Prognostic Gene Expression Tests for Early Stage Breast Cancer

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University of Utah/Huntsman Cancer Center
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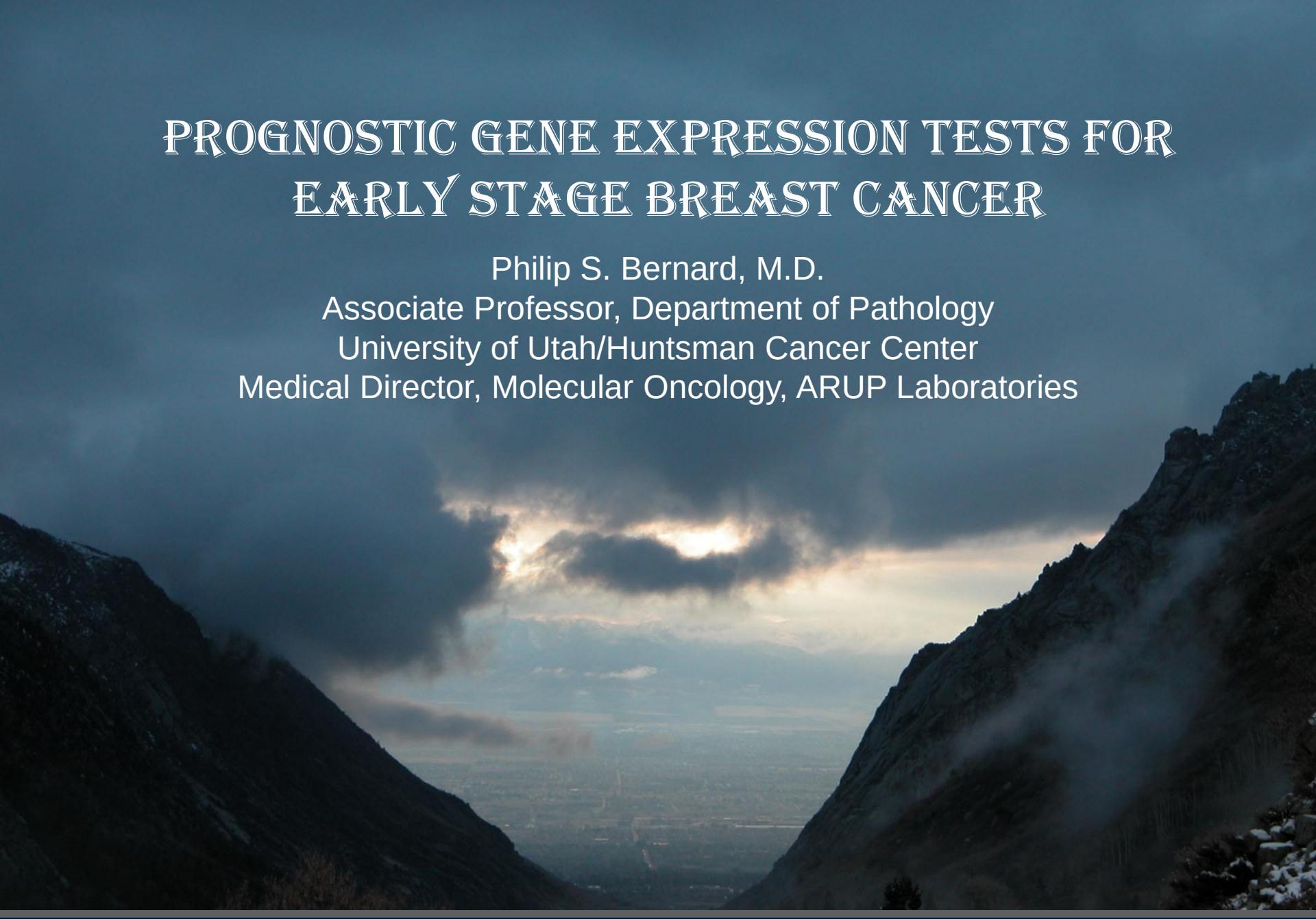


Conflict of Interest

- Dr. Bernard is an inventor of the PAM50 signature and a stakeholder in Bioclassifer LLC, a company that licensed the PAM50 know-how to Nanostring Inc for commercialization of Prosigna.

Learning Objectives

- Review differences between prognostic and predictive tests
- Review gene expression tests for prognosis in early stage breast cancer clinically available in 2014
- Review levels of evidence for using different tests based on clinical indications
- Review differences between research assays, Laboratory Developed Tests (LDT), and FDA-cleared tests

Prognostic vs Predictive

- **Prognostic** biomarkers provide information about the probability of survival (relapse or overall outcome) when patients are given the standard of care for their stage of disease
  - In early stage breast cancer, prognostic factors are used to determine who will have long-term survival without chemotherapy
    - Standard histopathologic staging: tumor size, node involvement, grade
    - Molecular: ER, PR, HER2, Ki67, and **gene expression tests**

- **Predictive** biomarkers provide information about who will respond to a *particular* therapy (e.g. HER2+ breast cancer predicts response to Trastuzumab)

Levels of Evidence for Biomarkers Becoming Standard of Care*

Category	Description	Main Challenge
Ia	Prospective clinical trial designed to test biomarker	Cost and time of running trial
Ib	Two or more clinical trials of similar design, well-annotated samples, long-term patient follow-up, retrospective sample collection, prospective statistical plan	Overcoming technical challenges of working with FFPE and identifying independent trials of similar design
II	Prospective trial(s), collected under clinical SOPs, and designed for therapeutic response	Trials powered for therapeutic response but not biomarker
III	Clinical trials run using research assays or observational studies using tissues collected under generic tissue banking protocols	Accuracy of data input and lack of targeted population

*Adapted from Simon R.M. et al, JNCI, 2009

Oncotype Dx: 21-gene Test to Determine Risk of Recurrence in Tamoxifen-Treated, Node-Negative Breast Cancer (NSABP-B14)

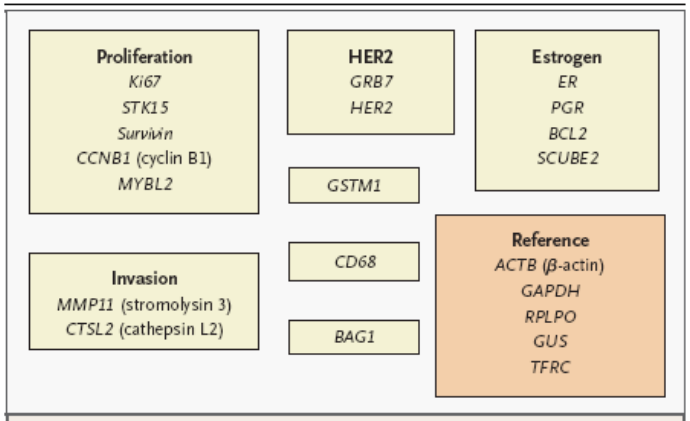
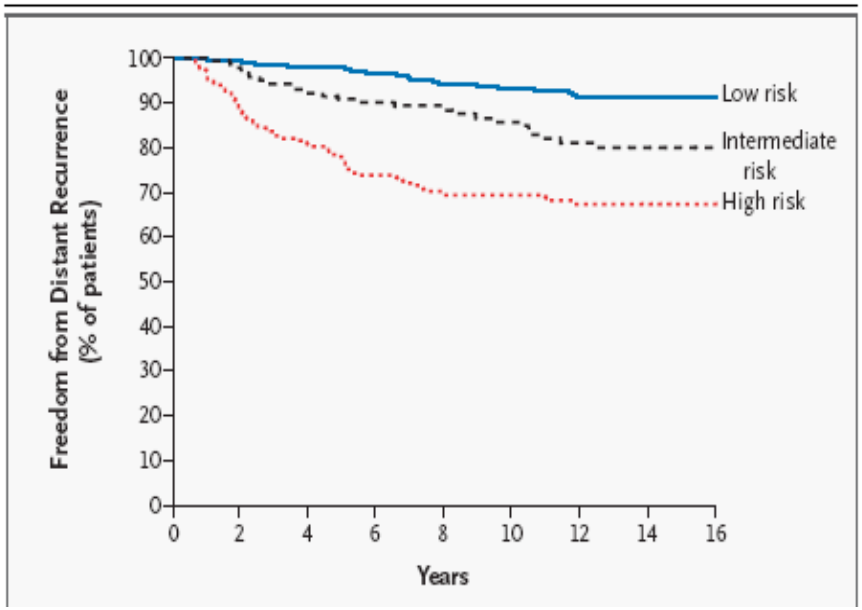


Figure 1. Panel of 21 Genes and the Recurrence-Score Algorithm.

The recurrence score on a scale from 0 to 100 is derived from the reference-normalized expression measurements in four steps. First, expression for each gene is normalized relative to the expression of the five reference genes (*ACTB* [the gene encoding β-actin], *GAPDH*, *GUS*, *RPLPO*, and *TFRC*). Reference-normalized expression measurements range from 0 to 15, with a 1-unit increase reflecting approximately a doubling of RNA. Genes are grouped on the basis of function, correlated expression, or both. Second, the *GRB7*, *ER*, proliferation, and invasion group scores are calculated from individual gene-expression measurements, as follows: *GRB7* group score = 0.9 × *GRB7* + 0.1 × *HER2* (if the result is less than 8, then the *GRB7* group score is considered 8); *ER* group score = (0.8 × *ER* + 1.2 × *PGR* + *BCL2* + *SCUBE2*) ÷ 4; proliferation group score = *Survivin* + *KI67* + *MYBL2* + *CCNB1* [the gene encoding cyclin B1] + *STK15*) ÷ 5 (if the result is less than 6.5, then the proliferation group score is considered 6.5); and invasion group score = (*CTSL2* [the gene encoding cathepsin L2] + *MMP11* [the gene encoding stromolysin 3]) ÷ 2. The unscaled recurrence score (RS_U) is calculated with the use of coefficients that are predefined on the basis of regression analysis of gene expression and recurrence in the three training studies[24-26]: RS_U = + 0.47 × *GRB7* group score − 0.34 × *ER* group score + 1.04 × proliferation group score + 0.10 × invasion group score + 0.05 × *CD68* − 0.08 × *GSTM1* − 0.07 × *BAG1*. A plus sign indicates that increased expression is associated with an increased risk of recurrence, and a minus sign indicates that increased expression is associated with a decreased risk of recurrence. Fourth, the recurrence score (RS) is rescaled from the unscaled recurrence score, as follows: RS = 0 if $RS_U < 0$; RS = 20 × (RS_U − 6.7) if $0 \le RS_U \le 100$; and RS = 100 if $RS_U > 100$.



Recurrences in Study:

28 Low Risk

25 Intermediate Risk

56 High risk

- Proliferation genes are incorporated in all prognostic tests for recurrence in early stage, ER+ breast cancer

Paik et al., The New England Journal of Medicine, 351:2817-26 (2004)

Prognosis in ER+ Breast Cancer (NSABP-B20)

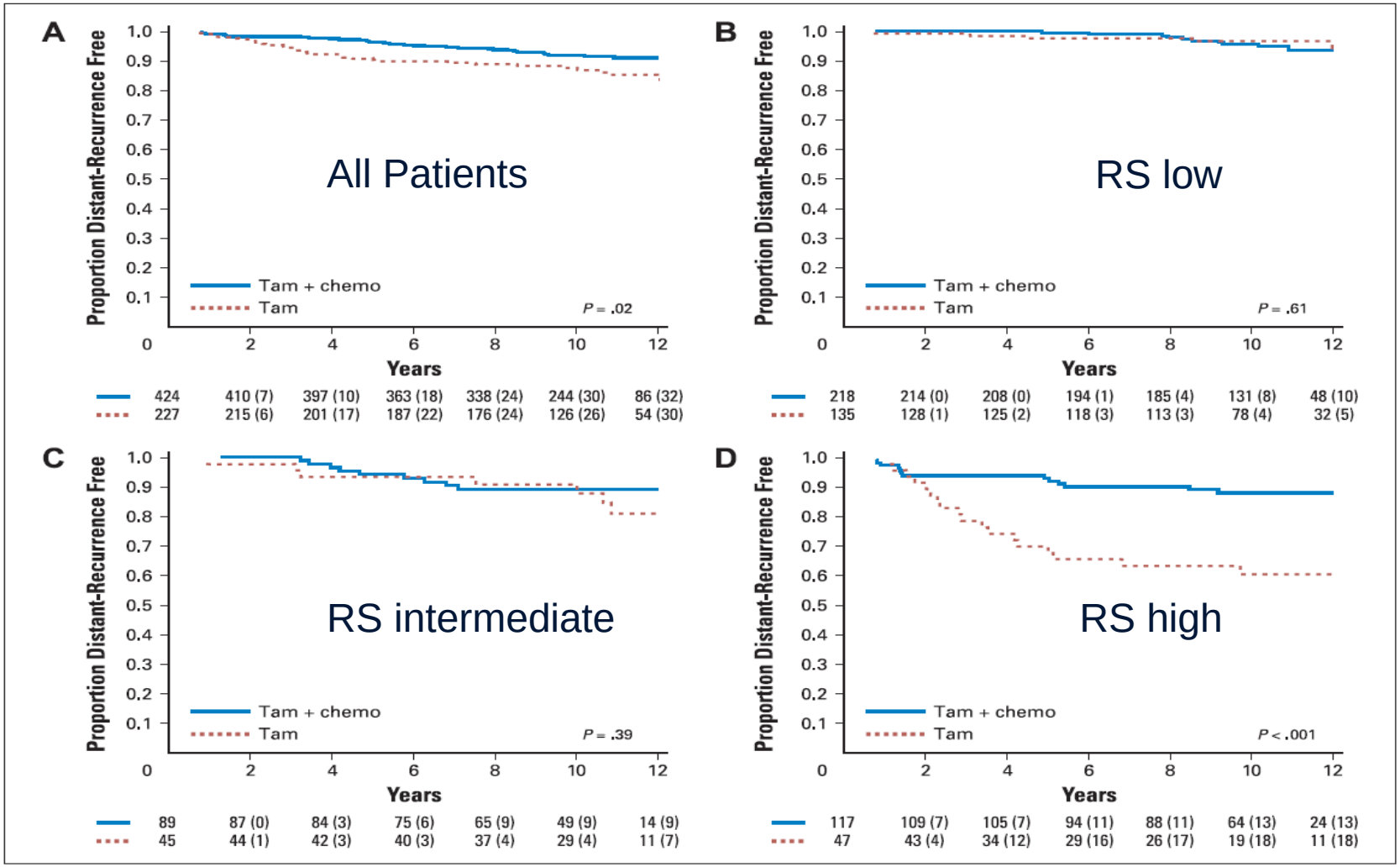


Fig 2. Kaplan-Meier plots for distant recurrence comparing treatment with tamoxifen (Tam) alone versus treatment with tamoxifen plus chemotherapy (Tam + chemo). (A) All patients; (B) low risk (recurrence score [RS] < 18); (C) intermediate risk (RS 18-30); (D) high risk (RS ≥ 31). The number of patients at risk and the number of distant recurrences (in parentheses) are provided below each part of the figure.

Paik et al., Journal of Clinical Oncology, 24: 1-12 (2006)

Gene expression profiling predicts clinical outomce of breast cancer
L. J. van 't Veer et al.
Nature 415, 530-536 (2002).

Sporadic breast tumours
patients <55 years
tumour size <5 cm
lymph node negative (LN0)
No chemotherapy given

5,000 genes differentially expressed across 78 tumors

supervised correlation analysis to identify genes that correlated with Bad versus Good prognosis
(230 genes)

Distant metastases
<5 years (2.5)
Bad prognosis

No distant metastases
>5 years (8.7)
Good Prognosis

Gene set optimized for determining which patients with early stage breast cancer do not require chemotherapy

mammaprint®

decoding breast cancer.

CUSTOMER		SPECIMEN		PATIENT	
Doctor:	Domen Ribnikar	Requisition #:	AG100583	Patient:	Ribnikar, Andreja
Account:	Institute of Oncology	Collection Date:			
		Date Received:	04-Jan-2012	DOB:	26-May-1957
Address:	Zaloška cesta 2	Report Date:	06-Jan-2012	Patient #:	2884/93
		Specimen Type:	FFPE Surgical Specimen	Gender:	Female
City, St., Zip:	Ljubljana 1000	Customer Ref.:	Andreja Ribnikar, 26-May-1957	SSN:	

Gene Profile Test Result LOW RISK

The breast cancer tissue sample submitted was analyzed by MammaPrint, an IVDMIA 70 - Gene Profile of Breast Cancer for Metastatic Risk that has been validated to correlate with high or low outcome risk for distant metastases in patients with invasive breast cancer.[1] In the reference group as published, "Low Risk" means that a lymph node negative breast cancer patient has a 10% chance (95% CI 4-15) that their cancer will recur within 10 years without any additional adjuvant treatment, either hormonal therapy or chemotherapy.[2]

blueprint™

molecular subtyping profile

CUSTOMER		SPECIMEN		PATIENT	
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Molecular Subtyping Test Result Luminal-type

Luminal-type breast cancers are characterized by gene expression of luminal epithelial cells that line the breast ducts and glands. The Luminal-type cancers are typically hormone receptor positive tumors and therefore responsive to hormonal therapy. A Luminal-type molecular subtyping result means that the tumor phenotype most closely resembles the Luminal-type intrinsic subtype. Patients classified as MammaPrint® 70-gene signature "Low Risk" and Luminal-type can be expected to have a clinical course similar to luminal A, usually treated with hormonal therapy, whereas those with a MammaPrint "High Risk" and Luminal-type, a clinical course similar to luminal B patients who usually benefit from more aggressive treatment which may include chemotherapy.

Discovery of the PAM50: Standardized Gene Set for Identifying Intrinsic/Biologic Subtypes of Breast Cancer

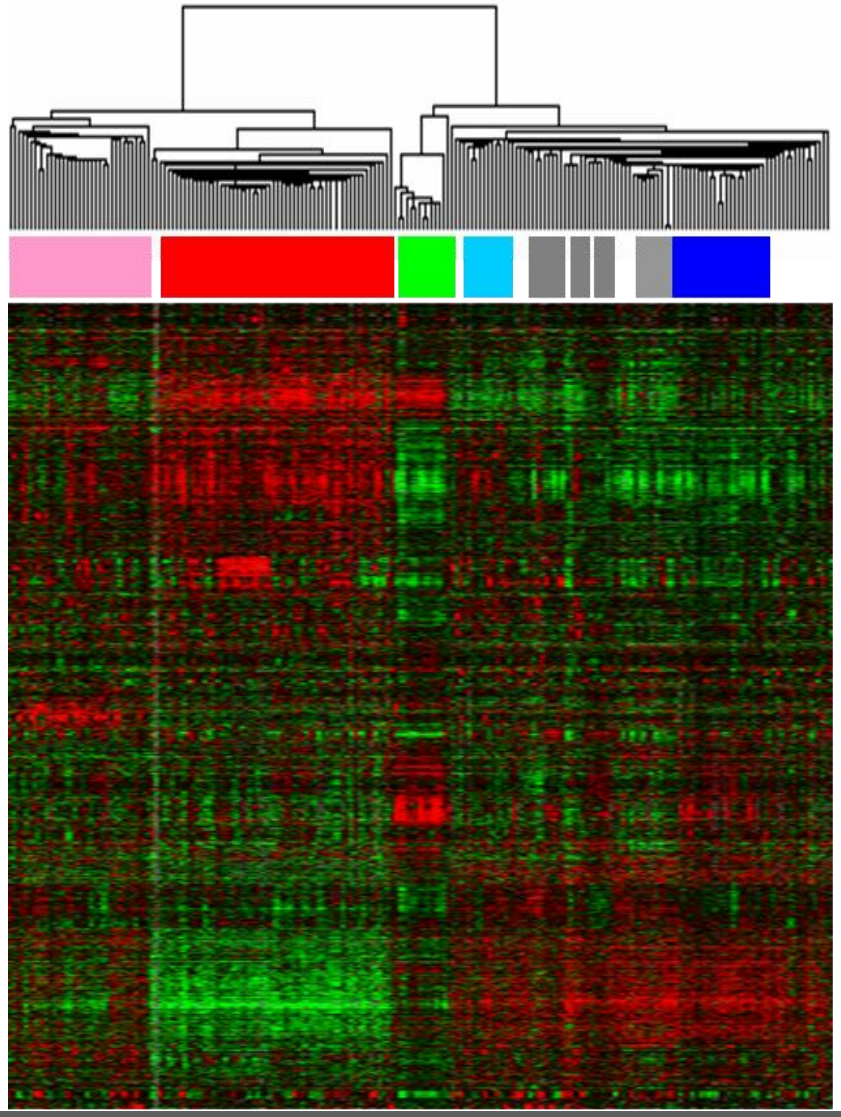
1. **Expression profiled 218 breast samples by full-genome microarrays (>25,000 genes) using RNA from fresh frozen tissues**

2. **Statistically identified 9 significant groups of invasive breast cancer and selected the common subtypes (LumA, LumB, HER2-enriched, Basal-like, and Normal-like) for training**

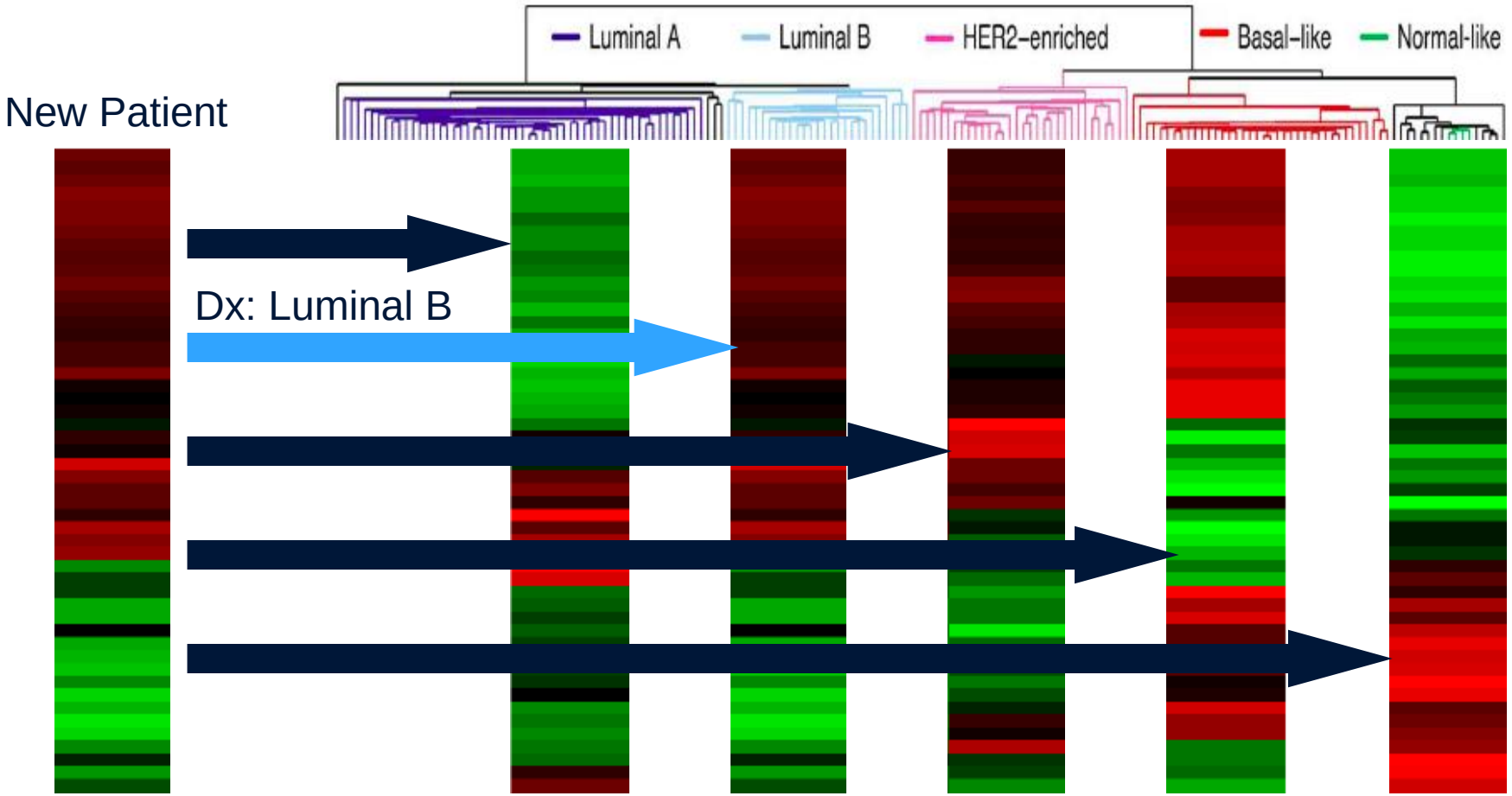
3. **Expression profiled the same tumors analyzed by microarray but using the corresponding FFPE blocks and RT-qPCR assays for 200 genes**

4. **Selected minimal gene set from RT-qPCR data that had the highest concordance to the microarray subtype assignment (i.e. PAM50)**

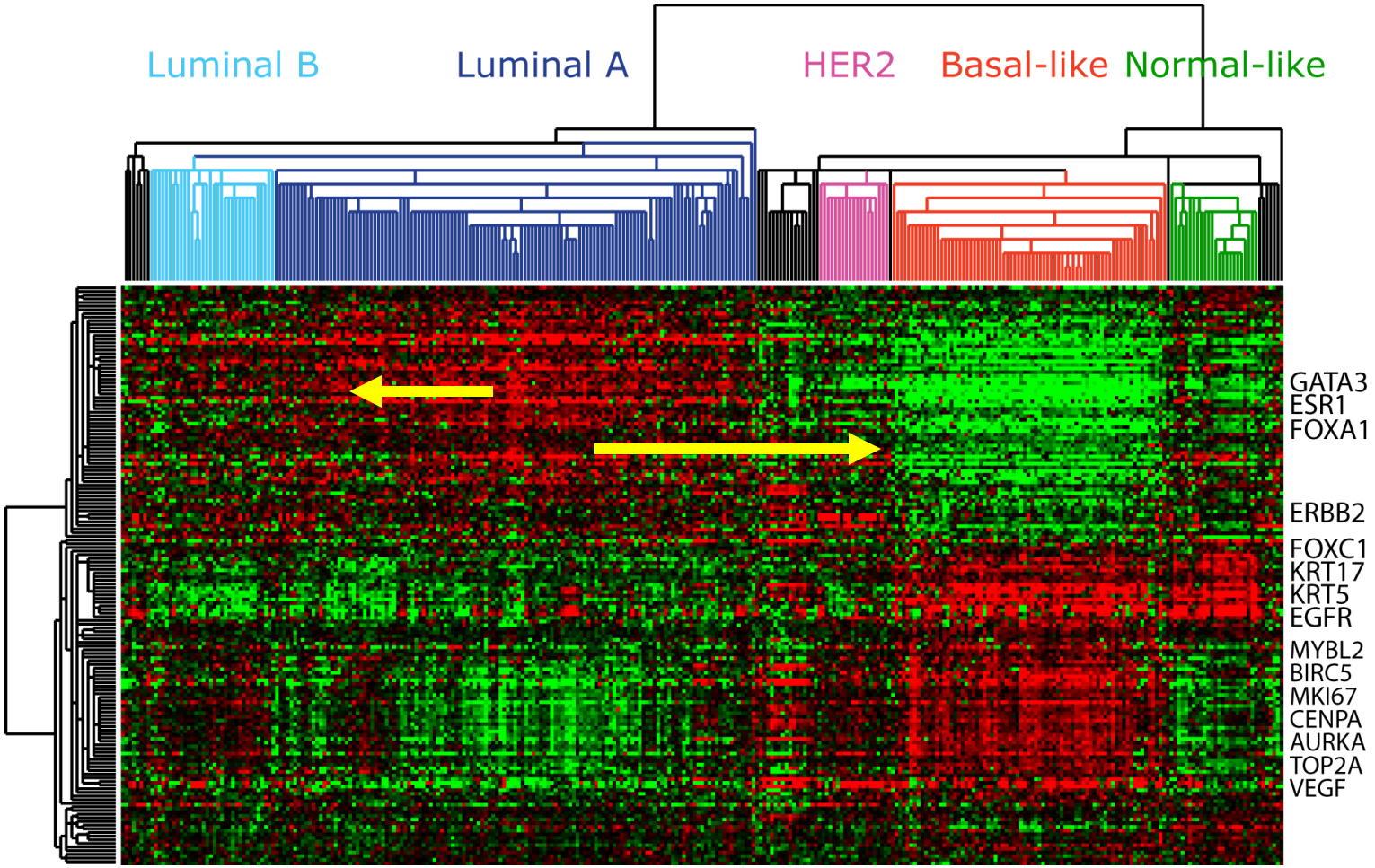
Parker et al., J Clin Oncol. 2009 Mar 10;27(8):1160-7



Supervised Predictor of PAM50 Subtypes



Developing a Risk Score Based on Correlation to Subtypes and Clinical Variables



Distance to each centroid as a genomic summary

Prognostic Risk Classification Strategy: Risk Of Relapse (ROR)

- **Similarity to the subtypes are used as variables in the prognostic model where the outcome is Risk of Relapse (ROR):**

$$\text{(Model 1) ROR-S} = \beta_{1*}\text{Basal} + \beta_{2*}\text{HER2} + \beta_{3*}\text{LumA} + \beta_{4*}\text{LumB}$$

$$\text{(Model 2) ROR-C} = \beta_{1*}\text{Basal} + \beta_{2*}\text{HER2} + \beta_{3*}\text{LumA} + \beta_{4*}\text{LumB} + \beta_{5*}\text{Tumor Size}$$

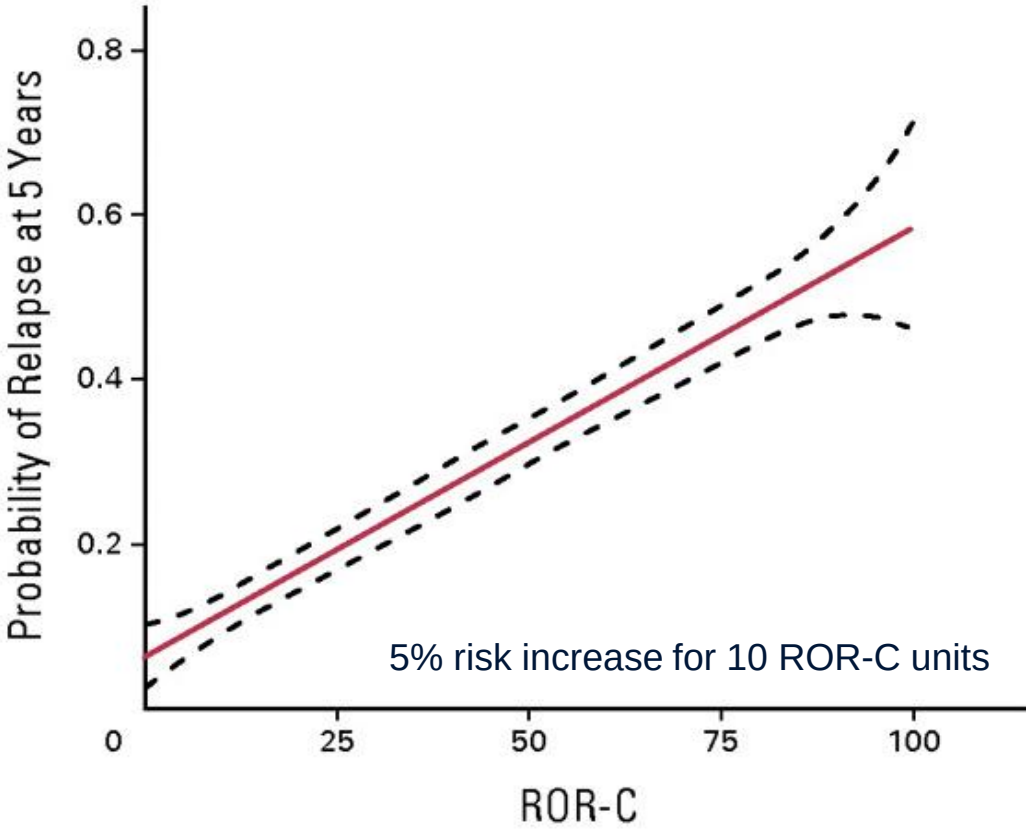
$$\text{(Model 3) ROR-X} = \beta_{1*}\text{Basal} + \beta_{2*}\text{HER2} + \beta_{3*}\text{LumA} + \beta_{4*}\text{LumB} + \beta_{5*}\text{Size} + \beta_{6*}\text{Node}$$

- **Weights for each term are learned from a training data set using a Cox model with Ridge Regression**

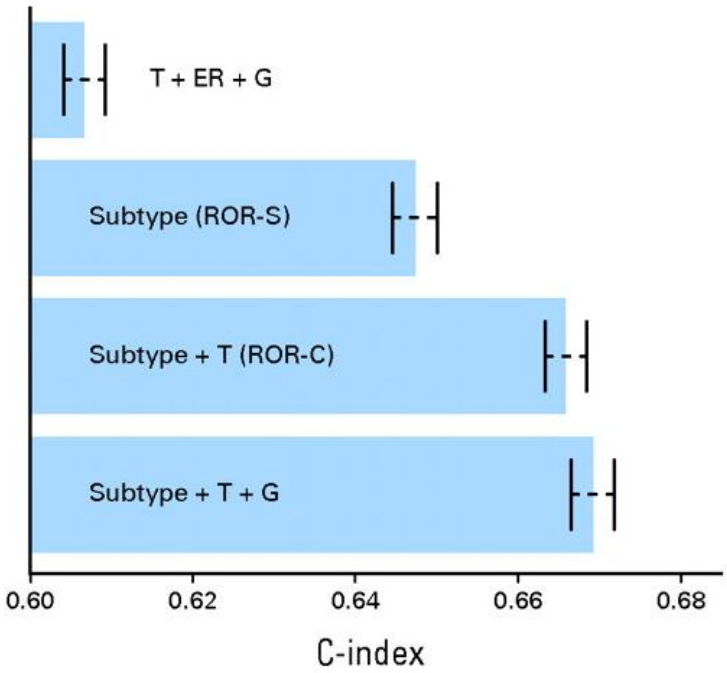
- **The weighted sum is assigned as the ROR score for a test case and a threshold may be applied for class assignment**

Ridge regression with Cox model: Tibshirani, Statistics in Medicine 1997
Comparative study: Bovelstad et al. Bioinformatics 2007

Prognosis in no Adjuvant Systemic Therapy (no AST): PAM50 ROR



N=558 no adjuvant systemic therapy and node negative test cases

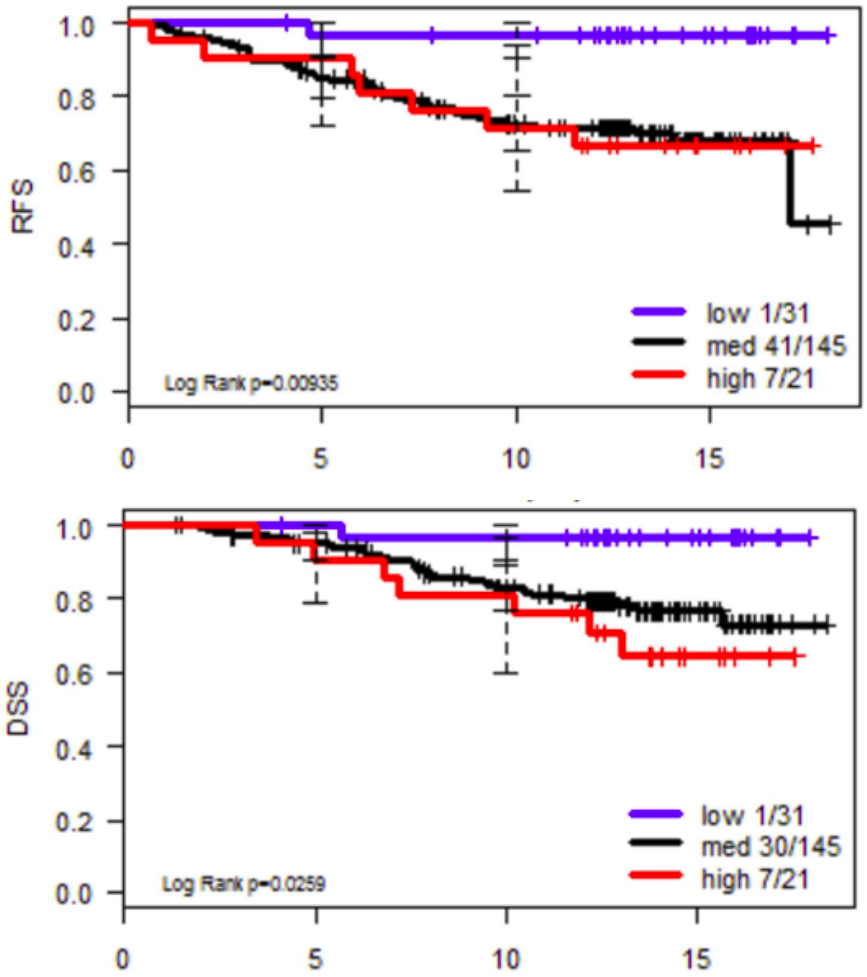


C-index: FE Harrell et al., *JAMA* 1982; 247(18).

"The c-index is the proportion of all pairs of subjects whose survival time can be ordered such that the subject with the higher predicted survival is the one who survived longer" (taken from Harrell, Regression Modeling Strategies, Springer Series in Statistics).

Parker, J. S. et al. J Clin Oncol; 27:1160-1167 2009

ROR Model in Tamoxifen Series from University of British Columbia



Subtype predictions weighted for for tumor size and proliferation identifies a patient subset that could forego chemotherapy!

Nielsen, et al, CCR, 2010 Nov 1;16(21):5222-32

Intrinsic Subtypes: Discovery and Research

Perou et al., Nature, 2000
Sorlie et al., PNAS, 2003

- Microarray discovery
- Prognostic significance of subtypes

Perreard et al., Breast Cancer Res, 2006
Mullins et al, Clin Chem, 2007

- Technical feasibility using RT-qPCR assays on FFPE tissues

Parker et al., JCO, 2009
Nielsen et al., CCR, 2010
Cheang et al., CCR, 2012
Bastien et al., BMC Med Genomics, 2012
Martin et al., Breast Cancer Res Treat, 2013

- Discovery of PAM50 for subtyping and ROR score
- ROR score for prognosis in ER+ disease
- Prognosis in chemo treated patients
- Anthracycline benefit in HER2-E disease
- Correlation of subtypes with standard markers

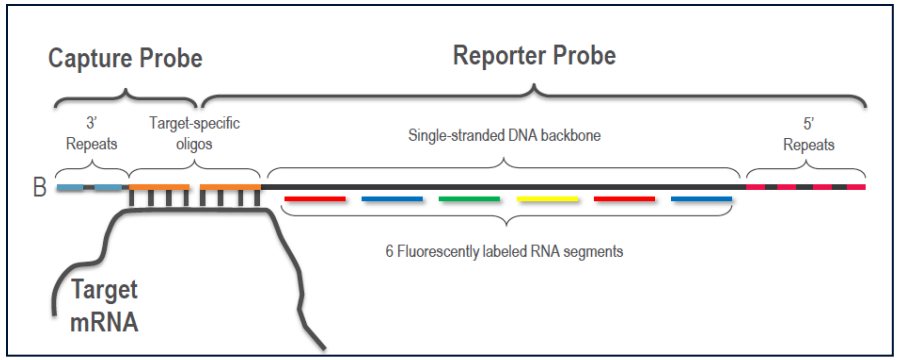
Sweeney et al., CEBP, 2014
Caan, et al, CEBP, 2014
Kroenke et al, Breast Cancer Res Treat, 2014

- Association of subtype with race and age
- Prognosis of subtypes in population-based study

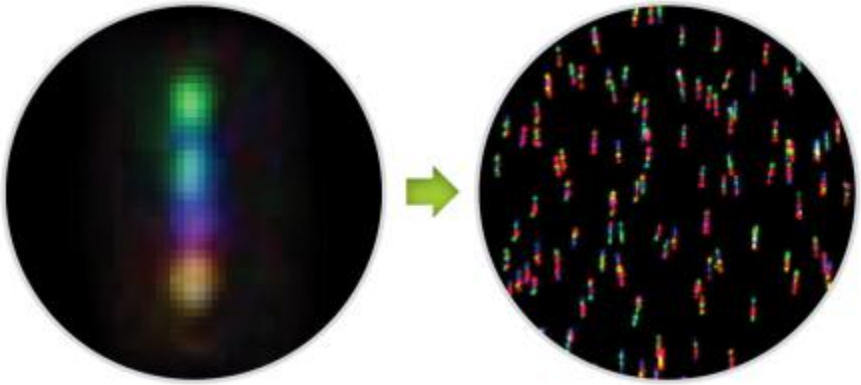
Transfer of RT-qPCR PAM50 Research Assay to nCounter Platform for FDA-clearance and Decentralization

6 Fluorescent spots are labeled RNA molecules complementary to ssDNA backbone

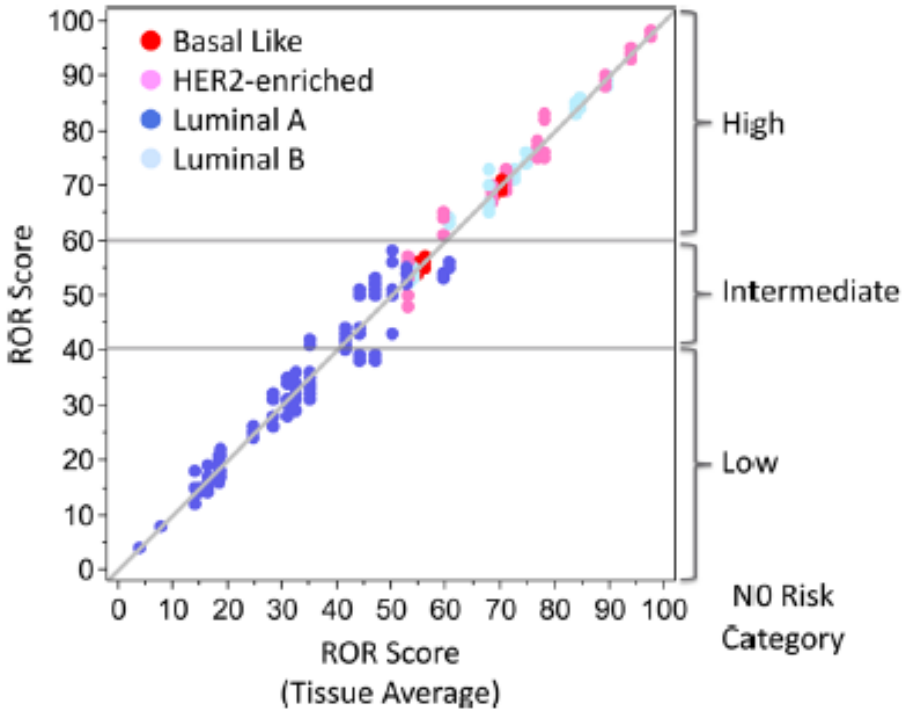
- ~50 fluorophores / spot generate very bright signal allowing for digital detection
- 4 colors, no consecutive spots of same color = 972 possible codes



Single molecule fluorescent barcodes,
each attached to an individual nucleic acid



Prosigna Multi-site Analytical Validation

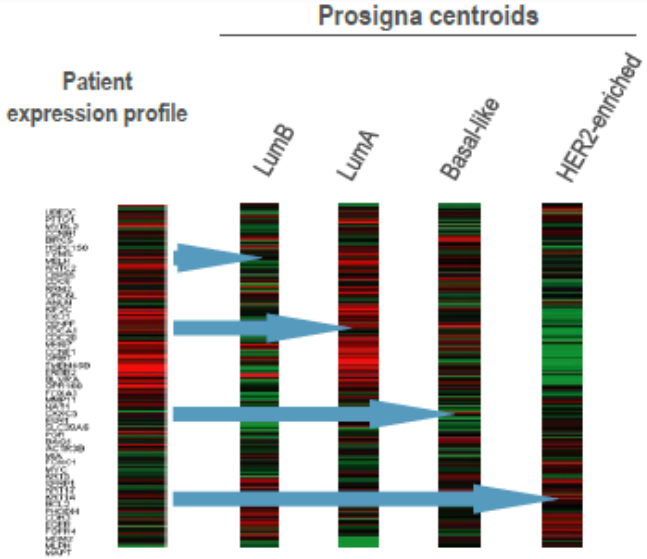


- Forty-three specimens shared across 3 sites
- All subtypes represented with large range of ROR scores
- Block processing, macrodissection, RNA extraction
- No samples changed from low-high risk

Nielsen TO, BMC Cancer, 2014

Generating a Prosigna Score

Determine intrinsic subtype through Pearson's correlation to centroids



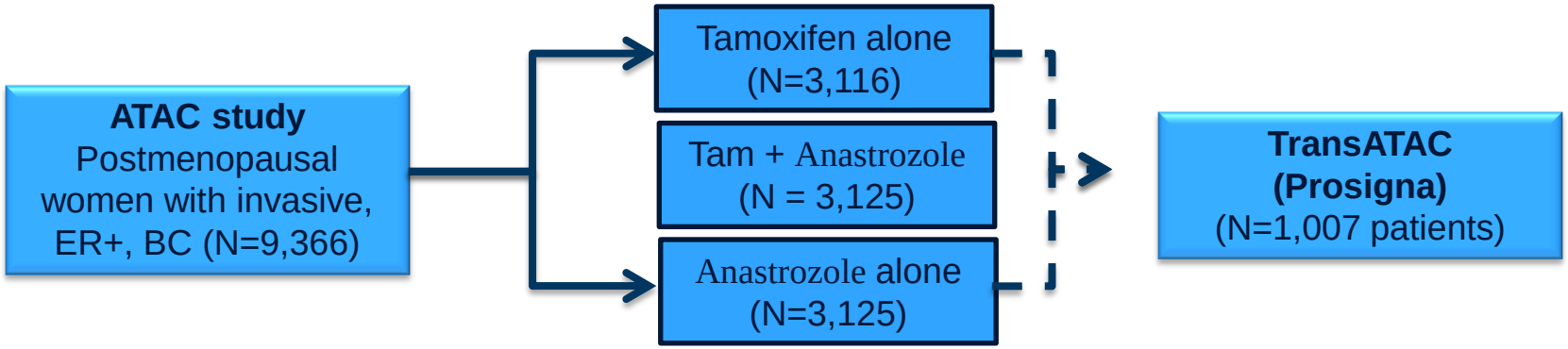
Calculate ROR (Prosigna Score)

$$ROR = aR_{LumA} + bR_{LumB} + cR_{Her2e} + dR_{Basal}$$ — Pearson's correlation to centroids

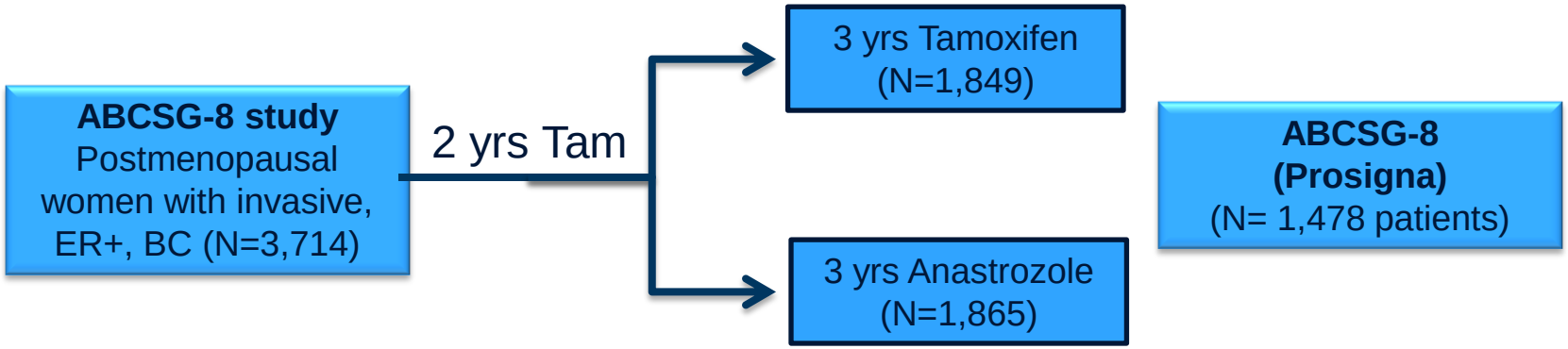
$$eP + fT$$ — Proliferation score, Tumor size

Prosigna Validation Studies

- **TransATAC Study**

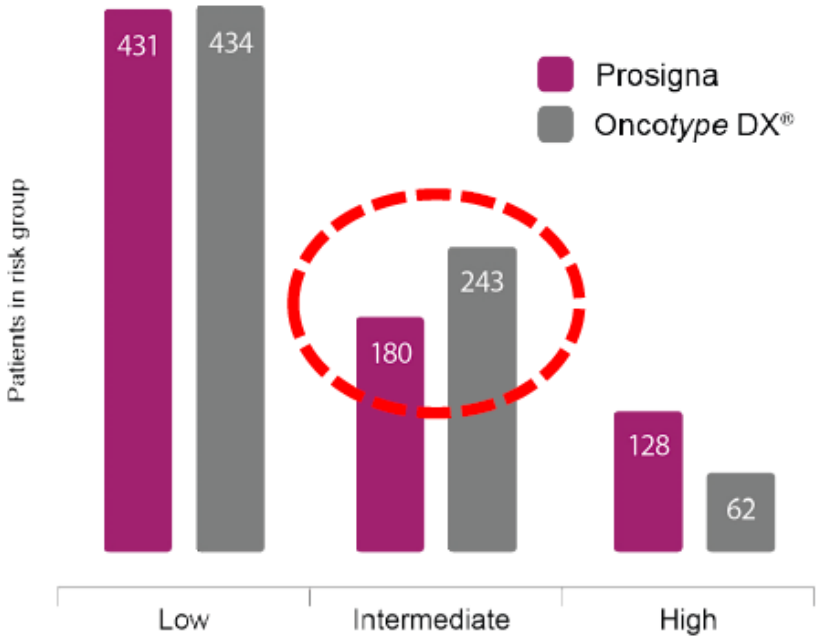


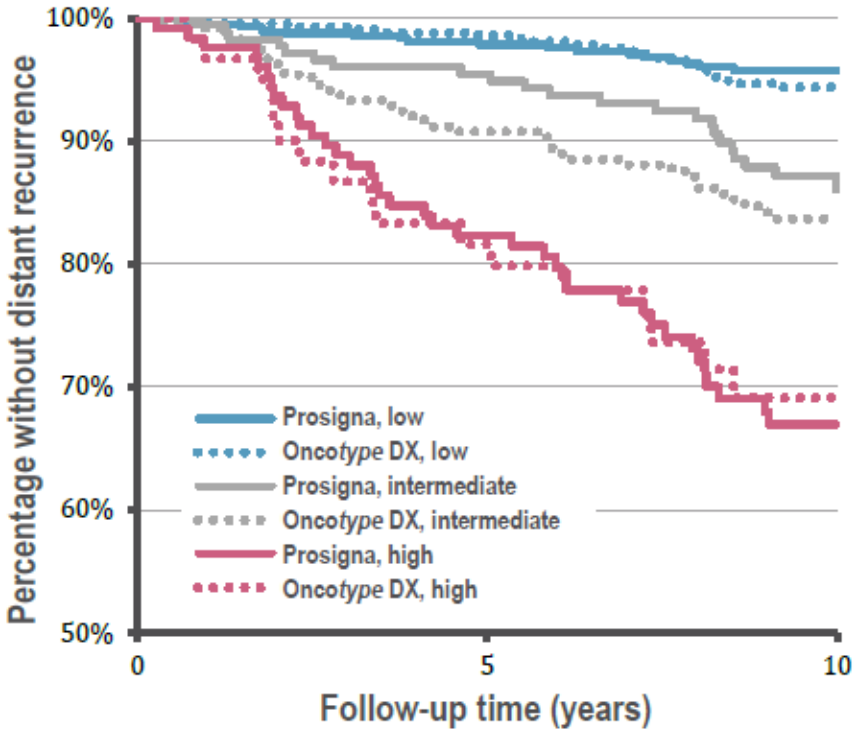
- **ABCSG-8 study**



Dowsett M, JCO, 2013
Filipits M, Clin Cancer Res, 2014

Oncotype Dx vs Prosigna: Head-to-Head Comparison in TransATAC

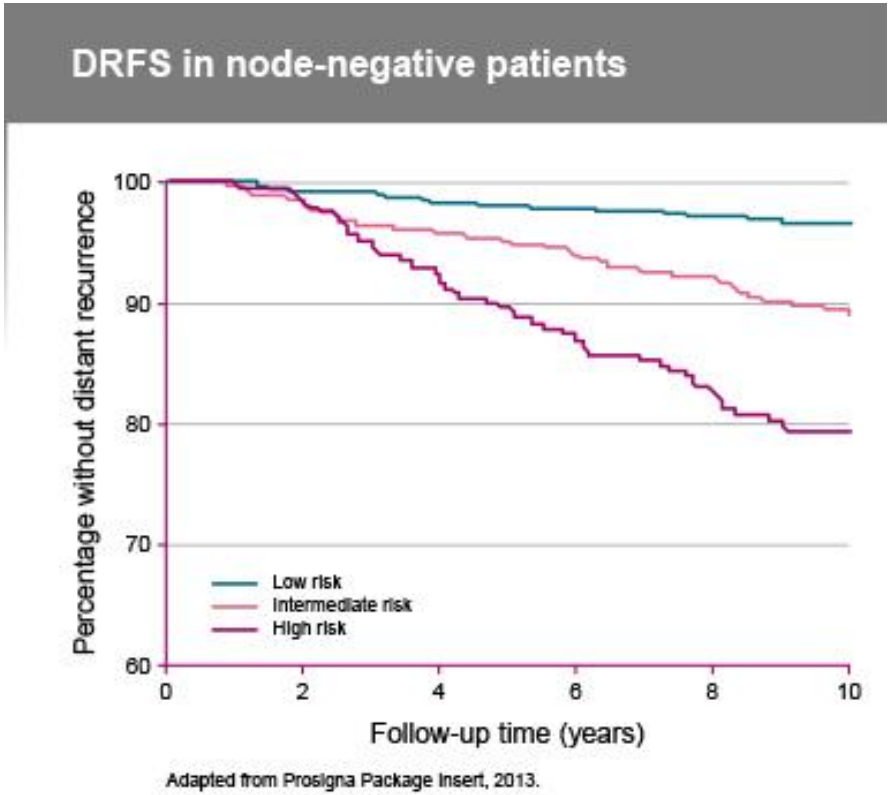


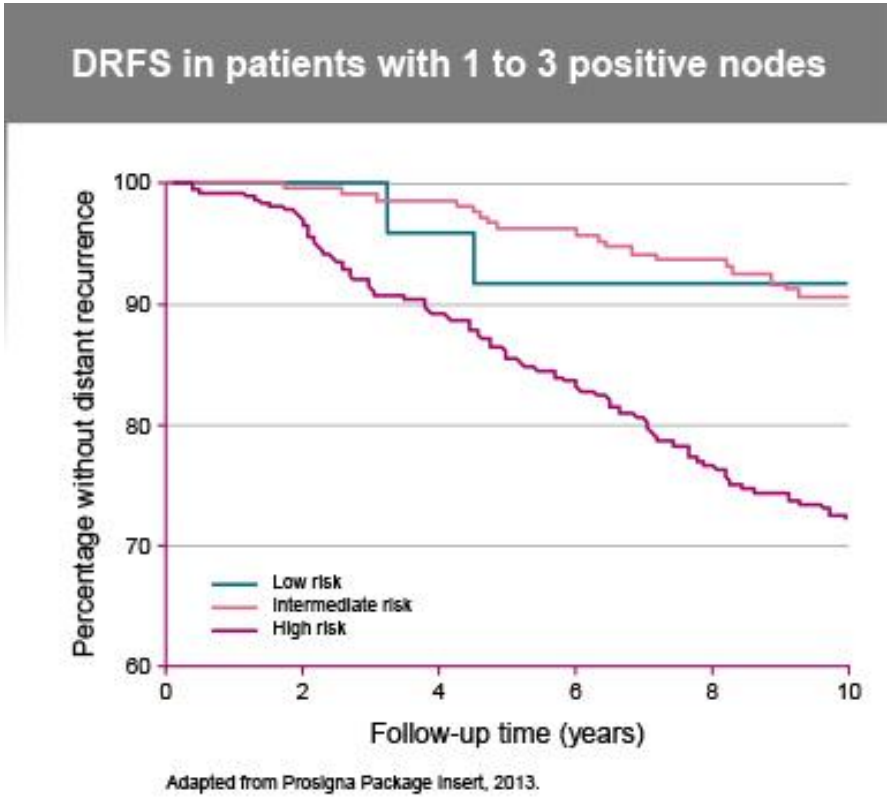


Node negative only; N=739

Dowsett M, JCO, 2013

Risk Stratification by Prosigna (ROR) Score in ABCSG-8: HR+/HER2-, N0 and N1, Adjuvant Endocrine Therapy Alone



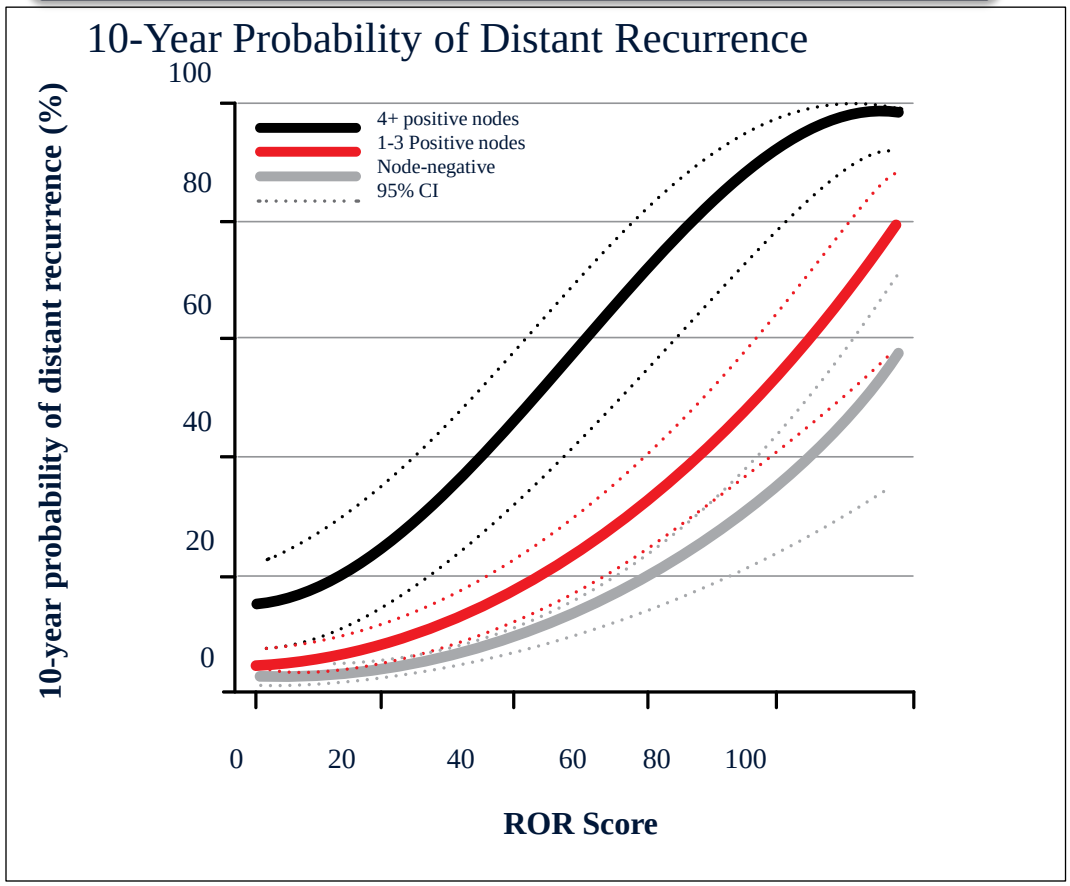


10-year probability of distant recurrence of < 10% is considered **low risk**

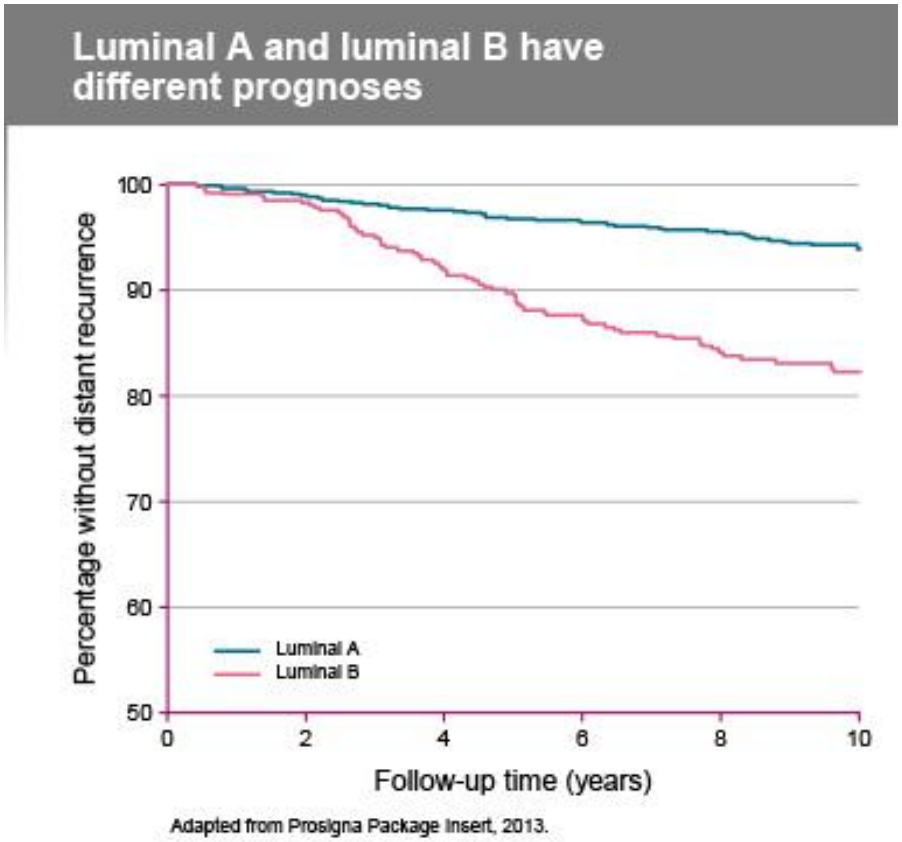
10-year probability of distant recurrence of > 20% is considered **high risk**

Risk Interpretation by Nodal Status

ROR: 0-100

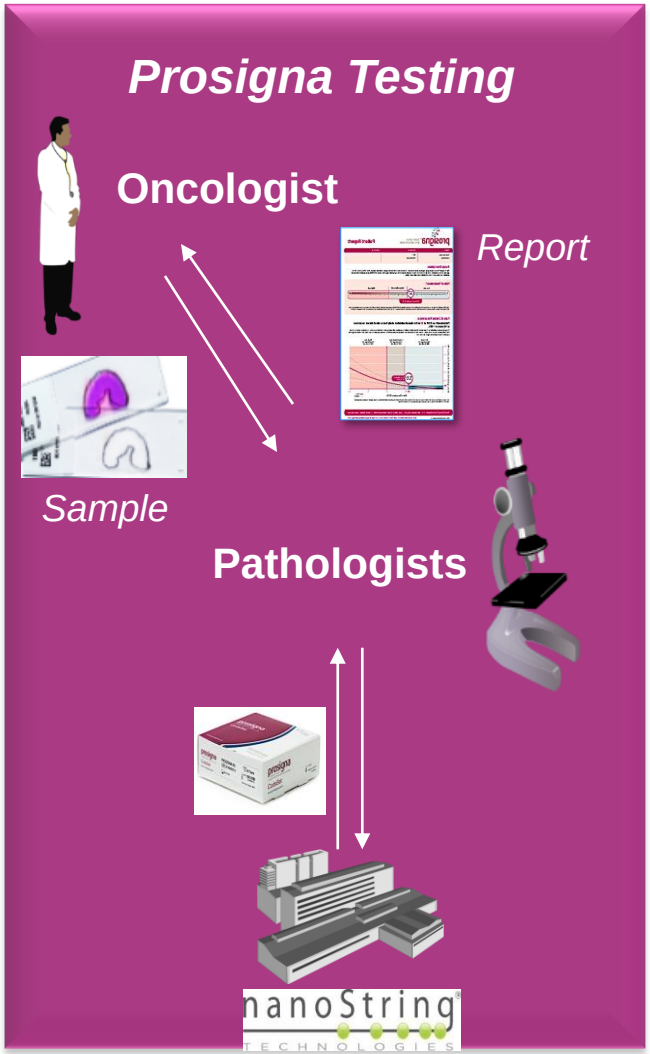


Risk Stratification by Luminal Subtypes (A/B) in ABCSG-8: HR+/HER2-, N0 and N1, Adjuvant Endocrine Therapy Alone



12-15% greater probability of distant relapse at 10 years if Luminal B compared to Luminal A

FDA Clearance Allows Decentralization of Prosigna



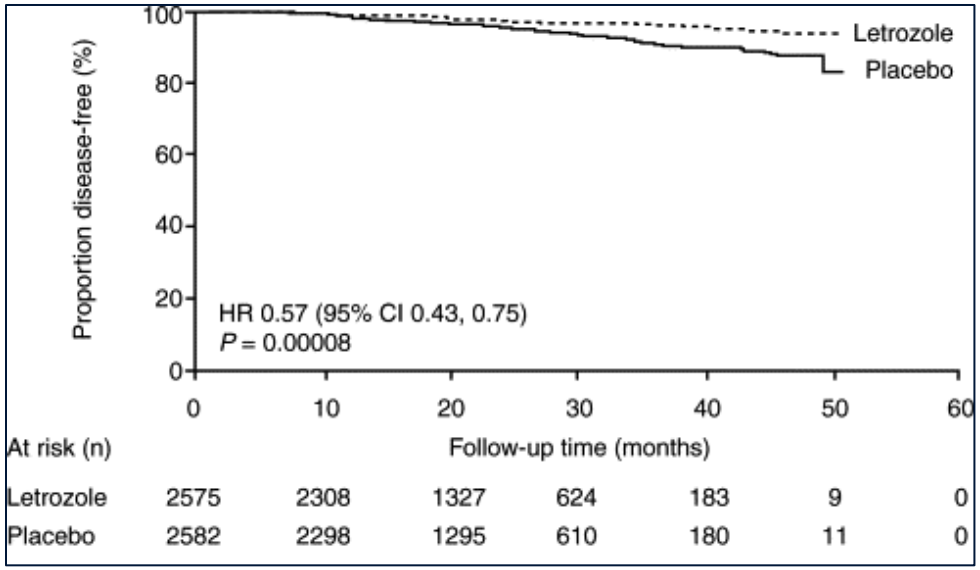
- Medicine is practiced close to the patient
- Pathologists remain integral to the decision-making
- Local clinical lab remains the service provider

Extended Adjuvant Endocrine Therapy

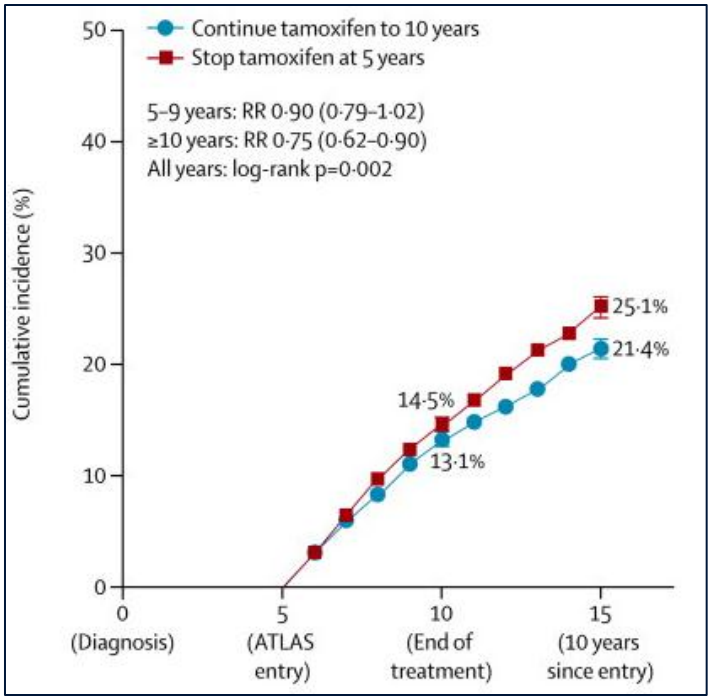
- **MA.17** and **ATLAS trials** demonstrate the benefit of extending endocrine therapy beyond 5 years

. . . but only a small percentage of patients benefit!

MA.17 Trial



ATLAS Trial



[1]Goss PE et al., N Engl J Med 2003;349. [2]Davies C et al, Lancet. 2013 Mar 9;381(9869):805-16

Breast Cancer Index (BCI): Early Stage ER+ Breast Cancer

- BCI is a laboratory developed test using RT-qPCR for measuring 7 genes and housekeepers
- Developed by combining biomarkers from two complementary gene expression signatures:

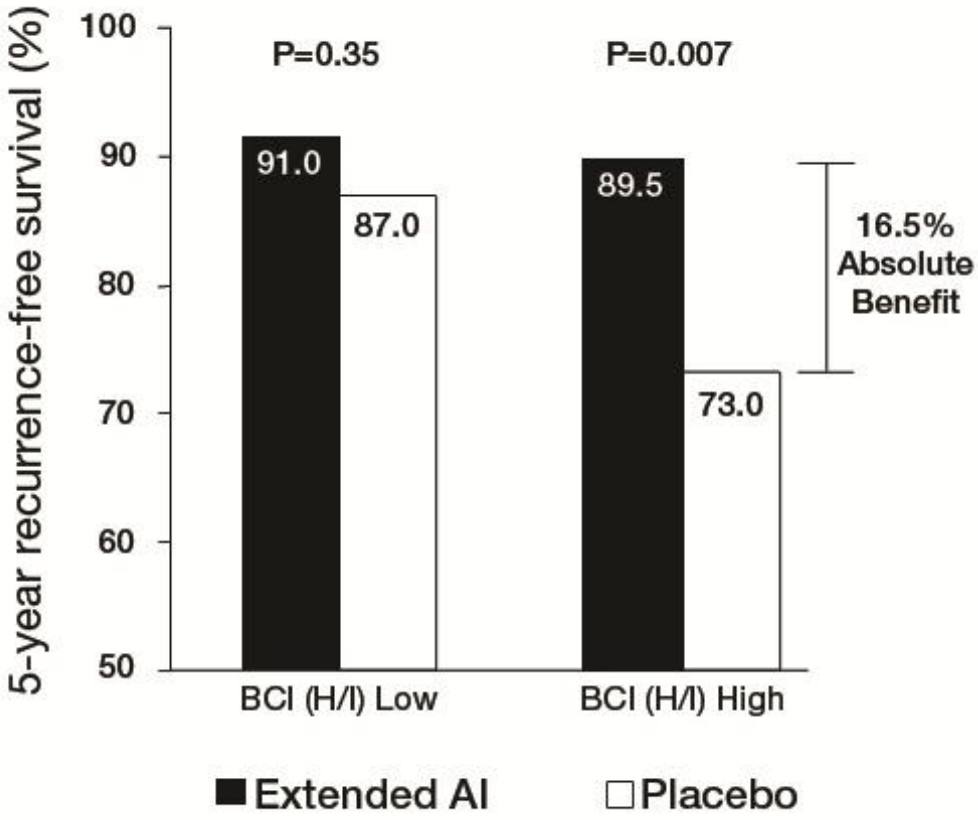
HoxB13/IL17BR (H/I)	Molecular Grade Index (MGI)
− Gene expression ratio − Estrogen signaling-related	− 5 cell cycle genes − Assesses tumor proliferation

- BCI Test Report provides two key pieces of information:
  1) Risk of recurrence over 10 yrs from diagnosis
  2) Risk of recurrence after 5 yrs endocrine therapy

BCI Validation in MA.17: Who Benefits from Extended Endocrine Therapy

MA.17 Study Data

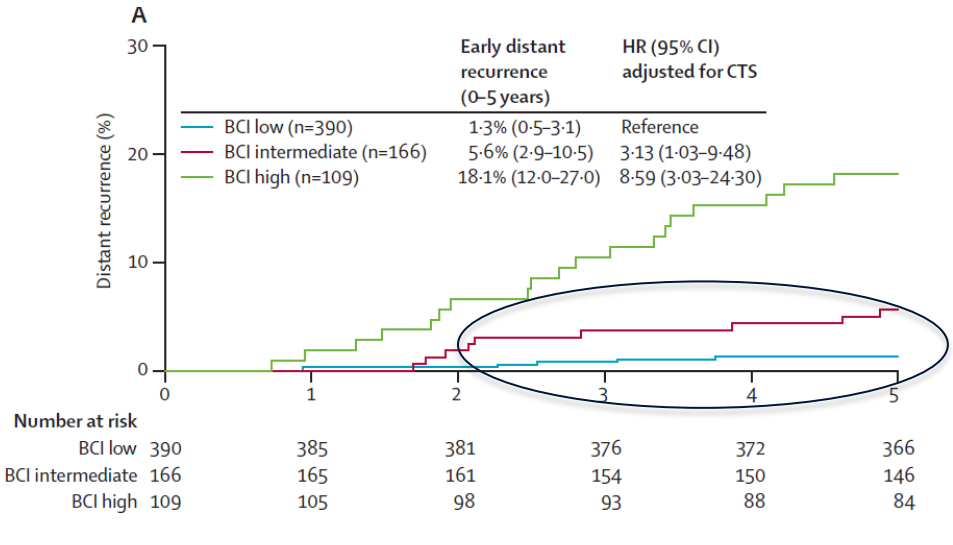
- Nested case-control design of 83 recurrences matched to 166 nonrecurrences
- Patients with high BCI (H/I) had a 5yr absolute benefit of 16.5% from extended endocrine therapy with letrozole (p=0.007)
- Patients with low BCI (H/I) had no significant benefit from extended endocrine therapy with letrozole (p = 0.35)

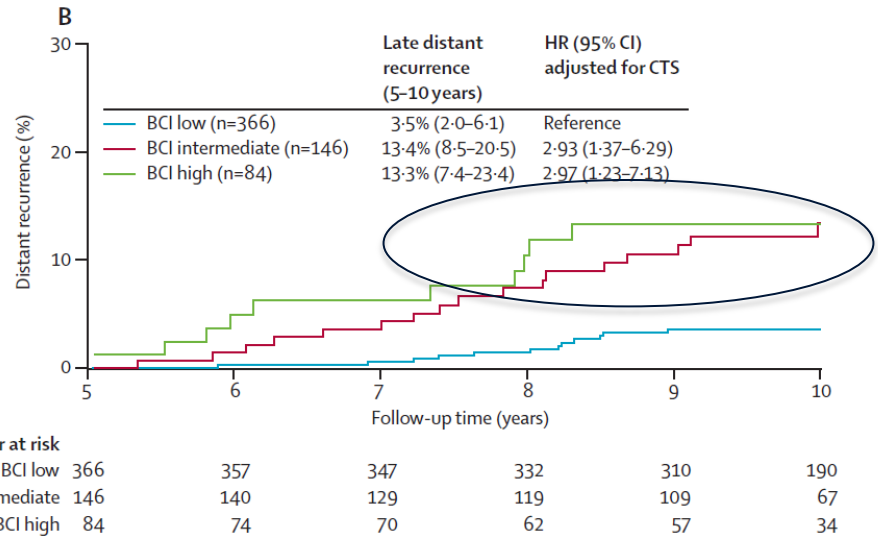


Sgroi DC, JNCI, 2013

BCI Validation in TransATAC: Key Results

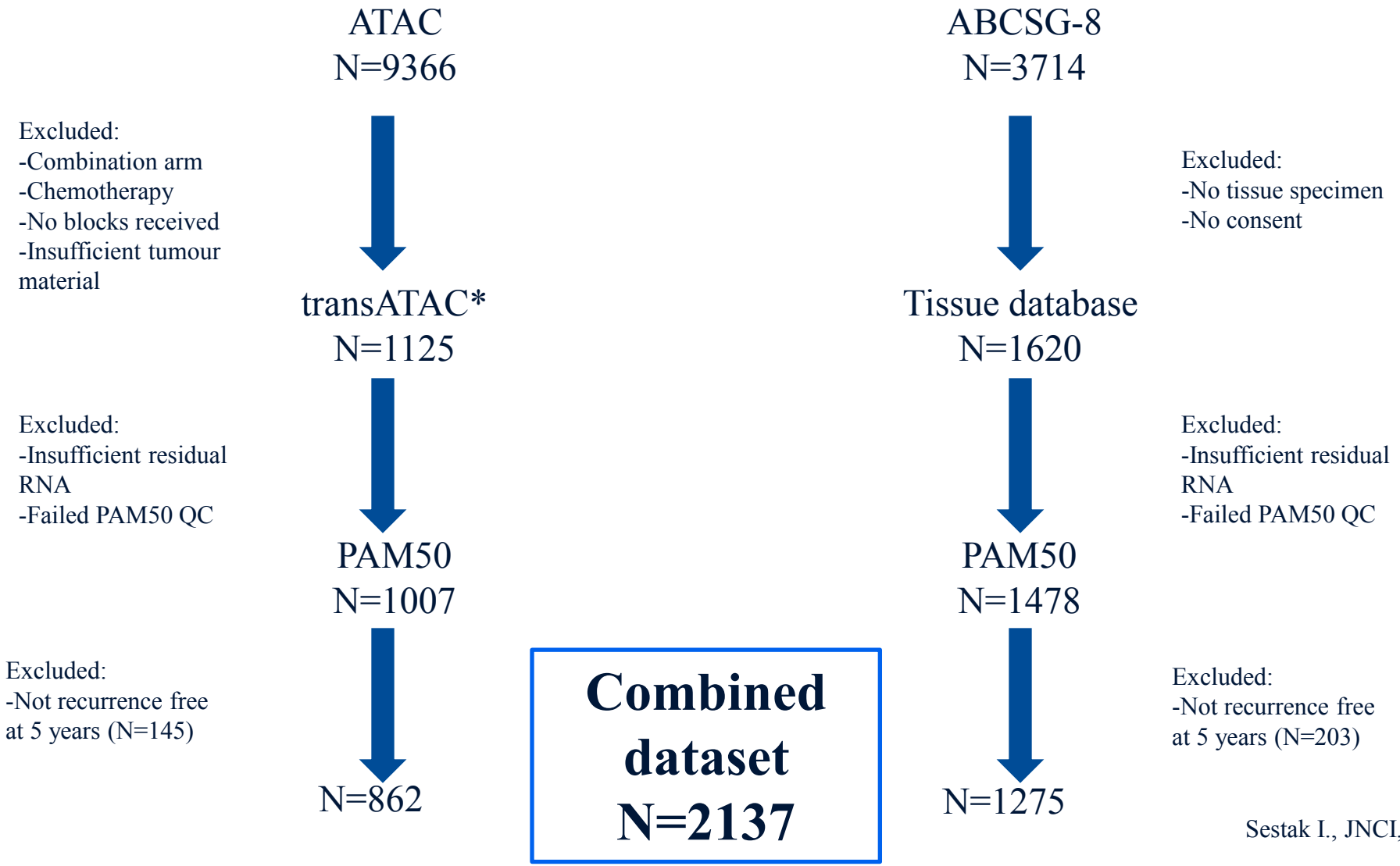
- BCI significantly predicted recurrence beyond clinical and pathologic factors for both early and late recurrence
  - At diagnosis: **Low/Intermediate** and **High Risk**
  - At 5 years and recurrence free: **Low** and **Intermediate/High Risk**
    - In comparison with Oncotype Dx and IHC4, BCI was the only biomarker able to predict late recurrences





Sgroi DC, Lancer Oncol, 2013

Late Recurrence: Population Evaluated by Prosigna

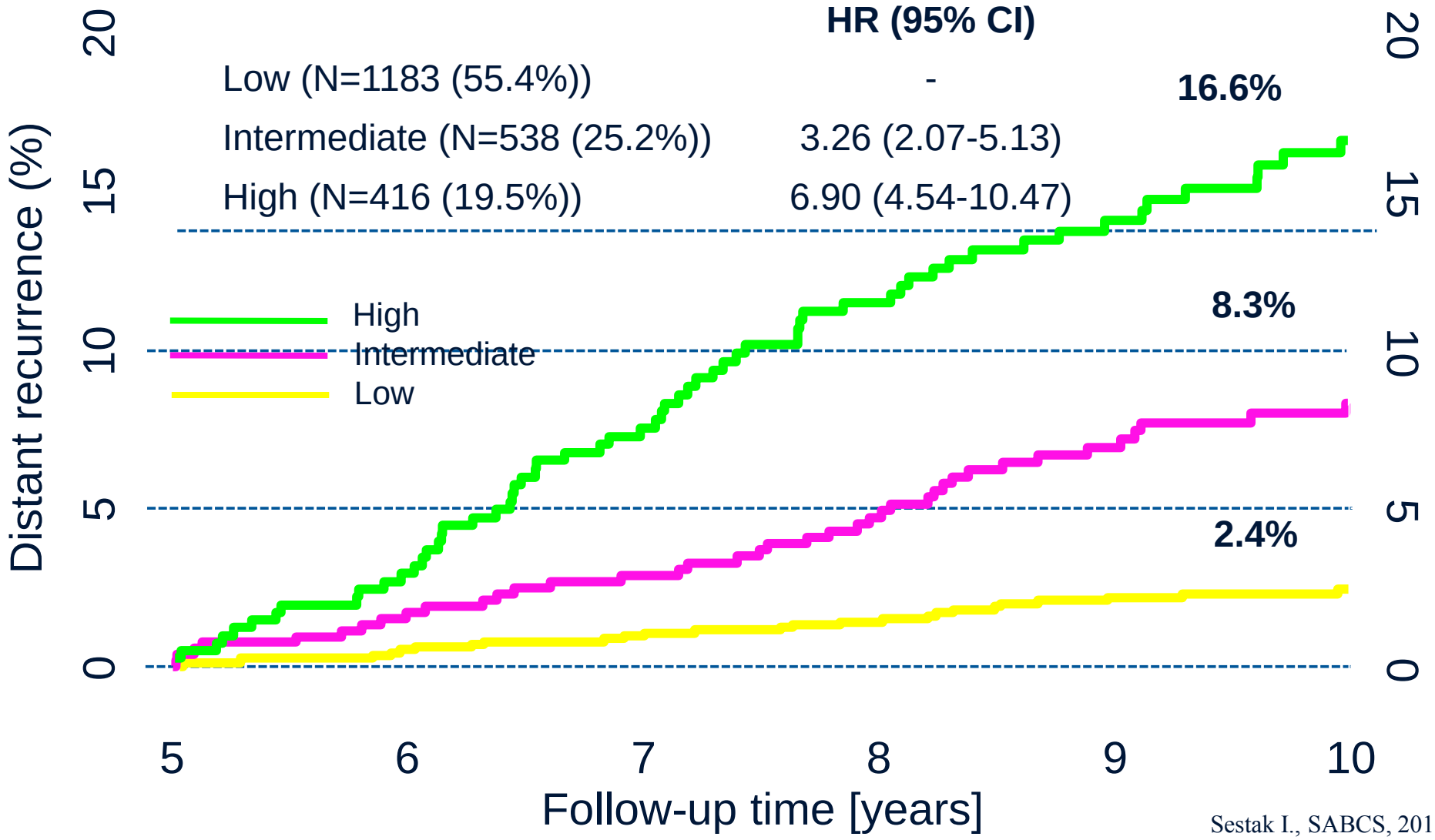


Sestak I., JNCI, 2013

Patients Characteristics

	transATAC (N=862)	ABCSG-8 (N=1275)
Median follow-up, years (IQR)	10.0 (9.1-10.1)	10.3 (8.8-12.4)
Age > 65 years	41.5%	39.3%
Node positive	24.9%	26.8%
Tumour size, mm (mean, SD)	19.0 (10.1)	16.7 (8.3)
Grade		
Well	195 (22.6%)	242 (19.0%)
Moderate	519 (60.2%)	1033 (81.0%)
Poor	148 (17.2%)	-
Distant recurrence	80 (9.3%)	68 (5.3%)

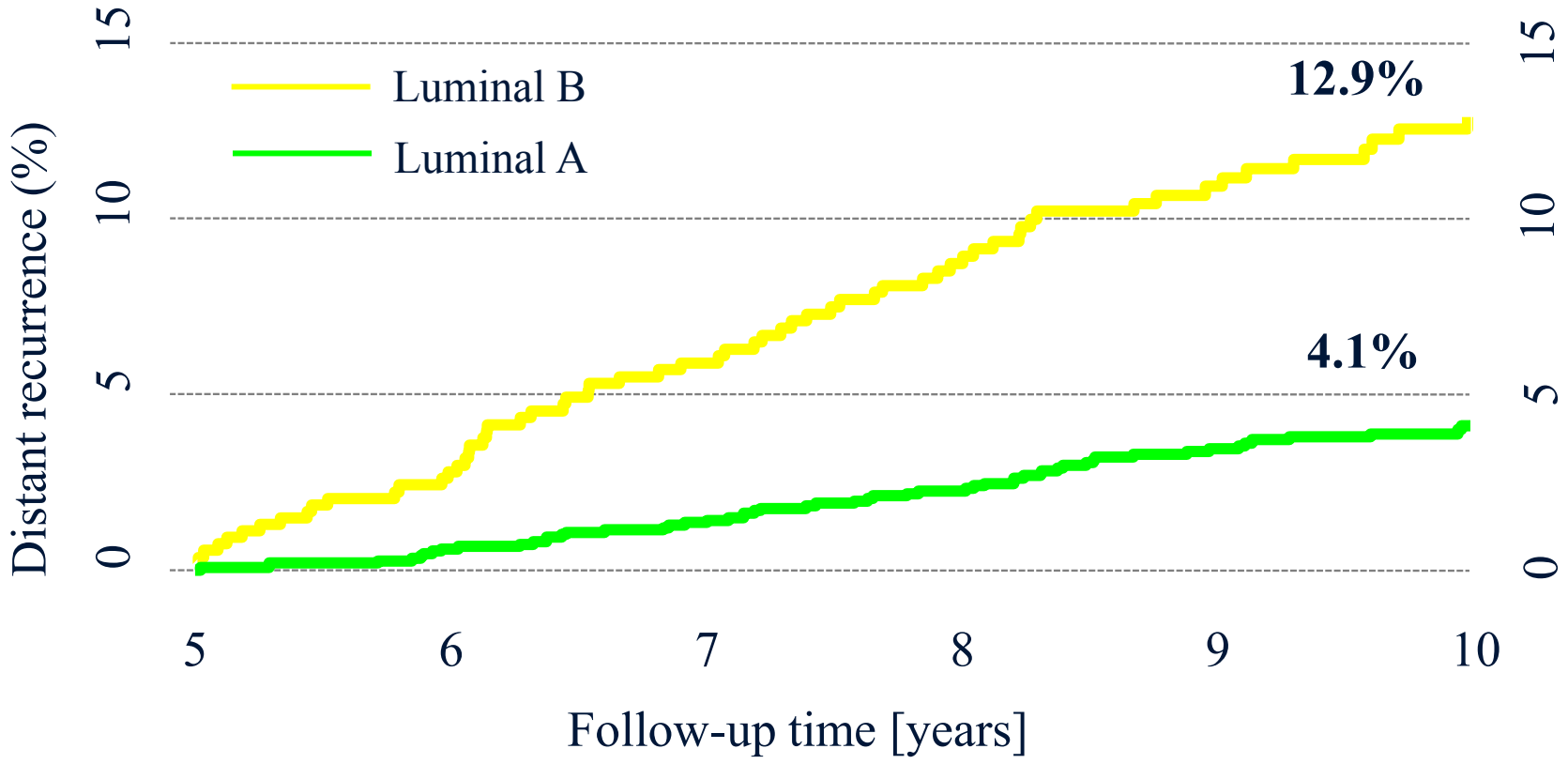
Risk groups – ROR score



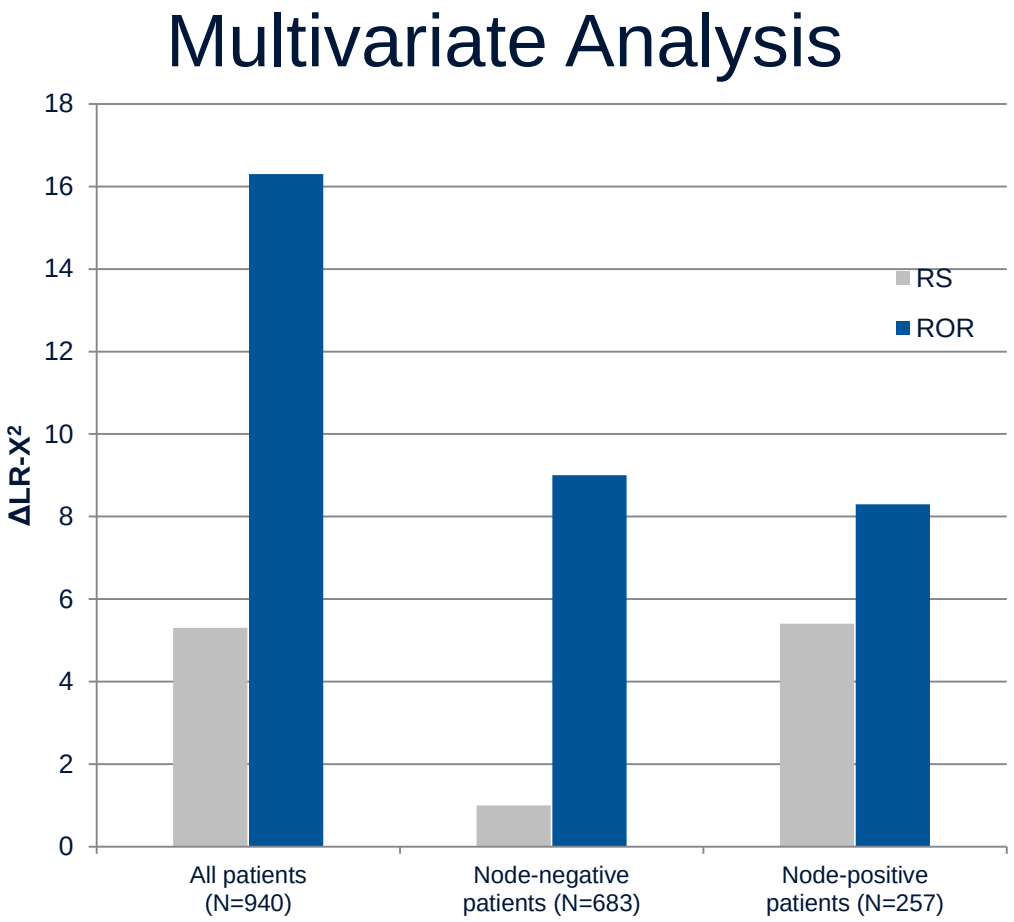
Sestak I., SABCS, 2013

Luminal A vs Luminal B

Luminal A (N=1530 (71.6%))	-	-
Luminal B (N=542 (25.4%))	**2.89 (2.07- 4.02)**	**<0.0001**



ROR (Prosigna) vs RS (Oncotype) for Late Recurrence in TransATAC



ROR provides additional prognostic information in multivariate analyses

Gene Expression Tests for Recurrence in ER+ Breast Cancer Receiving Endocrine Therapy Alone

Test Name	Number Classifiers	Platform	FDA-cleared	Decentralized Testing	Recommended for HER2+	Validated in N0 and N1	Utility in Late Recurrence
Oncotype Dx	16 genes	qPCR	No	No	No	Yes	No
EndoPredict	8 genes	qPCR	No	No	No	Yes	Yes
Breast Cancer Index	7 genes	qPCR	No	No	No	No	Yes
Mammaprint	70 genes	Microarray	Yes	No	Yes	No	No
Prosigna*	50 genes	nCounter	Yes	Yes	No (US only)	Yes	Yes

*Used for prognosis in ER+ breast cancer in US under FDA regulations but all cancers outside the US

Levels of Evidence for Biomarkers Becoming Standard of Care*

Category	Description	Examples in Clinical Breast Cancer Tests
Ia	Prospective clinical trial designed to test biomarker	TAILORx MINDACT RxPONDER (node positive, 1-3 nodes)
Ib	Two or more clinical trials of similar design, well-annotated samples, long-term patient follow-up, retrospective sample collection, prospective statistical plan	Prosigna (TransATAC, ABCSG-8) Oncotype (NSABP-B14, NSABP-B20) EndoPredict (ABCSG-6, ABCSG-8) BCI (MA.17, TransATAC)
II	Prospective trial(s), collected under clinical SOPs, and designed for therapeutic response	Oncotype (NSABP20) - CMF vs CEF Oncotype (SWOG8814) - CAF
III	Clinical trials run using research assays or observational studies using tissues collected under generic tissue banking protocols	Primarily research assays and training sets

*Adapted from Simon R.M. et al, JNCI, 2009


THANK YOU