Virtual Journal Club
Expanding Options for Gene Expression Profiling and Risk Assessment in Patients With Early Breast Cancer

Discussants

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Introduction and Background

- >2/3 of breast cancers are hormone receptor–positive[1]
- Most patients have early-stage disease and good prognosis[1]
- However, ~50% of relapses occur >5 years after initial diagnosis and treatment[1]
- Gene expression profiling tests may be used to help guide treatment decisions for patients with hormone receptor–positive early breast cancer (EBC)

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). *Lancet*. 2011;378(9793):771-784.

First-Generation Gene Expression Profile 70-Gene Assay (Mammaprint®)

- Originally fresh frozen only, but now also formalin-fixed paraffin-embedded (FFPE) tissue
- DNA microarray analysis evaluates 70 genes to evaluate early recurrence
- Must be performed at a central laboratory
- Prognostic
  - Good signature – low risk of disease recurrence without adjuvant therapy
  - Poor signature – high risk of disease recurrence without adjuvant therapy

van de Vijver MJ, et al. *N Engl J Med.* 2002;347(25):1999-2009.

First-Generation Gene Expression Profile 21-Gene Recurrence Score (OncotypeDX®)

- Uses FFPE tissue
- RT-PCR assay of 21 prospectively selected genes (16 cancer-related genes and 5 reference genes)
- Must be performed at a single central laboratory
- Produces a recurrence score that stratifies patients into 3 risk groups:
  - Low risk (RS <18) – endocrine therapy only
  - Intermediate risk (RS ≥18 and <31)
  - High risk (≥31) – needs chemotherapy
- Validated in node-negative and node-positive disease
- Predicts likely chemotherapy benefit

Paik S, et al. *N Engl J Med.* 2004;351(27):2817-2826; Albain K, et al. *Lancet Oncol.* 2010;11(1):55-65.

First-Generation Gene Expression Profiles: Limitations

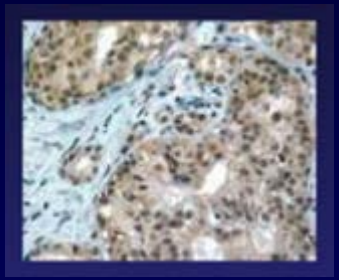
- Access requires submission of samples to a centralized laboratory
- Turnaround time is relatively slow
- Do not take into account tumor size and nodal status
- Large group of intermediate-risk tumors with Oncotype DX®

Prosigna™ Breast Cancer Prognostic Gene Signature Assay

- **Based on the PAM50 gene expression profile**
  - **Measures expression levels of 50 genes in a surgically resected breast cancer sample**
- ***In vitro* diagnostic assay performed using the nCounter® Analysis System suitable for local pathology laboratory use**
- **Test outputs:**
  - **Risk of recurrence (ROR) score that correlates with probability of distant recurrence within 10 years**
  - **Risk category (low, intermediate, or high)**
  - **Intrinsic subtype (luminal A/B, HER2, basal-like)**

Prosigna™ Technology

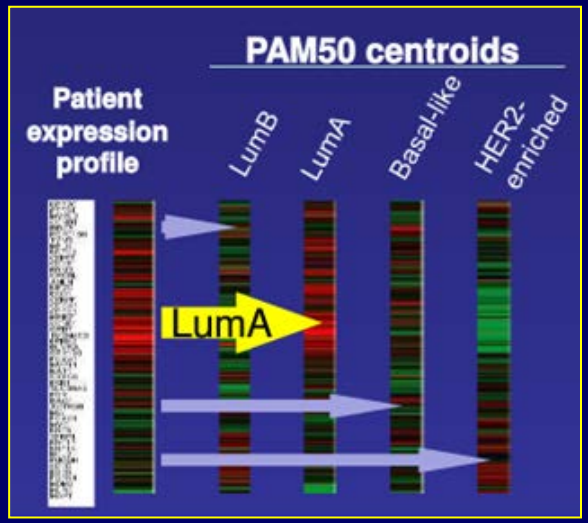
Extract RNA from FFPE tumor sample



Run RNA & PAM50 CodeSet on nCounter Analysis System

Capture patient expression profile

Determine intrinsic subtype through Pearson's correlation to centroids



Calculate risk of recurrence (ROR) score

$ROR = aR_{LumA} + bR_{LumB} + cR_{Her2e} + dR_{Basal} + eP + fT$

- aR_{LumA}, bR_{LumB}, cR_{Her2e}, dR_{Basal}: Pearson's correlation to centroids*
- eP: Proliferation score (19 genes)
- fT: Tumor Size

Validation of Prosigna™ ROR Scoring in Early Breast Cancer

- Relatively few molecular classifiers are reproducible[1]
- Validation of the precision and accuracy of Prosigna™ across multiple laboratories is needed
- Reproducibility is important because Prosigna™ is meant to be decentralized
- Prosigna™ offers the opportunity for local labs to communicate directly with local physicians, instead of requiring central processing of samples[2]

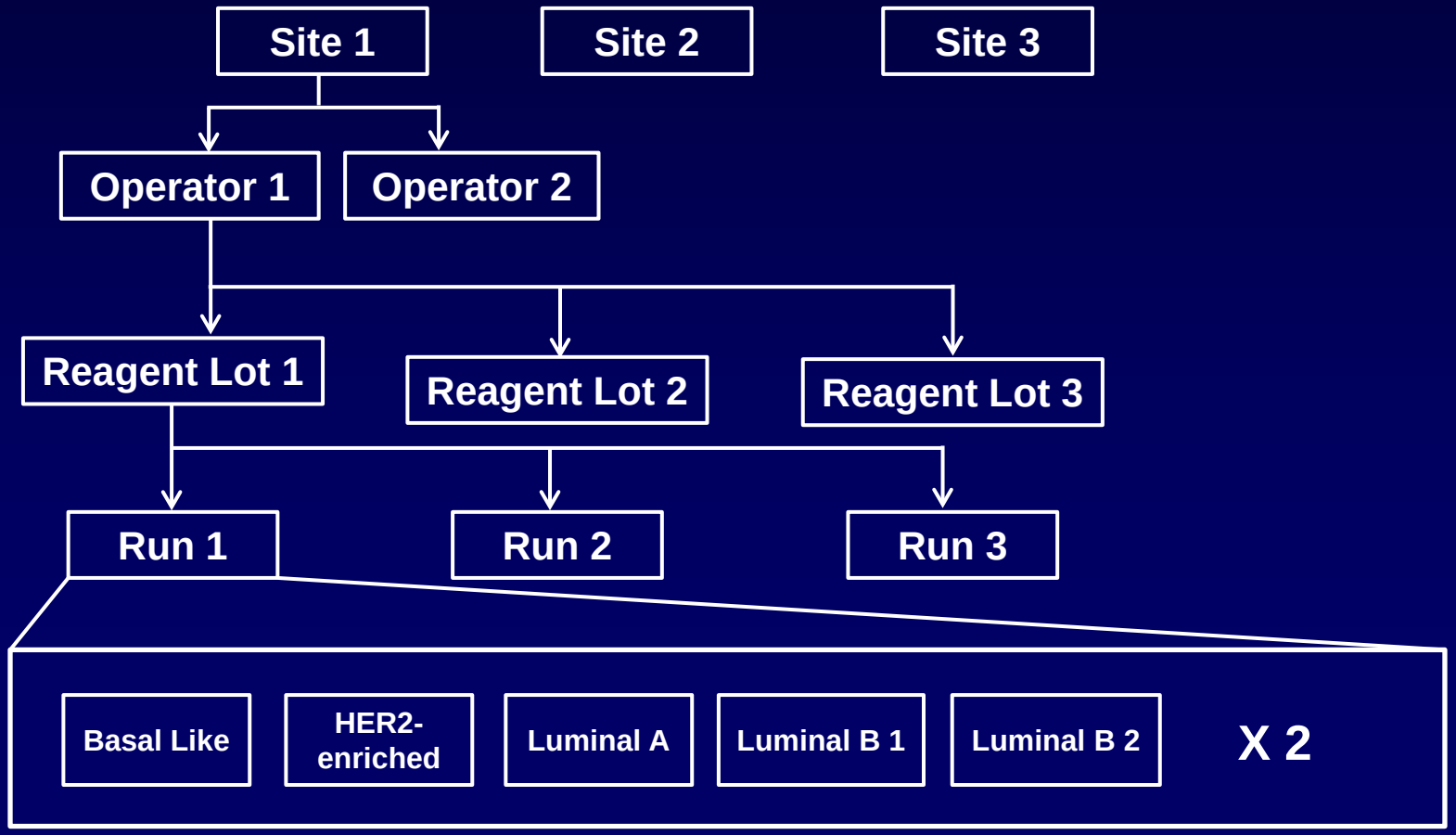
1. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. *Genet Med*. 2009;11(1):66-73.
2. Nielsen T, et al. *BMC Cancer*. 2014;14:177.

Analytical Validation of the Prosigna™ Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System Using Formalin-Fixed Paraffin-Embedded Breast Tumor Specimens

Nielsen T, et al. *BMC Cancer*. 2014;14:177.

RNA Precision Validation Study

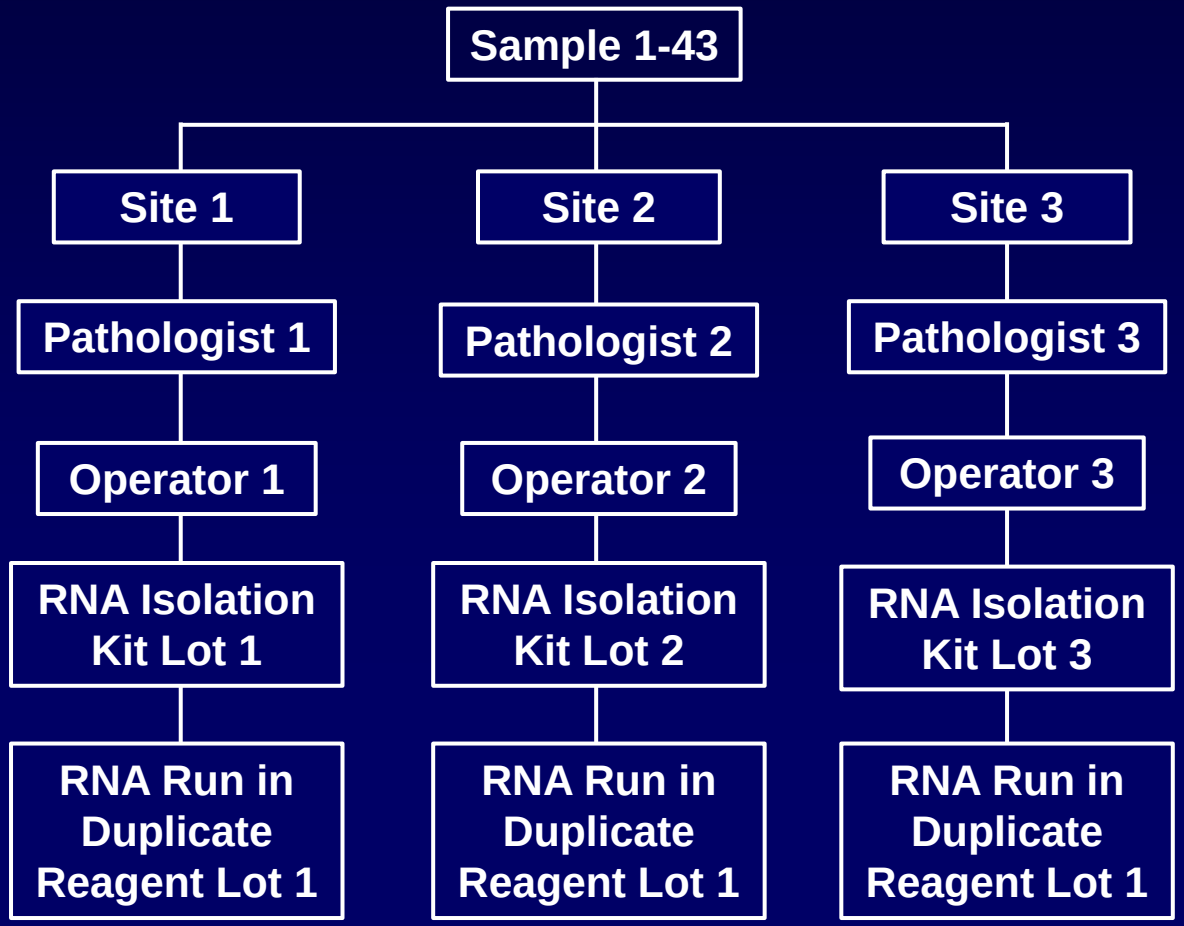
5 pooled breast tumor RNA samples were tested across several sites, operators, reagent lots, and runs



Nielsen T, et al. *BMC Cancer*. 2014;14:177.

Tissue Reproducibility Validation Study

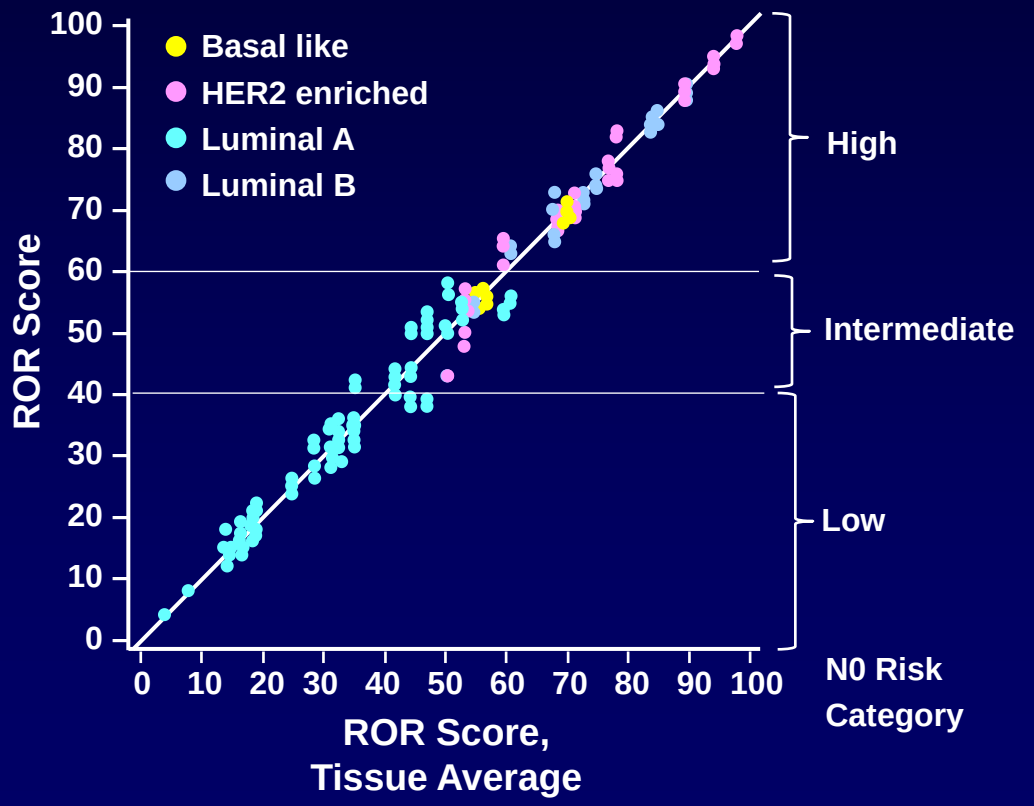
Tissue samples (1-43) were processed in parallel across different sites, pathologists, operators, and RNA isolation kits



Nielsen T, et al. *BMC Cancer*. 2014; 14:177.

Risk of Recurrence (ROR) Score Is Reproducible Across Different Samples and Molecular Subtypes

- Repeated measurements of RNA FFPE tissue samples demonstrate the reproducibility of PAM50
- Tissue processing represented >90% of total variance
- Total standard deviation of 2.9 indicates that a difference between two ROR scores of 6.75 can be detected with 95% confidence



Nielsen T, et al. *BMC Cancer*. 2014;14:177.

Results Are Highly Concordant Across Multiple Testing Sites

Comparison Type	Pairwise Concordance [95% CI][1]			Average Concordance
	Site 1 vs Site 2 (N = 40)	Site 1 vs Site 3 (N = 41)	Site 2 vs Site 3 (N = 40)	
Subtype	96.3% [86.4%-99.5%]	98.8% [91.0%-100%]	95% [83.1%-99.3%]	97%
Risk Category (Node Negative)	87.5% [73.2%-95.8%]	92.7% [80.1%-98.4%]	90% [76.4%-97.2%]	90%
Risk Category (Node Positive)	90.0% [76.9%-96.0%]	95.1% [83.9%-98.7%]	95.0% [83.5%-98.6%]	93%

Measurements were also concordant with previously-reported results from centralized lab tests[2]

1. Nielsen, et al. *BMC Cancer.* 2014;14:177.
2. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. *Genet Med*. 2009;11(1):66-73.

Conclusions

- Results of Prosigna™ ROR tested in multiple labs are similar to those from central lab tests
- Prosigna™ ROR is reproducible despite process variables and different test sites

Nielsen T, et al. *BMC Cancer*. 2014;14:177.

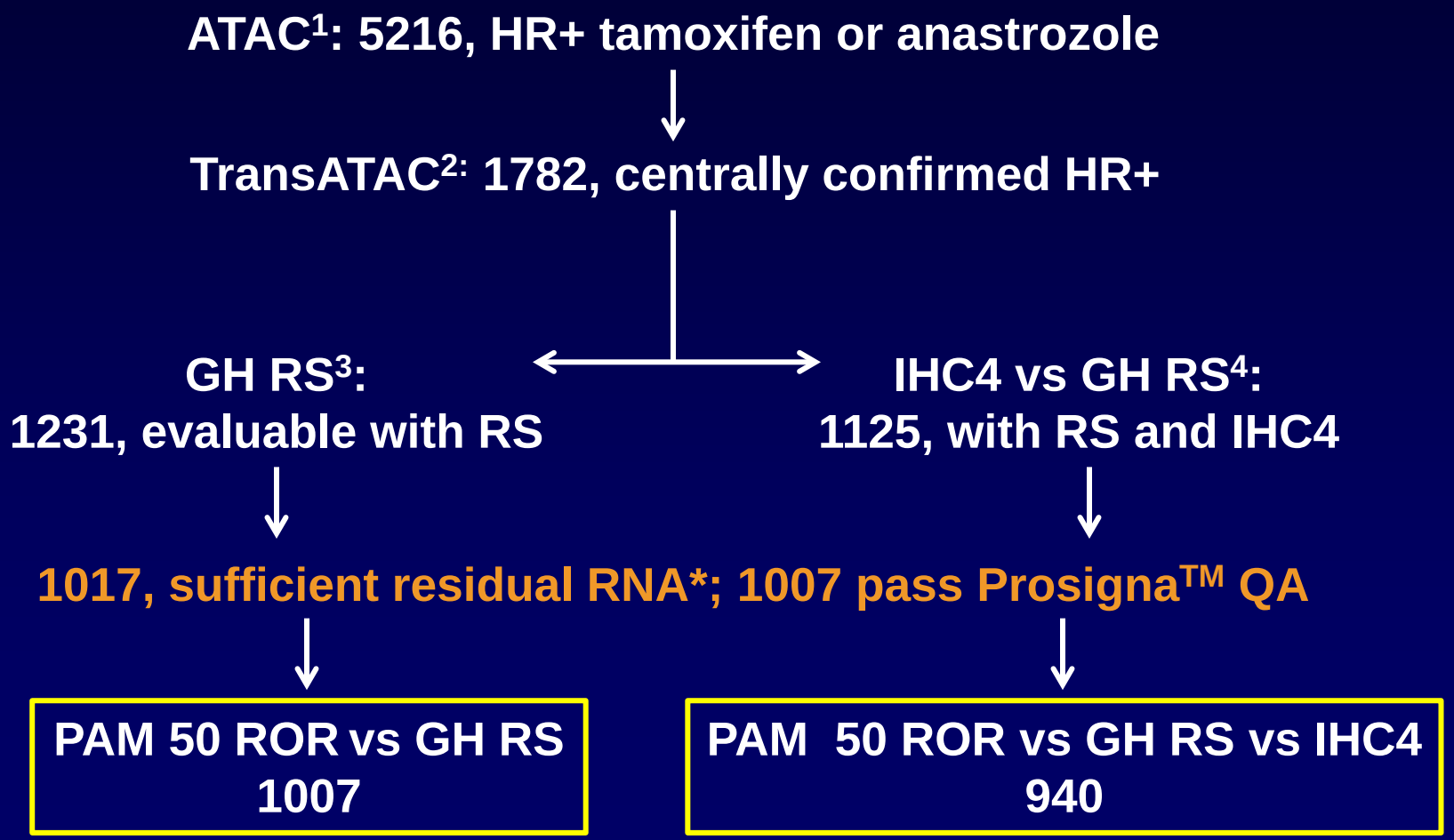
Comparison of Prosigna™ Risk of Recurrence Score With RS and IHC4 for Predicting Risk of Distant Recurrence After Endocrine Therapy

Background

- ATAC trial (anastrozole, tamoxifen alone or combined) samples were retrospectively analyzed by Oncotype DX, Prosigna™ risk of recurrence (ROR) score, or IHC4 (prognostic score computed by immunohistochemically measured markers: ER, PR, Ki67, and HER2)
- Evaluated the ability of ROR score to add prognostic information beyond clinical treatment score (CTS: includes nodes, grade, tumor size, age, treatment)
- Compared ROR with RS and IHC4 in predicting risk of distant recurrence after endocrine therapy

Dowsett, et al. *J Clin Oncol.* 2013;31(22):2783-2790.

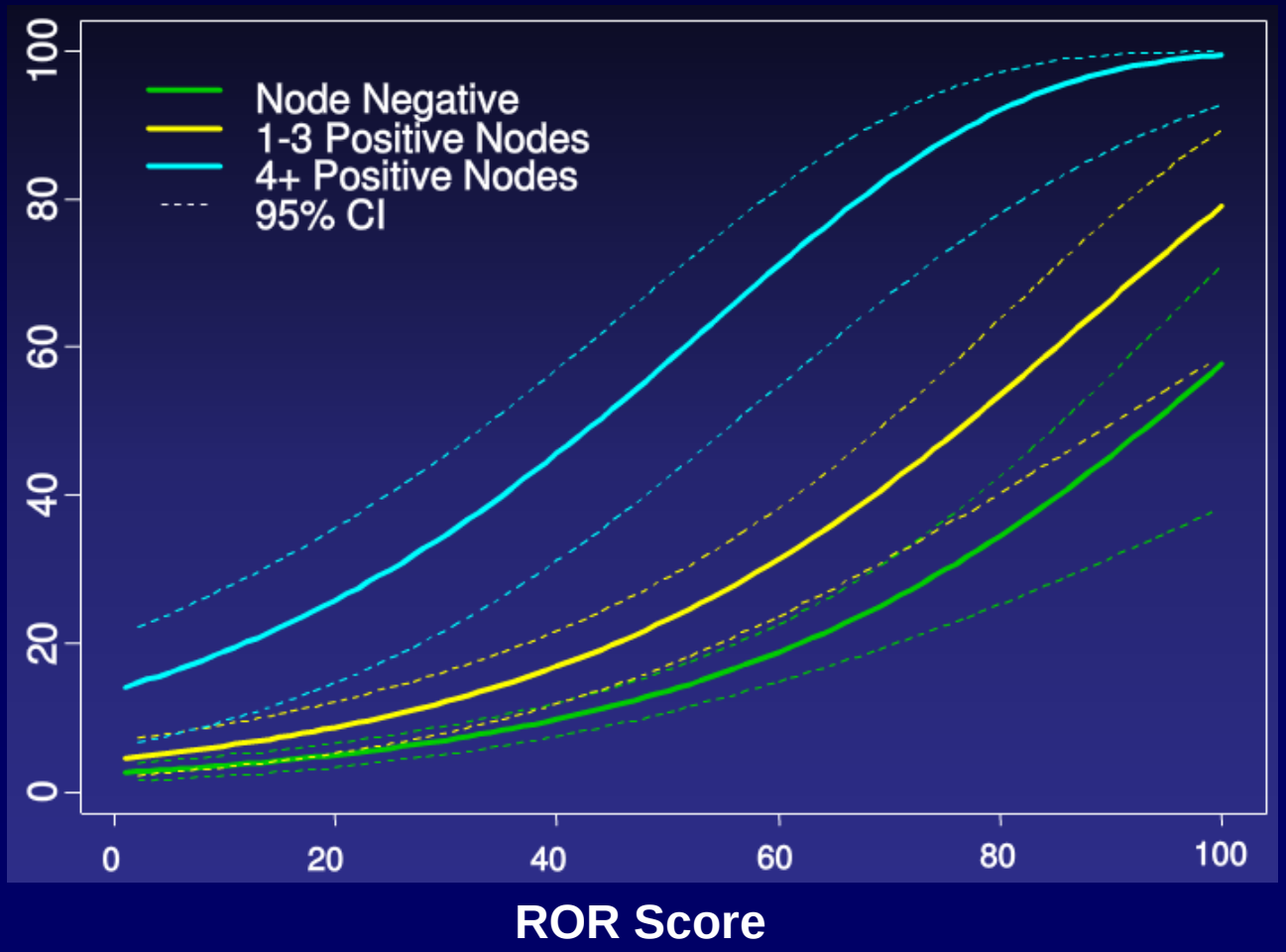
Samples Analyzed



*RNA extracted by GHI

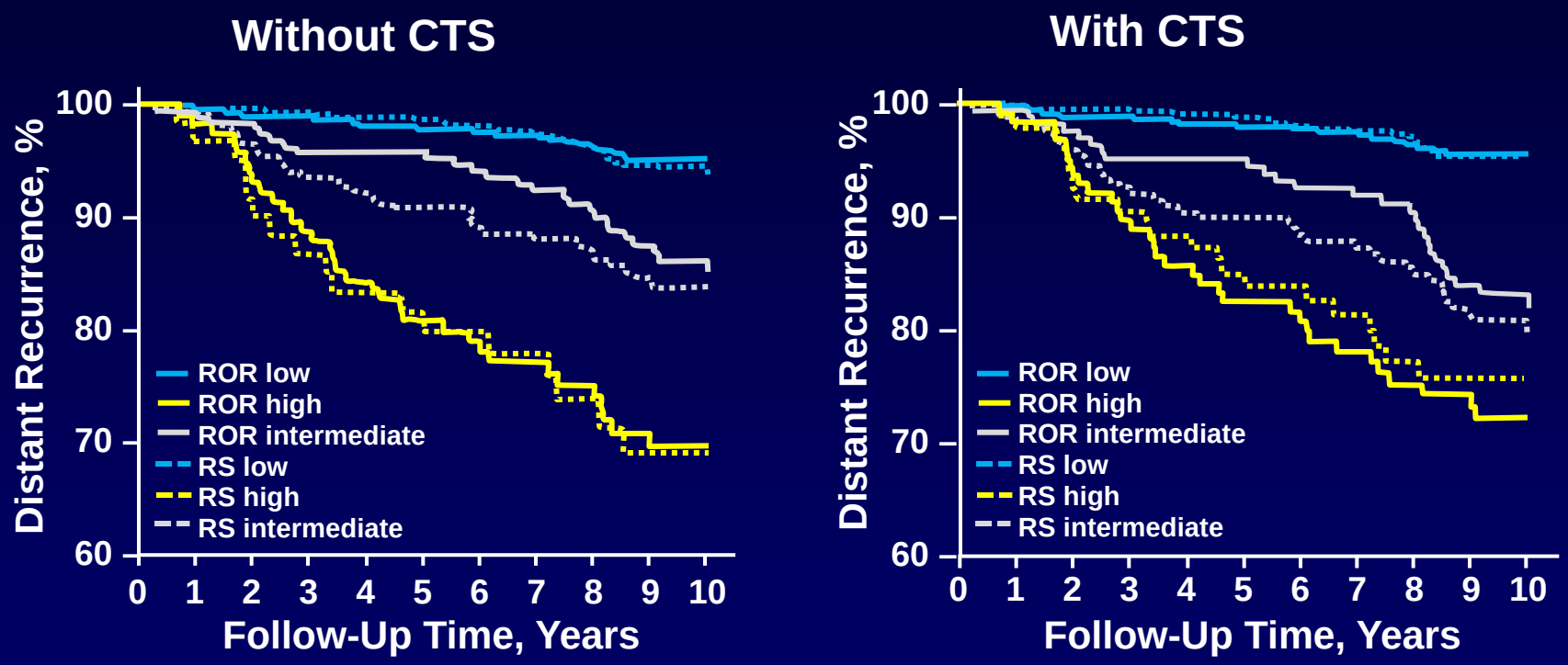
Cuzick J, et al. *Lancet Oncol.* 2010;11(12):1135-1141. Dowsett M, et al. *J Clin Oncol.* 2008;26(7):1059-1065. Dowsett M, et al. *J Clin Oncol.* 2010;28(11):1829-1834. Cuzick M, et al. *J Clin Oncol.* 2011;29(32):4273-4278.

Ten Year Predicted Risk of Distant Recurrence Using ROR Score



Dowsett M, et al. *J Clin Oncol.* 2013;31(22):2783-2790.

ROR Score Categorized Fewer Patients As Intermediate Risk Than RS



- With or without factoring in tumor size, ROR yields similar rates of stratification into low-risk (<10% rate of distant relapse by ten years) compared with RS
- ROR categorized fewer patients as intermediate risk

Dowsett M, et al. *J Clin Oncol*. 2013; 31(22):2783-2790.

Prosigna™ ROR Provides More Prognostic Information Than RS

	# of pts	# of DRs	ROR+RS vs RS		ROR+RS vs ROR		ROR+CTS vs CTS		RS+CTS vs CTS		ROR+CTS+RS vs CTS+RS		ROR+CTS+RS vs CTS+ROR	
			LR $\Delta\chi^2$	*P*	LR $\Delta\chi^2$	*P*	LR $\Delta\chi^2$	*P*	LR $\Delta\chi^2$	*P*	LR $\Delta\chi^2$	*P*	LR $\Delta\chi^2$	*P*
All patients	1007	160	50.2	<.001	1.1	.3	34.3	<.001	22.7	<.001	14.5	<.001	2.9	.09
Node-negative pts	739	79	28.5	<.001	1.6	.2	23.7	<.001	15.0	<.001	10.9	.001	2.2	.1
Node-positive pts	268	81	11.8	<.001	0.3	.6	10.1	.002	6.3	.01	4.5	.03	0.7	.4
HER2-negative pts	888	131	45.6	<.001	0.8	.4	29.5	<.001	16.0	<.001	15.2	<.001	1.7	.2
HER2-neg/node-neg pts	649	62	28.1	<.001	1.1	.3	23.3	<.001	10.2	.001	13.9	<.001	0.8	.4

DR, distant recurrence; RS, recurrence score; measured by Oncotype DX

- ROR added significant prognostic information beyond clinical treatment score (CTS) in node-negative and node-positive patients
- ROR added significant prognostic information beyond RS and CTS

Dowsett M, et al. *J Clin Oncol*. 2013; 31(22):2783-2790.

Conclusions

- Prosigna™ ROR score provides more prognostic information than RS in patients with ER-positive, node-negative disease
- Prosigna™ ROR score is better able than RS to differentiate patients with intermediate or high risk of recurrence within 10 years of endocrine therapy

Dowsett M, et al. *J Clin Oncol*. 2013; 31(22):2783-2790.

Use of Prosigna™ in Analysis of the ABCSG-8 Study

Background

- ABCSG-8 study assessed the sequencing of therapy for hormone receptor–positive early breast cancer[1]
- FFPE samples from 1478 patients in the ABCSG-8 study were analyzed using the Prosigna™ ROR scoring system to differentiate patients who have low, intermediate, or high risk of late distant recurrence (after 5 years of initial endocrine therapy)[2]

1. Dubsky PC, et al. *J Clin Oncol*. 2012;30(7):722-728.
2. Gnant M, et al. *Ann Oncol.* 2014;25(2):339-345.

Purpose

- **Does Prosigna™ ROR provide prognostic information beyond standard clinical variables for predicting distant recurrence-free survival (DRFS)?**

Prosigna™ ROR Provides Prognostic Information Beyond Standard Clinical Parameters

- Clinical linear predictor (CLP) accounts for nodal status, tumor grade, and tumor size
- CLP is a highly prognostic score on its own
- In most cases, Prosigna™ ROR or ROR risk groups provide significant prognostic information beyond the CLP

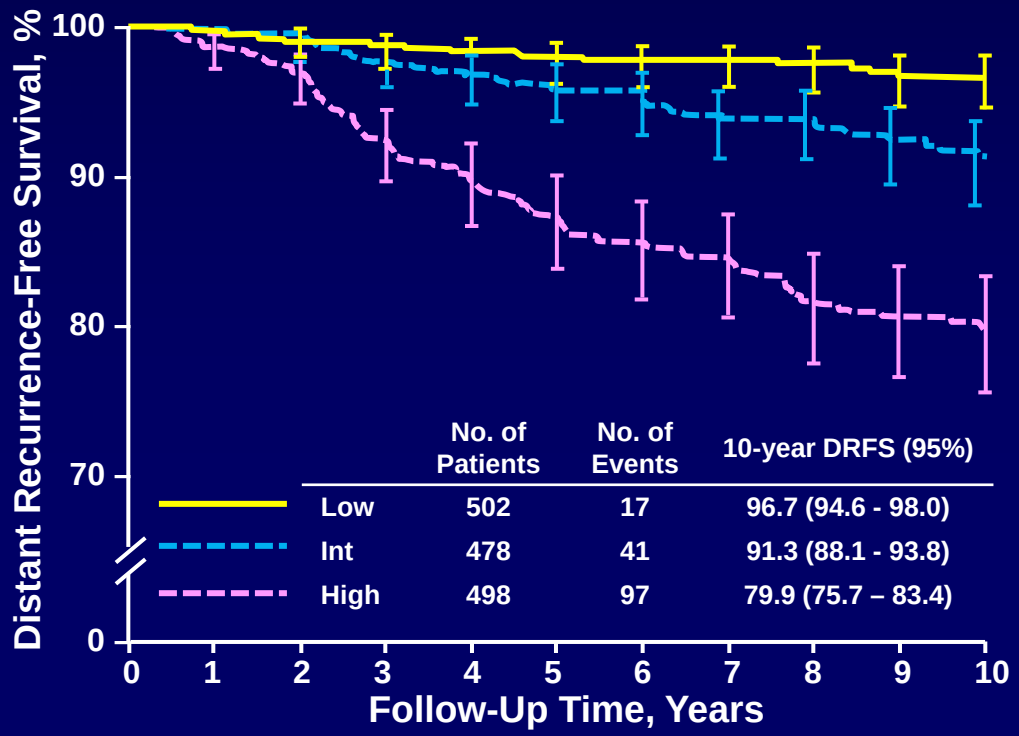
	# of pts	# of DRs	CLP + ROR vs CLP		CLP + risk groups vs CLP	
			LR $\Delta\chi^2$	*P*	LR $\Delta\chi^2$	*P*
All patients	1478	155	53.49	<.0001	34.12	<.0001
Node-negative pts	1047	86	25.57	<.0001	23.36	<.0001
Node-positive pts	431	69	29.61	<.0001	18.30	.0001
HER2-negative pts	1397	145	47.50	<.0001	29.94	<.0001
HER2-positive pts	77	10	5.34	.021	4.41	.111
HER2-neg/node-neg pts	984	79	21.69	<.0001	20.32	<.0001
HER2-positive/node-neg pts	59	7	2.76	.097	3.98	.137
HER2-neg/node-positive pts	413	66	27.65	<.0001	17.45	.0002
HER2-positive/node-positive pts	18	3	2.75	.098	0.53	.767

CLP, clinical linear predictor

Gnant M, et al. *Ann Oncol*. 2014;25(2):339-345.

Prosigna™ ROR Risk Groups Predict the Likelihood of DRFS

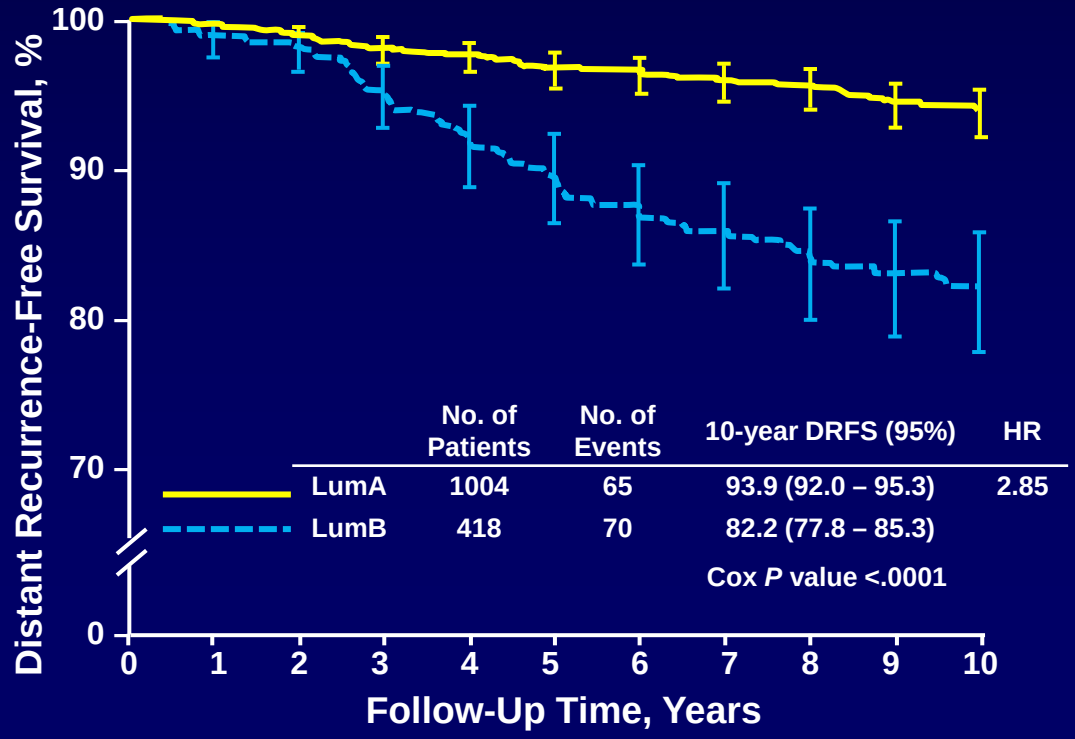
- **Probabilities for 10-year distant recurrence-free survival:**
  - **Low risk: 96.7%**
  - **Intermediate risk: 91.3%**
  - **High risk: 79.9%**
- **Additional chemotherapy may not benefit patients in the low-risk group**



Gnant M, et al. *Ann Oncol*. 2014;25(2):339-345.

DRFS Differs by Molecular Subtype

- Most patients had luminal A (67.9%) or luminal B (28.3%) breast cancer
- Luminal A and luminal B breast cancers, as assigned by the Prosigna™ test, had different outcomes at 10 years
- Luminal A disease showed a significantly greater rate of DRFS than did luminal B disease (HR 2.85, *P*<.0001)



DRFS, distant relapse-free survival

Gnant M, et al. *Ann Oncol*. 2014;25(2):339-345.

Conclusions

- Prosigna™ ROR accurately predicts individual risk of overall disease recurrence and adds significant prognostic information beyond classic disease characteristics
- Prognostic benefit of ROR score was evident in both node-negative and node-positive disease
- Molecular subtype predicted by ROR correlated with DRFS

Gnant M, et al. *Ann Oncol*. 2014;25(2):339-345.

Predicting Risk of Distant Recurrence After Year 5 of Endocrine Therapy in the ABCSG-8 Trial

Background

- Of 1478 patients previously assessed from ABCSG-8,[1] 232 were excluded
  - 87 had early (0 to 5 years) distant recurrence
  - 55 died either with no breast cancer or unknown breast cancer status
  - 90 had a secondary malignancy within 5 years
- The remaining 1246[2] patients were stratified into low-, intermediate-, and high-risk groups for late (after 5 years) distant recurrence

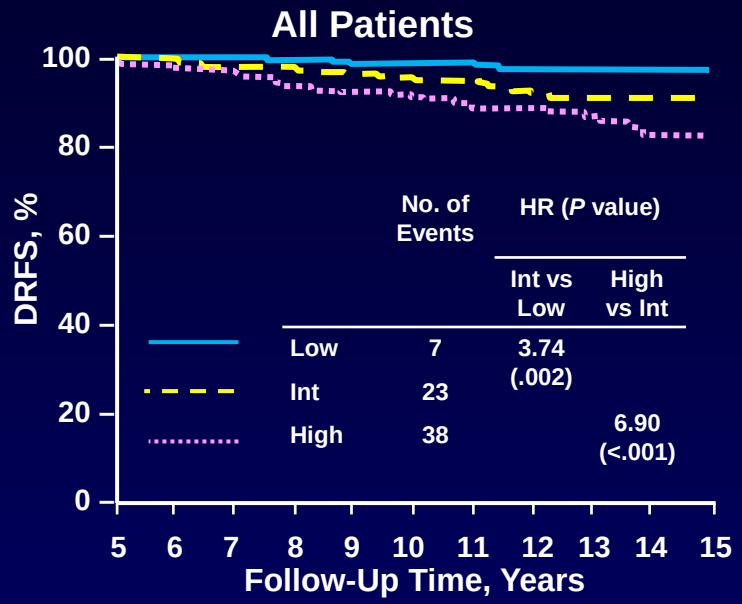
1. Gnant M, et al. *Ann Oncol*. 2014;25(2):339-345.
2. Filipits M, et al. *Clin Cancer Res*. 2014;20(5):1298-1305.

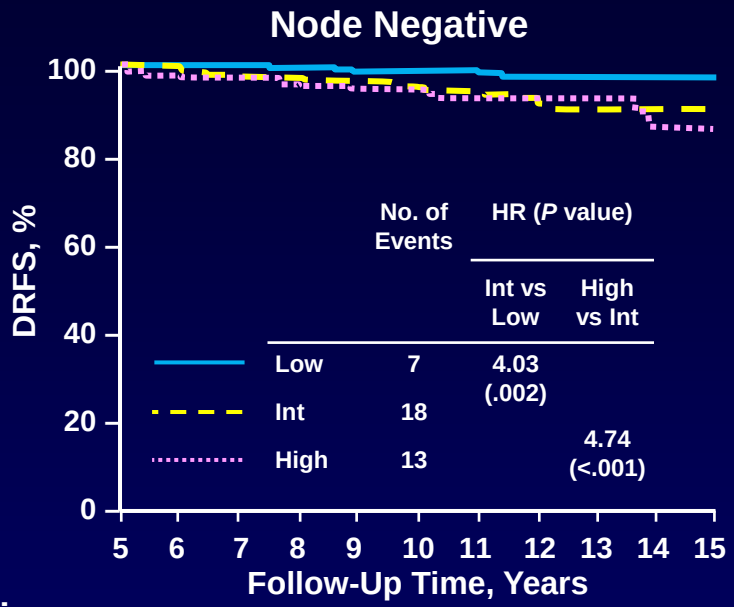
Prosigna™ ROR Score and Molecular Subtype Contribute to 15-Year Risk of Recurrence

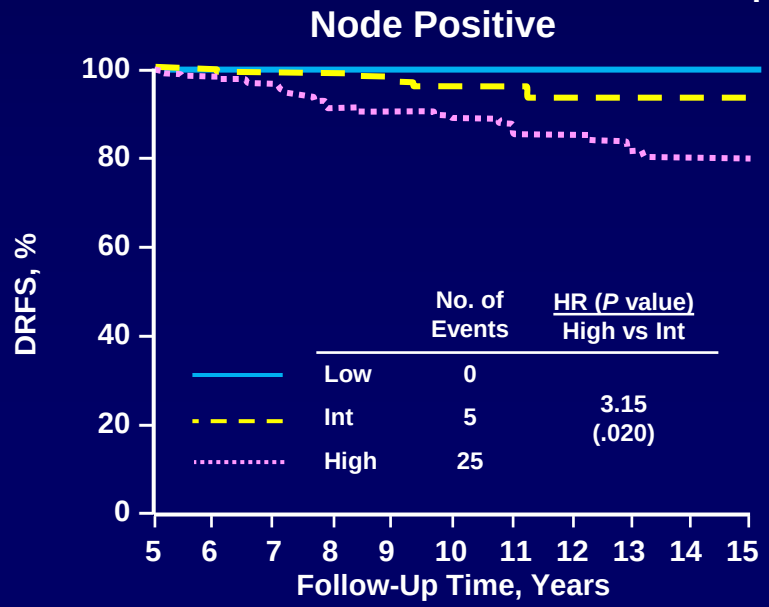
- **Cumulative risk of distant relapse for all patients at 15 years:**
  - **Low-risk pts: 2.4%**
  - **High-risk pts: 17.5%**
- **Luminal A and luminal B subtypes have a different long-term outcome**

Filipits M, et al. *Clin Cancer Res.* 2014;20(5):1298-1305.

Prosigna™ ROR Score and Molecular Subtype Contribute to 15-Year Risk of Recurrence







DRFS, distant recurrence-free survival

Filipits M, et al. *Clin Cancer Res.* 2014;20(5):1298-1305.

Prosigna™ ROR Contributes Significant Prognostic Information Beyond Clinical Factors

	LR $\Delta\chi^2$	*P*
PAM50 ROR score		
All pts	15.32	<.001
Node-negative pts	7.40	.007
Node-positive pts	8.94	.003
ROR-based risk groups		
All pts	14.83	<.001
Node-negative pts	11.96	.003
Node-positive pts	5.92	.05
Luminal molecular subtypes		
All pts	8.73	.003
Node-negative pts	4.17	.04
Node-positive pts	4.82	.03

Filipits M, et al. *Clin Cancer Res*. 2014;20(5):1298-1305.

Conclusions

- Prosigna™ ROR risk groups showed differences in terms of 5- and 10-year risk of distant recurrence
- ROR score is able to differentiate risk groups at times beyond those segregated by clinicopathologic risk factors
- ROR is beneficial for predicting risk of both early[1] and late recurrence[2]
- Results from Prosigna™ can help determine the need for chemotherapy or prolonged endocrine therapy

1. Gnant M, et al. *Ann Oncol.* 2014;25(2):339-345.
2. Filipits M, et al. *Clin Cancer Res*. 2014;20(5):1298-1305.

Factors Predicting Late Recurrence for Estrogen Receptor–Positive Breast Cancer

Background

- Previous studies have determined risk of late relapse in women receiving adjuvant hormone therapy for early ER+ breast cancer
- Recently-developed scoring systems may help to predict late recurrence
- Ability to predict recurrence between years 5 and 10 was unknown for the scoring systems
- TransATAC cohort had RS, ROR, and IHC4 tested

Sestak I, et al. *J Natl Cancer Inst*. 2013;105(19):1504-1511.

Purpose

- **Four different scoring systems (CTS, IHC4, RS, and ROR) used to predict distant recurrence both in the 0 to 5–year range and in the 5 to 10–year range**

- **No studies to date investigated IHC markers in the rate of late recurrence after 5 years**

Sestak I, et al. *J Natl Cancer Inst*. 2013;105(19):1504-1511.

Prosigna™ ROR Is Superior to RS and IHC4 in the Late Follow-Up Period

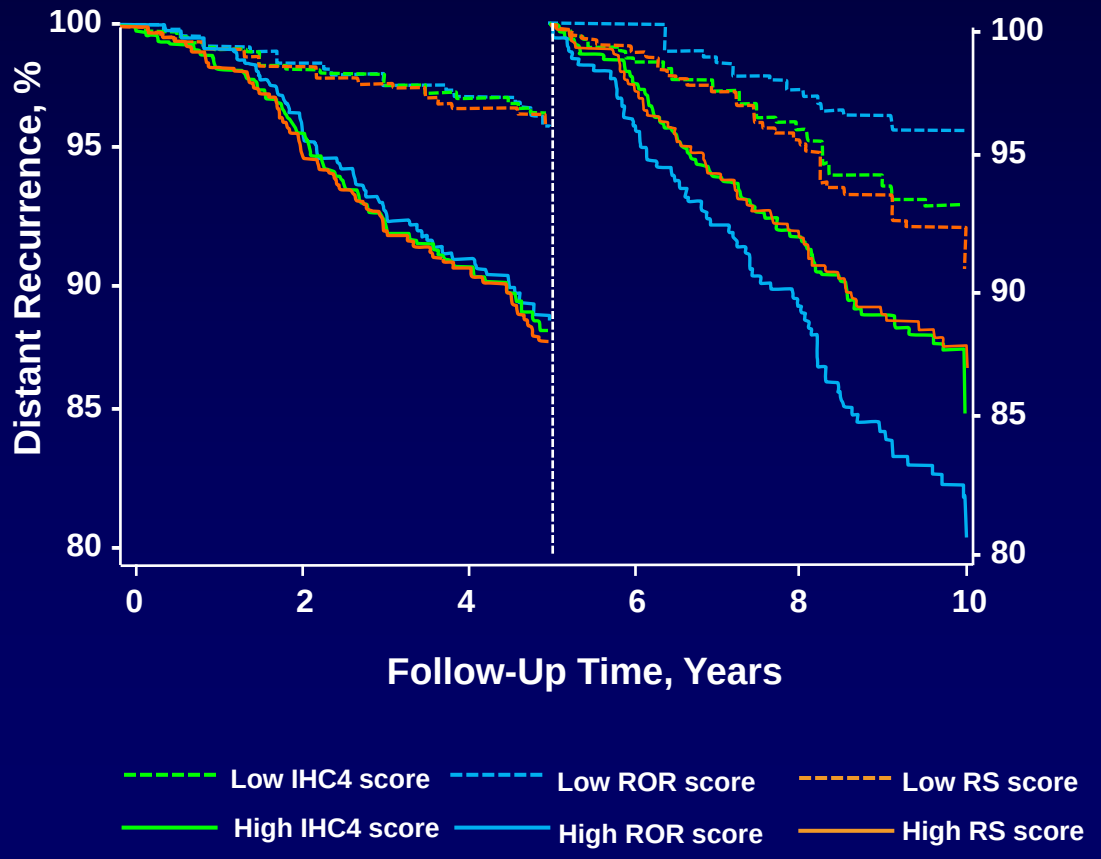
- Multivariate analysis shows that ROR adds significant prognostic information beyond the clinical treatment score (CTS) in years 5 to 10
- RS and IHC4 did not add significant prognostic information beyond CTS in node-negative patients in years 5 to 10
- Tumor size and nodal status were also significantly associated with late recurrence ($\Delta\chi^2$ 21.72 and 10.52, respectively)

	0 to 5 years		5 to 10 years	
	$\Delta\chi^2$	*P*	$\Delta\chi^2$	*P*
Node-negative pts (N = 683)				
IHC4	27.19	<.001	1.98	.20
RS	14.52	<.001	1.01	.30
ROR	10.41	.001	8.93	.003
Node-positive pts (N = 257)				
IHC4	1.38	.20	6.05	.01
RS	0.81	.40	5.17	.02
ROR	1.33	.20	8.37	.004
HER2-negative pts (N = 845)				
IHC4	14.61	<.001	5.67	.02
RS	10.35	.001	2.81	.09
ROR	8.69	.003	18.18	<.001
HER2-negative/node-positive pts (N = 230)				
IHC4	3.94	.05	1.44	.20
RS	4.01	.05	0.38	.50
ROR	1.96	.20	4.78	.03

Sestak I, et al. *J Natl Cancer Inst*. 2013; 105(19):1504-1511.

Prosigna™ ROR Is the Strongest Predictor of High- and Low- Risk for Recurrence in Years 5 to 10

- In years 0 to 5, difference between high- and low-risk groups for rates of distant recurrence (DR) was ~7%, and this did not differ with the scoring groups
- In years 5 to 10, the difference between rates of DR in high- and low-risk groups was greater for ROR (15.1%) than for RS (5.4%) and for IHC4 (9.8%)



Sestak I, et al. *J Natl Cancer Inst*. 2013; 105(19):1504-1511.

Conclusions

- Clinical variables are strong prognostic factors in years 0 to 5
- Beyond 5 years, only nodal status and tumor size were prognostic clinical variables
- IHC4, RS, and ROR each added prognostic information in years 5 to 10, but ROR provided the most prognostic information in this timespan
  - IHC4 and RS were more weakly prognostic than ROR
- Prosigna™ ROR was the most effective for segregating high-risk and low-risk patients in years 5 to 10

Sestak I, et al. *J Natl Cancer Inst*. 2013;105(19):1504-1511.

Implications for Clinical Practice

- **Prosigna™ has been cleared by the FDA due to analytical and clinical validation studies**
- **Prosigna™ ROR provides valuable prognostic information for both early (<5 years of therapy) and late (>5 years) recurrence and helps guide adjuvant therapy decisions**
- **Prosigna™ ROR can aid in the identification of patients who have a low risk of late distant recurrence and who are unlikely to benefit from extended adjuvant endocrine therapy**