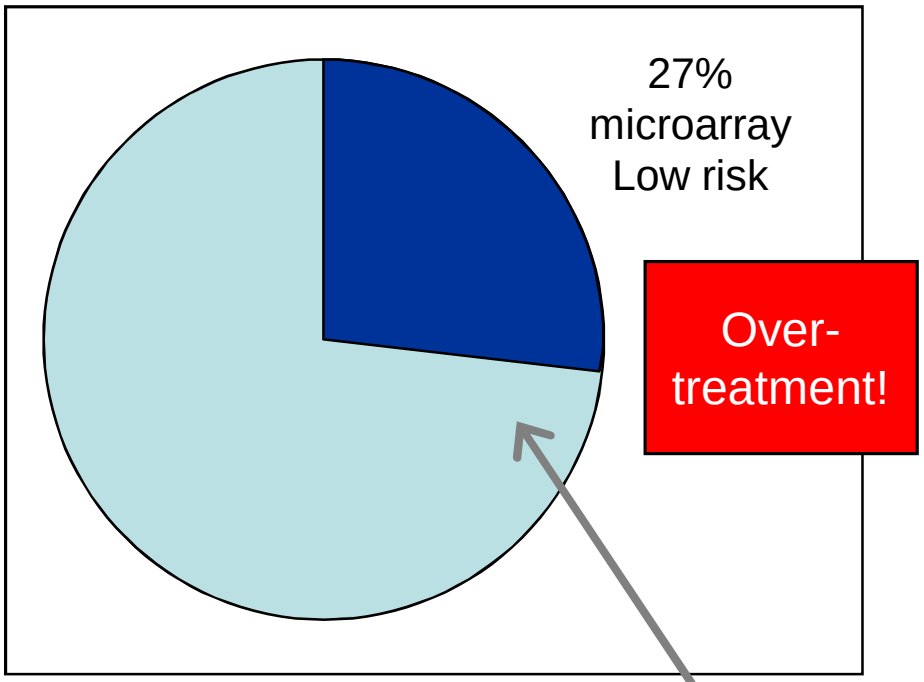
Low risk Adjuvant!

High risk Adjuvant!

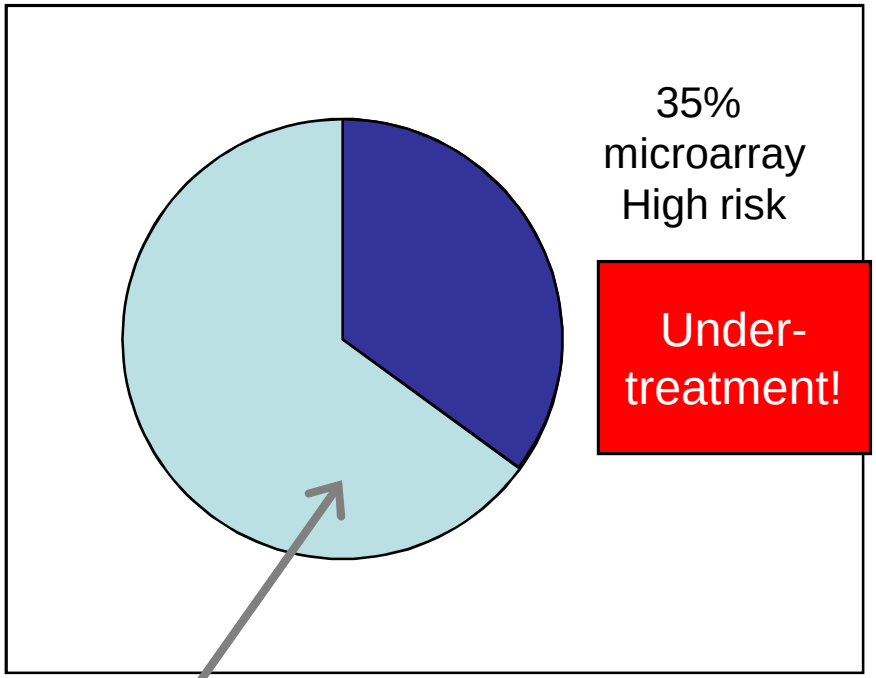
Independent External Validation:

Microarray outperforms all clinical risk assessment

High clinical risk
Adjuvant on line! N=222
73%



Low clinical risk
Adjuvant on line! N=80
27%



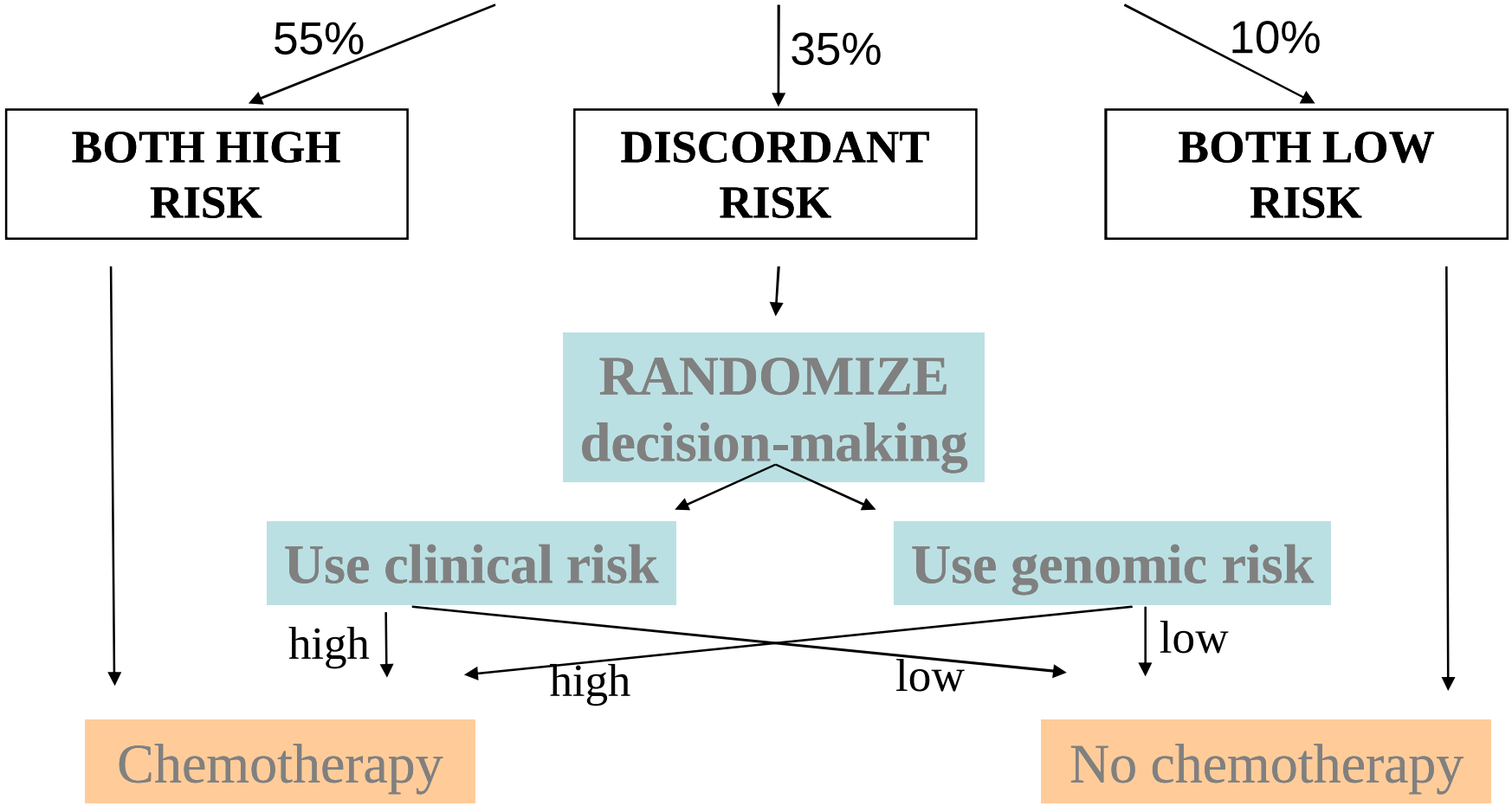
Buyse et al JNCI 2006

35% Discordant cases!

MINDACT design

6000 patients, <70 YRS, 1-3 POS NODES

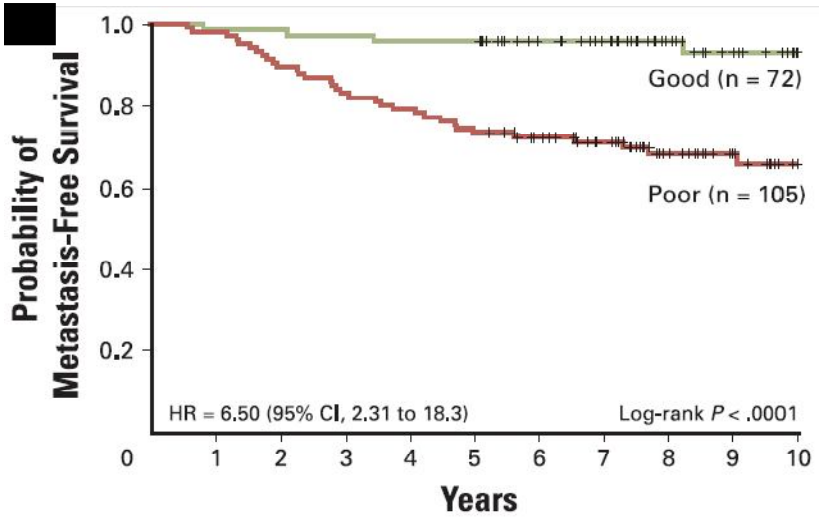
ASSESS CLINICAL RISK AND GENOMIC RISK
(adjuvant!online; 70-gene signature)

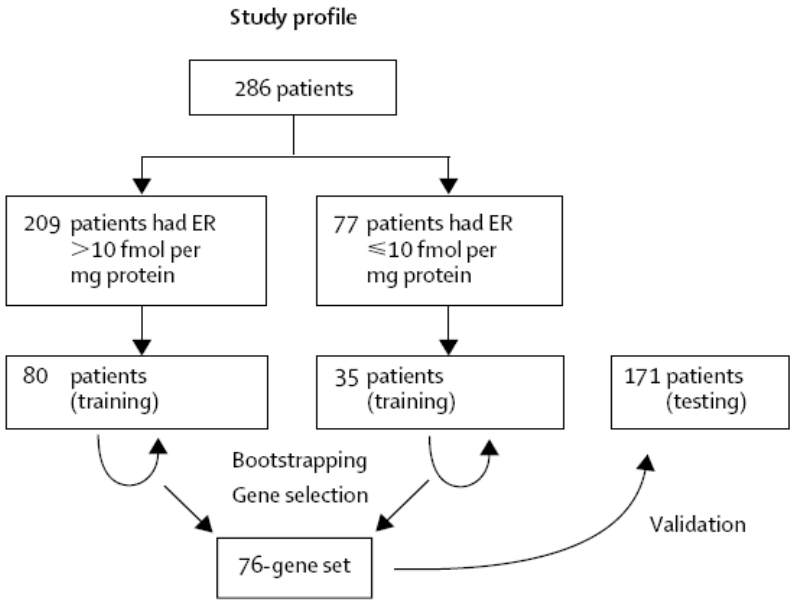


Another Supervised Prognostic Signature[1, 2]

- **286 lymph-node-negative patients who had not received adjuvant systemic treatment.**
- **Identified genes that were associated with relapse separately for the ER-negative and ER-positive subsets of patients (1).**
- **The markers that were selected from each group were then combined to form a single 76-gene prognostic signature (1).**

This predictor performed well when tested on 180 independent cases (2).



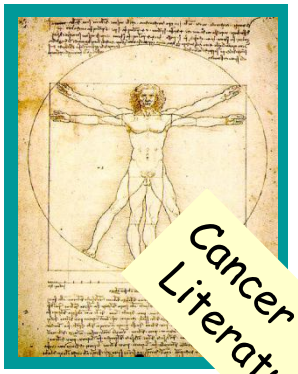


(1) Wang et al. Lancet 2005;365:671–679.
(2) Foekens et al. J Clin Oncol 2006;24:1665–1671.

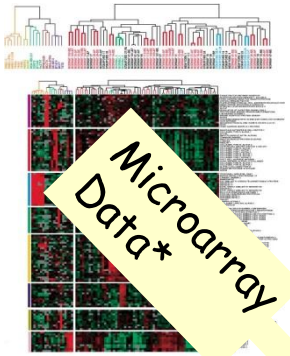
ONCOTYPE DXTM

A Multigene RT-PCR Assay for Predicting the Likelihood of Breast Cancer Recurrence and Response to Adjuvant Treatment

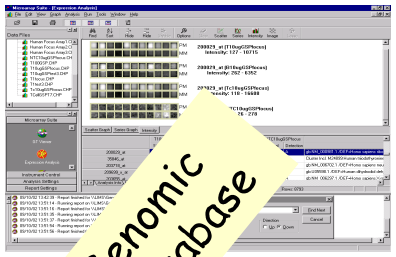
From ~25,000 genes:



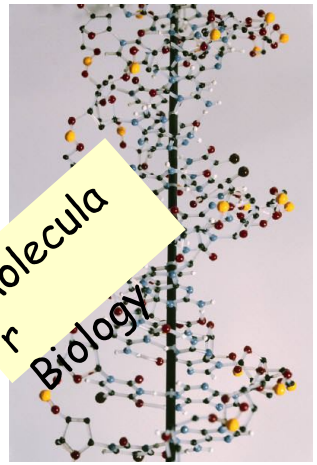
Cancer Literature



Microarray Data*



Genomic Databases



Molecular Biology

250 cancer-related candidate genes

ONCOTYPE DXTM

A Multigene RT-PCR Assay for Predicting the Likelihood of Breast Cancer Recurrence and Response to Adjuvant Treatment

- **Predictive gene signature based assay for ER-positive, LN-negative tamoxifen treated breast cancer tumors.**

Researchers studied 447 patients from 3 independent clinical studies to test the relationship between expression of 250 candidate cancer related genes and recurrence.

21 genes (16 genes + 5 reference genes)

Recurrence Category	RS (0-100)
Low risk	<18
Intermediate risk	18-30
High risk	≥31

Recurrence Score (RS) algorithm

ONCOTYPE DXTM

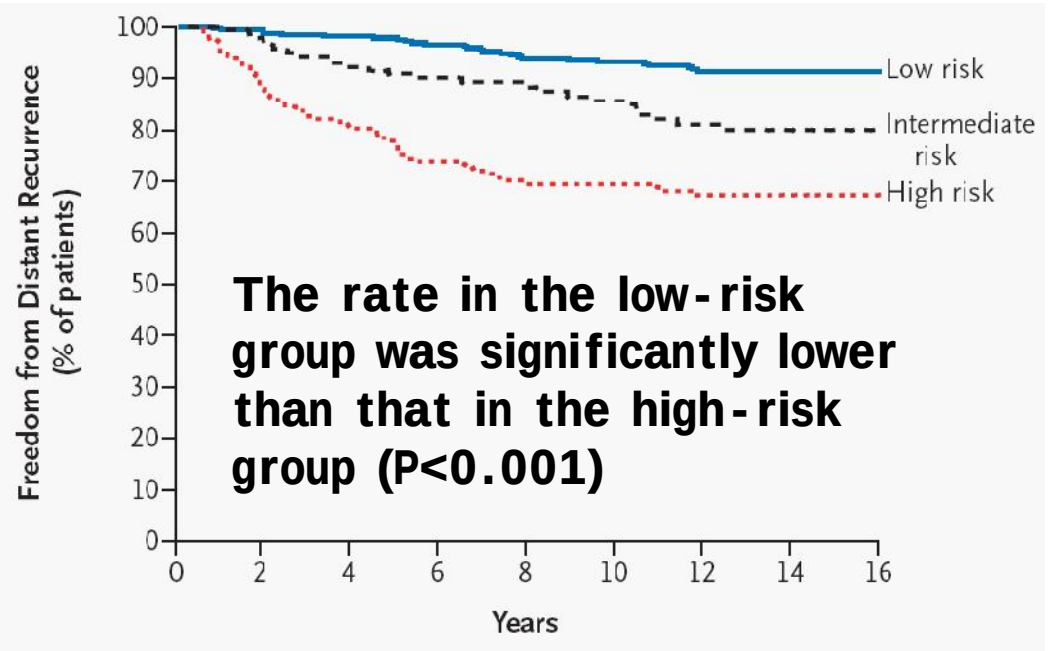
A Validation as Predictor of Recurrence/Survival NSABP B-14 (1)

Objective: Prospectively validate RS as predictor of distant recurrence in 668 N-, ER+, tam-treated patients

Table 1. Kaplan–Meier Estimates of the Rate of Distant Recurrence at 10 Years, According to Recurrence-Score Risk Categories.*

Risk Category	Percentage of Patients	Rate of Distant Recurrence at 10 Yr (95% CI)†
		percent
Low	51	6.8 (4.0–9.6)
Intermediate	22	14.3 (8.3–20.3)
High	27	30.5 (23.6–37.4)‡

Confirmed results in the Northern California Kaiser Permanente validation study (2)



RS and AdjuvantOnline! predicted outcomes correlated relatively weakly (concordance = 48%)

(1) Paik et al. N Engl J Med. 2004;351:2817-26.
(2) Habel et al. Br Cancer Res Treatment 2004, 88:s118.

ONCOTYPE DXTM

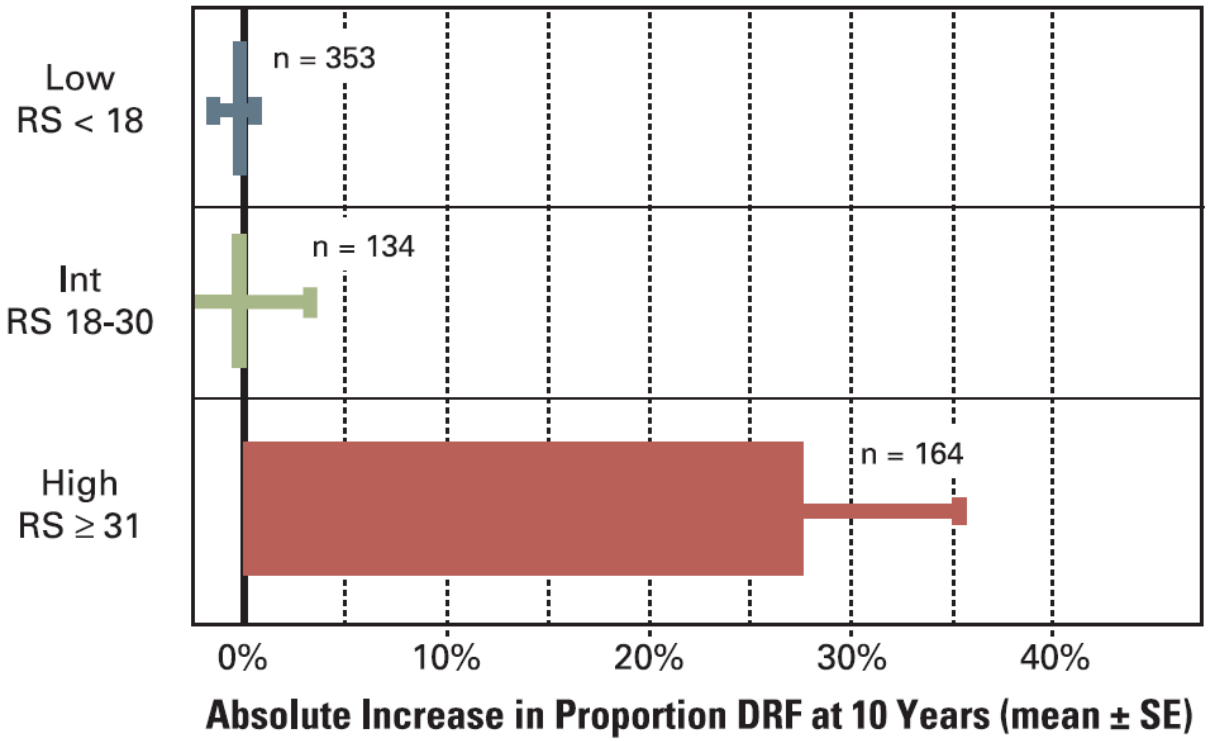
Prediction of Chemotherapy Benefit

NSABP B-20 Adjuvant Study

- **Low RS associated with no chemotherapy benefit**
- **High RS associated with large chemotherapy benefit**

CMF + TAM vs TAM

651 patients assessed

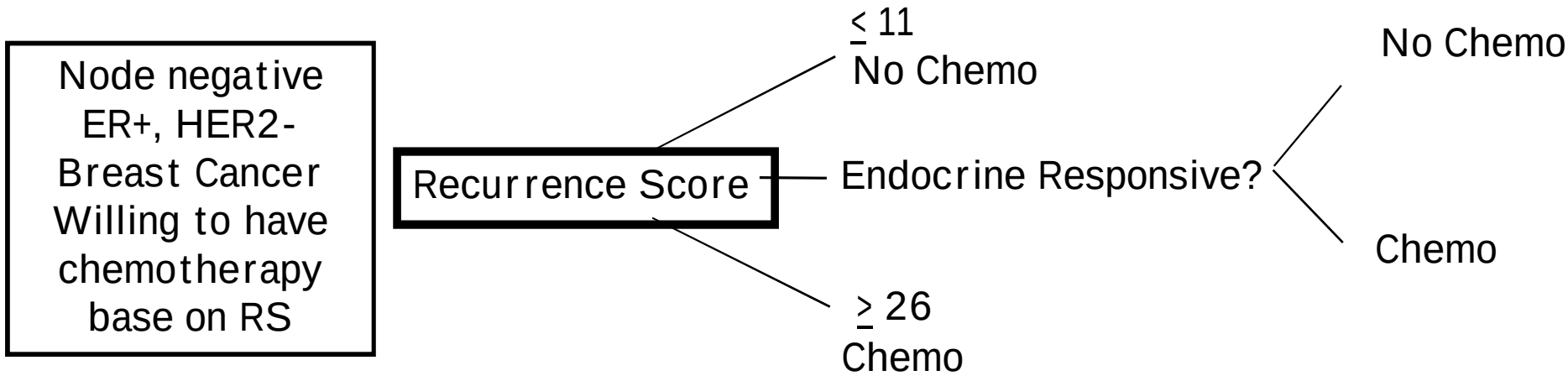


Paik et al. J Clin Oncol. 2006 Aug 10;24(23):3717-8.

ONCOTYPE DX™

Into Clinical Decision Making

- **TAILORx trial** will incorporate OncoType DX into clinical decision making



Gene Expression Profiling as a Predictor of Sensitivity to Preoperative Chemotherapy

Rationale

ER(-) and high grade tend to indicate more chemotherapy-sensitive cancer.

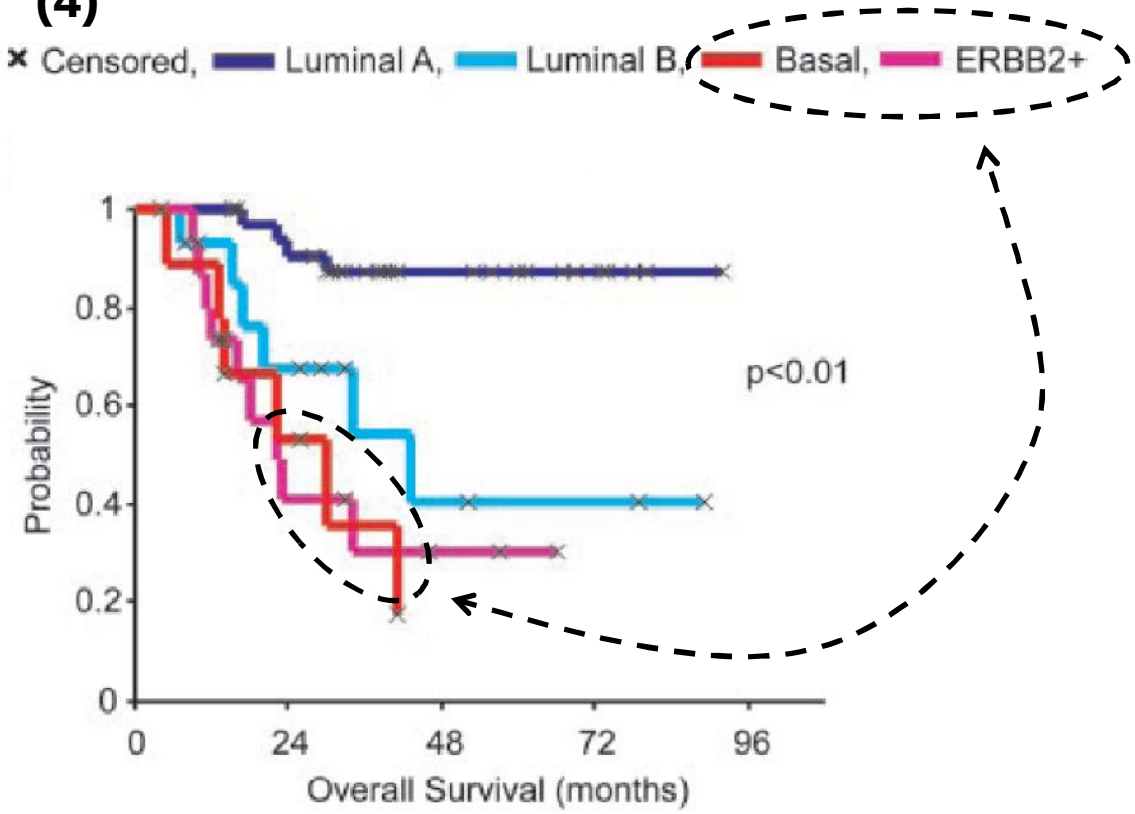
These variables predict general chemotherapy sensitivity; little potential to guide selection of a specific regimen.

pCR is associated with excellent long-term DFS.

Is gene expression profiling a potential tool to predict who may achieve pCR to preoperative chemotherapy?

Molecular Subtypes of Breast Cancer and Responses to Preoperative Chemotherapy

- **At least four major molecular classes of breast cancer exist (1-3).**
- **Basal-like and HER-2-positive tumors are more sensitive to chemotherapy (4)**



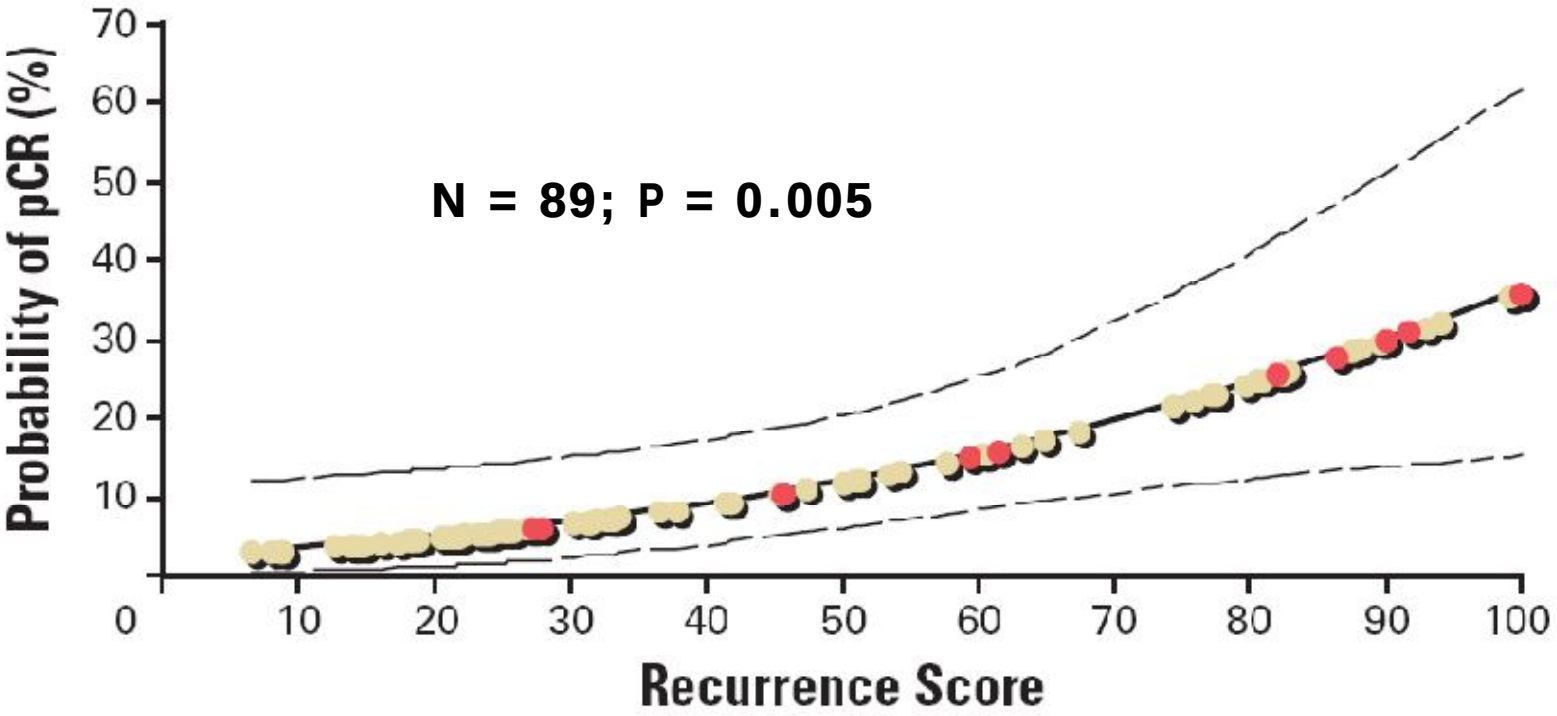
	pCR (4)	ER (4)
Luminal-like	7%	63%
Normal-like	0%	60%
HER-2 (+)	45%	55%
Basal-like	45%	5%

(1) Perou et al. Nature 2000;406:747–752.
(2) Pusztai L et al. Clin Cancer Res 2003;9:2406–2415.
(3) Sorlie et al. Proc Natl Acad Sci U S A 2003;100:8418–8423
(4) Rouzier et al. Clin Cancer Res. 2005 Aug 15;11(16):5678-85.

ONCOTYPE DXTM

Prediction of Response to Chemotherapy in Women With Locally Advanced Breast Cancer

- **Higher RS → Higher likelihood of pCR in patients treated with neoadjuvant AT**



Gianni et al. J Clin Oncol. 2005 Oct 10;23(29):7265-77.

Gene Expression Profiling for the Prediction of Therapeutic Response to Docetaxel[1, 2]

- Both studies have shown that gene profiling can be used to accurately predict response to neoadjuvant docetaxel.

  88%[1] and 80%[2] accuracy

  Used Microarray analysis[1] and RT-PCR technique[2]

Prediction Strengh (2)	Responders	Non-responders
High	11	4
Low	1	10

(1) Chang et al. Lancet 2003, 32:280-287.
(2) Iwao-Koizumi et al. J Clin Oncol 2005, 23:422-431.

The molecular basis of breast cancer: application in the daily clinical routine

CONCLUSION


We
ShouldStart Using
Gene
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CONCLUSION

We Should Start Using Gene Signatures to Guide Treatment Decisions in Breast Cancer


GRACIAS