The Role of Onco*type* DX® in Breast Cancer Management

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Genomic Health, Inc

Assistant Professor of Pathology
University of California, San Francisco

Objectives

- Brief overview of the Onco*type* DX® Breast Cancer assay and reports
- Review assay development strategy and supporting studies
  - Technical feasibility studies
  - Gene discovery and refinement studies
  - Analytical validation studies
- Review clinical validation studies in women with breast cancer
  - Prognostic studies
  - Predictive studies
- Discuss Oncotype DX Breast Cancer Assay in Node Positive Patients

Case Study Presentation

- A 55-year old post-menopausal woman presents with a moderately differentiated ductal carcinoma
  - Tumor size 1.0 cm
  - ER/PR IHC positive
  - HER2 IHC negative
  - Sentinel lymph node negative
  - Excellent overall health

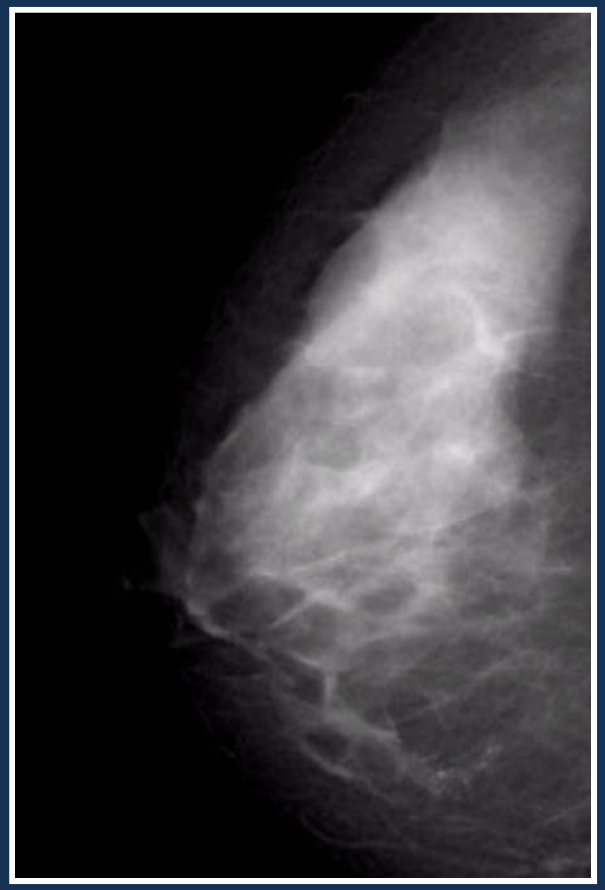
How should this patient be evaluated for treatment?
What is her risk of disease recurrence?
How likely is she to benefit from hormonal or chemotherapy?

Breast Cancer Treatment in the United States (2009)

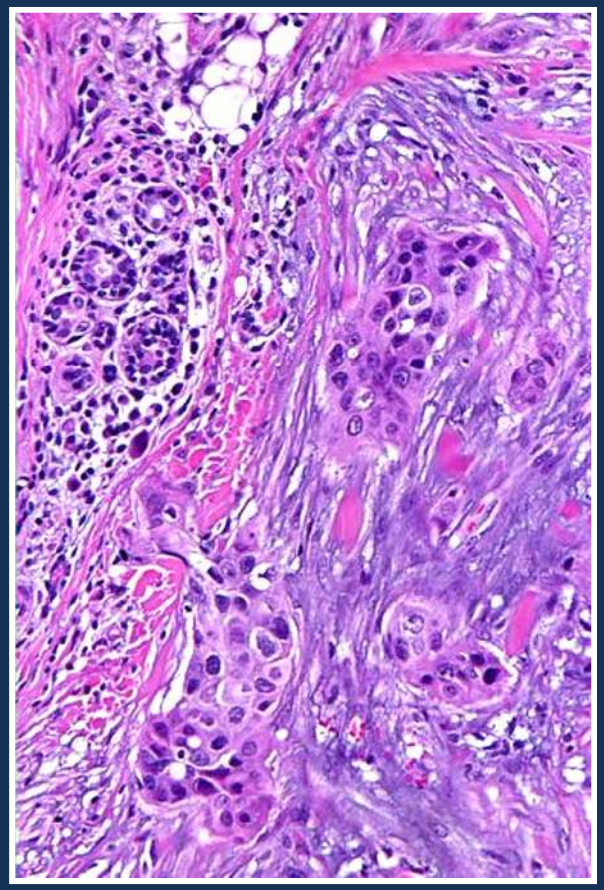
- Approximately 110,000 women with ER+, lymph node-negative breast cancer are diagnosed annually in the United States
  - This represents ~50% of newly diagnosed patients today
  - Many women are offered chemotherapy, yet few benefit

Better identification of disease markers is needed to help make therapeutic decisions

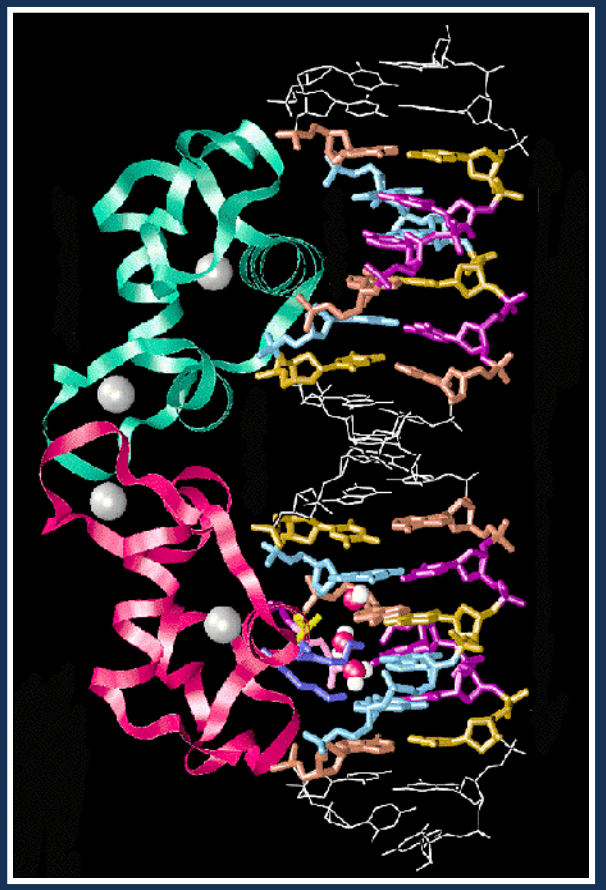
Standard Clinical & Pathologic Metrics



Size
Age



Phenotype
Nodal status



Protein
DNA

Prognostic & Predictive Markers Utilized in Breast Cancer Management

Prognostic (recurrence risk)

- Axillary node status
- Histologic type/grade
- Tumor size
- Patient age
- Lymphatic/Vascular invasion
- ER/PR status
- HER2 *neu* status
- Onco*type* DX

Predictive (treatment benefit)

- ER/PR status
- HER2 *neu* status
- Onco*type* DX

These markers can be used to estimate the risk of disease recurrence

These markers can be used to predict treatment benefit

Cianfrocca and Goldstein. *Oncologist*. 2004;9(6):606-616;
Lonning PE. *Ann Oncol*. 2007;18(suppl 8):viii3-viii7.

Oncotype DX® Assay

- Quantitatively predicts the likelihood of breast cancer recurrence in women with newly diagnosed, early stage invasive breast cancer
- Assesses the likely benefit from both hormonal therapy and chemotherapy
- Is the only multi-parameter gene expression assay to show clinical utility in breast cancer
- Is recommended by both ASCO and NCCN clinical practice guidelines

Harris L, et al. *J Clin Oncol*. 2007;33(25):5287-5312.
NCCN Clinical Practice Guidelines in Oncology. Breast Cancer. Version 2. 2008.
Available at: http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf. Accessed December 8, 2008.

Oncotype DX® Report Samples

genomic health | oncotype DX Breast Cancer Assay

Genomic Health, Inc.
301 Penobscot Drive
Redwood City, CA 94063
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www.oncotypeDX.com

PATIENT REPORT

Patient: Doe, Jane
Sex: Female
DOB: 01/01/1950

Requisition: R00003G
Order Received: 10/15/2008
Date Reported: 10/23/2008

RESULTS

Recurrence Score = 6

Test Results should be interpreted using the Clinical Experience information contained in this report which is derived from clinical studies involving patient populations with specific clinical features as noted in each section of the Clinical Experience. It is unknown whether the findings summarized in the Clinical Experience are applicable to patients with features different from those described.

CLINICAL EXPERIENCE: PROGNOSIS AND CHEMOTHERAPY BENEFIT FOR NODE POSITIVE, HR-POSITIVE PATIENTS

The following results are from a clinical study involving 367 patients from the SWOG 8814 Study. This study included post-menopausal female patients with Node Positive, Hormone Receptor (HR)-Positive breast cancer. Patients were randomized to either tamoxifen alone or CAF chemotherapy followed by tamoxifen (CAF-T). The endpoint for this study was disease-free survival (time to local or distant recurrence, new primary breast cancer, or death from any cause) and outcomes after 5 years of follow-up were presented. Note that this differs from the endpoint and follow-up time used in the two NSABP studies of Node Negative, ER-Positive patients. For patients in the pre-specified group with Recurrence Scores ≥ 31 and 1–3 positive nodes, the group average 5-year rates (95% CI) of recurrence or death were 31% (17%, 52%) for Tam alone and 28% (15%, 46%) for CAF-T. For patients in the pre-specified group with Recurrence Scores ≥ 31 and ≥ 4 positive nodes, the group average 5-year rates (95% CI) of recurrence or death were 52% (33%, 74%) for Tam alone and 32% (20%, 50%) for CAF-T. *San Antonio Breast Cancer Symposium 2007* Abstract #10.

Recurrence Score vs Recurrence or Death in NODE POSITIVE, HR-Positive Breast Cancer
Prognosis and Chemotherapy Benefit (Difference Between Rates)

Laboratory Director: Patrick Joseph, MD
CLIA Number 05D1018272

This test was developed and its performance characteristics determined by Genomic Health, Inc. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. These results are adjunctive to the ordering physician's workup.

301 Penobscot Drive Redwood City, CA 94063 (866) ONCOTYPE (866-662-6897) www.oncotypeDX.com

GH004 Rev015

- Oncotype DX® provides valuable information on:
  - Clinical prognosis
  - Predicted chemotherapy benefit
  - Quantitative data on ER / PR / HER2
- Node positive report contains an additional page with prognosis and predicted chemo benefit information specific to node-positive patients

Oncotype DX® Technology
Development Overview

Agenda
Development Overview

Technical Feasibility

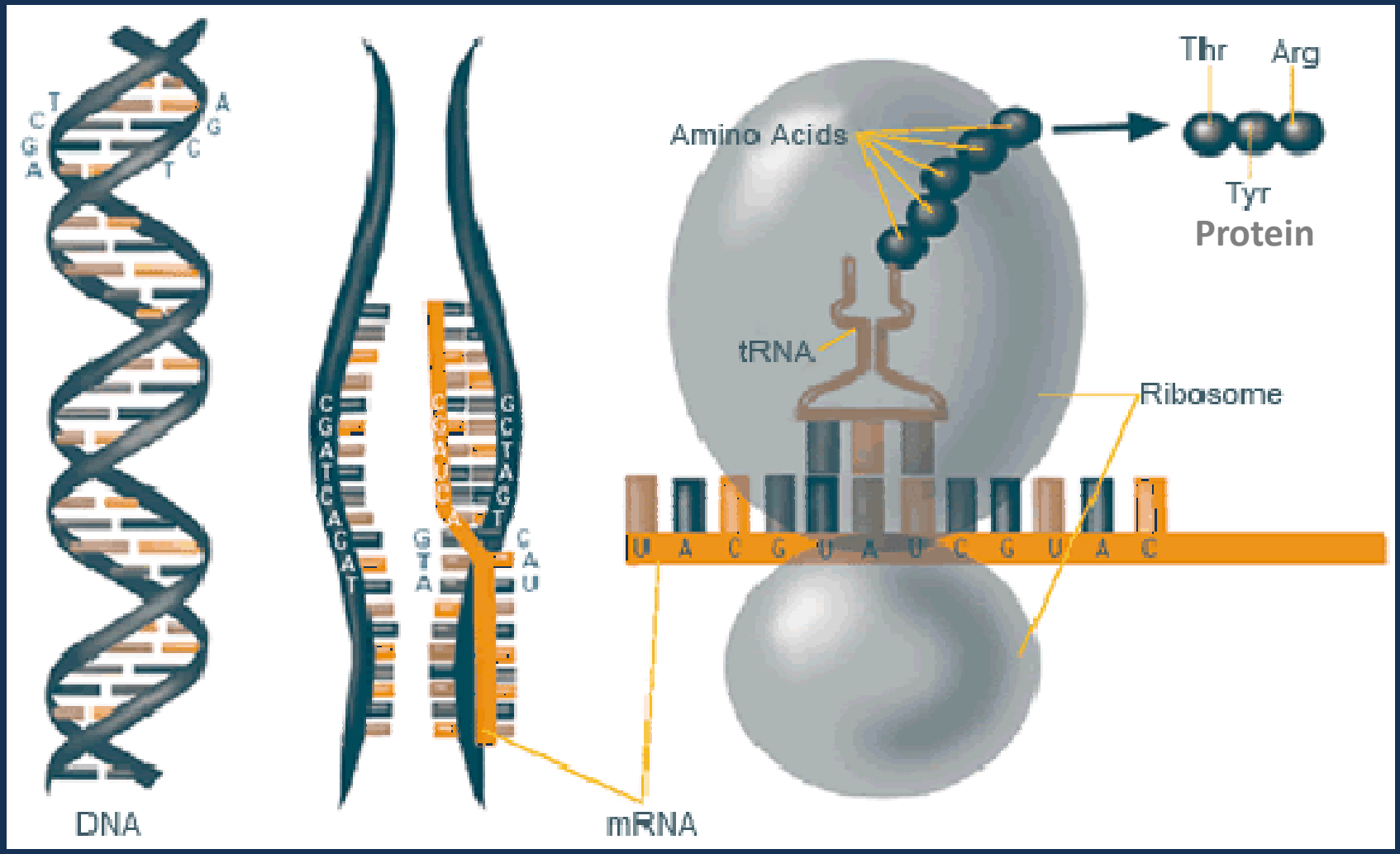
Gene Discovery & Refinement

Analytical Validation

Clinical Validation (prognostic)

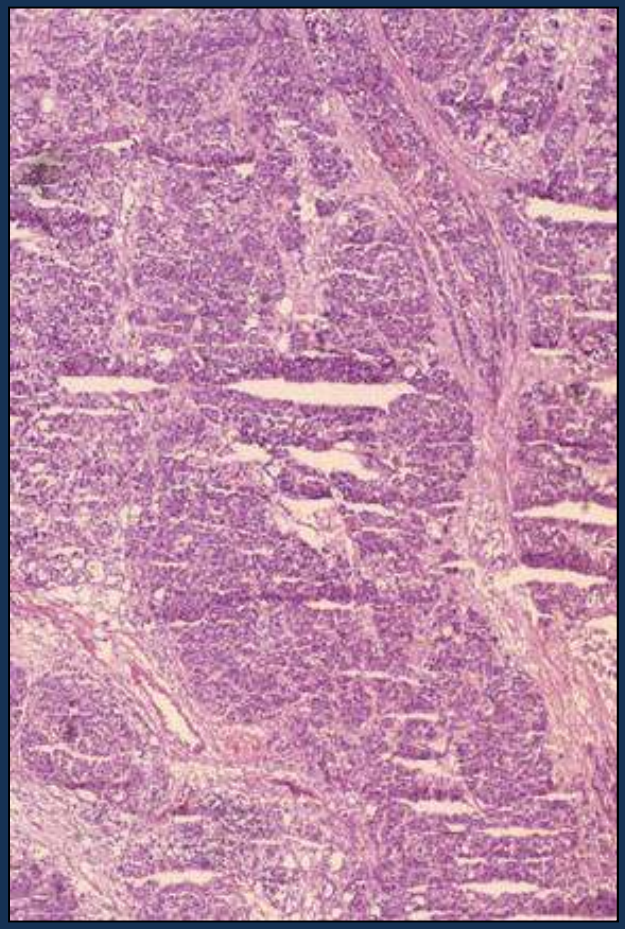
Clinical Validation (predictive)

Oncotype DX® uses RT-PCR Technology for quantitation of mRNA

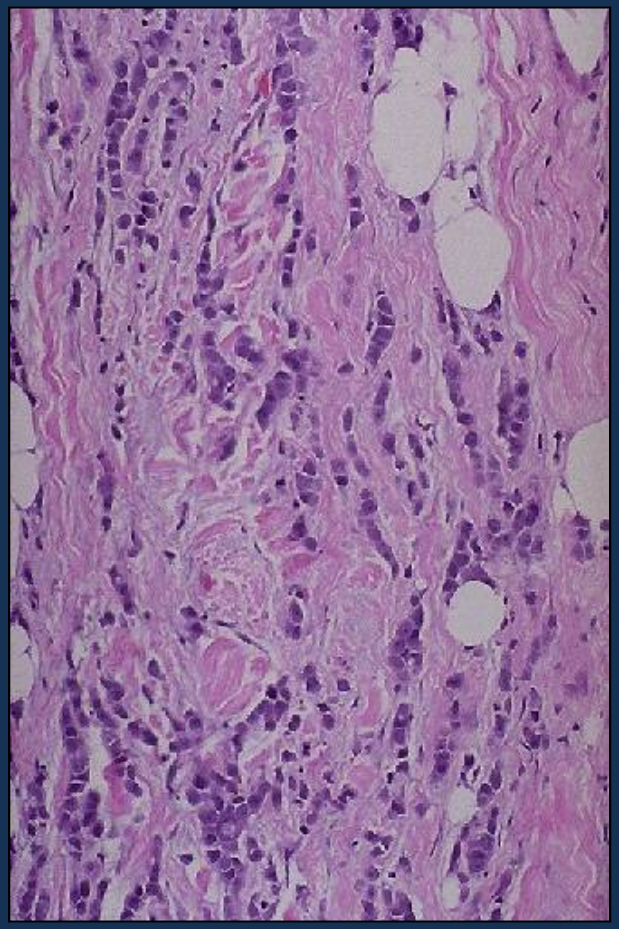


http://people.eku.edu/ritchisong/301images/Protein_synthesis_gov.gif

Morphologic Benefit of Formalin Fixed Paraffin Embedded Tissue



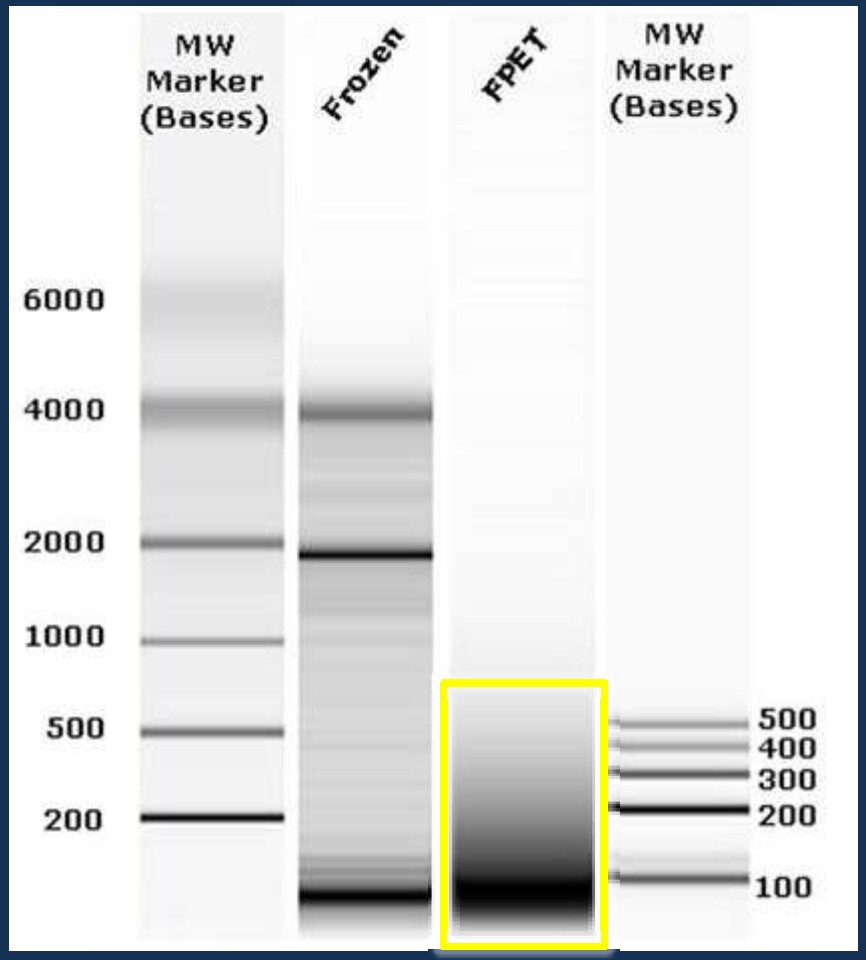
Frozen block
Poor morphology



FPET block
Excellent morphology

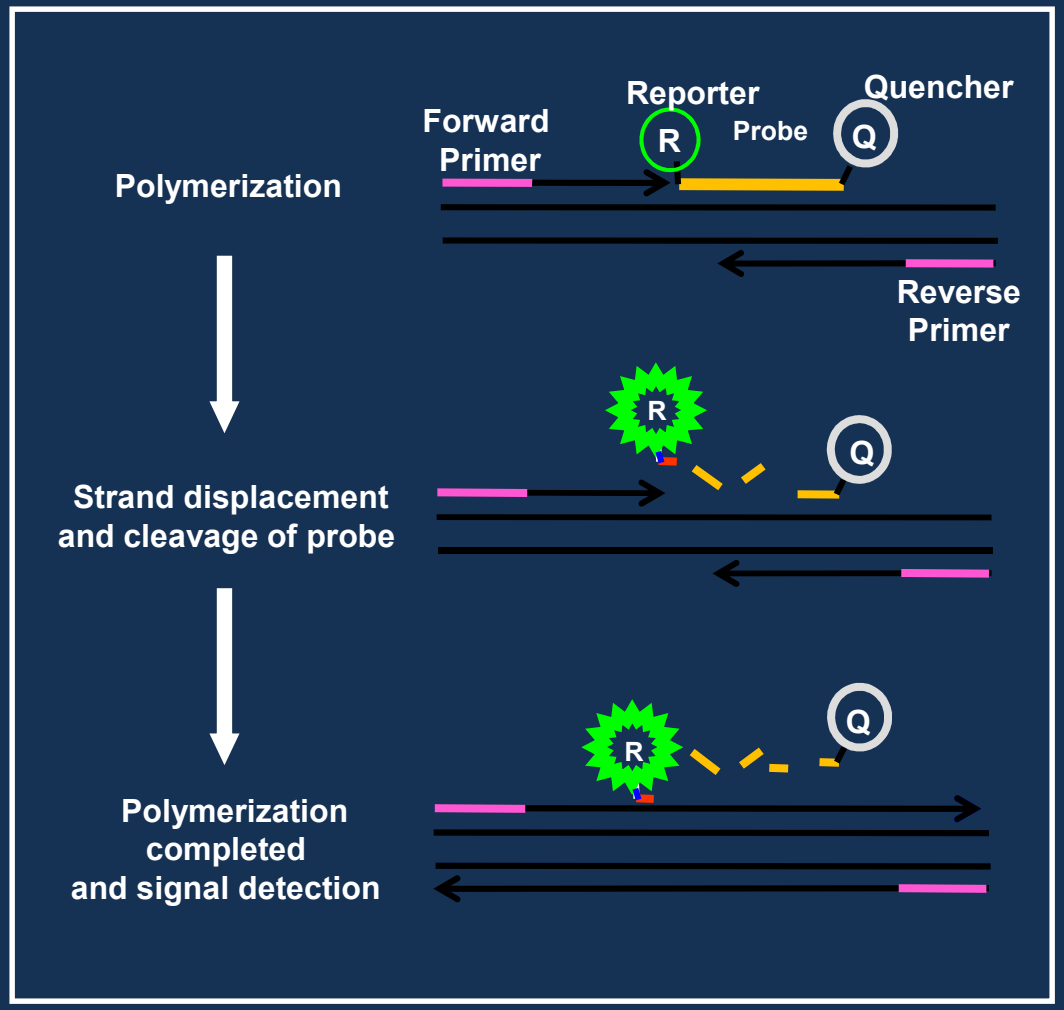
Oncotype DX® Process
Standardized quantitative RT-PCR

- Optimized for the small RNA fragments extracted from fixed paraffin embedded tissue (FPET)
- Optimized to be robust with regard to sources of pre-analytic variability such as
  - Delay to fixation
  - Duration of fixation
  - Fixative type
  - Sample age



Cronin et al. *Am J Pathol.* 2004;164:35-42

Oncotype DX® Uses RT-PCR Technology



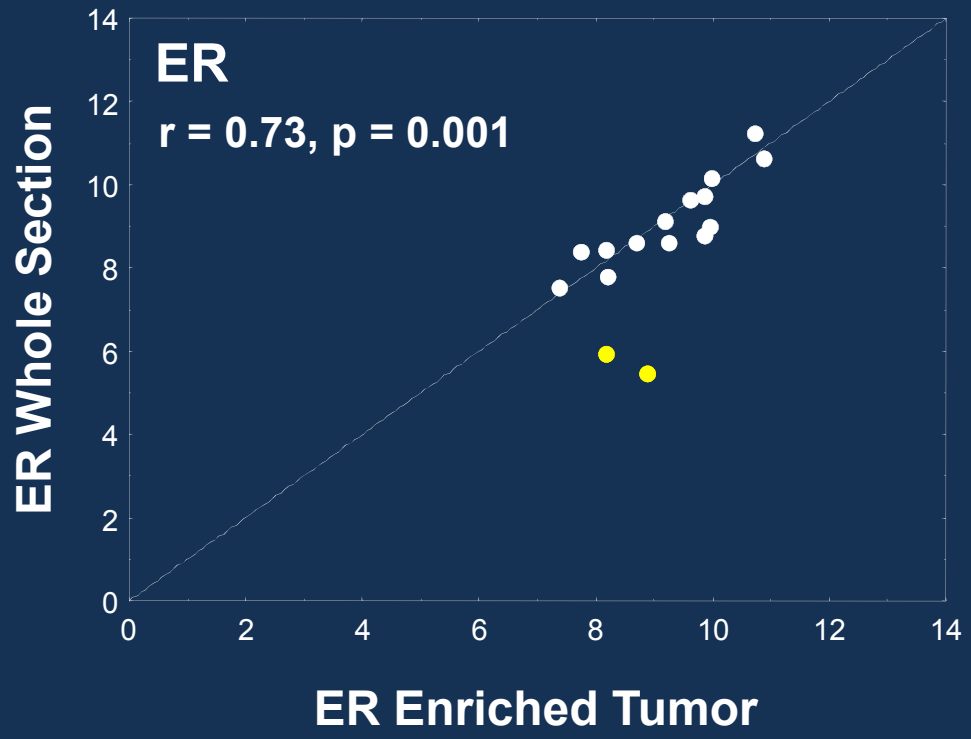
- RT-PCR provides >65,000-fold range of measurement
  - Maximizes ability to discriminate the full range of gene expression differences among individual samples
- RT-PCR reactions can be repeated with high quantitative precision
  - Provides required reliability for individualized reporting
- RT-PCR works well with RNA from formalin-fixed paraffin-embedded tissue

Technical Feasibility Studies were Designed to Assess

- RNA yield and the quality of RNA after extraction from FPET tissues
- Gene expression differences and similarities between whole section and enriched tumor tissue sections
  - To establish criteria for manual microdissection
- Gene expression heterogeneity within breast tumor tissues
  - Assess within block and between block gene expression heterogeneity
- Selection of reference genes (important for normalization of pre-analytical factors)
  - Delay to fixation, duration of fixation, choice of fixative

Importance of Manual Microdissection

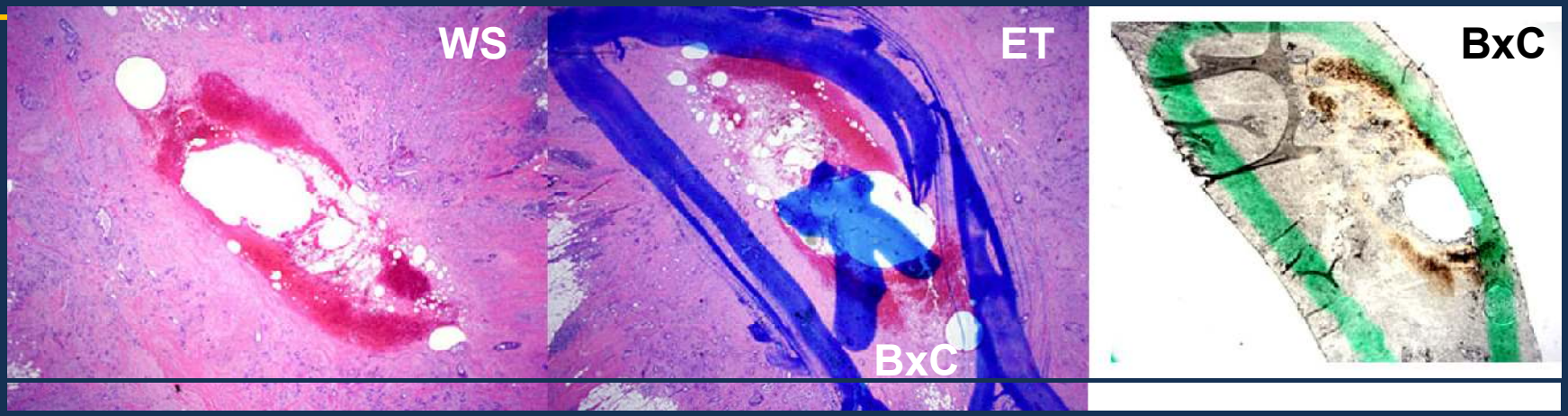
Example from study of 16 breast cancer blocks for ER expression



- **Most cases show minimal differences in ER expression between WS and ET**
- **Some tumors contain significant amounts of non-tumor elements (e.g., biopsy cavities, skin, smooth muscle) which require manual microdissection**
- **Thus, if <50% invasive carcinoma, manual microdissection is always performed**

ER = estrogen receptor
Differences in non-tumor tissue may impact single gene assessment

Importance of Manual Microdissection



A. WS: including BxC before dissection (H&E stained)

B. Marked BxC; ET labeled (H&E stained)

C. BxC after dissection (unstained)

- Of the 16 cancer-related genes there were statistically significant differences in reference normalized gene expression between ET and WS in 12 genes
  - The largest magnitudes of change were in CD68, ER, SCUBE2 and Stromelysin 3

Poster presented at: ASCO Breast Annual Meeting; October, 2009; San Francisco, CA

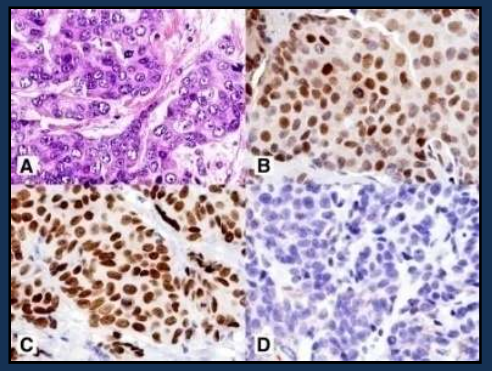
Evaluation of Tumor Gene Expression Heterogeneity

Example of the differences in gene expression within & among 3 FPET blocks from two patients

3 Blocks from spatially distinct tumor regions

Block 1 2 3

ER/PR/HER2 IHC Status



H&E **ER+**

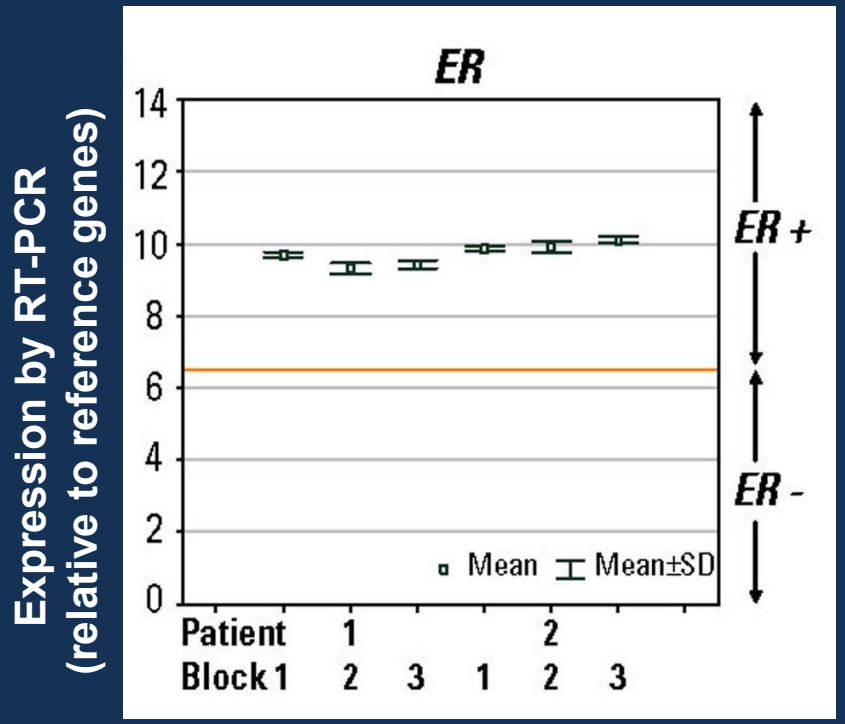
PR+ **HER2-**

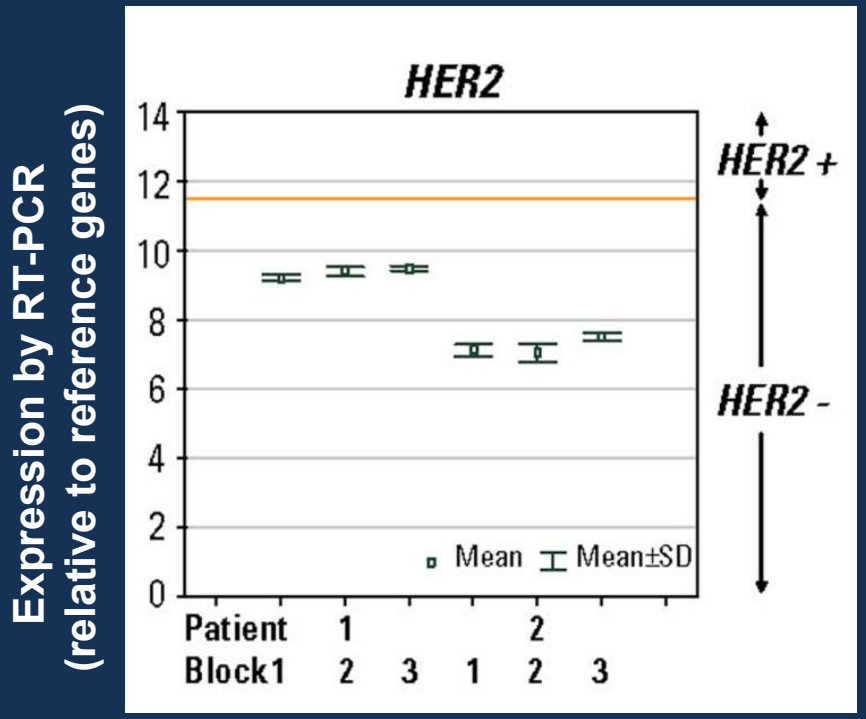
- The three FPET blocks were step sectioned at five different levels
- Quantitative RT-PCR was performed on all 15 samples

Poster presented at: United States-Canadian Academy of Pathology 93rd Annual Meeting; March, 2004; Vancouver, British Columbia.

Importance of Standardized Quantitative Measurement using RT-PCR:

Minimal Gene Expression Heterogeneity Within & Among Tumor Blocks





Reproducibility:

- Within block expression: standard deviation < 0.5 normalized expression units
- Among block expression: standard deviation < 1.0 normalized expression units

Poster presented at: United States-Canadian Academy of Pathology 93rd Annual Meeting; March, 2004; Vancouver, British Columbia.

Oncotype DX® Technology
Development Overview

Technical Feasibility

Gene Discovery & Refinement

Analytical Validation

Clinical Validation (prognostic)

Clinical Validation (predictive)

Oncotype DX® Gene Panel Was Developed from Clinical Trial Evidence

- 250 cancer-related genes were selected from a number of sources:
  - Scientific literature, microarray data, genomic databases, molecular biology
- Genes were analyzed for expression and relapse-free interval correlations across 3 independent studies of 447 breast cancer patients

Study Site	N	Node Status	ER Status	Treatment
NSABP B-20, Pittsburgh, PA	233	N-	ER+	Tamoxifen (100%)
Rush University, Chicago, IL	78	≥10 positive nodes	ER+/-	Tamoxifen (54%) Chemotherapy (80%)
Providence St. Joseph's Hospital, Burbank, CA	136	N+/-	ER+/-	Tamoxifen (41%) Chemotherapy (39%)

From these studies 21 genes were selected

Paik et al. SABCS 2003. Abstract #16.
Cobleigh et al. *Clin Cancer Res.* 2005;11(24 Pt 1):8623-8631.
Esteban et al. *Proc ASCO 2003.* Abstract #3416.

Oncotype DX® Recurrence Score

Calculated from 21 Different Genes

16 CANCER RELATED GENES

Estrogen	Proliferation	HER2	Invasion	Others
ER PR Bcl2 SCUBE2	Ki-67 STK15 Survivin Cyclin B1 MYBL2	GRB7 HER2	Stromelysin 3 Cathepsin L2	CD68 GSTM1 BAG1

5 REFERENCE GENES

Beta-actin	GAPDH	RPLPO	GUS	TFRC

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX® Recurrence Score Calculation and Risk Categories

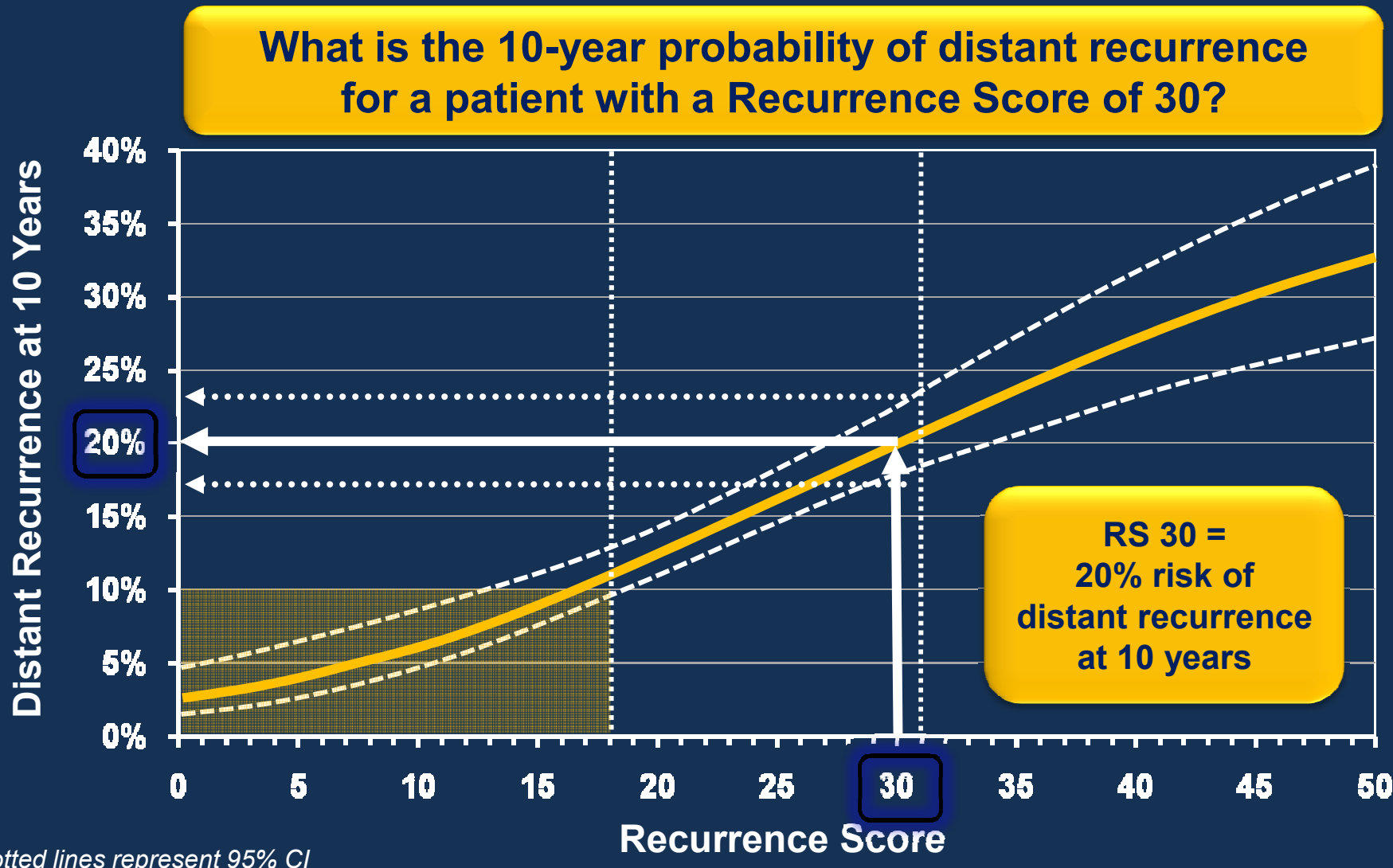
Recurrence Score =

+ 0.47 x HER2 Group Score
- 0.34 x Estrogen Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

Risk Group	*Recurrence Score*
Low risk	<18
Intermediate risk	18 - 30
High risk	≥31

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

The Oncotype DX® Recurrence Score is a Continuous Predictor of Recurrence Risk



Agenda
Development Overview

Technical Feasibility

Gene Discovery & Refinement

Analytical Validation

Clinical Validation (prognostic)

Clinical Validation (predictive)

Oncotype DX® is Analytically Validated

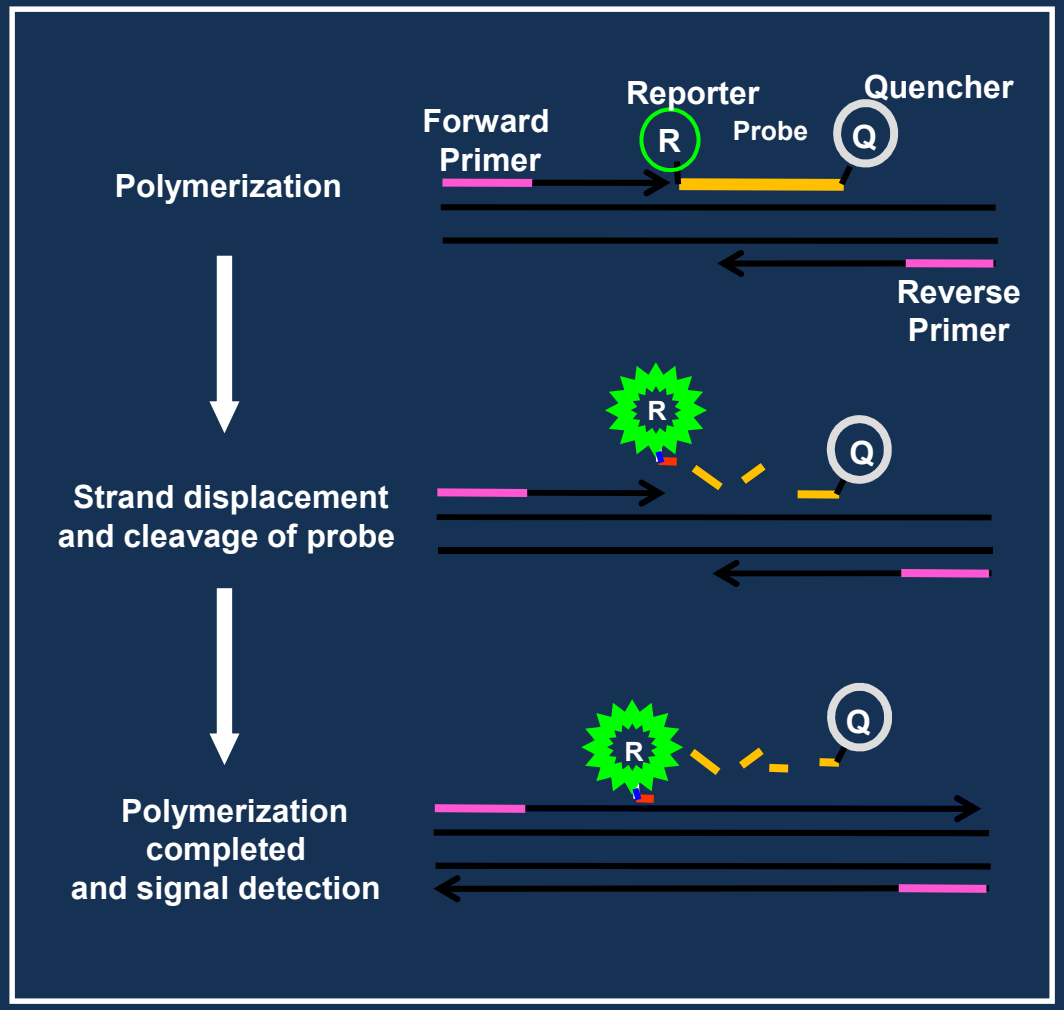
Analytical validation is the assessment of assay performance characteristics and the optimal conditions to generate accuracy, precision and reproducibility

Elements of Analytic Validation

- Analytical sensitivity (limits of detection and quantitation)
- Assay precision and linear dynamic range
- Analytical reproducibility
- PCR amplification efficiency
- Sample and reagent stability
- Reagent calibration
- Instrument validation and calibration

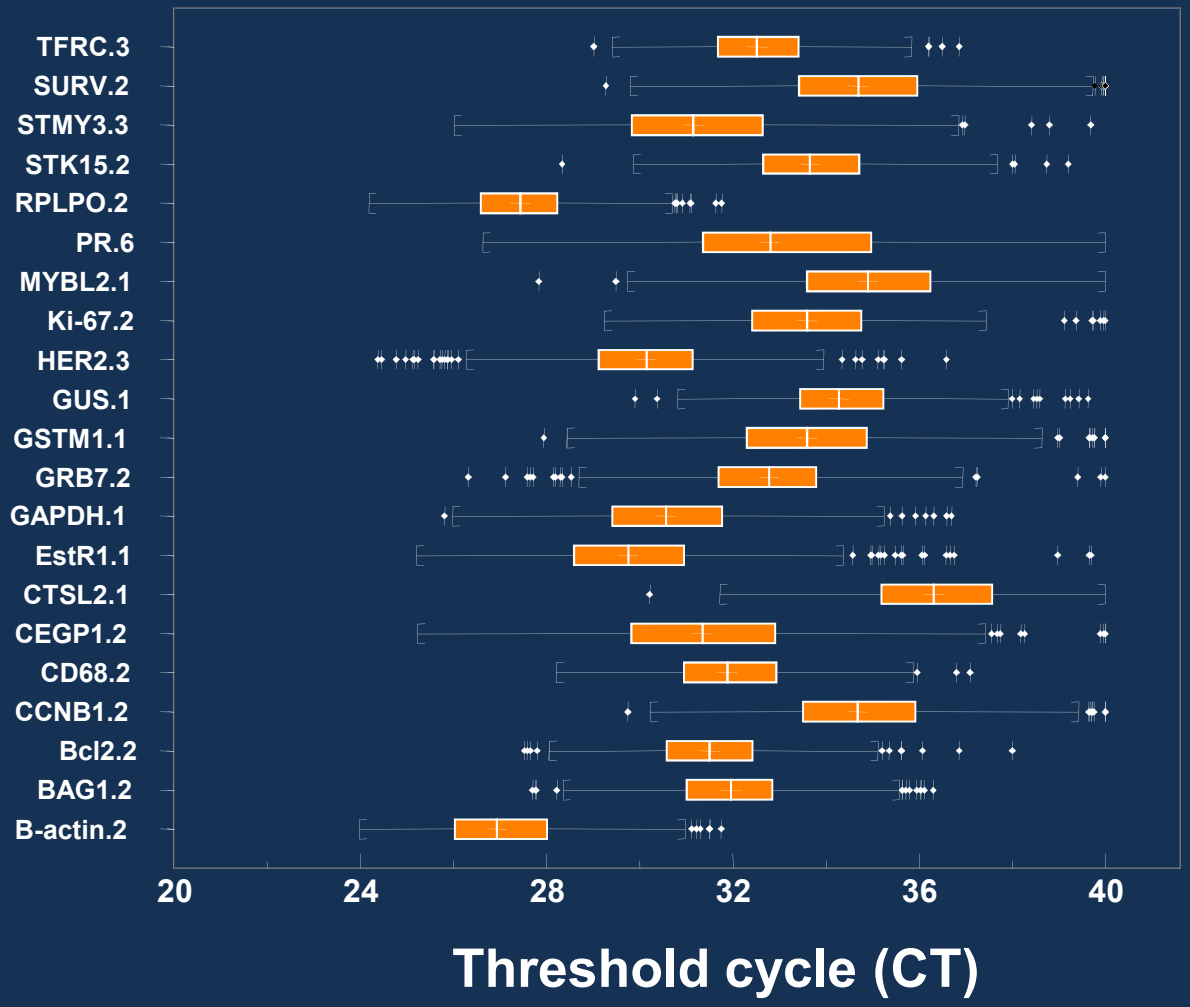
Chau CH, et al. *Clin Cancer Res.* 2008;14(19):5967-5976.

Oncotype DX® Uses RT-PCR Technology



- RT-PCR provides >65,000-fold range of measurement
  - Maximizes ability to discriminate the full range of gene expression differences among individual samples
- RT-PCR reactions can be repeated with high quantitative precision
  - Provides required reliability for individualized reporting
- RT-PCR works well with RNA from formalin-fixed paraffin-embedded tissue

RT-PCR Process Used by Oncotype DX® Has a Wide Dynamic Range



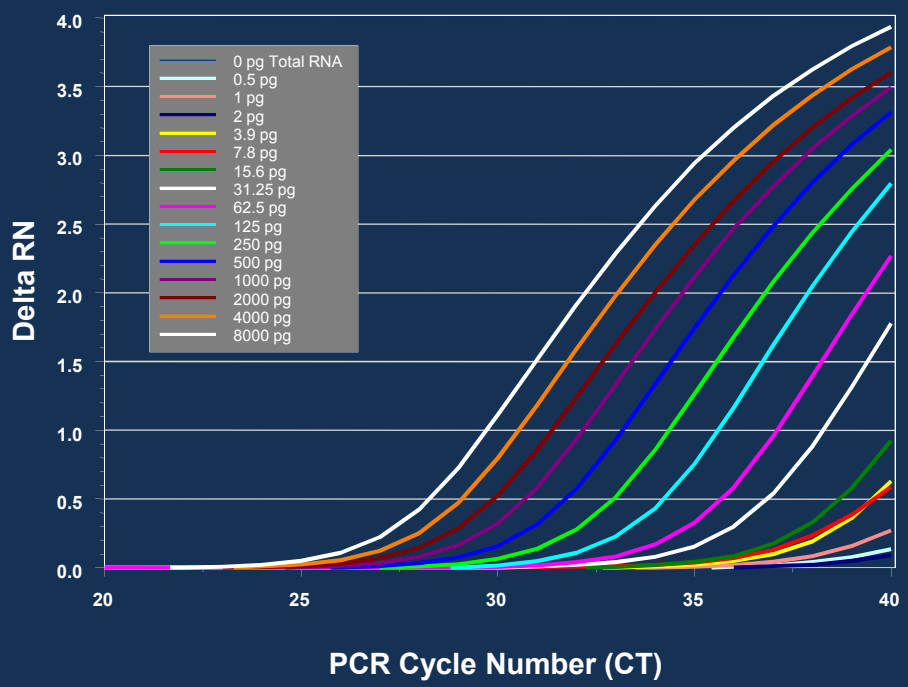
Dynamic range of quantitative expression for 21 Oncotype DX genes

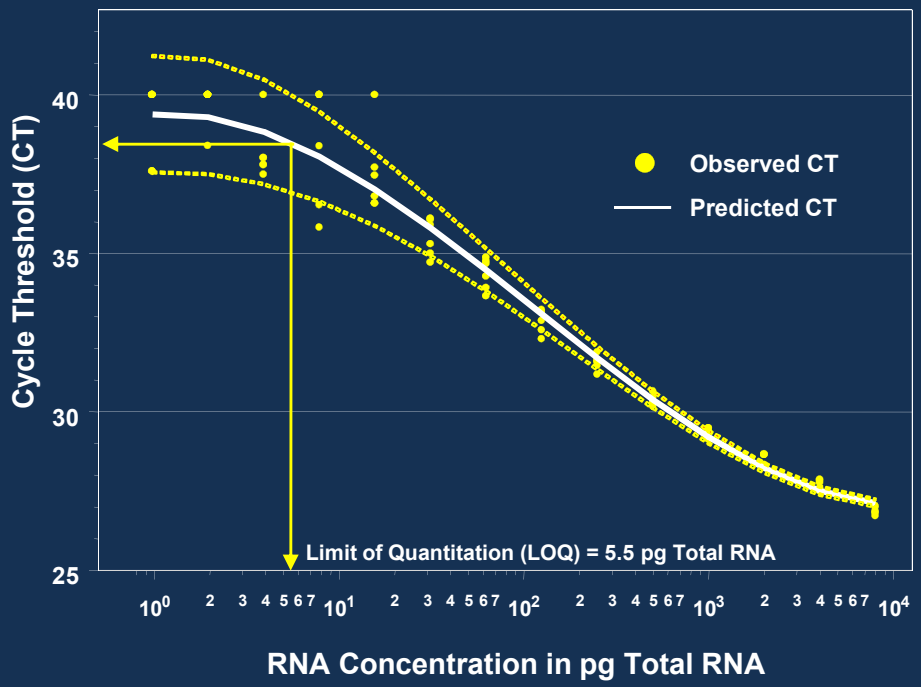
2^{16} = 65,536 fold

Oncotype DX® Technology

Assay Sensitivity

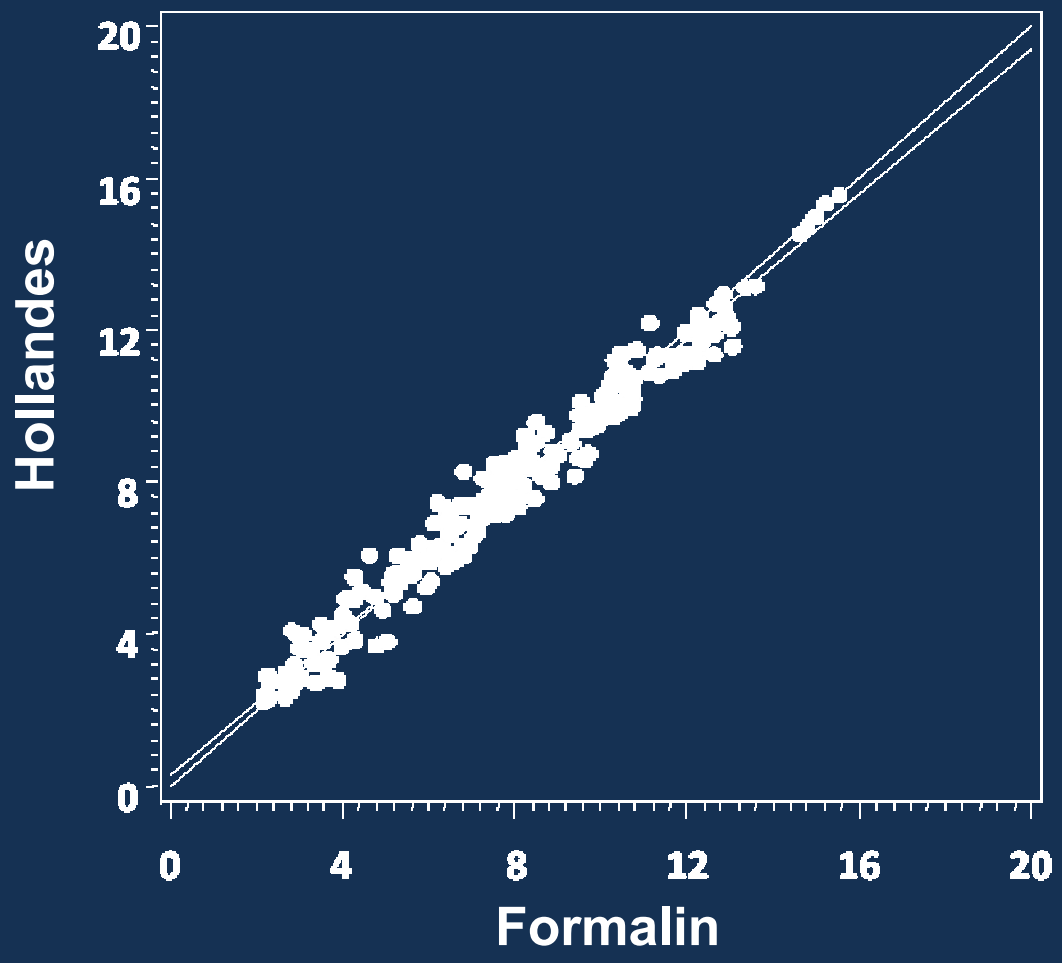
(RT-PCR Analysis) - LOQ for HER2 mRNA





Increasing amounts of total RNA quantified using gene-specific primers/probes for HER2

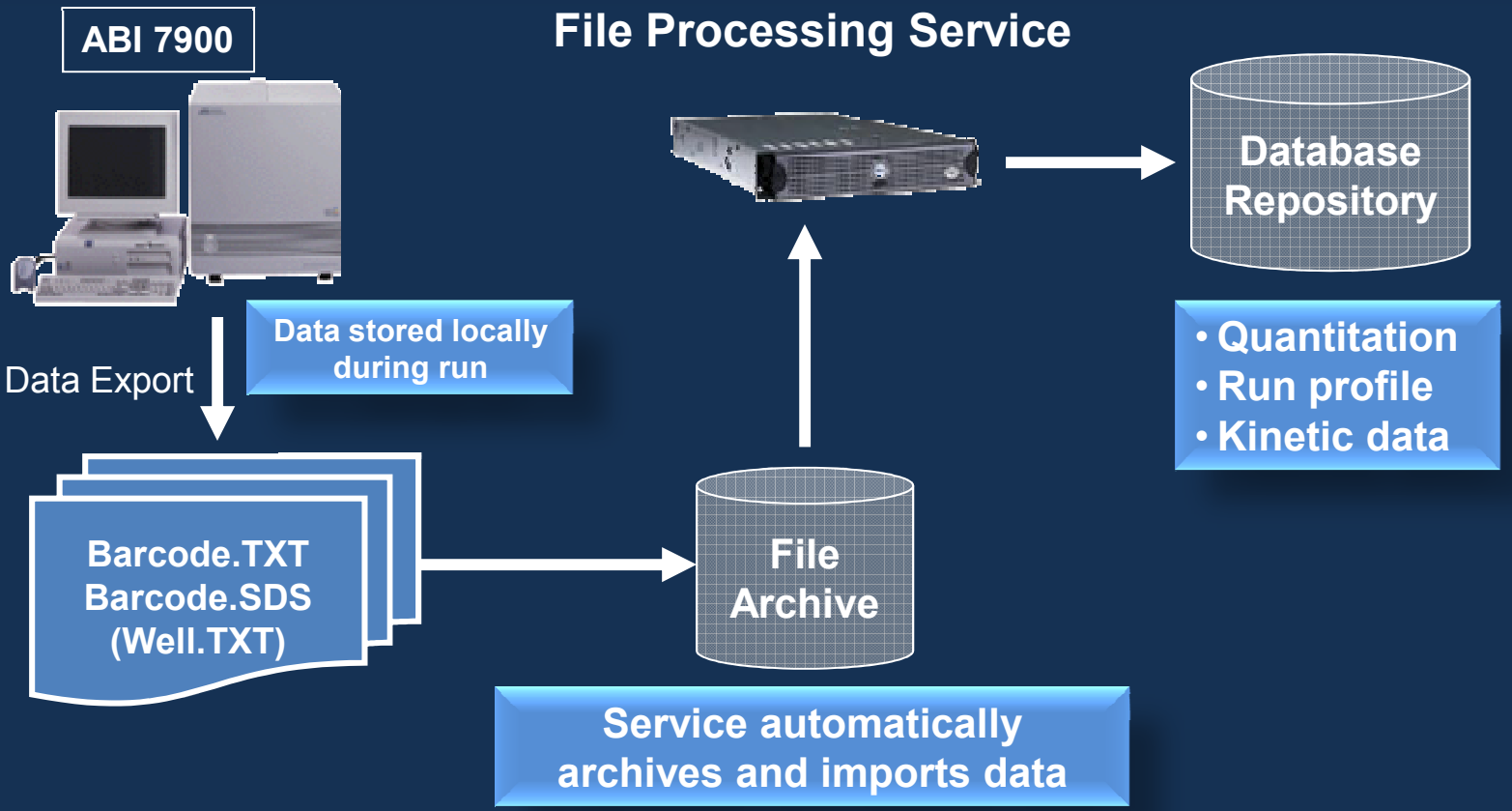
Normalization Accounts for All Sources of Preanalytic Variability



- Delays to fixation, duration of fixation, different fixatives and sample age can affect RNA quality
- Reference normalization compensates for these differences in sample processing and sample age

Quantitative PCR Data Acquisition

Data Import Services acquire, validate, and load data as laboratory runs complete



Oncotype DX® Assay Process Steps

1) **PRE-ANALYTIC**
   - Pathology review of the FPET sample by a Board Certified Anatomic Pathologist with breast surgical pathology expertise
   - Determine whether manual microdissection for tumor enrichment is required (~40% of submissions are microdissected for tumor enrichment)

2) **ANALYTIC**
   - RNA extraction and quantitation (Ribogreen® method)
   - qPCR test for residual genomic DNA
   - Reverse transcription
   - TaqMan PCR
   - Data quality control

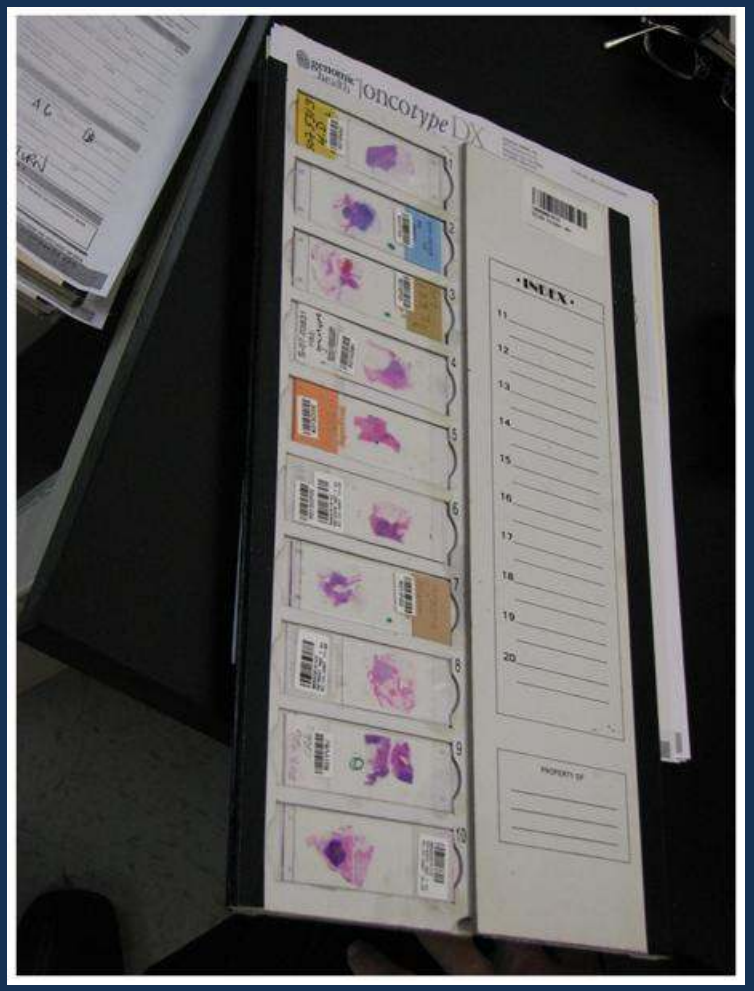
3) **POST-ANALYTIC**
   - Calculation of Recurrence Score®
   - Report preparation and approval

Pre-analytic Processing

All FPET blocks are barcoded before entering histology

Pre-analytic Processing

All tumors assessed by surgical pathologists with breast expertise



Pathology review to assess:

- **Is tumor present?**
- **Is there sufficient tumor?**

Patient Samples are Barcode Tracked from Submission to Report

Automation is Central to Laboratory Processes

Patient Sample Tracking

LIMS bar-coding integrates reagents and robots for tracking and process control

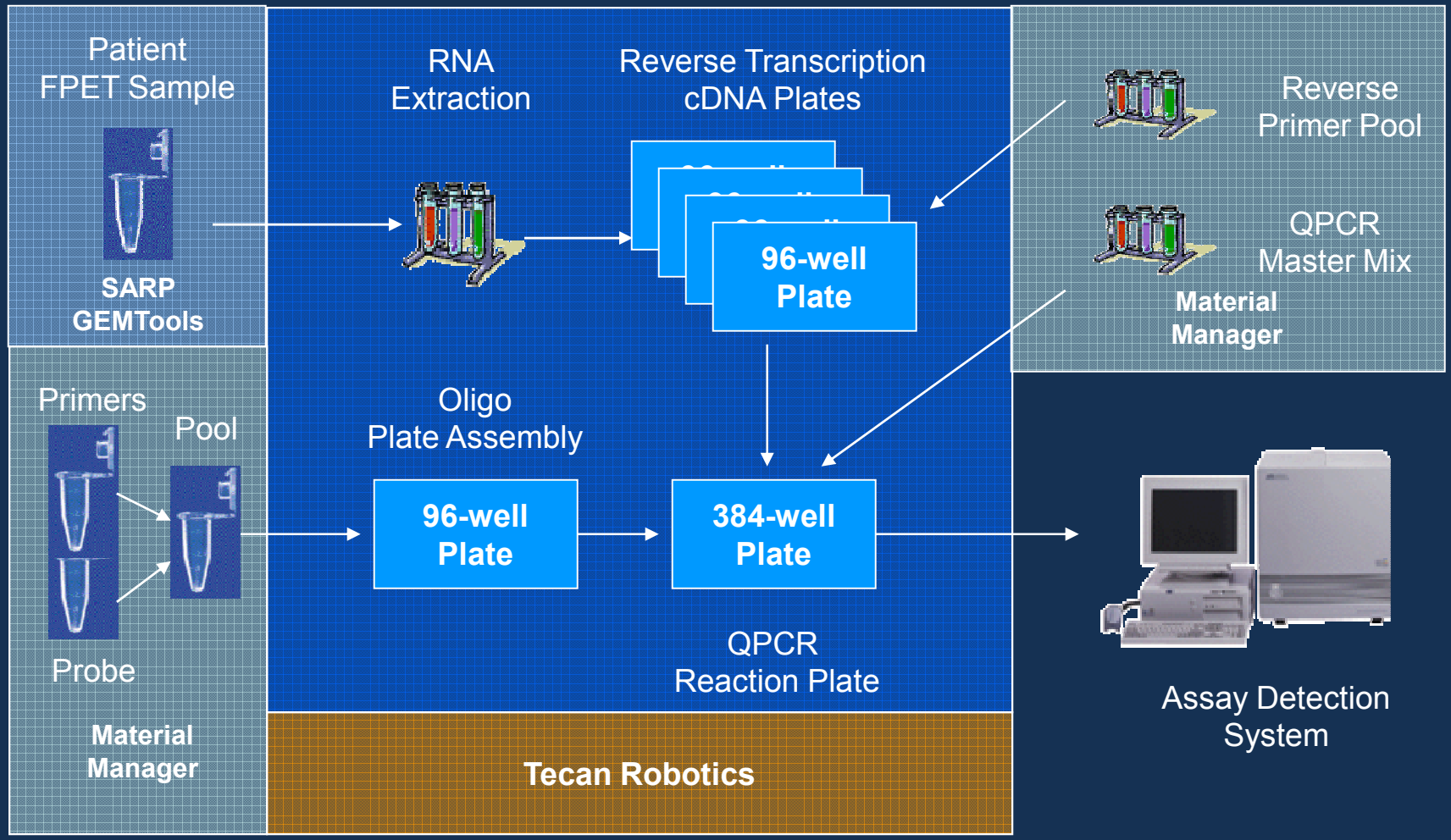
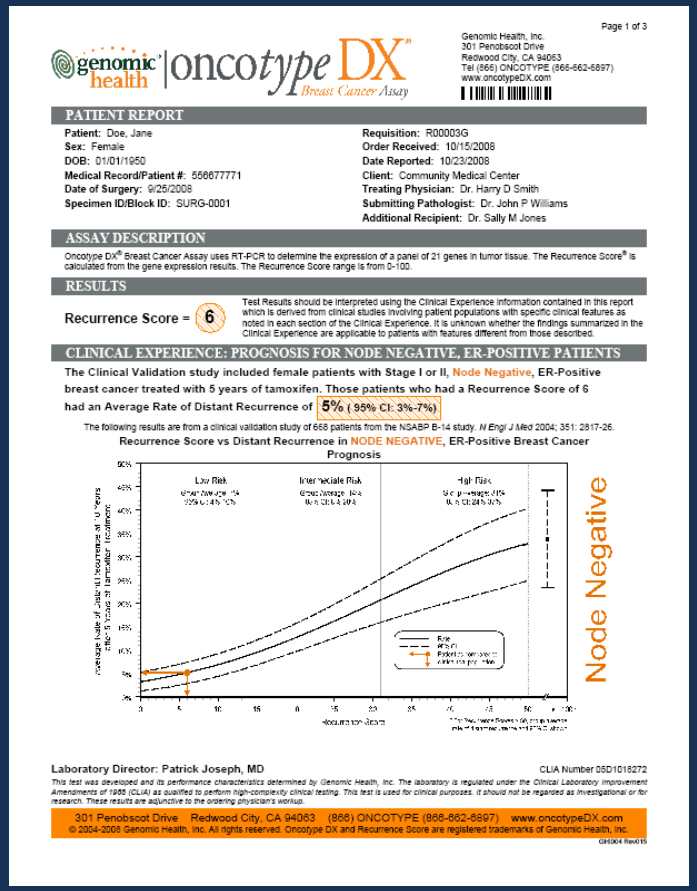


Plate Layout Template and Assembly for Primers and Samples

Patient Report Delivery

Automated Output and Delivery



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genomic health | oncotype DX® Breast Cancer Assay

Genomic Health, Inc.
301 Penobscot Drive
Redwood City, CA 94063
Tel (866) ONCOTYPE (866-662-6897)
www.oncotypeDX.com

PATIENT REPORT

Patient: Doe, Jane
Sex: Female
DOB: 01/01/1950
Medical Record/Patient #: 556677771
Date of Surgery: 9/25/2008
Specimen ID/Block ID: SURG-0001

Requisition: R00003G
Order Received: 10/15/2008
Date Reported: 10/23/2008
Client: Community Medical Center
Treating Physician: Dr. Harry D Smith
Submitting Pathologist: Dr. John P Williams
Additional Recipient: Dr. Sally M Jones

ASSAY DESCRIPTION

Oncotype DX® Breast Cancer Assay uses RT-PCR to determine the expression of a panel of 21 genes in tumor tissue. The Recurrence Score® is calculated from the gene expression results. The Recurrence Score range is from 0-100.

RESULTS

Recurrence Score = 6

Test Results should be interpreted using the Clinical Experience information contained in this report which is derived from clinical studies involving patient populations with specific clinical features as noted in each section of the Clinical Experience. It is unknown whether the findings summarized in the Clinical Experience are applicable to patients with features different from those described.

CLINICAL EXPERIENCE: PROGNOSIS FOR NODE NEGATIVE, ER-POSITIVE PATIENTS

The Clinical Validation study included female patients with Stage I or II, Node Negative, ER-Positive breast cancer treated with 5 years of tamoxifen. Those patients who had a Recurrence Score of 6 had an Average Rate of Distant Recurrence of 5% (95% CI: 3%-7%)

The following results are from a clinical validation study of 668 patients from the NSABP B-14 study. *N Engl J Med* 2004; 351: 2817-26.

Recurrence Score vs Distant Recurrence in NODE NEGATIVE, ER-Positive Breast Cancer Prognosis

Laboratory Director: Patrick Joseph, MD
CLIA Number 05D1018272

This test was developed and its performance characteristics determined by Genomic Health, Inc. The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. These results are adjunctive to the ordering physician's workup.

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GH004 Rev015

PDF Report
w/ Electronic Signature Approval

Report Distribution Service

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- **Web**

Agenda
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Technical Feasibility

Gene Discovery & Refinement

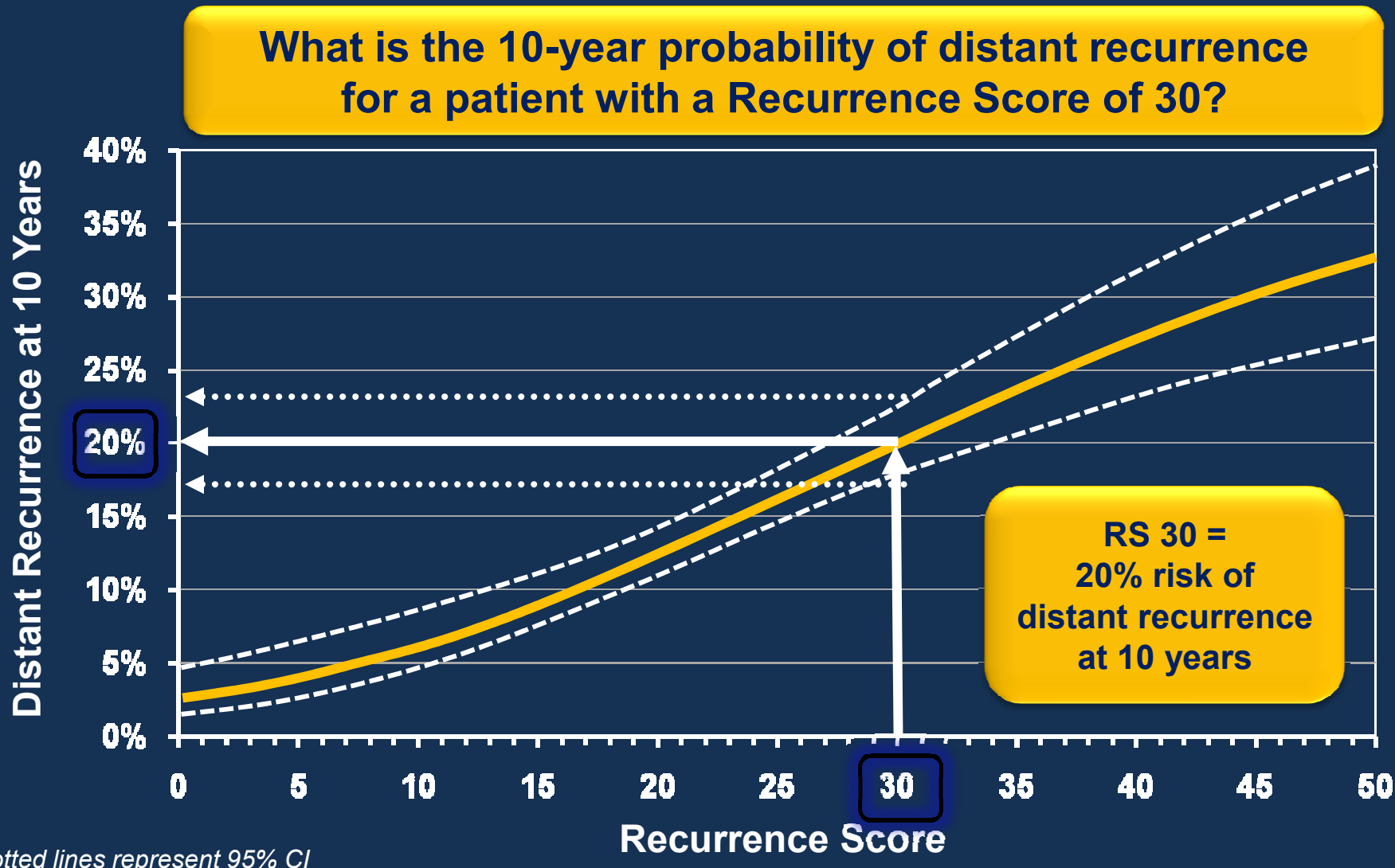
Analytical Validation

Clinical Validation (prognostic)

Clinical Validation (predictive)

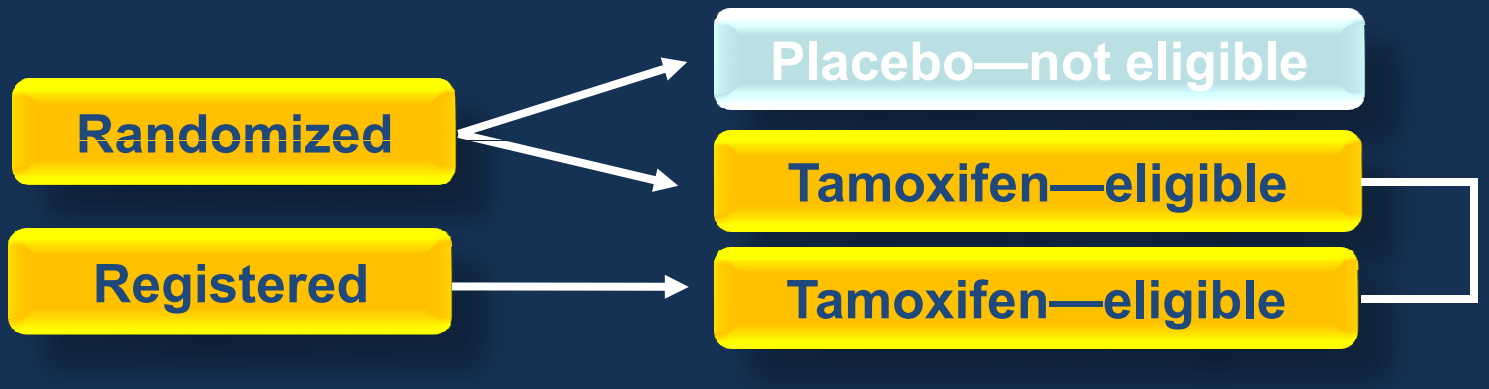
Clinical Validation of Onco*type* DX®
in Node Negative Disease

Validation of the Oncotype DX® Recurrence Score as a Continuous Predictor of Recurrence Risk



Oncotype DX® Clinical Validation: NSABP B-14

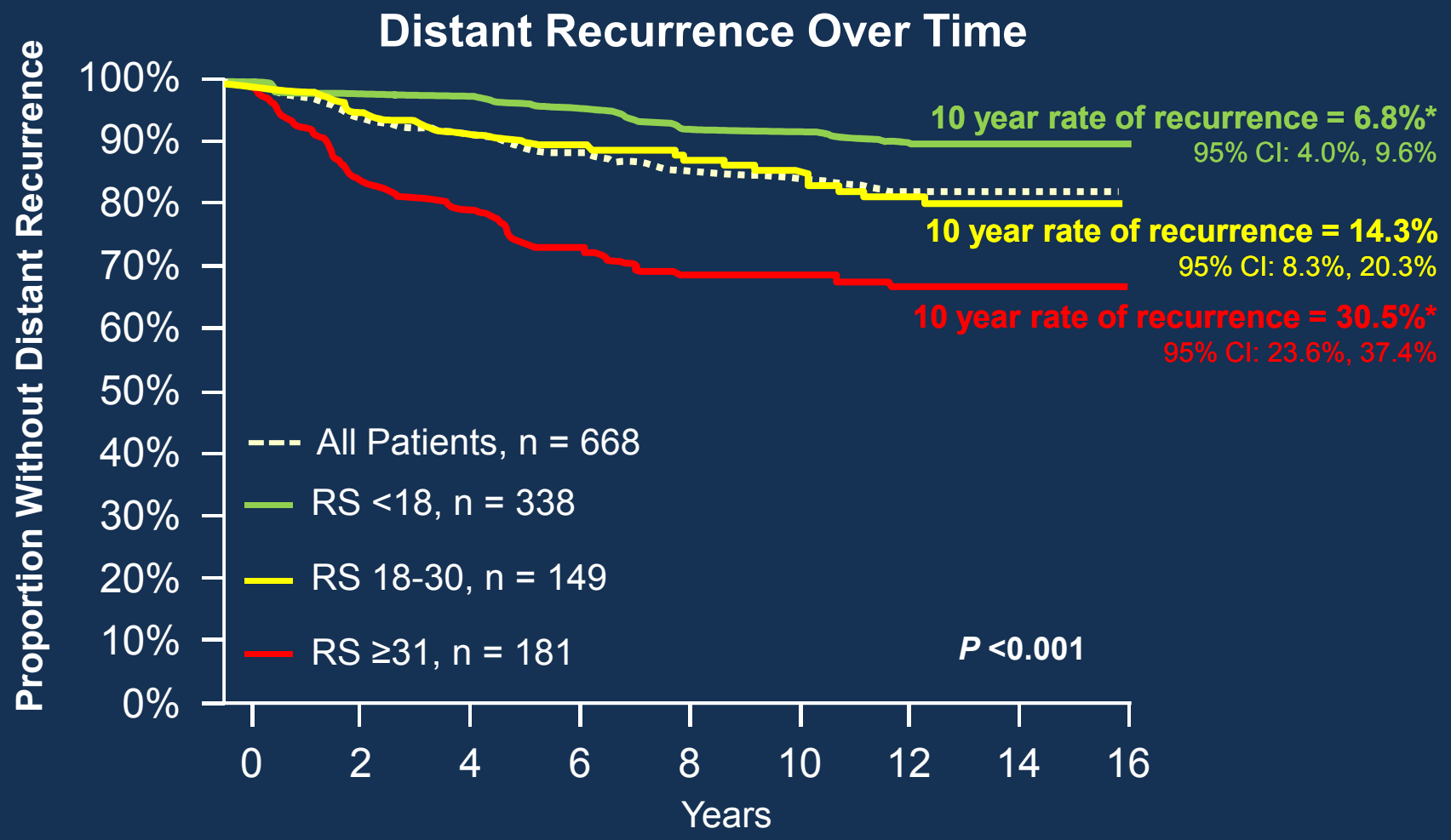
- Objective: Prospectively validate Recurrence Score as predictor of distant recurrence in N–, ER+ patients



- Multicenter study with pre-specified 21-gene assay, algorithm, endpoints, analysis plan

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX® Clinical Validation:
NSABP B-14 – Distant Recurrence



*10-year Distant Recurrence comparison between low-and high-risk groups: *P* <0.001

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

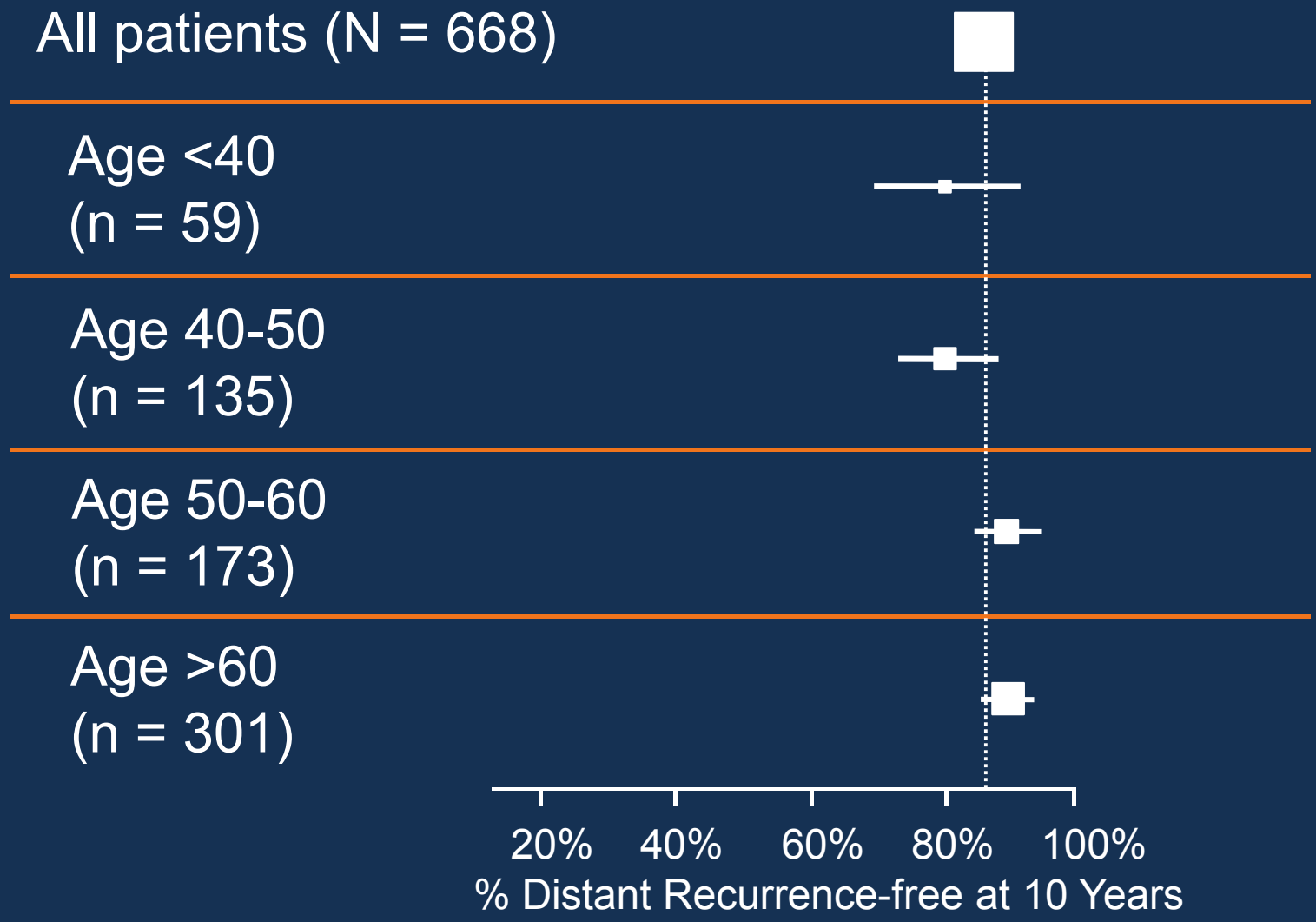
Multivariate Cox Proportional Hazards Regression of Age, Tumor Size, Tumor Grade and Recurrence Score in Relation to Likelihood of Distant Recurrence (NSABP B-14)

Variable	Analysis without Recurrence Score		Analysis with Recurrence Score	
	P value	HR	*P* value	HR
Age at surgery	0.1	0.7	0.22	0.76
Clinical tumor size	0.13	1.35	0.38	1.19
Tumor grade				
Moderately differentiated	0.04	1.87	0.15	1.55
Poorly differentiated	<0.001	5.14	<0.001	3.34
HER2 amplification	0.89	1.04	0.06	0.51
Estrogen-receptor protein				
50-99 fmol/mg	0.23	0.71	0.32	0.75
100-199 fmol/mg	0.38	0.78	0.72	0.9
>200 fmol/mg	0.9	0.97	0.94	1.02
	-	-	<0.001	2.81

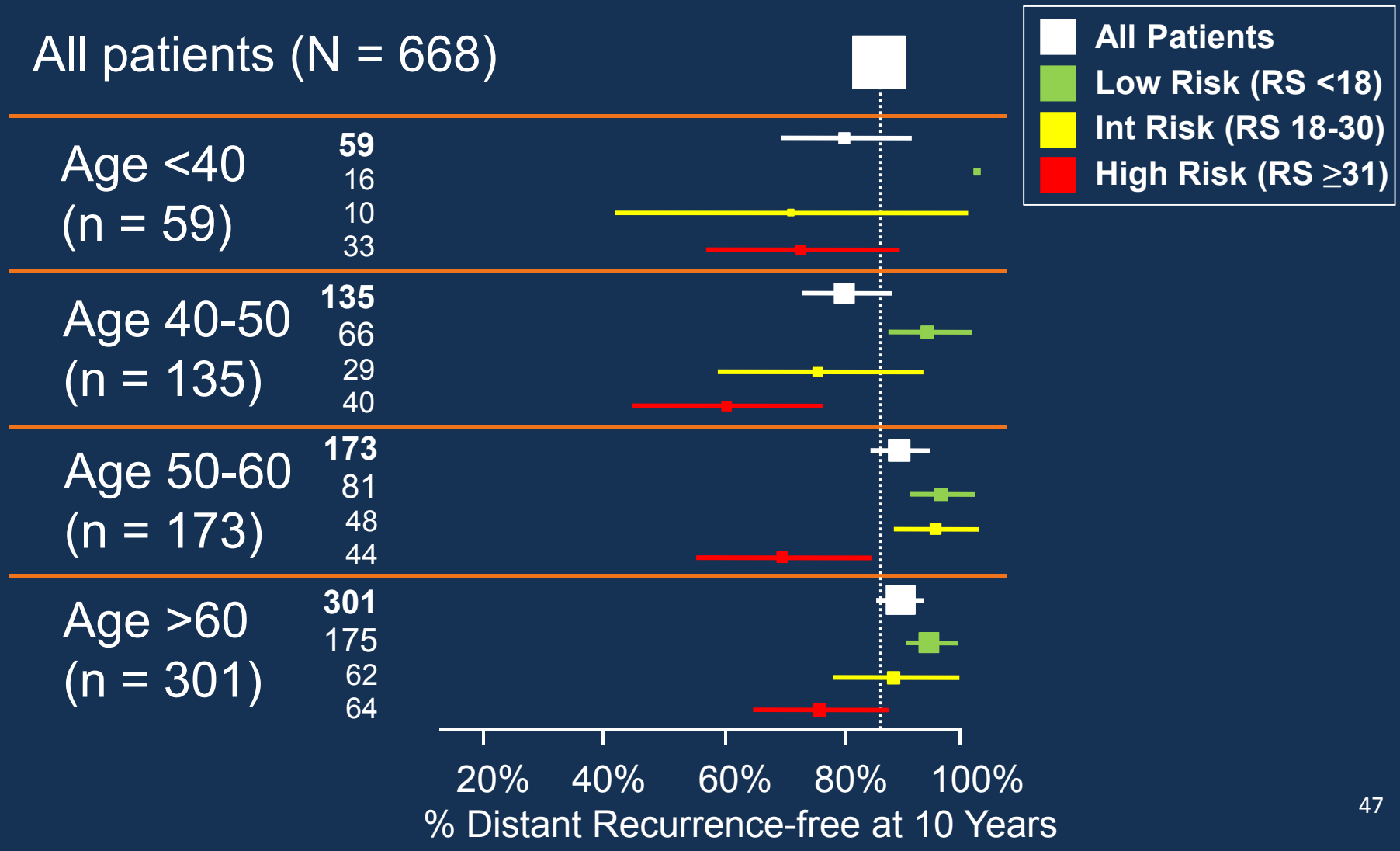
Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX®
NSABP B-14 Subgroup Analysis

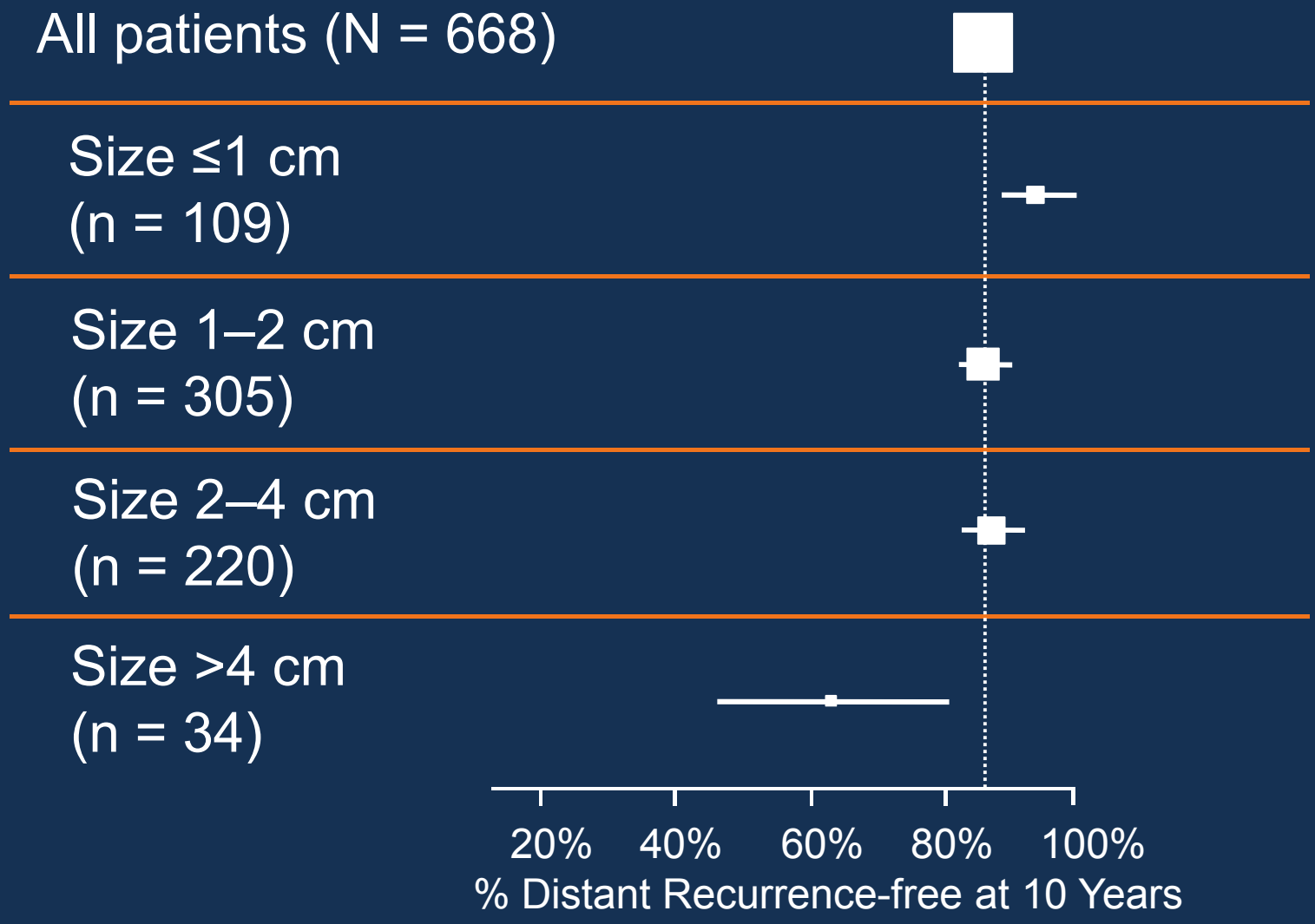
Oncotype DX® NSABP B-14: Patient Age Subgroups



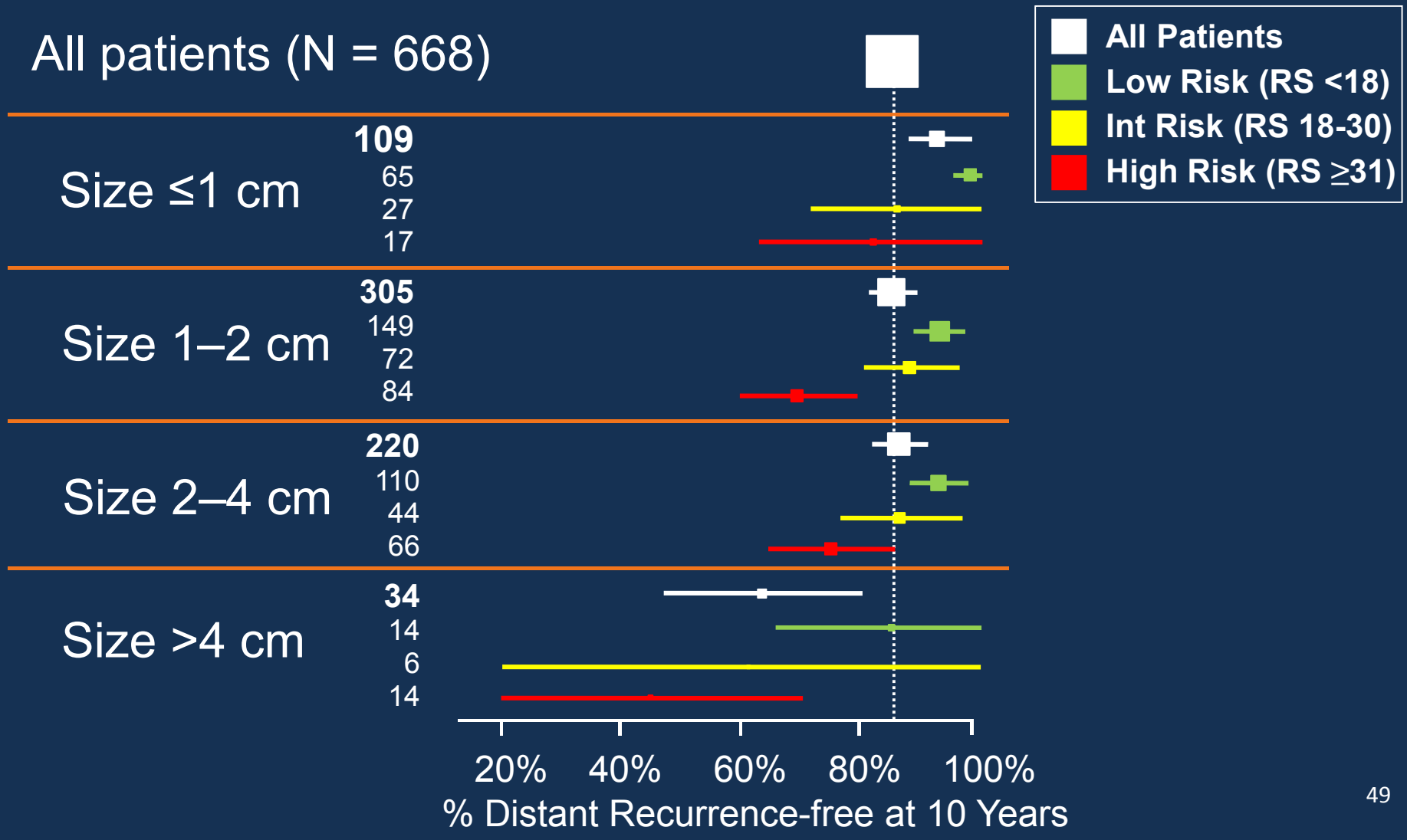
Oncotype DX® NSABP B-14: RS Subgroups by Patient Age



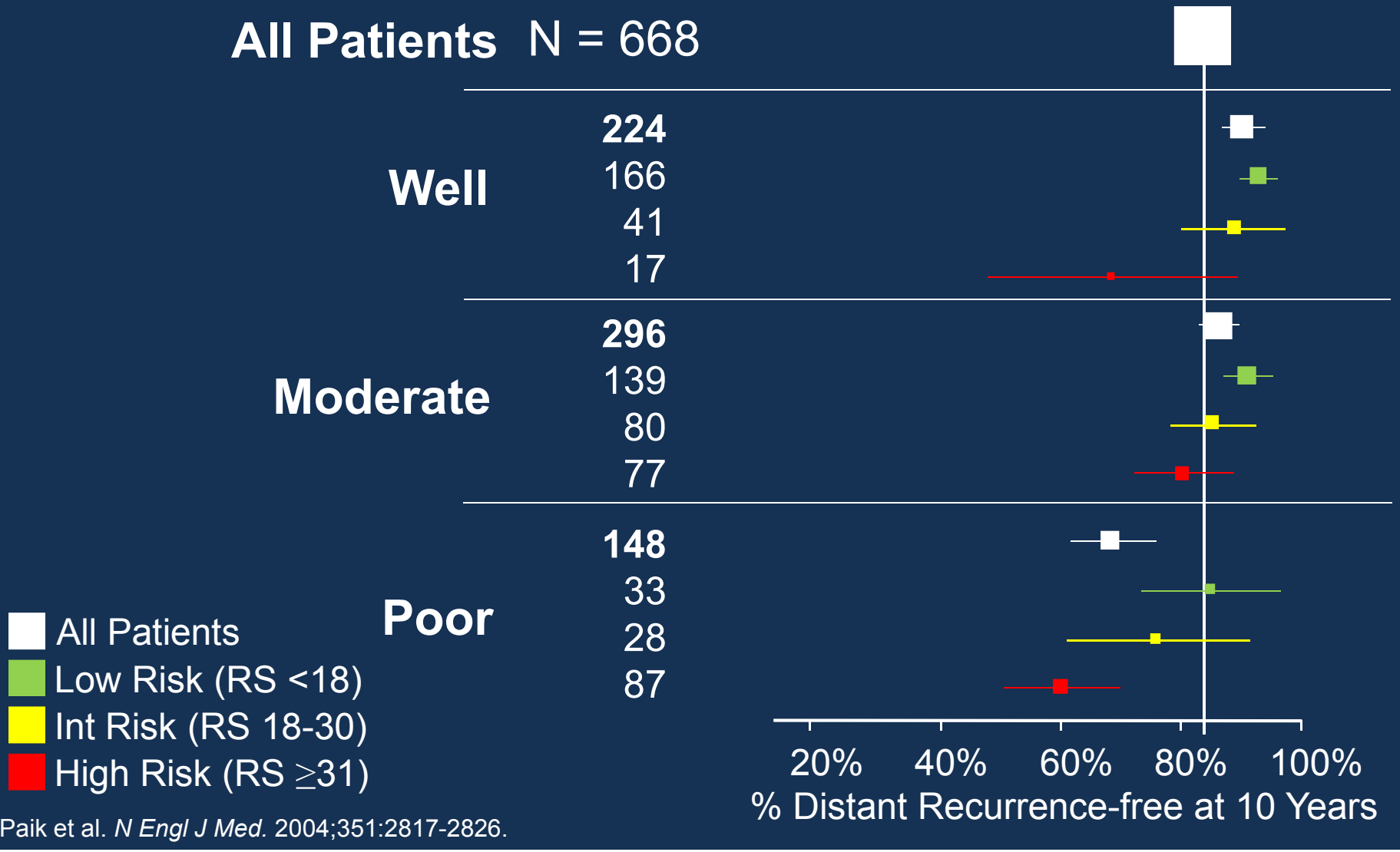
Oncotype DX® NSABP B-14: Tumor Size Subgroups



Oncotype DX® NSABP B-14: RS Subgroups by Tumor Size



Oncotype DX® NSABP B-14: RS Subgroups by Tumor Grade



Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX® Clinical Validation: Conclusions – NSABP B-14

- Oncotype DX® RS validated as predictor of recurrence in node-negative, ER+ patients
- Oncotype DX® RS performance exceeds standard measures (patient age, tumor size, and tumor grade)
- Oncotype DX® RS (based on tumor gene expression) more accurately quantifies the risk of distant recurrence than do the NCCN guidelines (based on patient age, tumor size, and tumor grade)

Oncotype DX® Clinical Validation: The Kaiser Permanente Study

Study Design	Matched case-control
Study Population (N = 4964)	Kaiser Permanente patients <75 years old in 14 Northern California hospitals diagnosed with node-negative breast cancer between 1985-1994, no adjuvant chemotherapy Cases: Deaths from BC (n = 220) Controls: Randomly selected, matched on age, race, diagnosis year, KP facility, tamoxifen (n = 570)
Data Sources	Cancer registry, medical records, archived diagnostic slides, and tumor blocks

Habel LA, et al. *Breast Cancer Res*. 2006;8(3):R25.

The Kaiser Permanente Study:
Risk of BC Death at 10 Years: ER+, Tam-treated Patients

Risk Classification (Recurrence Score)	10-year Absolute Risk[1] Kaiser	10-year Absolute Risk[1] NSABP B-14
Low	2.8%	3.1%
Intermediate	10.7%	12.2%
High	15.5%	27.0%

[1]*Based on methods by Langholz and Borgan, Biometrics 1997;53:767-774.*

- The RS has now been shown to be strongly associated with risk of breast cancer-specific mortality among LN–, ER+, tam-treated patients participating in a clinical trial and among similar patients from the community setting.
- Results from our study suggest that combining Recurrence Score, tumor grade, and tumor size provides better risk classification than any one of these factors alone.

Habel LA, et al. *Breast Cancer Res*. 2006;8(3):R25.

Agenda
Development Overview

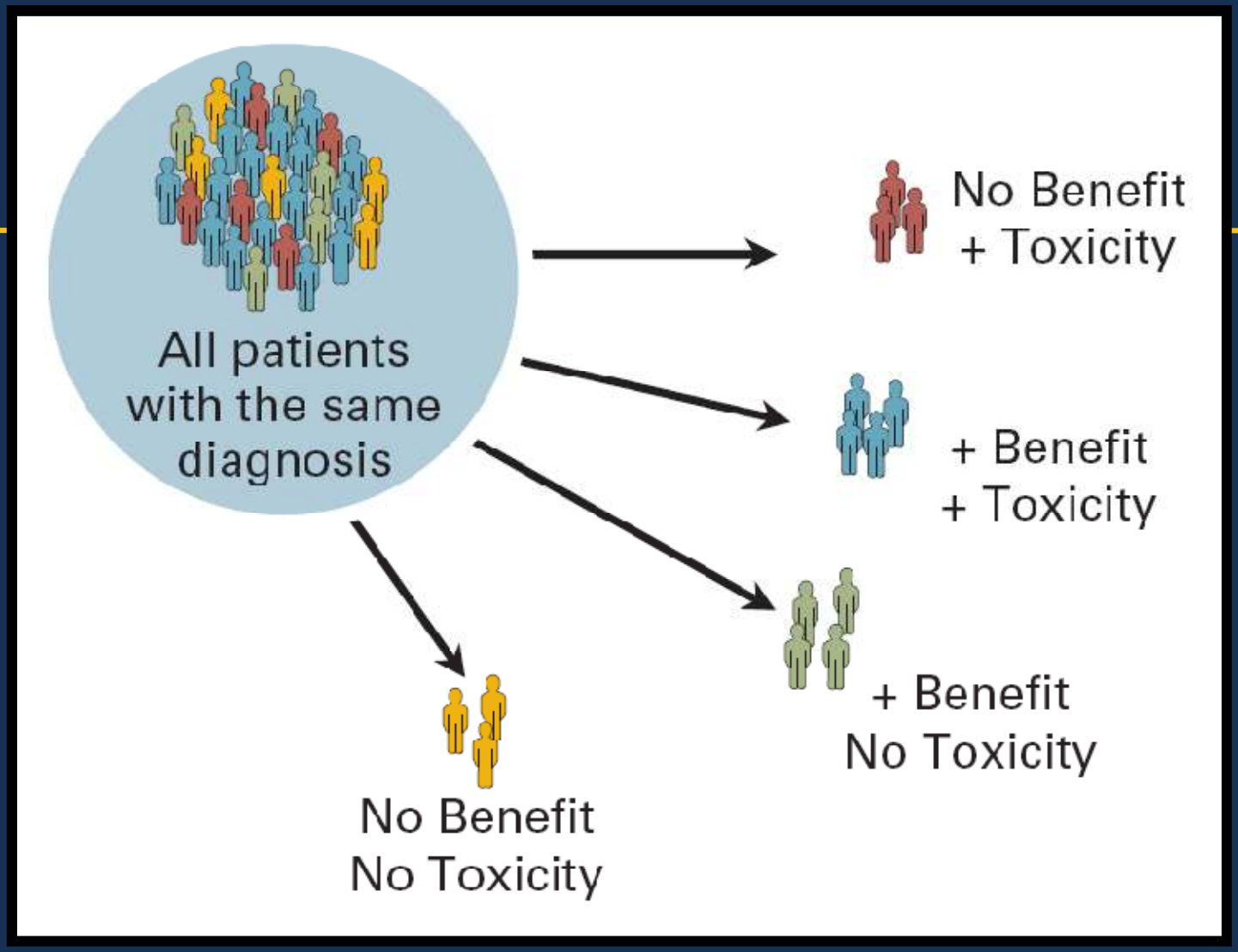
Technical Feasibility

Gene Discovery & Refinement

Analytical Validation

Clinical Validation (prognostic)

Clinical Validation (predictive)

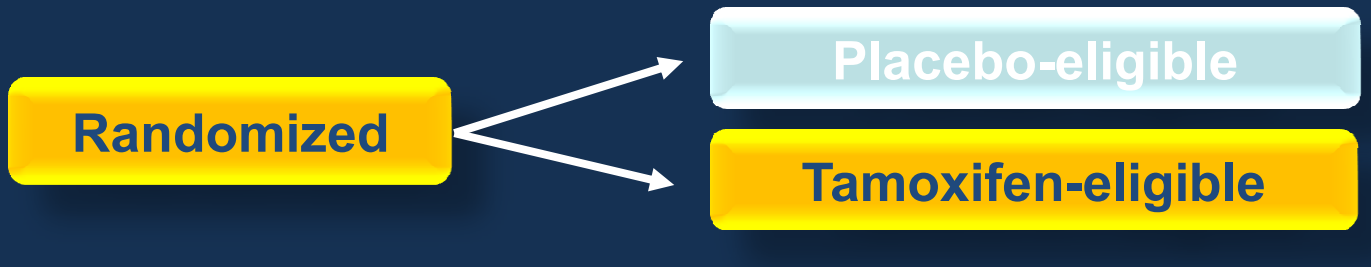


- Breast cancer patient populations are treated as if they are homogenous
- Better segregated into those that will or will not have a benefit from a therapy
- Further divide into those that will or will not have a toxic response to a therapy

JCO; 23:7342-7349 2005

Tamoxifen Benefit & Oncotype DX®

NSABP B-14 Tamoxifen Benefit Study in N–, ER+ Patients

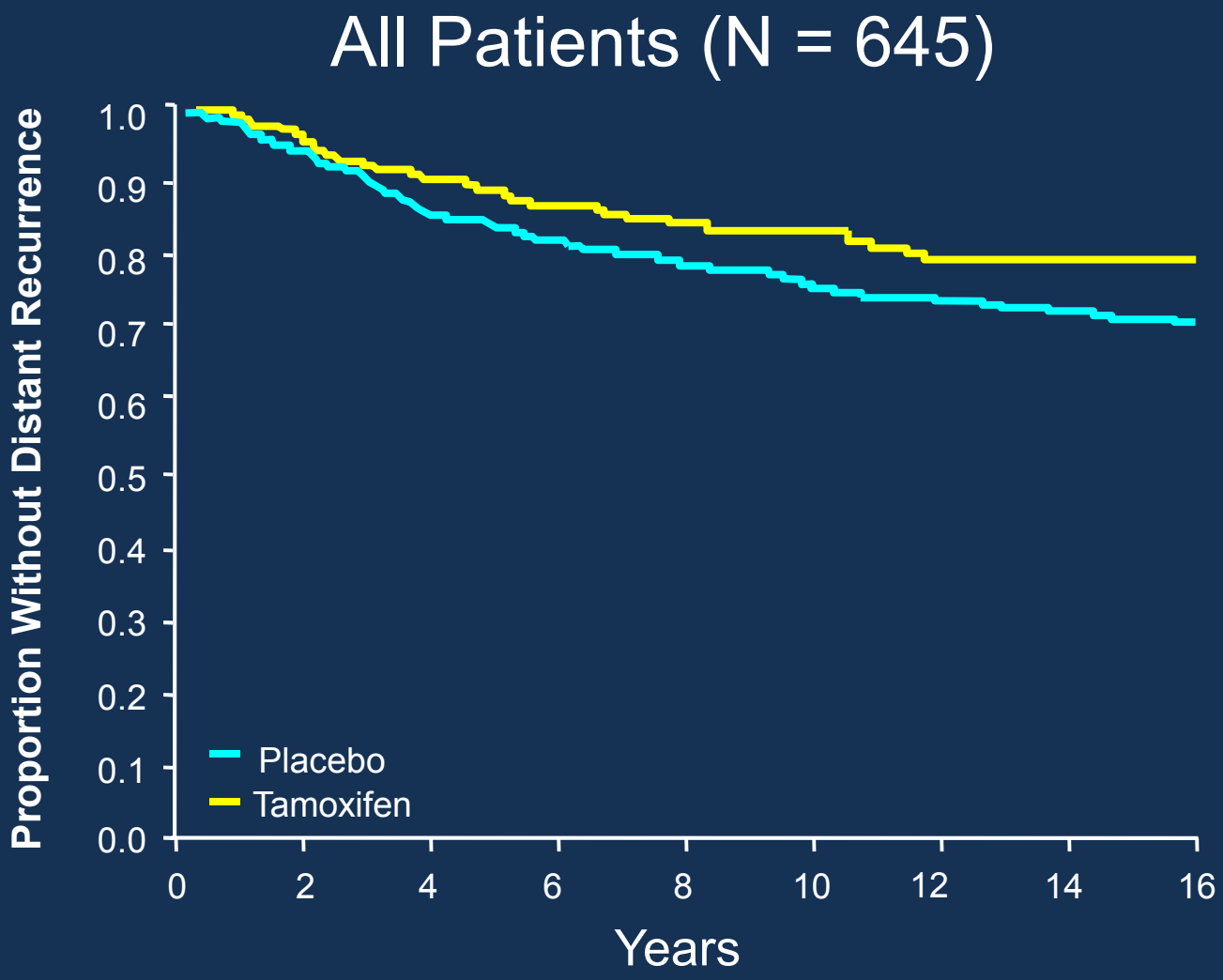


Objective: determine whether Oncotype DX® provides information on

1) Prognosis (likelihood of recurrence)
2) Response to tamoxifen (change in likelihood of recurrence with tamoxifen)
3) Both

Paik et al. *ASCO* 2004; Abstract 510.

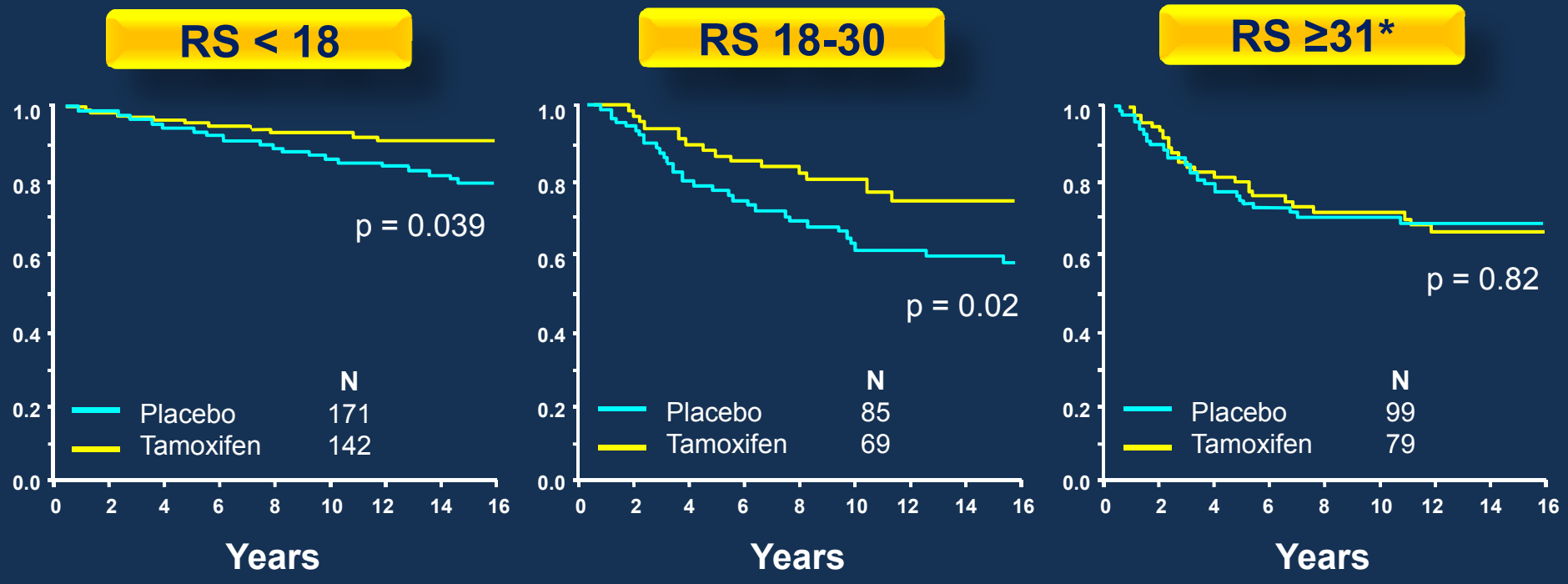
B-14 Overall Benefit of Tamoxifen



Paik et al. *ASCO* 2004; Abstract 510.

B-14 Benefit of Tamoxifen By Recurrence Score Risk Category

DISTANT RECURRENCE FREE INTERVAL

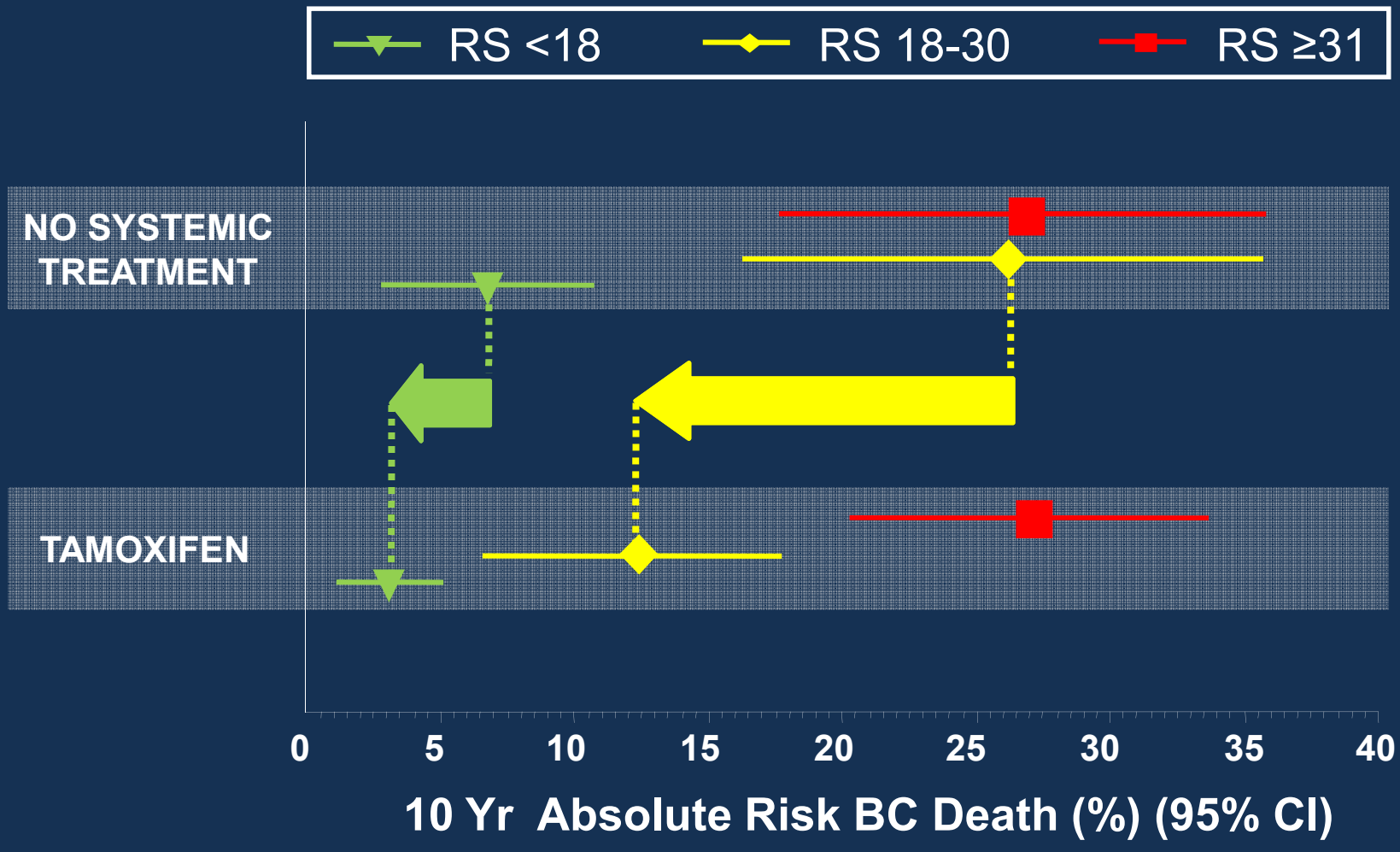


Interaction *P* = 0.06

***Results should not be used to indicate that tamoxifen should not be given to the high-risk group**

Paik et al. *ASCO* 2004; Abstract 510.

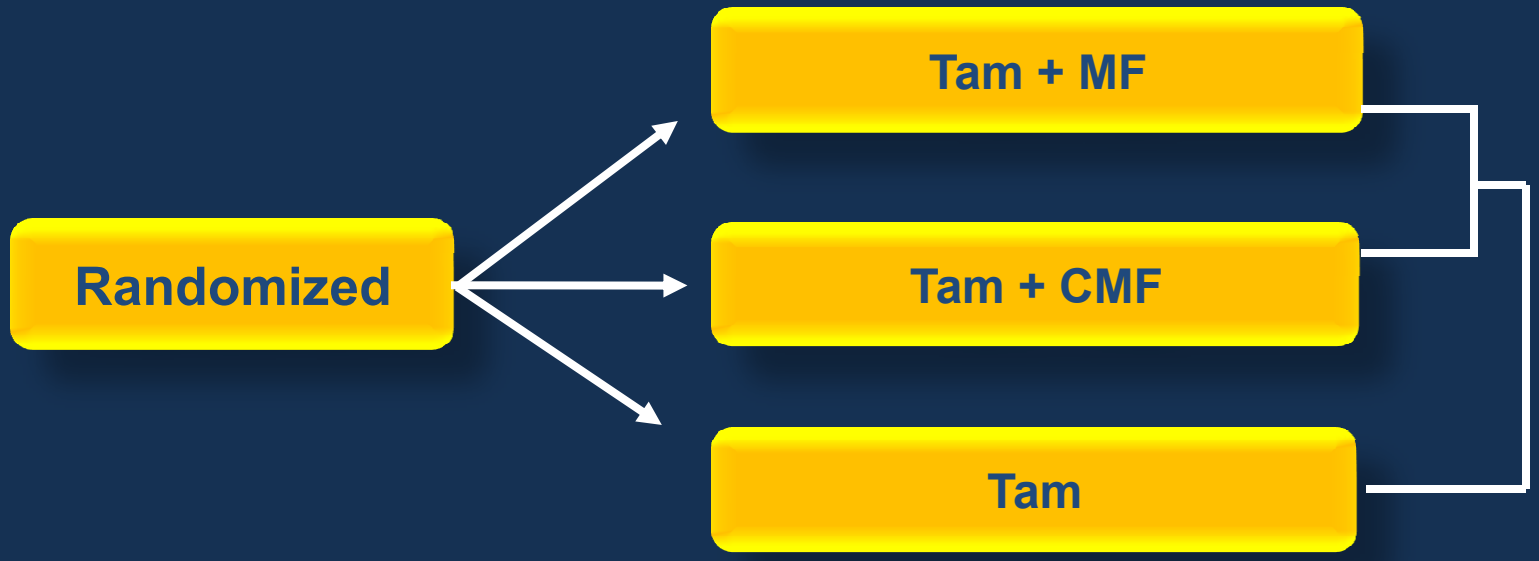
Largest Benefits of Tamoxifen Observed in Low and Intermediate Risk Groups (NSABP B-14)



Paik et al. *ASCO* 2004; Abstract 510.

Oncotype DX® Clinical Validation: NSABP B-20

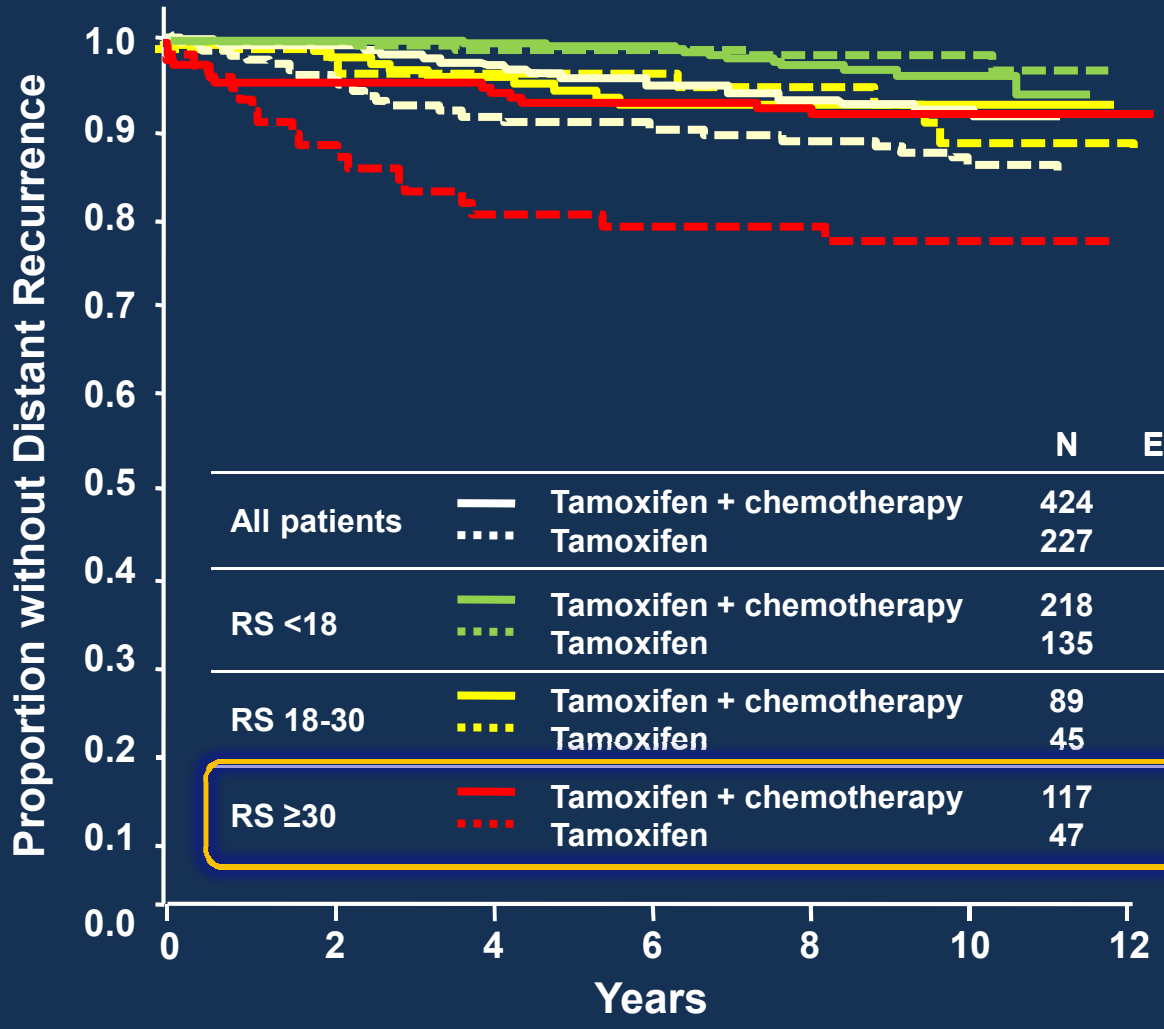
- Objective: To determine the relationship between RS and chemotherapy benefit in N-, ER+ patients



- Multicenter study with pre-specified 21-gene assay, algorithm, endpoints, analysis plan

Paik S, et al. *J Clin Oncol*. 2006;24:3726-3734.

High RS Correlates with Greater Benefit from Chemotherapy (NSABP B-20)



PATIENTS WITH HIGH RS
28% absolute benefit from tamoxifen + chemotherapy

		N	Events	
All patients	Tamoxifen + chemotherapy	424	33	p=0.02
	Tamoxifen	227	31	
RS <18	Tamoxifen + chemotherapy	218	8	p=0.61
	Tamoxifen	135	4	
RS 18-30	Tamoxifen + chemotherapy	89	9	p=0.39
	Tamoxifen	45	4	
RS ≥30	Tamoxifen + chemotherapy	117	13	p<0.001
	Tamoxifen	47	18	

4.4% absolute benefit from tamoxifen + chemotherapy

Paik S, et al. *J Clin Oncol*. 2006;24:3726-3734.

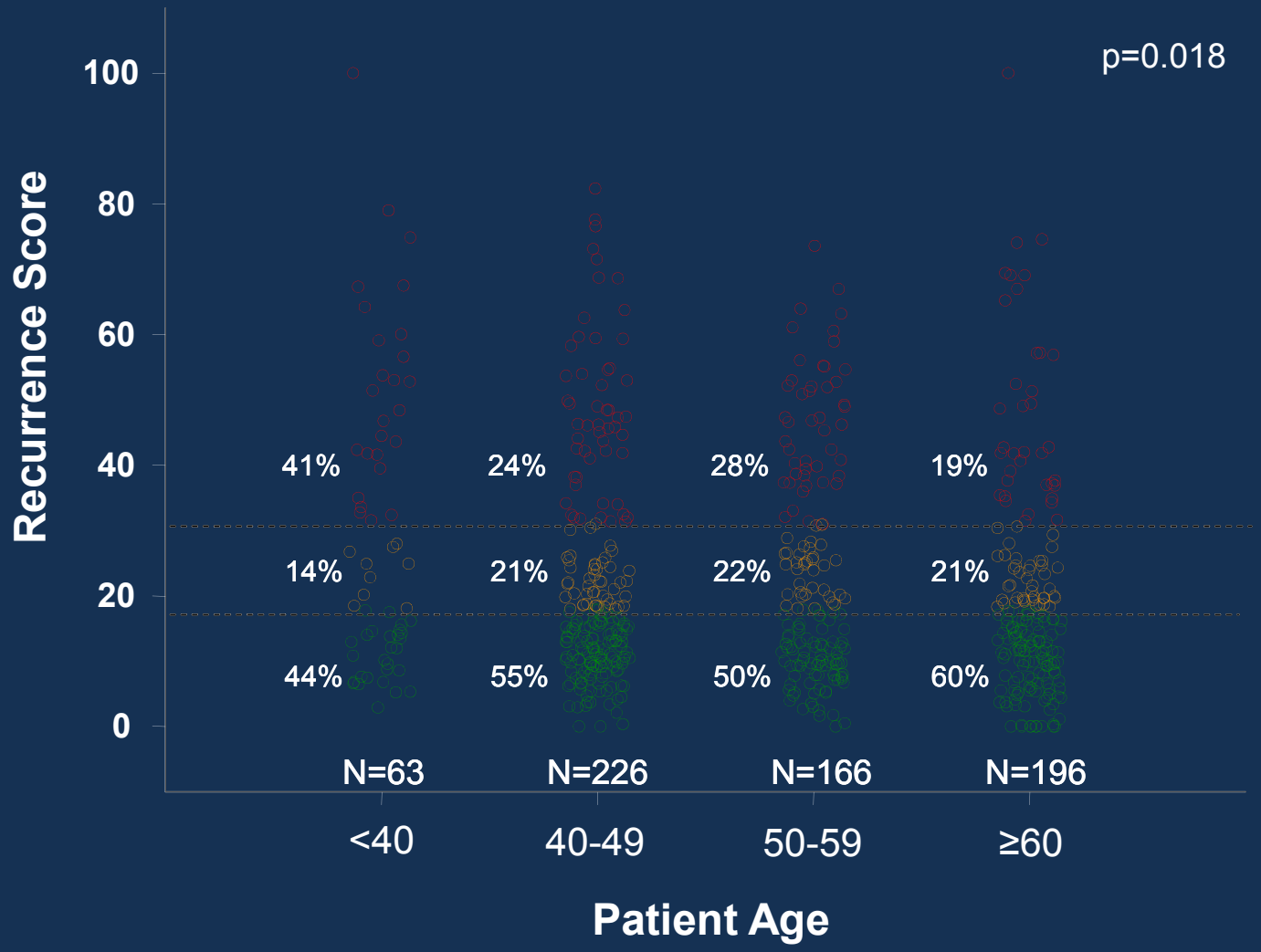
Recurrence Score Can Add Prognostic Discrimination Not Always Provided by Traditional Prognostic Factors

- Age
  - 44% of patients <40 years old had low RS (i.e., there is a large fraction of younger patients for whom chemotherapy benefit may be minimal)
- Tumor size
  - 46% of patients with large tumors (>4 cm) had low RS
  - Some patients with small tumors (<1 cm) had intermediate or high RS
- Tumor grade
  - Assessment by local pathologists revealed that, even for poorly differentiated tumors, 36% of patients had low RS
  - Approximately 20% of poorly differentiated tumors still had a low RS

Paik S, et al. *J Clin Oncol*. 2006;24:3726-3734.

Oncotype DX® NSABP B-20:

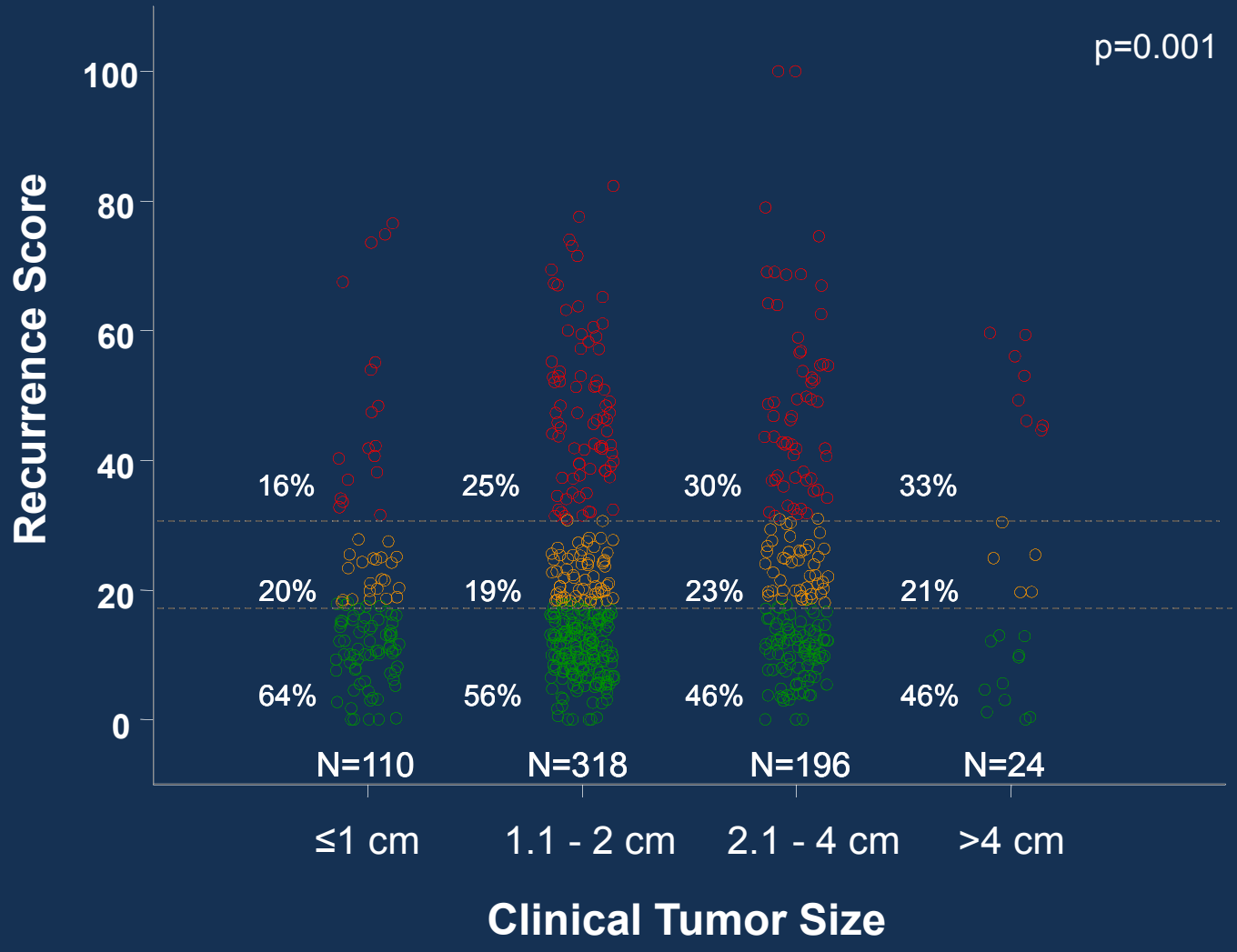
Many Younger Patients Have Low Recurrence Scores



Paik S, et al. *J Clin Oncol*. 2006;24:3726-3734.

Oncotype DX® NSABP B-20:

Many Small Tumors Have Intermediate to High Recurrence Scores

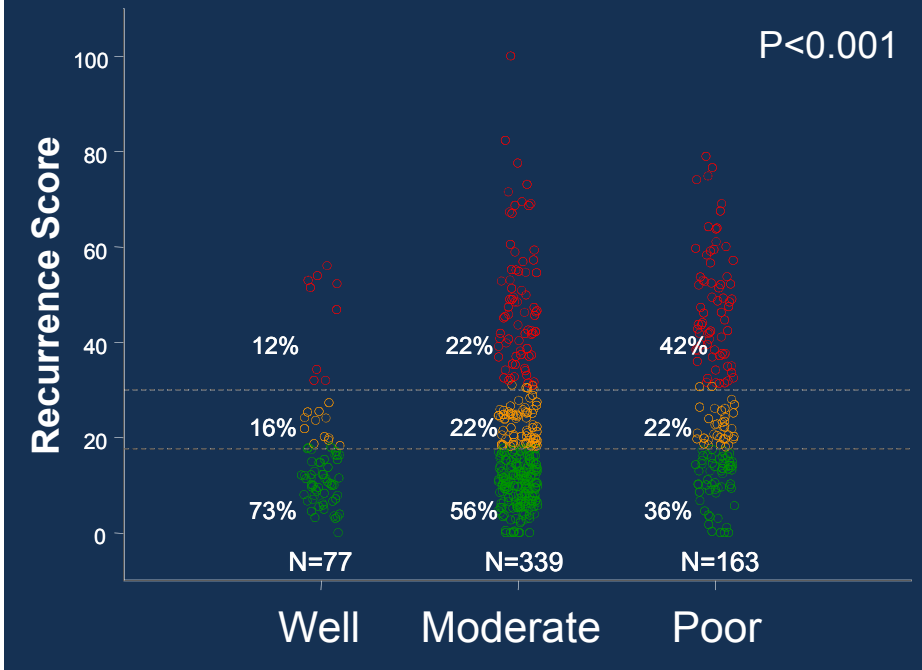


Paik S, et al. *J Clin Oncol*. 2006;24:3726-3734.

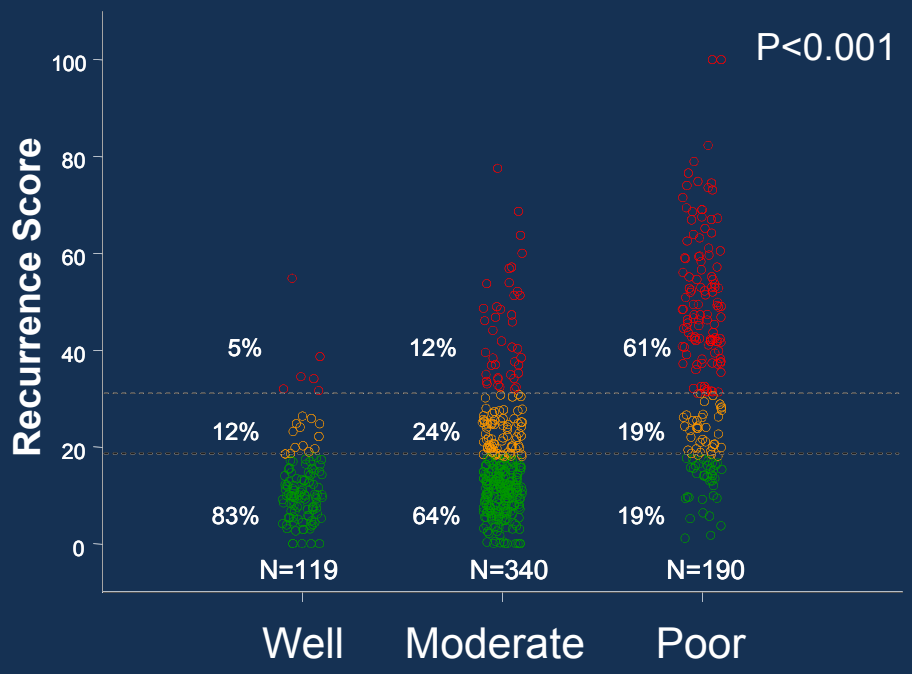
Oncotype DX® NSABP B-20:

Significant Proportion of High-Grade Tumors Have Low Recurrence Scores (NSABP B-20)

Grading by pathologist at local clinical trial site

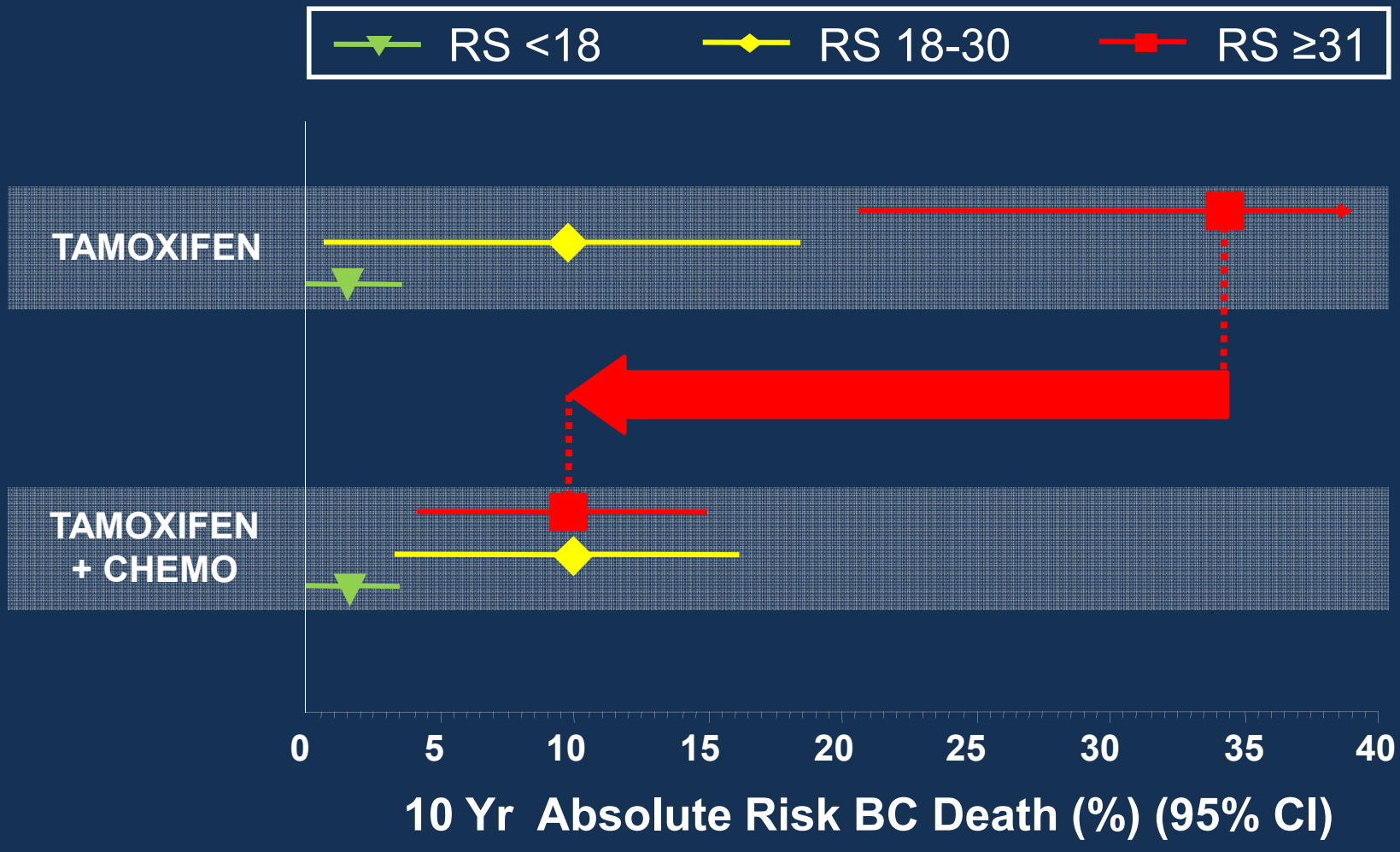


Grading by pathologist at central lab



Paik S, et al. *J Clin Oncol*. 2006;24:3726-3734.

Largest Benefits of Chemotherapy Observed in High Risk Groups (NSABP B-20)



Agenda
Development Overview

- ASCO & NCCN Guidelines
- Lymph Node Positive Studies
- transATAC Study Results
- SWOG 8814 Study Results
- Quantitative Single Gene Reporting
- ER, PR and HER2 Concordance Data

Agenda
Development Overview

- **ASCO & NCCN Guidelines**
- Lymph Node Positive Studies
- transATAC Study Results
- SWOG 8814 Study Results
- Quantitative Single Gene Reporting
- ER, PR and HER2 Concordance Data

ASCO Guidelines on the Use of Tumor Markers in Breast Cancer

- Onco*type* DX® can be used to determine prognosis in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer who will receive tamoxifen

To predict risk of recurrence in patients considering treatment with tamoxifen

To identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy

Patients with high recurrence scores appear to achieve relatively more benefit from adjuvant chemotherapy (specifically CMF) than tamoxifen

- Conclusions may not be generalizable to hormonal therapies other then tamoxifen, or to other chemotherapy regimens

Harris L, et al. *J Clin Oncol*. 2007;33(25):5287-5312.

NCCN Clinical Practice Guidelines

Hormone Receptor Positive, HER2 Negative Disease

pT1, pT2, or pT3 and pN0 or pN1m

- Tumor ≤ 0.5 cm or
- MicroInvasive or
- Tumor 0.6-1.0 cm, well differentiated, no unfavorable features

→ pN0 (no adjuvant therapy)

→ pN1mi (consider adjuvant endocrine therapy)

- Tumor 0.6-1.0 cm with moderate, poorly differentiated, or unfavorable features
- Tumor > 1cm

→ Consider Oncotype DX®

No test	Adjuvant endocrine therapy ± adjuvant chemotherapy
RS <18	Adjuvant endocrine therapy
RS 18-30	Adjuvant endocrine therapy ± adjuvant chemotherapy
RS ≥31	Adjuvant endocrine therapy + adjuvant chemotherapy

Node Positive
(micromets: >.2mm to ≤2mm)

→ Adjuvant endocrine therapy + adjuvant chemotherapy

Adapted from NCCN Practice Guidelines in Oncology – v.2.2008

Onco*type* DX®
in Node-Positive Disease

Oncotype DX® Clinical Validation in Node-Positive Patients (ECOG trial 2197)

ECOG TRIAL 2197

Operable Breast Cancer
0-3 Positive Nodes
T.1cm if Node Negative
N=2885 Eligible patients

AC Doxorubicin 60 mg/m^2 Cyclophosphamide 600 mg/m^2 Every 3 weeks x 4 cycles	AT Doxorubicin 60 mg/m^2 Docetaxel 60 mg/m^2 Every 3 weeks x 4 cycles
Tamoxifen x 5 years If HR-Positive (Amended to Allow AIs) Plus RT if indicated	Tamoxifen x 5 years If HR-Positive (Amended to Allow AIs) Plus RT if indicated

776 samples with genomic data, including Recurrence Score

Paraffin blocks with cancer cells occupying <5% of the section area excluded

Manual micro-dissection

RNA extraction

- No difference between arms
- Median follow-up 76 months
- 96.8% reported follow up until death or for at least 5 years

Goldstein LJ, et al. ASCO 2007. Abstract 526.

Patients with 1-3 Positive Nodes and Low RS do well without chemotherapy*

5-Year Event Rates by Nodal Status & RS

RS	Nodes	RFI (%)	DFS (%)	OS (%)
<18	Negative	96	93	95
	Positive	95	91	97
18-30	Negative	86	87	97
	Positive	87	77	86
≥ 31	Negative	87	80	92
	Positive	75	61	72

**Including micrometastases (pN1mi)*

- **Low RS (<18) in patients with 1-3 positive axillary nodes may eventually be used to select individuals for a short course of chemotherapy plus hormonal therapy**
- **Elevated RS (≥18) may eventually be used to select individuals for participation in clinical trials evaluating novel treatment strategies, or for more aggressive chemotherapy regimens**

Goldstein LJ, et al. ASCO 2007. Abstract 526.

Oncotype DX® Clinical Validation in Node-positive Patients (SWOG 8814 sub-analysis)

SWOG 8814

Postmenopausal, node positive, ER positive breast cancer
N=1477

Tamoxifen x 5 yrs n=361	CAF x 6 + tamoxifen n=550	CAF x 6 → tamoxifen n=566

SUB ANALYSIS

Patients with samples (n=666)

RT-PCR obtained (n=601)
- Tamoxifen alone (n=148)
- CAF + T (n=243)
- CAF → T (n=219)

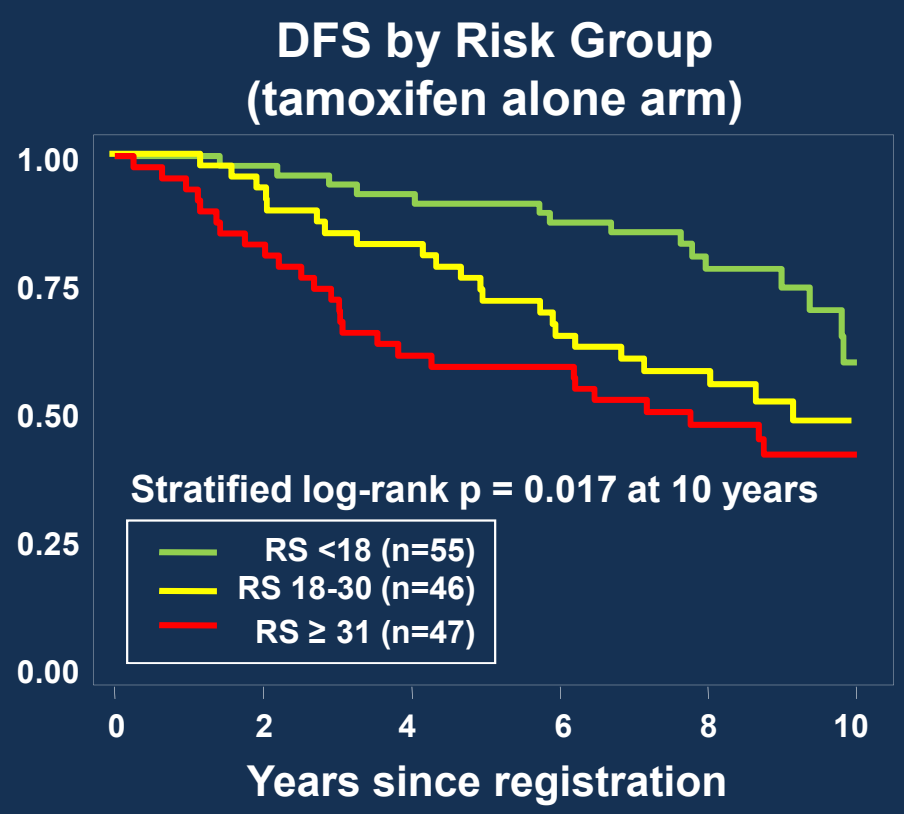
Sample for primary analysis
- 148 + 219 = 367

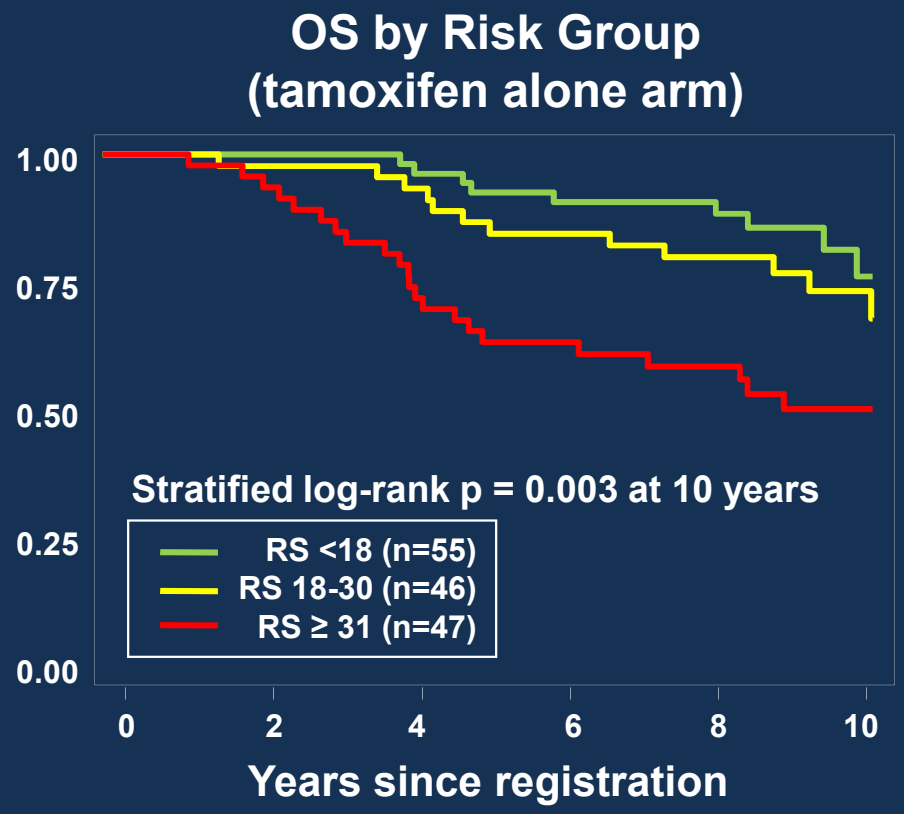
(40% of parent trial)

Superior Disease-Free Survival and Overall Survival over 10 Years

Albain, SABCS 2007, Abstract #10

Recurrence Score is Prognostic for Node-Positive Patients (Tamoxifen Arm)





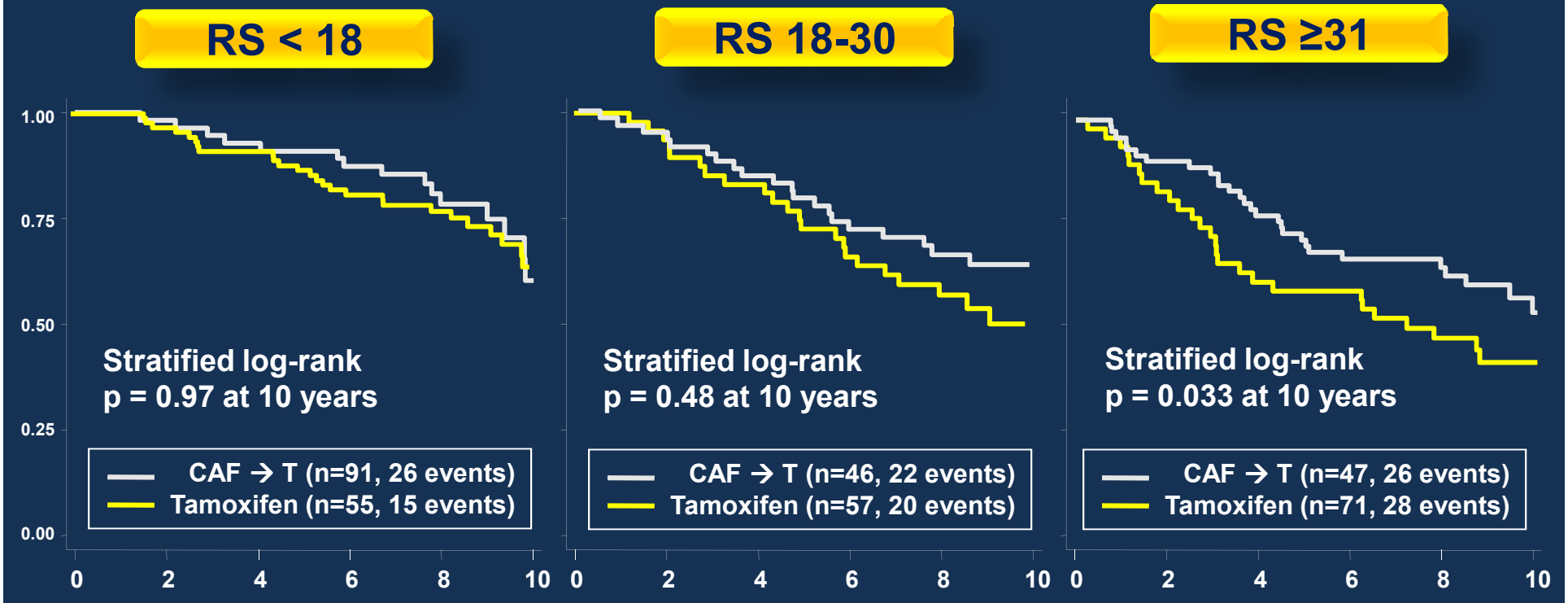
10-year DFS: 60%, 49%, 43%

10-year OS: 77%, 68%, 51%

Albain, SABCS 2007, Abstract #10

High Recurrence Score Predictive of Chemotherapy Benefit in Node-Positive Patients

DFS BY TREATMENT & RS GROUP



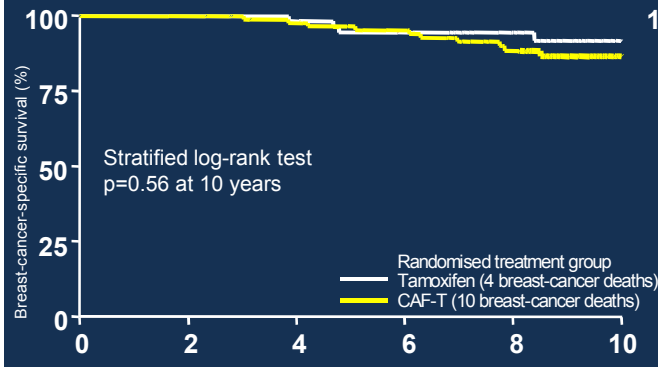
No benefit to CAF over time if low RS

Strong benefit if high RS

Albain, SABCS 2007, Abstract #10

Breast Cancer Specific Survival of Node-positive Patients by Treatment and Recurrence Score® (RS) Group

RS < 18

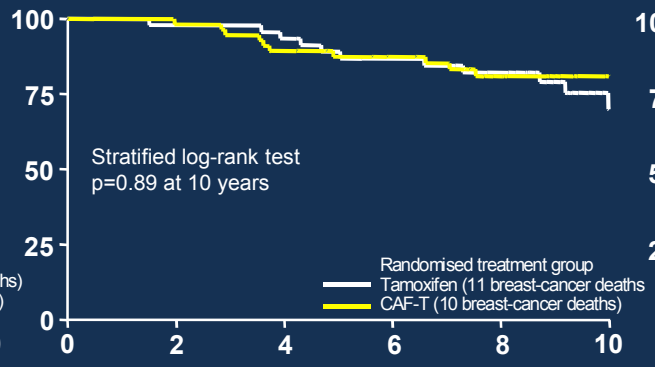


Years since registration

10 yr BCSS
T: 92% vs CAF-T: 87%

No benefit to CAF over time if low RS

RS 18-30

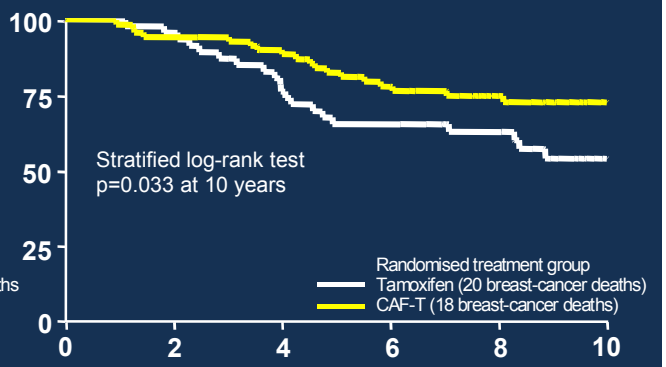


Years since registration

10 yr BCSS
T: 70% vs CAF-T: 81%

Interaction p=0.021

RS ≥31



Years since registration

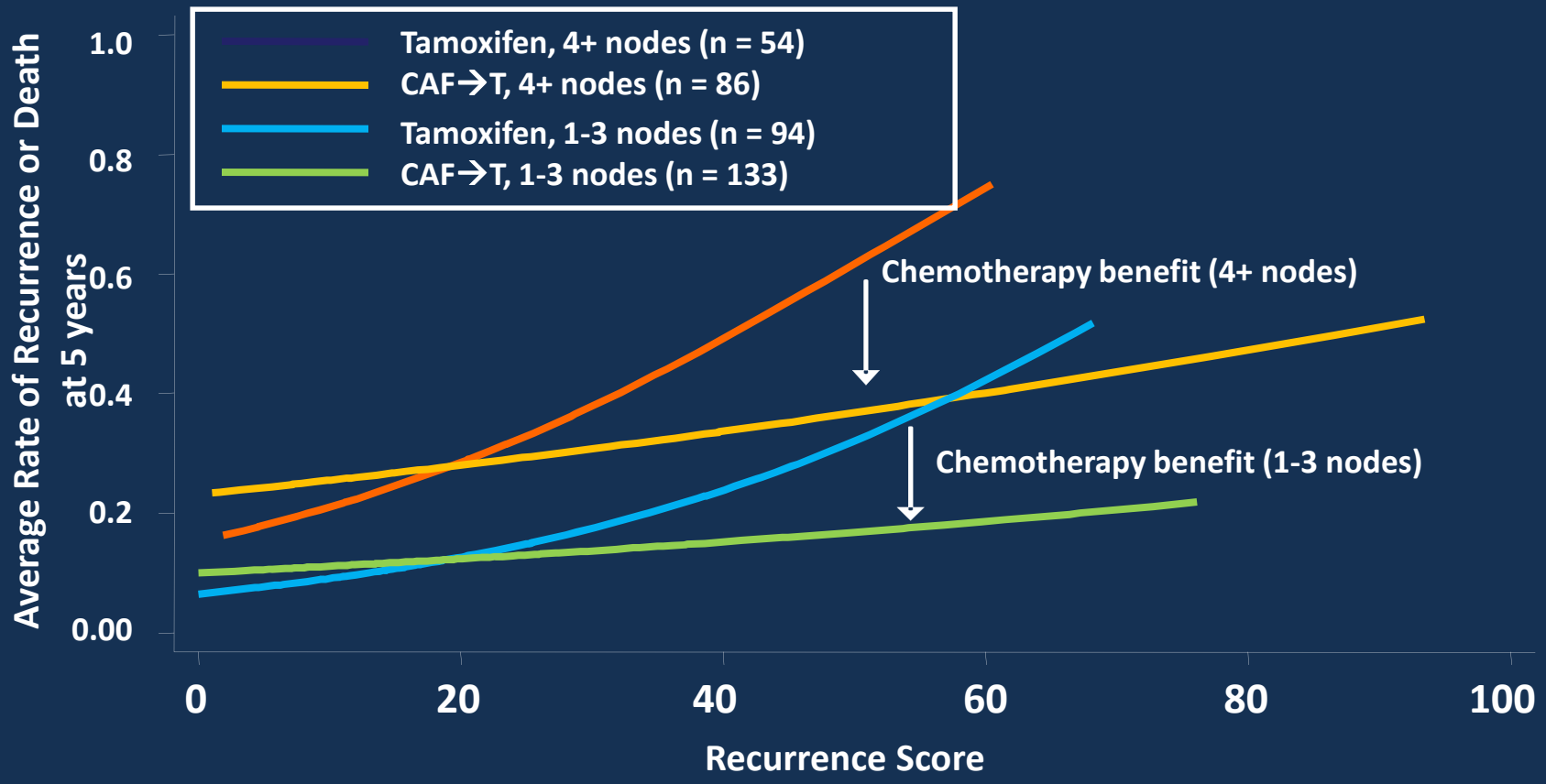
10 yr BCSS
T: 54% vs CAF-T: 73%

Strong benefit if high RS

Albain KS et al. *Lancet Oncol.* 2009 Dec: Epub ahead of print.

SWOG 8814: Chemotherapy Benefit Greatest in Patients With Higher Recurrence Score®, Regardless of Number of Positive Nodes

5-year Probability of Death or Disease Recurrence



Albain K, et al. SABCS. 2007; Abstract 10.

Comparative Distribution of RS
SWOG 8814: Less Low RS, More High RS

Study	Low Risk (RS < 18)	Int. Risk (RS 18-30)	High Risk (RS ≥ 31)
NSABP B14*	51%	22%	27%
NSABP B20*	54%	21%	25%
Kaiser controls*	56%	19%	25%
ECOG 2197**	49%	31%	20%
SWOG 8814***	40%	28%	32%

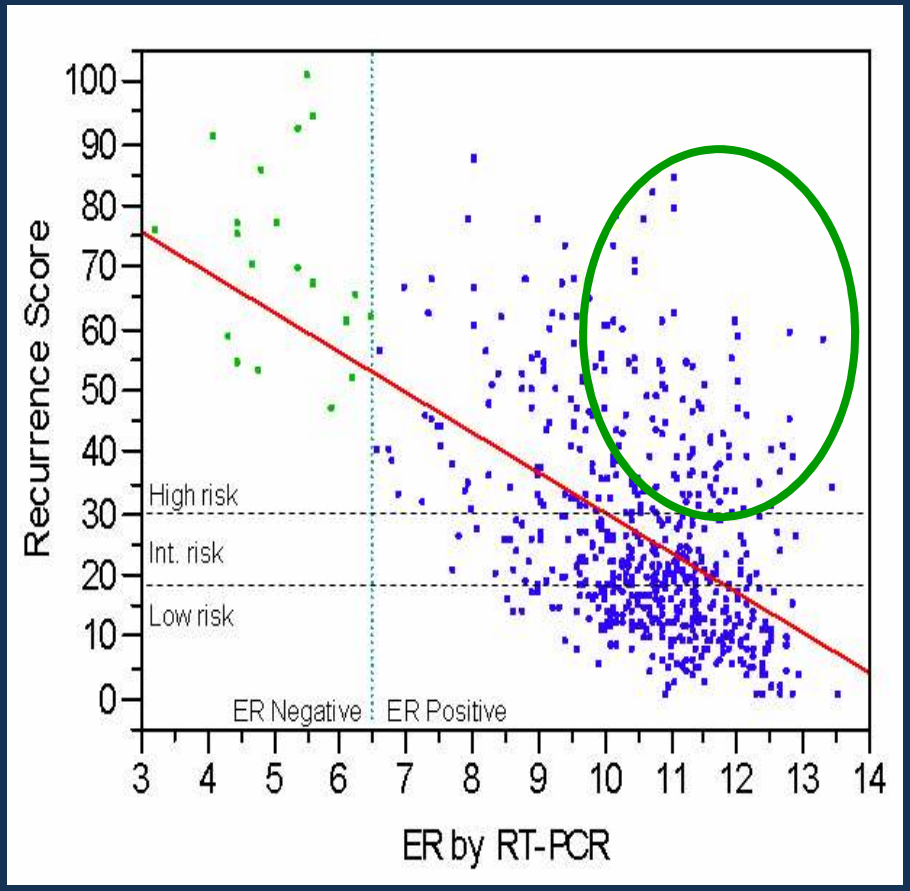
*node(-): Paik, et al. NEJM 2004 & JCO 2006; Habel, et al. Breast Ca Res Treat 2006

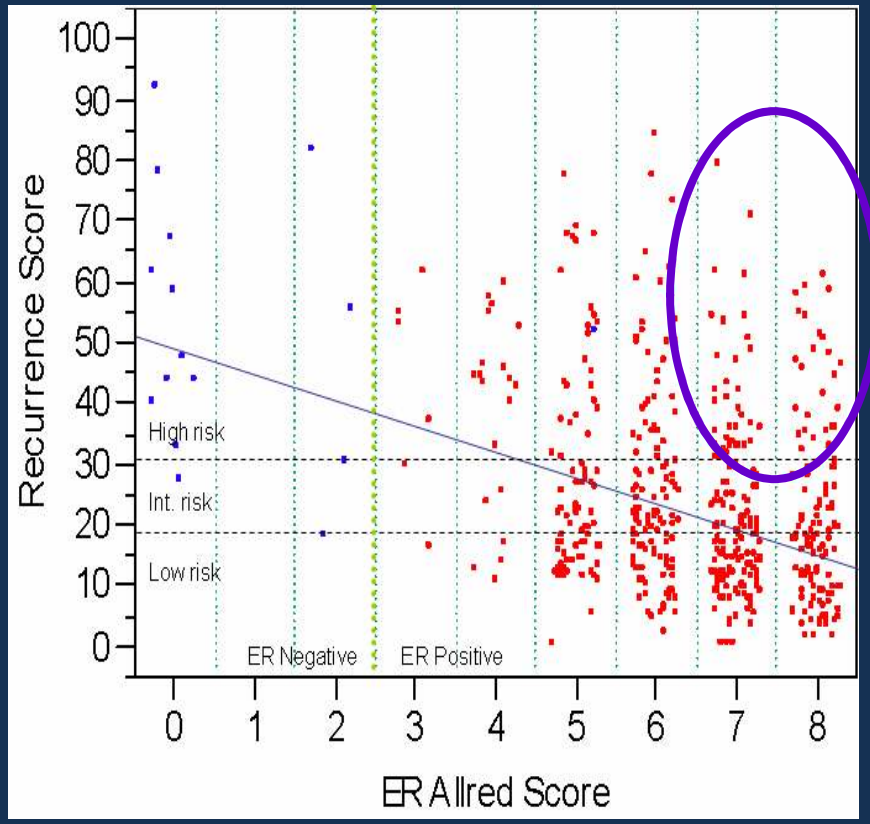
**node- or 1-3+: Goldstein, et al. Proc ASCO 2007

***node+, postmenopausal: this analysis - no difference by age

Single Gene Analyses might "Misclassify" the Dominant Biology of the Tumor

Example: High ER (by either Allred Score or RT-PCR) could have High RS





Summary of Findings

- Recurrence Score® (RS) was prognostic in tamoxifen-alone group
  - p=0.006; HR 2.64 (95% CI 1.33-5.27)
- No apparent benefit of CAF in patients with RS<18
  - log-rank p=0.97; HR 1.02 (0.54-1.93)
- CAF improved DFS in patients with high RS ≥ 31
  - log-rank p=0.033; HR 0.59 (0.35-1.01), after adjusting for number of positive nodes
- RS by CAF treatment interaction was significant in first 5 yrs (p=0.029) but not additionally predictive beyond 5 yrs (p=0.58) although the cumulative benefit remained at 10 yrs.
- Results were similar for OS and BCSS

Risk of Distant Recurrence Using Onco*type* DX® in Postmenopausal Primary Breast Cancer Patients Treated with Anastrozole or Tamoxifen: a TransATAC Study

Dowsett M *et al* on behalf of the ATAC Trialists' Group

San Antonio Breast Cancer Symposium. 2008;
Abstract 53.

Study Overview

ATAC Study Population (N=9366)

- Tamoxifen
- Anastrozole
- Tamoxifen + Anastrozole *(combination arm not examined)*

Primary Analysis: To determine whether Oncotype DX® significantly adds to a proportional hazards model for time to distant recurrence (age, tumor size, grade, treatment) in N-, HR+, patients with no adjuvant chemotherapy

- Secondary analyses:
  - Determine whether the relationship between continuous RS and time to distant recurrence differs by nodal status or treatment arm
  - Determine the relationship of predefined RS groups with time to distant recurrence by nodal status and treatment arm
  - Evaluate whether RS adds to the Adjuvant! Online estimate of risk

Dowsett M, et al. SABCS 2008; abstract 53.

Number of Evaluable Patients and Distant Events by Nodal Status

	Node negative	Node positive	Node unknown	Total
All	**890**	**363**	**55**	**1308**
Adjuvant chemo	*-9*	*-55*	*-1*	*-65*
HR negative	*-4*	*-0*	*-0*	*-4*
Didn't start T or A	*-5*	*-2*	*-1*	*-8*
Evaluable Patients	**872 (71%)**	**306 (25%)**	**53 (4%)**	**1231 (100%)**
Number of distant events	**72**	**74**	**6**	**152**

Distributions of the clinical variables in the 1231 evaluable (non-N Am) patients were similar to those in the 2929 ATAC (non-N Am) patients who were not included in this study

Dowsett M, et al. SABCS 2008; abstract 53.

Primary Analysis: Time to Distant Recurrence and Recurrence Score Adjusted for Clinical Covariates

(Node Negative Patients, Both Treatment Arms)

Variable	HR (95% CI)*	*P* value
Recurrence Score / 50*	**5.25 (2.84, 9.73)**	**<0.001**
Tumor Size: >2 vs. ≤2 cm	**2.78 (1.70, 4.57)**	**<0.001**
Central Grade		**0.270**
Moderate vs. Well	**1.70 (0.75, 3.86)**	
Poor vs. Well	**2.06 (0.82, 5.17)**	

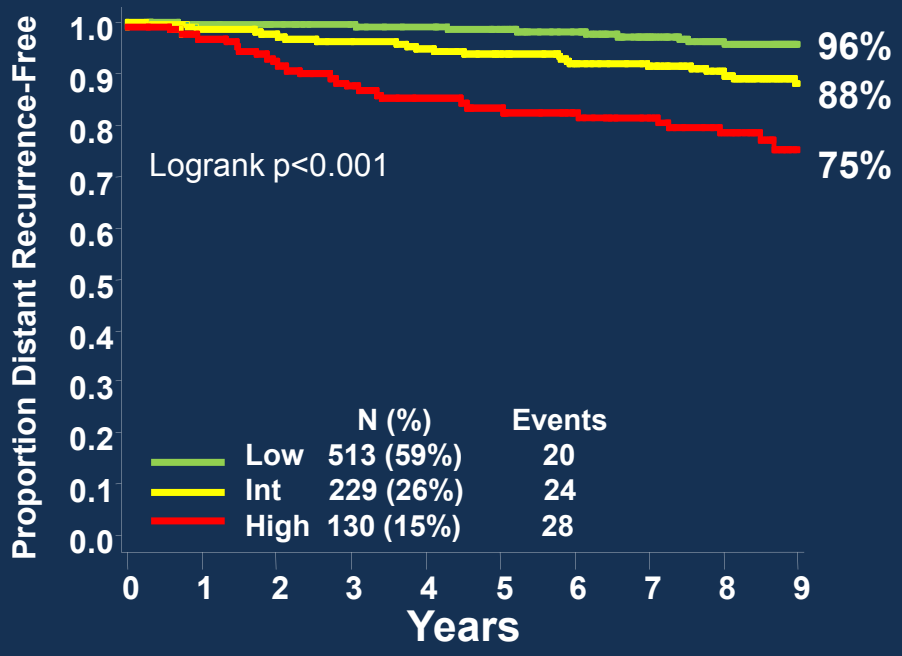
Multivariate analysis adjusted for treatment arm and patient age
**Hazard Ratio for a 50-point increment in Recurrence Score*

Multivariate analysis confirms that the Onco*type* DX® Recurrence Score as a continuous variable is a highly significant predictor of time to distant recurrence

Dowsett M, et al. SABCS 2008; abstract 53.

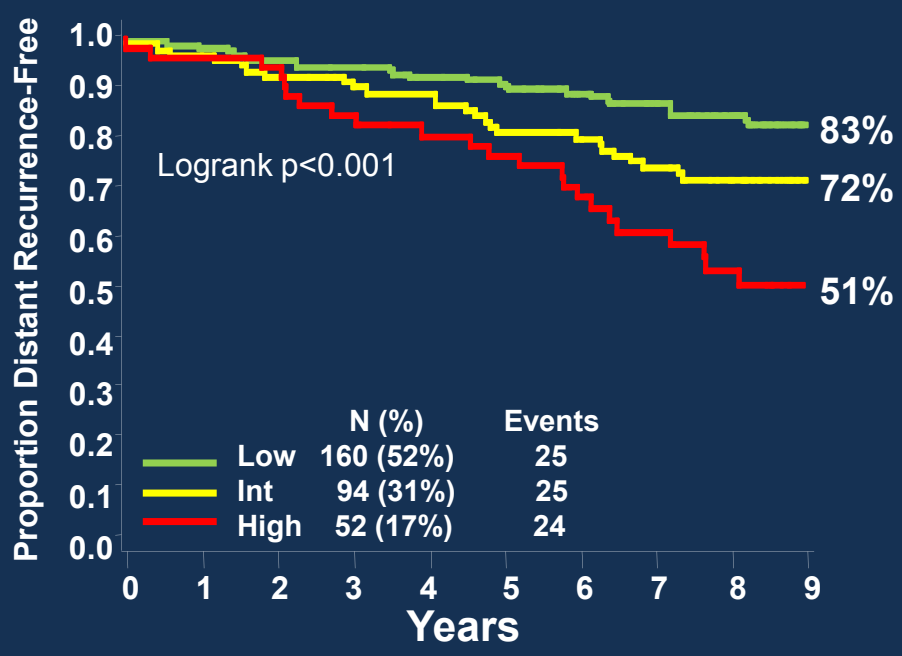
Time to Distant Recurrence by Recurrence Score Group

Node Negative (n=872) (both treatment arms)



RS Group	HR* (95% CI)
High vs Low	5.2 (2.7 – 10.1)
Int vs Low	2.5 (1.3 – 4.5)

Node Positive (n=306) (both treatment arms)

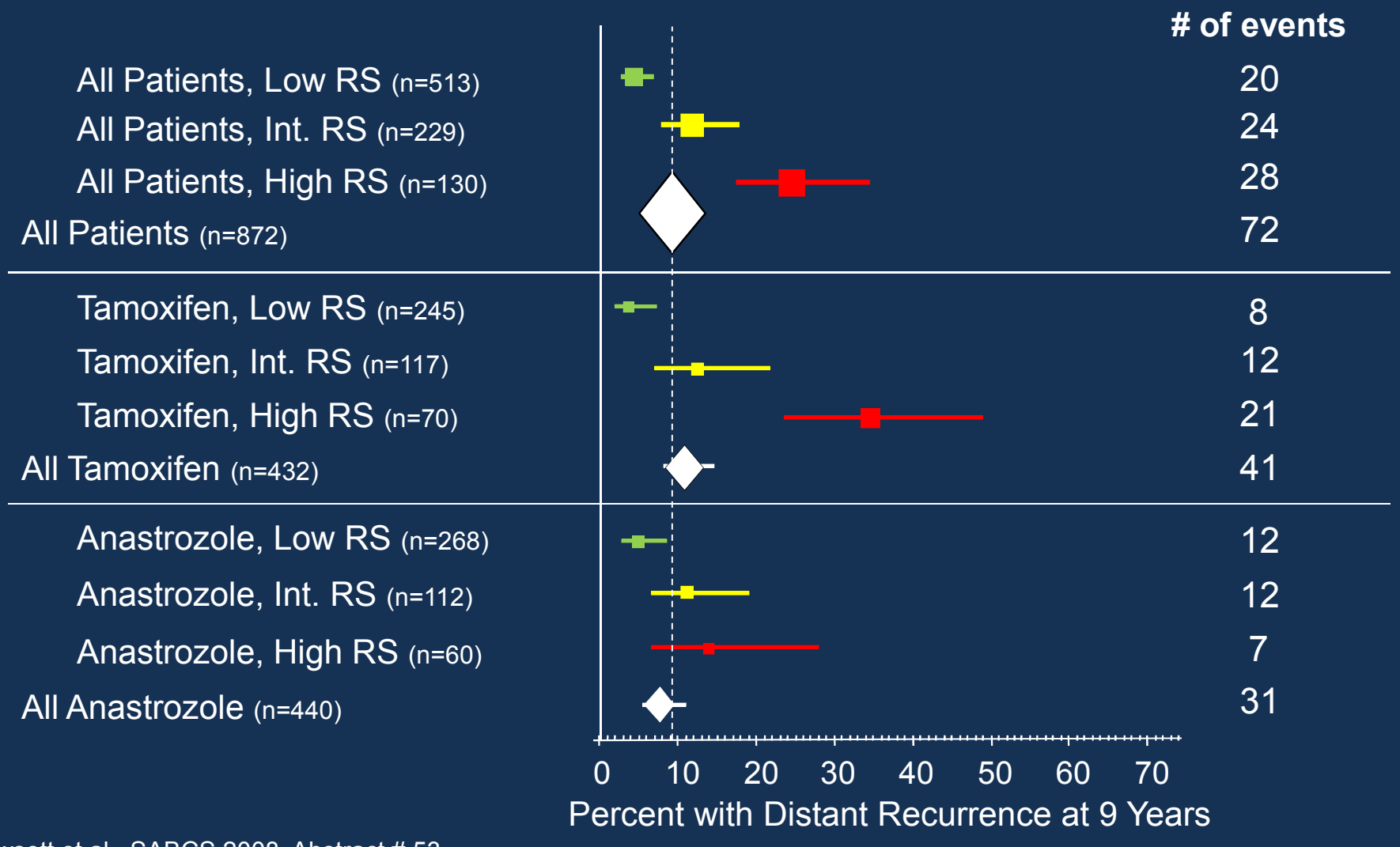


RS Group	HR* (95% CI)
High vs Low	2.7 (1.5 – 5.1)
Int vs Low	1.8 (1.0 – 3.2)

***Hazard Ratio for RS Group adjusted for tumor size, grade, age and treatment**

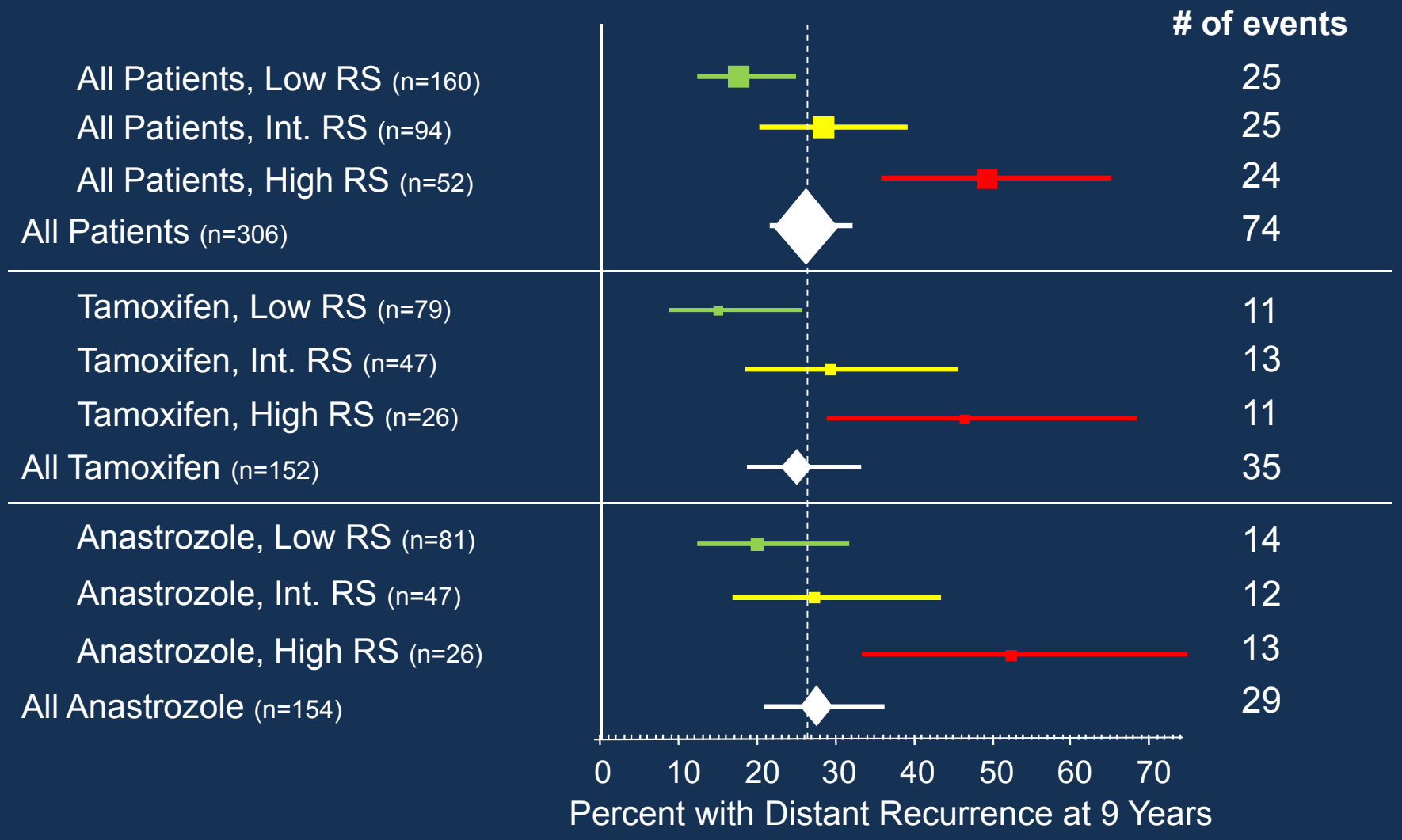
Dowsett M, et al. SABCS 2008; abstract 53.

Percent with Distant Recurrence at 9 Years (Node Negative)



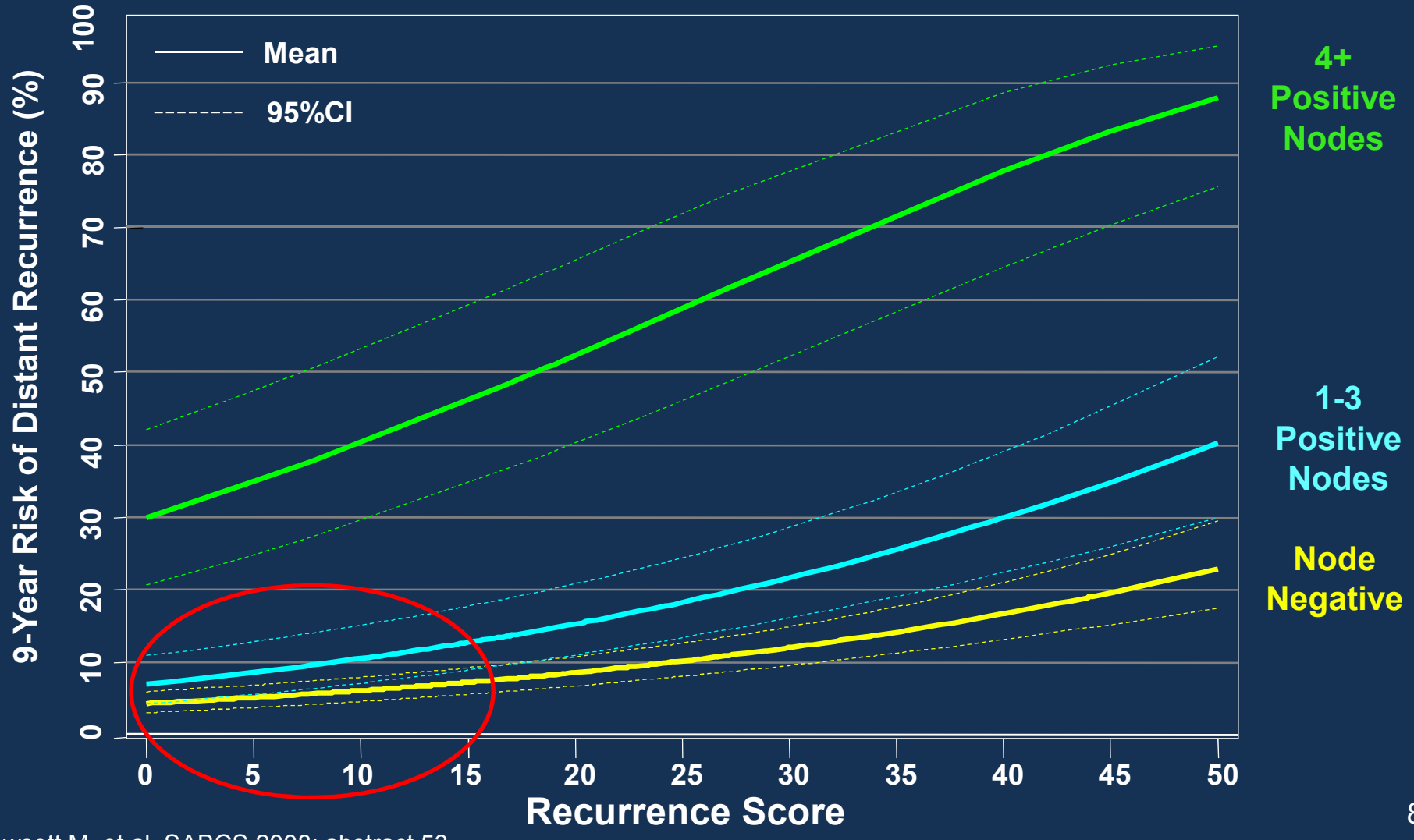
Dowsett et al., SABCS 2008, Abstract # 53

Percent with Distant Recurrence at 9 Years (Node Positive)



Dowsett et al., SABCS 2008, Abstract # 53

Rate of Distant Recurrence Increases with the Number of Positive Nodes for all Recurrence Scores



Dowsett M, et al. SABCS 2008; abstract 53.

ATAC Conclusions

- Confirms performance of Onco*type* DX® Recurrence Score in postmenopausal HR+ patients treated with tamoxifen in a large contemporary population
- Demonstrates for the first time that the Onco*type* DX® Recurrence Score is an independent predictor of distant recurrence in node negative and node positive HR+ patients treated with anastrozole
- The established relationship between Onco*type* DX® Recurrence Score and distant recurrence for tamoxifen may be applied for anastrozole with adjustment for the lower risk of distant recurrence with the aromatase inhibitor

Dowsett M, et al. SABCS 2008; abstract 53.

Reproducible Clinical Validation Essential in Changing Standard of Care

More than 4,000 Patients Studied in 12 Trials

Study	Type	No. Pts	Nodal Status
Providence	Exploratory	136	Neg
Rush*	Exploratory	78	Pos
NSABP B-20	Exploratory	233	Neg
NSABP B-14*	Prospective	668	Neg
MD Anderson*	Prospective	149	Neg
Kaiser Permanente*	Prospective Case-Control	790 cases/controls	Neg
NSABP B-14	Prospective Placebo vs Tam	645	Neg
Milan*	Exploratory	89	Neg/Pos
NSABP B-20*	Prospective Tam vs Tam+Chemo	651	Neg
ECOG 2197*	Exploratory and Prospective	776	Neg/Pos
SWOG 8814	Prospective Tam vs Tam+Chemo	367	Positive
ATAC	Prospective Tam vs AI	1231	Neg/Pos

*Published studies

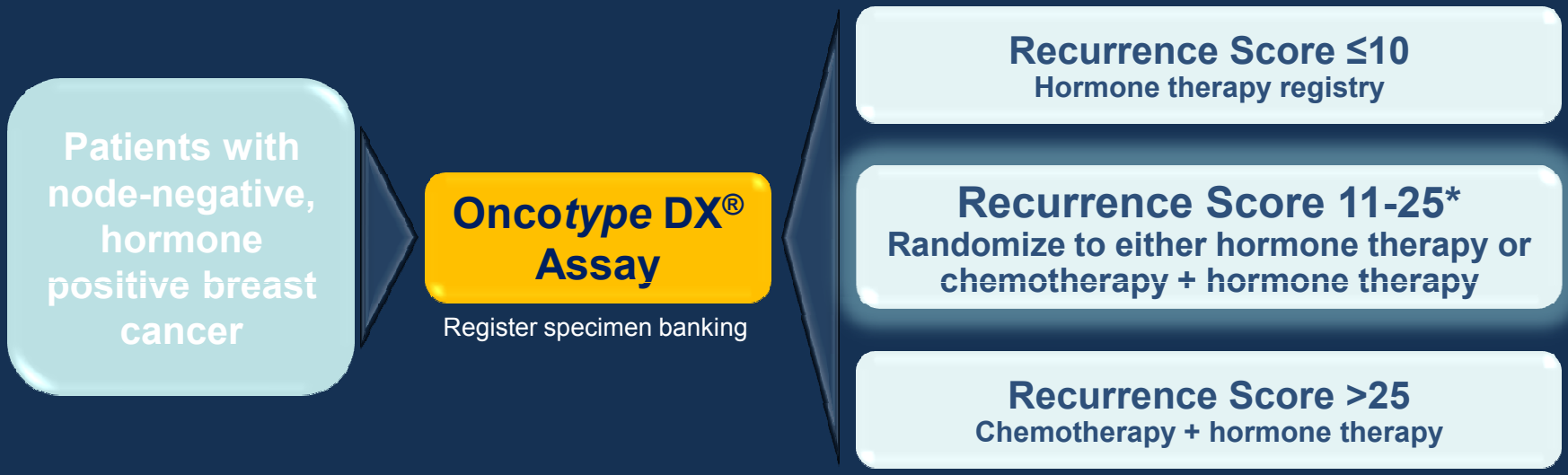
TAILORx Study

Trial Assigning Individualized Options for Treatment

- Primary objective is to determine whether adjuvant hormonal therapy is not inferior to adjuvant chemohormonal therapy for patients with RS 11-25
  - Correlates with 10-20% risk of distance recurrence at 10 years
- Potential implications
  - Reduce chemotherapy overtreatment in those likely to be treated with hormonal therapy alone
  - Reduce inadequate treatment by identifying individuals who derive great benefit from chemotherapy
  - Evaluate benefit of chemotherapy where uncertainty still exists about its utility

TAILORx Schema

Trial Assigning Individualized Options for Treatment



***Primary Study Group:** Recurrence Score 11-25 correlates with a 10-20% risk of distance recurrence at 10 years (upper 95% CI)