

Pharmacogenomics, personalized medicine and the drug development process.

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Topics

- The need for personalized medicine
- Drug development aspects
- Case studies
 - Genomic Health
 - PharmGKB
- Bioinformatics and statistics issues:
 - SNP's, expression, environment
 - Sample size, validation

The medical need for personalized medicine

- Problem: The narrow “therapeutic window” dosing range for drugs.
- Patients suffer from adverse events or ineffective drugs. Can we predict who and adjust therapy?
- Drugs fails for adverse events or lack of efficacy. Can we rescue drugs that benefit the great majority?

Drug development aspects of personalized medicine

- Drugs fail in clinical trials because of adverse events or lack of efficacy.
- Need: Identify the genetic and environmental factors that determine which patients have lack of efficacy, which will have adverse events.
- Bring safer, more effective drugs to market.
- Save drugs that might otherwise fail to gain approval.
- Design drugs for patients based on genetic & genomic information

Case studies

- *Genomic Health*
- PharmGKB

ORIGINAL ARTICLE

A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer

Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang, Ph.D.,
Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin, Ph.D.,
Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew Watson, Ph.D.,
Taesung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher, M.D.,
D. Lawrence Wickerham, M.D., John Bryant, Ph.D.,
and Norman Wolmark, M.D.

New England Journal of Medicine 2004; 351:2817-26.

Current Cancer Treatment

- 15% of women with node -ve, ER+ breast cancer will have a distant recurrence.
- But 50% of node -ve, ER+ women receive chemotherapy, with its attendant morbidity, mortality, and cost
- Can we identify those women who will not benefit from and don't need chemo?

Steps in assay development: how to get from thousands of genes to a validated algorithm?



Assay development and validation studies

- Three studies for gene selection and algorithm development:
 - Providence
 - Rush
 - NSABP B20
- One prospective validation study:
 - NSABP B14

Providence Medical Center Study: Tumor gene expression in early-stage breast cancer

In collaboration with J. Esteban et al
Providence-St. Joseph Medical Center, Burbank

Specific Objectives

- Explore correlation between RNA expression in primary tumor blocks for 185 candidate genes and likelihood of breast cancer recurrence
- Lead to design of a multi-gene assay to be used in large Clinical Validation studies

Study Design

- 136 eligible patients with sufficient sample
 - Invasive breast cancer
 - Surgery between 1/1/90 and 12/31/97
 - Primary tumor block available
 - Sufficient tumor (>20% of section invasive cancer)

Gene Expression and Prognosis

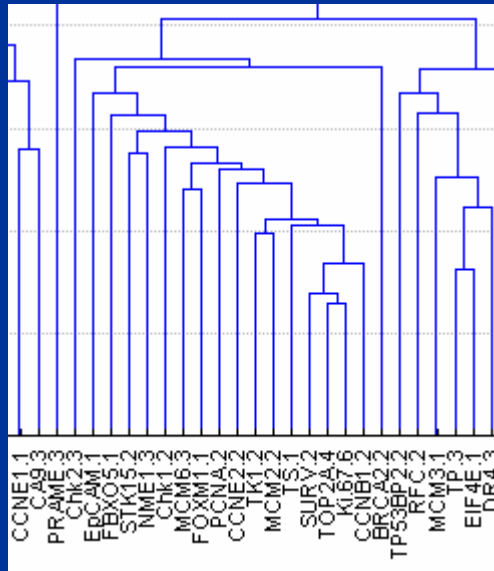
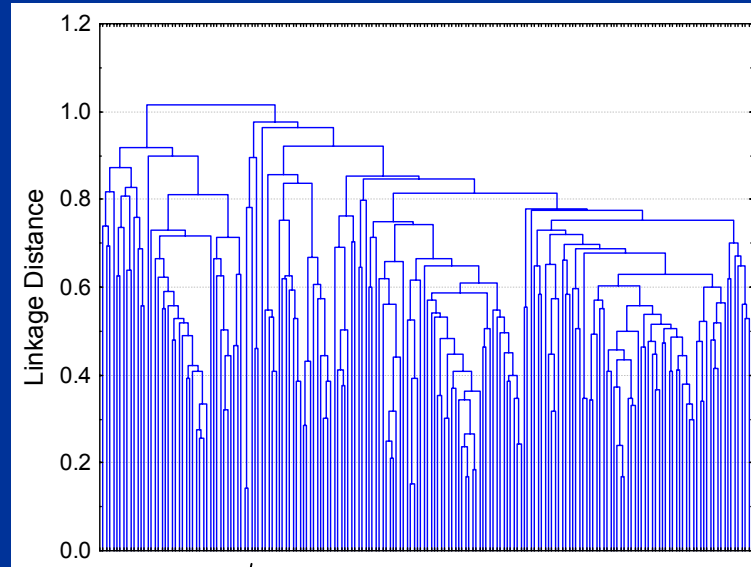
Cox proportional hazards survival analysis

45 genes prognostic of recurrence
($p < 0.05$)

Direction of gene expression is, in general, biologically plausible

Cluster Analysis--Genes

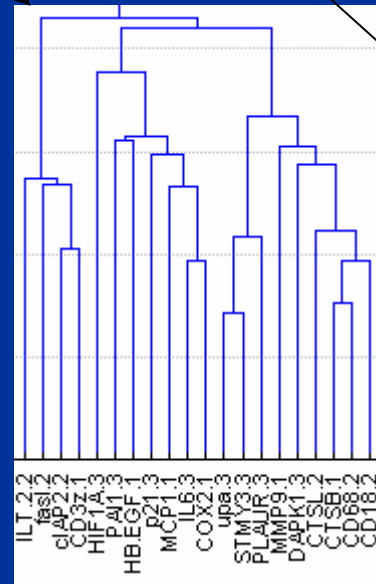
Expected clusters of co-expressed genes were found



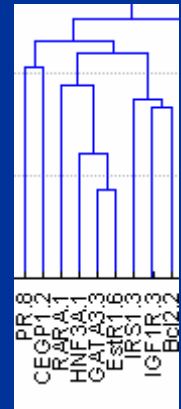
Proliferation



HER2



Immune/Invasion



ER

**Rush study:
Tumor gene expression in breast
cancer patients with 10 or more
positive nodes**

In collaboration with
Melody A. Cobleigh et al.
Rush-Presbyterian-St Luke's Med Ctr

Specific Objective

- Rush-Genomic Health Study is second of three Clinical Testing studies that:
 - Explore correlation between RNA expression in primary tumor blocks for 187 candidate genes and likelihood of breast cancer recurrence
 - Lead to design of a multi-gene assay to be used in large Clinical Validation studies

Rush: Univariate Cox proportional hazards analysis

21 genes predict likelihood of recurrence ($p < 0.05$)

Includes related genes and signaling pathways such as:

ER (e.g., PR, Bcl2, ER, CEGP1)

HER2 (e.g., HER2, Grb7)

Effect of gene expression is generally in the “right” direction

Higher expression of the HER2 and Grb7 are associated with higher risk

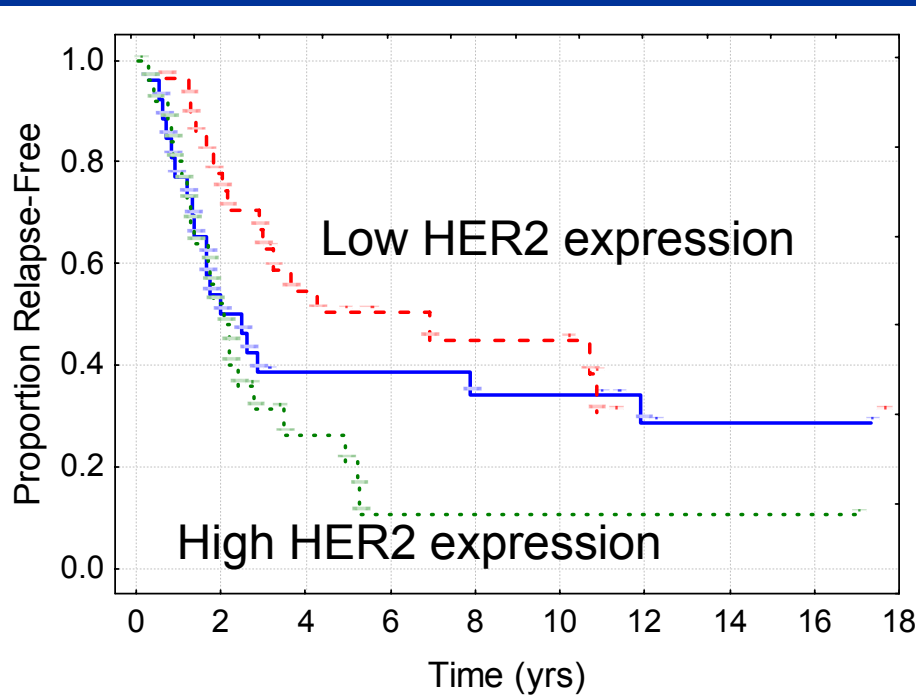
Higher expression of the ER genes are associated with lower risk

Rush: Clinical Variables, Gene Expression and Prognosis

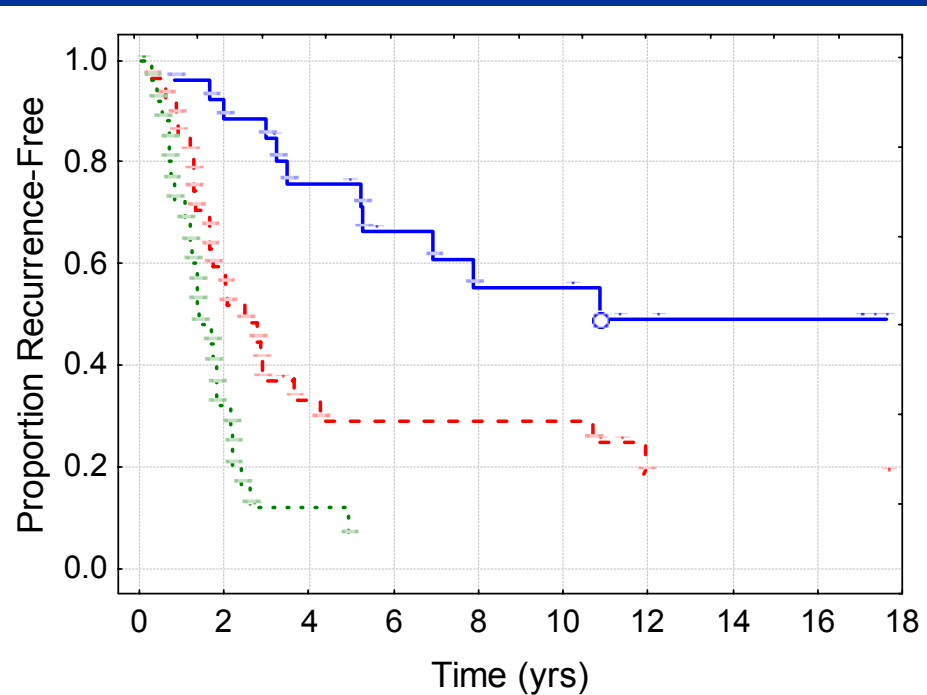
Variables	Relative Risk	95% CI RR Lower bound	95% CI RR Upper bound	p-value
Nodes Involved	0.99	0.95	1.03	0.755
Tumor Grade (Rush)	1.24	0.70	2.19	0.466
Adjuvant Tamoxifen	0.93	0.49	1.75	0.811
Chemotherapy	0.59	0.29	1.20	0.145
Tumor Size	1.04	0.95	1.14	0.429
Age At Surgery	1.00	0.97	1.02	0.732
GRB7	1.28	1.08	1.52	0.004
Bcl2	0.59	0.45	0.77	<0.001
DIABLO	2.91	1.74	4.88	<0.001
CTSL	2.10	1.30	3.38	0.002

Gene expression is the strongest predictor of outcome, independent of clinical variables, including the number of involved nodes

Rush: Gene Expression and Prognosis



Single Gene Model
(pts separated into tertiles
by HER2 expression)



Multi-Gene Model
(pts separated
into tertiles)

NSABP B20 study

- Third screening study to identify candidate genes
- Results were combined with Rush and Providence to identify genes that were significant predictors across all studies
- Led to development of Recurrence Score to predict breast cancer recurrence

Algorithm development I

- Determine appropriate number of terms in final model using bootstrap and stepwise variable selection in B20
- Select statistically significant genes in Cox survival analyses in three studies
- Create new variables from correlated genes (proliferation, ER, Her2 groups)

Algorithm development II

- Based on analysis of Martingale residuals, create non-linear (threshold) functional forms
- Fit model to NSABP B20 data with specified number of terms and specified functional forms.
- Apply Bayesian parameter adjustments.
- Define thresholds for low, moderate, and high risk groups

Three Breast Cancer Studies Used to Select 16 Cancer and 5 Reference Genes

PROLIFERATION

Ki-67
STK15
Survivin
Cyclin B1
MYBL2

HER2

GRB7
HER2

ESTROGEN

ER
PGR
Bcl2
SCUBE2

INVASION

Stromolysin 3
Cathepsin L2

GSTM1

CD68

BAG1

REFERENCE

Beta-actin
GAPDH
RPLPO
GUS
TFRC

**Best RT-PCR performance
and most robust predictors**

Three Breast Cancer Studies Used to Develop Recurrence Score (RS) Algorithm

$$\begin{aligned} \text{RS} = & + 0.47 \times \text{HER2 Group Score} \\ & - 0.34 \times \text{ER Group Score} \\ & + 1.04 \times \text{Proliferation Group Score} \\ & + 0.10 \times \text{Invasion Group Score} \\ & + 0.05 \times \text{CD68} \\ & - 0.08 \times \text{GSTM1} \\ & - 0.07 \times \text{BAG1} \end{aligned}$$

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

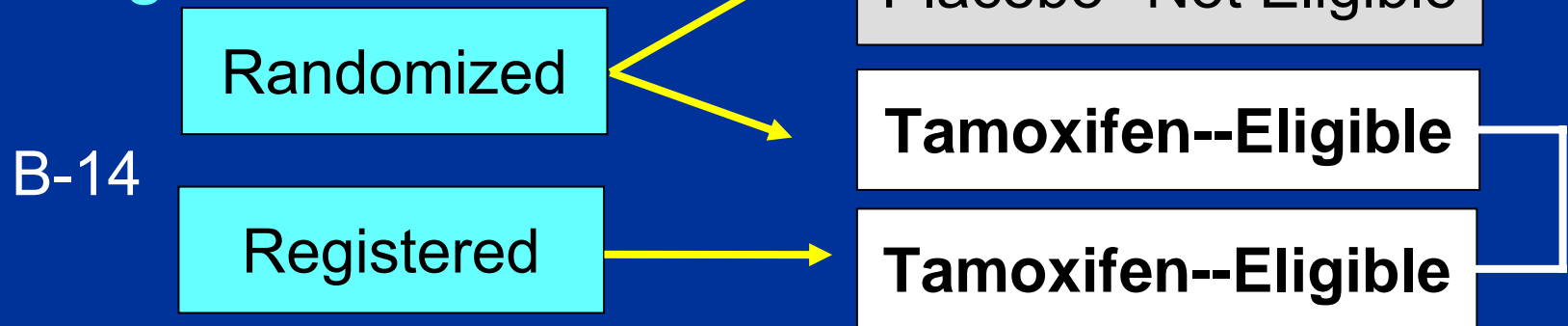
Genomic Health-NSABP B-14

Prospective Clinical Validation Study

- Objective

- Validate Recurrence Score as predictor of distant recurrence in N-, ER+, tamoxifen-treated patients

- Design



- Pre-specified 21 gene assay, algorithm, endpoints, analysis plan
- Blinded laboratory analysis of three 10 μ sections

B-14 - Subjects

Evaluable Patients

- 2617 tamoxifen-treated eligible patients in B-14
- 675 pathology eligible patients and RT-PCR performed—block never obtained or insufficient tumor in block in remaining cases
 - Insufficient RNA or RT-PCR outside of specifications
 - 7 pts (1%)
 - Evaluable patients in final analysis
 - 668 pts (99%)

B-14 Evaluable Patients (n=668) Similar to All Patients (n=2617)

	Eval (%)	All (%)	
Tumor Size (cm)			
0 - 1	16	19	} <i>p=0.23</i>
> 1 - 2	46	45	
> 2 - 4	33	32	
> 4	5	4	
 Patient Age (yr)			
< 50	29	34	} <i>p=0.16</i>
≥ 50	71	66	

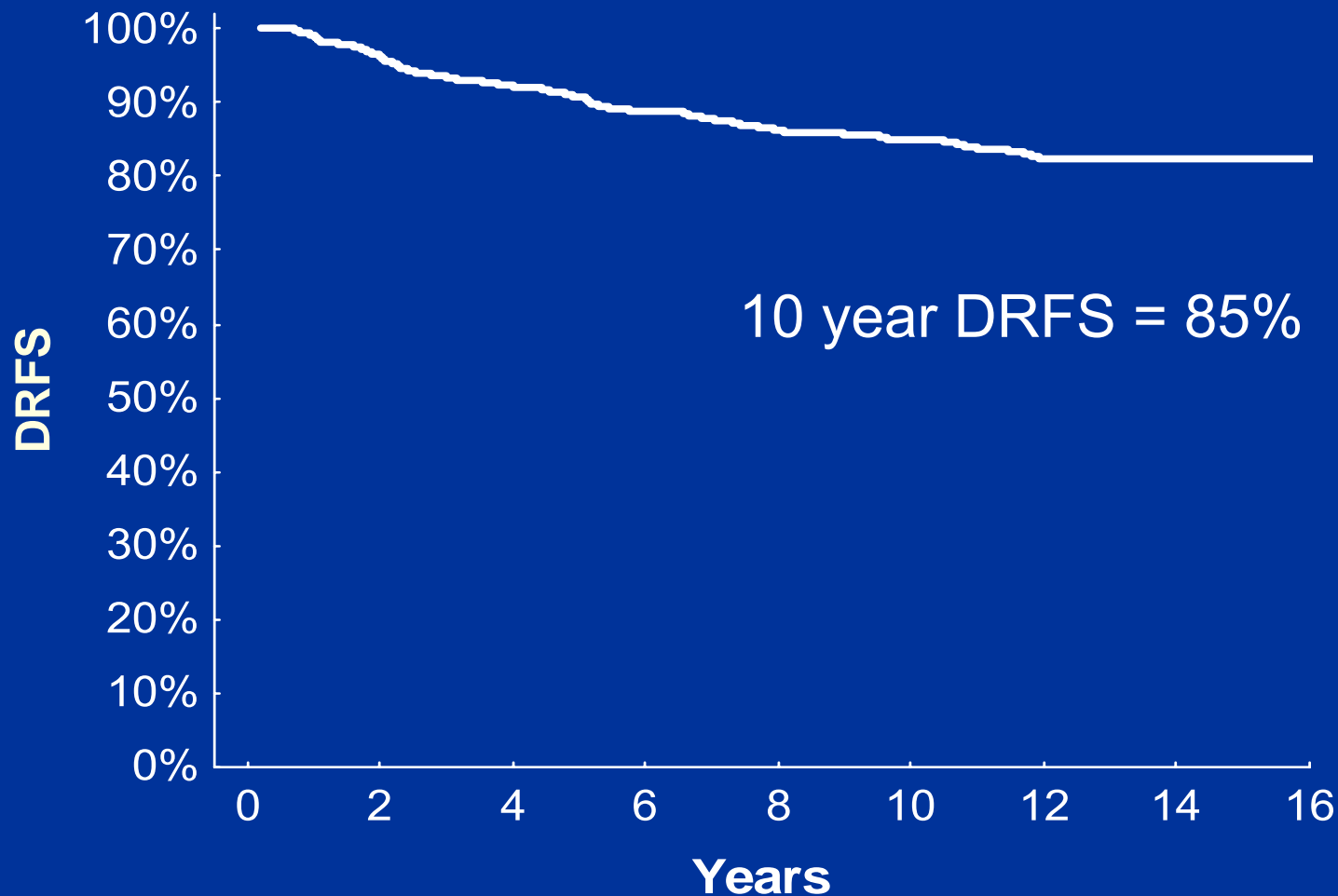
B-14 Pre-Specified Endpoints

- Primary
 - Distant Recurrence-Free Survival (DRFS)*
- Secondary
 - Relapse-Free Survival (RFS)
 - Overall Survival (OS)

*For primary analysis, patients censored at time of development of contralateral breast cancer, second non-breast cancer, or death without breast cancer recurrence

B14-Results

DRFS—All 668 Patients



B-14 Results

- First Primary Objective
 - Validate that 10 year DRFS in the low risk group ($RS < 18$) is significantly better than 10 year DRFS in the high risk group ($RS \geq 31$)

B-14 Results

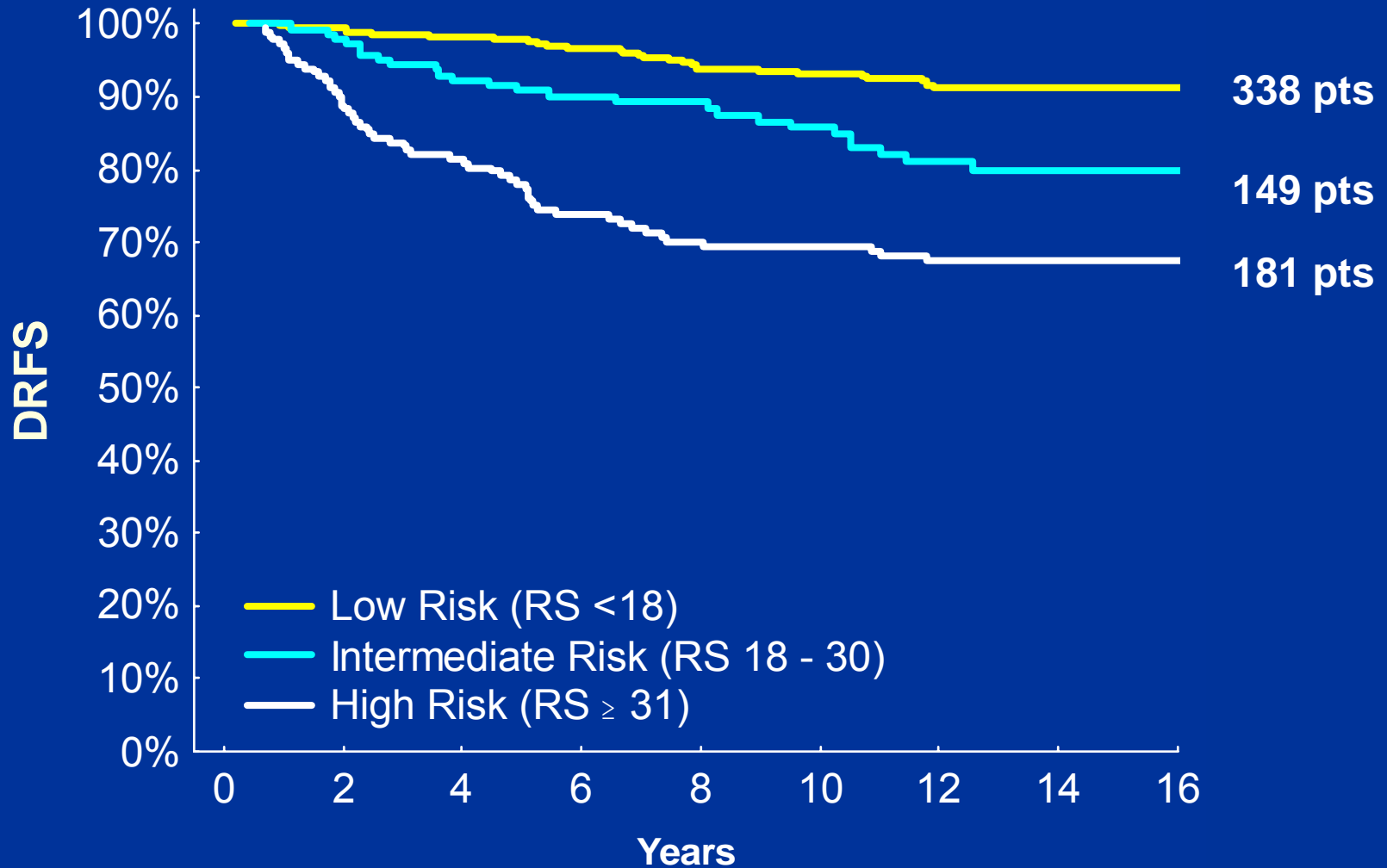
DRFS—Low, Intermediate and High RS Groups

Risk Group	% of Patients	10-yr Rate Recurrence	95% CI
Low (RS<18)	51%	6.8%	4.0%, 9.6%
Intermediate (RS 18-30)	22%	14.3%	8.3%, 20.3%
High (RS≥31)	27%	30.5%	23.6%, 37.4%

Test for the 10-year DRFS comparison between the Low and High risk groups: $p < 0.00001$

B14-Results

DRFS—Low, Intermediate, High RS Groups



B-14 Results

- Second Primary Objective
 - Validate that Recurrence Score remains a significant predictor of DRFS, after accounting for age and tumor size

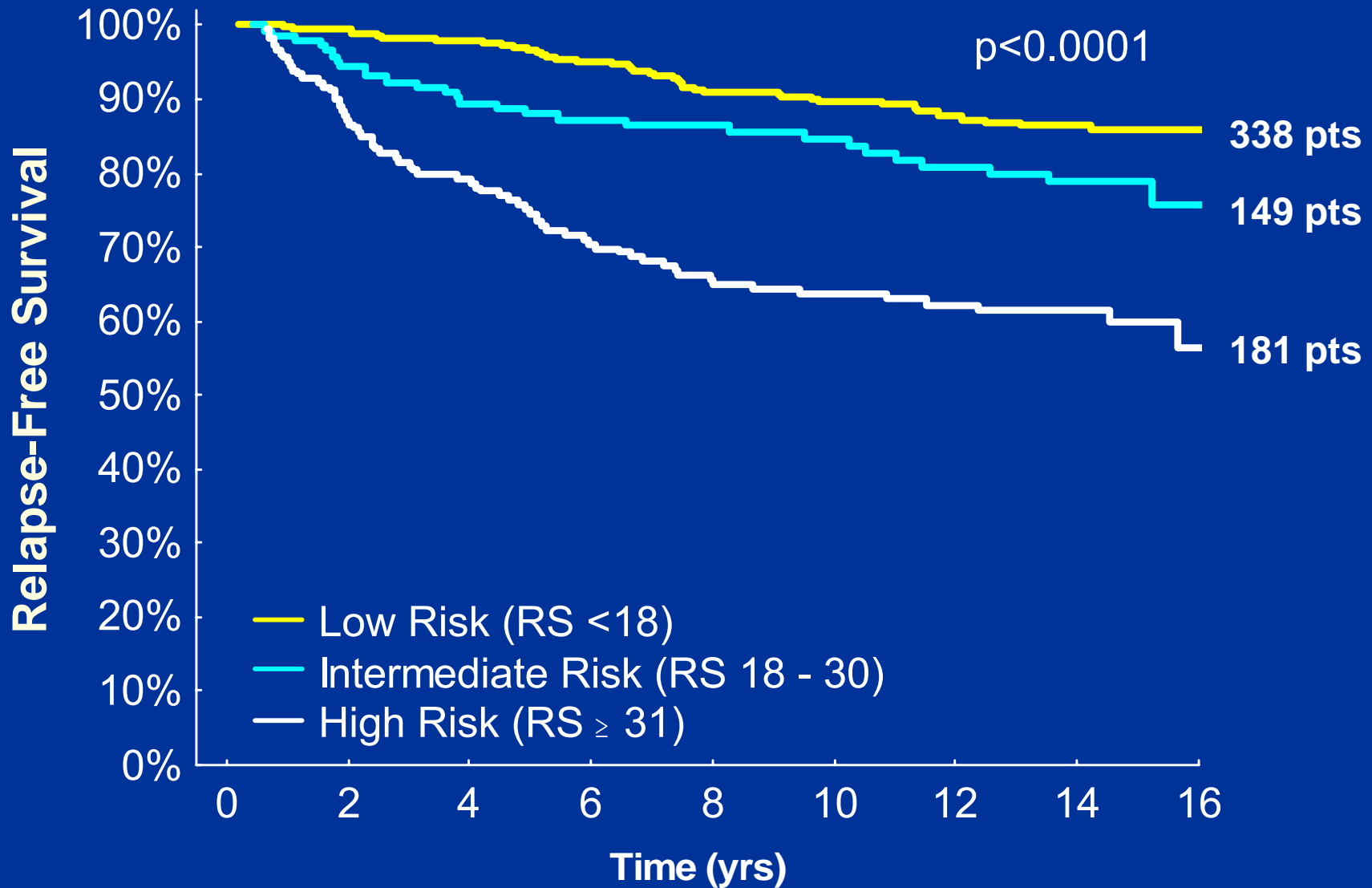
B-14 Results

Cox Models for DRFS—Age, Size Alone vs. Age, Size + RS

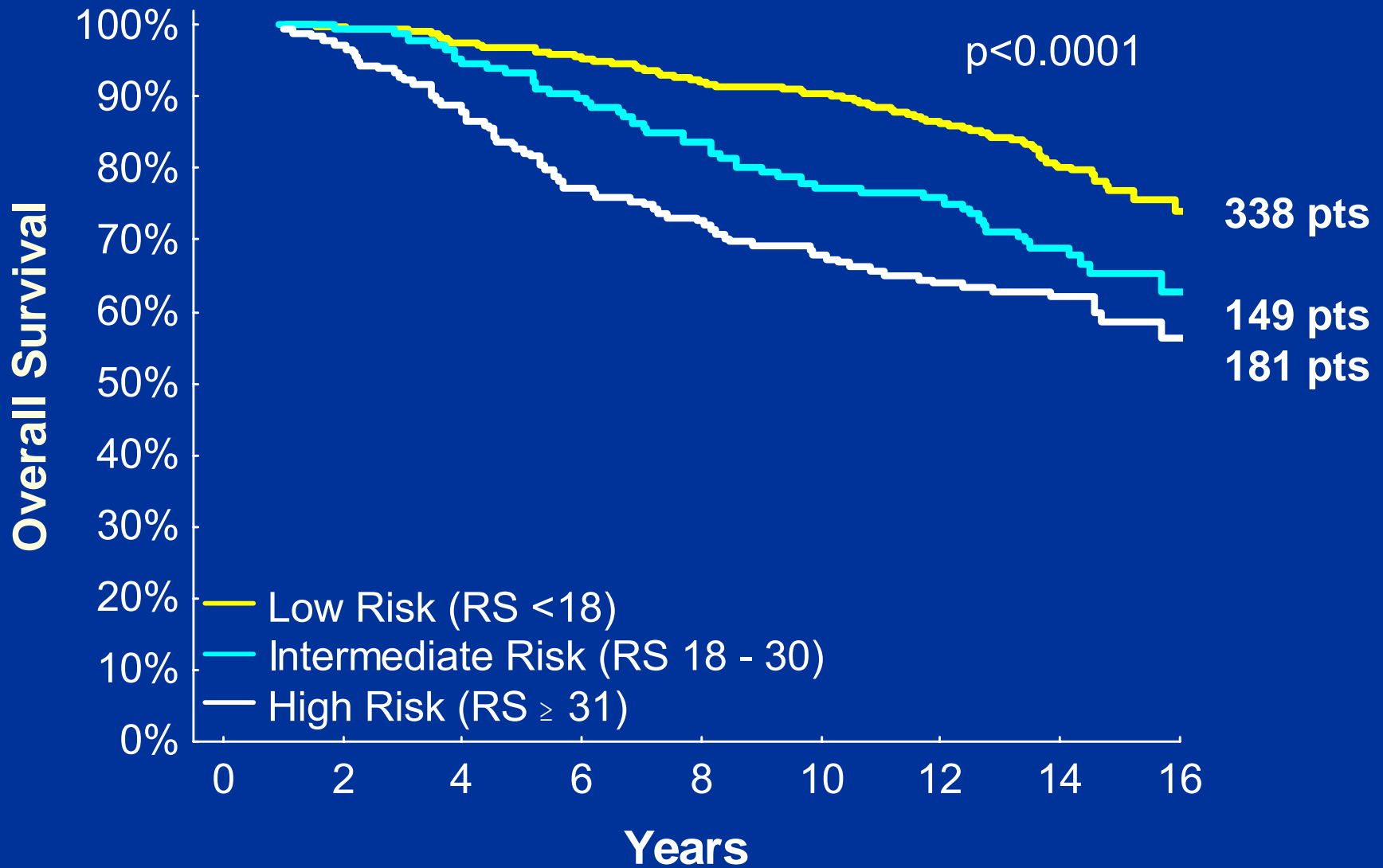
Variable	Hazard Ratio	95% CI	p-value
<i>Age ≥ 50</i>	0.57	(0.39, 0.83)	0.004
<i>Size > 2.0 cm</i>	1.44	(0.99, 2.11)	0.058
<i>Age ≥ 50</i>	0.71	(0.48, 1.05)	0.084
<i>Size > 2.0 cm</i>	1.26	(0.86, 1.85)	0.231
<i>Recurrence Score</i>	3.21	(2.23, 4.61)	<0.00001

} **p<0.00001**

B-14 Results—Relapse-Free Survival



B-14 Results—Overall Survival

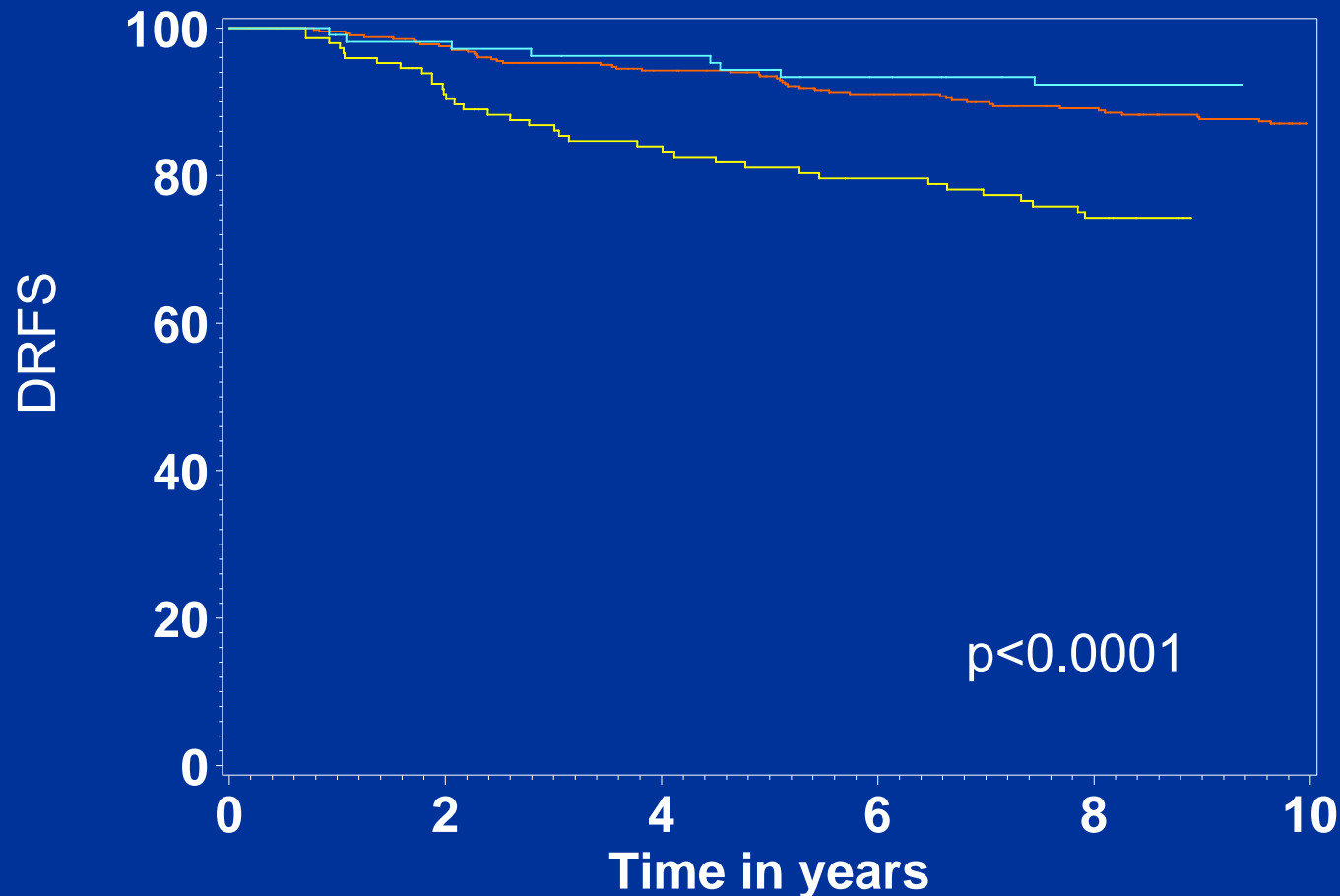


Recurrence Score and Tumor Grade

- Tumor grade is subjective and varies between different readers
- RS and tumor grade correlate, but only modestly
- RS is more powerful, objective and reproducible

Tumor Grade Correlates with Recurrence

Tumor Grade and DRFS in B-14 (n = 668)



Tumor Grade Concordance 43% Among Three Pathologists for B-14

NSABP, UCSF, Stanford Pathologists

		Pathologist B		
		Well	Moderate	Poor
Pathologist A	Well	105	114	5
	Moderate	24	241	31
	Poor	3	82	63

Concordance = 61%

		Pathologist B		
		Well	Moderate	Poor
Pathologist C	Well	56	50	1
	Moderate	74	309	30
	Poor	2	78	68

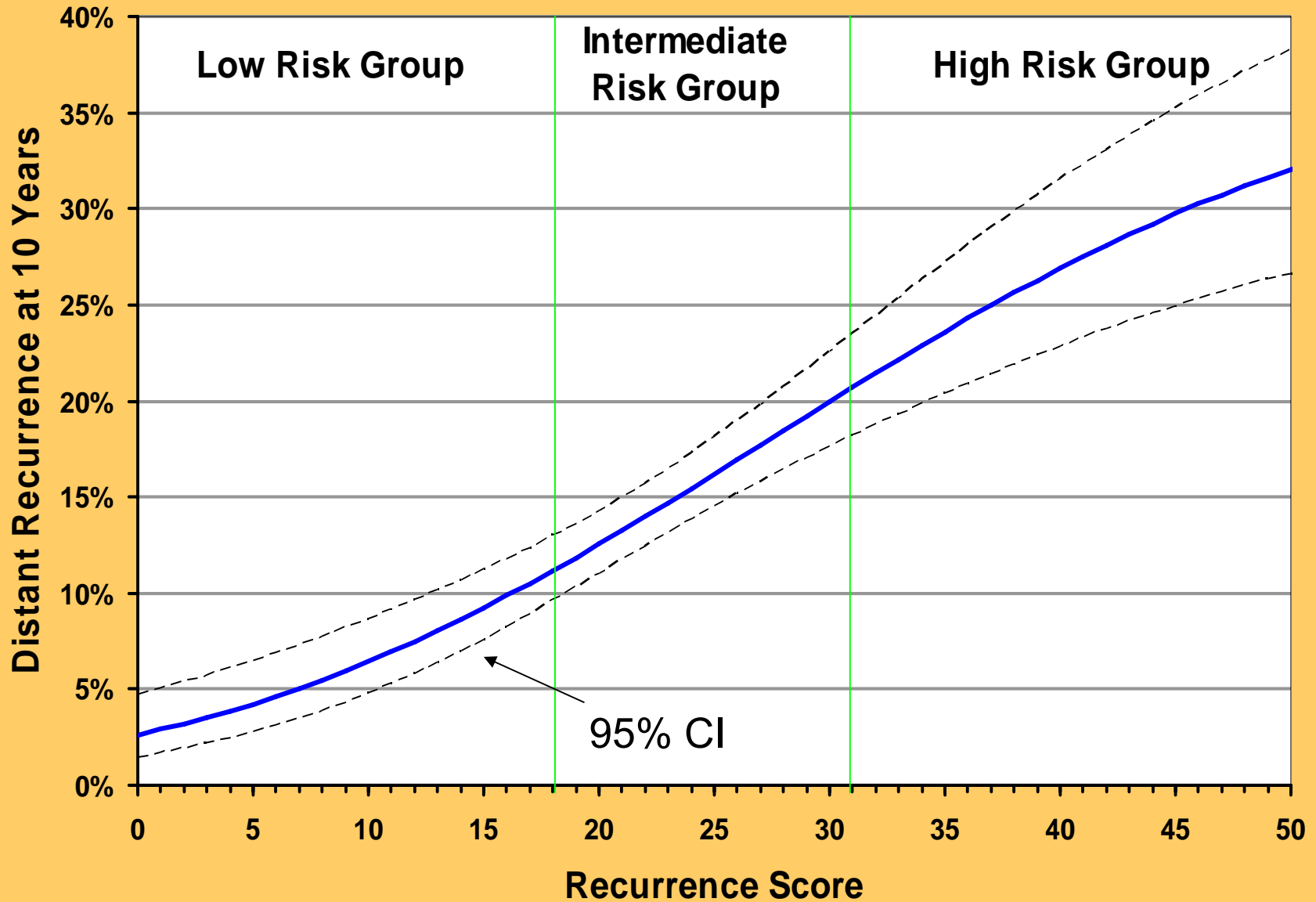
Concordance = 65%

		Pathologist A		
		Well	Moderate	Poor
Pathologist C	Well	76	31	0
	Moderate	140	221	52
	Poor	8	44	96

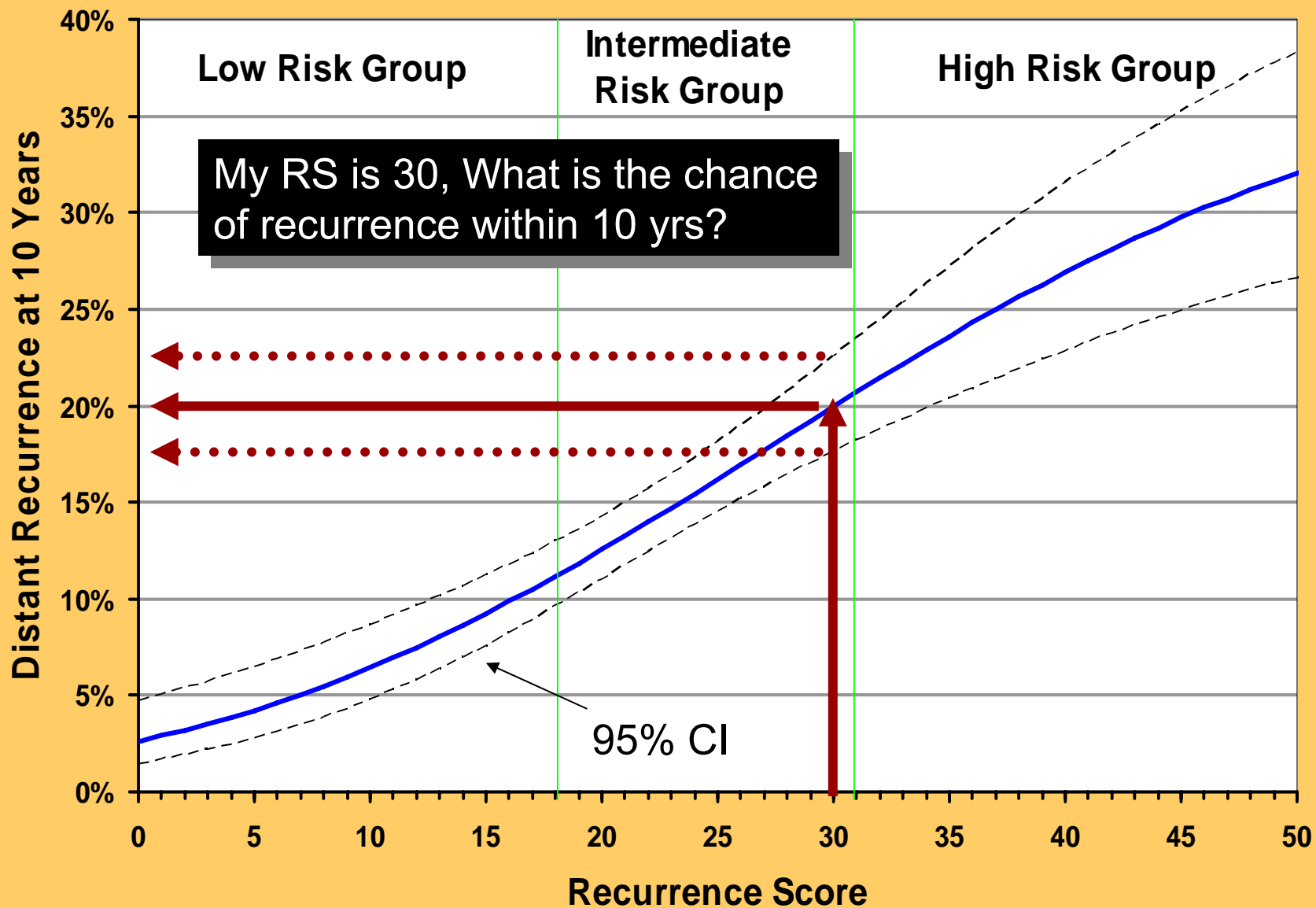
Concordance = 59%

Overall Concordance = 43%

Recurrence Score as a Continuous Predictor



Recurrence Score as a Continuous Predictor



My RS is 30, What is the chance of recurrence within 10 yrs?

Summary

- The NSABP B-14 study shows that the Recurrence Score identifies a set of women, comprising over 50% of node-, ER+, tamoxifen-treated patients, who are at low risk of recurrence and are less likely to benefit from chemotherapy.
 - Met its prospectively defined endpoints
 - Assay success rate in this prospective multi-center study was 99%
 - Validates results of prior NSABP B-20 in similar population
- Recurrence Score performance exceeds standard measures, such as age, tumor size, and tumor grade either in prognostic power or in reproducibility

Case studies

- Genomic Health
- *PharmGKB*

PharmGKB

- **PharmGKB (Pharmacogenomics Knowledge Base)**
- **Curated database of genotype and phenotype information**
- **Shared resource for researchers**
- **Developed at Stanford**
 - Russ Altman
 - Teri Klein

Bioinformatics and biostatistics issues

Phenotype (disease or drug response) is a function of:

- Several million SNP's
- Expression of 30,000 + genes
- Environment
- Interactions among these variables

- With current technology, we rarely have enough data to understand these factors.
- Can only look at a small number of variables.
- Usually explain only a very small part of the phenotypic variability.
- How much clinical utility?

- A given SNP variant's effects are often cancelled out or masked by the effects of other SNP variants
- Gene expression compensates for SNP variants
 - Drug metabolizing enzymes

- Genotype (SNP) studies have a long record of irreproducible results.
- Don't hold up when replicated in other populations.

Nonvalidation of Reported Genetic Risk Factors for Acute Coronary Syndrome in a Large-Scale Replication Study

Thomas M. Morgan, MD

Harlan M. Krumholz, MD, MS

Richard P. Lifton, MD, PhD

John A. Spertus, MD, MPH

COMPELLING EVIDENCE FROM twin and epidemiological studies suggests a genetic basis for atherosclerotic heart disease and acute coronary syndromes (ACS), including unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI).^{1,2} To date, numerous candidate genes have

Context Given the numerous, yet inconsistent, reports of genetic variants being associated with acute coronary syndromes (ACS), there is a need for comprehensive validation of ACS susceptibility genotypes.

Objective To perform an extensive validation of putative genetic risk factors for ACS.

Design, Setting, and Participants Through a systematic literature search of articles published before March 10, 2005, we identified genetic variants previously reported as significant susceptibility factors for atherosclerosis or ACS. Restricting our analysis to white patients to reduce confounding from racial admixture, we identified 811 patients who presented from March 2001 through June 2003 with ACS at 2 Kansas City, Mo, university-affiliated hospitals. During 2005-2006, we genotyped the 811 patients along with 650 age- and sex-matched controls for 85 variants in 70 genes and attempted to replicate previously reported associations. We further explored possible associations without prior assumption of specific risk models and used the Sign test to search for weak associations.

Main Outcome Measures Compare each prespecified gene variant associated with ACS risk among cases and controls. A surplus of associations would imply that some are associated with ACS.

**A Quantitative Trait Locus Not
Associated With Cognitive Ability in
Children:
A Failure to Replicate**

Hill, L. et al.

Psychological Science 13 (6), 561–562.

The most likely reasons for failure to replicate

- Complex traits are due to many genes and many variations of each gene, each having a very small effect on the trait.
- Different study populations may not have the same variations.

- The individual genes and the individual variations within genes may interact, so that no one variation will affect the trait uniformly.
- Looking at variants one at a time will not detect such interactions, and the sample size available will usually be inadequate to examine interactions.

- Gene expression tends to integrate the effects of SNP variants, environment, and expression of other genes.
- Maybe easier to find clinically significant, reproducible, relationship to phenotypes using gene expression or proteins than SNPs.

- Genotyping studies will have much more chance of success when we can look at a large set of SNPs, gene expression, protein levels, and environmental factors in **very** large numbers of individuals.

Statistical issues

- Failure to replicate
- Too many variables, too few subjects
- Too many interactions, too few subjects
- Power and sample size are inadequate