LIVING BEYOND
BREAST CANCER®

Biomarker testing for early-stage breast cancer

Mass General Brigham
Mass General Cancer Center

Molecular Profiling for Early Stage HR+/HER2- Breast Cancer

April 11th, 2025
Living Beyond Breast Cancer Webinar

Seth A. Wander, MD, PhD
Assistant Professor of Medicine
Harvard Medical School
Massachusetts General Hospital
swander@mgh.harvard.edu

Disclosures

- Consulting/Advisory Board: Foundation Medicine, Veracyte, Hologic, Eli Lilly, Biovica, Pfizer/Arvinas, Puma Biotechnology, Novartis, AstraZeneca, Genentech, Regor Therapeutics, Menarini

- Education/Speaking: Eli Lilly, Guardant Health, 2ndMD

- Institutional Research Support: Genentech, Eli Lilly, Pfizer/Arvinas, Nuvation Bio, Regor Therapeutics, Sermonix

Molecular Profiling for HR+ Early Breast Cancer

- General principles and therapeutic approach
- Mammaprint Assay (Agendia)
- Oncotype Risk Score (Exact Sciences)
- Prosigna Assay (Veracyte)
- Summary and Future Directions

Molecular Profiling for HR+ Early Breast Cancer

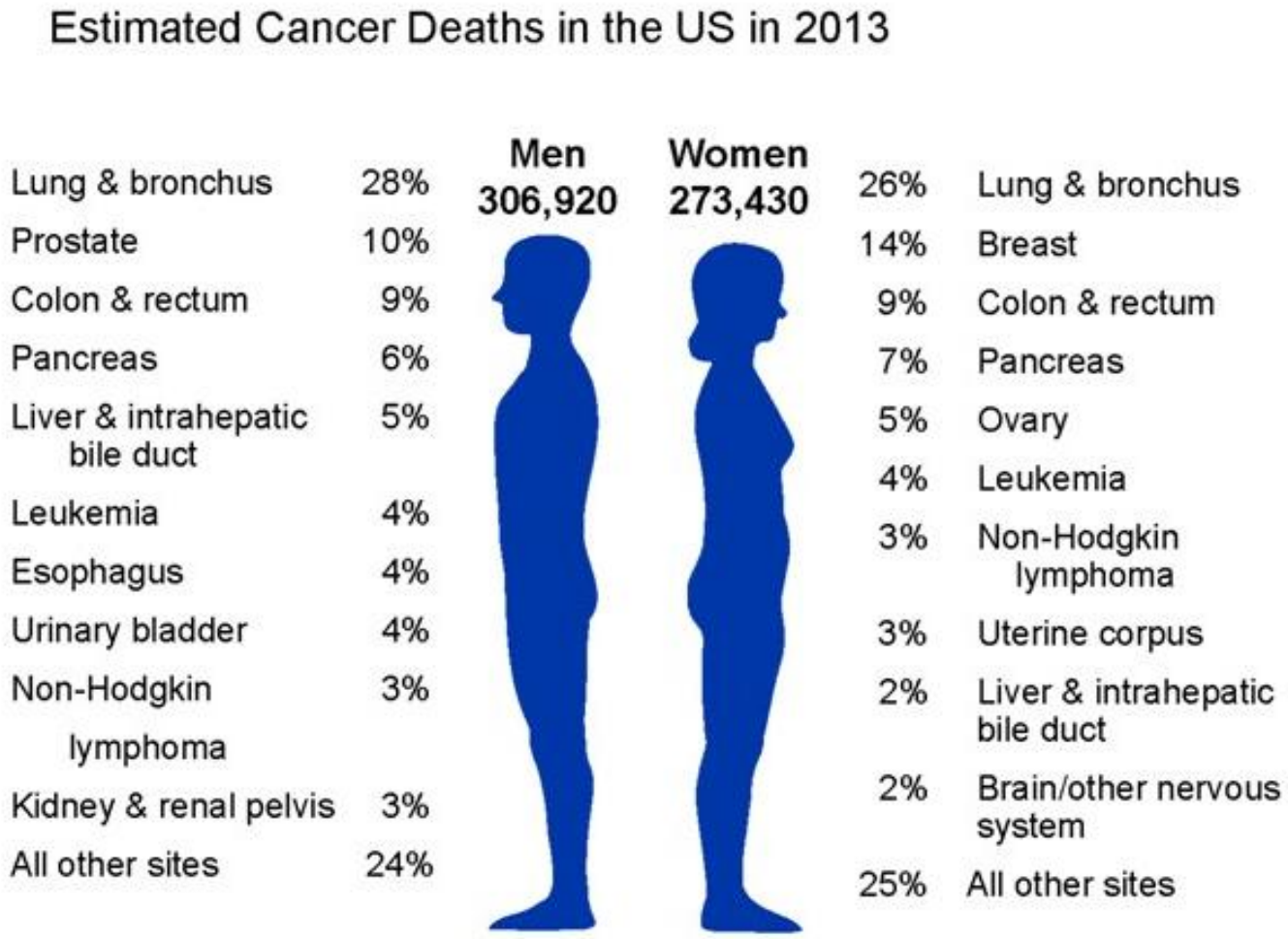
- General principles and therapeutic approach
- Mammaprint Assay (Agendia)
- Oncotype Risk Score (Exact Sciences)
- Prosigna Assay (Veracyte)
- Summary and Future Directions

Epidemiology

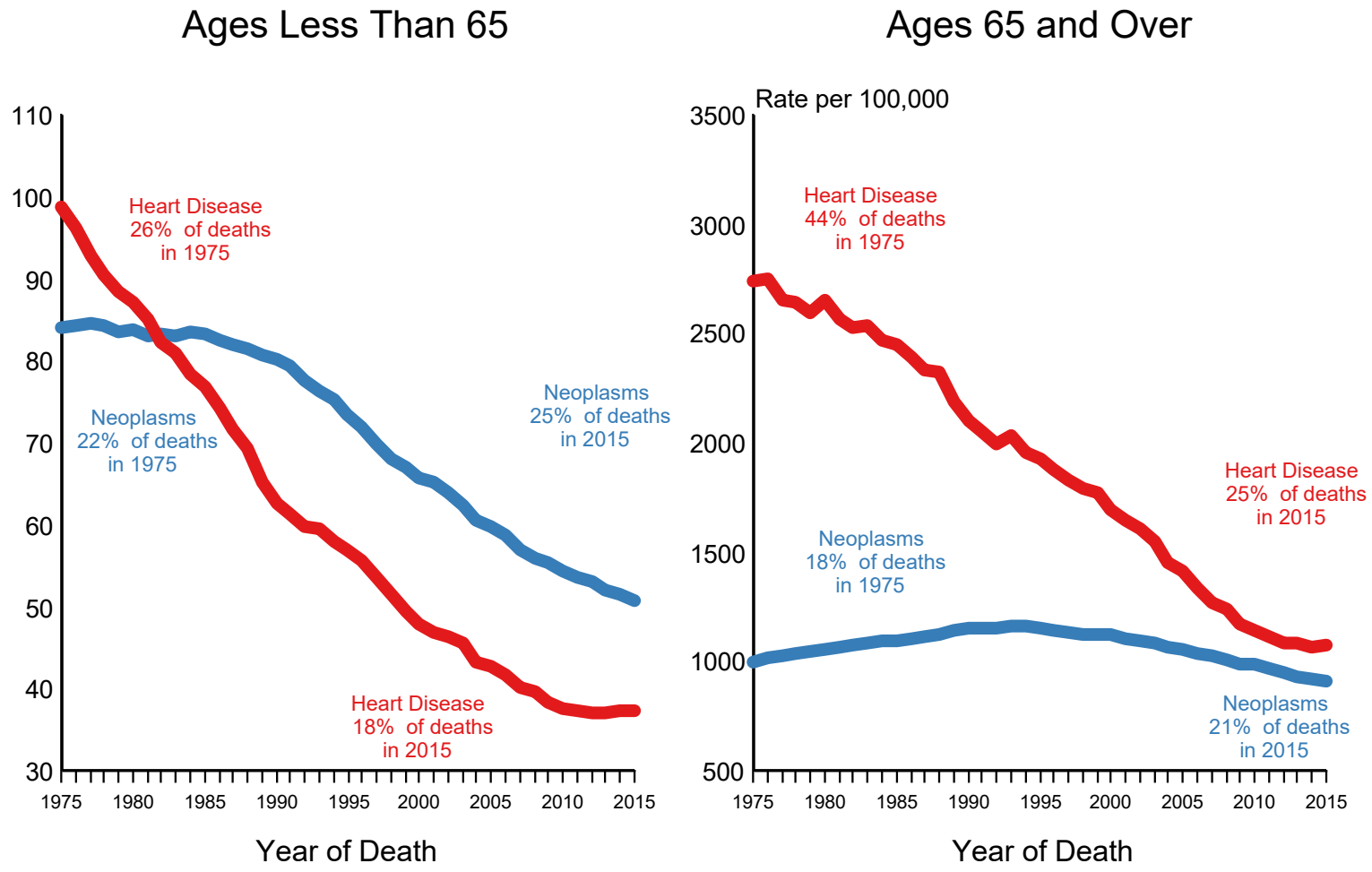
Breast cancer rank (Female):

Incidence – 1st

Mortality – 2nd

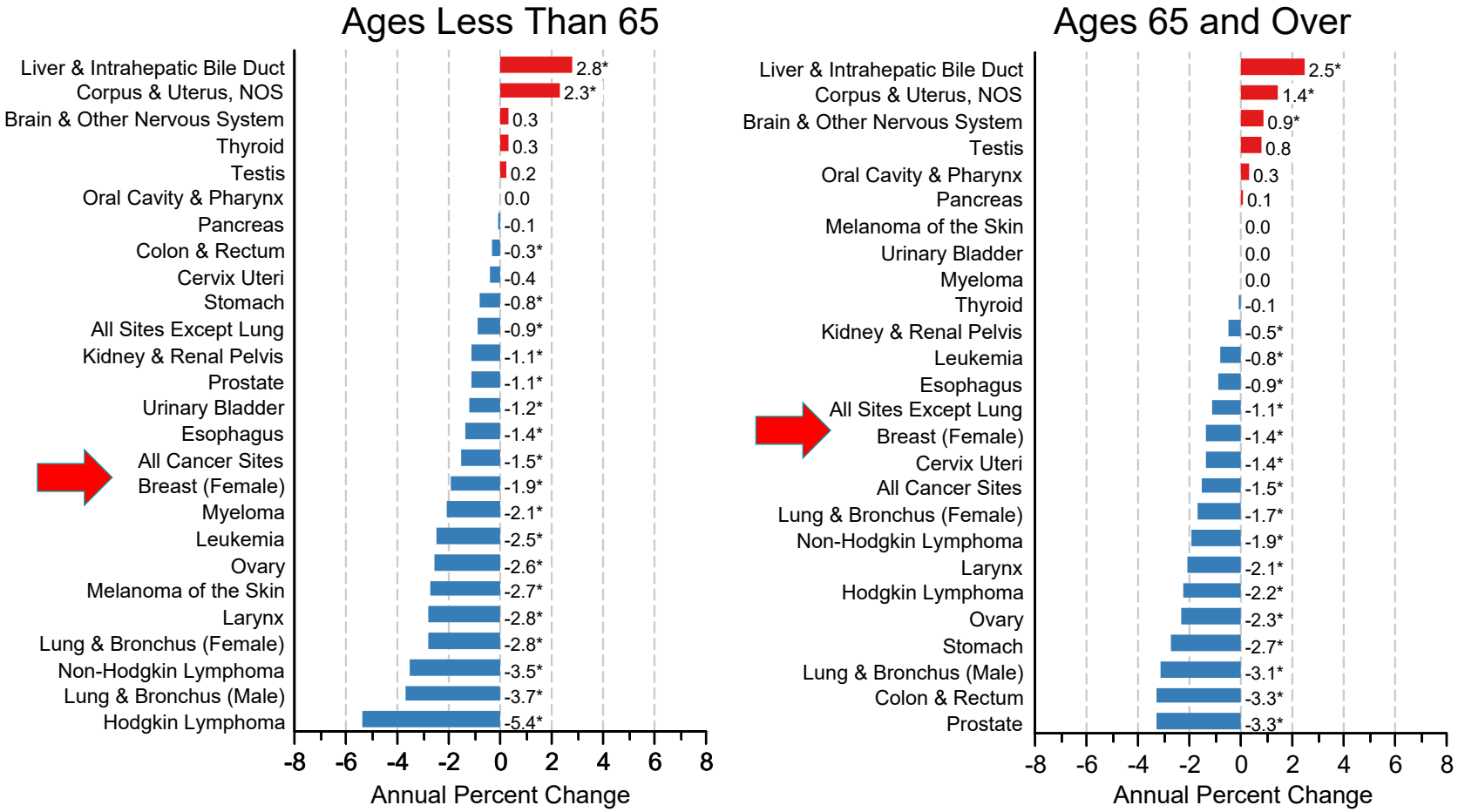


Us Death Rates, 1975-2015
Heart Disease compared to Neoplasms, by age at death



Source: US Mortality Files, National Center for Health Statistics, Centers for Disease Control and Prevention.
Rates are per 100,000 and age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1103).

Trends in US Death Rates by Age Group and Primary Cancer Site 2006-2015



Source: US Mortality Files, National Center for Health Statistics, Centers for Disease Control and Prevention.
Underlying rates are per 100,000 and age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1103).
For sex-specific cancer sites, the population was limited to the population of the appropriate sex.
* The APC is significantly different from zero (p<.05).

Staging

Grade ≠ Stage

Grade – pathologic assessment of tumor differentiation

Stage –

- Tumor size (T)
- Lymph node status (N)
- Distant metastatic disease (M)
- PLUS (2018): receptor status (ER, PR, HER2), genomic recurrence risk score, grade

Local Therapy: Surgery, Radiation

Mastectomy = Lumpectomy + XRT

Indications for Mastectomy:

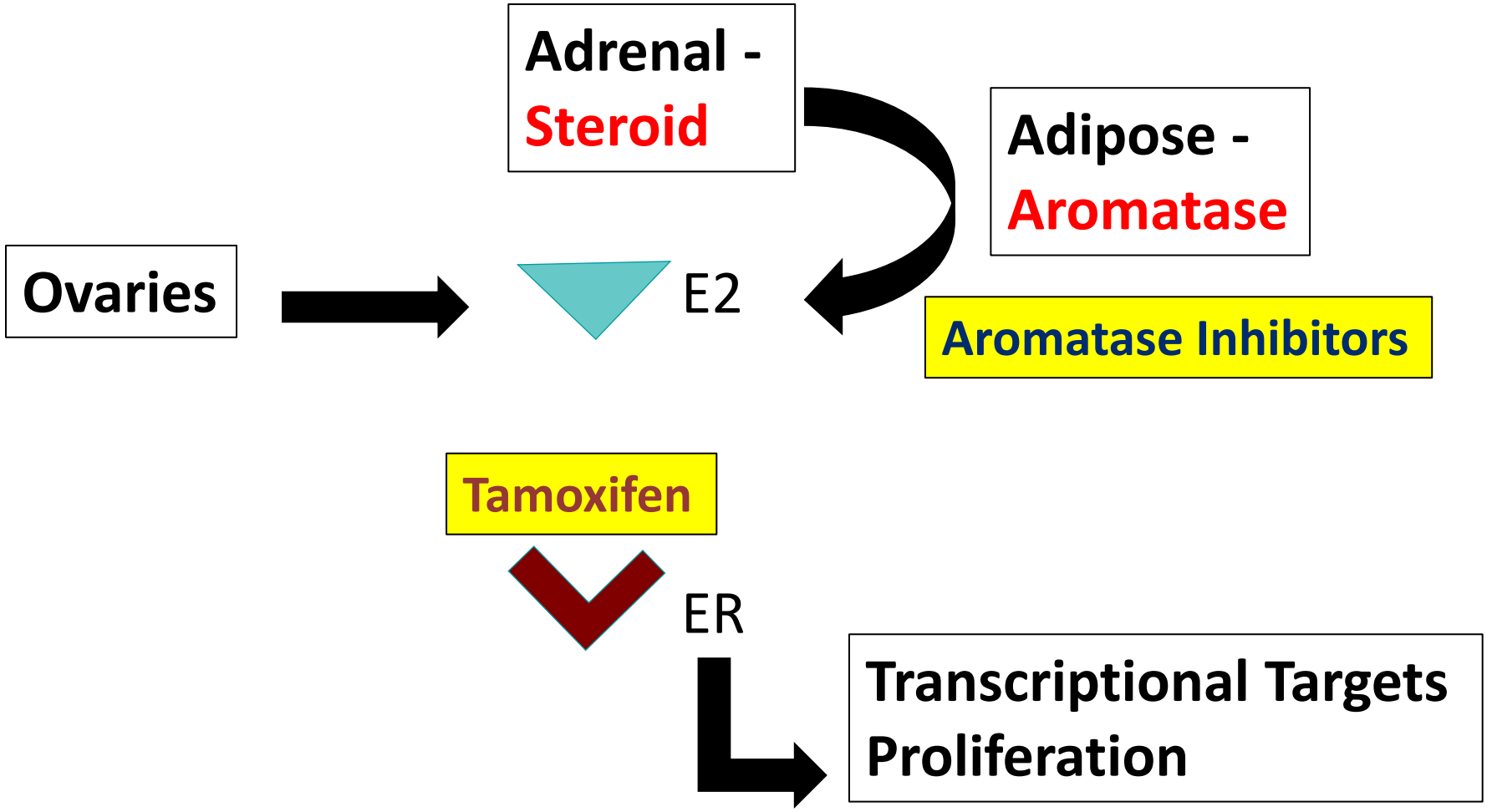
- Extent of disease v cosmetic outcome
- BRCA carrier (prophylactic bilateral)
- Inability to achieve negative margins
- Patient preference

Post-Mastectomy Radiation (PMRT):

- Positive margins (often chest wall)
- Positive lymph nodes

Systemic Therapy: Antiestrogens

Adjuvant antiestrogen = 50% reduction in long-term recurrence risk



Systemic Therapy: Antiestrogens

Tamoxifen

Pre or Post-menopausal

Aromatase Inhibitors

Post-menopausal

- Hot Flashes
- Vaginal Dryness
- Fatigue

Transaminitis

DVT

Secondary uterine malignancy

Myalagia

Osteopenia

Key Questions:

- What is the recurrence risk for any given patient/tumor?
- Who gets benefit from chemotherapy in addition to antiestrogens?
- Who benefits from extended duration antiestrogen therapy (10y v 5y)?
- Which pre-menopausal patients should have ovarian suppression?

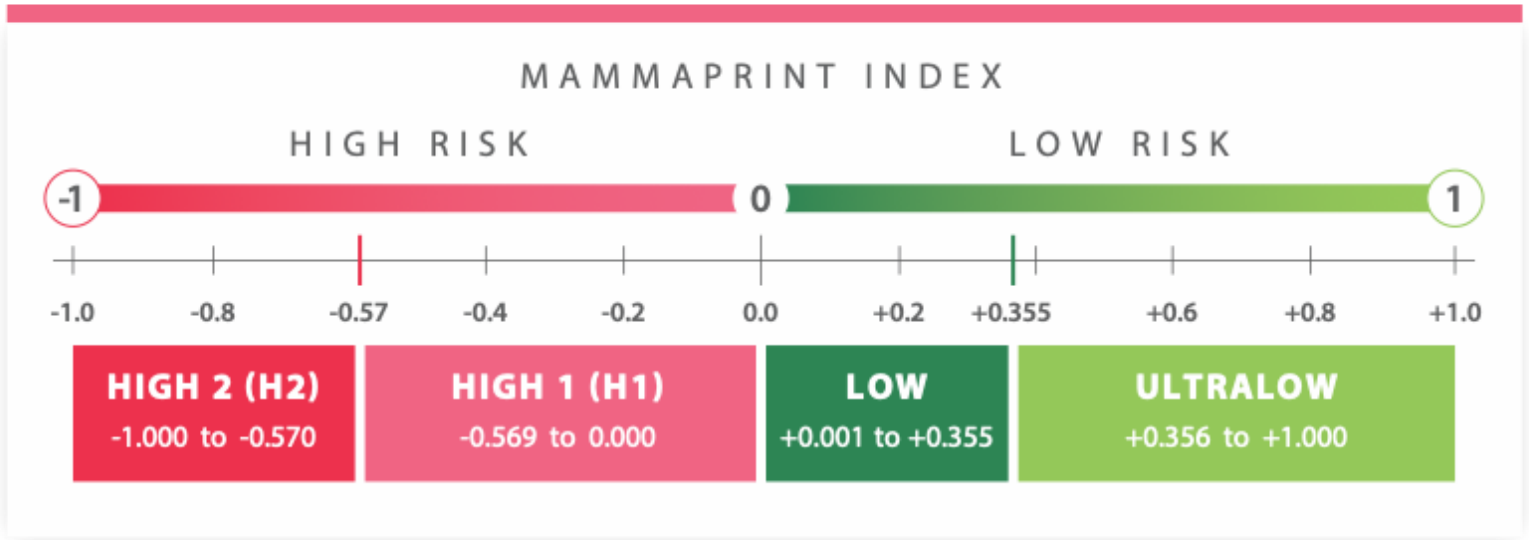
Molecular Profiling for HR+ Early Breast Cancer

- General principles and therapeutic approach
- Mammaprint Assay (Agendia)
- Oncotype Risk Score (Exact Sciences)
- Prosigna Assay (Veracyte)
- Summary and Future Directions

The MammaPrint Index (MPI)

MammaPrint:
70 gene recurrence risk panel.

4 MammaPrint Risk Categories

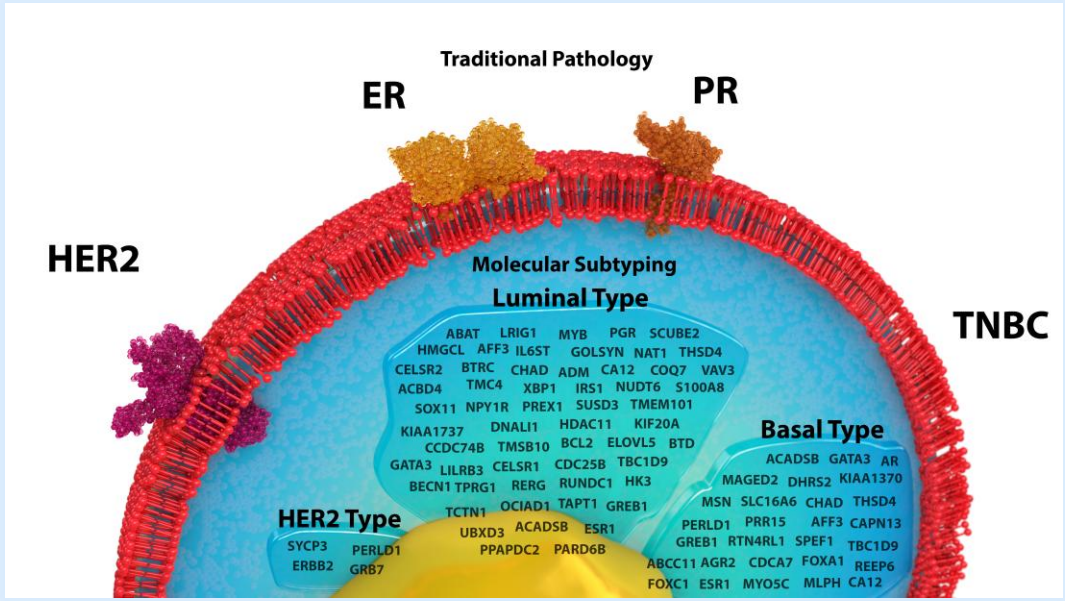


More likely to recur early

Less likely to recur early

80-gene molecular subtyping assay

BluePrint:
80 gene panel exploring signaling pathways driving tumor growth.



BluePrint Genomic Subtypes

BASAL-TYPE	HER2-TYPE	LUMINAL-TYPE
Basal-type (ER-)	HER2-type	Luminal-type A
Basal-type (ER+)		Luminal-type B

BluePrint stratifies up to 23% of tumors *into a different molecular subtype compared with IHC/FISH*

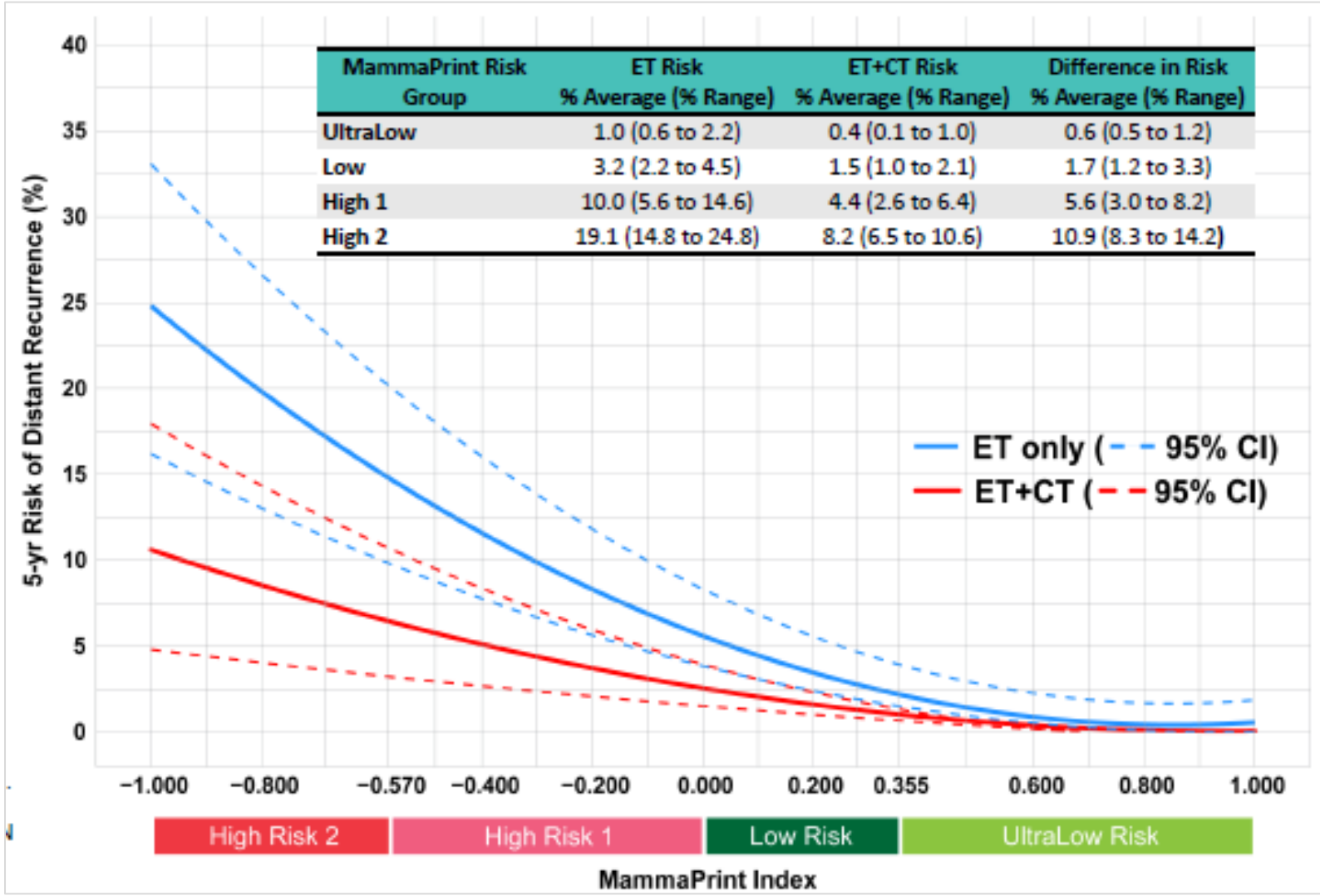
Whitworth P et al Ann Surg Oncol 2022

Mammaprint and Adjuvant Chemotherapy early-stage breast cancer

FLEX Study: **positive predictive utility of MammaPrint** in predicting adjuvant chemo sensitivity

Risk of 5-year DRFI for patients receiving ET vs ET+CT across the MammaPrint Index

MammaPrint Risk Group	ET Risk % Average (% Range)	ET+CT Risk % Average (% Range)	Difference in Risk % Average (% Range)
UltraLow	1.0 (0.6 to 2.2)	0.4 (0.1 to 1.0)	0.6 (0.5 to 1.2)
Low	3.2 (2.2 to 4.5)	1.5 (1.0 to 2.1)	1.7 (1.2 to 3.3)
High 1	10.0 (5.6 to 14.6)	4.4 (2.6 to 6.4)	5.6 (3.0 to 8.2)
High 2	19.1 (14.8 to 24.8)	8.2 (6.5 to 10.6)	10.9 (8.3 to 14.2)

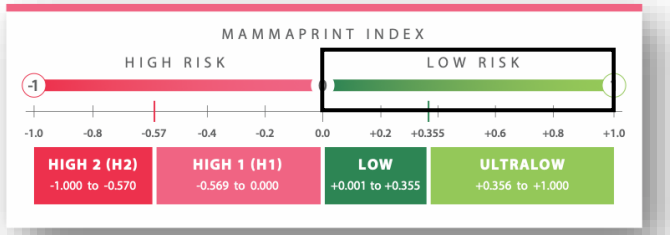


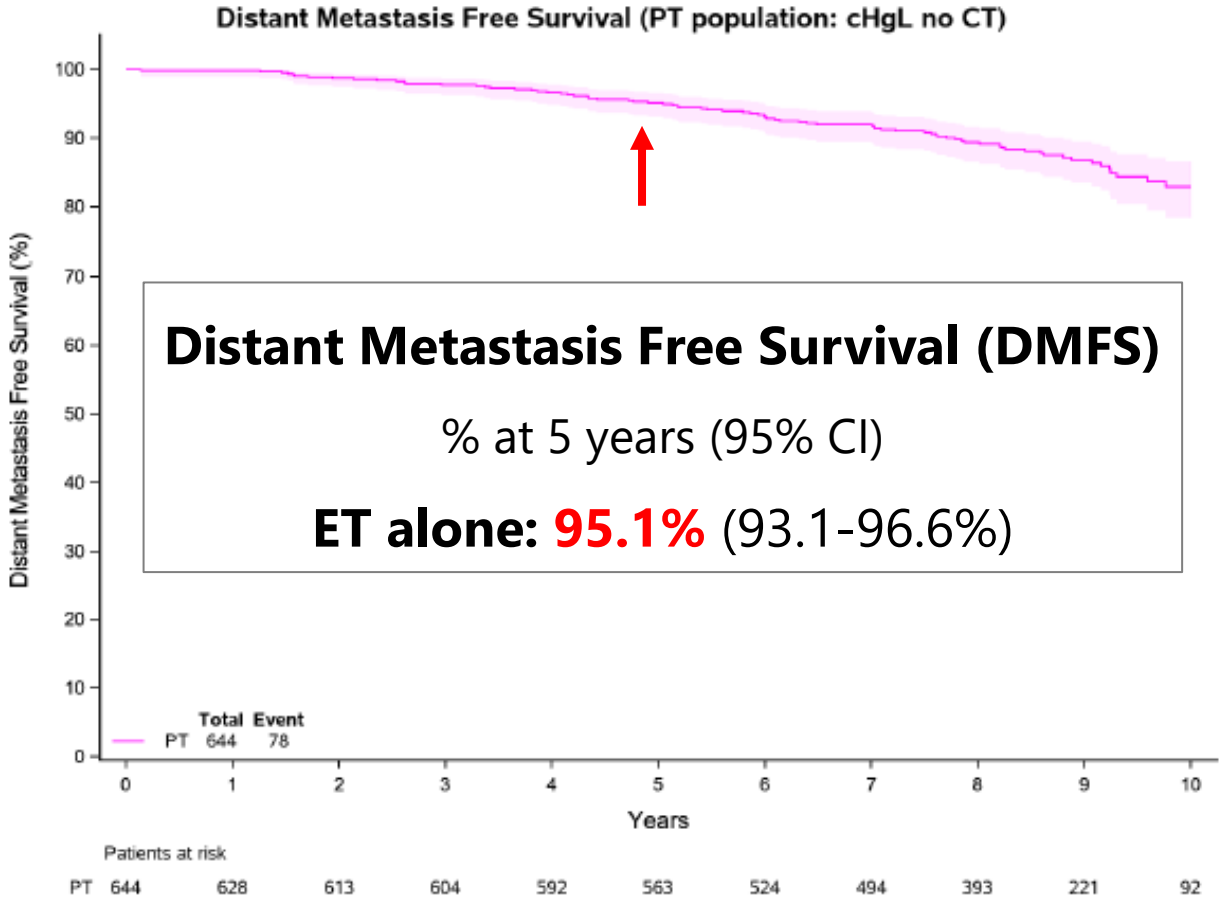
FLEX Study Real World Evidence prospective, propensity score matched study (n = 1002)

Brufsky et al SABCS 2024

Mammaprint and lack of adjuvant CT benefit

<u>Level 1A Evidence</u> from the MINDACT Trial show MammaPrint Low Risk may safely avoid chemotherapy





Distant Metastasis Free Survival (DMFS)

% at 5 years (95% CI)

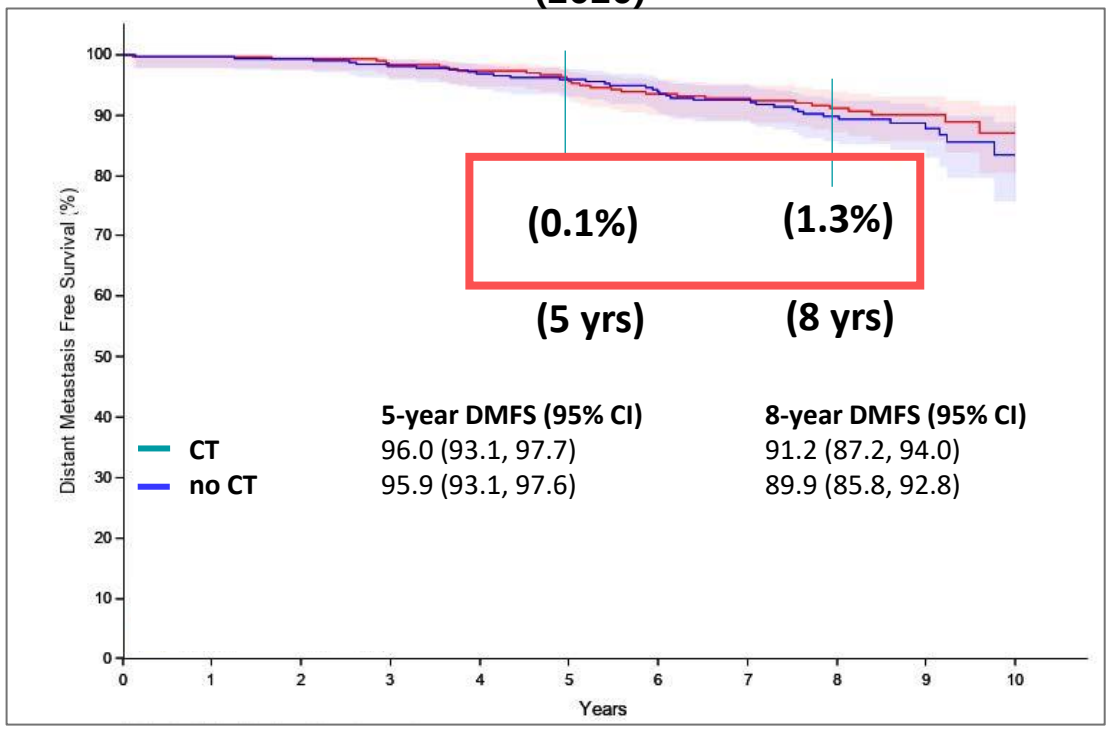
ET alone: 95.1% (93.1-96.6%)

Cardoso at al ASCO 2020
Piccart et al Lancet Oncol 2021

Distant Metastasis-Free Survival, HR+/HER2-, LN+

Clinical high risk/MammaPrint Low Risk

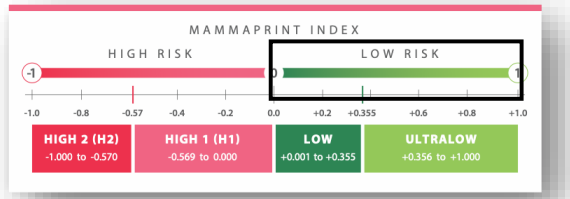
(2020)



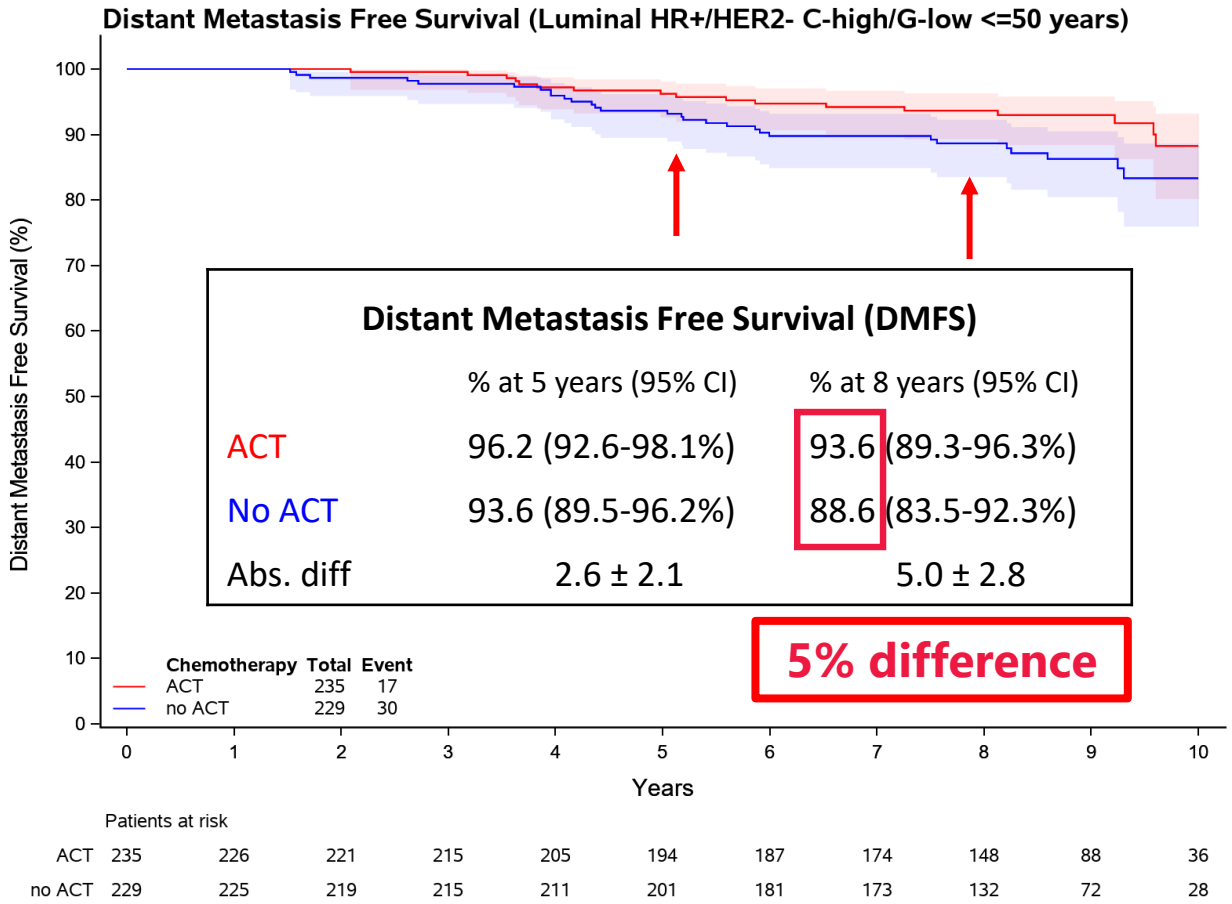
First prospective, randomized data with <u>8+ years</u> of follow-up in 1-3 LN+ (2020)

Mammaprint and lack of adjuvant CT benefit

Does Age Affect Benefit of Chemotherapy in MammaPrint Low Risk?



Age ≤50 years

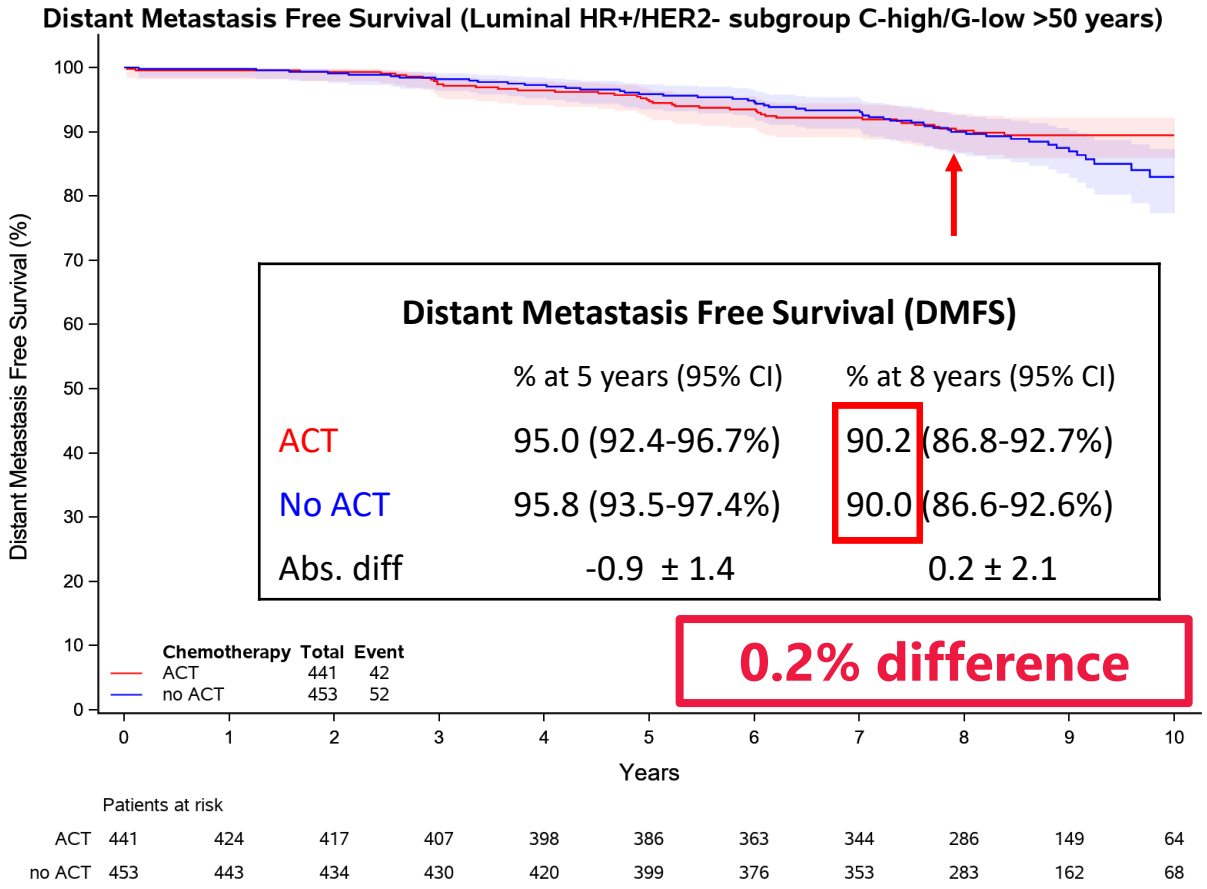


Distant Metastasis Free Survival (DMFS)

	% at 5 years (95% CI)	% at 8 years (95% CI)
ACT	96.2 (92.6-98.1%)	93.6 (89.3-96.3%)
No ACT	93.6 (89.5-96.2%)	88.6 (83.5-92.3%)
Abs. diff	2.6 ± 2.1	5.0 ± 2.8

5% difference

Age >50 years



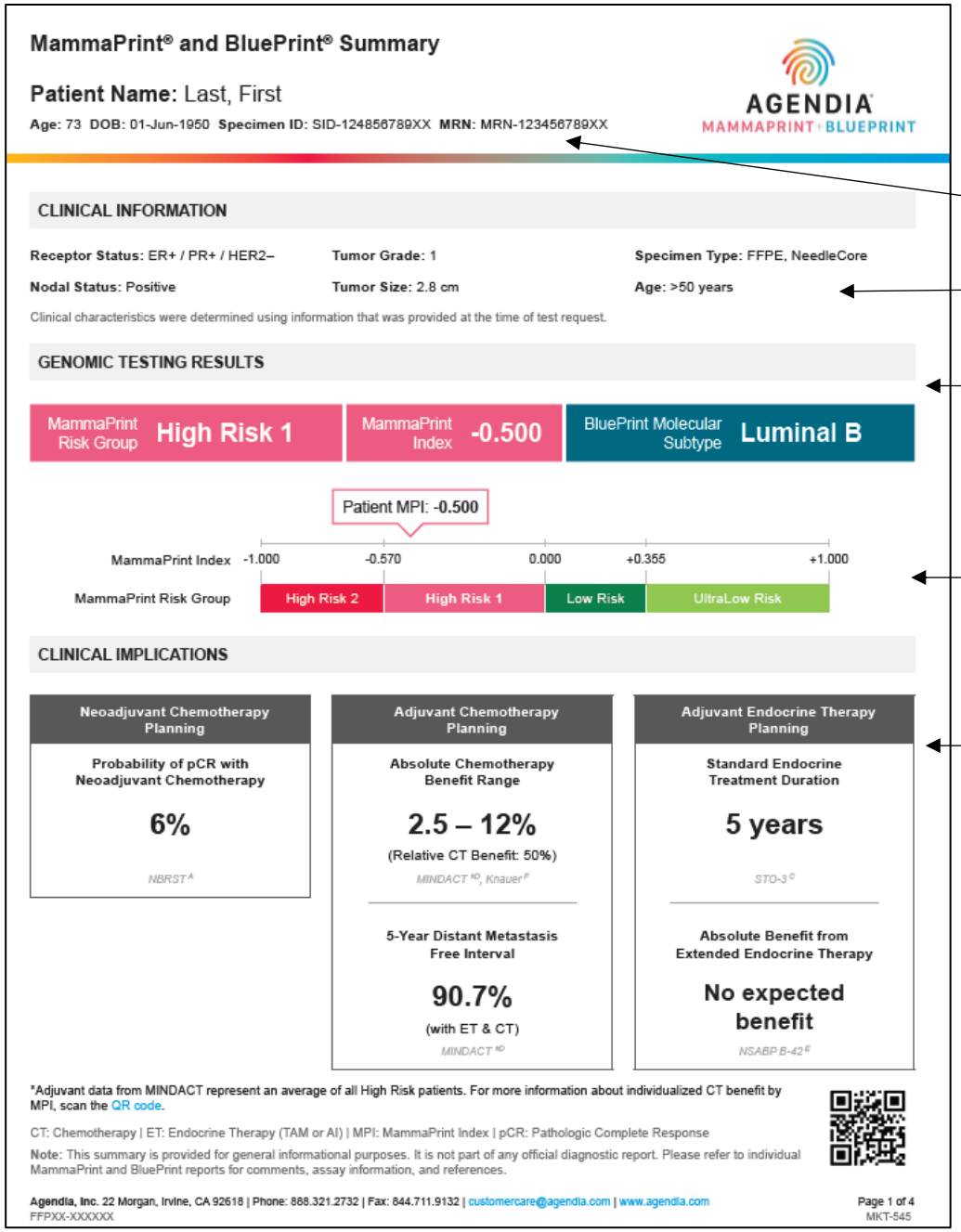
Distant Metastasis Free Survival (DMFS)

	% at 5 years (95% CI)	% at 8 years (95% CI)
ACT	95.0 (92.4-96.7%)	90.2 (86.8-92.7%)
No ACT	95.8 (93.5-97.4%)	90.0 (86.6-92.6%)
Abs. diff	-0.9 ± 1.4	0.2 ± 2.1

0.2% difference

Cardoso at al ASCO 2020
Piccart et al Lancet Oncol 2021

Sample of a MammaPrint High 1, Luminal B summary variation



MammaPrint® and BluePrint® Summary

Patient Name: Last, First

Age: 73 **DOB:** 01-Jun-1950 **Specimen ID:** SID-124856789XX **MRN:** MRN-123456789XX

AGENDIA MAMMAPRINT + BLUEPRINT

CLINICAL INFORMATION

Receptor Status: ER+ / PR+ / HER2– **Tumor Grade:** 1 **Specimen Type:** FFPE, NeedleCore

Nodal Status: Positive **Tumor Size:** 2.8 cm **Age:** >50 years

Clinical characteristics were determined using information that was provided at the time of test request.

GENOMIC TESTING RESULTS

MammaPrint Risk Group	MammaPrint Index	BluePrint Molecular Subtype
High Risk 1	-0.500	Luminal B

Patient MPI: -0.500

MammaPrint Index	-1.000	-0.570	0.000	+0.355	+1.000
MammaPrint Risk Group	High Risk 2	High Risk 1	Low Risk	UltraLow Risk	

CLINICAL IMPLICATIONS

Neoadjuvant Chemotherapy Planning	Adjuvant Chemotherapy Planning	Adjuvant Endocrine Therapy Planning
Probability of pCR with Neoadjuvant Chemotherapy: 6% (NBRST[A])	Absolute Chemotherapy Benefit Range: 2.5 – 12% (Relative CT Benefit: 50%) (MINDACT[*D], Knauer[F])	Standard Endocrine Treatment Duration: 5 years (STO-3[G])
	5-Year Distant Metastasis Free Interval: 90.7% (with ET & CT) (MINDACT[*D])	Absolute Benefit from Extended Endocrine Therapy: No expected benefit (NSABP B-42[E])

*Adjuvant data from MINDACT represent an average of all High Risk patients. For more information about individualized CT benefit by MPI, scan the QR code.

CT: Chemotherapy | ET: Endocrine Therapy (TAM or AI) | MPI: MammaPrint Index | pCR: Pathologic Complete Response

Note: This summary is provided for general informational purposes. It is not part of any official diagnostic report. Please refer to individual MammaPrint and BluePrint reports for comments, assay information, and references.

Agendia, Inc. 22 Morgan, Irvine, CA 92618 | Phone: 888.321.2732 | Fax: 844.711.9132 | customercare@agendia.com | www.agendia.com

Page 1 of 4

FFPXX-XXXXXX MKT-545

Page 1: Summary of MammaPrint + BluePrint assay results

Patient Information

Clinical Information (from provided pathology

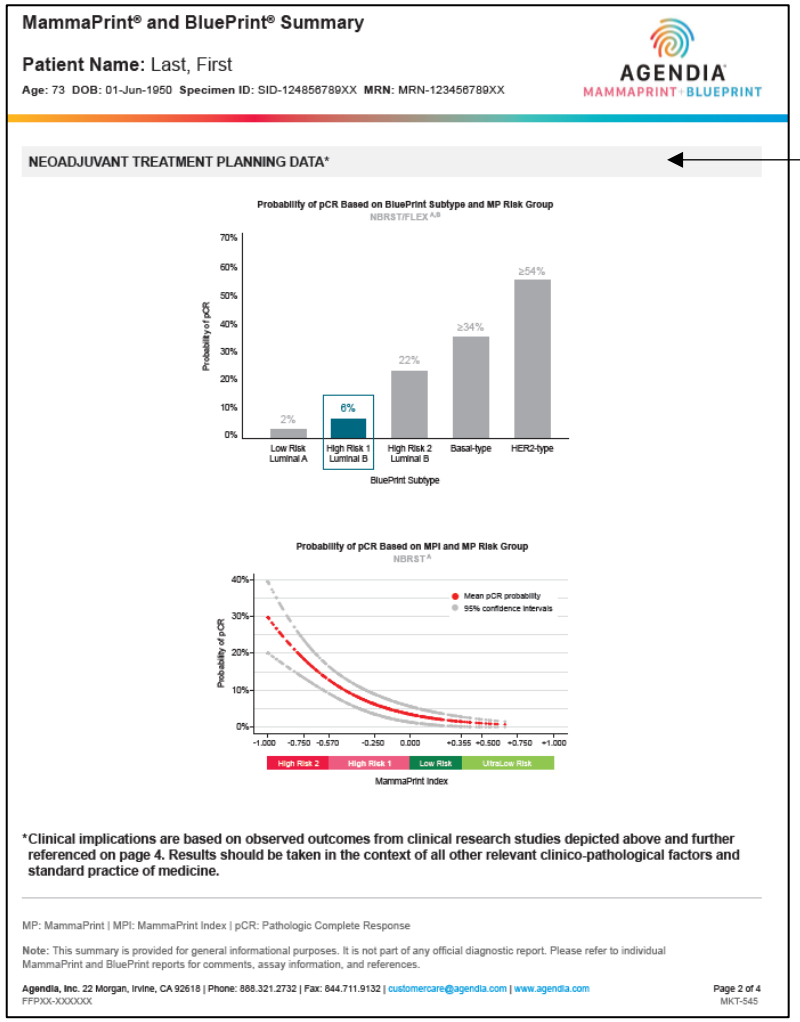
MammaPrint Risk Group, MammaPrint Index, BluePrint Molecular Subtype (if ordered)

Patient-specific MammaPrint Index

Clinical implications derived from genomic analysis dependent upon nature of sample received

Sample of a MammaPrint High 1, Luminal B summary variation

Page 2: Neoadjuvant Treatment Planning Data *(pCR by BluePrint, if ordered)*



MammaPrint® and BluePrint® Summary

Patient Name: Last, First

Age: 73 **DOB:** 01-Jun-1950 **Specimen ID:** SID-124856789XX **MRN:** MRN-123456789XX

AGENDIA MAMMAPRINT + BLUEPRINT

NEOADJUVANT TREATMENT PLANNING DATA*

Probability of pCR Based on BluePrint Subtype and MP Risk Group

NBRST/I/FLEX[4,5]

Probability of pCR Based on MPI and MP Risk Group

NBRST[4]

*Clinical implications are based on observed outcomes from clinical research studies depicted above and further referenced on page 4. Results should be taken in the context of all other relevant clinico-pathological factors and standard practice of medicine.

MP: MammaPrint | MPI: MammaPrint Index | pCR: Pathologic Complete Response

Note: This summary is provided for general informational purposes. It is not part of any official diagnostic report. Please refer to individual MammaPrint and BluePrint reports for comments, assay information, and references.

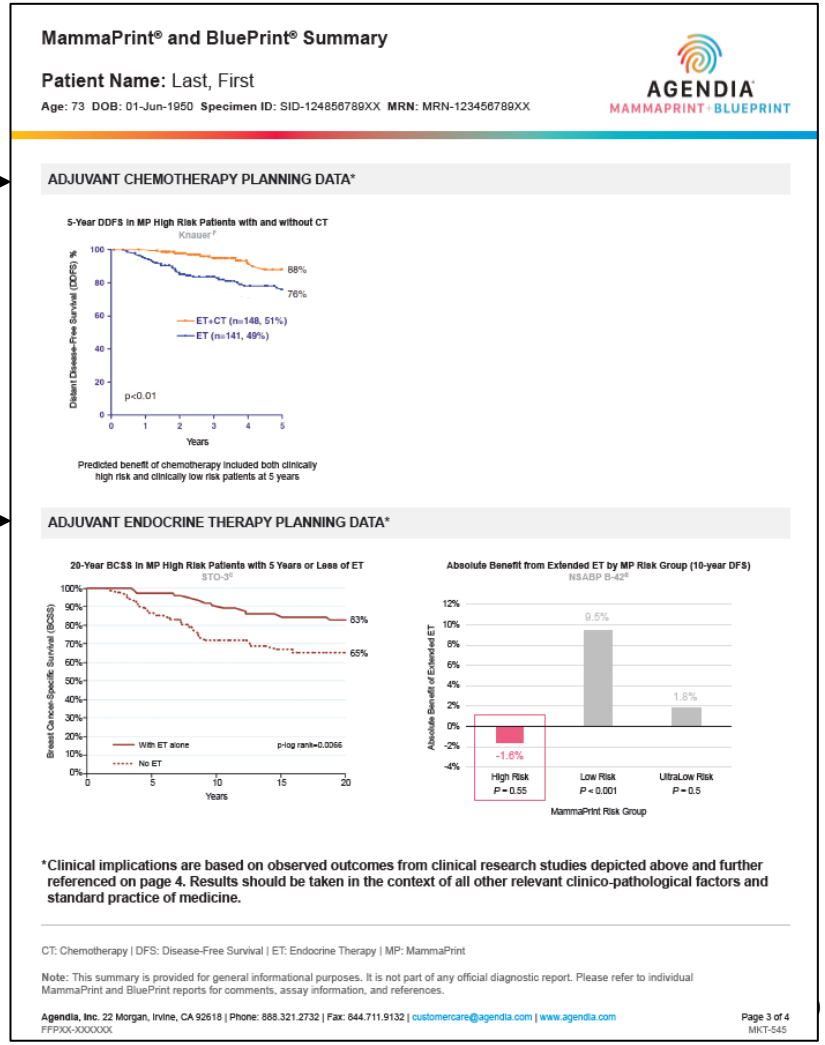
Agendia, Inc. 22 Morgan, Irvine, CA 92618 | Phone: 888.321.2732 | Fax: 844.711.9132 | customercare@agendia.com | www.agendia.com

Neoadjuvant Treatment Planning

Adjuvant CT Planning Data

Adjuvant Endocrine Therapy Planning Data

Page 3: Adjuvant CT & ET/EET Treatment Planning Data



MammaPrint® and BluePrint® Summary

Patient Name: Last, First

Age: 73 **DOB:** 01-Jun-1950 **Specimen ID:** SID-124856789XX **MRN:** MRN-123456789XX

AGENDIA MAMMAPRINT + BLUEPRINT

ADJUVANT CHEMOTHERAPY PLANNING DATA*

5-Year DDFS in MP High Risk Patients with and without CT

Knauer[7]

Predicted benefit of chemotherapy included both clinically high risk and clinically low risk patients at 5 years

ADJUVANT ENDOCRINE THERAPY PLANNING DATA*

20-Year BCSS in MP High Risk Patients with 5 Years or Less of ET

STO-3[6]

Absolute Benefit from Extended ET by MP Risk Group (10-year DFS)

NSABP B-42[8]

*Clinical implications are based on observed outcomes from clinical research studies depicted above and further referenced on page 4. Results should be taken in the context of all other relevant clinico-pathological factors and standard practice of medicine.

CT: Chemotherapy | DFS: Disease-Free Survival | ET: Endocrine Therapy | MP: MammaPrint

Note: This summary is provided for general informational purposes. It is not part of any official diagnostic report. Please refer to individual MammaPrint and BluePrint reports for comments, assay information, and references.

Agendia, Inc. 22 Morgan, Irvine, CA 92618 | Phone: 888.321.2732 | Fax: 844.711.9132 | customercare@agendia.com | www.agendia.com

Molecular Profiling for HR+ Early Breast Cancer

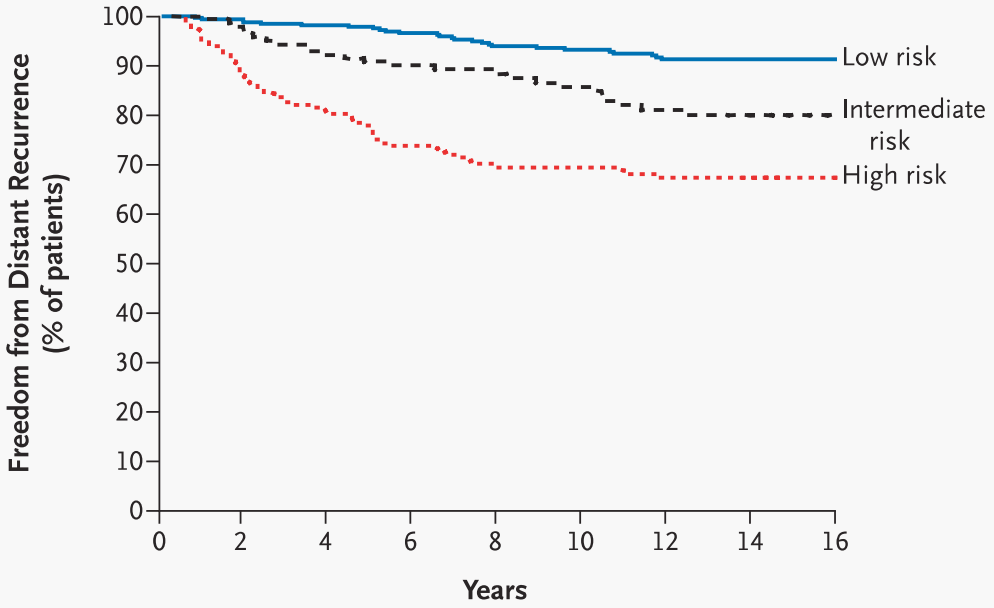
- General principles and therapeutic approach
- Mammaprint Assay (Agendia)
- Oncotype Risk Score (Exact Sciences)
- Prosigna Assay (Veracyte)
- Summary and Future Directions

Oncotype Corresponds to Distant Recurrence

- Oncotype: 21 gene expression assay (genomic mediators of ER/PR expression and cellular proliferation, among others)
- Increasing rates of distance recurrence with rising oncotype score
- Valid in multivariate analysis (independent of age, tumor size)

Table 1. Kaplan–Meier Estimates of the Rate of Distant Recurrence at 10 Years, According to Recurrence-Score Risk Categories.*

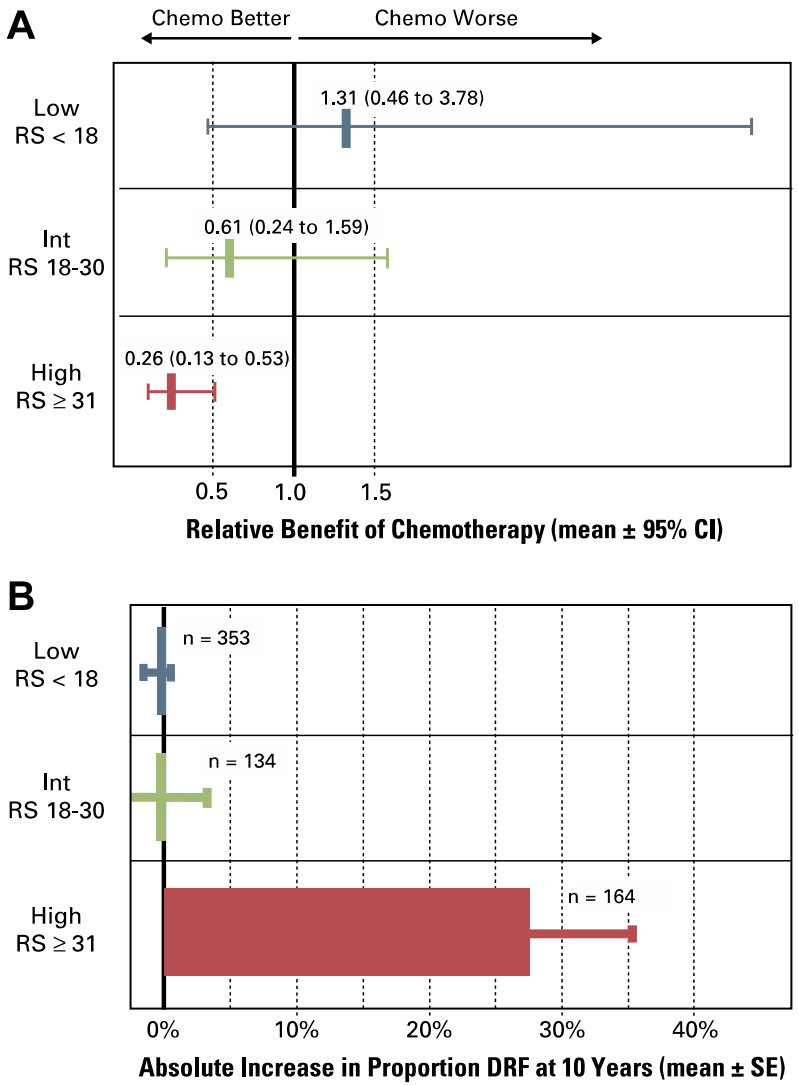
Risk Category	Percentage of Patients	Rate of Distant Recurrence at 10 Yr (95% CI)†
		percent
Low	51	6.8 (4.0–9.6)
Intermediate	22	14.3 (8.3–20.3)
High	27	30.5 (23.6–37.4)‡



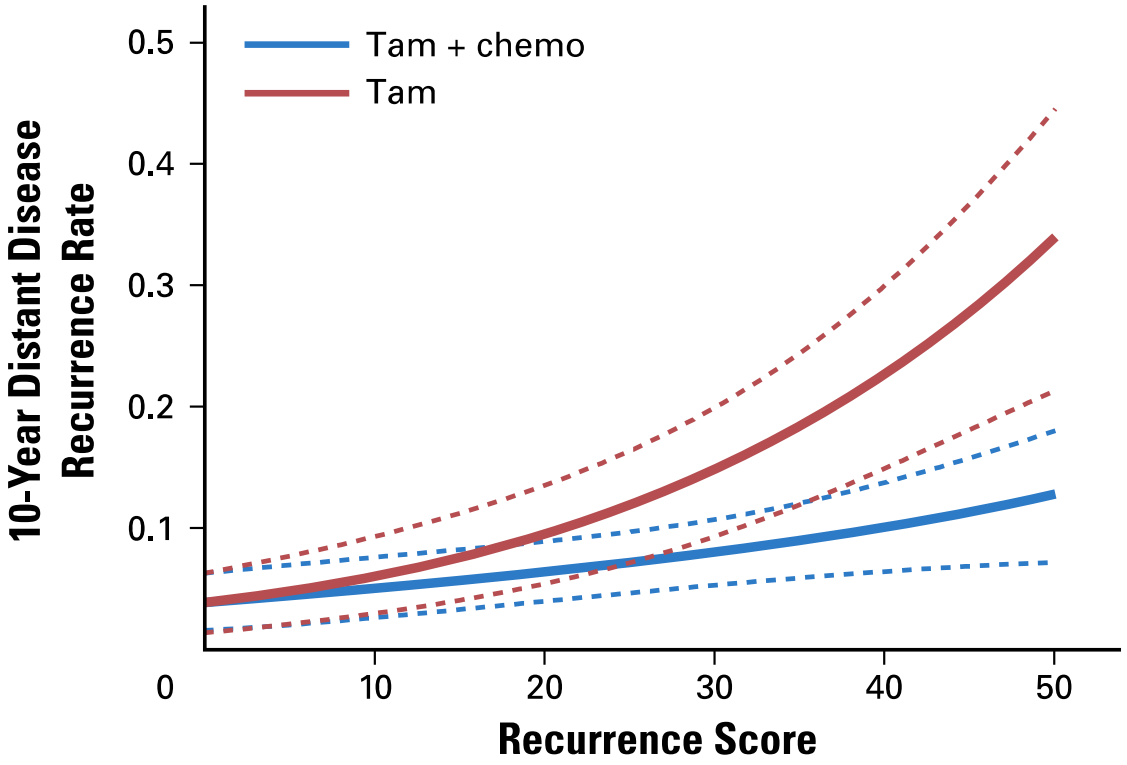
No. at Risk									
Low risk	338	328	313	298	276	258	231	170	38
Intermediate risk	149	139	128	116	104	96	80	66	16
High risk	181	154	137	119	105	91	83	63	13

Paik S et al NEJM 2004

Oncotype and Chemotherapy Benefit (retrospective)



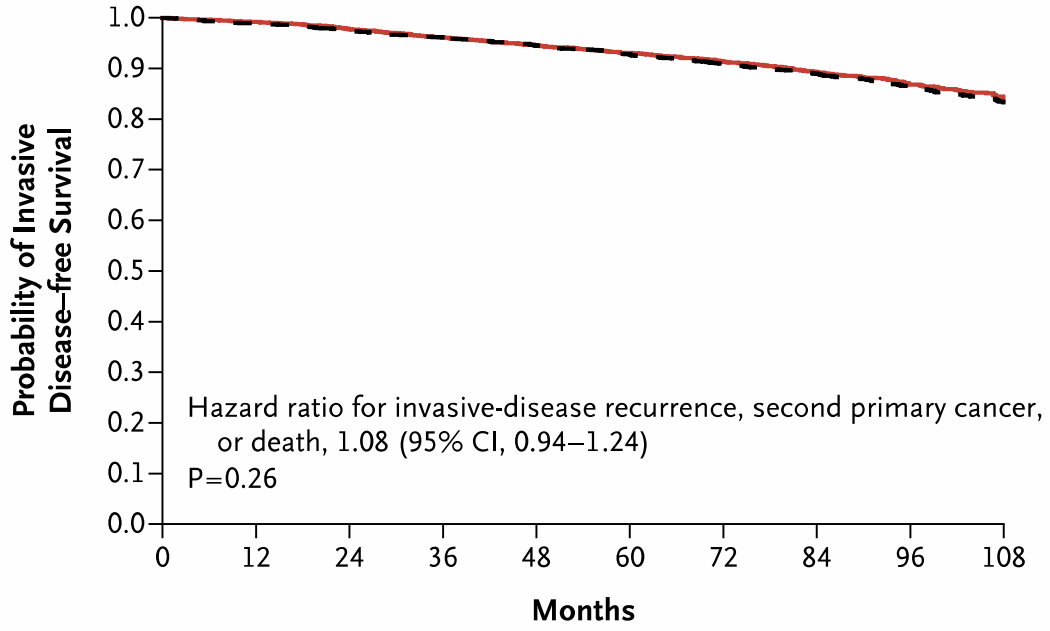
- Oncotype is an independent predictor of chemo benefit on multivariate analysis



Paik S et al JCO 2006

TAILORx: Chemotherapy impact for oncotype <25

A **Invasive Disease–free Survival**



No. at Risk

Chemoendocrine therapy	3312	3204	3104	2993	2849	2645	2335	1781	1130	523
Endocrine therapy	3399	3293	3194	3081	2953	2741	2431	1859	1197	537

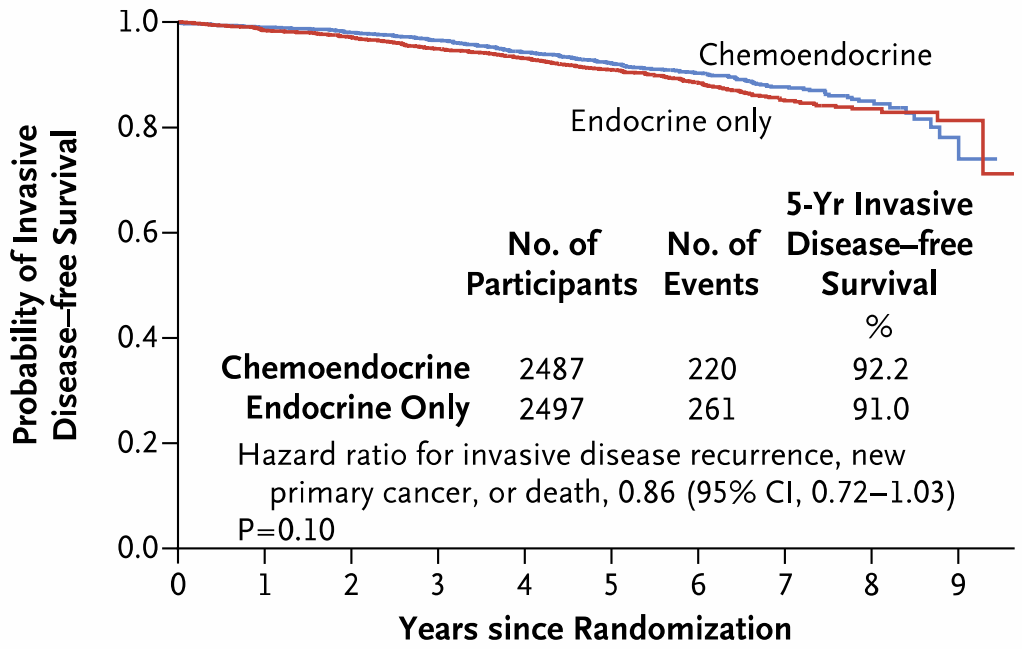
Table 3. Estimated Survival Rates According to Recurrence Score and Assigned Treatment among Women 50 Years of Age or Younger in the Intention-to-Treat Population.*

End Point and Treatment Group	Rate at 5 Yr	Rate at 9 Yr
	percent	
Invasive disease–free survival†		
Score of ≤10, endocrine therapy	95.1±1.1	87.4±2.0
Score of 11–15, endocrine therapy	95.1±1.1	85.7±2.2
Score of 11–15, chemoendocrine therapy	94.3±1.3	89.2±1.9
Score of 16–20, endocrine therapy	92.0±1.3	80.6±2.5
Score of 16–20, chemoendocrine therapy	94.7±1.1	89.6±1.7
Score of 21–25, endocrine therapy	86.3±2.3	79.2±3.3
Score of 21–25, chemoendocrine therapy	92.1±1.8	85.5±3.0
Score of ≥26, chemoendocrine therapy	86.4±1.9	80.3±2.9
Freedom from recurrence of breast cancer at a distant site		
Score of ≤10, endocrine therapy	99.7±0.3	98.5±0.8
Score of 11–15, endocrine therapy	98.8±0.6	97.2±1.0
Score of 11–15, chemoendocrine therapy	98.5±0.7	98.0±0.8
Score of 16–20, endocrine therapy	98.1±0.7	93.6±1.4
Score of 16–20, chemoendocrine therapy	98.9±0.5	95.2±1.3
Score of 21–25, endocrine therapy	93.2±1.7	86.9±2.9
Score of 21–25, chemoendocrine therapy	96.4±1.2	93.4±2.3
Score of ≥26, chemoendocrine therapy	91.1±1.6	88.7±2.1

Sparano JA et al NEJM 2018

Oncotype is Valid in Node-Positive Post-menopausal

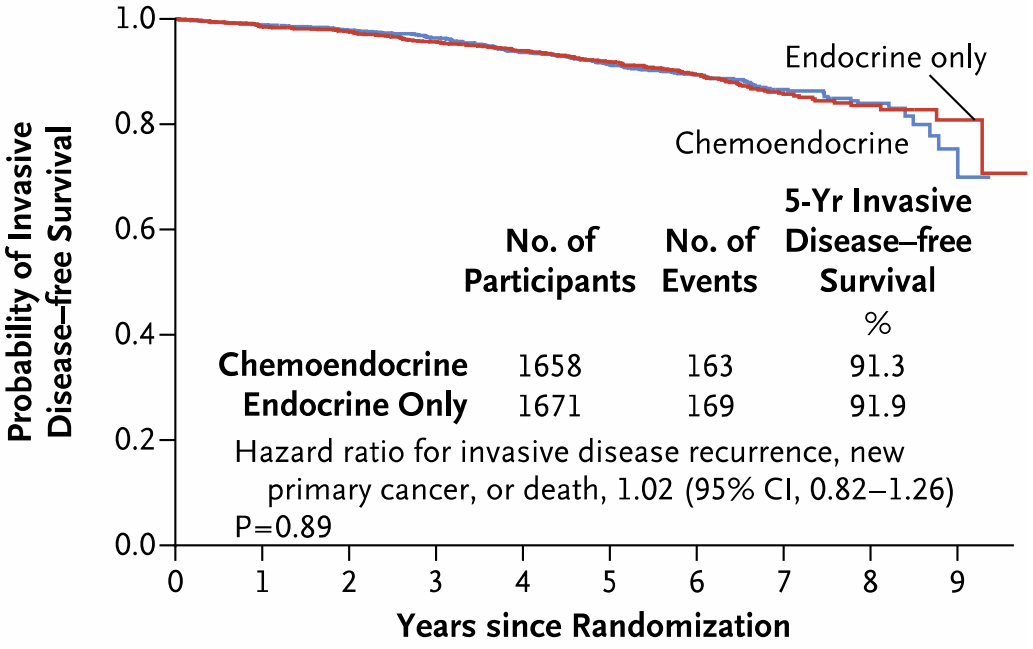
A Invasive Disease–free Survival, All Participants



No. at Risk

Chemoendocrine group	2487	2279	2123	1940	1691	1477	971	511	175	19
Endocrine-only group	2497	2328	2177	1965	1738	1493	969	502	181	23

B Invasive Disease–free Survival, Postmenopausal Participants

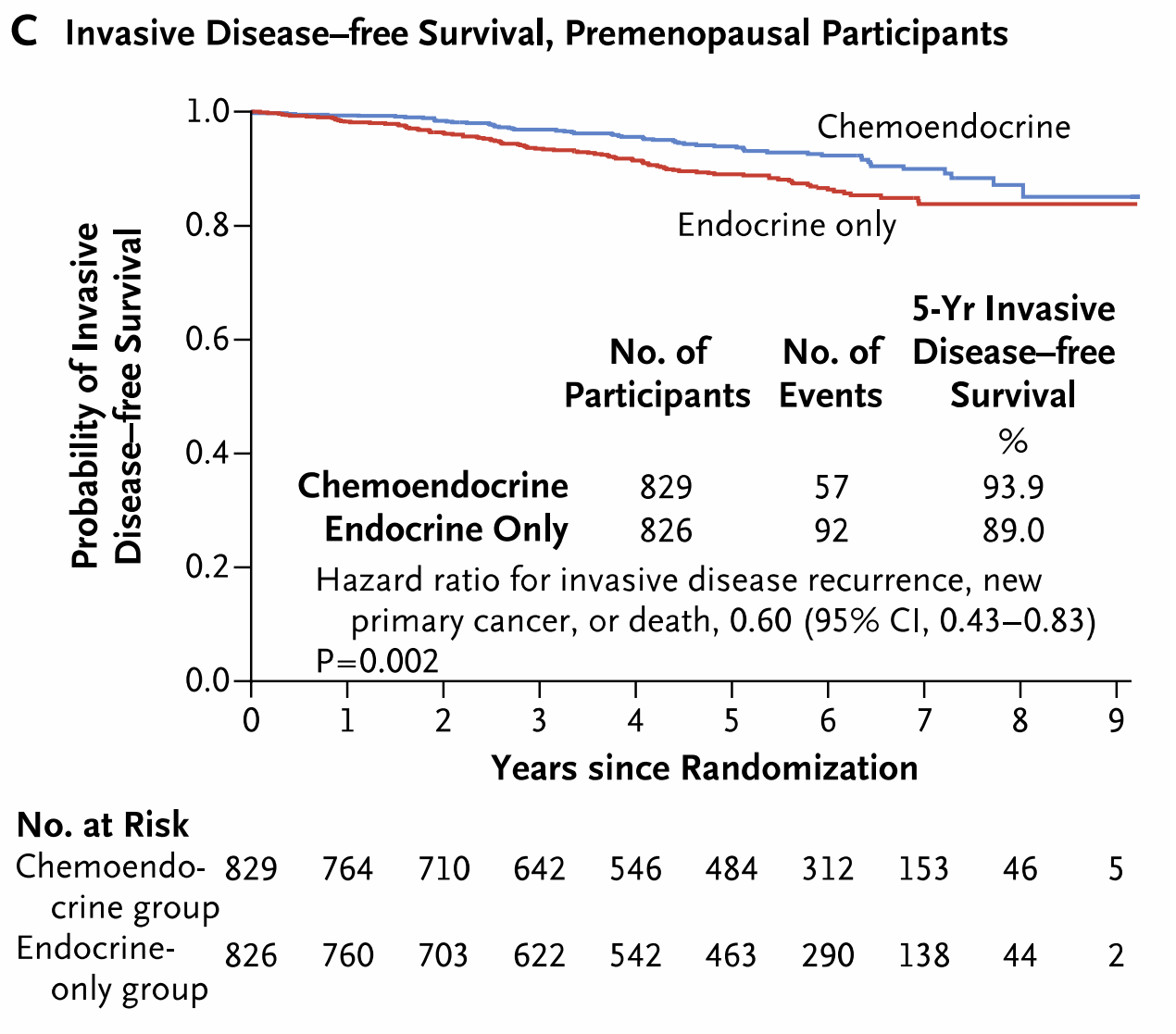


No. at Risk

Chemoendocrine group	1658	1515	1413	1298	1145	993	659	358	129	14
Endocrine-only group	1671	1568	1474	1343	1196	1030	679	364	137	21

Kalinsky K et al NEJM 2021

Controversy re: Oncotype in N+ PRE-menopausal



Kalinsky K et al NEJM 2021

Shows the individualized risk of distant recurrence when treated with endocrine therapy alone.

Sample Report

Oncotype DX Breast Recurrence Score® Report
Node Negative

EXACT SCIENCES

DOE, JANE ELIZABETH
Date of Birth: 01 - Jan - 1951 Gender: Female Report Number: OR123456789-01 Report Date: 13-July-2022
Specimen Source/ID: Breast/SP-16_0123456
Ordering Physician: Dr. First-Name I. Ordering-Physician-Last-Name

Based on a continuous scale of 0-100 and reflects individual tumor biology. The higher the patient's score, the higher the risk of distant recurrence and likelihood of chemotherapy benefit.

Recurrence Score® (RS) Result	Distant Recurrence Risk at 9 Years	Group Average Absolute Chemotherapy (CT) Benefit*
13	With AI or TAM Alone 4% 95% CI (3%, 5%) TAILORx	RS 11-25 All Ages <1% 95% CI (-1%, 2%) TAILORx

Represents the likelihood that adding chemotherapy will reduce the risk of distance recurrence.

Exploratory Subgroup Analysis for TAILORx and NSABP B-20:
Absolute CT Benefit for Distant Recurrence by Age and RS

This table provides additional data about potential chemotherapy benefit based on age and Recurrence Score® (RS) result group.

Age	RS 0-10	RS 11-15	RS 16-20	RS 21-25	RS 26-100
>50 years	No CT Benefit (<1%)				>15% CT Benefit
≤50 years	No CT Benefit (<1%)		~1.6% CT Benefit	~6.5% CT Benefit	>15% CT Benefit

Quantitative Single-Gene Scores[1]

10.0 ER Positive | 8.7 PR Positive | 8.8 HER2 Negative

Provides ER, PR and HER2 gene expression levels based upon RT-PCR.

PAGE 1 OF 2

1. ER Score based on quantitative ESR1 expression (estrogen receptor); PR Score based on quantitative PGR expression (progesterone receptor); HER2 Score based on quantitative ERBB2 expression.

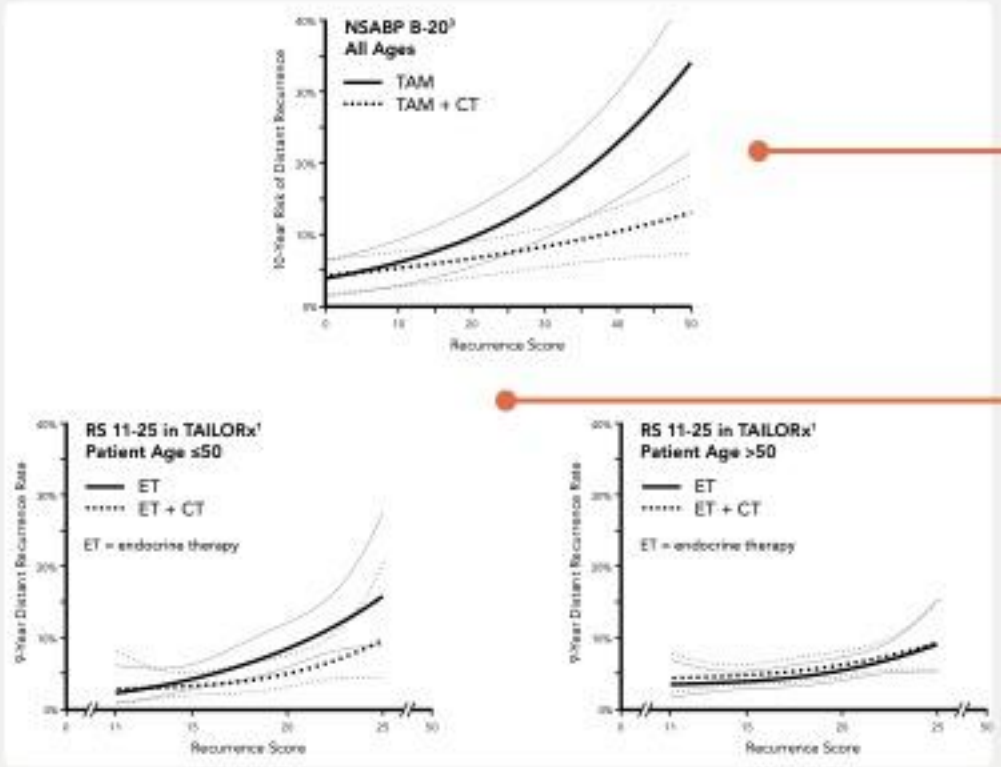
Sample Report

Oncotype DX Breast Recurrence Score® Report
Node Negative

EXACT SCIENCES

DOE, JANE ELIZABETH
Date of Birth: 01 - Jan - 1951 Gender: Female Report Number: OR123456789-01 Report Date: 13-July-2022
Specimen Source/ID: Breast/SP-16_0123456
Ordering Physician: Dr. First-Name I. Ordering-Physician-Last-Name

Estimated Chemotherapy Benefit for Individual Recurrence Score Results
second line for localization



Recurrence Score ranges shown above reflect randomized patients in NSABP B-20 and TAILORx.

PAGE 2 OF 2

The NSABP B-20 study randomized patients to treatment with TAM alone or TAM + CT and was the first validation study to demonstrate that the Breast Recurrence Score® test is predictive of chemotherapy benefit in node-negative patients.

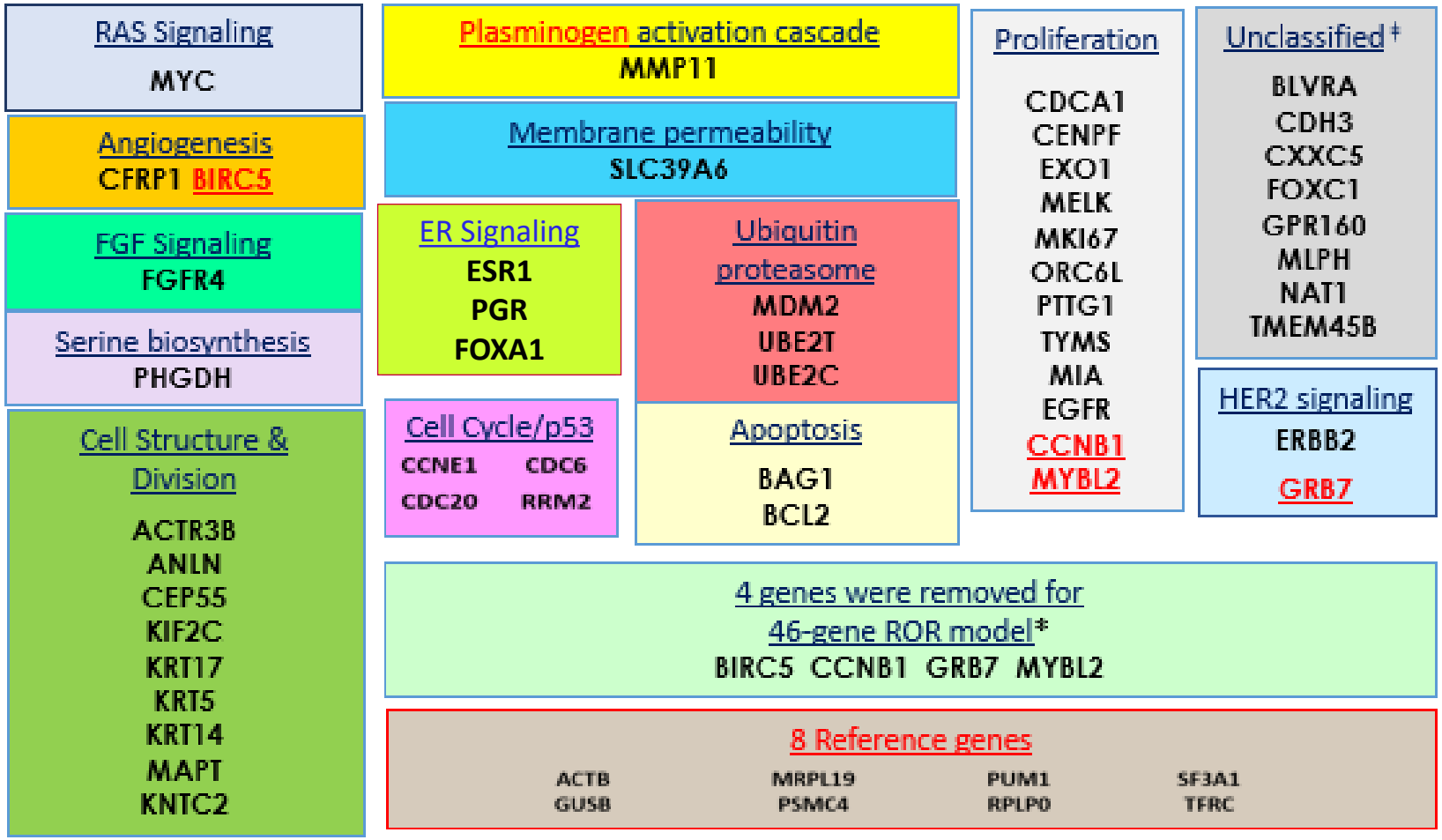
The TAILORx study is a prospective, randomized trial in which patients with Recurrence Score® results between 11-25 were randomly assigned to ET alone or CT + ET. Treatment with ET alone was found to be non-inferior to CT + ET across patients of all ages.

An exploratory subgroup analysis in patients ≤50 years of age showed increasing chemotherapy benefit with increasing Recurrence Score® result.

Molecular Profiling for HR+ Early Breast Cancer

- General principles and therapeutic approach
- Mammaprint Assay (Agendia)
- Oncotype Risk Score (Exact Sciences)
- Prosigna Assay (Veracyte)
- Summary and Future Directions

Prosigna Assay: extensively researched 50 genes (PAM50) used for determination of molecular subtypes



- Single tube hybridization
- Digital counting
- FDA cleared for consistent performance across assay sites
- Robustness allows an approved kit formulation
- Over 400 publications using PAM50 in PubMed

‡ Genes that are involved in many different pathways and it was difficult to classify them into a single pathway. Data on file.

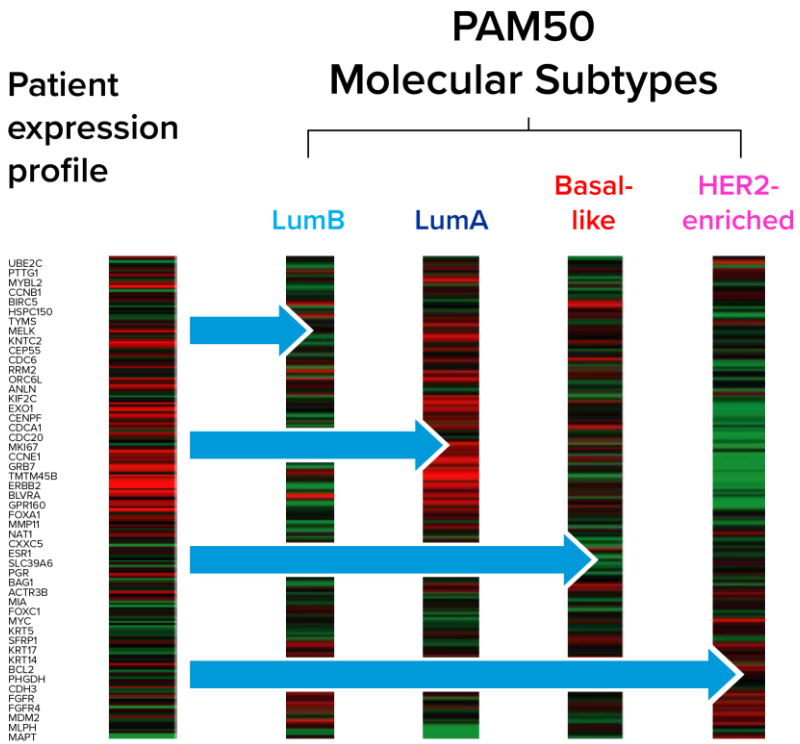
46 of the 50 genes used to calculate the Prosigna ROR Score

Prosigna incorporates clinical and pathological features for individualized risk assessment

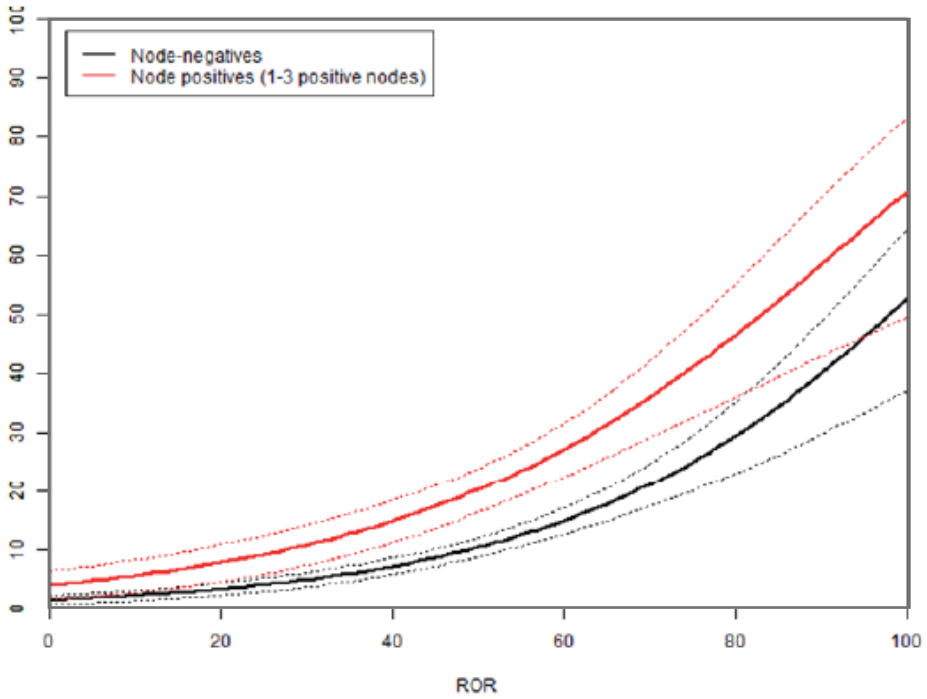
- Gene expression data weighted with clinical variables
- Scale of 1-100 related to probability of distant recurrence

Calculated correlation to PAM50 intrinsic subtypes
Add proliferation score and tumor size

Determine intrinsic (molecular) subtype



Estimate 10y Risk of Distant Recurrence on Endocrine Therapy Alone: N- and N+

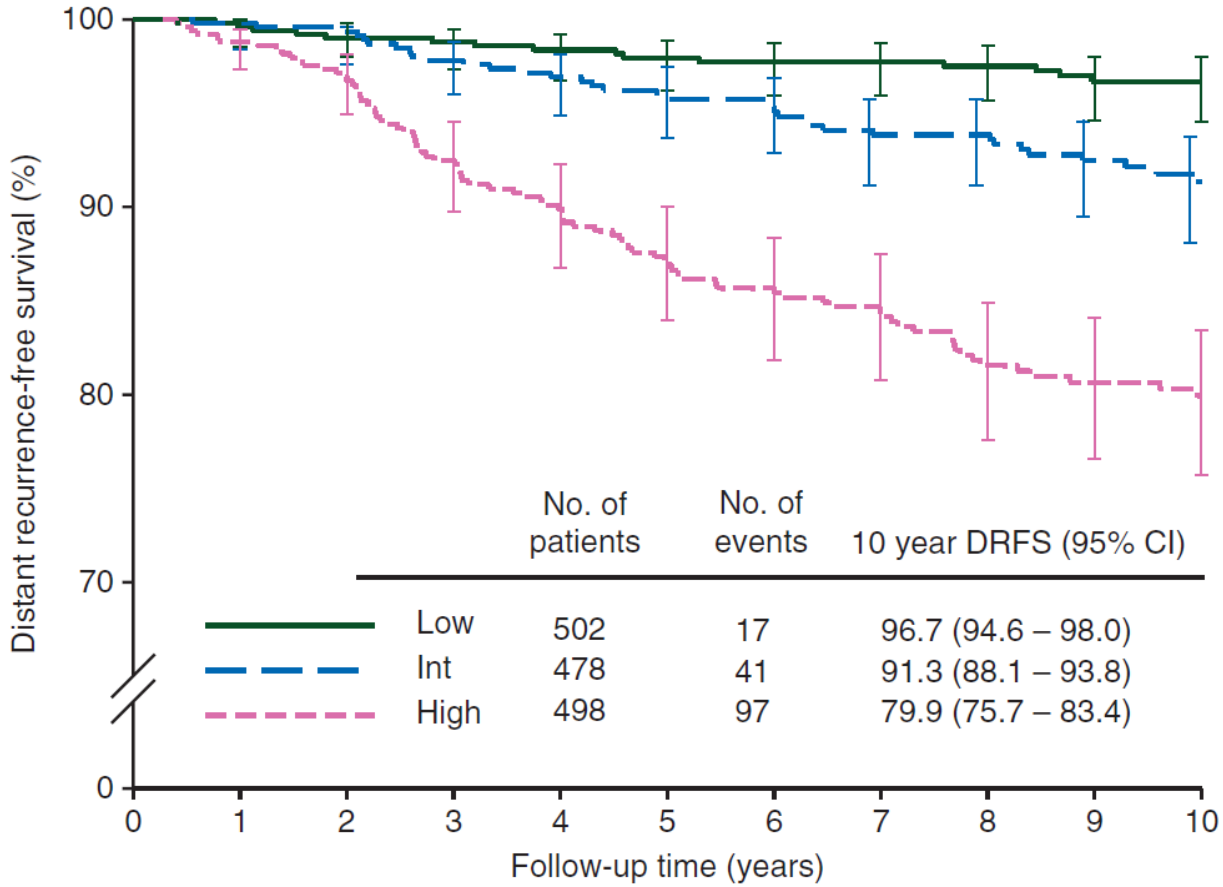


Adapted from Wallden et al BMC Medical Genomics 2015

ABCSG8 trial: Prosigna assay discriminates distinct recurrence risk

- **Cohort**
  - 1,047 node-negative
  - 431 node-positive patients
  - Postmenopausal
- **Methodology:** FDA consulted, prospective study with a Primary endpoint of distant recurrence free survival (DRFS)
- **Follow up:** 10.8 years median follow-up
- **The Prosigna Score adds prognostic information on DRFS**

Distribution of DRFS for all patients by Prosigna score: N = 1478 (95% CI)



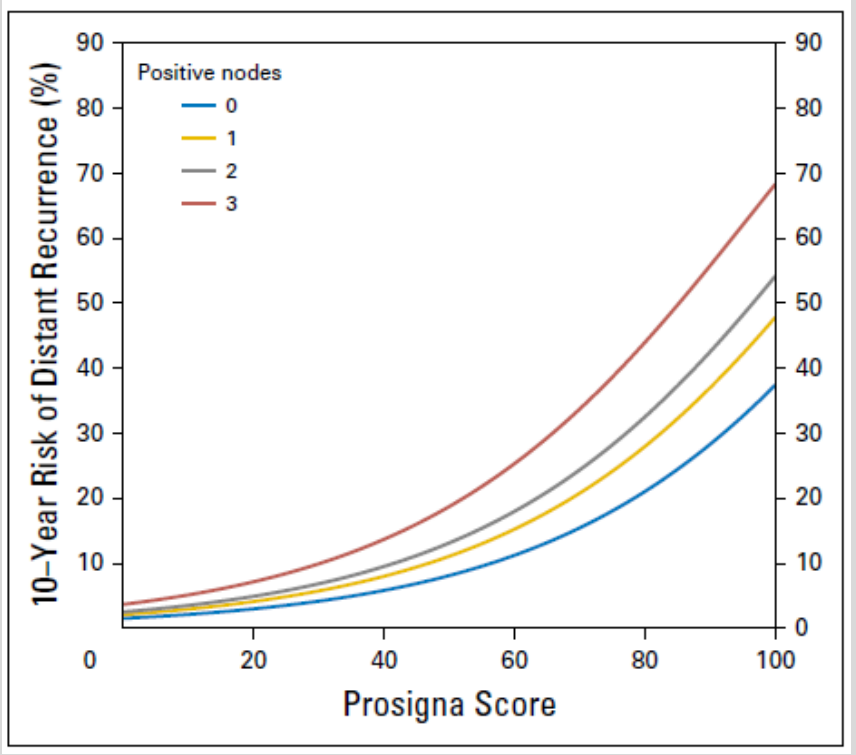
Gnant M et al Ann Oncol 2014

Population cohort trial of Prosigna's prognostic performance for DR at 10y

"At 10 years, patients with any nodal status (ie, 0-3 positive nodes) and a low Prosigna score who, without chemotherapy, were allocated to 5 years of adjuvant endocrine treatment had a DR rate of 4.3%."

- **Population-based Cohort**
  - 1,163 node-negative
  - 1,395 node-positive patients
  - Postmenopausal
- **Methodology:** Prospectively-defined analysis plan with primary endpoint of time to DR
- **Follow up:** 9.25-year median follow-up

Continuous relationship between the 10-year risk of DR and the Prosigna Score by number of positive nodes



DR by Prosigna Risk Group Separated by Nodal Status

Nodal Status	Risk Category	10-Year DR [95% CI]	P values	
			Any Difference	Difference from Intermediate
Node-Negative (n=1132)	High	17.8 [14.0-22.0]	<0.0001	<0.0001
	Intermediate	7.3 [4.8-10.6]		
	Low	**5.0 [2.9-8.0]**		0.1543
Node-Positive (1-3 nodes) (n=1358)	High	21.9 [18.9-25.1]	<0.0001	NA
	Low (ROR ≤ 40)	**4.8 [3.1-6.9]**		

Laenkholm et al J Clin Oncol 2018

Intrinsic Subtypes: Methodology Matters

PAM50- and immunohistochemistry-based subtypes of breast cancer and their relationship with breast cancer mortality in a population-based study (Long Island Breast Cancer Study)

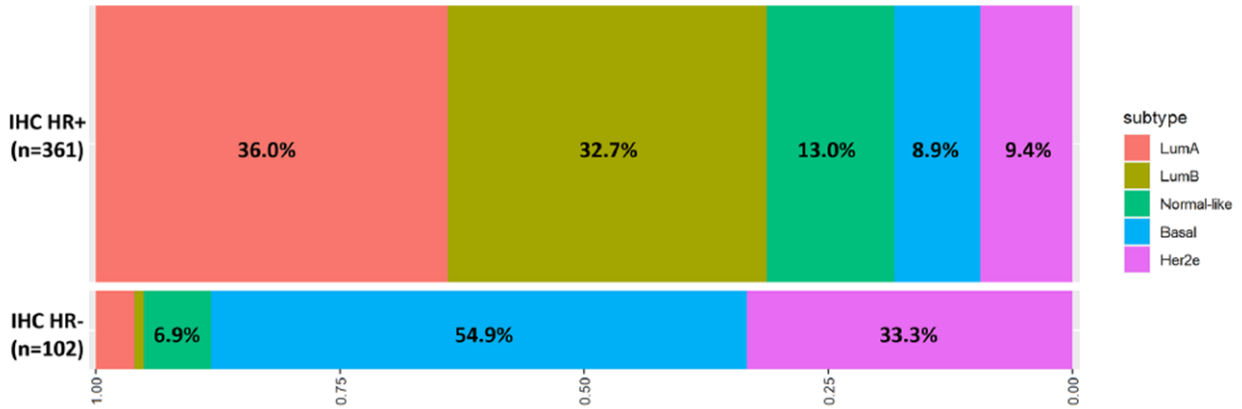
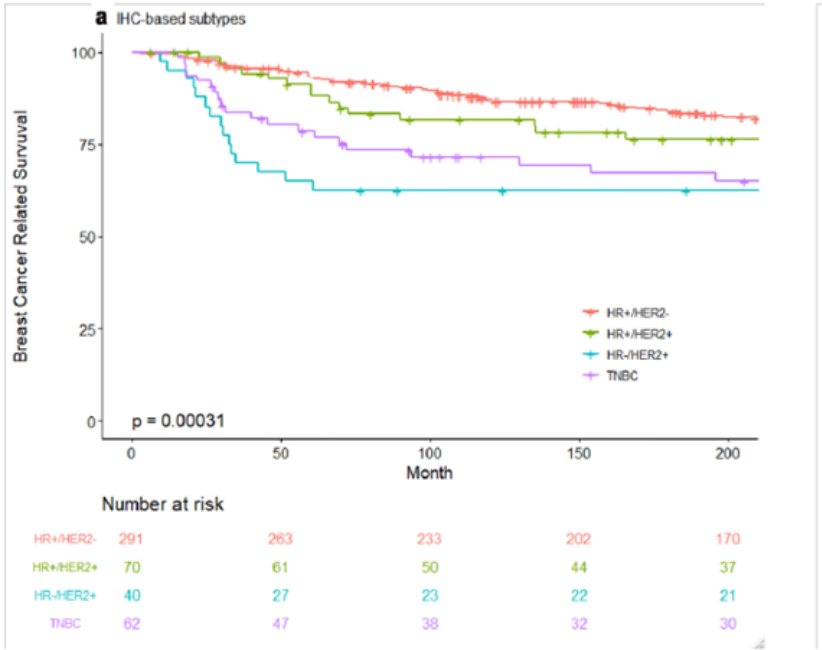
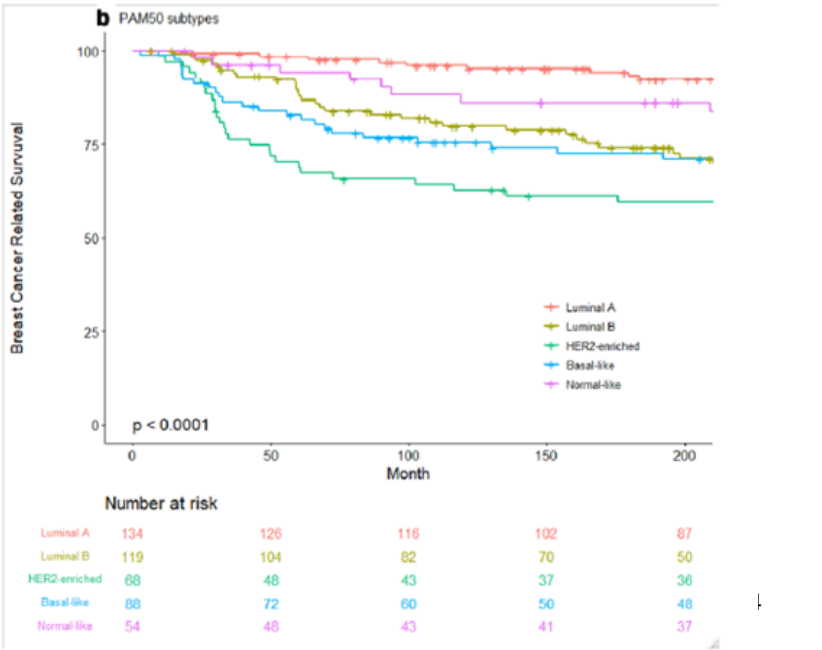


Fig. 2 Distribution of PAM50 subtypes by IHC-based HR status

Subtyping by nCounter based assay.





Wang et al Breast Cancer 2021

Patient	Specimen	Run Set ID: Prosigna Sample 2
Tumor Size: <= 2cm	**ID #:** n0-l2-70-HR-LB	**Comments:** Comment for n0-l2-70-HR-LB
Lymph Nodes: node-negative	**Date Reported:** September 20, 2017	

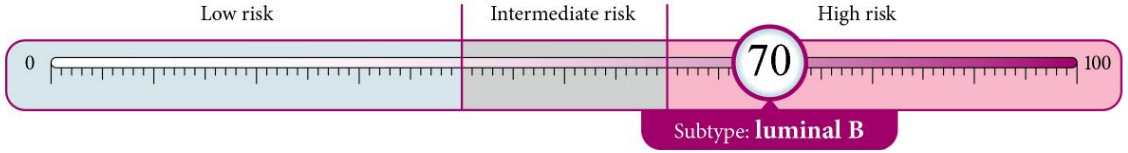
Prosigna®

Breast Cancer Assay

ID #: n0-l2-70-HR-LB **Tumor Size:** <= 2cm **Lymph Nodes:** node-negative

Assay Description: The Prosigna® breast cancer gene signature assay measures the expression of 50 different genes to identify subtype and report a Risk of Recurrence Score (ROR), which is used to assign the patient to a predefined risk group. These results are derived from a proprietary algorithm based on the PAM50 gene signature, intrinsic subtype, and clinical variables including tumor size and nodal status.

Risk of Recurrence*:

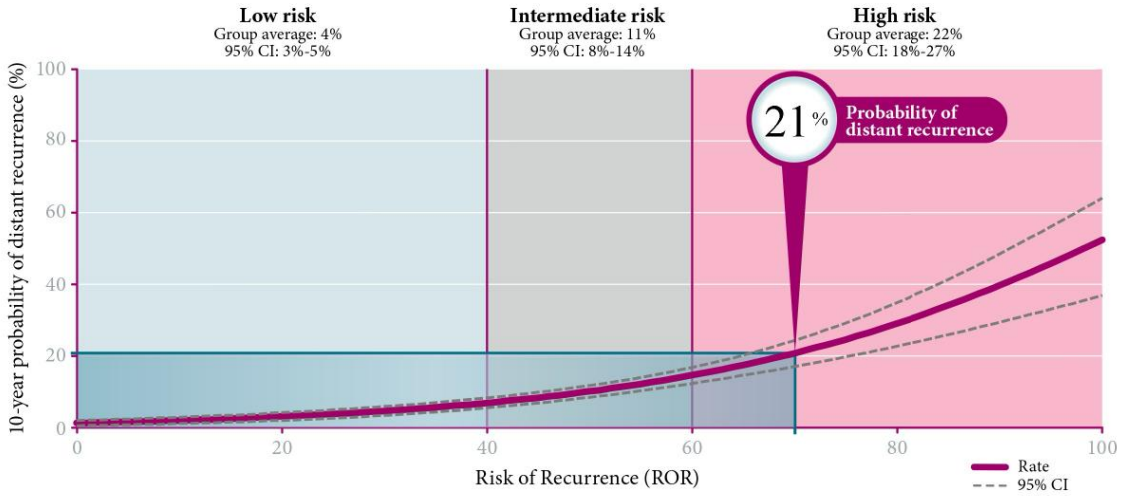


* The ROR ranges from 0 through 100 and correlates with the probability of distant recurrence (DR) in the tested patient population. The risk classification is provided to guide the interpretation of the ROR using cutoffs related to clinical outcome.

Probability of Distant Recurrence:

In the clinical validation studies, patients who were node-negative, luminal B subtype, with an ROR score of 70 were in the high-risk group. This group averaged a 22% probability of distant recurrence at 10 years.

The Prosigna® algorithm has been validated by 2 random ized clinical trials including more than 2400 patients with varying rates of distant recurrence. An analysis of these 2 clinical validation studies shows that the probability of distant recurrence for the high-risk population is 22%.†



For more information, visit PROSIGNA.com or e-mail info@prosigna.com

†Data apply to patients being treated with hormone therapy for 5 years as in the tested patient population. See Package Insert for further information on therapy regimens and teste d patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

Patient	Specimen	Run Set ID: Prosigna Sample 2
Tumor Size: <= 2cm	**ID #:** n0-l2-70-HR-LB	**Comments:** Comment for n0-l2-70-HR-LB
Lymph Nodes: node-negative	**Date Reported:** September 20, 2017	

Prosigna
BREAST CANCER ASSAY

ID #: n0-l2-70-HR-LB **Tumor Size:** <= 2cm **Lymph Nodes:** node-negative

Clinical Validation Studies: Prognosis for node-negative, luminal B, high-risk breast cancer patients was determined based on the rate of distant recurrence (DR) of this population in 2 prospective-retrospective clinical studies. These studies analyzed more than 2400 samples from postmenopausal women with early stage, hormone receptor-positive breast cancer, using a prospectively defined analysis plan. The data shown are for postmenopausal women with early stage, hormone receptor-positive breast cancer who received 5 years of endocrine therapy after surgical resection of the primary tumor.

Rate of Distant Recurrence (DR) for Node-Negative Patients

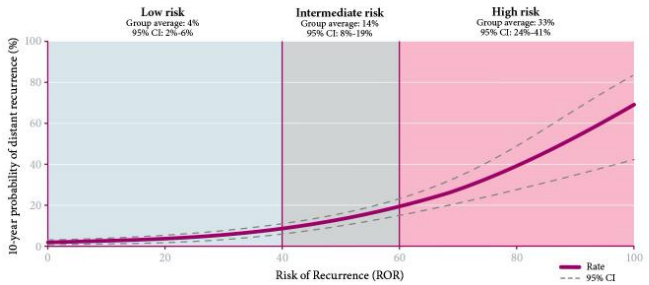
Subtype	Luminal A [95% CI]	Luminal B [95% CI]	HER2-enriched	Basal-like
Rate of DR	**5%** [4%-7%]	**18%** [15%-22%]	*	*

*There were insufficient numbers of basal-like and HER2-enriched patients in these studies to produce data.

Subtype and Prognosis:

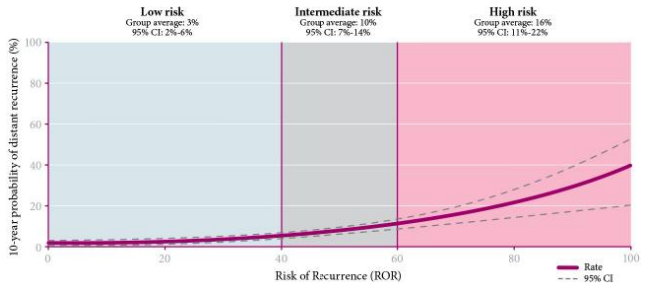
Intrinsic subtype is related to prognosis in the tested patient population. The most common subtypes of breast cancer are the luminal subtypes: luminal A and luminal B. In the combined analysis of 2 clinical validation studies of hormone receptor-positive patients, 68% of the tested patient population was found to be luminal A, and 27% was luminal B.[1] The gene expression pattern of these subtypes resembles the luminal epithelial component of the breast.[3] These tumors are characterized by high expression of estrogen receptor (ER), progesterone receptor (PR), and genes associated with ER activation.[3] Luminal A breast cancers exhibit low expression of genes associated with cell cycle activation and generally have a better prognosis than luminal B.

TransATAC clinical validation study[1]:



The TransATAC study analyzed 1007 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

ABCSG-8 clinical validation study[2]:



The ABCSG-8 study analyzed 1478 samples using a prospectively defined analysis plan. Data shown are for postmenopausal stage I or II, node-negative, hormone receptor-positive breast cancer patients that received 5 years of endocrine therapy.*

For more information, visit PROSIGNA.com or e-mail info@prosigna.com

*See Package Insert for further information on therapy regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules.

REFERENCES:

1. Dowsett M, Lopez-Knowles E, Sidhu K, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: A TransATAC Study. Program and abstracts of the 34th Annual San Antonio Breast Cancer Symposium; December 6-10, 2011; San Antonio, Texas. Abstract S4-5.

2. Gnant M, et al., P2-10-02, Clinical Validation of the PAM50 risk of recurrence (ROR) score for predicting residual risk of distant-recurrence (DR) after endocrine therapy in postmenopausal women with HR+ early breast cancer (EBC): An ABCSG study, SABCS 2012.

3. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27(8):1160-1167

OPTIMA Study Design

optima@warwick.ac.uk

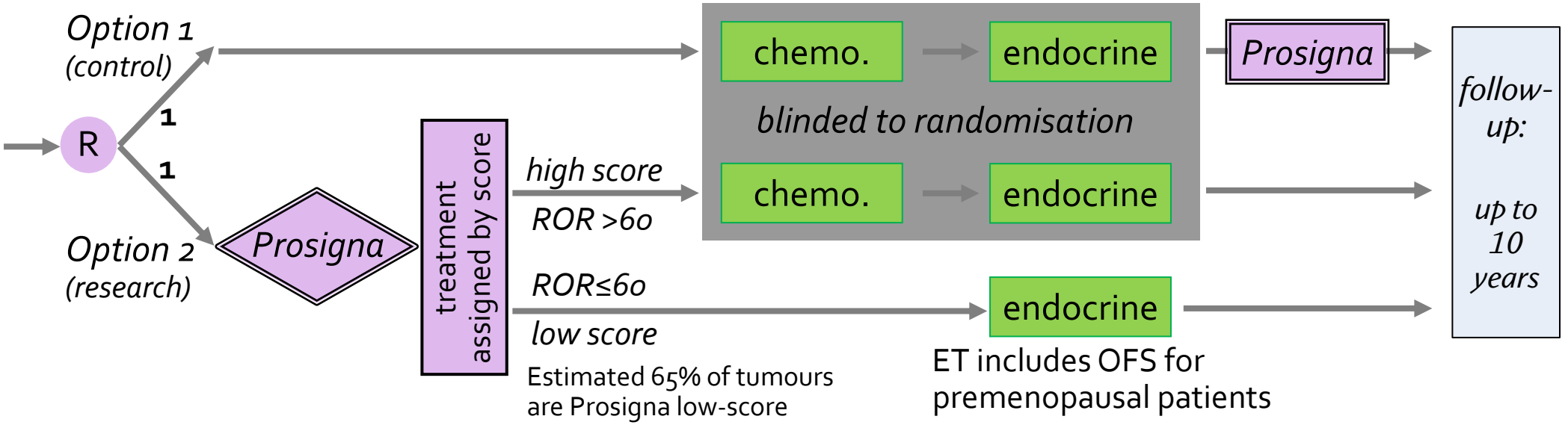
optima
personalised treatment of breast cancer

Main eligibility criteria

- Women or Men age ≥40
- "Adequate surgery"; ANC not mandatory
- ER-pos (>10%) HER2-neg (local lab)
- Size & nodes: one of the following
  - pN0 & pT≥30mm; pN1mi & pT≥20mm; pN1-2
- Multiple ipsilateral and "non-significant" contralateral tumours permitted

Main exclusion criteria

- Advanced stage – pN3/ IM node involvement
- Neoadjuvant chemotherapy
- Previous IBC; locally treated DCIS permitted



Efficacy analysis uses non-inferiority design | Health economics analysis | Sample size = 4,500 (+ 412 OPTIMA prelim)

Molecular Profiling for HR+ Early Breast Cancer

- General principles and therapeutic approach
- Mammaprint Assay (Agendia)
- Oncotype Risk Score (Exact Sciences)
- Prosigna Assay (Veracyte)
- Summary and Future Directions

Molecular Profiling for HR+ Early Breast Cancer

- Breast cancer is the most common malignancy in woman; HR+/HER2- is the most common subtype
- Foundational approaches to curative intent: local + systemic therapy
- Antiestrogen therapy is critical to reducing long-term local/distant recurrence risk

- Genomic risk assays help provide further insights and personalized care
- Prognostic: likelihood of distant relapse
- Predictive: impact of chemotherapy (in addition to antiestrogen treatment)

- Further work is important and ongoing:
- How to optimize care for premenopausal patients with node+ disease?
- Impact of emerging assays (eg. minimum residual disease/MRD assays)?

Thank You!