Radiation Oncology Updates In Breast Cancer:

Molecularly based signatures for radiation decision making

Where are we now and where are we going?

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Midwest Regional SABCS Review

Disclosures

Corey Speers MD, PhD- Co-founder and unpaid consultant for PFS Genomics

Educational Objectives

- Review the recent updates of radiation-related studies from SABCS 2019
- Review the development of prognostic and predictive gene signatures to guide systemic chemotherapy decisions
- Review the development of prognostic and predictive gene signatures to guide radiotherapy decisions
- Summarize all ongoing genomically stratified clinical trials, including those for radiation omission

Question

Which of the following molecular signatures has been validated as a prognostic biomarker for women with breast cancer?

(A) Oncotype Dx
(B) MammaPrint
(C) ProSigna
(D) All of the above
(E) None of the above

Question

Which of the following molecular signatures has been validated as a predictive biomarker of radiation response for women with breast cancer?

(A) DBCG-RT
(B) Radiation Sensitivity Index (RSI)
(C) Radiation and Immune-based Signature
(D) Radiotype Dx
(E) All of the above
(F) None of the above

Question

There are currently numerous ongoing genomically stratified clinical trials for radiation omission

(A) True
(B) False

Oral presentation at 2019 SABCS of radiation-related trials

San Antonio Breast Cancer Symposium®, December 10-14, 2019

Accelerated partial breast or whole breast irradiation after breast conservation surgery for patients with early breast cancer

10-year follow up results of the APBI IMRT Florence randomized phase 3 trial

Icro Meattini[1,2], Calogero Saieva[3], Sara Lucidi[1], Monica lo Russo[1], Vieri Scotti[2], Isacco Desideri[1,2], Livia Marrazzo[2], Gabriele Simontacchi[2], Monica Mangoni[1,2], Carlotta Becherini[1], Lisa Paoletti[4], Erika Moretti[5], Luca Triggiani[6], Marco Bernini[2], Lorenzo Orzalesi[1,2], Luis Sanchez[2], Jacopo Nori[2], Stefania Pallotta[1,2], Simonetta Bianchi[1,2], and Lorenzo Livi[1,2]

[1]University of Florence, Florence; [2]Azienda Ospedaliero-Universitaria Careggi, Florence; [3]Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO), Florence; [4]Ospedale Santa Maria Annunziata - Azienda Usl Toscana centro, Florence; [5]Ospedale S. Stefano - Azienda Usl Toscana centro, Prato; [6]University of Brescia, Brescia; Italy

Background

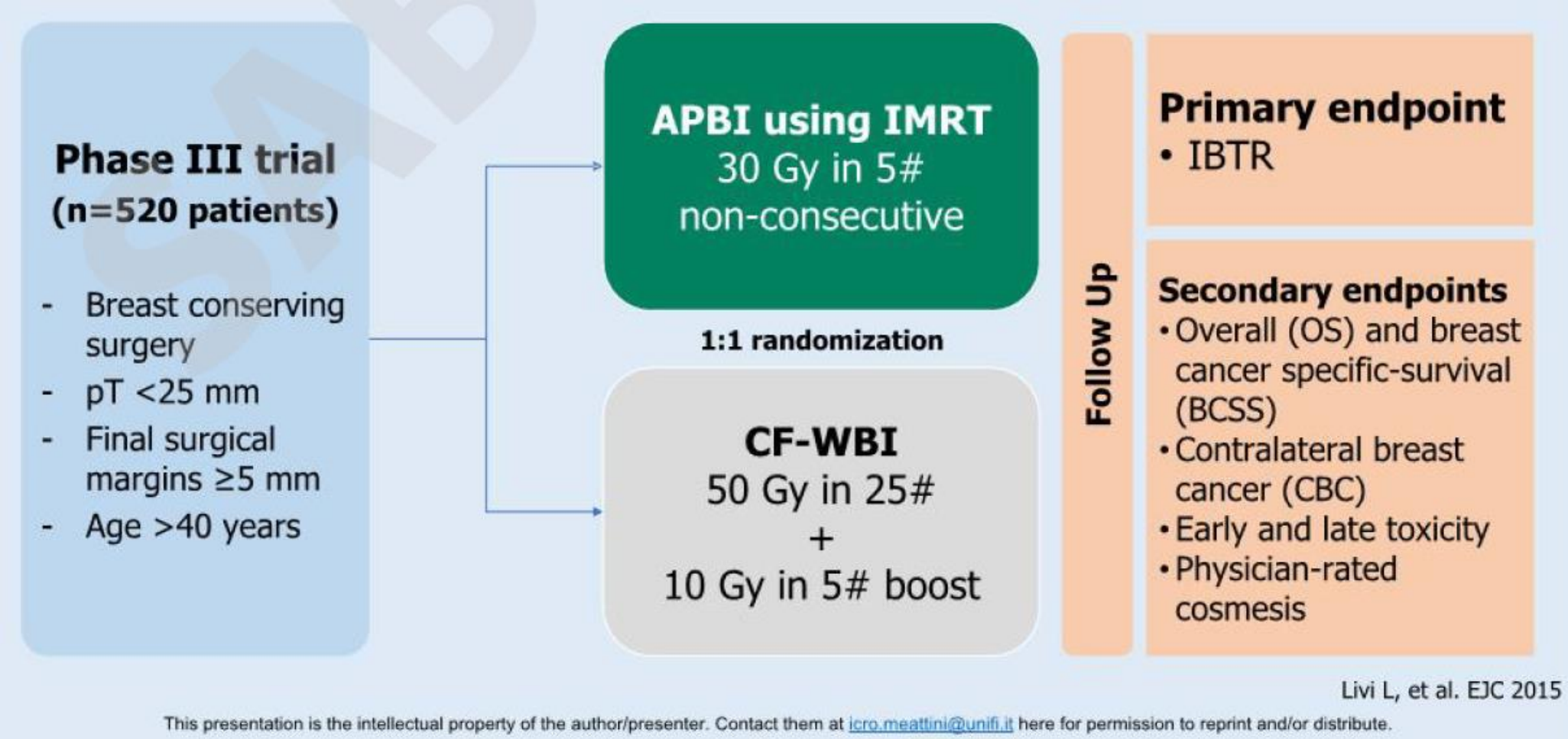
ESTRO and ASTRO recommendation identified suitable patients for PBI outside clinical trials

Patient Group	Risk Factor	2009	2016 Update
ASTRO Suitable	Age	≥60	≥50
	Margins	≥ 2 mm	≥ 2 mm
	Nodal status	pN0	pN0
	T stage	T1	Tis or T1
	ER/PgR	Positive	
	DCIS Lobular invasive	Not allowed Not allowed	G1-2; ≤2.5 cm

Patient Group	Risk Factor	2010
ESTRO Low Risk	Age	≥ 50
	Margins	≥ 2 mm
	Nodal status	pN0
	T stage	T1-2
	ER/PgR	Any
	DCIS Lobular Invasive	Not allowed Not allowed

Polgar C, et al. R&O 2010
Smith BD, et al. IROBP 2009
Correa C, et al. PRO 2016

Trial design- APBI IMRT Florence



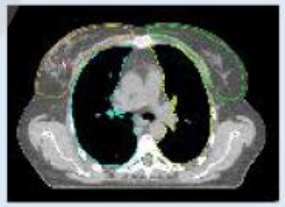
Technical Details of the Radiation Delivery

San Antonio Breast Cancer Symposium®, December 10-14, 2019

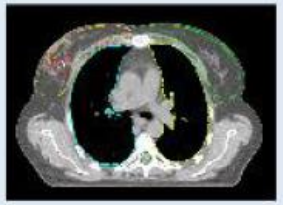
APBI USING S&S IMRT TECHNIQUE

Target Delineation and OARs dose thresholds

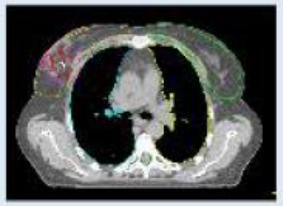
Surgical Clips
(4 mandatory)
CTV identification



CTV
Surgical Clips + 1 cm



PTV
CTV + 1 cm
limiting to 3 mm from skin and to 4 mm intrusion in homolateral lung



OAR	Constraint
Contralateral Lung	V5 <10%
Homolateral Lung	V10 <20%
Heart	V3 <10%
Homolateral breast (uninvolved tissue)	V15 <50%
Contralateral Breast	Max 1 Gy in each point

Livi L, et al. IJROBP 2010
Livi L, et al. EJC 2015

Patient characteristics

	APBI N (%)	WBI N (%)
Age		
≤50	41 (15.8)	45 (17.3)
51-59 60-69 ≥70	**84.2**	**82.7**
Grade		
G1-2	**90.0**	**87.3**
G3	26 (10)	33 (12.7)
pT stage		
pTis	23 (8.8)	32 (12.3)
pT1	**85.8**	**81.9**
pT2	14 (5.4)	15 (5.8)
No. N+		
None	**89.2**	**81.9**
1-3	19 (7.3)	33 (12.7)
No ALDN	9 (3.5)	14 (5.4)
ER status		
Positive	**95.4**	**95.8**
Negative	12 (4.6)	11 (4.2)

	APBI N (%)	WBI N (%)
Ki67 index		
<20%	193 (72.2)	174 (72.2)
≥20%	50 (20.6)	67 (27.8)
Molecular Subtype		
Luminal A-like Luminal B-like	**94.9**	**92.8**
HER2+ (non luminal)	6 (2.8)	13 (6.2)
Triple negative	5 (2.3)	2 (1)
Systemic treatment		
None	**35.8**	**28.8**
Endocrine therapy (ET) only	155 (59.6)	162 (62.3)
Chemotherapy (CT) only	5 (1.9)	3 (1.2)
CT and ET	7 (2.7)	20 (7.7)
Risk Class		
ASTRO Suitable	133 (51.2)	113 (43.5)
ASTRO Intermediate-Unsuitable	127 (48.8)	147 (56.5)
ESTRO Low Risk	190 (73.1)	166 (63.8)
ESTRO Medium-High Risk	70 (26.9)	94 (36.2)

Results – Ipsilateral Breast Tumour Recurrence

No differences in locoregional recurrence, distant metastasis, disease specific survival, or contralateral breast cancers

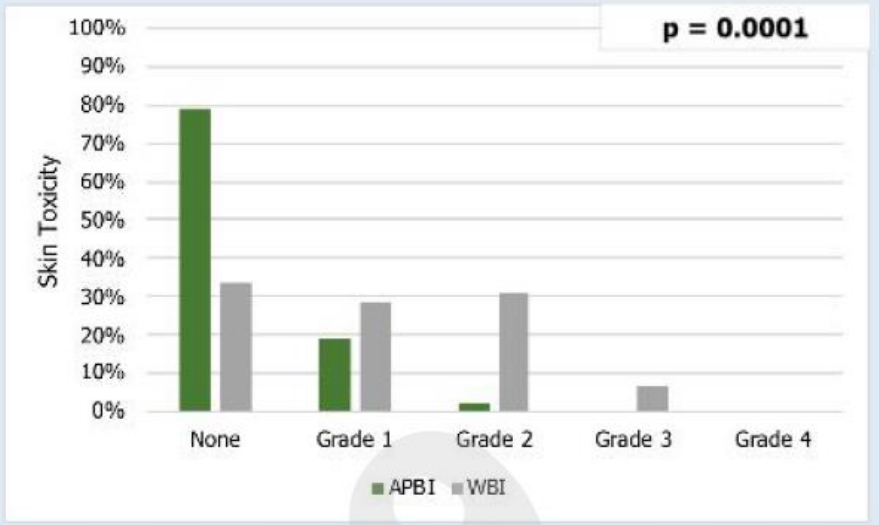
Acute reactions- skin toxicity

San Antonio Breast Cancer Symposium®, December 10-14, 2019

Results - Acute Adverse Events

RTOG & EORTC acute toxicity scale

	APBI (n=246) N	APBI (n=246) %	WBI (n=274) N	WBI (n=274) %	p-value
Any skin toxicity					
None	194	78.9	87	33.5	
Yes, any Grade	52	21.1	173	66.5	**0.0001**
None	194	78.9	87	33.5	
Grade 1	47	19.1	75	28.8	
Grade 2	5	2.0	81	31.2	
Grade 3	-	-	17	6.5	
Grade 4	-	-	-	-	**0.0001**
Grade 0-1	241	98.0	162	62.3	
Grade ≥2	5	2.0	98	37.7	**0.0001**



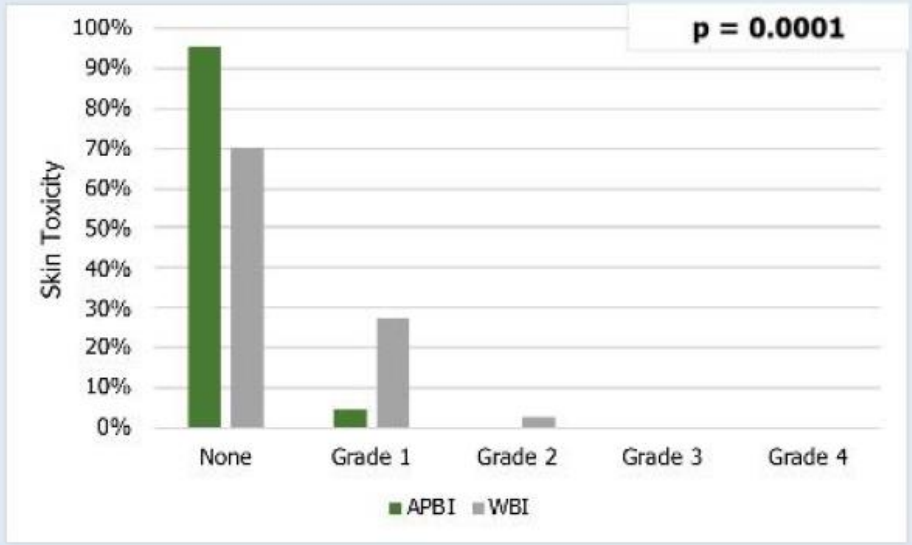
Late reactions- skin toxicity

San Antonio Breast Cancer Symposium®, December 10-14, 2019

Results - Late Adverse Events

RTOG & EORTC late toxicity scale

	APBI (n=246) N	%	WBI (n=274) N	%	p-value
Any skin toxicity					
None	235	95.5	182	70.0	
Yes, any Grade	11	4.5	78	30.0	**0.0001**
None	235	95.5	182	70.0	
Grade 1	11	4.5	71	27.3	
Grade 2	-	-	7	2.7	
Grade 3	-	-	-	-	
Grade 4	-	-	-	-	**0.0001**
Grade 0-1	246	100	253	97.3	
Grade ≥2	0	-	7	2.7	**0.015**

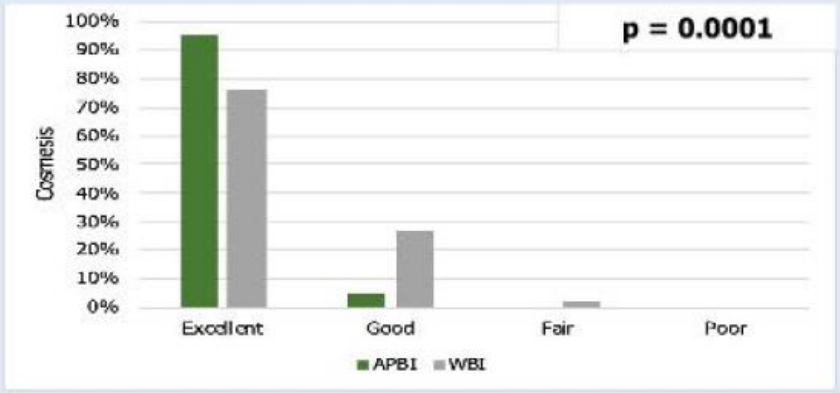


Cosmesis

San Antonio Breast Cancer Symposium®, December 10-14, 2019

Results – Cosmesis
Harvard Breast Cosmesis Scale

	APBI (n=246) N	%	WBI (n=274) N	%	p-value
Physician					
Excellent	235	95.5	199	76.5	
Good	11	4.5	70	26.9	
Fair	-	-	5	1.9	
Poor	-	-	-	-	**0.0001**



	APBI (n=246) N	%	WBI (n=274) N	%	p-value
Patient					
Excellent	44	17.9	14	5.4	
Good	200	81.3	220	84.6	
Fair	2	0.8	40	15.4	
Poor	-	-	-	-	**0.0001**

Other trials of APBI reported at SABCS 2018

These results add to the previously presented and published data on APBI

- The Canadian/NZ/Australian **RAPID** trial (2,135 pts) using 3D conventional treatment (APBI using 38.5 Gy in 10 fractions BID vs. standard fractionation/hypofractionated RT)
  - Similar rates of IBTR with worse late cosmesis

- RTOG 0413/NSABP **B-39** trial (4,216 pts) of 38.5 Gy in 10 fractions BID vs. standard fractionation (3D or brachytherapy).
  - Numerically higher rates of IBTR and non-equivalence for treatment effect between the treatment arms
  - Slightly higher rates of late Grade 3 and Grade 4-5 toxicity in PBI patients

- Numerous other brachytherapy trials

Outline

Molecular signatures in Breast Cancer

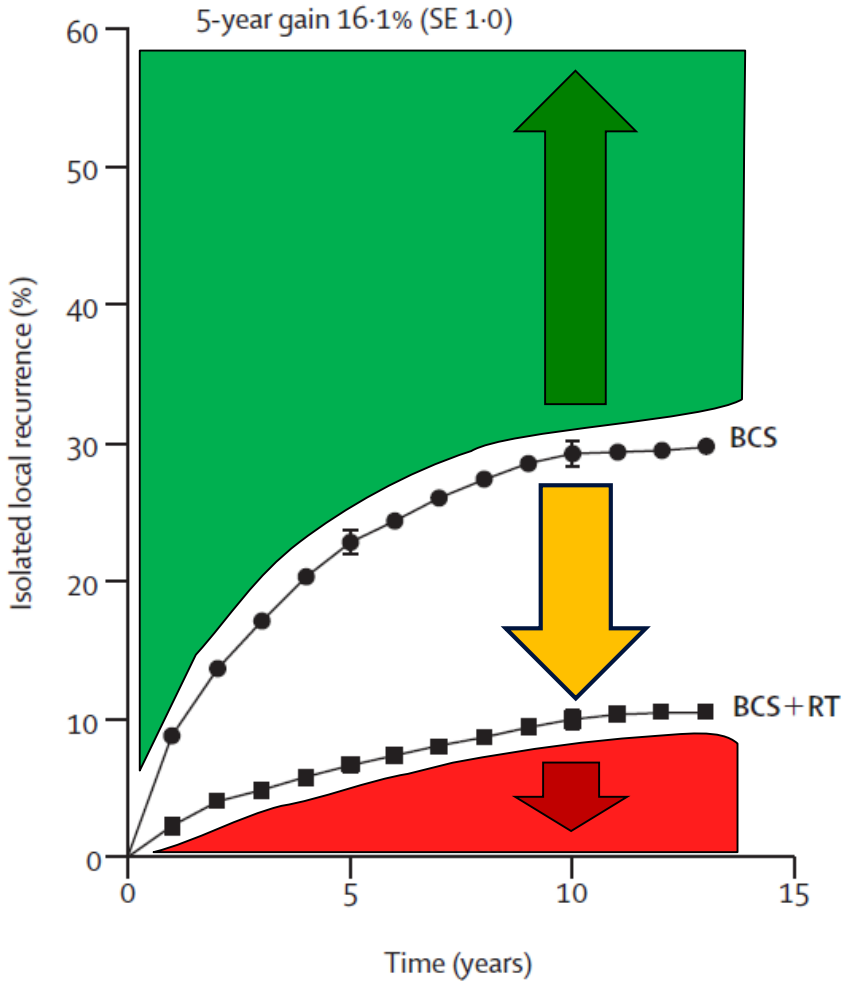
- Prognostic and predictive signatures and the relationship to chemotherapy response
- Radiation response signatures for invasive breast cancer
- Ongoing radiation trials for invasive disease using molecularly stratified inclusion criteria

Two ends of the treatment spectrum

- For certain patients, more effective surgical and systemic therapies have made adjuvant radiation therapy unnecessary

- For other patients, current multi-modality therapy is ineffective in prevent disease recurrence and/or progression
    - Ineffective therapies
    - inadequate risk stratification

EBCTCG Oxford Meta-analysis



70% need no additional radiation

2/3 relative risk reduction with RT

10% develop recurrence despite standard therapy

>6000 women treated with breast conserving surgery *EBCTCG, Lancet* 2005;366:2087-2106

Who needs adjuvant radiotherapy?

Which individual (rather than which group) will benefit from adjuvant therapy?

Up to 40% of patients with a poor prognosis as defined by conventional clinicopathological parameters will remain disease free without adjuvant radiation therapy

The benefit from adjuvant radiotherapy for patients with lymph node–negative (LN-) disease is not uniform; some patients relapse despite therapy (10%) and others are already cured by local treatment (60-70%).

Prognostic and Predictive Signatures

The likelihood of developing distant recurrence in this patient population is only 15% at 10 years, which means 85% of patients are overtreated if they all receive chemotherapy.

Who benefits from chemotherapy?
Who can we safely spare?

Oncotype DX® was developed to quantify the likelihood of disease recurrence in women with ER+, LN- breast cancer and was found to be useful in predicting response to chemotherapy.

Oncotype Dx® Breast Recurrence Score

Sixteen cancer-related genes and five reference genes were selected from the candidate genes. The 16 cancer-related genes were then used to develop an algorithm based on the expression levels of these genes, thus allowing a Recurrence Score™ (RS) to be computed for each specimen. This RS correlated with the rate of distant recurrence at 10 years

Sixteen Cancer and Five Reference Genes

PROLIFERATION	INVASION	HER2	ESTROGEN	REFERENCE
Ki-67 STK15 Survivin Cyclin B1 MYBL2	Stromelysin 3 Cathepsin L2 Best RT-PCR performance and most robust predictors	GRB7 HER2 GSTM1 CD68 BAG1	ER PGR Bcl2 SCUBE2	Beta-actin GAPDH RPLPO GUS TFRC

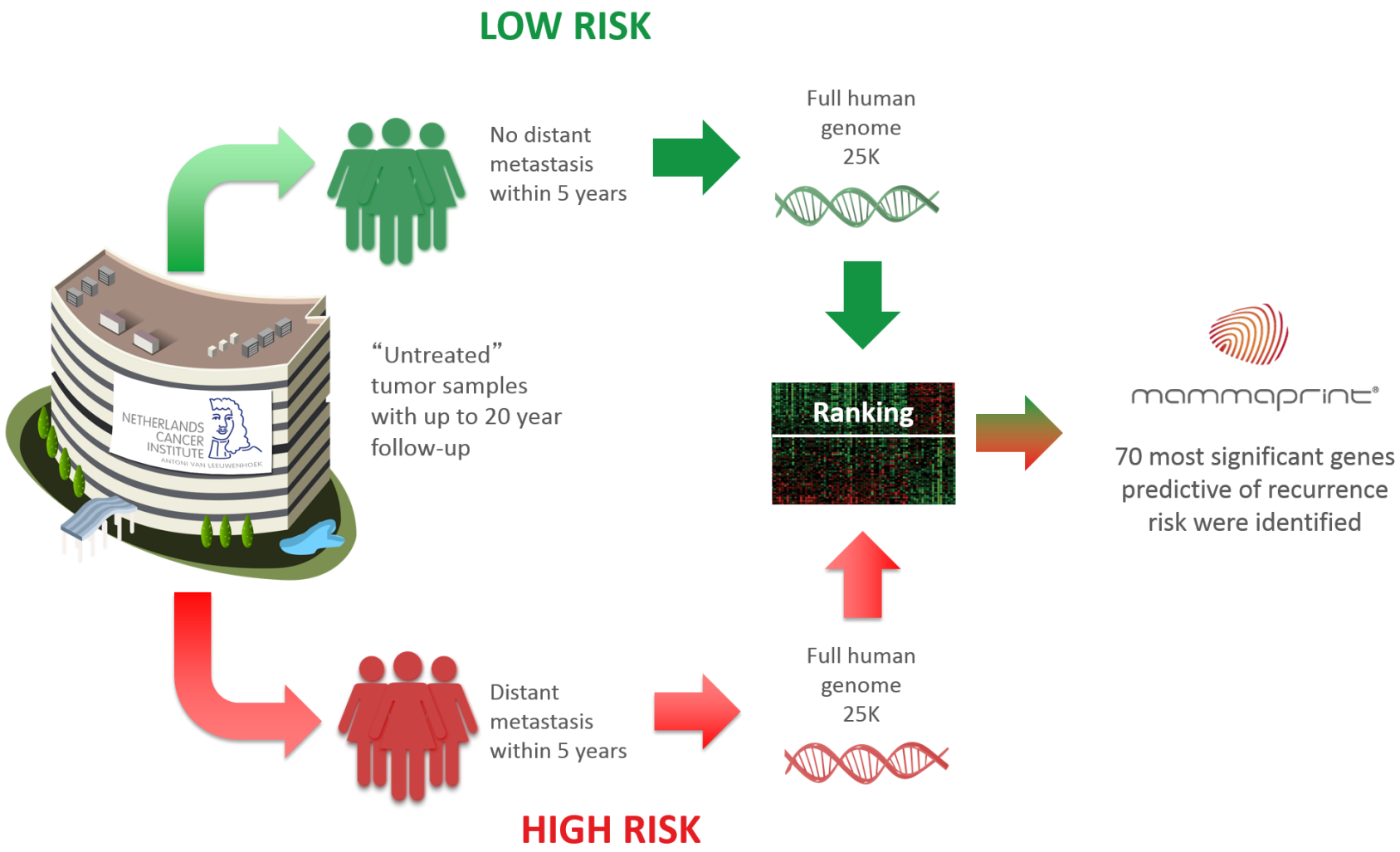
Oncotype Dx® Validation

NSABP Study B-20

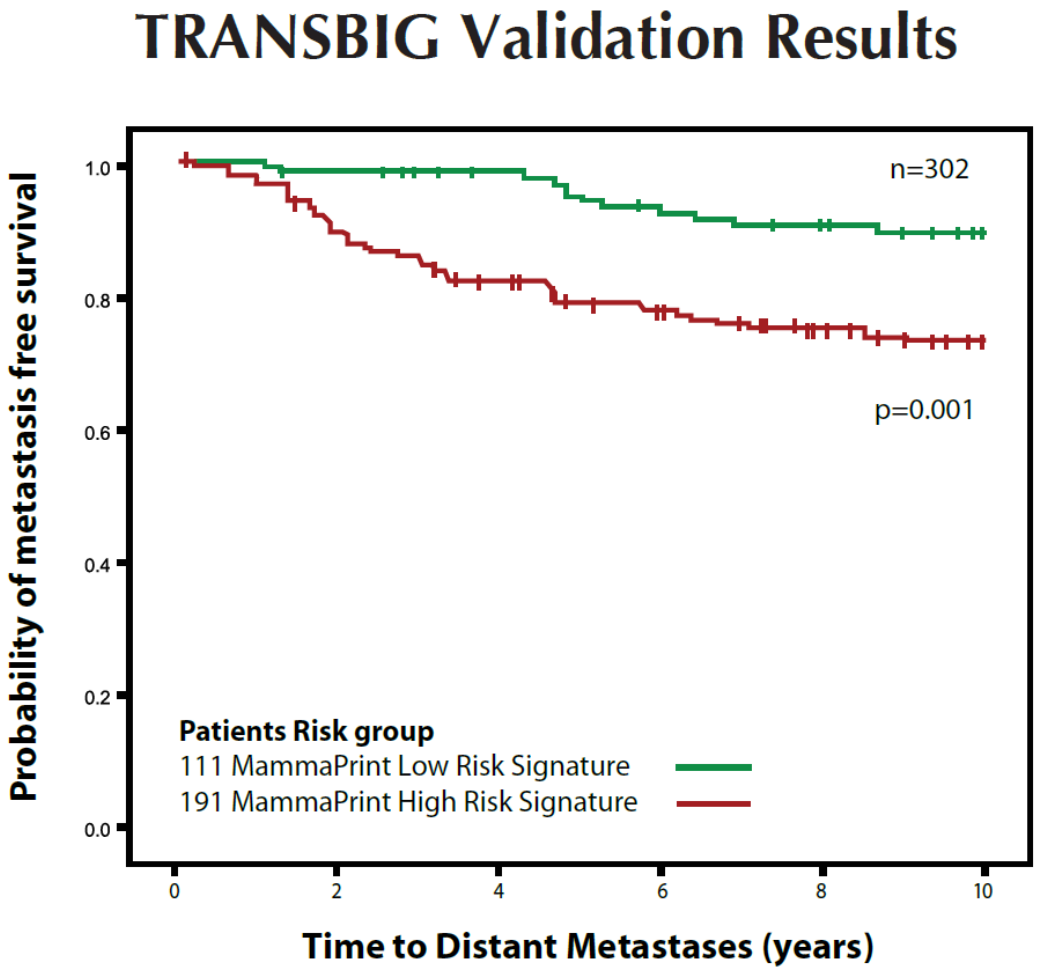
The Oncotype Dx® Recurrence Score assay not only quantifies the likelihood of breast cancer recurrence in women with N-, ER+ breast cancer, but also predicts the magnitude of chemotherapy benefit.

Additional NSABP studies showing predictive in LN-positive patients (and SWOG 8814) and lack of chemotherapy benefit in patients with an intermediate risk score (RS= 11-25) (TAILORx)

MammaPrint® - Agendia



MammaPrint® Validation



MINDACT validation in patients with discordant clinical and genomic risks

Prosigna® Breast Cancer Prognostic Gene Signature Assay- Nanostring

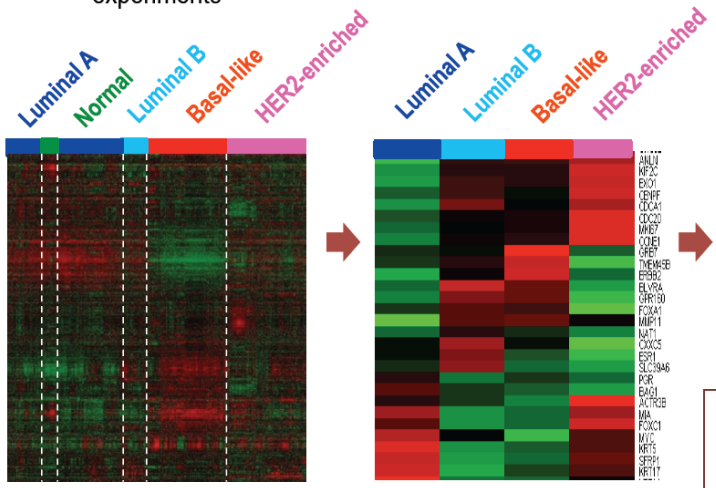
Development of Prosigna™ is Based on PAM50 Gene Signature

2000
Researchers first describe breast cancer intrinsic subtypes based on microarray experiments

2009
Researchers first describe "PAM50" gene expression signature

2010
NanoString exclusively licenses PAM50 gene expression signature

2012/13
Prosigna launches after receiving CE Mark for Europe & Israel; FDA 510k clearance in US



prosigna™
Breast cancer prognostic gene signature assay

PAM50 developed by a consortium of four academic breast cancer experts

- Charles Perou, PhD, University of North Carolina
- Dr. Matt Ellis, Washington University School of Medicine
- Torsten Nielsen, MD, PhD, Pathologist, BC Cancer Agency
- Philip Bernard, MD, University of Utah / Huntsman Cancer Institute

Source: *Molecular portraits of breast cancer.* Nature. 2000 May 25;.

Source: *Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes, JCO.2009*

Question

Which of the following molecular signatures has been validated as a prognostic biomarker for women with breast cancer?

(A) Oncotype Dx
(B) MammaPrint
(C) ProSigna
(D) All of the above
(E) None of the above

What about signatures to predict radiation response?

Several under development, but what about the previously derived signatures for chemotherapy benefit?

Prognostic and Predictive Signatures for Personalized Radiation Decisions

Previously derived

- Oncotype Dx®
- Oncotype Dx® for DCIS
- IHC surrogates for subtype

Radiation specific signatures

Invasive disease

- Danish Breast Cancer Group (DBCG-RT)
- Radiation Sensitivity Index (RSI)
- Radiosensitivity and Immune Gene Signature
- Radiation Sensitivity Signature (RSS or Radiotype Dx®) and Adjuvant RadioTherapy Intensification Classifier (ARTIC)

Previously derived signatures applied to radiation questions

Previously derived

- Oncotype Dx®
- Oncotype Dx® for DCIS
- IHC surrogates for subtype

Previously derived signatures applied to radiation questions

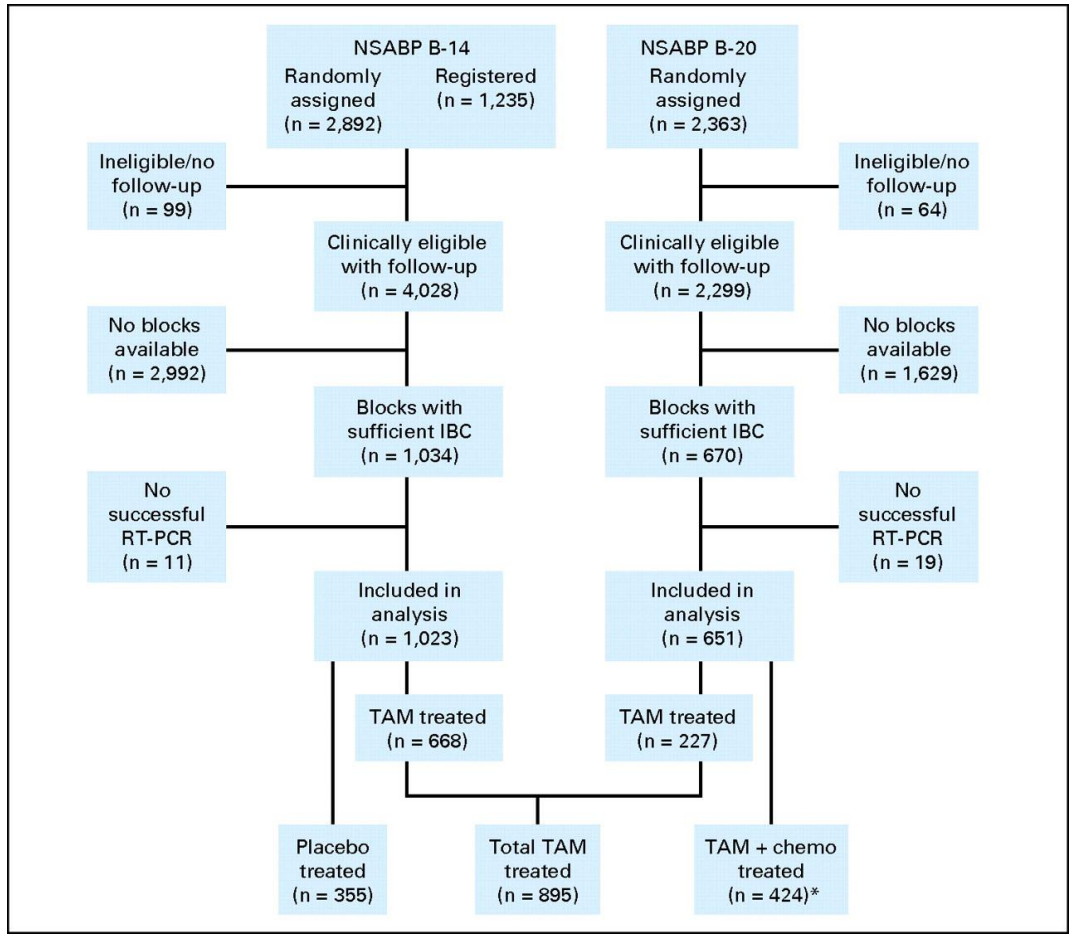
Previously derived

- Oncotype Dx®
- Oncotype Dx® for DCIS
- IHC surrogates for subtype

Locoregional recurrence using Oncotype Dx® in node-negative patients

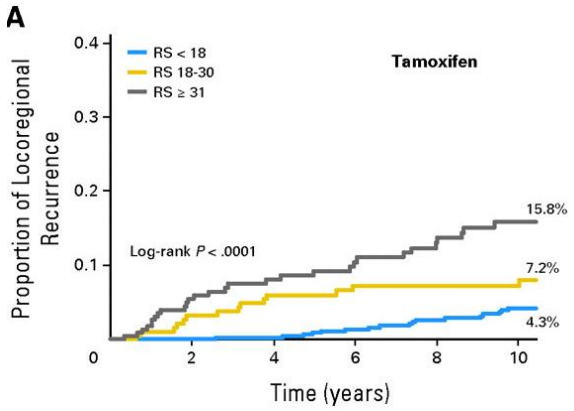
NSABP B-14: ER+, node-negative patients s/p TM or lumpectomy, ALND, and RT randomized to +/- tamoxifen

NSABP B-20: ER+, node-negative patients s/p TM or lumpectomy, ALND, and RT randomized to chemo + tamoxifen vs tamoxifen alone

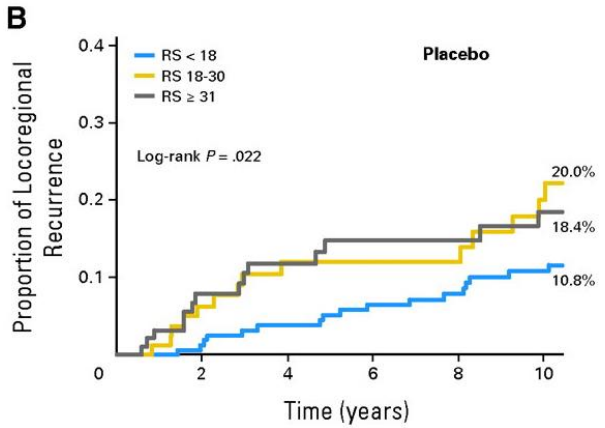


EP Mamounas et al., *JCO* **2010,** 28, 1677-1683.

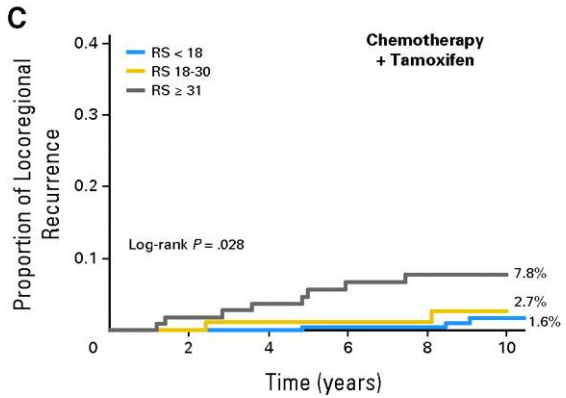
Locoregional Recurrence using Oncotype Dx® in Node-negative patients



B14 and B20 pts tam pts



B14 placebo pts

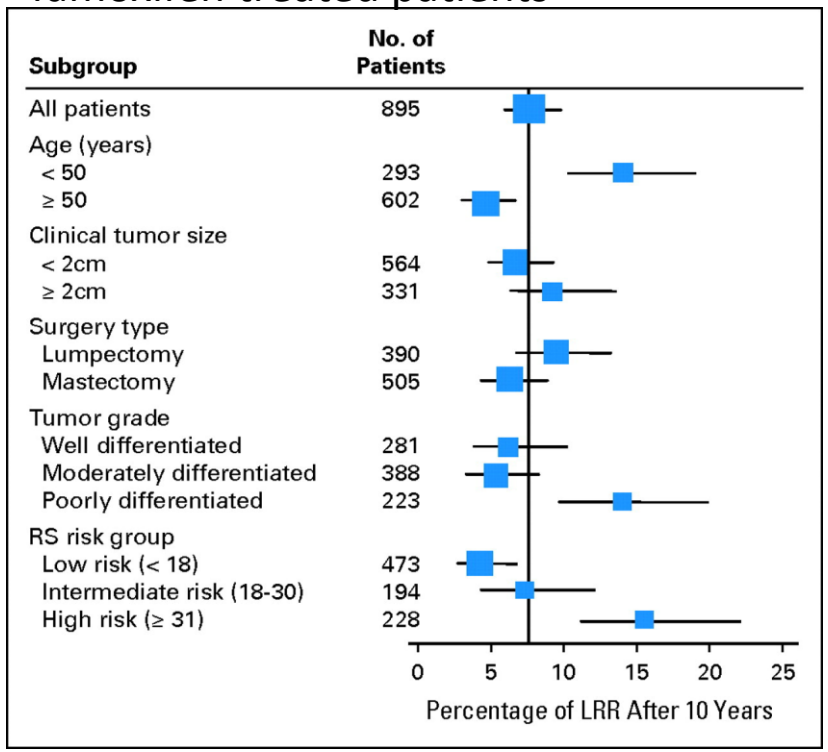


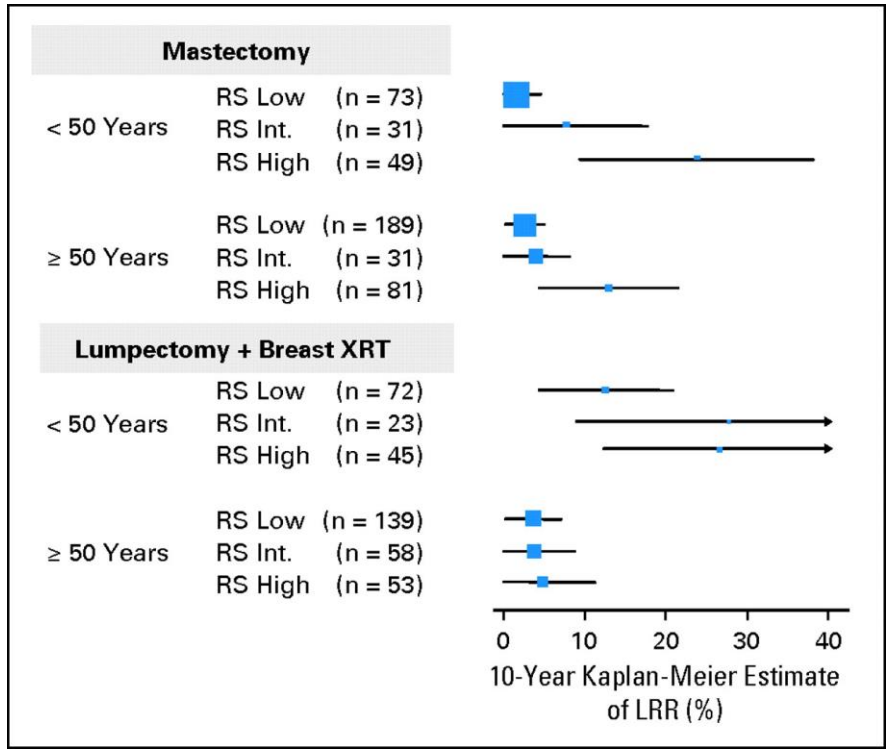
B20 chemo+tam pts

EP Mamounas et al., *JCO* **2010,** 28, 1677-1683.

Factors associated with locoregional recurrence in NSABP B-14 and B-20

Tamoxifen treated patients





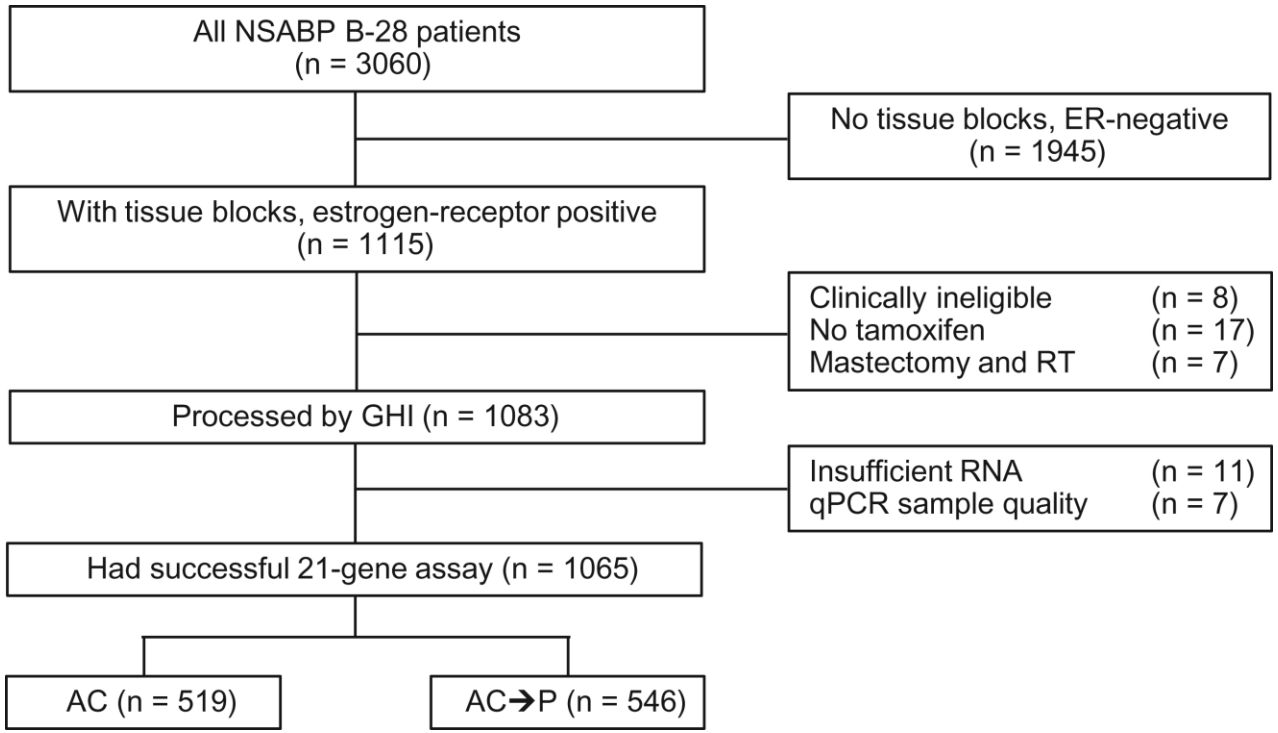
Importantly, there is no data regarding recurrence rates by recurrence score in women treated with lumpectomy *WITHOUT* RT, nor are there differences in LRR rates by RS score in RT treated pts.

EP Mamounas et al., *JCO* **2010,** 28, 1677-1683.

Locoregional Recurrence using Oncotype Dx® in Node-positive patients

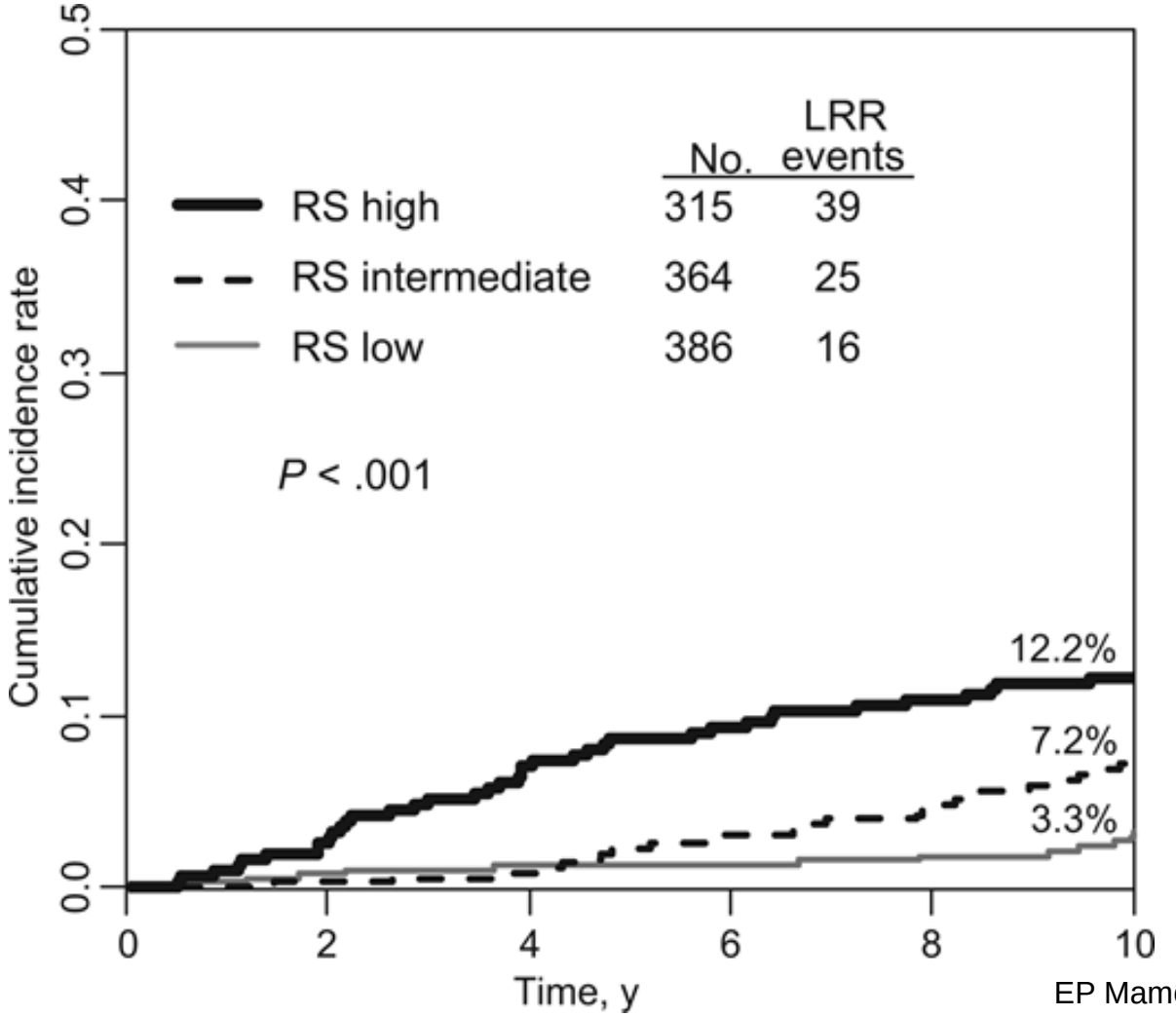
NSABP B-28: AC x 4 cycles vs. AC x 4 + T x 4 in N+ patients.

- **tamoxifen in young ER+ and older than 50 yr old patients**
- **RT for lumpectomy patients, not given to mastectomy patients**



EP Mamounas et al., J Natl Cancer Inst. 2017;109(4)

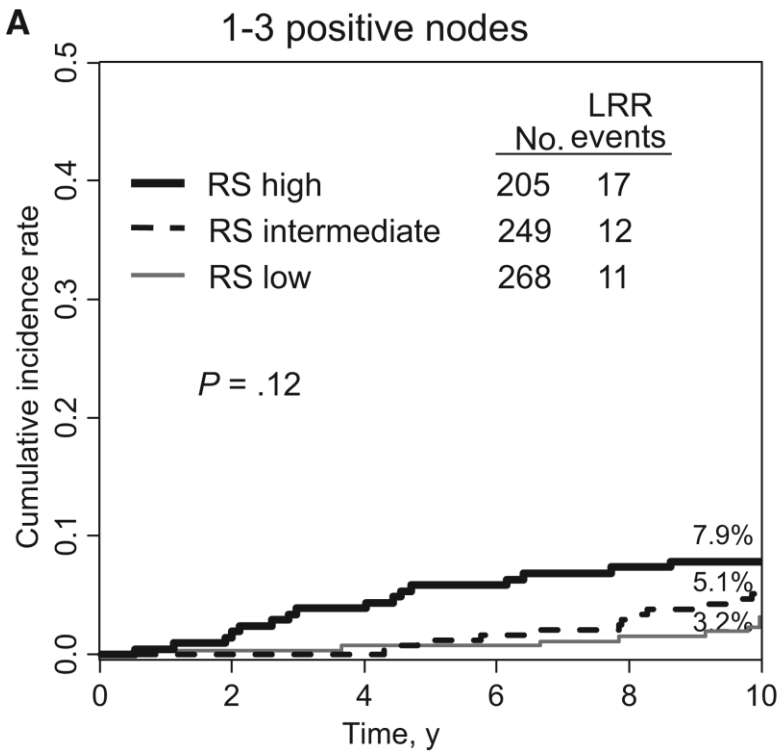
Locoregional Recurrence using Oncotype Dx® in Node-positive patients

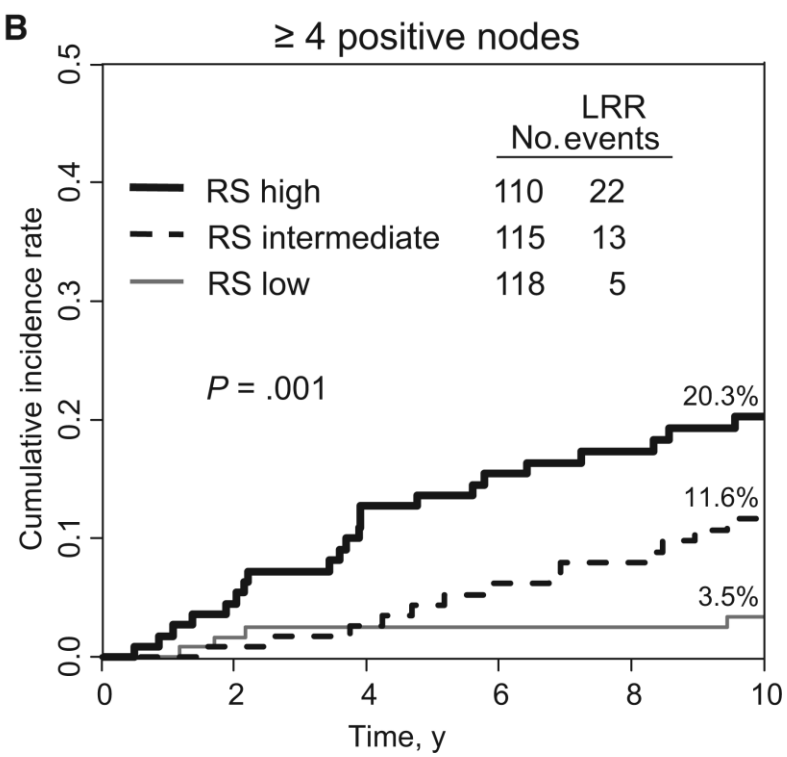


Mix of mastectomy (no radiation-604 pts) and lumpectomy (received RT-461 pts)

EP Mamounas et al., J Natl Cancer Inst. 2017;109(4)

Locoregional Recurrence using Oncotype Dx® in Node-positive patients





All patients

EP Mamounas et al., J Natl Cancer Inst. 2017;109(4)

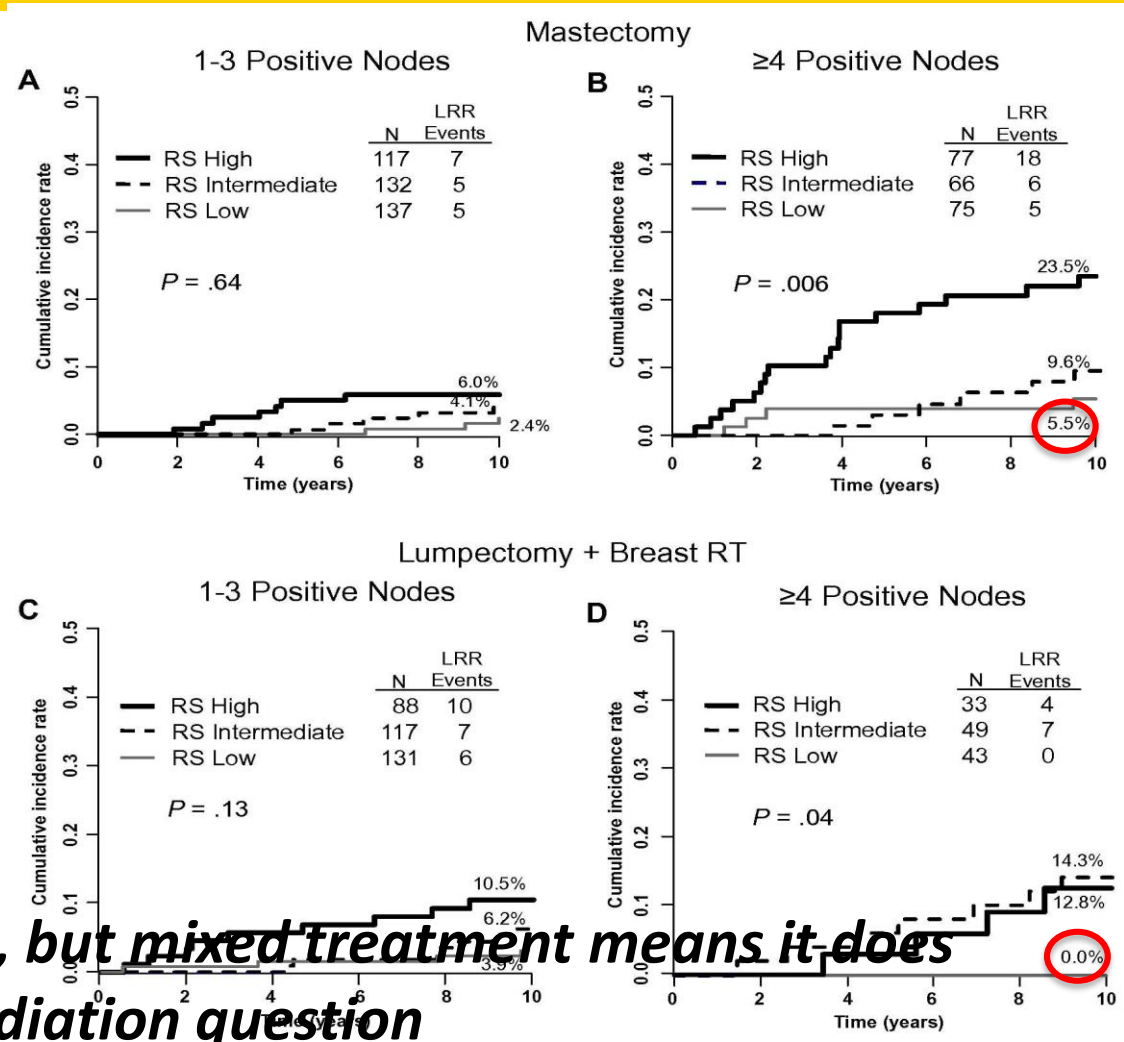
Locoregional Recurrence using Oncotype Dx® in Node-positive patients- association with RT

Not significant in patients treated with mastectomy and 1-3 nodes positive (only 4 or more)

Not significant in BCT+RT patients with 1-3 nodes positive (only 4 or more)

Intriguing in that subset of post-mastectomy patients with 4+ nodes but low RS, radiation may not be necessary

AVOID OVERTREATMENT



Important and useful data, but mixed treatment means it does not directly address the radiation question

EP Mamounas et al., J Natl Cancer Inst. 2017;109(4)

Locoregional Recurrence using Oncotype Dx® in Node-positive patients- association with RT

SWOG 8814 assessment of LRR by Recurrence Score
(led by Wendy Woodward at MD Anderson CC)

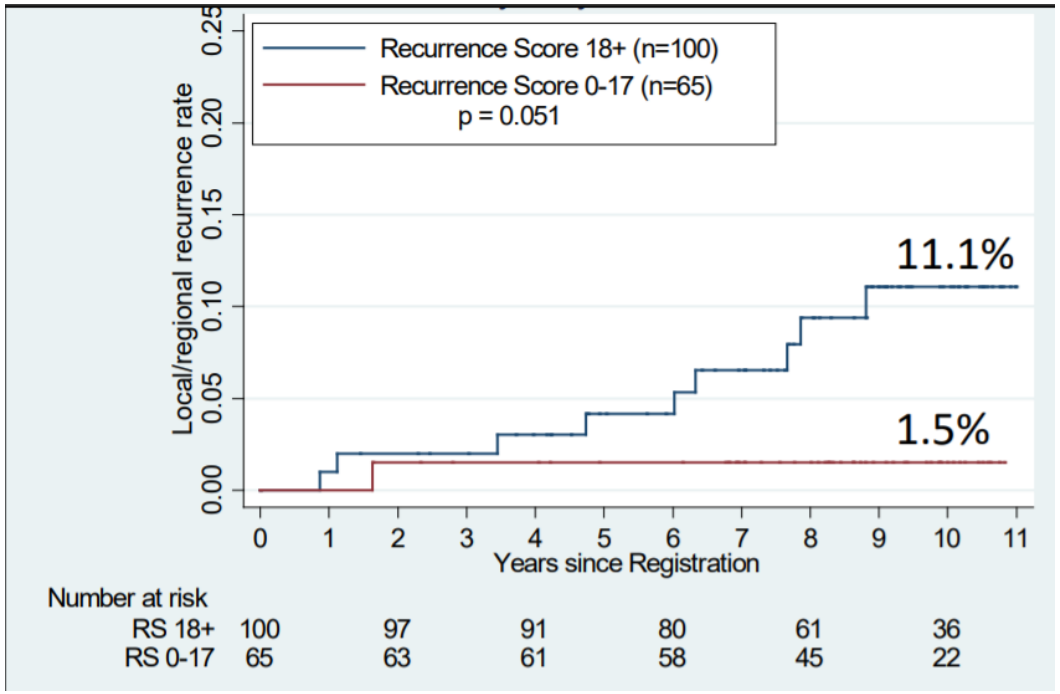
SWOG 8814: Tamoxifen With or Without Combination Chemotherapy in Postmenopausal Women Who Have Undergone Surgery for Breast Cancer

- **Randomized phase III trial, N = 1477**
- **ER and/or PR+, Node+, post-menopausal randomized to tamoxifen alone vs. Tamoxifen then CAF vs. Concurrent Tamoxifen + CAF**
- **RS determined using RT-PCR**

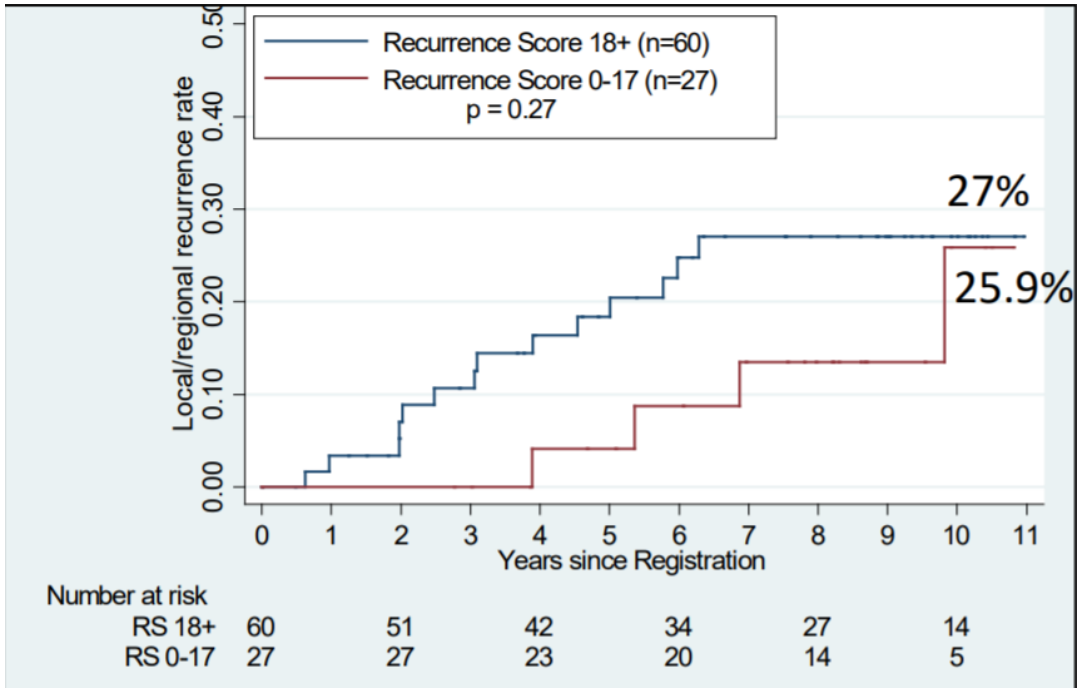
Locoregional Recurrence using Oncotype Dx® in Node-positive patients- association with RT

SWOG 8814: Post mastectomy patients without RT

1-3 nodes positive



>3 nodes positive



Prognostic and Predictive Signatures for Personalized Radiation Decisions

Previously derived

- Oncotype Dx®
- Oncotype Dx® for DCIS
- IHC surrogates for subtype

Radiation specific signatures

Invasive disease

- Danish Breast Cancer Group (DBCG-RT)
- Radiation Sensitivity Index (RSI)
- Radiosensitivity and Immune Gene Signature
- Radiation Sensitivity Signature (RSS or Radiotype Dx®) and Adjuvant RadioTherapy Intensification Classifier (ARTIC)

Radiation specific signatures for treatment decisions

- Danish Breast Cancer Group (DBCG-RT)- radiation necessity signature based on benefit of **post-mastectomy** radiation on Danish 82b/c trials[1] – IDENTIFIES RADIATION BENEFIT GROUP
- Radiation Sensitivity Index (RSI)- pan cancer radiation signature to predict benefit of radiation based on radiation sensitivity of NCI-60 cell lines[2] – IDENTIFIES RADIATION RESISTANT GROUP
- Radiation Sensitivity and Immune Signature- public datasets used to predict benefit of radiation[3]– IDENTIFIES RADIATION BENEFIT AND RESISTANT GROUP
- Radiation Sensitivity Signature (RSS or Radiotype Dx®)- breast cancer cell line-specific signature to predict utility and efficacy of radiation in women treated with **RT after lumpectomy**[4] - IDENTIFIES RADIATION BENEFIT AND RESISTANT GROUP
- Adjuvant RadioTherapy Intensification Classifier (ARTIC)[5]- IDENTIFIES RADIATION BENEFIT AND RESISTANT GROUP

1. Tramm T, et al., Clin Cancer Res. 2014 Oct 15;20(20):5272-80
2. Torres-Roca JF et al., Cancer Res 2005: 65(16):7169-76
3. Cui Y, et al., Clin Cancer Res. 24(19) October 1, 2018
4. Speers C, et al., Clin Cancer Res 2015 Aug 15;21(16):3667-77
5. Sjöström et al, JCO Oct. 16, 2019; JCO.19.00761

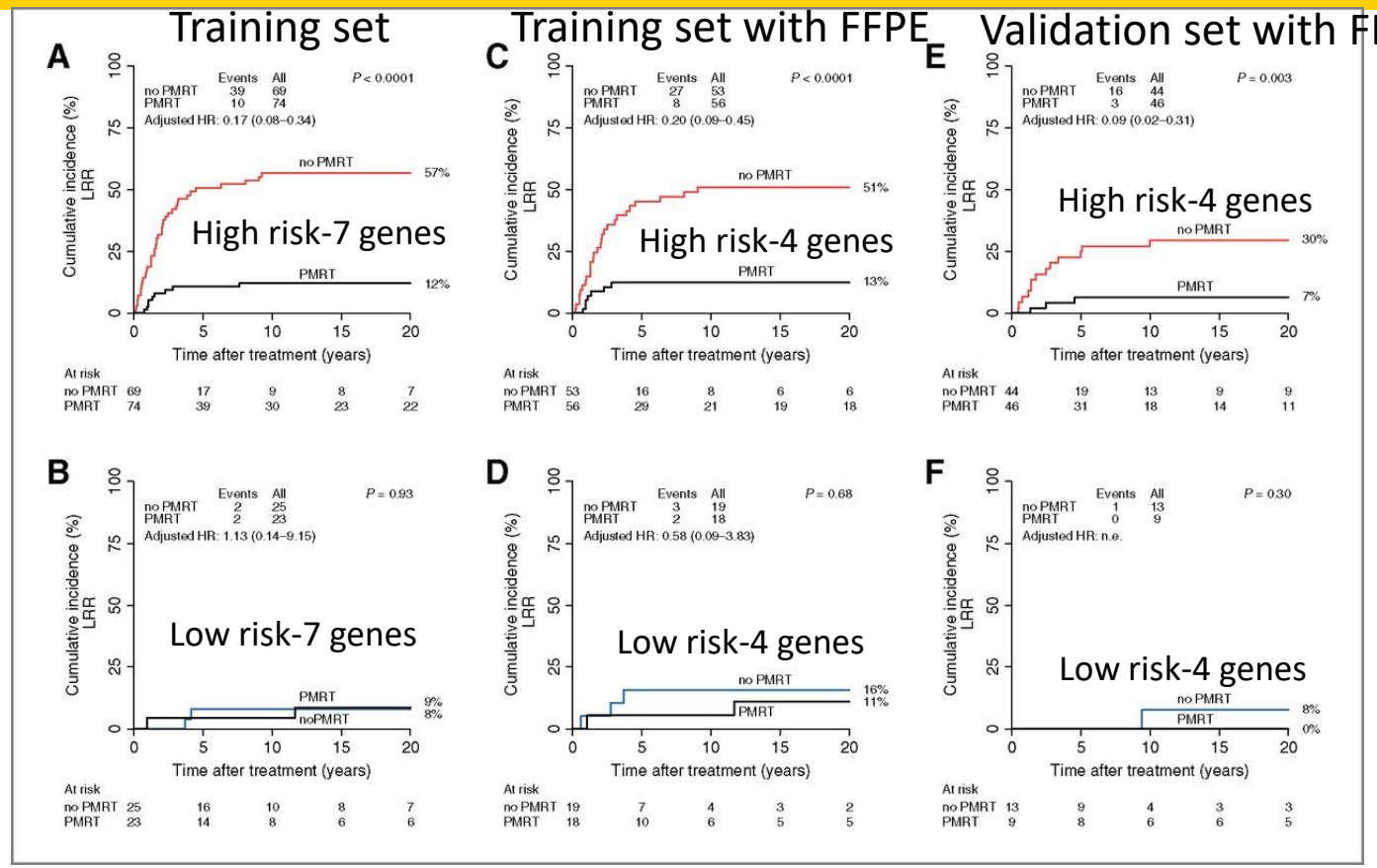
Danish Breast Cancer Group (DBCG-RT)

A DBCG-RT gene profile was identified and validated within the same patient cohort. Discovery using fresh frozen tissue, validated in FFPE

7 genes (*HLA-DQA, RGS1, DNALI1, hCG2023290, IGKC, OR8G2, and ADH1B*) were identified, and the derived DBCG-RT profile divided the 191 patients into "high LRR risk" (75% of the cohort) and "low LRR risk" groups (25% of the cohort). Mix of +/- PMRT in the training cohort to evaluate for RT interaction. Then transferred to FFPE, lost 3 genes and ended with **4** gene signature (*IGKC, RGS1, ADH1B*, and *DNALI1*)

PMRT significantly reduced risk of LRR in "high LRR risk" patients **RT benefit**
PMRT did NOT reduce LRR risk in the "low LRR risk" patients **No RT benefit**

Danish Breast Cancer Group (DBCG-RT) for post-mastectomy RT benefit



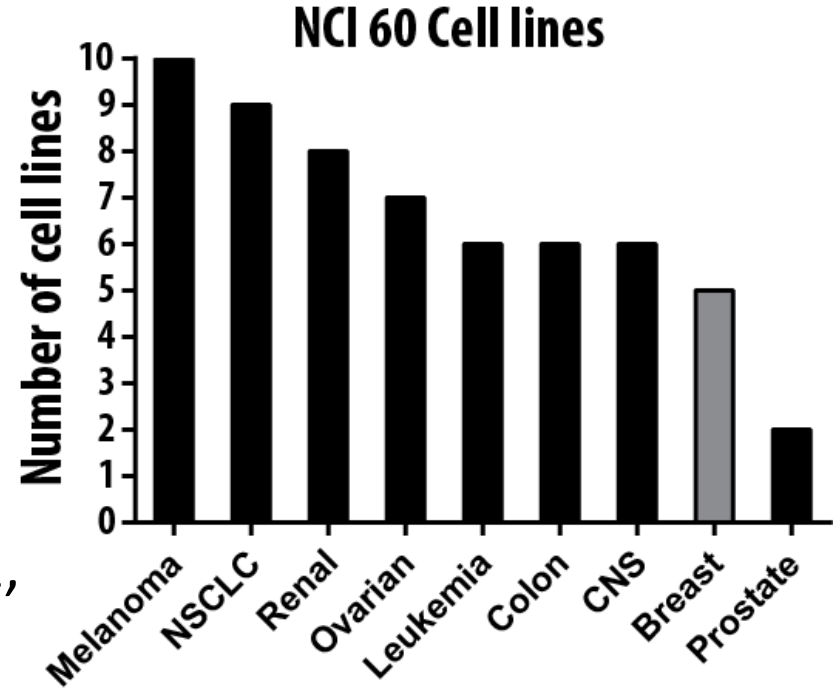
Predictive impact of the identified DBCG-RT profile is presented in the training set of 191 patients (A and B), in the subset of 146 patients from the training set, where FFPE was available (C and D), and in 112 patients with high-risk breast cancer patients in the validation dataset

Trine Tramm et al. Clin Cancer Res 2014;20:5272-5280

Radiation Sensitivity Index (RSI)

Radiosensitivity molecular signature (RSI) which was developed as a biomarker of cellular radiosensitivity (NCI-60 cell lines, mostly non-breast)

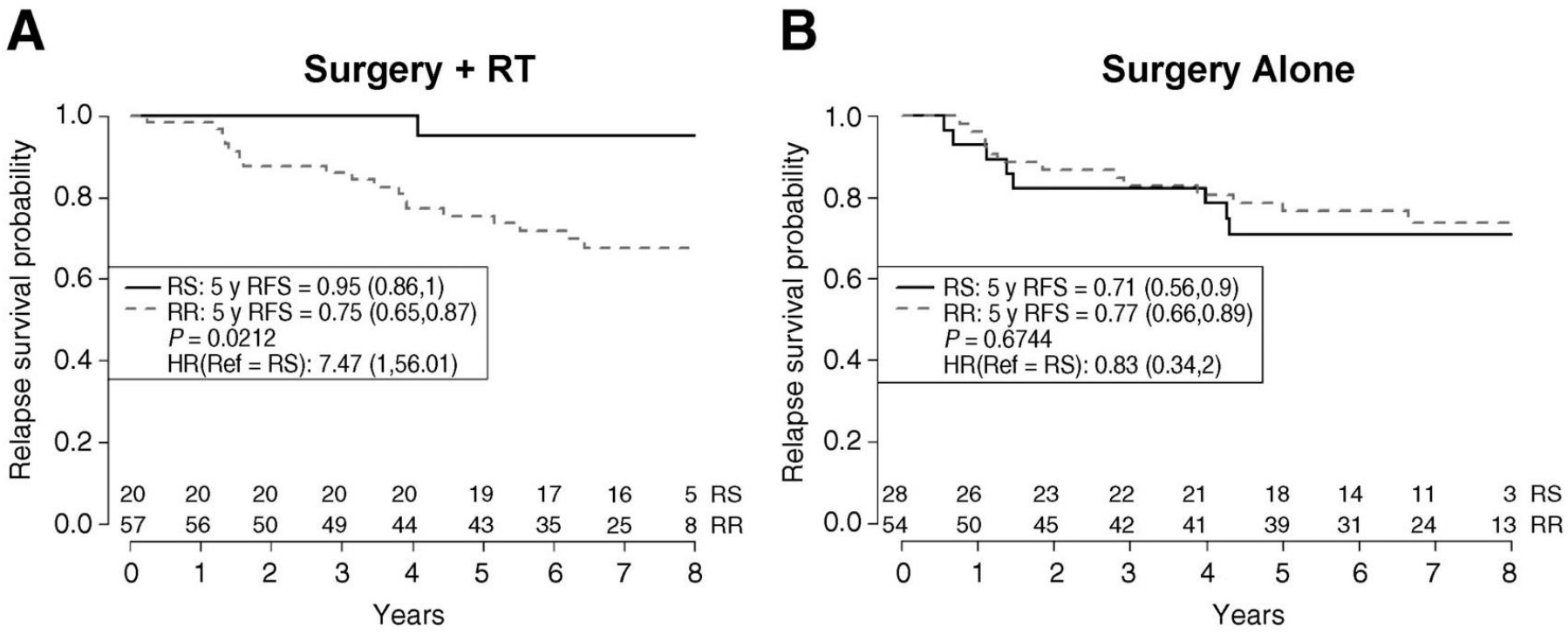
Signature is based on gene expression for 10 specific genes (AR, cJun, STAT1, PKC, RelA, cABL, SUMO1, CDK1, HDAC1, IRF1)



Initially evaluated in rectal, esophageal, and H&N SCC; extended into breast cancer evaluation in Swedish and Dutch cohorts

SA. Eschrich et al, Clin Can Res 2012 DOI: 10.1158/1078-0432.

Radiation Sensitivity Index (RSI)

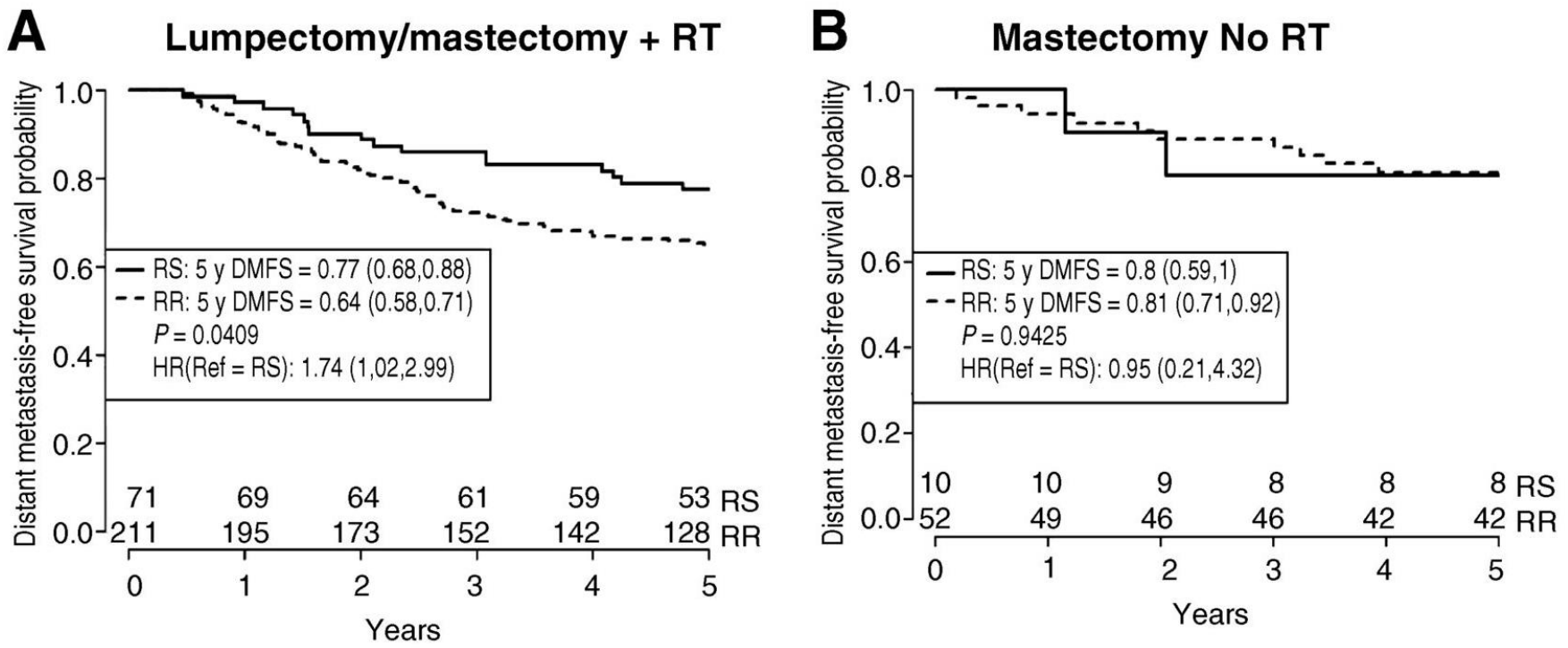


Karolinska dataset: Post BCS; mostly predicted radiation resistant (RR)

Signature identifies radiation benefit, not prognostic or predictive in non-irradiated patients

SA. Eschrich et al, Clin Can Res 2012 DOI: 10.1158/1078-0432.

Radiation Sensitivity Index (RSI)

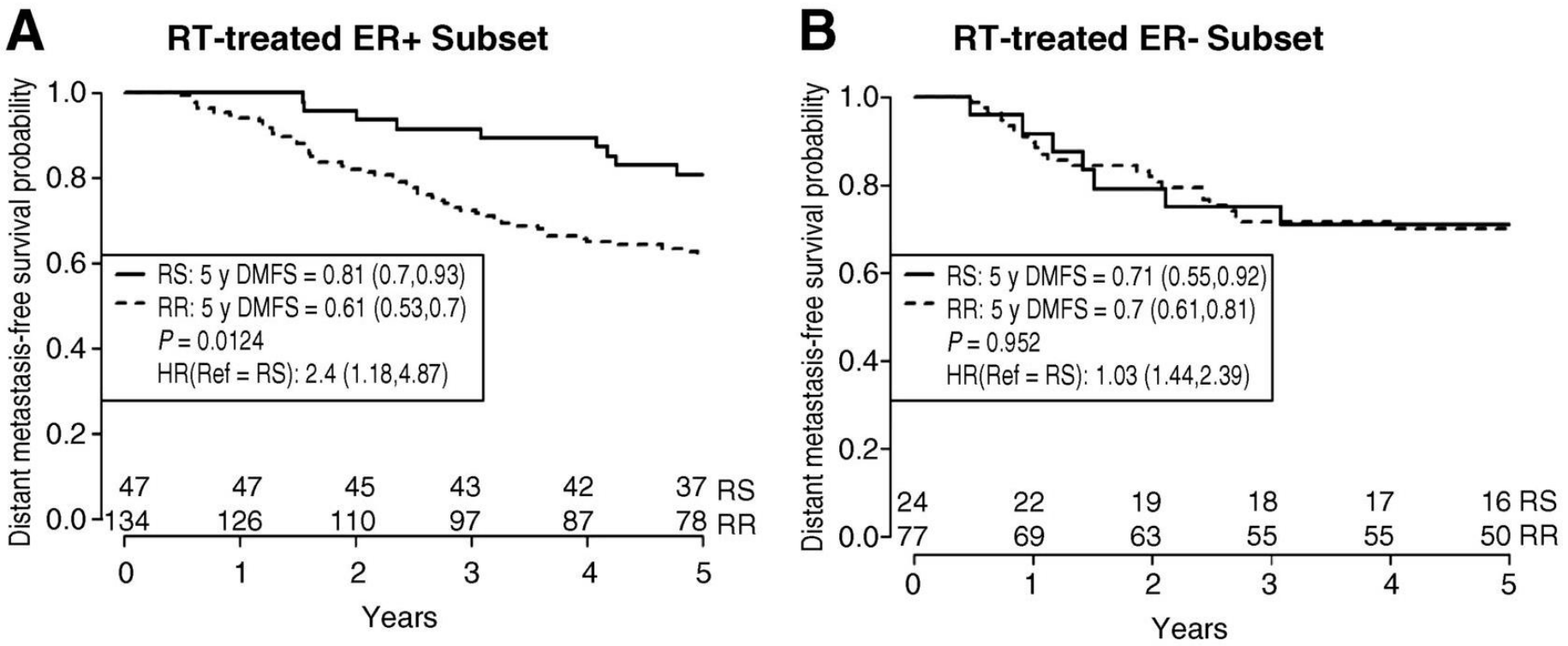


Erasmus dataset: Postmastectomy; mostly predicted radiation resistant (RR)

Signature identifies radiation benefit, not prognostic or predictive in non-irradiated patients

SA. Eschrich et al, Clin Can Res 2012 DOI: 10.1158/1078-0432.

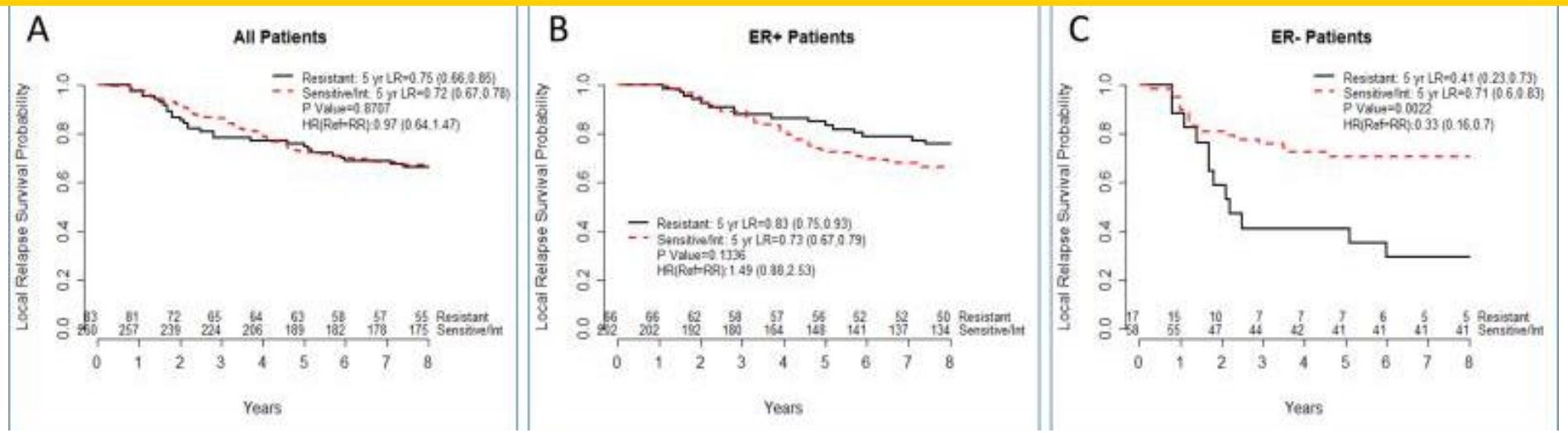
Radiation Sensitivity Index (RSI)



Predictive value seen in ER+ patients but not ER- in this cohort

SA. Eschrich et al, Clin Can Res 2012 DOI: 10.1158/1078-0432.

Radiation Sensitivity Index (RSI) validation



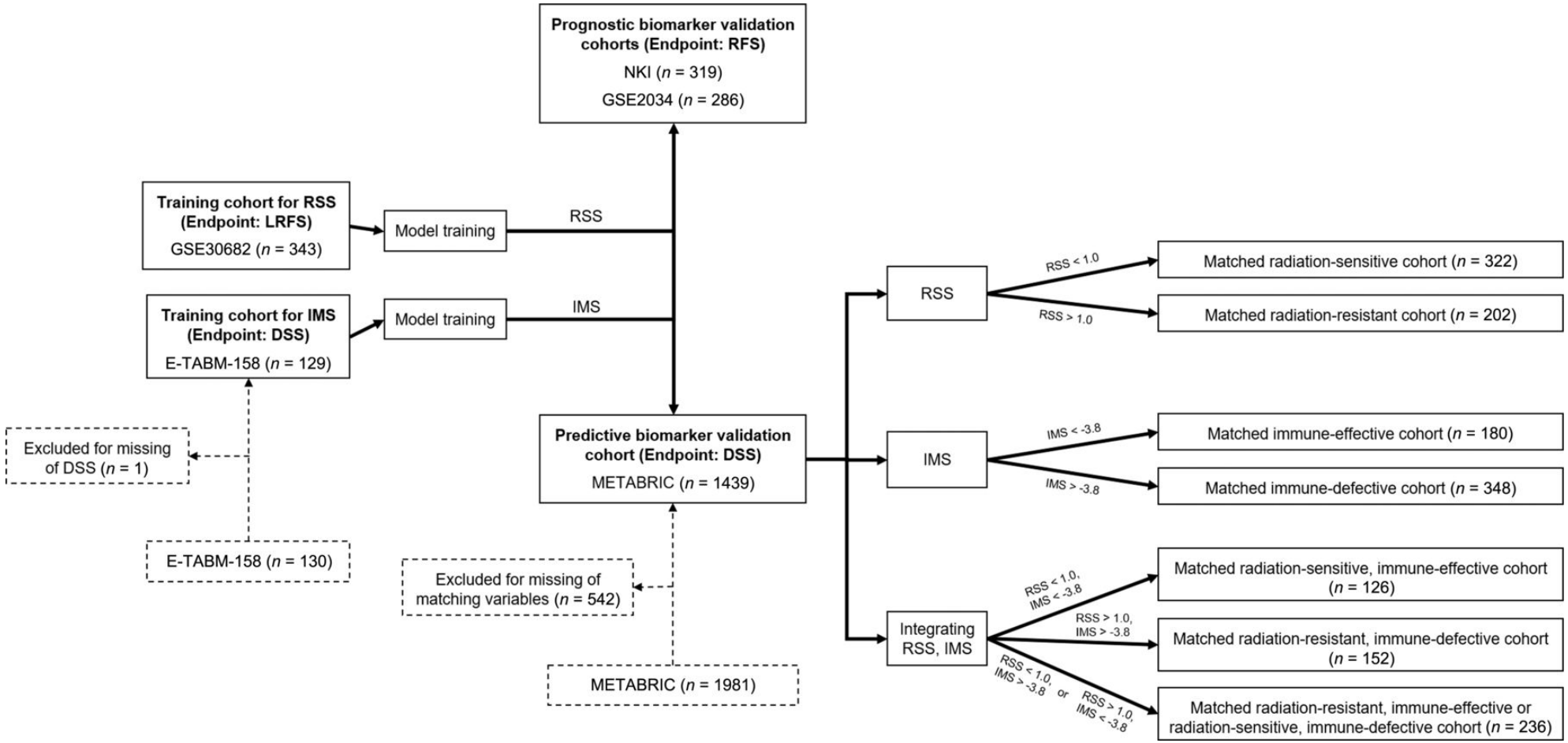
Unlike previous study, predictive value seen in ER- patients but not ER+ in this cohort (4 Dutch and 1 French non-randomized cohorts)

Went on to look at value of determining a genomic adjusted radiation dose (GARD) for breast and other types of cancers

While RSI did not uniformly predict for local recurrence across the entire cohort, it may identify a sub-population of TNBC (RSI-determined radioresistant patients) with the highest risk of local recurrence.

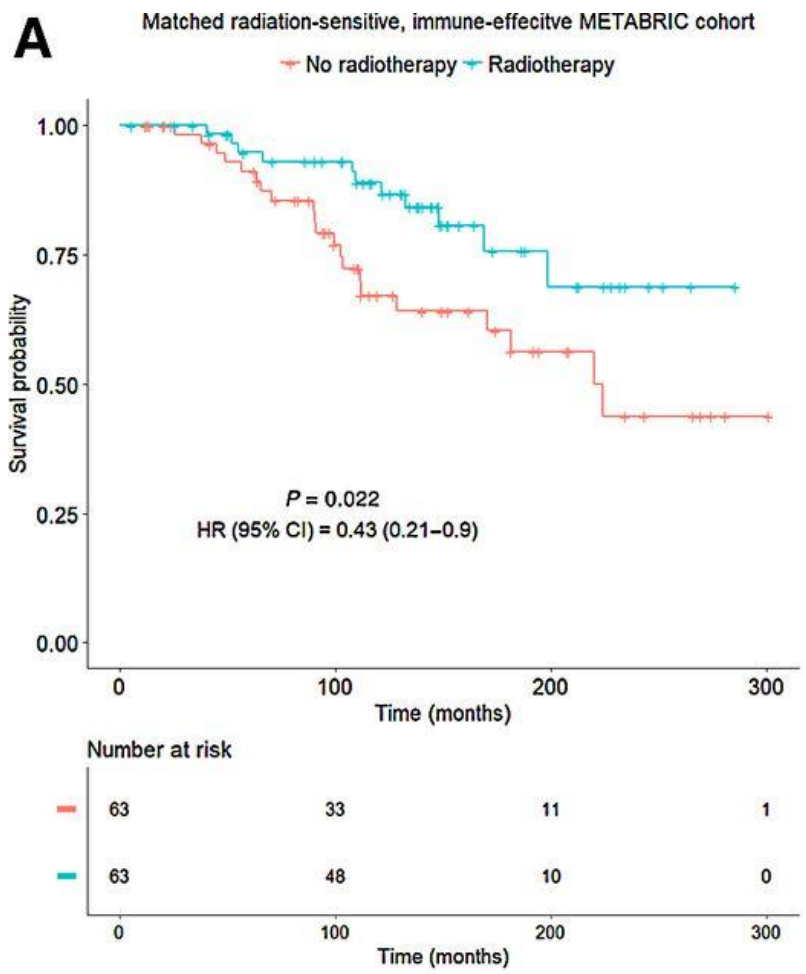
J Torres-Rocha, Int J Radiat Oncol Biol Phys. 2015 Nov 1; 93(3): 631–638.

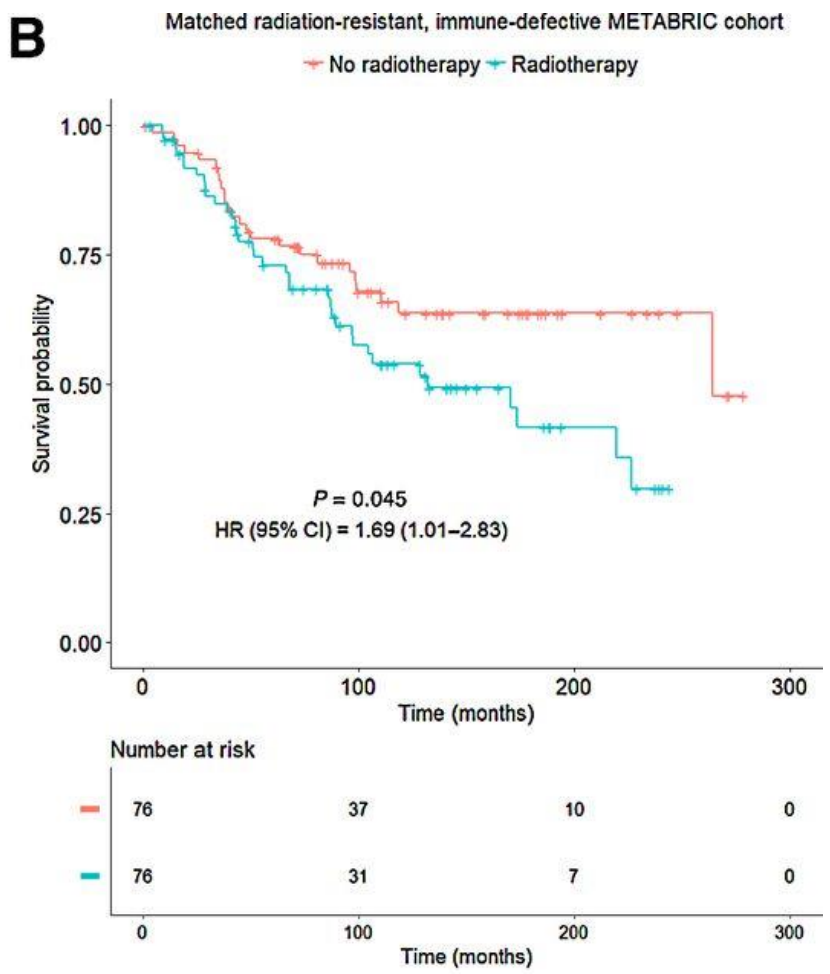
Radiation and Immune Gene Signature



Cui et al., Clin Cancer Res; 24(19) October 1, 2018

Radiation and Immune Gene Signature validation in METABRIC



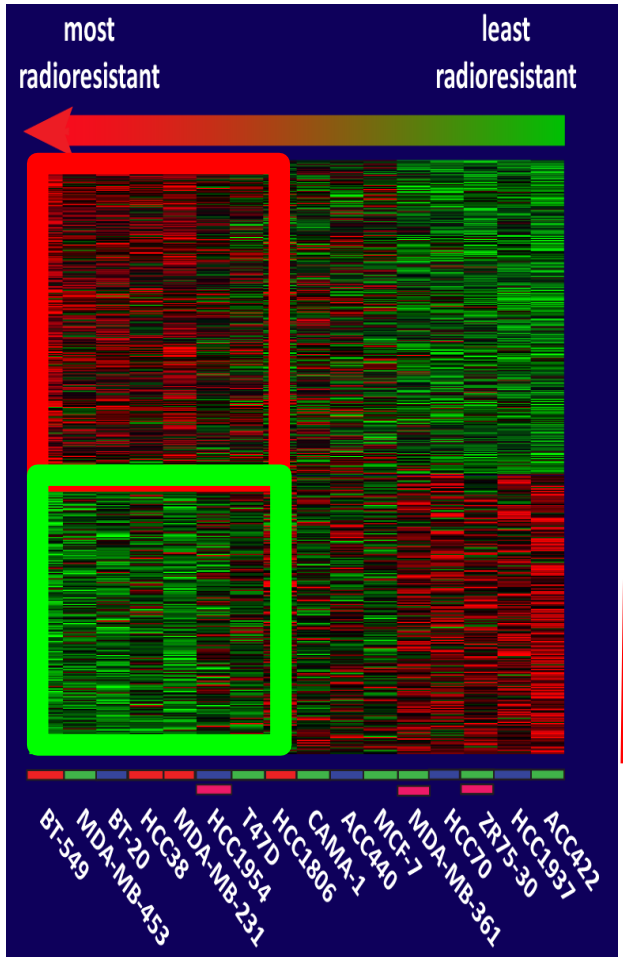


Combined radiation/immune signature shows improved DSS for the “predictive sensitive” group in RT treated patients, but worse DSS with RT in the “predicted resistant” groups

Neither RSI nor Oncotype DX showed a significant interaction with radiotherapy as continuous variables in multivariate Cox regression analyses.

Cui et al., Clin Cancer Res; 24(19) October 1, 2018

Radiotype Dx development



147 Genes Correlated with Radiation Sensitivity

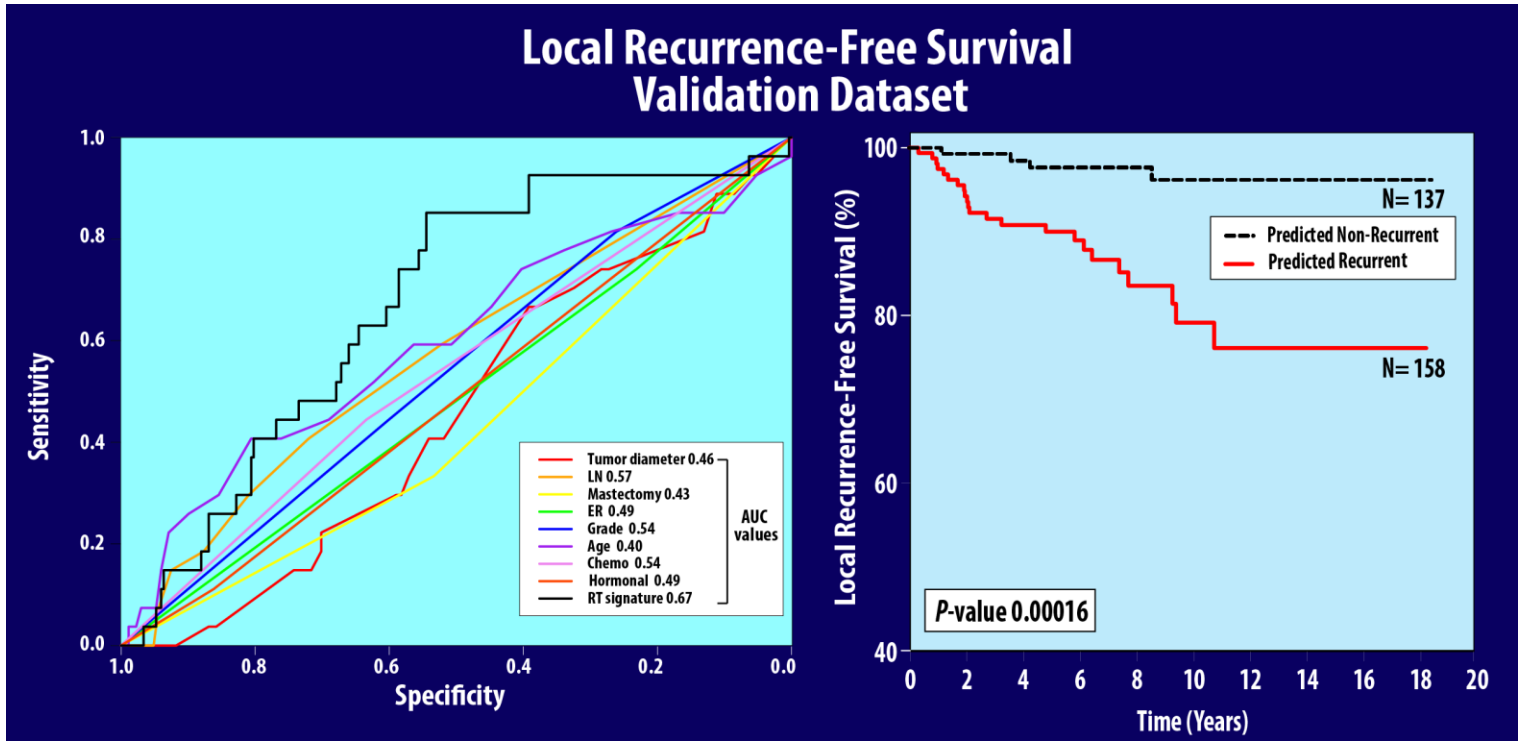
67 genes increased in radioresistant cell lines

80 Genes decreased in radioresistant cell lines

- Basal claudin-low
- Basal
- Luminal
- HER2

Speers et al., Clin Cancer Res. 2015 Aug 15;21(16):3667-77.

Radiotype Dx validation

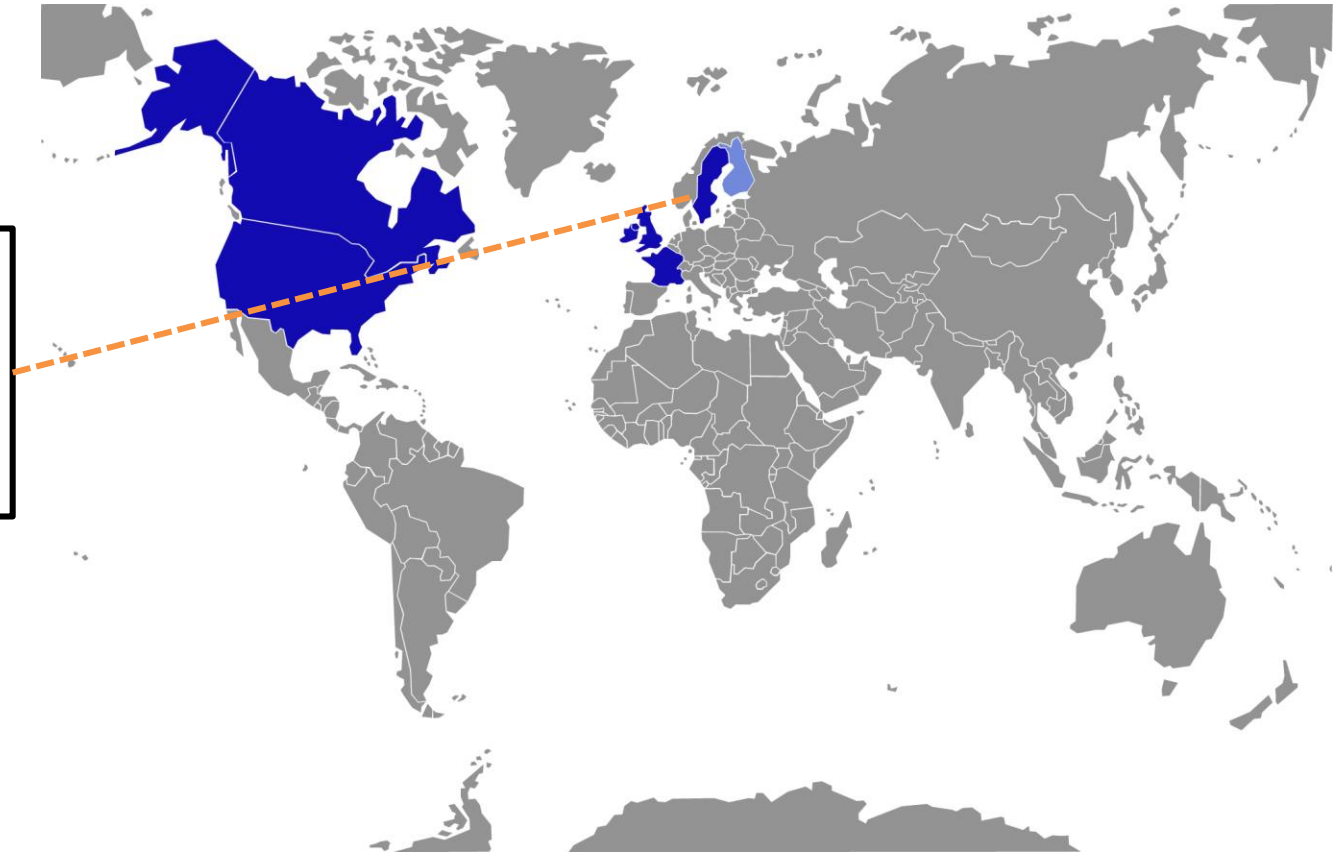


Sensitivity for recurrence: 85%
Negative Predictive Value: 97%
Log-rank P-value <0.001
Hazard Ratio: 6.1 (95% CI 4.48-12.65)

Speers et al., Clin Cancer Res. 2015 Aug 15;21(16):3667-77.

Efforts to improve Radiotype Dx

SweBCG (1991): 1185 pts



Development of **A**djuvant **R**adio**T**herapy **I**ntensification **C**lassifier (ARTIC)

Training of Model

16 matched samples from Lund FF and SweBCG 91-RT

- For each gene, correlate expression of Lund FF to SweBCG 91-RT
- Calculate variance for expression for each gene in Lund FF and SweBCG 91-RT, separately

Training cohort: Servant, N=343

- Vary
  - Threshold for gene expression correlation between fresh frozen and FFPE samples
  - Threshold for gene expression variance
  - Threshold for the univariable Cox p-value as calculated only within training cohort to the local recurrence endpoint
- Train ridge-penalized Cox models

Testing cohorts
vandeVijver, N=228
Sjöström et al, N=102

- Model selected by choosing model that minimized the product of the p-values in a Cox proportional hazards model in the testing cohorts
- As patient age was the strongest clinical factor for the endpoint in the training dataset, the linear model was retrained to include patient age as a variable in addition to the genes

Final locked model

Final Model (ARTIC)
27 genes and patient age

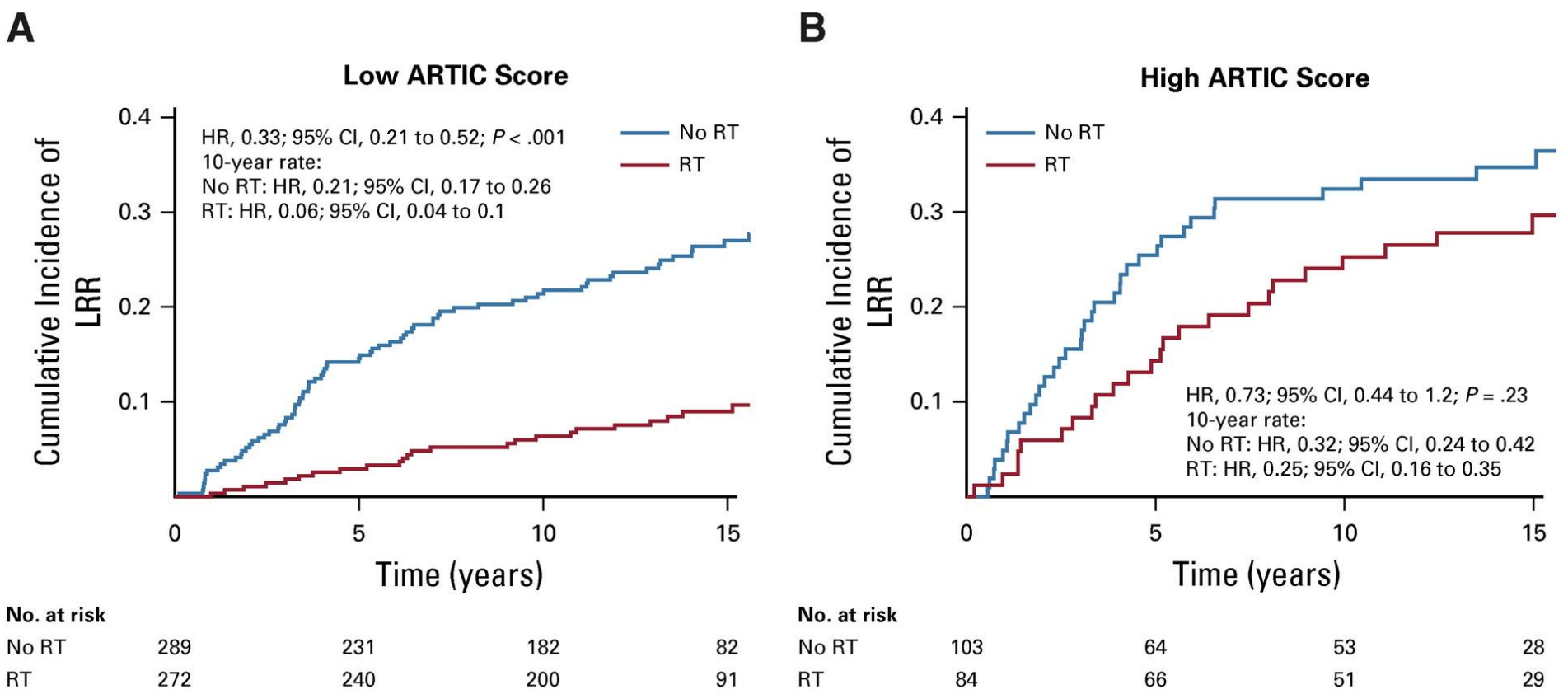
Validation cohort
SweBCG 91-RT, N=748

External validation of locked model in phase III randomized trial of +/- RT

Performed by L. Chang

Performed by M. Sjöström

Performance of ARTIC

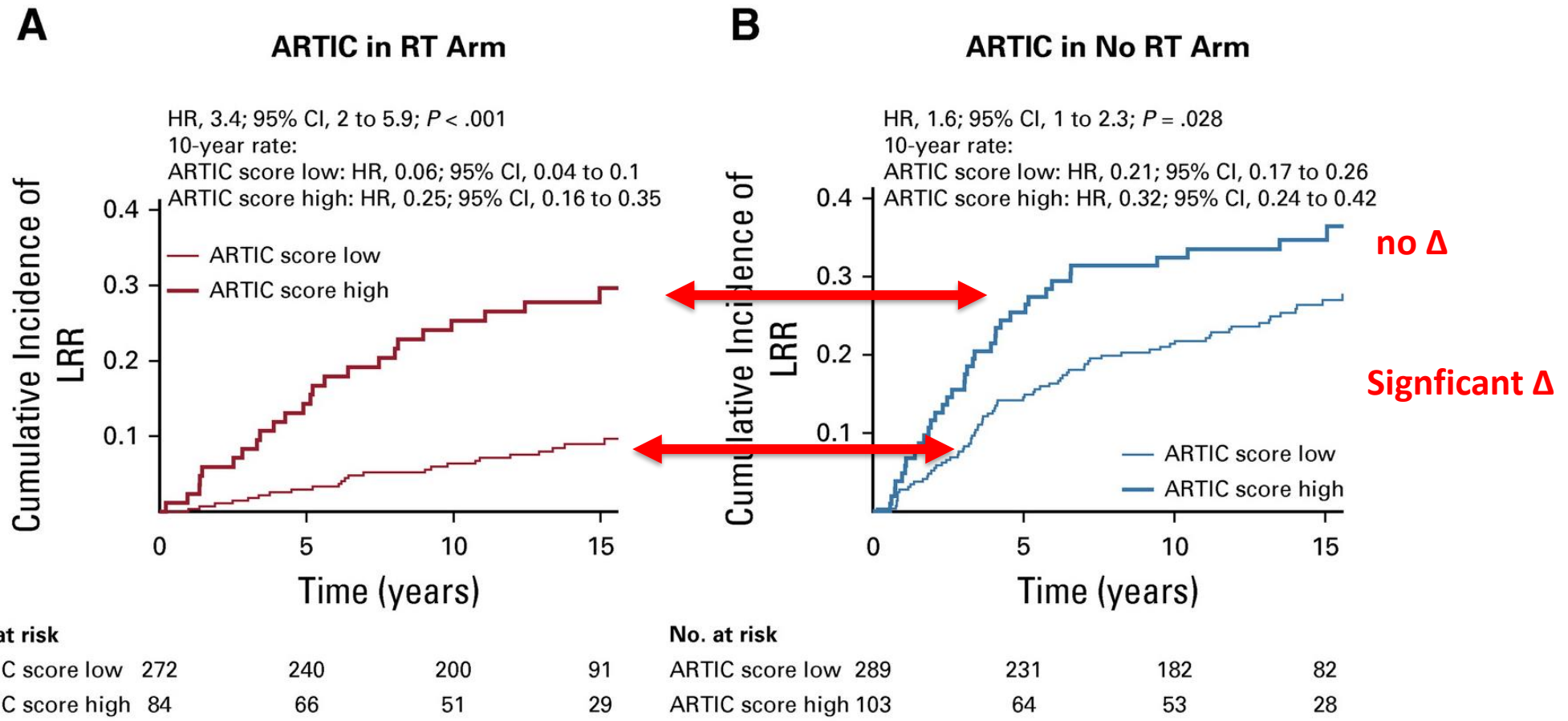


Performance of ARTIC for prognostication of locoregional recurrence and treatment prediction for adjuvant radiotherapy in the SweBCG91-RT validation cohort.

Cumulative incidence of locoregional recurrence for high and low classifier scores (as split by the 75th percentile score) and interaction with RT.

Sjöström et al, JCO Oct. 16, 2019; JCO.19.00761

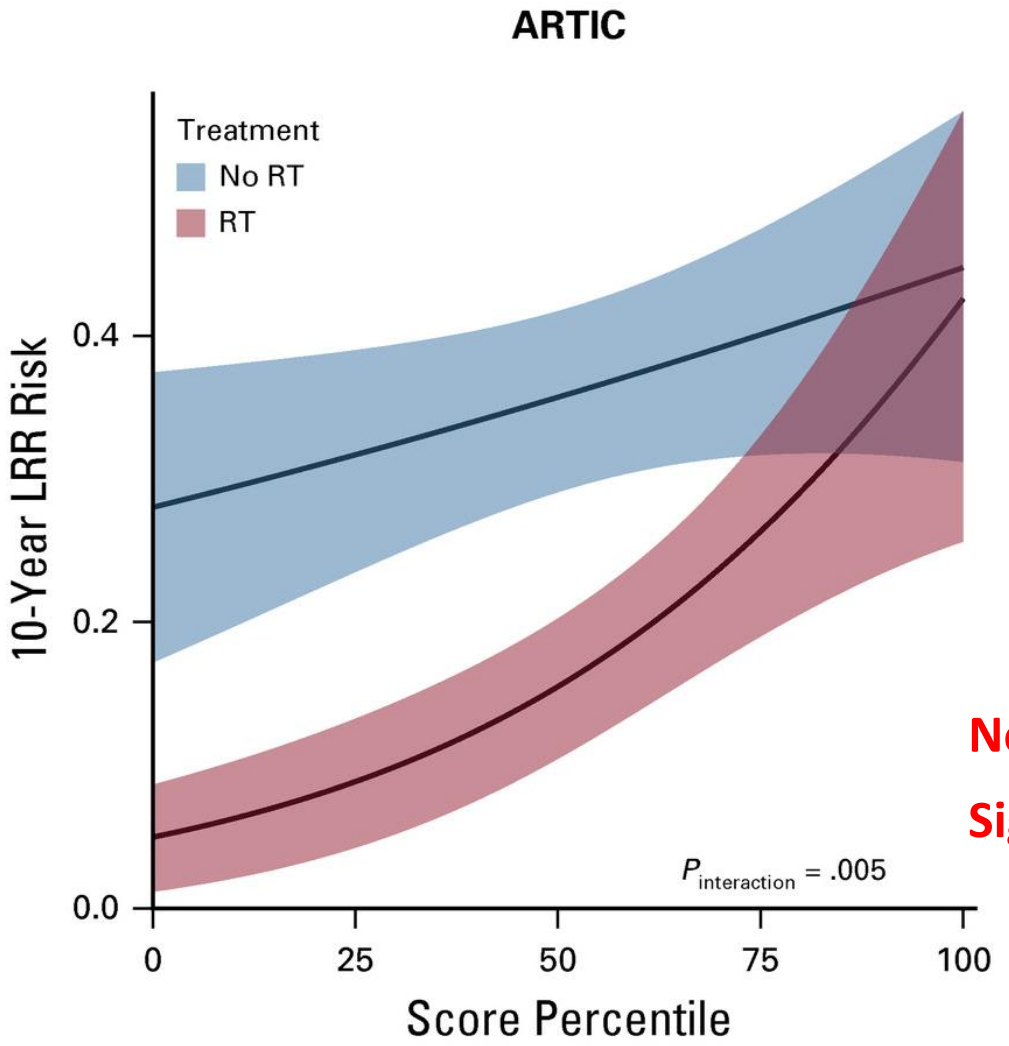
Performance of ARTIC- Prognostic



Prognostic performance of ARTIC in the SweBCG91-RT validation cohort.
Cumulative incidence of locoregional recurrence for patients split by the 75th percentile score in the radiation therapy treated arm (A) and in the no radiation therapy arm (B).

Sjöström et al, JCO Oct. 16, 2019; JCO.19.00761

Performance of ARTIC- Interaction Test



Interaction of radiation therapy and ARTIC

Continuous classifier scores are presented with the risk for locoregional recurrence with or without radiation therapy. The 10-year LRR free interval risk was calculated by fitting a cause-specific Cox regression model to time-to-LRR using the interaction of calculated ARTIC scores and RT status. Predicted survival curves and variances were generated using Efron's approach and the confidence intervals were constructed using the log approach.

No interaction with RT (i.e not predictive)= parallel lines

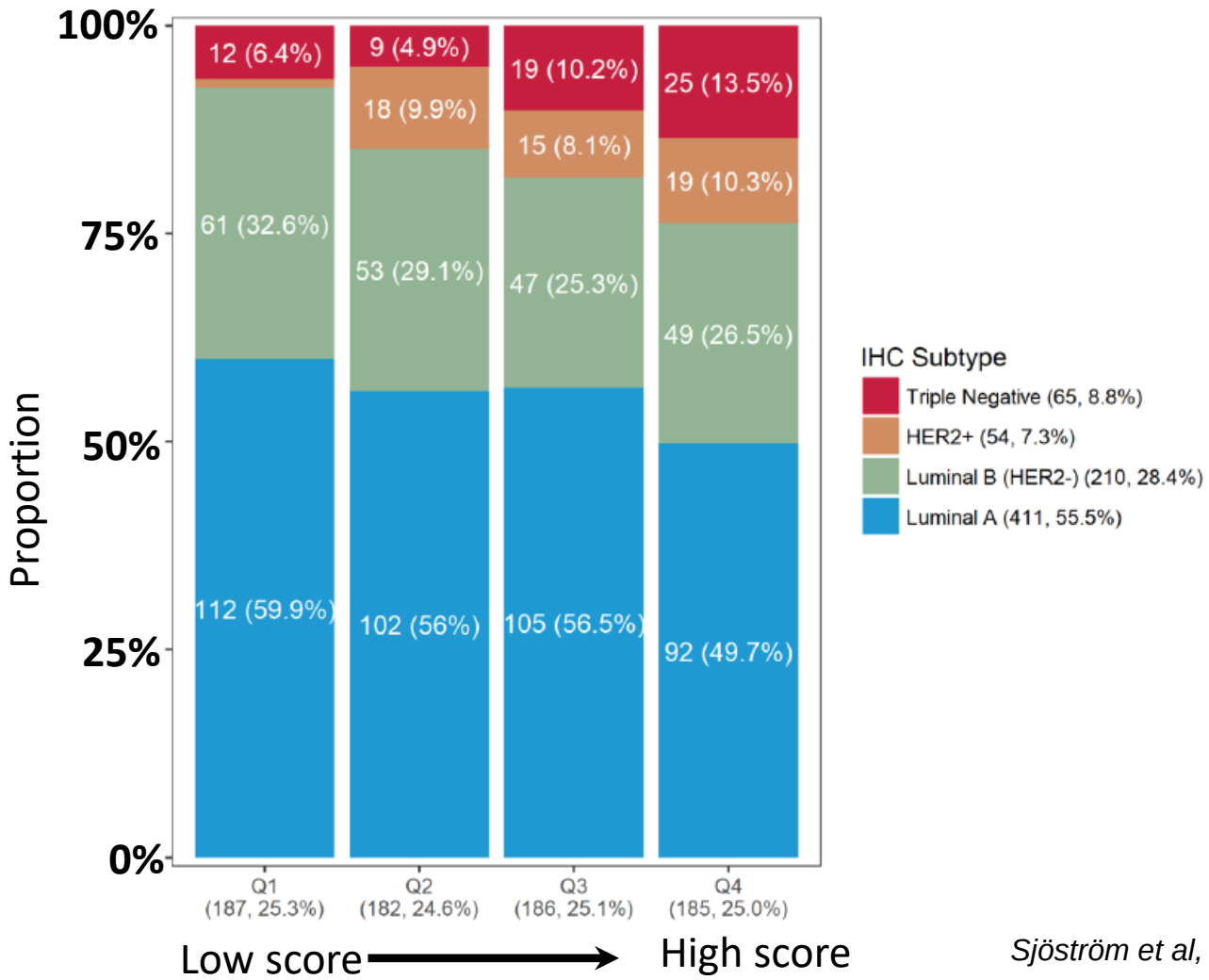
Significant interaction with RT (i.e IS predictive)= lines converge

Sjöström et al, JCO Oct. 16, 2019; JCO.19.00761

ARTIC does not just recapitulate intrinsic BC subtypes

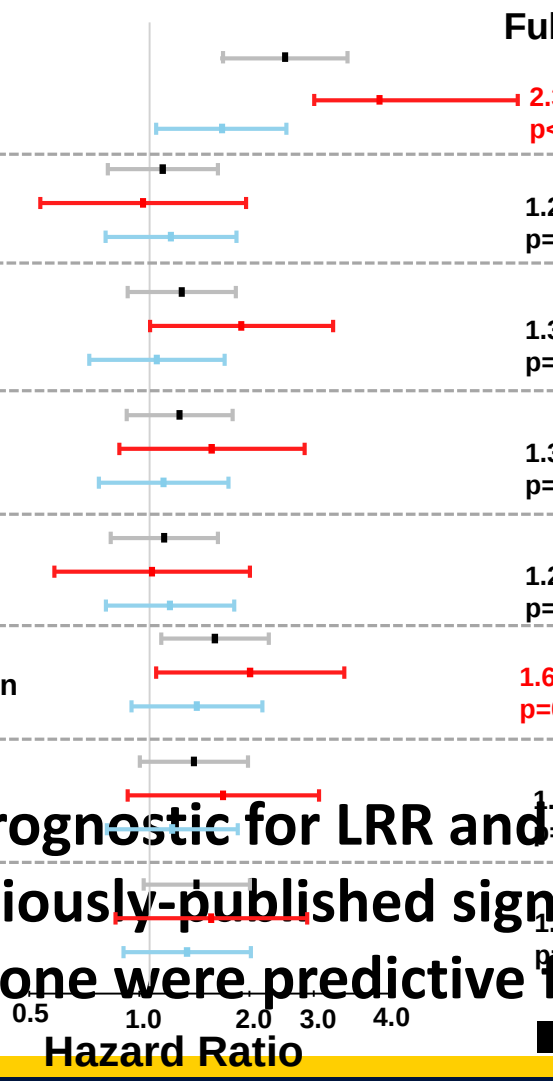
Entire cohort

Subtype proportions by ARTIC quartiles



Sjöström et al, JCO Oct. 16, 2019; JCO.19.00761

Comparative performance of other signatures



Signature	Full Cohort	RT Arm	No RT Arm	Interaction P
ARTIC	2.3 [1.8-4.4], p<0.001*	3.4 [2.5-9.0], p=<0.0001*	1.6 [1.1-2.3], p=0.03*	0.005*
21−gene like (OncotypeDx)	1.2 [0.8-1.6], p=0.41	1 [0.5-2.0], p=0.94	1.2 [0.8-1.8], p=0.35	0.97
70−gene like (Mammaprint)	1.3 [0.9-1.8], p=0.13	1.9 [1.1-3.4], p=0.024*	1.1 [0.7-1.7], p=0.62	0.51
Cui 2018	1.3 [0.9-1.8], p=0.14	1.6 [0.9-2.8], p=0.13	1.2 [0.8-1.7], p=0.47	0.54
Eschrich 2009 (RSI)	1.2 [0.8-1.6], p=0.36	1.1 [0.6-2.0], p=0.8	1.2 [0.8-1.8], p=0.35	0.46
Sjostrom 2018 Intensification	1.6 [1.1-2.3], p=0.0058*	2.1 [1.2-3.9], p=0.012*	1.5 [.97-2.2], p=0.07	0.1
Sjostrom 2018 Omission	1.4 [1,2], p=0.04	1.6 [0.9-2.9, p=0.13	1.4 [0.9-2.1], p=0.1	0.6
Zhang 2016	1.4 [1,2], p=0.034	1.6 [0.9-2.9], p=0.14	1.3 [0.9-2.0], p=0.14	0.59

*Statistically significant

ARTIC validates as prognostic for LRR and predictive for RT benefit. Conversely, while 2 of the 7 previously-published signatures were prognostic for the LRR endpoint (p<0.05), none were predictive for benefit from RT in SweBCG 91-RT

Full Cohort RT Arm No RT Arm

Radiation specific signatures for treatment decisions

Others generated previously but not under clinical development

- Preclinically derived signatures:
  - Radiation induction signature[1], Interferon-based signature[2] (that worked for chemo and radiation), Wound-response signature[3]
- Clinically derived signatures:
  - Swedish signature in breast cancer[4], Dutch signature in breast cancer[5]

Challenge has been lack of validation, or

inability to validate in external datasets

1. BD Piening et al., Jour of Rad Res, vol. 171, no. 2, pp. 141–154, 2009.
2. R.Weichselbaum et al., PNAS, vol. 105, no. 47, pp. 18490–18495, 2008
3. DS Nuyten et al., Breast Cancer Research, vol. 8, no. 5, article no. R62, 2006
4. E. Nimeus-Malmstrom et al., Breast Cancer Research, vol. 10, no. 2, 2008
5. B. Kreike et al., Clin Can Res vol. 15, no. 12, pp. 4181–4190, 2009

Question

Which of the following molecular signatures has been validated as a predictive biomarker of radiation response for women with breast cancer?

(A) DBCG-RT
(B) Radiation Sensitivity Index (RSI)
(C) Radiation and Immune-based Signature
(D) Radiotype Dx
(E) All of the above
(F) None of the above

What about signatures for radiation benefit in DCIS?

Previously derived signatures applied to radiation questions

Previously derived

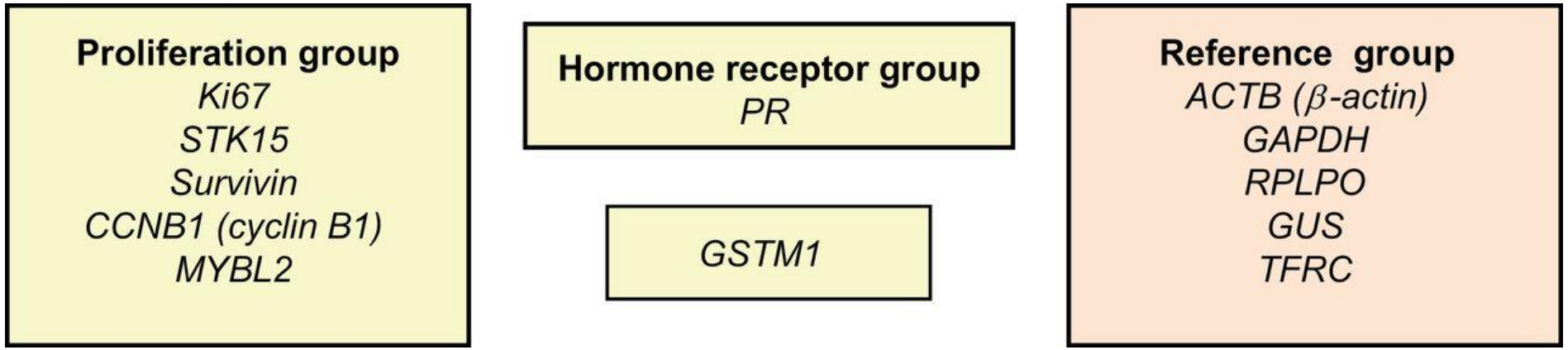
- Oncotype Dx®
- Oncotype Dx® for DCIS
- IHC surrogates for subtype

What about radiation omission for non-invasive disease (DCIS)

Oncotype Dx® for DCIS: Developed in 5 cohorts that included studies of 1) either DCIS only or both DCIS and invasive breast carcinoma, but without clinical outcome data; or 2) invasive breast carcinoma with clinical outcome data.

Validated in ECOG E5194 which was a trial of DCIS who were selected for low-risk clinical and pathologic characteristics. Patients were enrolled onto one of two study cohorts (not randomly assigned): cohort 1: low- or intermediate-grade DCIS, tumor size 2.5 cm or smaller (n = 561); or cohort 2: high-grade DCIS, tumor size 1 cm or smaller (n = 104). Negative margins at least 3 mm. Tamoxifen (not randomly assigned) was given to 30% of the patients.

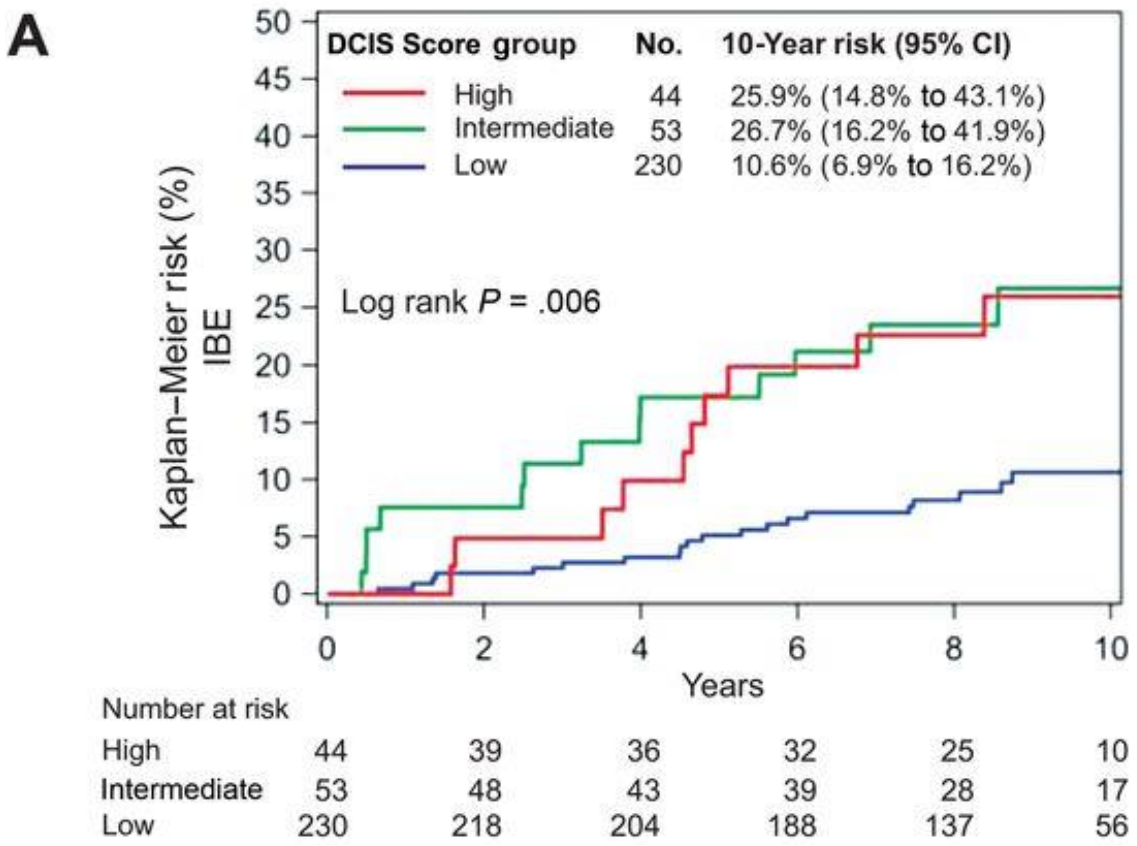
No RT



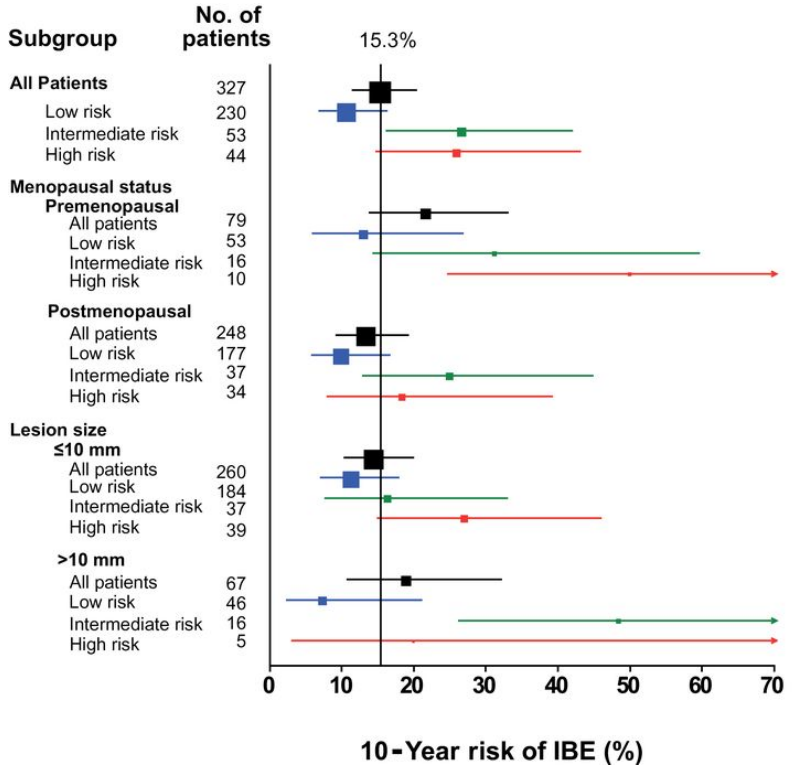
16 cancer related genes in Oncotype Dx®
7 cancer related genes for Oncotype Dx for DCIS®

LJ Solin et al., JNCI: Volume 105, Issue 10, 15 May 2013, Pages 701–710,

Development of Oncotype Dx® for DCIS



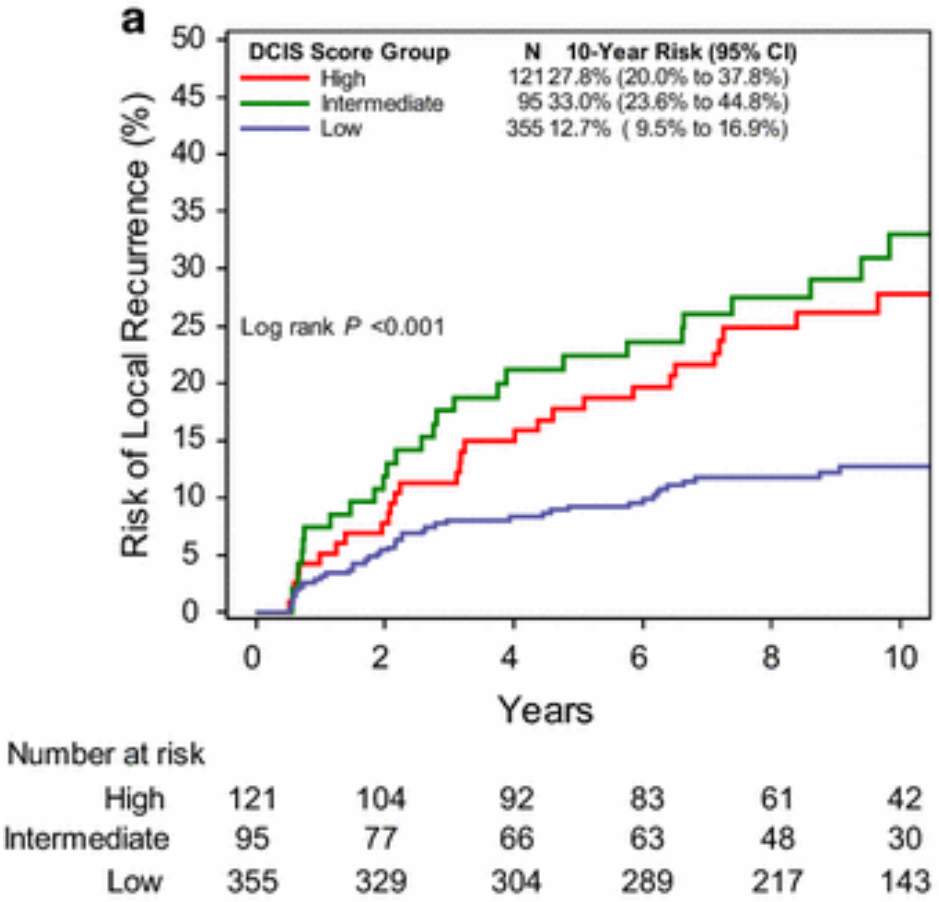
- Mix of 5 cohorts for training (invasive and DCIS cohorts)
- ECOG E5194 for validation



LJ Solin et al., JNCI: Volume 105, Issue 10, 15 May 2013, Pages 701–710,

Validation of Oncotype Dx® for DCIS

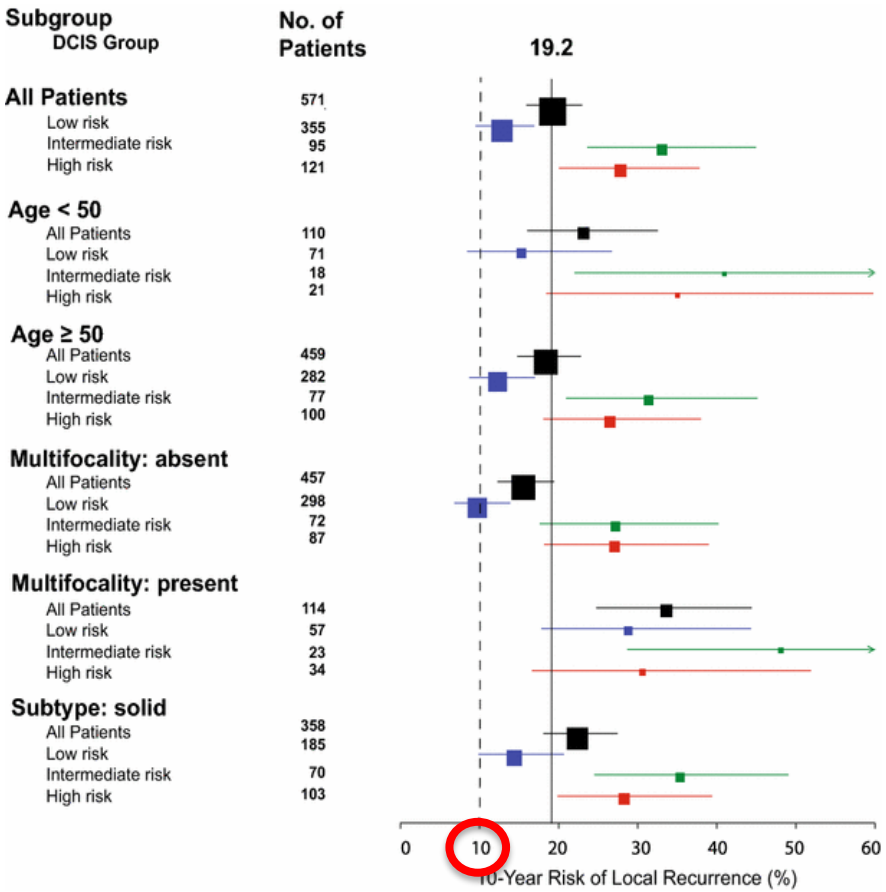
Population-based Canadian cohort of individuals diagnosed with DCIS treated with BCS alone from 1994 to 2003 (571 patients with negative margins.)

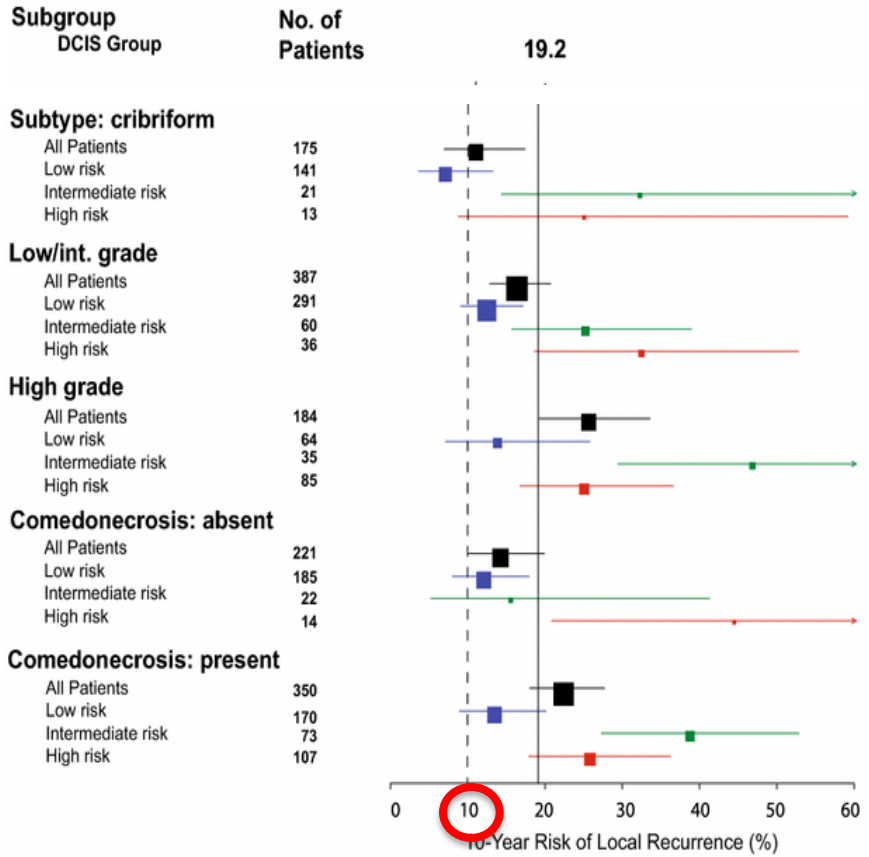


E Rakovitch et al., Breast Cancer Res Treat (2015) 152: 389

subsequent comparison with BCS+RT by E Rakovitch *JNCI*, Volume 109, Issue 4, 1 April 2017

Validation of Oncotype Dx® for DCIS





Want 10 year risk of recurrence <10%

Radiation specific signatures for DCIS treatment decisions

- DCISionRT® from PreludeDx™[1]

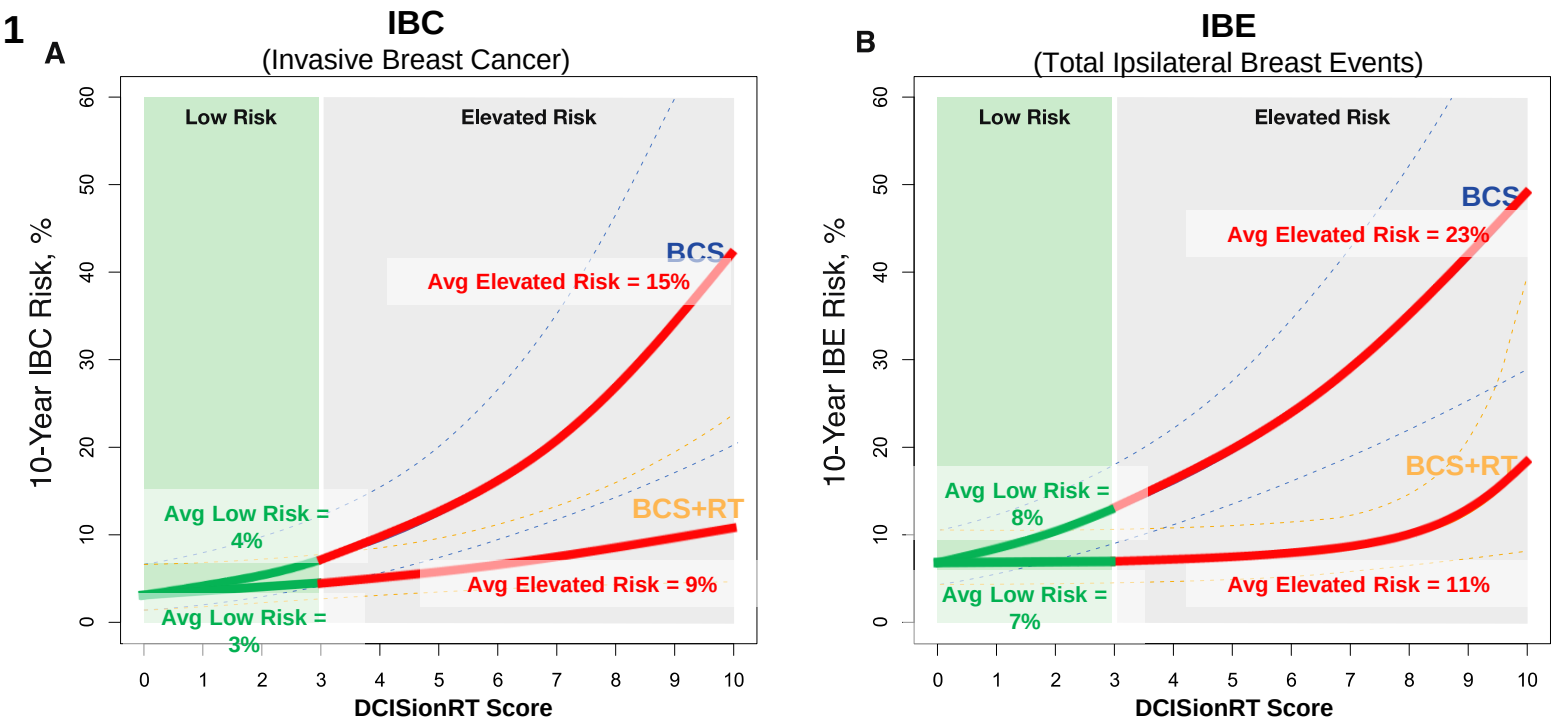
[1]Bremer TM, et al. Clin Cancer Res. July 2018:clincanres.0842.2018. doi:10.1158/1078-0432.CCR-18-0842

Cancer Pathway Comparison

	Hormone Receptor	HER2	Proliferation / Cell Cycle	Stress Response	Invasion	Clin / Path & Other factors
DCISionRT™	PR [FOXA1]	HER2	Ki-67	COX2 SIAH2	FOXA1 p16/INK4A [Ki-67]	Age Size Margin Palpability
OncotypeDX DCIS	PR	⊘	Ki-67 STK15 Survivin Cyclin B1 MYBL2	GSTM1	⊘	⊘

DCISionRT validation

Figure 1



- **Low risk group is clinically low risk**
  - Similar to contralateral risk
- **Elevated risk group is clinically high risk**
  - Similar to risk for women with BRCA mutations

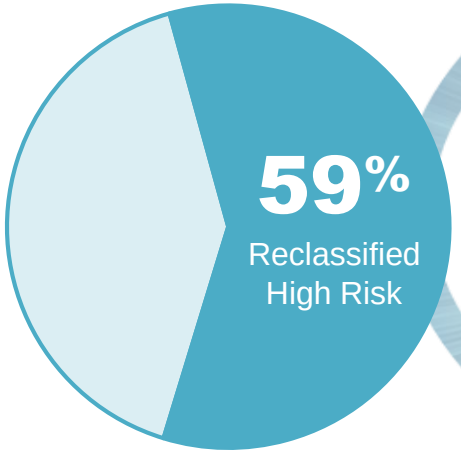
Non-randomized cohort from Uppsala and UMass

[1]Bremer TM, et al. Clin Cancer Res. July 2018:clincanres.0842.2018. doi:10.1158/1078-0432.CCR-18-0842

DCISionRT reclassification in US cohort

Low Risk
Clin/Path Patients

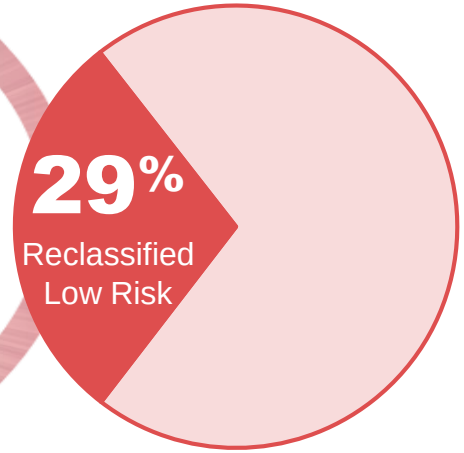
Reclassified by **DCISionRT** Kaiser Permanente Network Study



DCISionRT
upstaged **59%** of patients
in the Clin/Path low risk
as elevated risk

High Risk
Clin/Path Patients

Reclassified by **DCISionRT** Kaiser Permanente Network Study



DCISionRT
downgraded **29%** of patients
in the Clin/Path high risk
as low risk

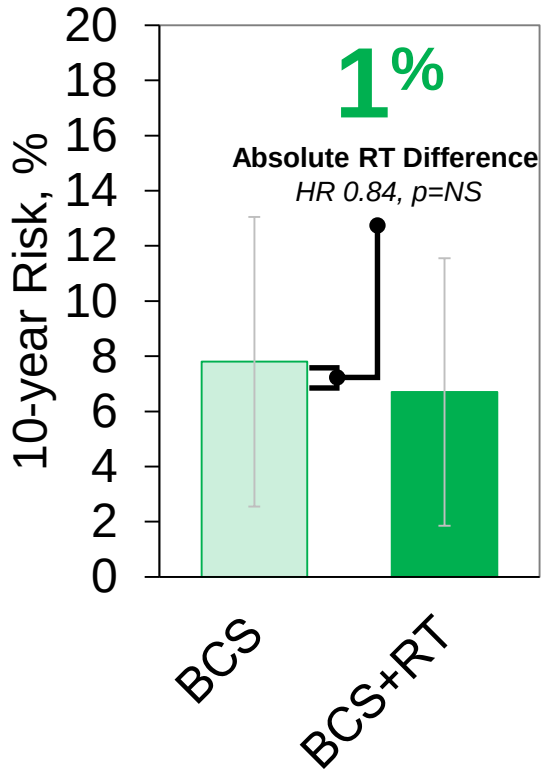
DCISionRT™
Reclassifies
Clin/Path Risk Groups

MSKCC Factors in Analysis:
- Age
- Family Hx
- Presentation
- Grade
- Margin
- No. of Excisions

Median follow-up 10.4 years, n = 455

Validation of DCISionRT in the SweBCG trial

Low Risk Group
(DS≤3 †)



SweDCIS: 10-Year Invasive Breast Cancer Risk

Complete Assay Data with Clear Margins, (1986-1999), n=506

AKADEMISKA SJUKHUSET

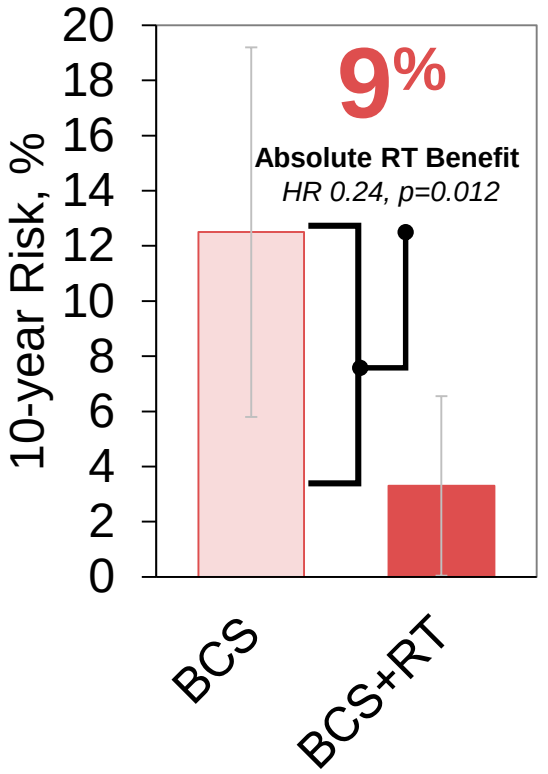
UPPSALA UNIVERSITET

DCISionRT®

PREDICTIVE TEST
for
RADIATION THERAPY

†DS = DCISionRT Score (Scale from 0 to 10)

Elevated Risk Group
(DS>3†)



Wärnberg F, et al. SABCS 2017. Publication Number GS5-08 – AACR; Cancer Res 2018;78(4 Suppl):Abstract nr GS5-08.

Are any of these signatures ready for “prime time”

- Numerous studies showing Oncotype Dx Recurrence Score or breast cancer intrinsic subtype (ProSigna) associated with local recurrence risk
  - Mamounas EP, Tang G, Fisher B., et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: Results from NSABP B-14 and NSABP B-20. J Clin Oncol. 2010;2810:1677–1683.
  - Nuyten DS, Kreike B, Hart AA., et al. Predicting a local recurrence after breast-conserving therapy by gene expression profiling. Breast Cancer Res. 2006;85:R62.
  - Nguyen PL, Taghian AG, Katz MS., et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. J Clin Oncol. 2008;2618:2373–2378.
  - Voduc KD, Cheang MC, Tyldesley S., et al. Breast cancer subtypes and the risk of local and regional relapse. J Clin Oncol. 2010;2810:1684–1691.
  - Solin LJ, Gray R, Goldstein LJ., et al. Prognostic value of biologic subtype and the 21-Gene Recurrence Score relative to local recurrence after breast conservation treatment with radiation for early stage breast carcinoma: Results from the Eastern Cooperative Oncology Group E2197 study. Breast Cancer Res Treat. 2012;1342:683–692.
- In those subsets with a very low risk of recurrence, can this information be used to omit radiation?

AVOID OVERTREATMENT

Genomically stratified ongoing clinical trials for radiation omission

Same general design, lumpectomy and endocrine therapy alone: For whom is radiation omission appropriate?

1. **I**ndividualized **D**ecisions for **E**ndocrine Therapy **A**lone (**IDEA**)-OncotypeDx (*PI: Dr. Reshma Jagsi, Univ. of Michigan*) Non-randomized, 202 pts
   - 50-69 yo women with RS ≤18, 1° endpoint rates of locoregional recurrence at 5 yrs
2. **Pr**ofiling **E**arly Breast **C**ancer for Radiotherapy **O**missio**n** (**PRECISION**)- ProSigna (*PI: Dr. Jennifer Bellon, Dana Farber*) Non-randomized phase II, 690 pts
   - 50-75 yo women with low risk, 1° endpoint rates of ipsilateral locoregional recurrence at 5 yrs.
3. **EX**amining **PE**rsonalised **R**adiation **T**herapy for Low-risk Early Breast Cancer (**EXPERT**)- (*Study Chair: Dr. Boon Chua, Prince of Wales Hospital; International Breast Cancer Study Group (IBCSG*) Randomized phase III, 1167 pts
   - ≥50 yo Luminal A pts by Prosigna (PAM50) with ROR score ≤60, 1° endpoint 10 yr LR
4. **LUMINA**- IHC (*PIs: Dr. Tim Whelan-OCOG and Dr. Sally Smith-BCCA*) Non-randomized observational, 500 pts
   - Luminal A patients by ER/PR/Her2/Ki67, 1° endpoint 5 yr IBTR
5. **PRIMETIME**- IHC4 (PI: *Dr. Charlotte Coles, Univ. of Cambridge*) Non-randomized observational, 2,400 pts
   - Luminal A patients by ER/PR/Her2/Ki67, , 1° endpoint 5 yr IBTR

***IHC 4, ProSigna (subtype), and Oncotype RS NOT the same**

Additional genomically stratified ongoing clinical trials for radiation omission in N+ patients

Same general design, but for N+ patients: For whom is radiation omission appropriate?

1. **MA.39 (TAILOR RT)** -OncotypeDx (*PI: Dr. Timothy Whelan on behalf of Canadian Cancer Trials Group)* Randomized phase III, 2140 pts
   - ≥40 yo women with Oncotype Dx RS ≤18
   - 1-3 positive axillary nodes (macrometastases, > 2 mm) with ALND, 1-2 positive LN with SLNB
   - Includes BCS and mastectomy treated pts, randomized to +/- RT
     - For BCS pts: Whole breast irradiation (WBI) +/- regional nodal RT (supraclavicular, non-dissected axillary, and internal mammary)
     - For mastectomy pts: +/- chest wall and regional nodal RT
   - 1° endpoint: BCRFS between patients that received regional RT or not

One additional trial similar to previous in patients with node-negative disease progressing through the US cooperative groups, led by NRG

Question

There are currently numerous ongoing genomically stratified clinical trials for radiation omission

(A) True
(B) False

Summary

- APBI a reasonable option for women at low risk of recurrence
- Genomic-based signatures now commonplace in guiding systemic therapy decisions
- Genomic-based signatures not yet validated for clinical use to guide radiation decisions– though we are getting close
- Validation awaited for:

Invasive disease

- Oncotype Dx®
- ProSigna
- IHC based subtyping
- Radiation-specific signatures (DBCG-RT, RSI, Radiotype Dx, ARTIC, etc.)

DCIS

Oncotype Dx® for DCIS
DCISionRT-

Questions ?