日本乳がん情報ネットワーク

Japan Comprehensive Cancer Network, Breast (JCCNB)

Luminal A and B
Where are we?

(or lost in translation?)

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NKI-AVL

How to determine adjuvant or neo-adjuvant treatment for Luminal A or Luminal B cancers? (as a surgeon)

The Netherlands Cancer Institute
Antoni Van Leeuwenhoek Hospital
Amsterdam

NKI-AVL

Disclosures

- No financial interest in a pharmaceutical or diagnostic company
- Not a member of speakers bureau or advisory board
- I’m a surgical oncologist/breast cancer specialist with specific interest in translational research

NKI-AVL

And some about us

The Netherlands Cancer Institute

The clinical issue

- The prognosis of the patient.
- What is Luminal A?
- What is Luminal B?
- Or how to distinguish Luminal A vs B
- How to treat pts with Luminal A vs Luminal B cancers?

  Should treatment for Luminal A cancers be different from Luminal B cancers

Or….

Is it simpler?

- ER strong +ve & low risk: HT?
- ER +ve plus risk factor: HT + chemotherapy?

Where it all started….

Molecular ('intrinsic') subtypes

letters to nature

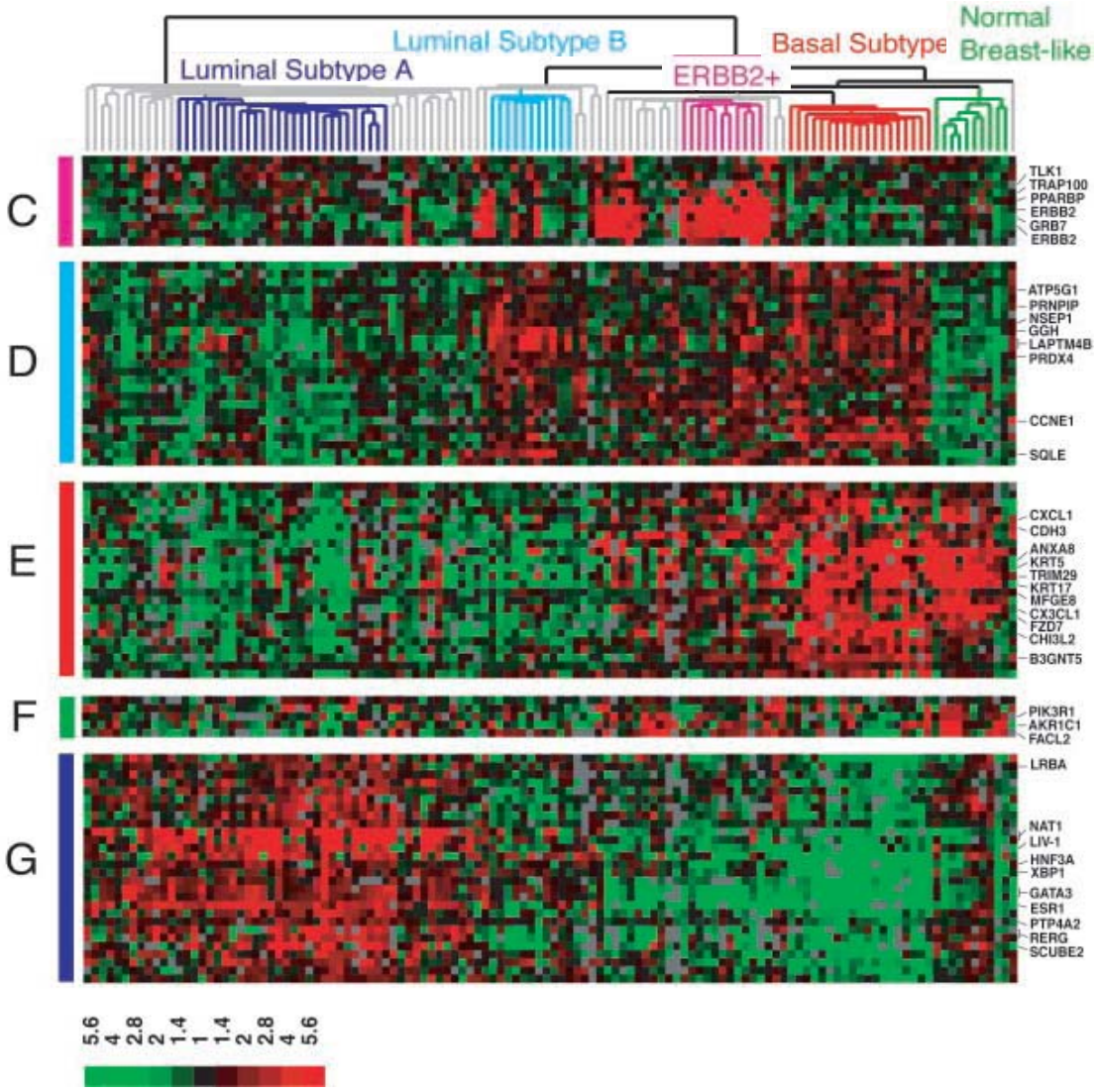
Molecular portraits of human breast tumours

Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning*†, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*

- Specimens from 65 tumors from 42 patients

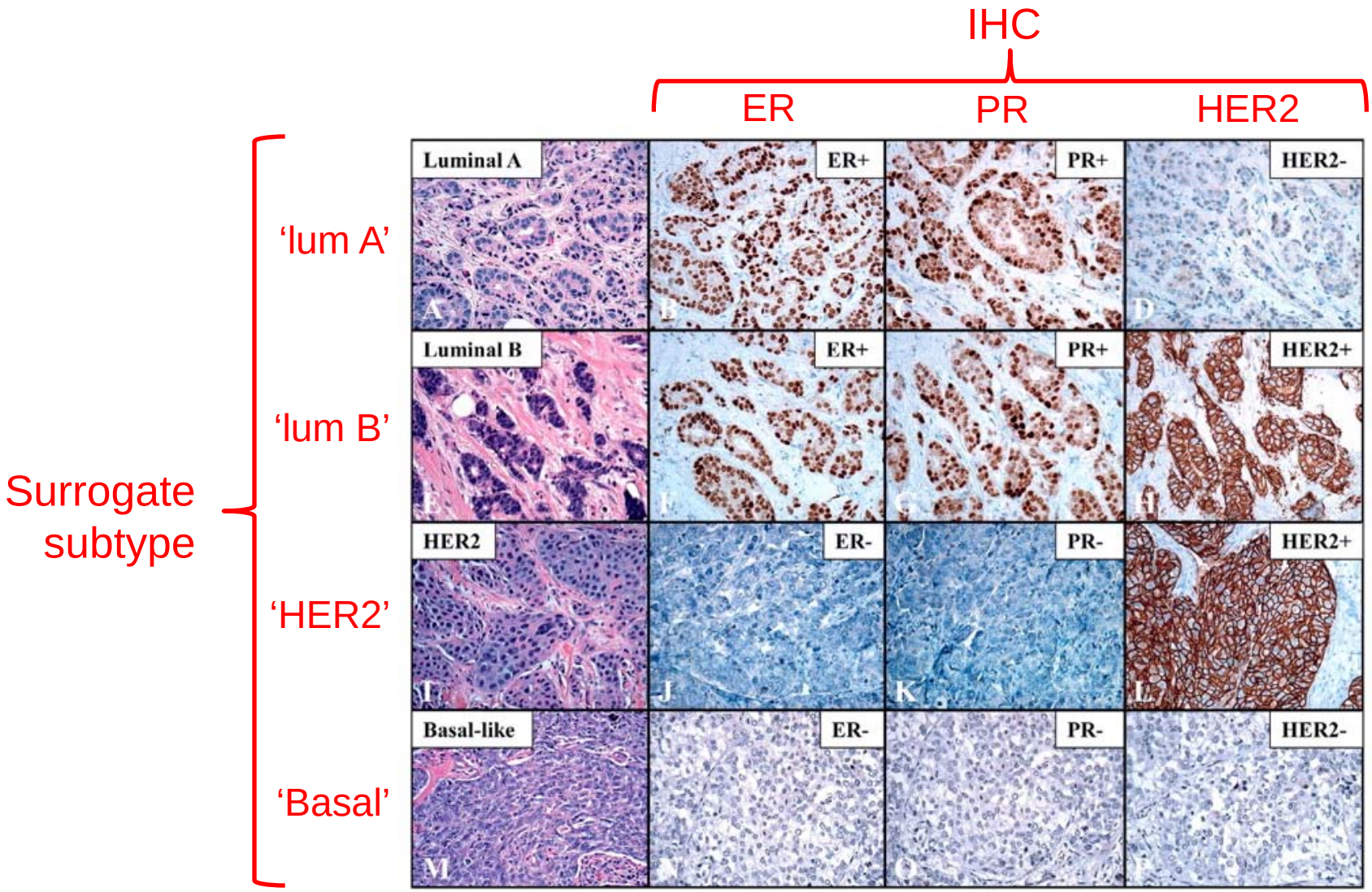
Perou et al., Nature (2000) 406:747

Molecular ('intrinsic') subtypes



Sørlie et al., PNAS (2003) 100:8418

Immunohistochemistry ('surrogate subtypes')



Molecular Subtypes provide insight on which therapies to select (St. Gallen, May 2011)

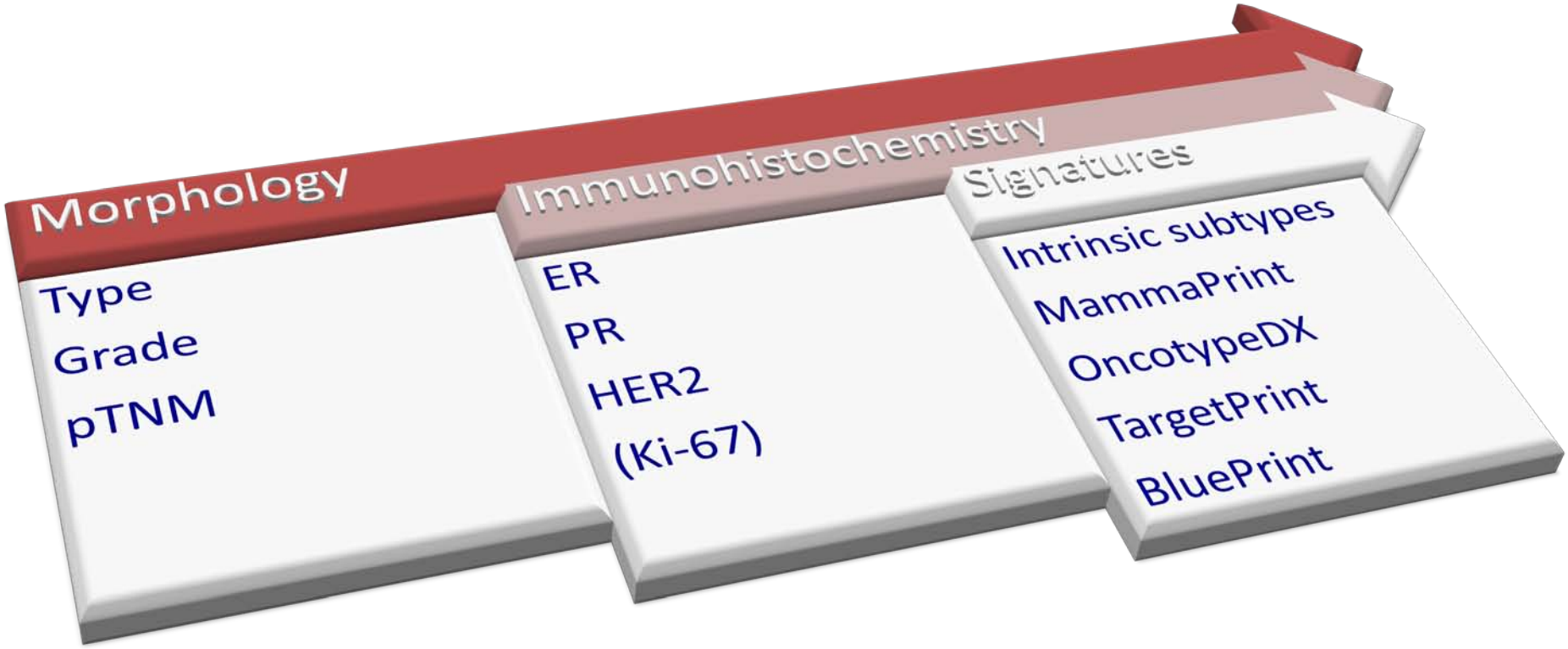
Table 2 | 2011 St Gallen consensus recommendations of systemic treatment[4]

IHC Subtype	Definition	Type of adjuvant therapy
Luminal A	HR+/HER2–/Ki67low	Endocrine therapy alone*
Luminal B	HR+/HER2–/Ki67high	Endocrine therapy ± cytotoxic therapy
Luminal B	HR+/HER2+	Cytotoxics + anti-HER2 + hormonal therapy
HER2-positive	HR–/HER2+	Cytotoxics + anti-HER2 therapy
Triple-negative	HR–/HER2–	Cytotoxic therapy

*A few patients require cytotoxics (such as high nodal status or other indicator of risk). Abbreviation: HR, hormone receptor.

Classification of breast cancer

Potentials, limitations, challenges



The clinical issue.
Think step by step

Step 1: The very low risk cancer: is chemotherapy indicated anyway?

Step 2: Is ER+ve really ER +ve? (or: do you trust your specimen work up system?)

Step 3: If ER +ve is reliably proven, and there is some risk of relapse: adjuvant anti estrogen treatment

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)

The clinical issue.
Think step by step

Step 1:

Is prognosis so good that survival advantage of adjuvant chemotherapy outweighs the disadvantages & serious late side effects?

Clinical determinate cases

High Risk	Low Risk
ER negative	ER positive
Lymph Node positive	Lymph Node negative
HER2 positive	HER2 negative
Grade III	Grade I
Larger tumor size	Small tumor size

Half of our patients are somewhere in between!

What to do?

Interobserver agreement morphology and IHC

- Kappa statistics local vs. central assessment
  - Tumor type 0.56
  - Grade 0.50
  - ER 0.85
  - PR 0.72
  - HER2 0.81

Degree of agreement:

0.00-0.20	slight
0.21-0.40	fair
0.41-0.60	moderate
0.61-0.80	substantial
0.81-1.00	(almost) perfect

Bueno-de Mesquita et al.; Annals Oncol. (2010) 21: 40-44

Is determinate always determinate?

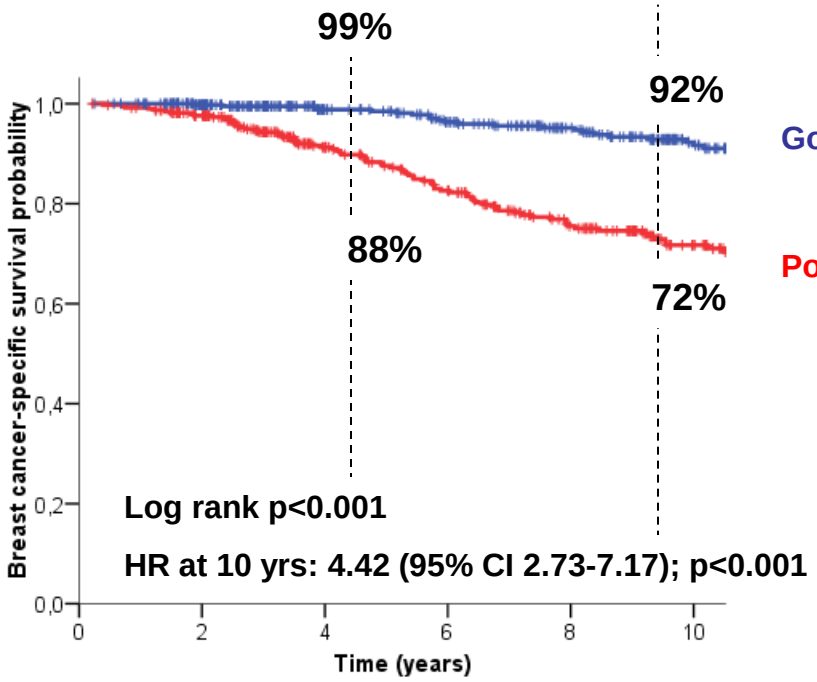
Some examples

- Small cancers good prognosis?
- Grade 1 good prognosis?

➢ There is an important and reproducible discordance between clinical-pathological risk estimates compared to newer techniques by tumor profiling

MammaPrint and Tumorsize T1c BCSS

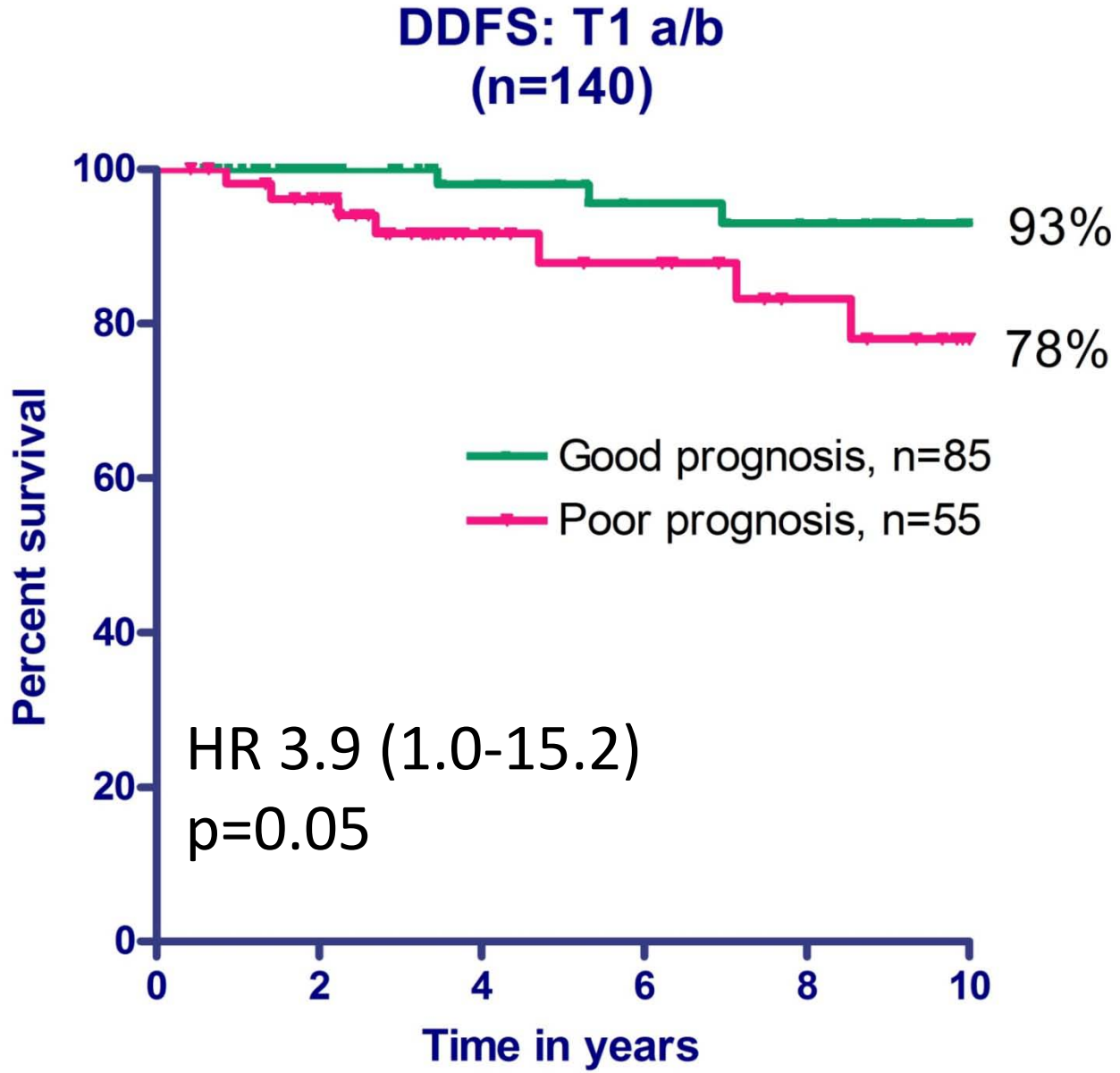
11 – 22 mm Tumors



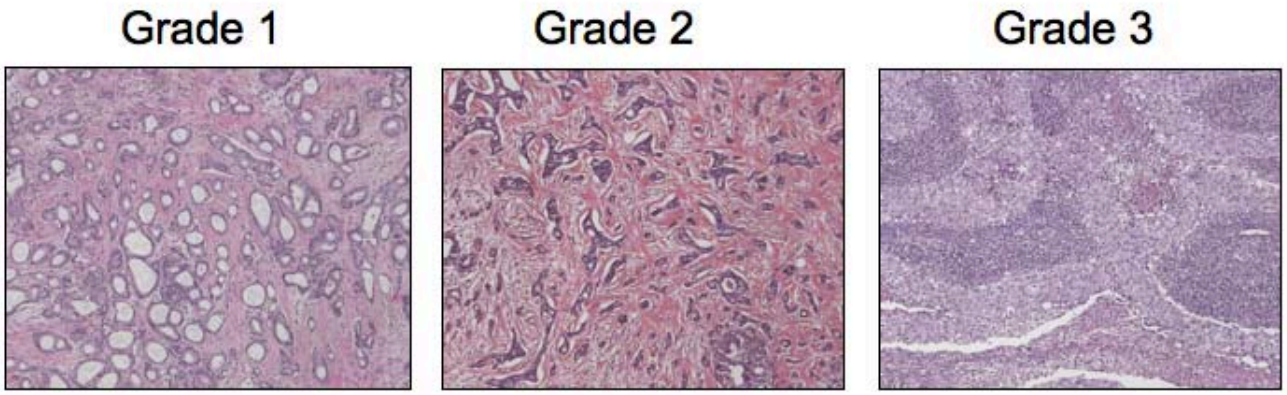
Good	441	392	294	261	218	154
Poor	384	350	256	215	166	114

T1c tumors derived from pooled database of all MammaPrint validation studies (all, n=1696)

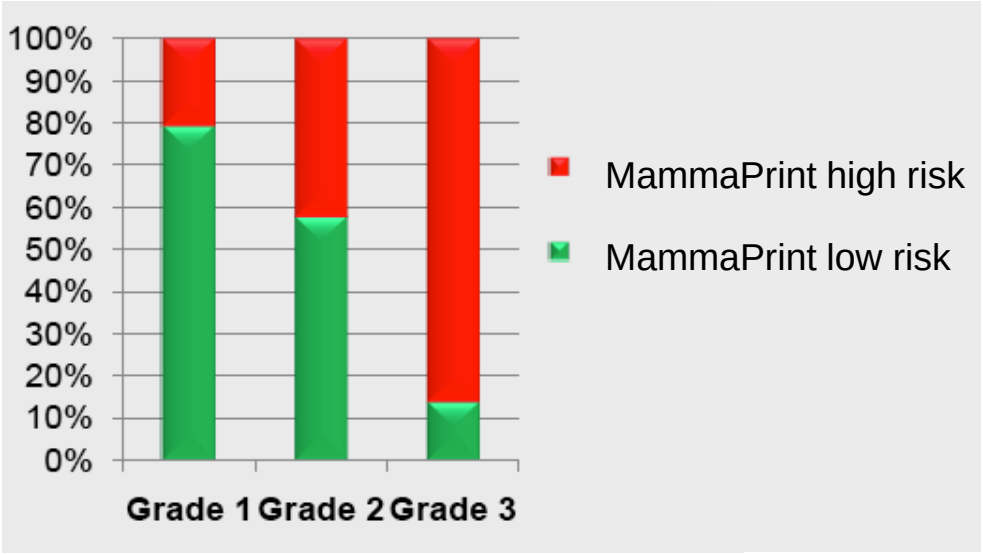
Mook et al, Ann Surg Oncol, 2010


DDFS: T1 a/b
(n=140)
100
80
60
40
20
0
Percent survival
93%
78%
Good prognosis, n=85
Poor prognosis, n=55
HR 3.9 (1.0-15.2)
p=0.05
0
2
4
6
8
10
Time in years

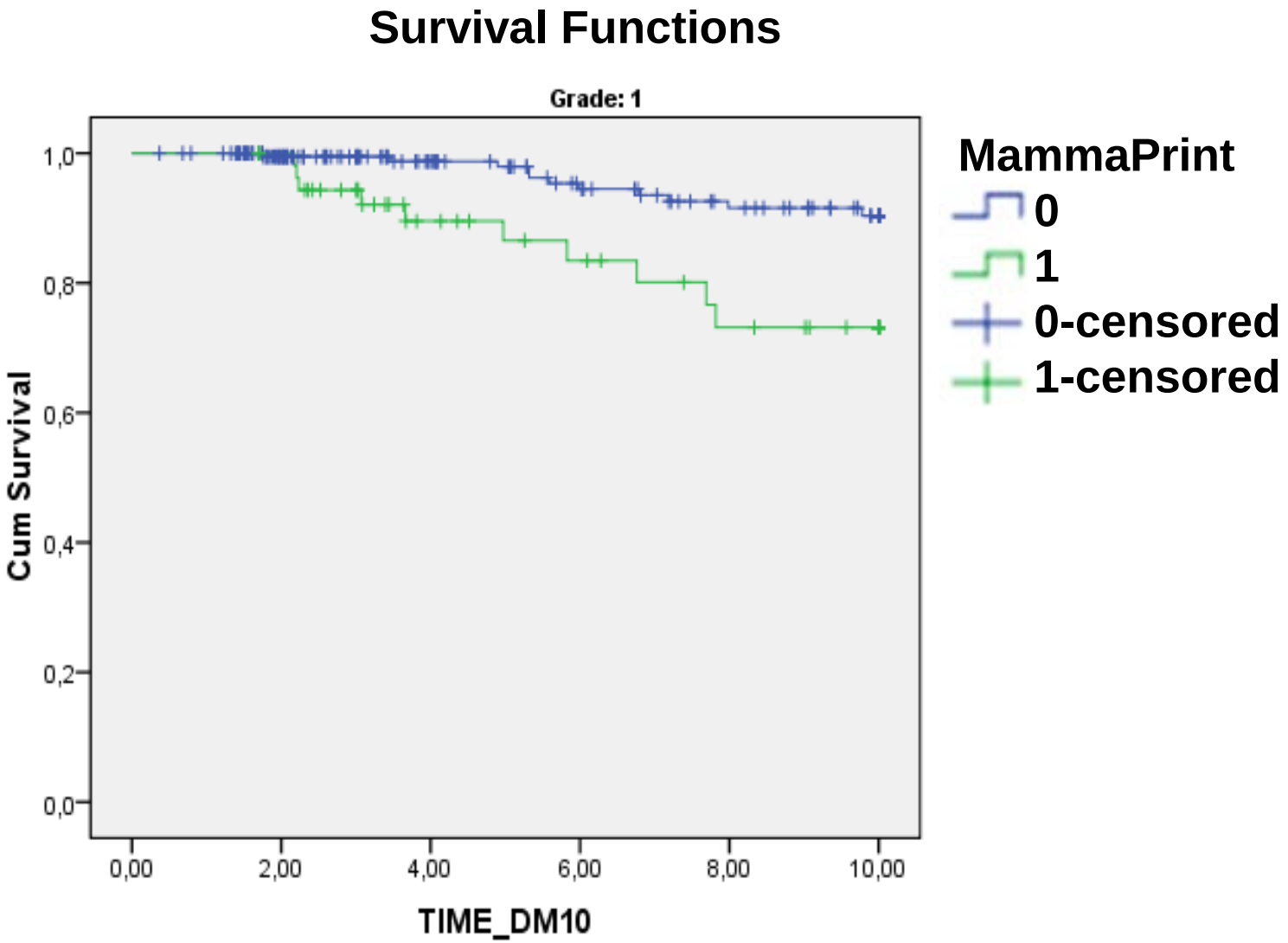
MammaPrint adds to grading of breast cancer



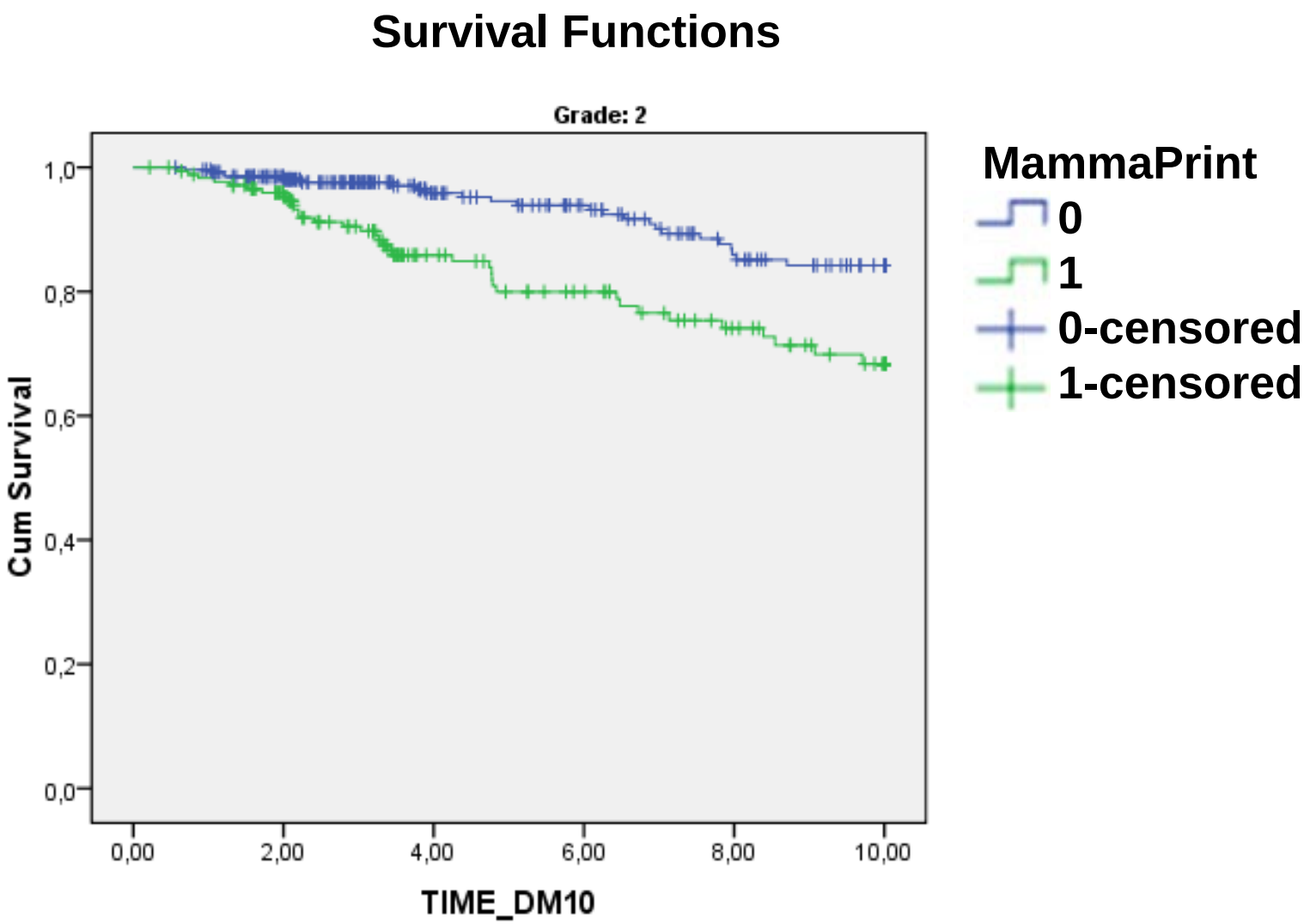
764 of 1630 patients (47%) were classified as good prognosis and 866 (53%) as poor prognosis by MammaPrint
Histological grading was centrally reviewed for all patients



DDFS N -ve


Survival Functions
Grade: 1
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0,00
2,00
4,00
6,00
8,00
10,00
TIME_DM10
MammaPrint
0
1
0-censored
1-censored

DDFS N-ve


Survival Functions
Grade: 2
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0,00
2,00
4,00
6,00
8,00
10,00
TIME_DM10
MammaPrint
0
1
0-censored
1-censored

Patient risk allocation

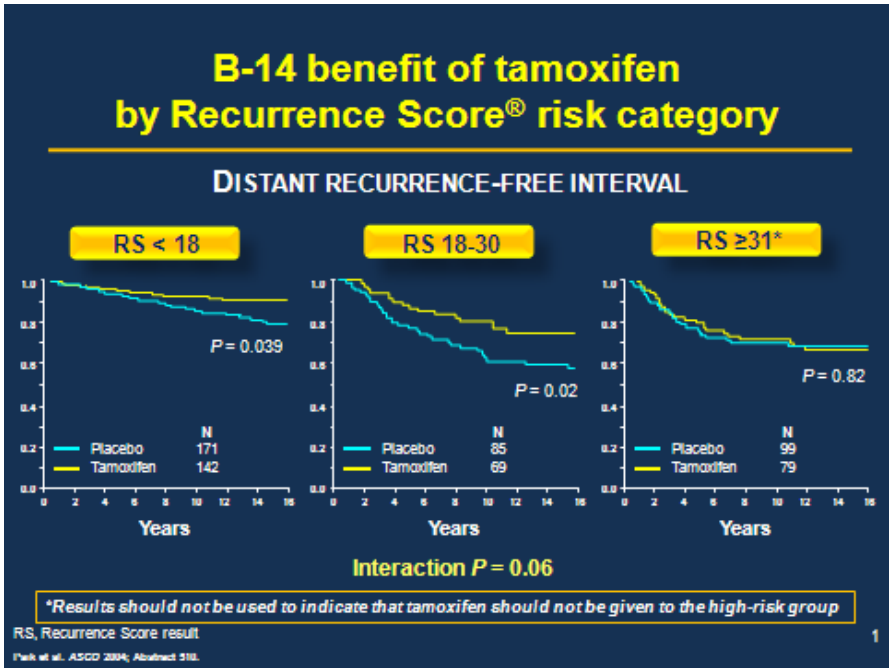
Clin-path risk and 70-gene risk at enrollment		Clinical-pathological risk		Total
		LOW N(%)	HIGH N(%)	
70-gene risk	LOW	2586 (40)	1436 (22)	4022 (62)
	HIGH	678 (10)	1827 (28)	2505 (38)
Total		3264 (50)*	3263 (50)*	N=6527

Discordant cases (10 + 22 = 32%) match protocol hypothesis

The absolute difference between C-HIGH / G-LOW and C-LOW / G-HIGH is 11.6%

*The 50-50 split is coincidental

Oncotype DX and low risk



B-14 Data NSABP	Untreated Population	Treated Population
Breast Cancer Mortality	(95%CI, 355 pat)	(85%CI) (290)
Low Risk (RS<18) (313 pat)	**14.1%** (19.5%, 8.64%) (171 pat)	**6.9%** (11.2%, 2.5%) (142 pat)
Int Risk (RS 18-30) (154 pat)	37.8% (48.9%, 26.8%) (85 pat)	20.5% (30.4%, 10.5%) (69 pat)
High Risk (RS≥31) (178 pat)	31.3% (40.9%, 21.8%) (99 pat)	29.7% (40.2%, 19.3%) (79 pat)

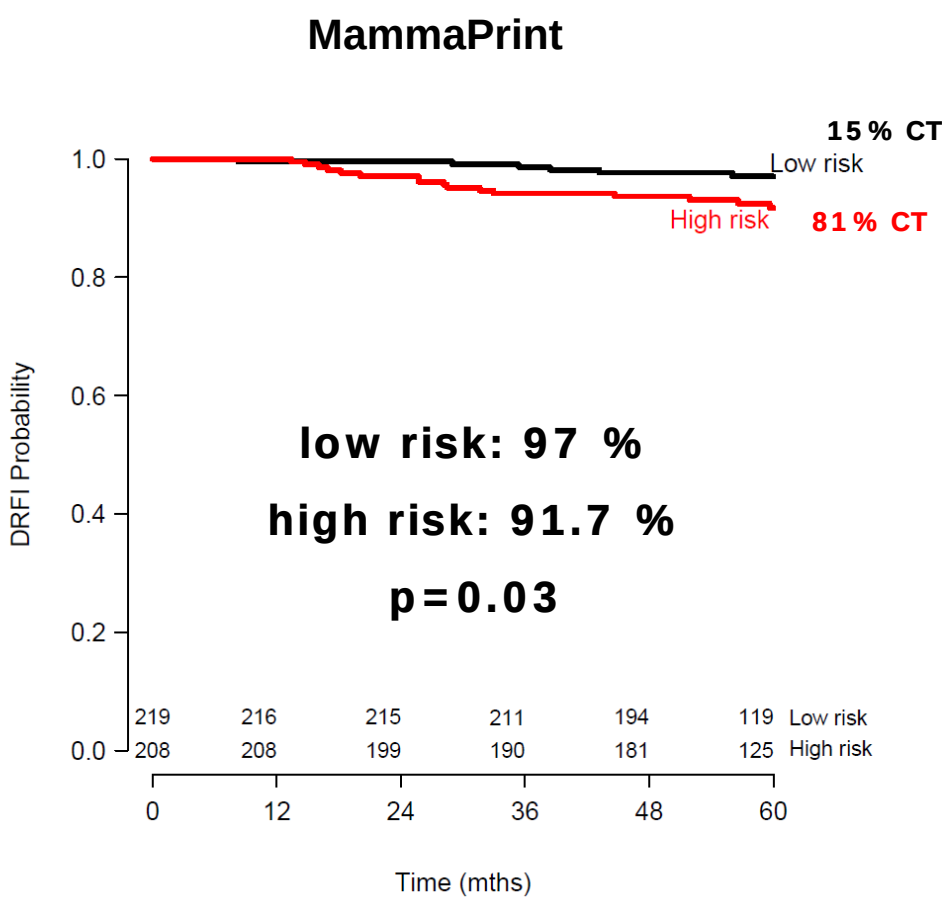
RASTER

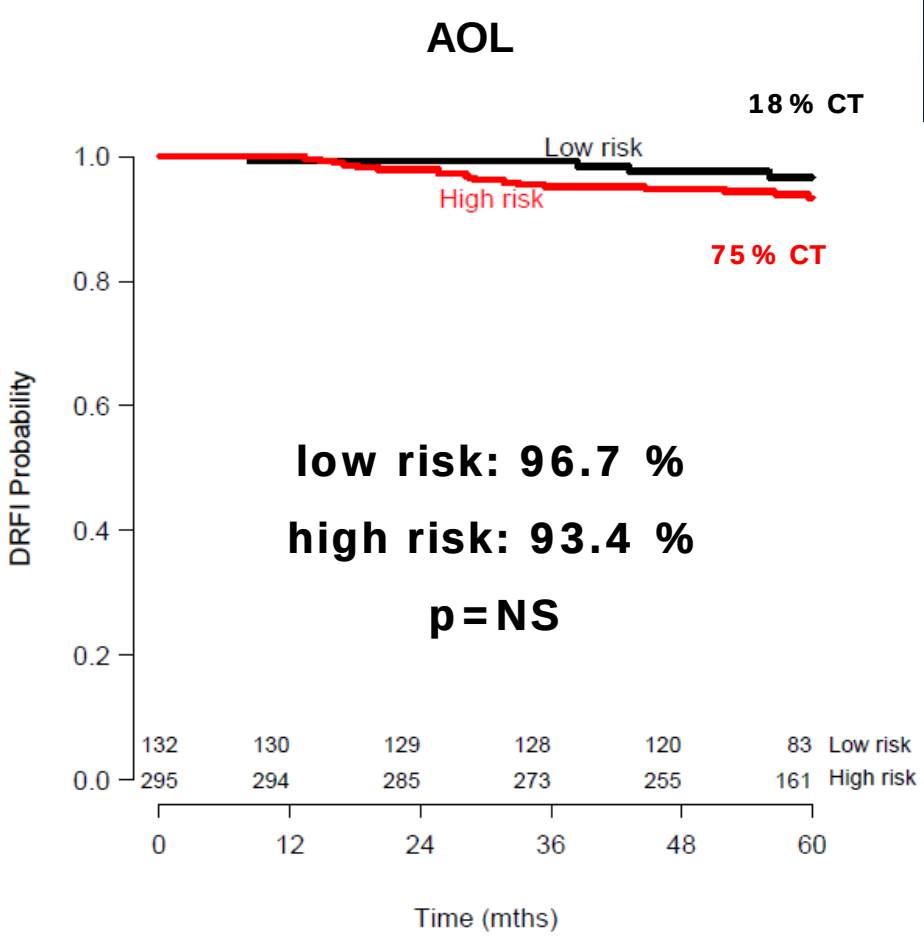
MICRO-ARRAY PROGNOSTIEK IN BORSTKANKER

70-Gene signature (MammaPrint) prospectively predicts prognosis of patients with node-negative breast cancer: 5 year follow-up of the RASTER study

S.C. Linn, C.A. Drukker, V.P. Retèl, J.M. Bueno-de-Mesquita, W.H. van Harten, H. van Tinteren, J. Wesseling, L.J. van ’t Veer, E.J.T. Rutgers, M.J. van de Vijver

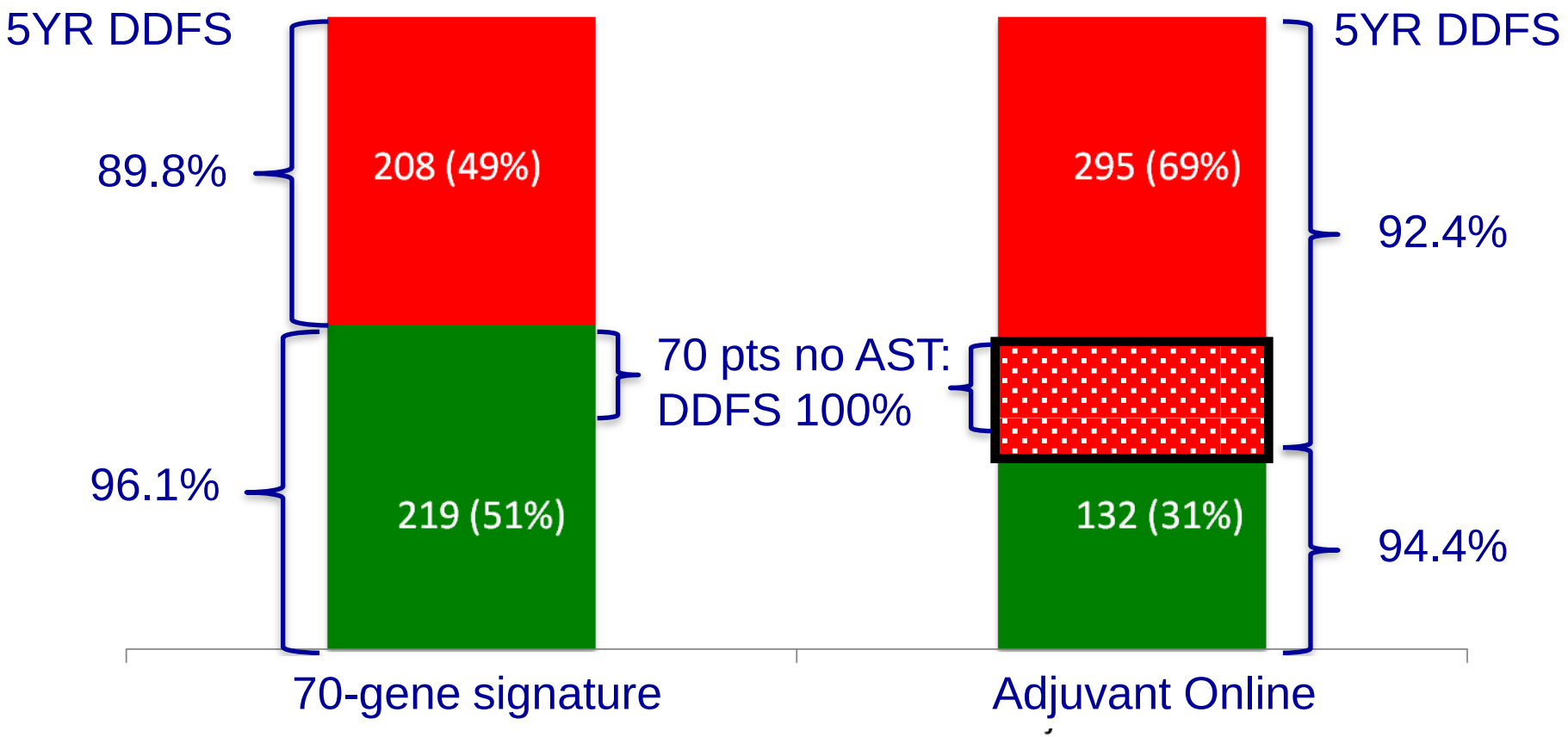
5-year distant recurrence-free interval





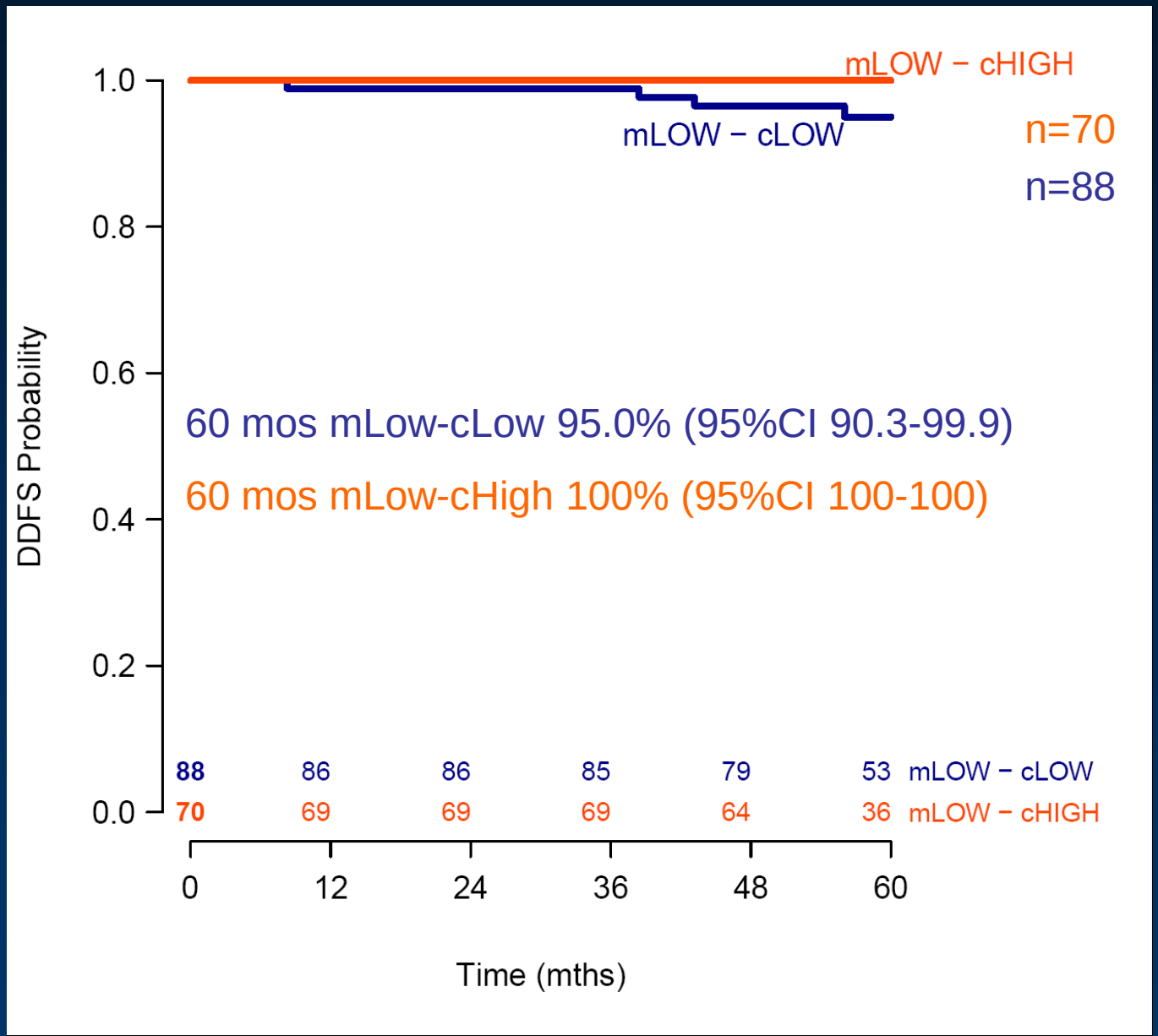
MammaPrint in observational prospective trial

RASTER study, 5-year DDFS of 427 patients according to 70GPS or AOL

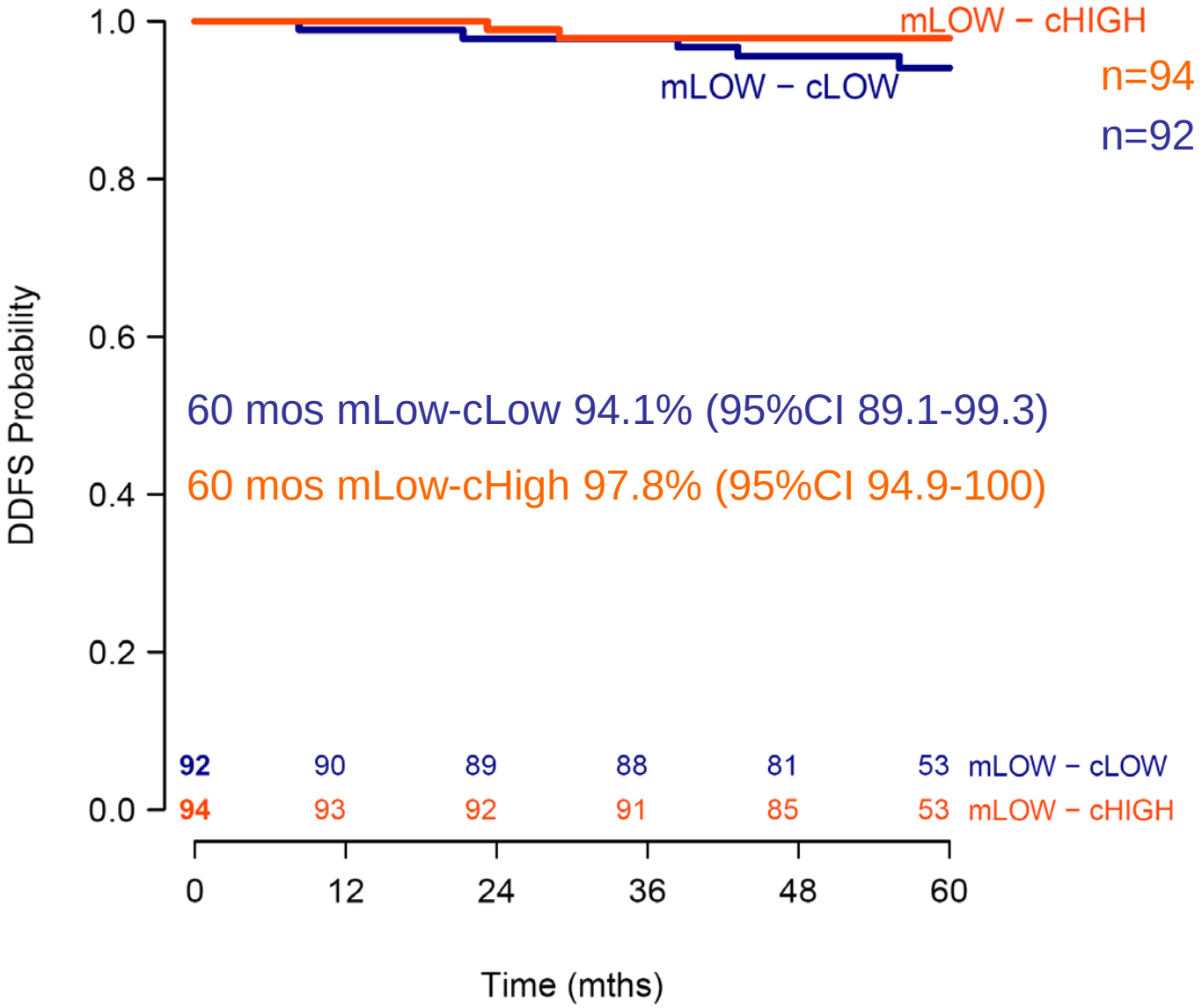


Linn, Rutgers, Drukker, et al., EBCC 2012

Discordant cases who received no AST

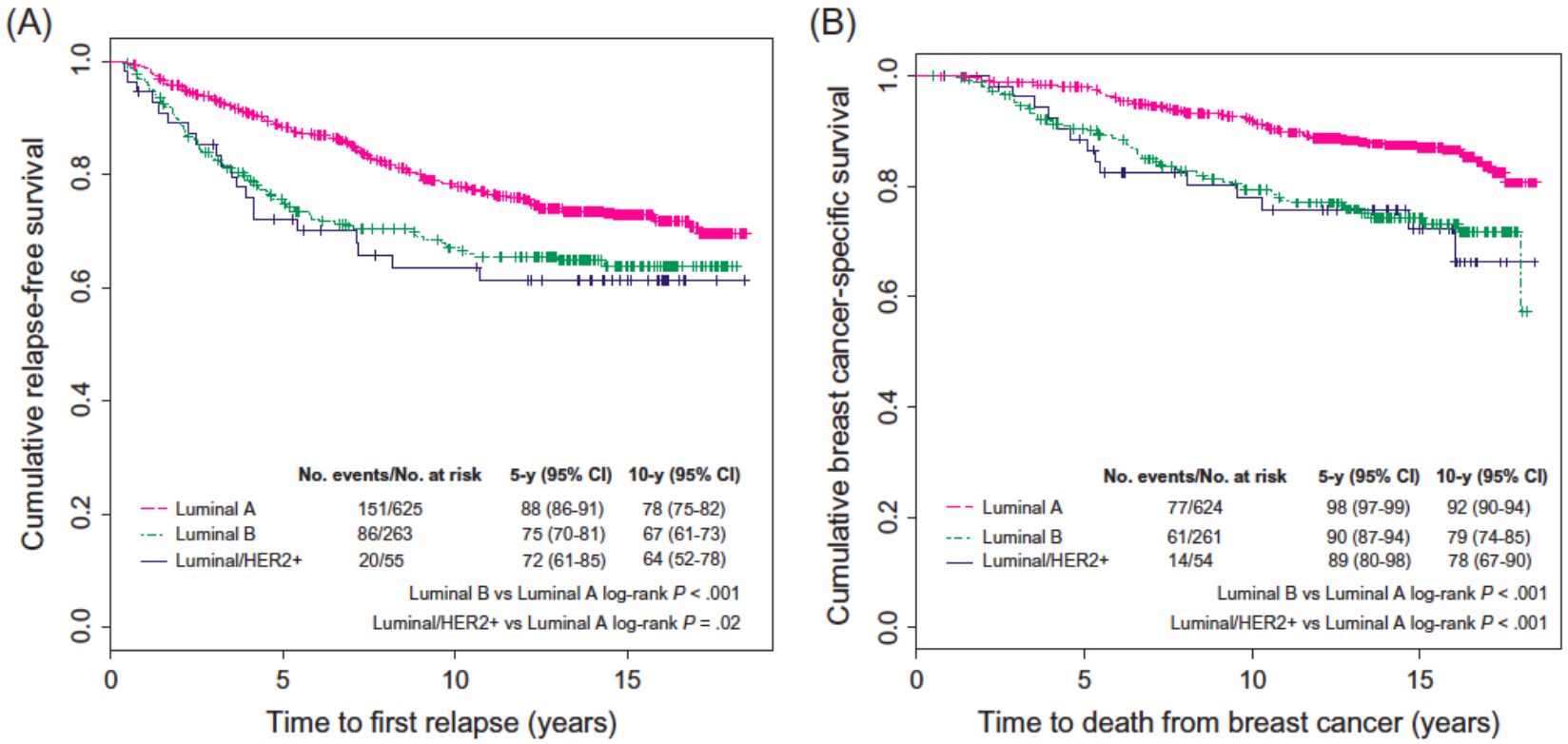


Discordant cases who received no AST or endocrine therapy only



Role of Ki-67

RFS luminal A vs. B based on Ki-67



Cheang et al., JNCI (2009) 101: 736-750

JNCI Journal of the National Cancer Institute Advance Access published September 29, 2011

DOI: 10.1093/jnci/djr393

COMMENTARY

Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group

Mitch Dowsett, Torsten O. Nielsen, Roger A'Hern, John Bartlett, R.Charles Coombes, Jack Cuzick, Matthew Ellis, N.Lynn Henry, Judith C. Hugh, Tracy Lively, Lisa McShane, Soon Paik, Frederique Penault-Llorca, Ljudmila Prudkin, Meredith Regan, Janine Salter, Christos Sotiriou, Ian E. Smith, Giuseppe Viale, Jo Anne Zujewski, Daniel F. Hayes

Manuscript received March 14, 2011; revised September 1, 2011; accepted September 2, 2011.

Mitch Dowsett (Mr. Ki-67):

- Ki-67 may identify luminal class with a cut-off level of 13.25% proposed to distinguish poorer prognosis luminal B cancers from luminal A
  - Lack of between laboratory standards limiting application as a surrogate marker
- Standardized methodologies for Ki-67 are lacking
  - ASCO Tumor Marker Guidelines Committee: clinical utility of Ki-67 insufficient to recommend routine use for prognostic purposes
  - In 2011, the International Ki-67 in Breast Cancer Working Group published recommendations for Ki-67 assessment in breast cancer

Sheri & Dowsett, Ann. Oncol. (2012) 23: 219-227

- Cut points for prognosis, prediction, and monitoring should only be applied if the results from local practice have been validated against those in studies that have defined the cutoff for the intended use of the Ki67 result.

Box 1. Recommendations for Ki67 assessment in breast cancer

Preanalytical

- Core-cut biopsies and whole sections from excision biopsies are acceptable specimens; when comparative scores are to be made, it is preferable to use the same type for both samples (eg, in presurgical studies).
- TMAs are acceptable for clinical trial evaluation or epidemiological studies of Ki67.
- Fixation in neutral buffered formalin should follow the same guidelines as published for steroid receptors (39,40).
- Once prepared, tissue sections should not be stored at room temperature for longer than 14 days. Results after longer storage must be viewed with caution.

Analytical

- Known positive and negative controls should be included in all batches; positive nuclei of nonmalignant cells and with mitotic figures provide evidence of the quality of an individual section.
- Antigen retrieval procedures are required. The best evidence supports the use of heat-induced retrieval most frequently by microwave processing.
- The MIB1 antibody is currently endorsed for Ki67.

Interpretation and scoring

- In full sections, at least three high-power (×40 objective) fields should be selected to represent the spectrum of staining seen on initial overview of the whole section.
- For the purpose of prognostic evaluation, the invasive edge of the tumor should be scored.
- If pharmacodynamic comparisons must be between core cuts and sections from the excision, assessment of the latter should be across the whole tumor.
- If there are clear hot spots, data from these should be included in the overall score.
- Only nuclear staining is considered positive. Staining intensity is not relevant.
- Scoring should involve the counting of at least 500 malignant invasive cells (and preferably at least 1000 cells) unless a protocol clearly states reasons for fewer being acceptable.
- Image analysis methods for Ki67 remain to be proven for use in clinical practice.

Data handling

- The Ki67 score or index should be expressed as the percentage of positively staining cells among the total number of invasive cells in the area scored.
- Statistical analysis should take account of the log-normal distribution generally followed by Ki67 measurement.
- The most appropriate endpoint in comparative studies of treatment efficacy or response is the percentage suppression of Ki67-positive cells.
- The most appropriate endpoint for assessing residual risk of recurrence is the on-treatment proportion of Ki67-positive cells.
- Cut points for prognosis, prediction, and monitoring should only be applied if the results from local practice have been validated against those in studies that have defined the cutoff for the intended use of the Ki67 result.

The clinical issue.
Think step by step

Step 1:

Is prognosis so good that survival advantage of adjuvant chemotherapy outweighs the disadvantages & serious late side effects?

Can we select those patients?

My conclusion:

- on the basis of standard clinical-pathological data only a few. Ki-67 is of limited help, only in the extremes
- You need to add extra information on the molecular tumor biology of the primary to be able to select a larger proportion (40% vs 10% of early node negative ER +ve breast cancers)

The clinical issue.
Think step by step

Step 2:

Is ER +ve really ER +ve?

Or:

- How reliable is your ER IHC scoring?
- Where is the cut-off?

Stages of IHC testing

- Transport
- Secretary support
- Tissue, type, and dimension
- Decalcification
- Preparation
- Fixation
  - Time, Type, Volume
- Section
  - Thickness
  - Storage & Drying

- Antigen retrieval
- Primary antibody
  - Clone
  - Dilution
  - Buffer
  - Time
  - Temperature
- Manual vs. Automated
- Development
- Visualization
- Sensitivity
- Specificity

- Interpretation
- Localization
- Cut-off
- Quantification
- Reporting
- Secretary support!
- Control
  - Internal
  - External
- Quality assessment

The perfect test is non-existent

- No 100% sensitivity
- No 100% specificity

ER-status based on IHC, mRNA, and signature
(n=456 FNAs)

mRNA **signature**

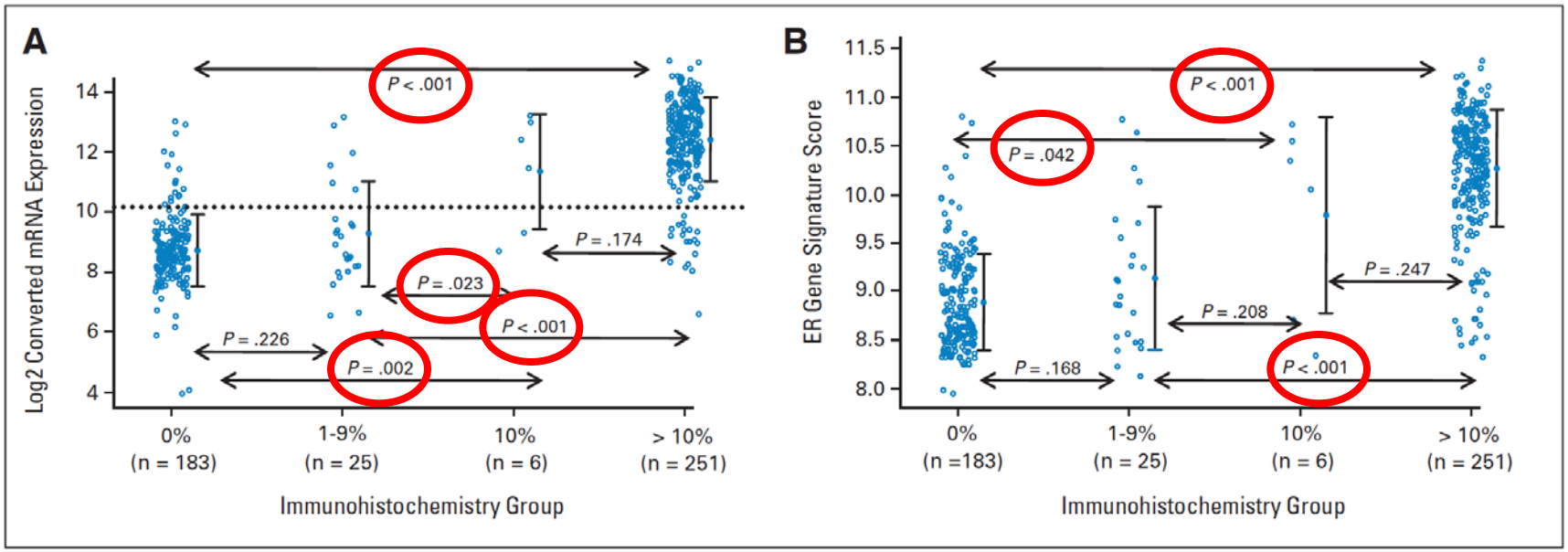
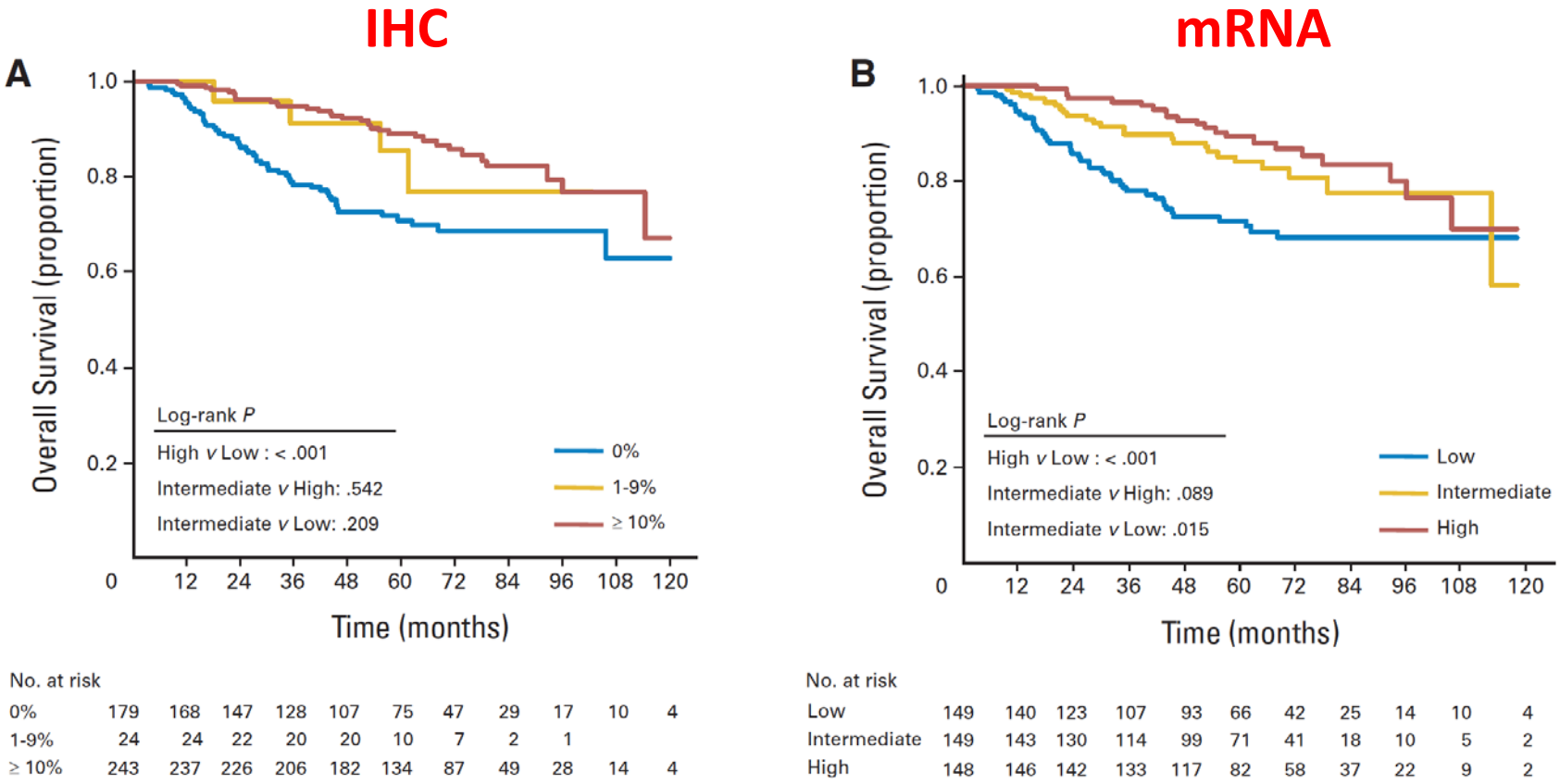


Fig 1. Estrogen receptor (ER) mRNA and ER-associated gene expression in four distinct immunohistochemistry groups. Immunohistochemistry groups were defined by the percentage of cells that were positive for nuclear ER staining. (A) Expression distribution of *ESR1* mRNA. (B) ER-associated gene signature refers to the average expression of 106 probe sets that are highly coexpressed with *ESR1*.[13] *P* values were calculated with the Wilcoxon test.

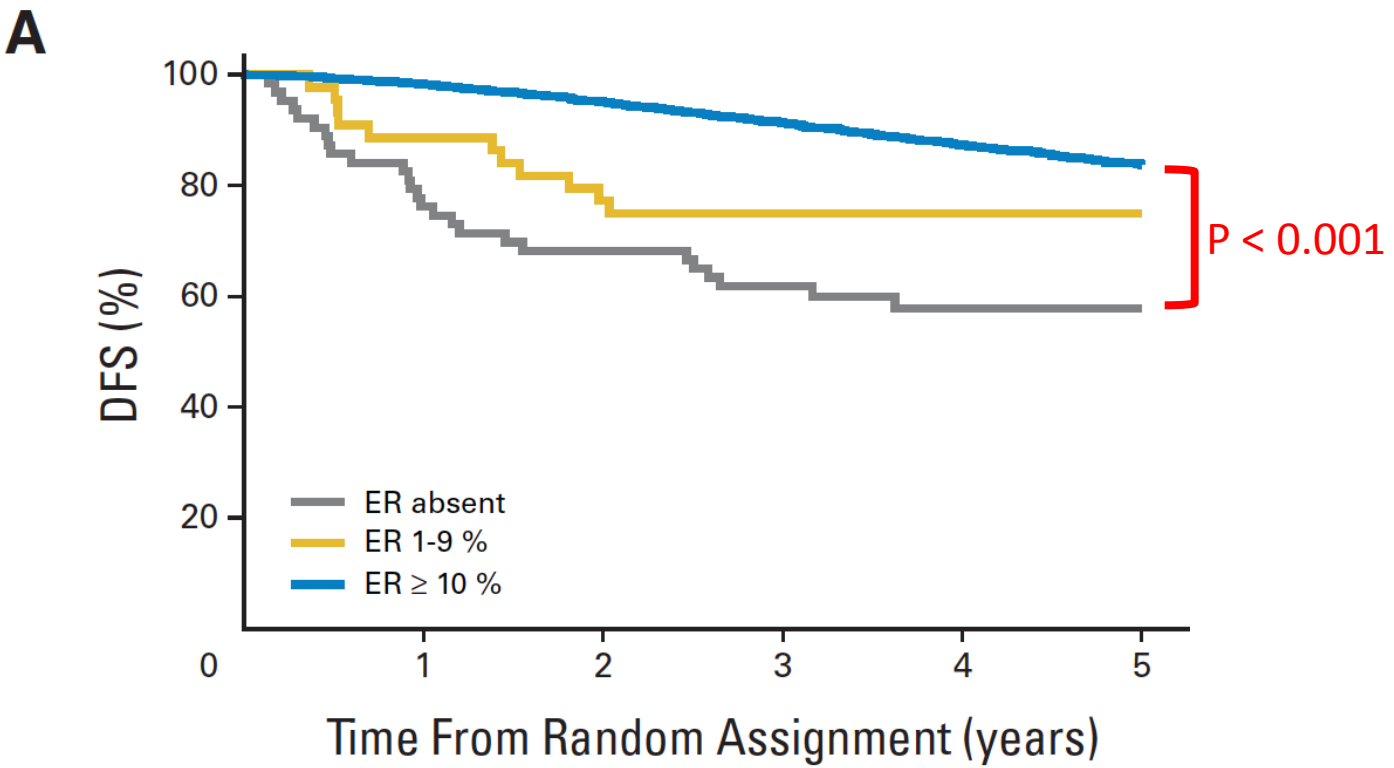
Iwamoto et al., J. Clin. Oncol. (2012) 30: 729-734

IHC and mRNA ER-status and OS



Iwamoto et al., J. Clin. Oncol. (2012) 30: 729-734

DFS and ER-status in BIG 1-98 trial



Central ER	No. at risk					
ER absent	63	48	43	34	28	22
ER 1-9%	44	39	34	27	24	20
ER ≥ 10%	3,489	3,424	3,294	2,548	1,546	938

Viale et al., J. Clin. Oncol. (2007) 25: 3846-3852

TargetPrint as second opinion

High concordance of protein (by IHC), gene (by FISH; HER2 only) and microarray readout (by TargetPrint) of ER/PR/HER2: results from the MINDACT trial

Giuseppe Viale[1], Jan Bogaerts[2], Leen Slaets[2], Laura van't Veer[3], Emiel Rutgers[3], Martine Piccart[4], Femke de Snoo[5], Kristel Engelen[2], Leila Russo[1], Patrizia Dell'Orto[1], Jeroen van den Akker[5], Annuska Glas[5], Fatima Cardoso[6]

on behalf of the TRANSBIG Consortium & the MINDACT investigators

1. European Institute of Oncology and University of Milan, Milan, Italy; 2. European Organisation of Research and Treatment of Cancer, Brussels, Belgium; 3. Netherlands Cancer Institute, Amsterdam, the Netherlands; 4. Institute Jules Bordet, Brussels, Belgium; 5. Agendia, Amsterdam, the Netherlands; 6. Champalimaud Cancer Center, Lisboa, Portugal

Background

This study was undertaken to further determine the correlation of microarray readout by TargetPrint with IHC/FISH assessment both locally and centrally determined in the 1st 800 pts enrolled in the MINDACT trial (Rutgers et al, 2011, EJC). This work is essential to determine the quality of biological data in the two risk assessment methods used in MINDACT based upon which adjuvant chemotherapy decision is made, in order to exclude bias.

Data overview

	local assessment	central review	TargetPrint
ER+	674	527	673
ER-	126	82	128
missing	0	101*	0
PR+	570	490	522
PR-	230	129	277
missing	2	101*	0
HER2+	92	74	79
HER2-	680	540	721
missing	28	186**	0

Methods

ER/PR/HER2 IHC assessment was performed on the primary breast cancers of the first 800 pts enrolled in the MINDACT study.
- Local assessment at each center (n=800)
- Central review at the EIO (n=626)
- Central ER/PR: Threshold at 1% positive staining
- Local ER/PR: Threshold at 1% positive staining or Allred >2 or ≥ 10 fmol/mg
- Threshold for HER2 3+ was 10% or more positive staining
- HER2 2+ cases: FISH for final HER2 status
- Gene expression for ER, PR and HER2 was obtained by TargetPrint (Roepman et al, CCR, 2009) (n=800)

Results

Local pathology assessment with central review

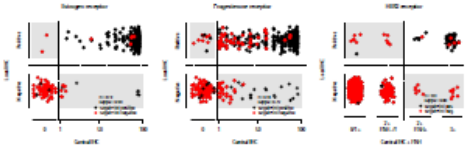
Comparison of local assessment (IHC & FISH for HER2) with central review (n=626) indicated highly similar results for receptor readout with a concordance of 98% (k=0.90) for ER; and 96% for HER2 (k=0.80) and slightly lower for PR (90% (k=0.72)).

central ER / central PR / central HER2

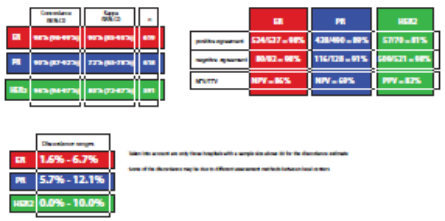
local ER	pos	neg
pos	524	2
neg	13	80

local PR	pos	neg
pos	438	12
neg	52	116

local HER2	pos	pos	neg	neg
pos	51	6	5	7
neg	7	6	100	409



The concordance for ER and HER2 shows the high quality of pathology assessments in the participating MINDACT hospitals. Please note however, discordance ranges up to 12% for PR and 10% for HER2.



Results

Central review with microarray readout by TargetPrint

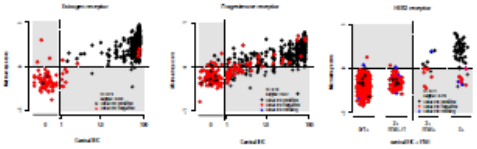
Comparison of central review (n=626) with microarray readout by TargetPrint indicated highly similar results for receptor readout with a concordance of 98% (k=0.90) for ER; and 96% for HER2 (k=0.78) and lower for PR (85% (k=0.62)).

central ER / central PR / central HER2

TargetPrint ER	pos	neg
pos	525	3
neg	12	79

TargetPrint PR	pos	neg
pos	408	11
neg	82	118

TargetPrint HER2	pos	pos	neg	neg
pos	47	6	2	3
neg	14	7	110	425



The positive and negative agreement of ER indicates TargetPrint to be a very stable and reliable assay for ER. PR concordance is lower. For HER2 the positive agreement indicates mRNA readout to be different from protein readout.

Acknowledgements

This trial has funding grants from the European Commission Framework Programme VI (FP6-LSHC-CT-2004-506410), the Breast Cancer Research Foundation, Novartis, F. Hoffman-La Roche, Sanofi-Aventis, the National Cancer Institute (NCI), the IBCC-Breast Cancer Working Group, the Jacqueline Seroussi Memorial Foundation, Fils Mais du Cancer du Sein, Susan G. Komen for the Cure, Fondation Belge Contre le Cancer, Dutch Cancer Society (KWF), Association Le Cancer du Sein, Parlons-en!, Deutsche Krebshilfe and the Grant Simpson Trust and Cancer Research UK. Whole genome analysis is provided in kind by Agendia.

MINDACT
Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy
EORTC 10041 / BIG 3-04
TRANSBIG

Variability

Inter-laboratory variability for ER, PR and HER2 has been reported and has initiated standardization protocols, indicating the need for a stable and reliable result for these prognostic parameters. To be considered acceptable, the results of the assay must be initially 90% concordant with those of the clinically validated assay for the ER- and PR-positive category and 95% concordant for the ER- or PR-negative category. For HER2, concordance in the positive category is important.

Conclusion

Locally and centrally assessed ER, PR and HER2 status in the first 800 (626 centrally assessed) MINDACT patient samples indicate a high quality level of pathology in the local participating hospitals. These results exclude any bias induced by a lower quality of traditional pathology results as compared to the centrally assessed MammaPrint, both used for risk assessment and adjuvant chemotherapy decision in the MINDACT trial. The microarray-based assessment of ER, PR and HER2 gives results comparable to IHC & FISH and provides an objective and quantitative assessment of tumor receptor status. These results indicate that TargetPrint can serve as a second pathology assessment for locally assessed parameters, especially since TargetPrint is part of a multi-profile platform for breast cancer treatment management.

B. Viale et al. (2011) ASCO

Results

Central review with microarray readout by TargetPrint

Comparison of central review (n=626) with microarray readout by TargetPrint indicated highly similar results for receptor readout with a concordance of 98% (k=0.90) for ER; and 96% for HER2 (k=0.78) and lower for PR (85% (k=0.62)).

central ER

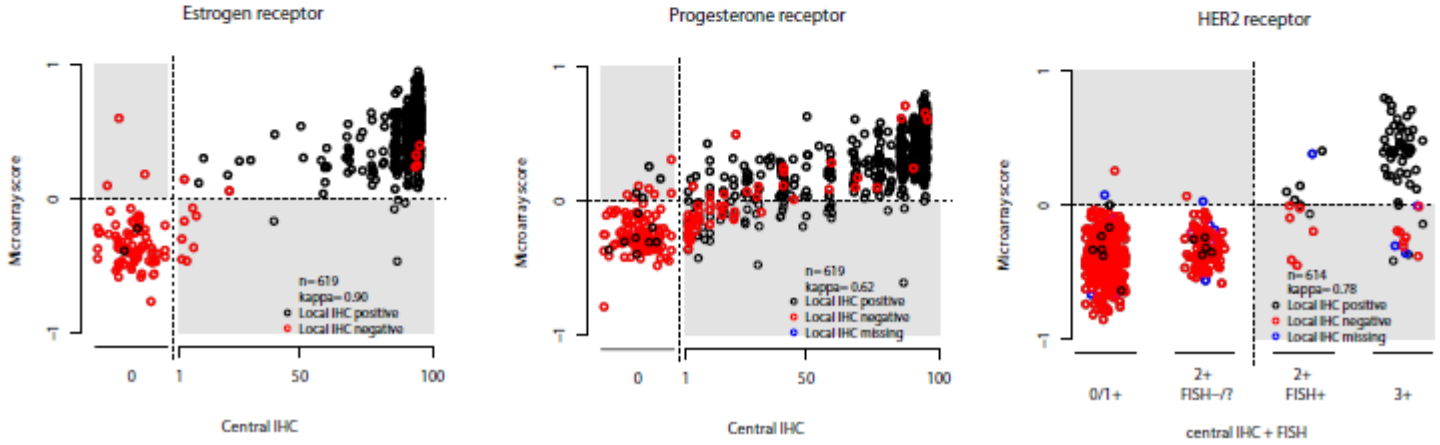
TargetPrint ER	ER n= 619*	pos ≥ 1%	neg < 1%
	pos	**525**	3
	neg	12	**79**

central PR

TargetPrint PR	PR n= 619*	pos ≥ 1%	neg < 1%
	pos	**408**	11
	neg	82	**118**

central HER2

TargetPrint HER2	HER2 n= 614*	pos (3+)	pos (2+/FISH+)	neg (2+/FISH-)	neg (0 or 1+)
	pos	**47**	**6**	2	3
	neg	14	7	**110**	**425**



High concordance for microarray based determination of ER, PR and HER2 receptor status and local IHC/FISH assessment worldwide in 827 patients

J. Wesseling[1], G. Cusumano[2], C. Tinterri[3], A. Sapino[4], F. Zanconati[5], M. Lutke-Holzik[6], B. Nguygen[7], K. Deck[8], P. Querzoli[9], T Perin[10], C. Giardina[11], G. Seitz[12], J. Guinebretiere[13], J. Barone[14], T. Watanabe[15]

1. Netherlands Cancer Institute, Amsterdam, Netherlands; 2. CHC, Liege, Belgium; 3. Instituto Clinico Humanitas, IRCCS, Rozzano, Italy; 4. Universita di Torino, Italy; 5. Università di Trieste, Trieste, Italy; 6. Medisch Spectrum Twente, Enschede, Netherlands; 7. Long Beach Memorial Health Care, Long Beach, California, United States; 8. Saddleback Memorial Medical Center, Laguna Hills, California; 9. Instituto di Patologia, Universita di Ferrara, Italy; 10. Centro di Riferimento Oncologico, Aviano, Italy; 11. Instituto di Anatomia Patologica, Iniverstita degli Studi di Bari, Italy; 12. Klinikum Bamberg, Germany; 13. Centre Rene Huguenin, Saint-Cloud, France; 14. Comprehensive Breast Care and Sharp Memorial Hospital, San Diego, California and 15. Hamamatsu Oncology Center, Japan

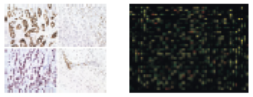
Background

- The level of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is predictive for prognosis and/or treatment response in breast cancer patients.
- Differences in fixation and subjective interpretation can substantially affect the accuracy and reproducibility of the results in IHC.
- The commercially available TargetPrint test measures the mRNA expression level of ER, PR and HER2 and provides an objective and standardized alternative to IHC.

Methods

- Tumor samples (n=831) from breast cancer patients (stage I to IV) were collected prospectively worldwide between 2008 and 2011 by core needle biopsy or from a surgical specimen
- The mRNA level of ER, PR and HER2 was assessed with TargetPrint
- IHC/FISH assessments were performed according to local standards at the participating hospitals
- HER2 IHC scores of 0 or 1+ were considered negative. An IHC score of 3+ was considered positive. IHC 2+ cases with an amplified FISH result were considered positive and none amplified FISH results negative
- HER2 IHC/FISH was unknown for 12 samples; ER/PR IHC unknown for 4
- IHC staining results were compared to the quantitative gene expression readouts (TargetPrint)
- Discordant cases were centrally reviewed for IHC/FISH assessment

IHC versus TargetPrint (microarray)



2x2 tables TargetPrint versus IHC/FISH

ER by TargetPrint	ER by IHC pos	ER by IHC neg
pos	681	15
neg	25	106

PR by TargetPrint	PR by IHC pos	PR by IHC neg
pos	493	51
neg	93	190

HER2 by TargetPrint	HER2 by IHC/FISH pos (3+)	HER2 by IHC/FISH pos (2+/FISH+)	HER2 by IHC/FISH neg (2+/FISH-)	HER2 by IHC/FISH neg (0 or 1+)
pos	65	18	6	9
neg	16	15	35	655

Overall comparison of IHC and gene expression (mRNA level) read out by TargetPrint shows a concordance of:

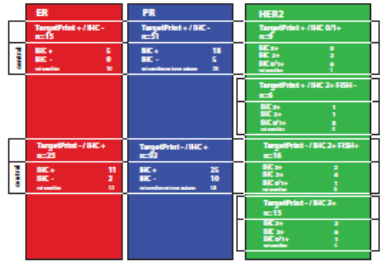
95% for ER; 83% for PR and 94% for HER2

Inter-institutional data: the concordance between centers ranged from: 88-100% for ER, 77-95% for PR, and 91-100% for HER2*

* ranges were calculated from institutes with more than 20 cases

Results central review of discordant cases

Central re-assessment (blinded for original results) for IHC ER-/TargetPrint ER+ cases indicates TargetPrint to be useful as second opinion in such cases. For HER2, microarray read-out shows true dicordance for a number of discordant cases. Further research is indicated.



Concordance & kappa statistics for ER, PR, and HER2

	Concordance Statistic	Concordance 95% CI	Kappa Statistic	Kappa 95% CI
ER	0.95	(0.93-0.96)	0.81	(0.75 - 0.87)
PR	0.83	(0.80-0.85)	0.60	(0.54 - 0.66)
HER2	0.94	(0.93-0.96)	0.75	(0.68 - 0.82)

Percent agreement for ER, PR, and HER2

	ER	PR	HER2
% Positive Agreement	681/ (681+25) = 97%	493/ (493+93) = 84%	83/ (83+31) = 73%
% Negative Agreement	106/ (106+15) = 88%	190/ (190+51) = 79%	690/ (690+15) = 98%
NPV/PPV	NPV = 81%	NPV = 67%	PPV = 85%

Conclusion

Microarray based readout of ER, PR and HER2 status using TargetPrint is highly comparable to local IHC and FISH analysis in 827 analyzed samples worldwide.

The results indicate mRNA expression read out for ER, PR and HER2 by TargetPrint provides high quality second opinion for local IHC/FISH assessment.

First central re-assessment of 103 discordant assessments are shown and discussed.

For further information on the comparison of local IHC read out and TargetPrint please view: poster P3-04-06 and P1-07-06

J. Wesseling et al. (2011) ASCO

Reliable ‘second opinion’

	Concordance (95% CI)	Kappa (95% CI)	n
ER	98% (96-99%)	90% (85-95%)	619
PR	85% (82-88%)	62% (55-69%)	619
HER2	96% (94-97%)	78% (70-86%)	614

	ER	PR	HER2
positive agreement	525/537 = 98%	408/490 = 83%	53/74 = 72%
negative agreement	79/82 = 96%	118/129 = 91%	535/540 = 99%
NPV/PPV	NPV = 87%	NPV = 59%	PPV = 91%

The clinical issue.
Think step by step

Step 2:

Is ER +ve really ER +ve?

My conclusion (for debate):

- Have your ER testing done by standard operational procedures, including quality control programs in sufficient case load labs.
- IHC is good
- Threshold: like ASCO-CAP guidelines: >1% consider anti ER therapy

The clinical issue.
Think step by step (the most easy one)

Step 3:

If ER +ve is reliably proven, and there is some risk of relapse

- ➢ Adjuvant anti-estrogen treatments: effect is proven
- At least 5 years
- Premenopausal: tamoxifen +/- ovarion ablation
- Postmenopausal at least 2-3 years AI (+ tamoxifen) or AI only
- In higher risk: extended to 7 (10?) years

Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

*Early Breast Cancer Trialists' Collaborative Group (EBCTCG)**

Summary

Background As trials of 5 years of tamoxifen in early breast cancer mature, the relevance of hormone receptor *Lancet* 2011; 378: 771–84

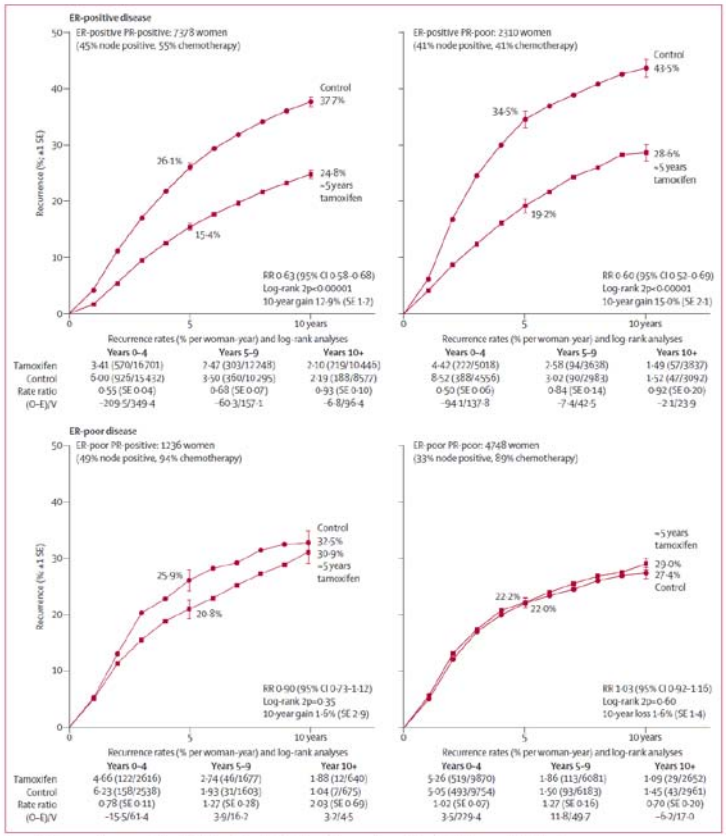


Figure 1: **Relevance of measured ER and PR status to the effects of about 5 years of tamoxifen on the 10-year probability of recurrence**
Outcome by allocated treatment in trials of about 5 years of adjuvant tamoxifen. Event rate ratio (RR) is from summed log-rank statistics for all time periods. Gain (and its SE) is absolute difference between ends of graphs. ER=oestrogen receptor. PR=progesterone receptor. O–E=observed minus expected, with variance V.

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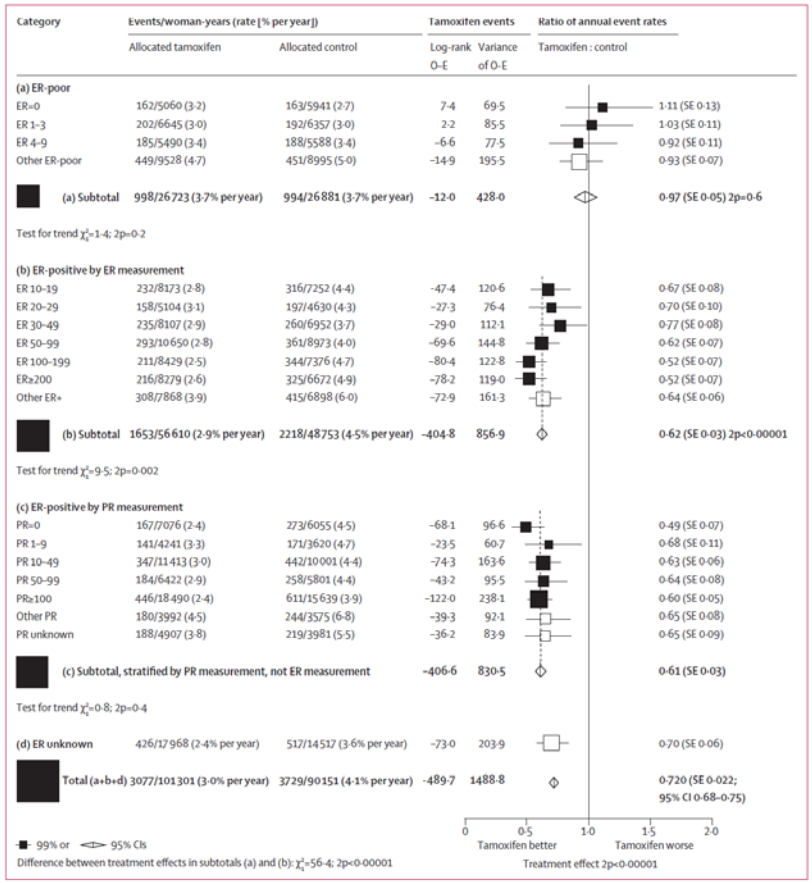

Figure 2: **Relevance of quantitative ER and PR measurement (fmol/mg cytosol protein) to the tamoxifen versus control recurrence rate ratio**
Outcome by allocated treatment in trials of about 5 years of adjuvant tamoxifen. Other ER poor includes ER-negative by immunohistochemistry and ER unspecified, but less than 10 fmol/mg. ER=oestrogen receptor. PR=progesterone receptor. O–E=observed minus expected.

Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

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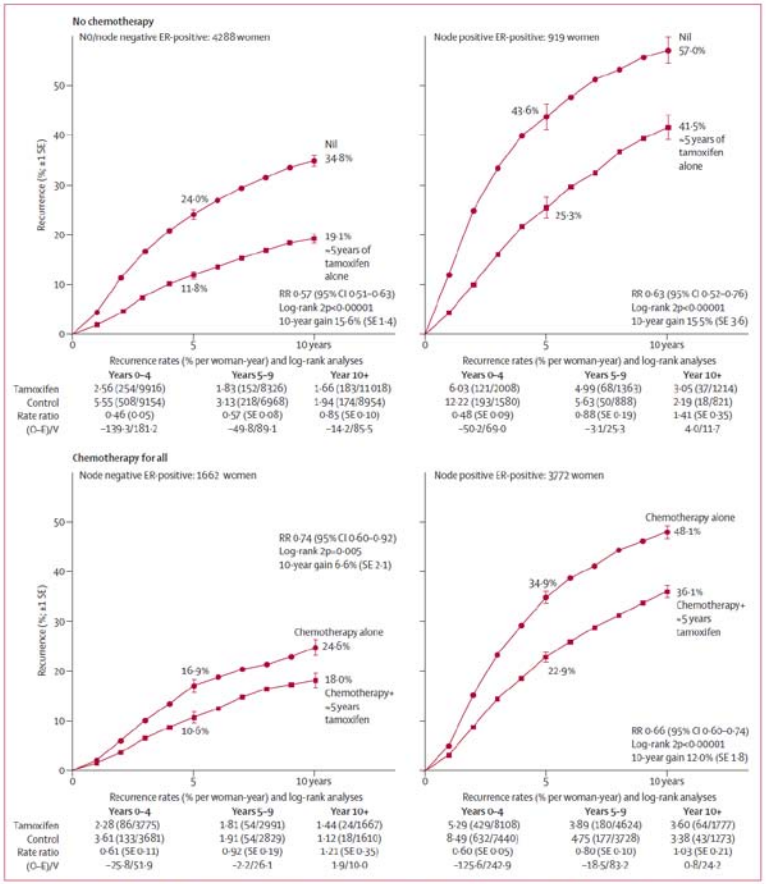


Figure 3: **Relevance of nodal status and of background chemotherapy to the effects of tamoxifen on the 10-year probability of recurrence, for ER-positive disease**
Outcome by allocated treatment in trials of about 5 years of adjuvant tamoxifen. Event rate ratio (RR) is from summed log-rank statistics for all time periods. Gain (and its SE) is absolute difference between ends of graphs. ER=oestrogen receptor. PR=progesterone receptor. O–E=observed minus expected, with variance V.

Step 3:

If ER +ve is reliably proven, and there is some risk of relapse

My conclusions:

- Adjuvant anti-estrogen treatments: effect is proven
- At least 5 years
- Premenopausal: tamoxifen +/- ovarion ablation(role await SOFT trial results)
- Postmenopausal at least 2-3 years AI (+ tamoxifen) or AI only
- In higher risk: extended to 7 (10?) years: see upcoming ATLAS trial results!

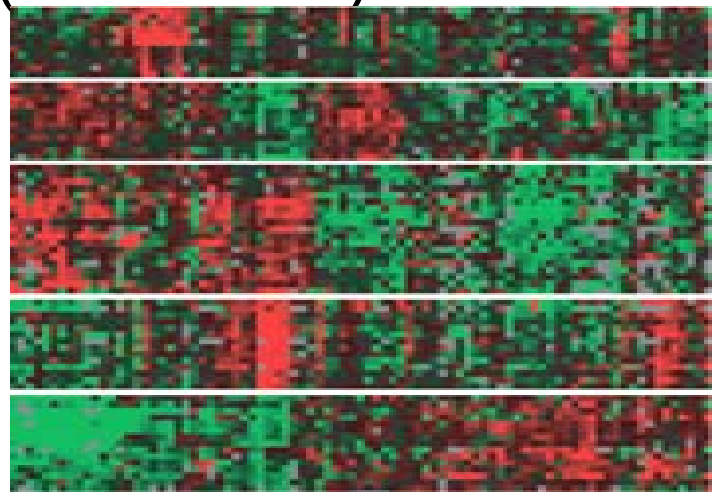
The clinical issue.
Think step by step
(the most difficult one)

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)

- Is every luminal A a luminal A?
- What makes luminal B a luminal B?
- What is the effect of chemotherapy: different for luminal A or B?

What are Intrinsic Molecular Subtypes?

- Molecular subtypes show which pathway drives cancer growth.
  - Luminal it is the estrogen pathway
  - ERB2 it is the HER2 pathway
  - Basal it is neither one of them
- There is approx 20% discordance between molecular subtypes and subtyping with IHC (Perou 2011)



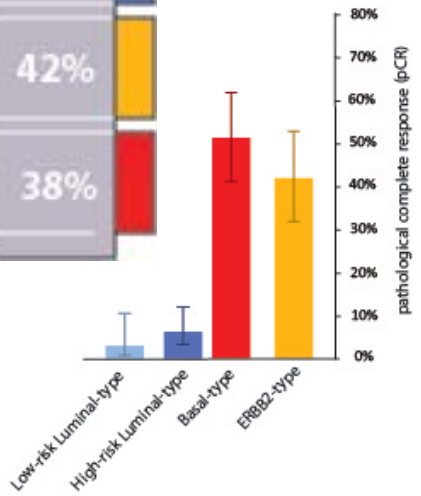
Red = Up-regulation
Green = Down-regulation

Is molecular subtyping useful in “fine tuning” your treatment decisions?

First some supportive data…

Response to neo-adjuvant chemotherapy in molecular subgroups

	Straver (1)		Somlo (2)		Krijgsman (3)		Total		
	n	pCR	n	pCR	n	pCR	n	pCR	%
Low Risk Luminal-type	21	0	14	1	29	1	64	2	3%
High Risk Luminal-type	67	3	16	0	53	6	136	9	7%
HER2-type	41	13	18	10	24	12	83	35	42%
Basal-type	38	13	20	4	27	15	85	32	38%



Breast Cancer Res Treat
DOI 10.1007/s10549-011-1683-z

PRECLINICAL STUDY

A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response

Oscar Krijgsman · Paul Roepman · Wilbert Zwart · Jason S. Carroll · Sun Tian · Femke A. de Snoo · Richard A. Bender · Rene Bernards · Annuska M. Glas

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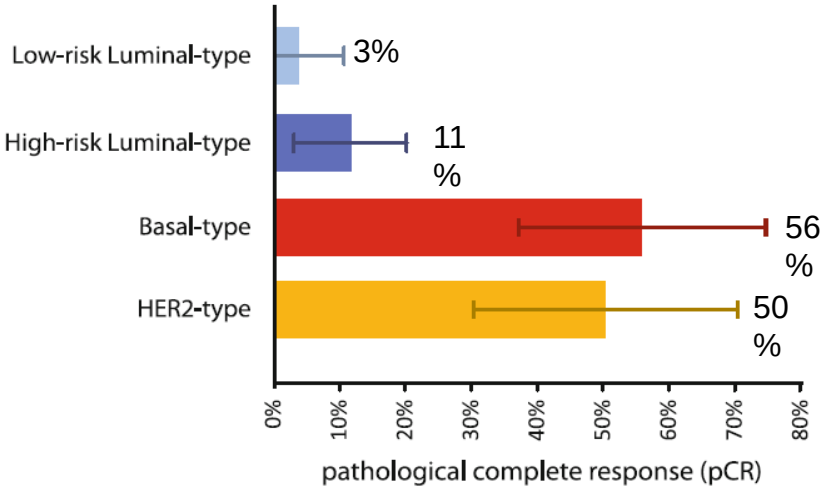
- PcR and 5yr follow-up of neoadjuvant patients confirms the very response to chemotherapy of Luminal Low Risk patients.
- PcR rates confirm that there is a benefit of chemotherapy in Luminal High Risk patients .
- PcR rates in Basal & HER2 are high stressing the importance of identifying the subtype in these two groups.



blueprint

Molecular Subtyping Signature

80-gene signature

Profiles Basal, Luminal and HER2 subtypes

ASCO - 2012

Response and long term outcomes after neo-adjuvant chemotherapy: Pooled dataset of patients stratified by molecular subtyping by MammaPrint and BluePrint

Stefan Glück[1], Femke de Snoo[2], Justine Peeters[2], George Somlo[3], Laura van 't Veer[4]

1. University of Miami/Sylvester Comprehensive Cancer Center, Miami, FL; 2. Agendia, Amsterdam, Netherlands; 3. City of Hope , Duarte, CA; 4. UCSF, San Francisco, CA

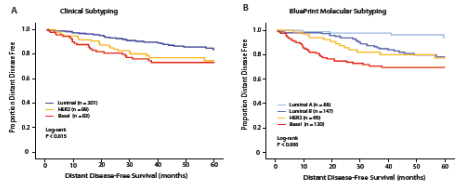
Background

Classification of breast cancers into molecular subtypes may be important for the appropriate selection of therapy for patients with early breast cancer. Previous analyses had shown that breast cancer subtypes have distinct clinical outcome (Sorlie, PNAS, 2001; Esserman, BCRT, 2011). In our study, we analyze using MammaPrint together with an 80-gene molecular subtyping profile (BluePrint) the response to neo-adjuvant chemotherapy and long term outcomes.

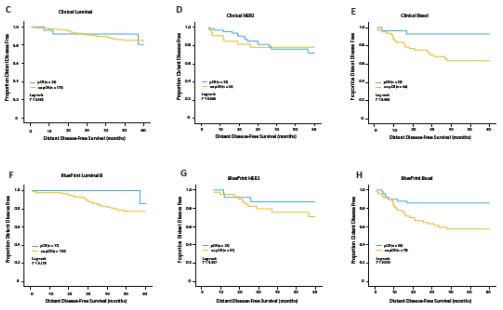
Methods

This study was carried out on data from 421 patients: 141 patients from the I-SPY I trial; 230 patients from biomarker discovery program at MD Anderson (131 and 99 respectively; Hess et al., 2006, JCO; Iwamoto et al., 2011, BCRT); and 50 patients from City of Hope (Somlo et al., ASCO, 2010). All patients were treated in the neo-adjuvant setting with chemotherapy. MammaPrint and BluePrint outcomes were determined from either 44K Agilent arrays run at Agendia or available through the I-SPY I data portal, or from Affymetrix U133A arrays. The combination of MammaPrint and BluePrint resulted in 4 distinct molecular groups: Luminal A (MammaPrint Low Risk/Luminal-type), Luminal B (MammaPrint High Risk/ Luminal-type), Basal-type and HER2-type.

Survival rates according to Clinical and Molecular Subtyping



Prognosis after pCR by Clinical and Molecular Subtyping



Added value of Molecular Subtyping

Luminal A patients (BluePrint Luminal/ MammaPrint Low Risk) have a good baseline prognosis with excellent survival and may have no benefit from chemotherapy.

A subset of clinical HER2+ patients are classified as Luminal-type by BluePrint. The BluePrint HER2-type pCR patients have a 5 yr DMFS of 87%, compared to clinical HER2+ pCR patients who have 78% 5 yr DMFS. A recent pooled analysis showed that pCR rate is low in clinical HER2+/Luminal patients and is not associated with outcome (von Minckwitz et al., 2012, JCO).

BluePrint classifies more patients as Basal-type (n=120) with higher pCR rate (42%), compared to clinical subtyping (n=93) with a pCR rate of 31%.

Clinical characteristics

	Molecular Subtyping: BluePrint and MammaPrint				
	Luminal A type (n=77)	Luminal B type (n=147)	HER2 type (n=65)	Basal type (n=120)	Total (n=411)
Age (median)	50 (26-75)	51 (23-79)	50 (32-73)	51 (24-71)	
Grade 1	12	76	2	0	90
Grade 2	53	72	19	21	165
Grade 3	21	58	44	78	201
Grade unknown	1	11	1	21	35
ER+ (IHC)	72	108	19	11	260
ER- (IHC)	16	34	37	84	171
ER unknown	0	5	9	5	19
PR+ (IHC)	59	74	22	30	186
PR- (IHC)	30	69	44	85	222
PR unknown	0	4	0	5	11
HER2 + (IHC/FISH)	9	34	17	29	89
HER2- (IHC/FISH)	63	104	28	87	289
HER2 unknown	0	9	1	10	20
triple negative (IHC/FISH)	11	15	9	29	64
MammaPrint Low Risk	66	0	0	0	66
MammaPrint High Risk	0	147	59	103	309
Anthracycline/non taxane	5	6	3	4	18
Taxane	72	106	61	78	288
HER2 targeted	3	6	13	12	34
treatment unknown	0	11	10	19	40

Summarizing tables

Clinical Subtyping	Chemosensitivity pCR/no pCR (%)	Prognosis 5 yr DMFS	Benefit from CT 5 yr DMFS pCR (CT responsive) 5 yr DMFS no pCR (CT non-responsive)
Luminal (ER and/or PR+; HER2-)	26/201 (15%)	93% (figure A)	pCR 80% (figure C) no pCR 94%
HER2 (HER2+)	33/99 (33%)	74% (figure A)	pCR 78% (figure D) no pCR 71%
HER2-type (malignancy without targeted treatment)	21/76 (30%)	78% (data not shown)	pCR 87% (data not shown) no pCR 73%
Basal-type (triple negative)	29/95 (31%)	71% (figure A)	pCR 83% (figure E) no pCR 63%

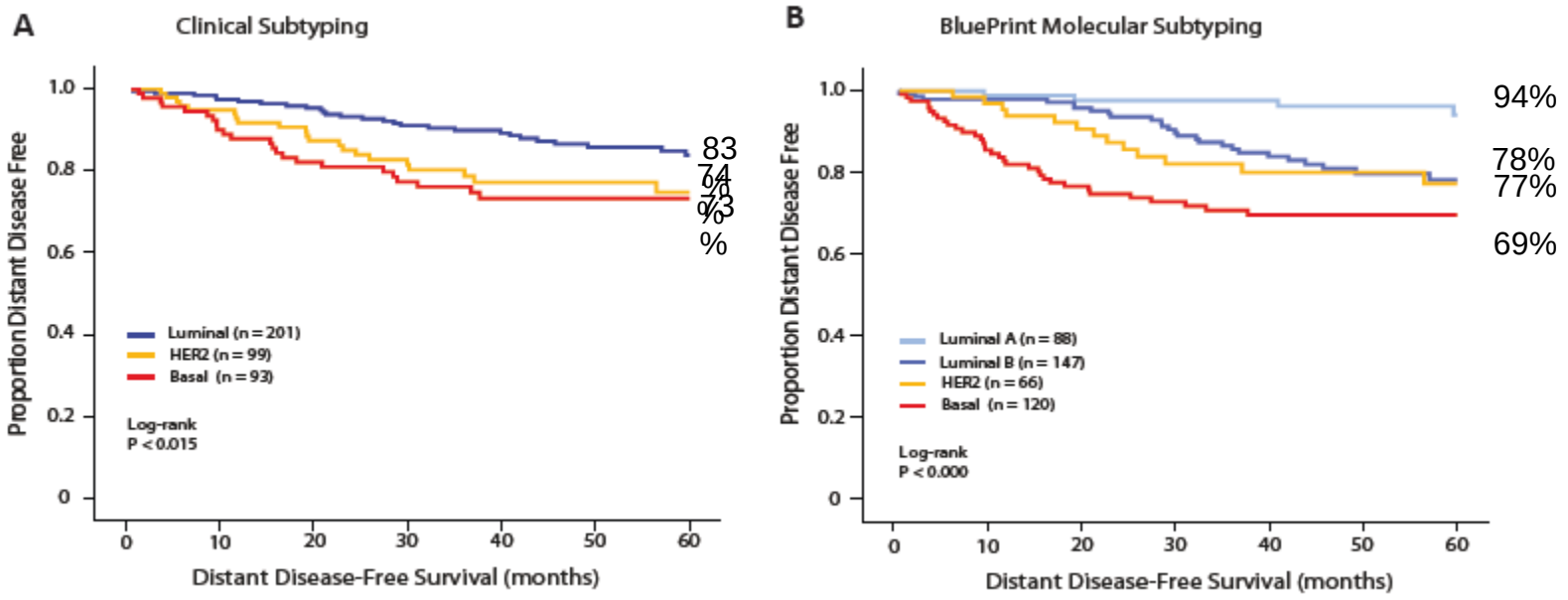
BluePrint Subtyping	Chemosensitivity pCR/no pCR (%)	Prognosis 5 yr DMFS	Benefit from CT 5 yr DMFS pCR (CT responsive) 5 yr DMFS no pCR (CT non-responsive)
LuminalA (MammaPrint Low Risk)	3/88 (3%)	94% (figure B)	good baseline prognosis (low pCR (n = 5)
LuminalB (MammaPrint High Risk)	12/147 (12%)	78% (figure B)	pCR 85% (figure F) no pCR 77%
HER2-type	25/66 (38%)	77% (figure B)	pCR 87% (figure G) no pCR 70%
HER2-type (malignancy without targeted treatment)	20/51 (39%)	77% (data not shown)	pCR 85% (data not shown) no pCR 72%
Basal-type	50/120 (42%)	69% (figure B)	pCR 86% (figure H) no pCR 57%

Summary

Molecular Subtyping can improve stratification of patients in the neo-adjuvant setting; MammaPrint Low Risk patients have a good baseline prognosis with excellent survival and may not benefit from chemotherapy. We observed marked differences in response and DMFS to neo-adjuvant treatment in groups stratified by MammaPrint and BluePrint. These findings confirm differences in chemotherapy response among molecular subgroups, and indicate that BluePrint and MammaPrint help to further establish a clinical correlation between molecular subtyping and treatment outcomes.

Key Findings:

- 5 year survival data suggests that a combination of MammaPrint and BluePrint more accurately identifies Luminal, Basal and ERB2 subtypes compared to IHC



Key Findings:

- 42% of patients that were classified as HER 2+ by IHC/ Fish were reclassified as Luminal's with Blue Print
- Luminal A's have a 5yr DMFS of 94%.
- If significant co-morbidites exist is it worth considering withholding Herceptin from Luminal A patients?

Clinical characteristics

	Molecular Subtyping: BluePrint and MammaPrint				
	Luminal A-type (n=88)	Luminal B-type (n=147)	HER2-type (n=66)	Basal-type (n=120)	Total (n=421)
Age (median)	50 (26-75)	51 (30-79)	53 (32-73)	51 (29-72)	
Grade 1	12	76	2	0	20
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ER+ (IHC)	72	108	29	31	240
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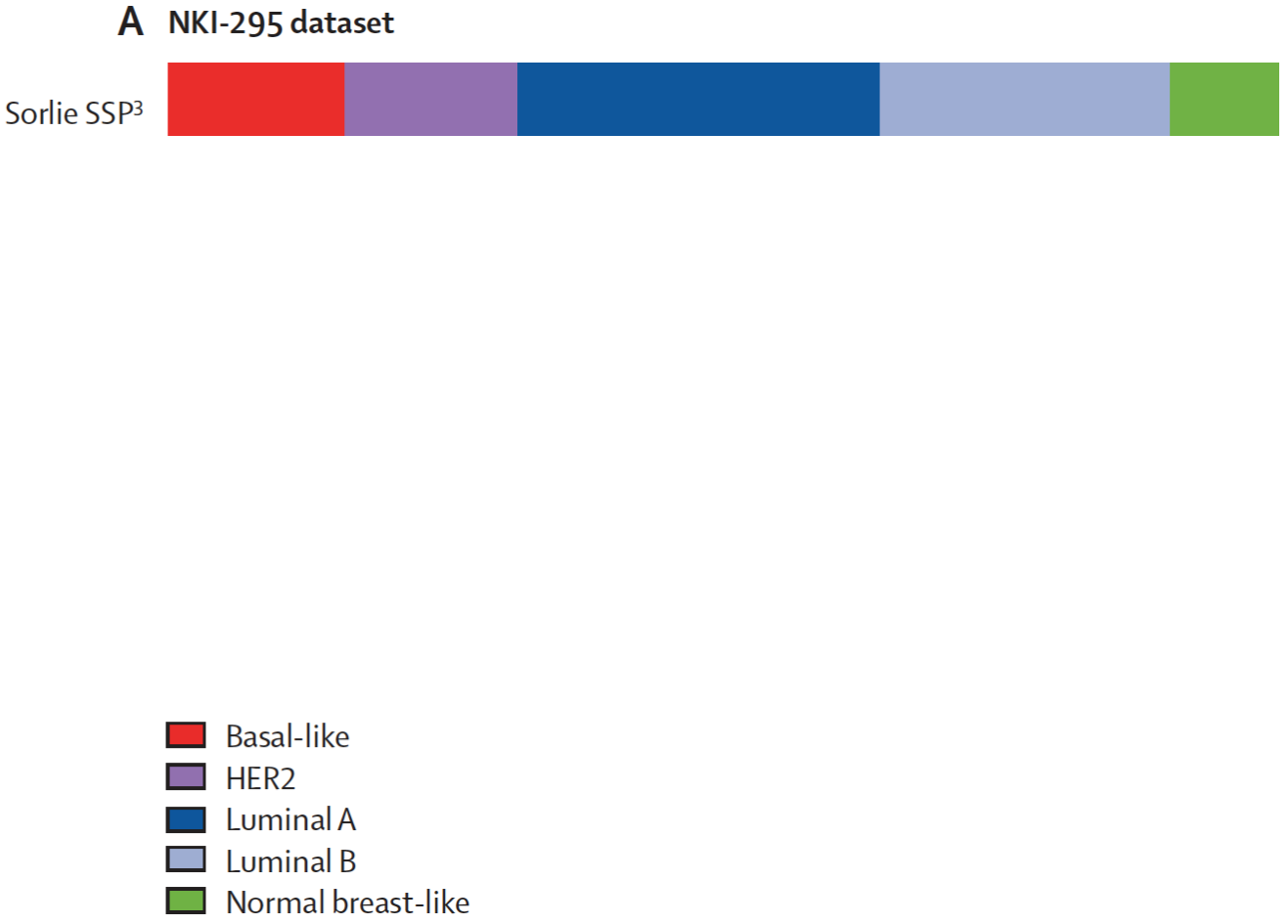
PR- (IHC)	30	63	44	85	222
PR unknown	0	8	0	5	13
HER2 + (IHC/FISH)	8	34	37	20	99
HER2- (IHC/FISH)	80	104	28	87	299
HER2 unknown	0	9	1	13	23
triple negative (IHC/FISH)	11	14	9	59	93
MammaPrint Low Risk	88	0	8	0	96
MammaPrint High Risk	0	147	58	120	325
Anthracycline/non taxane	5	4	2	4	15
Taxane	73	106	41	78	298
HER2 targeted	2	6	15	12	35
treatment unknown	9	33	10	29	81

Is molecular subtyping useful in “fine tuning” your treatment decisions?

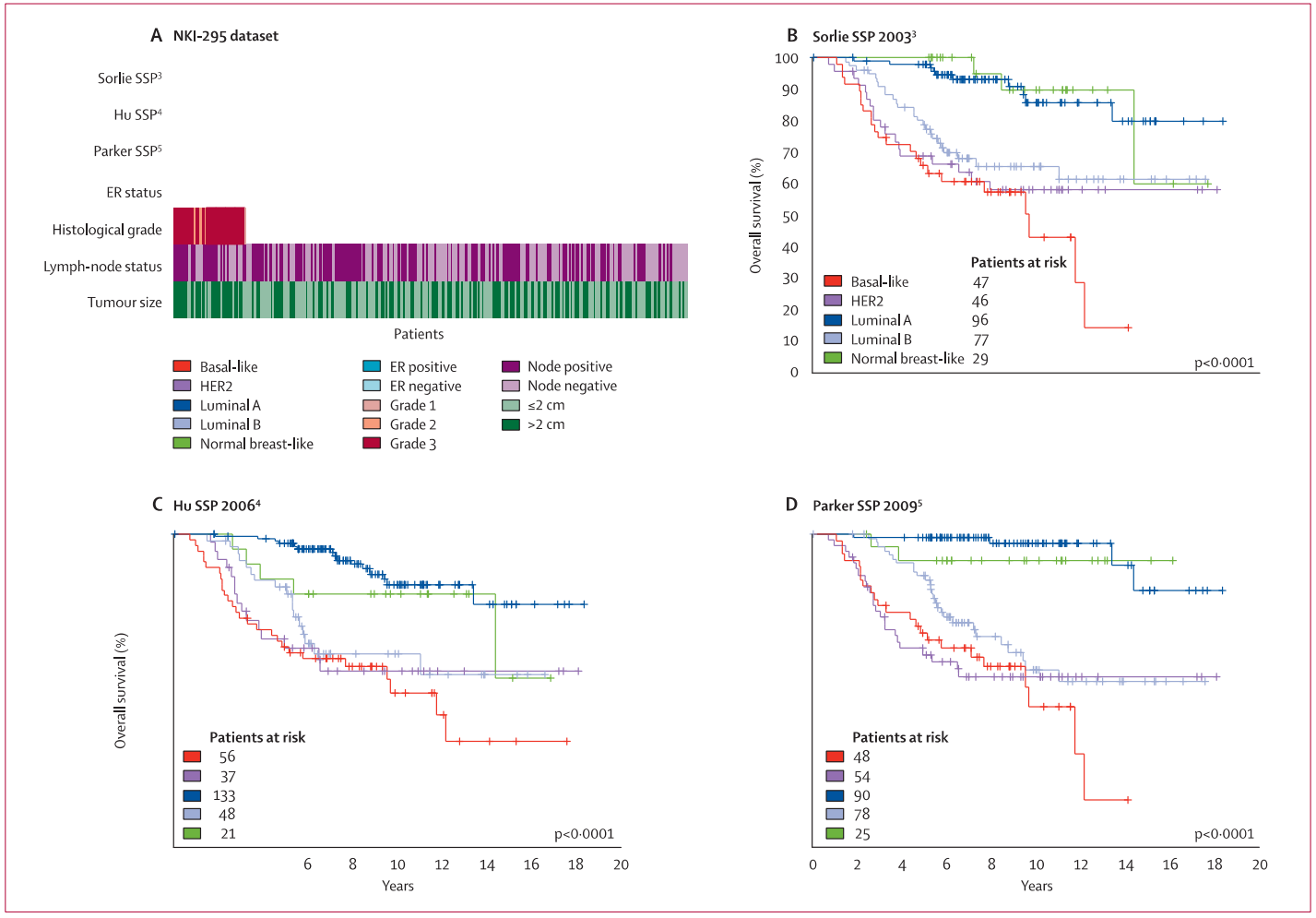
Than some sobering data….

Concordance single sample predictors (SSP)



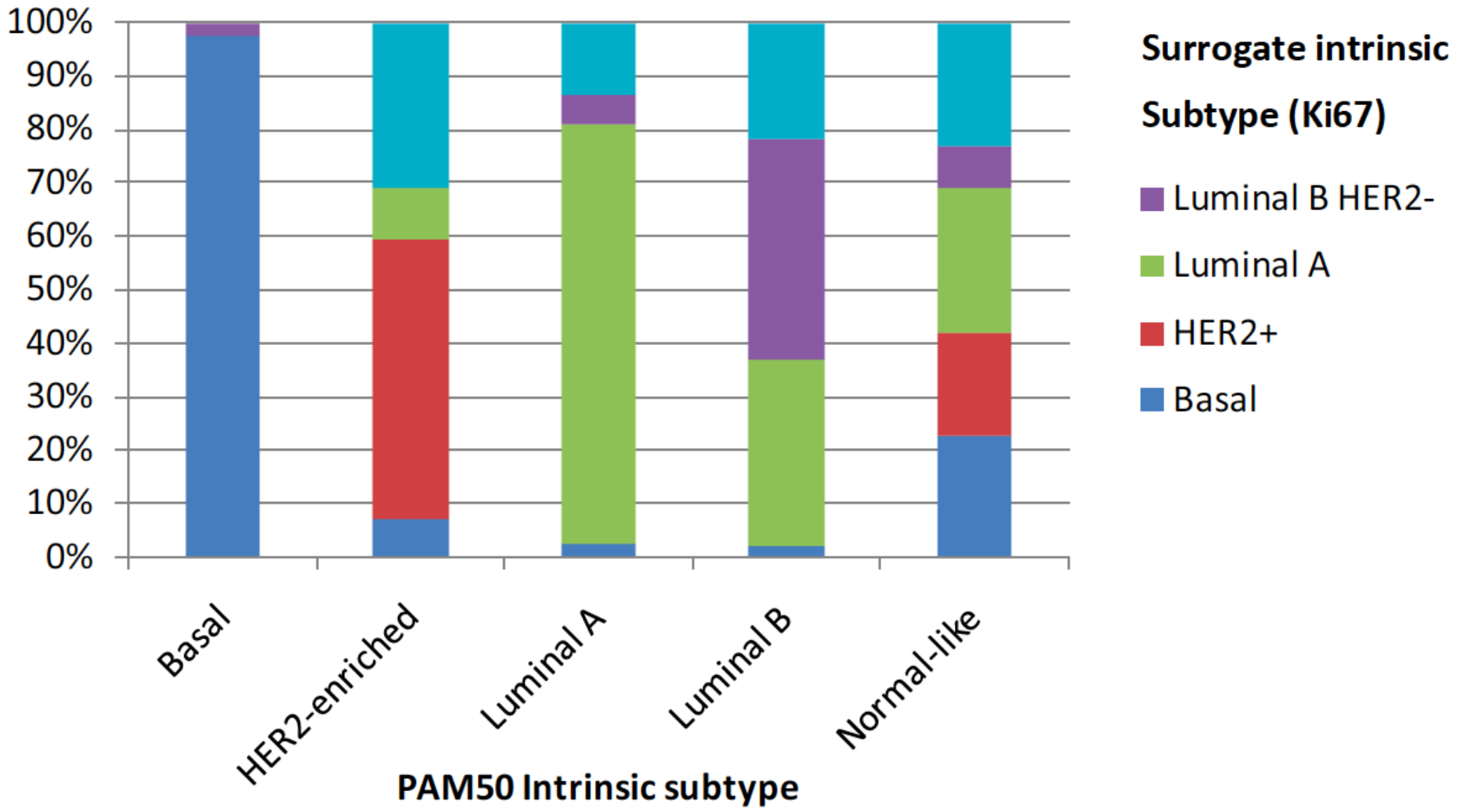
Weigelt et al., Lancet Oncology (2010) 11: 229

Concordance SSP algorithms



Weigelt et al., Lancet Oncology (2010) 11: 229

Concordance molecular vs. IHC subtyping (n=560)



Lips et al., submitted

ASCO - 2012

Comparison of molecular (BluePrint+MammaPrint) and pathological subtypes for breast cancer among the first 800 patients from the EORTC 10041/BIG 3-04 (MINDACT) trial

Giuseppe Viale[1], Leen Slaets[2], Femke de Snoo[3], Laura J. van 't Veer[4], Emiel J. Rutgers[5], Martine Piccart[6], Jan Bogaerts[2], Jeroen van den Akker[3], Kristel Engelen[2], Leila Russo[1], Patrizia Dell'Orto[1], Fatima Cardoso[7]

1. European Institute of Oncology and University of Milan, Milan, Italy; 2. European Organisation for Research and Treatment of Cancer, Brussels, Belgium; 3. Agendia, Amsterdam, Netherlands; 4. UCSF, San Francisco, US; 5. Netherlands Cancer Institute, Amsterdam, Netherlands; 6. Jules Bordet Institute, Brussels, Belgium; 7. Breast Unit, Champalimaud Cancer Center, Lisbon, Portugal

Background

Biology has become the main driver of breast cancer therapy. Intrinsic biological subtypes by gene expression profiling have been identified. Pathology can be used to define surrogates of these subtypes but these are not always concordant, which may lead to different treatment plans. We investigated the concordance between BluePrint + MammaPrint (micro array based) breast cancer subtypes and pathological surrogates (based on ER, PR, HER2 & Ki67). Contrary to the Perou gene set (evolved into PAM50), BluePrint was trained using pathological data.

Three ways to measure ER activity

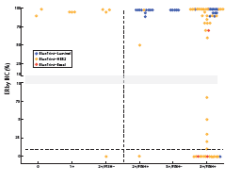
Methods

Using available data (centrally assessed pathology & genomic) from the MINDACT pilot phase (Rutgers et al, 2011) 621 tumors were analyzed. Patients were classified according to 4-category based pathology (ER, PR, HER2 and Ki67); additionally, classification was done adhering to the recent St. Gallen recommendations (Goldhirsch et al 2011) which recognizes an additional category (Luminal B HER2+). Based on BluePrint 3 subtypes are formed: Luminal, HER2 and Basal. The Luminal subtype is further split into Luminal A (MammaPrint Low Risk) and Luminal B (MammaPrint High Risk).

Substratification of the Luminal subgroup: Concordance MammaPrint versus Ki67

Ki67 is assumed to be a fairly reliable measure of proliferation. Generally, when multi-gene assay results are not available, Ki67 is often used as biomarker to distinguish Luminal A from Luminal B subgroups. The concordance between MammaPrint and centrally assessed Ki67 in Luminal-type patients is 71%, with a κ score of 0.35 (95% CI 0.26–0.45). The relatively high discordance with MammaPrint indicates that Ki67 and MammaPrint cannot reliably substitute for each other.

Molecular subtyping of HER2+ patients



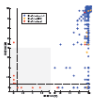
This figure depicts ER and HER2 clinical assessments for clinical HER2+ and/or BluePrint HER2 cases.
For visualization purposes, random trimmed noise is added to the HER2 assessments and ER scaling adjusted.

There is a relatively large group of clinical HER2+ cases that are BluePrint Luminal-type. BluePrint classifies these patients as Luminal-type despite being clinical HER2+, indicating the tumor's expression of the Luminal profile to be dominant over the expression of the HER2 profile. These patients have high IHC ER results and fall into the group that St Gallen separately defines as Luminal B HER2-type. These patients may have lower response to trastuzumab (von Minckwitz et al, 2012).

Subtyping with BluePrint/MammaPrint and IHC/FISH

4 category	St Gallen (5 category)	Luminal A	Luminal B	HER2	Basal	Total
Luminal A	Luminal A	263	19	4	1	287
Luminal B	Luminal B HER2-	111	70	4	11	196
HER2	Luminal B HER2+	25	3	31	1	60
	Erb-B2	1	0	13	2	16
Basal	Basal	0	0	1	61	62
Total		400	92	53	76	621

12 Clinical Luminal patients with BluePrint Basal-type



This figure depicts ER and PR IHC expression for clinical Luminal-type cases. For visualization purposes, random trimmed noise is added to a range of assessments and ER and PR scaling is adjusted.

The majority of the cases classified as Basal-type by BluePrint have low ER and PR expression (lower than 10%); indicating this to be a critical group in need of further research.

MINDACT
Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy
EORTC 10041 / BIG 3-04
TRANSBIG

Conclusions

- All pathological Basal cases are BluePrint Basal, apart from 1 BP HER2 case
- Of the BluePrint Basal cases, 20% are not pathological Basal (16% Luminal, 4% HER2). Of these 16% Luminal cases, the majority are IHC ER/PR borderline (≥1% and <10%)
- 97% of the pathological HER2+ cases that are BluePrint Luminal are ER+
- Most discordant cases are seen within the Luminal subtype, indicating that Ki67 distinguishes Luminal A from B differently than MammaPrint does
- The observed subtype discrepancies reveal potential important impact for treatment-decision making. MINDACT will provide such important information

References

Rutgers et al, 2011, European Journal of Cancer

Goldhirsch et al, 2011, Annals of Oncology

von Minckwitz et al, 2012, Journal of Clinical Oncology

Acknowledgements

This trial has funding grants from the European Commission Framework Programme VI (FP6-LSHC-CT-2004-503426), the Breast Cancer Research Foundation, Novartis, F. Hoffman La Roche, Sanofi-Aventis, the National Cancer Institute (NCI), the EBCC-Breast Cancer Working Group, the Jacqueline Seroussi Memorial Foundation, Prix Mois du Cancer du Sein, Susan G. Komen for the Cure, Fondation Belge Contre le Cancer, Dutch Cancer Society (KWF), Association Le Cancer du Sein, Pariono-en, Deutsche Krebshilfe and the Grant Simpson Trust and Cancer Research UK. Whole genome analysis is provided in kind by Agendia.

HER2+ and ER+ are often BP Luminal

- If you have patients with co-morbidities that you are concerned about treating with Herceptin, is there a subset of patients that you can withhold this drug?
- Large group of clinical HER2+ cases that are BluePrint Luminal type (46%).
- Indicating the tumor's expression of the Luminal profile to be dominate over the expression of the HER2+ profile.
- These patients may have a lower response to trastuzumab (von Minckwitz et al, 2012)

4 category	*St Gallen (5 category)*	Luminal A BluePrint Luminal MammaPrint Low Risk	Luminal B BluePrint Luminal MammaPrint High Risk	HER2 BluePrint HER2	Basal BluePrint Basal	Total
Luminal A ER+ and/or PR+ HER2-, Ki67 low	Luminal A idem	263	19	4	1	287
Luminal B ER+ and/or PR+ HER2-, Ki67 high	Luminal B HER2- idem	111	70	4	11	196
HER2 HER2+	Luminal B HER2+ ER+ and/or PR+ HER2+	25	3	31	1	60
	Erb-B2 ER-/PR-/HER2+	1	0	13	2	16
Basal ER-/PR-/HER2-	Basal idem	0	0	1	61	62
Total		400	92	53	76	621

Even the best KI67 assessment shows 30% discordance with MammaPrint

- Ki67 is assumed to be a fairly reliable measure of proliferation. Ki67 is utilized as a biomarker for chemotherapy
- The concordance between MammaPrint and centrally assessed Ki67 in Luminal-type patients is 71%, with a k score of 0.35 (95% CI 0.26-0.45).
- The relatively high discordance with MammaPrint indicates that Ki67 and MammaPrint cannot reliably substitute for each other.
- MammaPrint has a higher hazard ratio than KI67 and is a better indictor for prognosis

4 category	*St Gallen (5 category)*	Luminal A BluePrint Luminal MammaPrint Low Risk	Luminal B BluePrint Luminal MammaPrint High Risk	HER2 BluePrint HER2	Basal BluePrint Basal	Total
Luminal A ER+ and/or PR+ HER2-, Ki67 low	Luminal A idem	263	19	4	1	287
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Basal ER-/PR-/HER2-	Basal idem	0	0	1	61	62
Total		400	92	53	76	621

Key Findings:
20% of the Basal is IHC ER+

- These patients might take Endocrine therapy without effect
- Of the BluePrint Basal cases, 20% are not pathological Basal (16% Luminal, 4% HER2+
- Of the 16% Luminal cases, the majority (80% are IHC ER/PR borderline (≥ 1% and < 10%)

4 category	*St Gallen (5 category)*	Luminal A BluePrint Luminal MammaPrint Low Risk	Luminal B BluePrint Luminal MammaPrint High Risk	HER2 BluePrint HER2	Basal BluePrint Basal	Total
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Total		400	92	53	76	621

Are clinico-pathological data useful in “fine tuning” your treatment decisions towards adjuvant chemotherapy?

Then the basic & confusing data….

Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100 000 women in 123 randomised trials

Early Breast Cancer Trialists' Collaborative Group (EBCTCG)

Lancet 2012; 379: 432–44

Summary

Background Moderate differences in efficacy between adjuvant chemotherapy regimens for breast cancer are plausible,

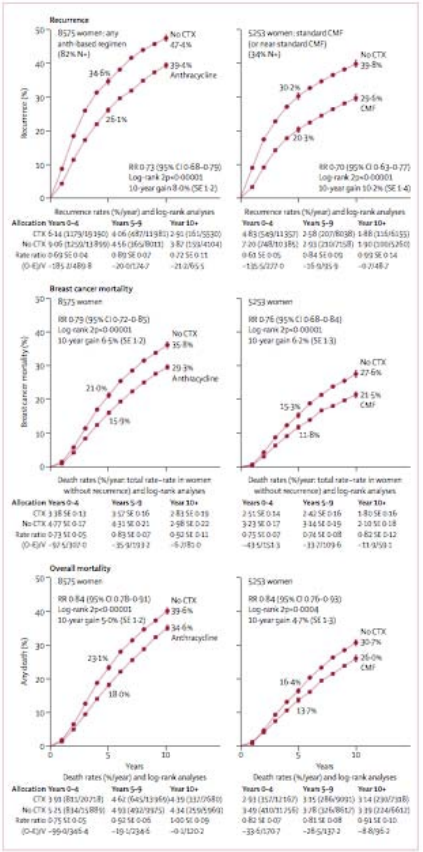

Figure 5: Time to recurrence, breast cancer mortality, and overall mortality for chemotherapy versus no adjuvant chemotherapy

Left: four or more cycles of any anthracycline (Anth)-based regimen—eg, standard 4AC. Right: standard or near-standard CMF. RR (and its 95% CI)=event rate ratio, from summed log-rank statistics for all time periods combined. Gain (and its SE)=absolute difference between ends of graphs. CTX=chemotherapy. Event rates, %/year, are followed by (first events/woman-years). Error bars show ±1 SE.

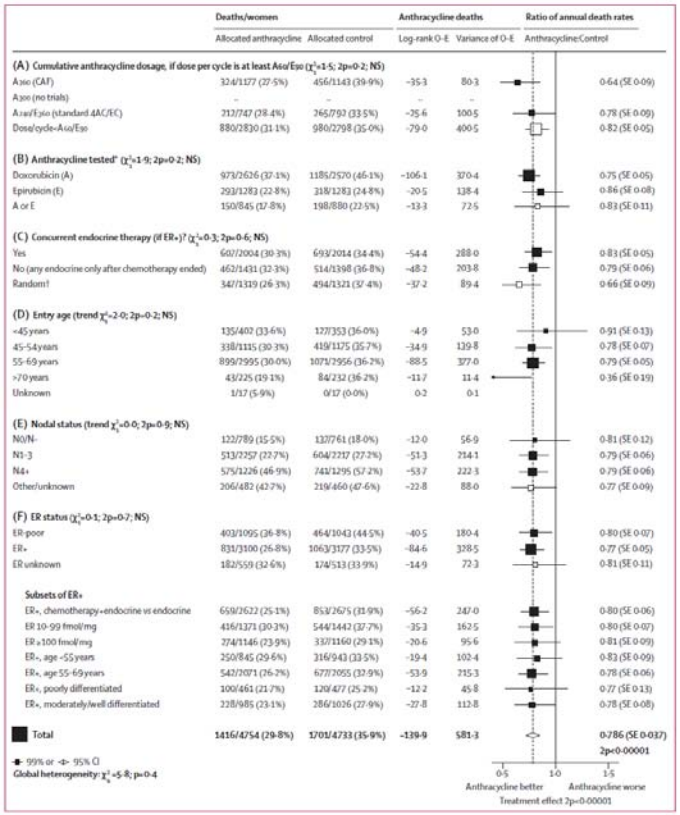

Figure 6: Subgroup analyses of breast cancer mortality (mortality with recurrence, by log-rank subtraction) for any anthracycline-based regimen versus no chemotherapy

A=doxorubicin (Adriamycin). E=epirubicin. Dose/cycle (and cumulative dosage) is given after the drug name in mg/m²: A60/E90 means 60 mg/m² of doxorubicin or 90 mg/m² of epirubicin. NS=not significant. ER=oestrogen receptor. *First four subgroups are as in the forest plots (webappendix pp 33–38) that give details of each trial's cytotoxic regimens. †In the SWOG 8814 trial of CAF in postmenopausal ER+ disease, tamoxifen started randomly with or after the chemotherapy.

Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100 000 women in 123 randomised trials

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(F) ER status (χ^2_1=0·1; 2p=0·7; NS)						
ER-poor	403/1095 (36·8%)	464/1043 (44·5%)	−40·5	180·4		0·80 (SE 0·07)
ER+	831/3100 (26·8%)	1063/3177 (33·5%)	−84·6	328·5		0·77 (SE 0·05)
ER unknown	182/559 (32·6%)	174/513 (33·9%)	−14·9	72·3		0·81 (SE 0·11)
Subsets of ER+						
ER+, chemotherapy+endocrine vs endocrine	659/2622 (25·1%)	853/2675 (31·9%)	−56·2	247·0		0·80 (SE 0·06)
ER 10–99 fmol/mg	416/1371 (30·3%)	544/1442 (37·7%)	−35·3	162·5		0·80 (SE 0·07)
ER ≥100 fmol/mg	274/1146 (23·9%)	337/1160 (29·1%)	−20·6	95·6		0·81 (SE 0·09)
ER+, age <55 years	250/845 (29·6%)	316/943 (33·5%)	−19·4	102·4		0·83 (SE 0·09)
ER+, age 55–69 years	542/2071 (26·2%)	677/2055 (32·9%)	−53·9	215·3		0·78 (SE 0·06)
ER+, poorly differentiated	100/461 (21·7%)	120/477 (25·2%)	−12·2	45·8		0·77 (SE 0·13)
ER+, moderately/well differentiated	228/985 (23·1%)	286/1026 (27·9%)	−27·8	112·8		0·78 (SE 0·08)

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	Deaths/women		Anthracycline deaths		Ratio of annual death rates
	Allocated anthracycline	Allocated CMF	Log-rank O–E	Variance of O–E	Anthracycline:CMF
(A) Cumulative anthracycline dosage, if dose per cycle is at least A60/E90 (trend χ^2_1=8·0; 2p=0·005)					
A_{360} or $E_{720\text{–}800}$ (eg, CAF/CEF)	378/2082 (18·2%)	475/2097 (22·7%)	−50·0	198·0	0·78 (SE 0·06)
A_{300} or $E_{400\text{–}480}$	396/2766 (14·3%)	472/2770 (17·0%)	−35·9	183·1	0·82 (SE 0·07)
A_{240} (standard 4AC)	877/2565 (34·2%)	886/2557 (34·6%)	−8·5	405·6	0·98 (SE 0·05)
Dose/cycle<A_{60}/E_{90}	358/1530 (23·4%)	357/1502 (23·8%)	−11·1	160·1	0·93 (SE 0·08)
(B) Cyclophosphamide in CMF oral/iv (χ^2_1=0·9; 2p=0·3; NS)					
$C_{100\times14}$ oral/cycle	1651/6530 (25·3%)	1834/6525 (28·1%)	−98·8	788·8	0·88 (SE 0·03)
$C_{600\times2}$ iv/cycle	358/2413 (14·8%)	356/2401 (14·8%)	−6·6	157·9	0·96 (SE 0·08)
(C) Concurrent endocrine therapy (if ER+)[?] (χ^2_1=0·0; 2p=1·0; NS)					
Yes	57/502 (11·4%)	62/502 (12·4%)	−2·9	29·0	
No (any endocrine only after chemotherapy ended)	1952/8441 (23·1%)	2128/8424 (25·3%)	−102·5	917·8	0·89 (SE 0·03)
(D) Entry age (trend χ^2_1=0·0; 2p=0·9; NS)					
<45 years	871/3398 (25·6%)	991/3454 (28·7%)	−54·8	422·8	0·88 (SE 0·05)
45–54 years	738/3399 (21·7%)	773/3356 (23·0%)	−30·6	344·3	0·91 (SE 0·05)
55–69 years	375/1961 (19·1%)	396/1920 (20·6%)	−20·2	169·3	0·89 (SE 0·07)
>70 years	18/106 (17·0%)	25/112 (22·3%)	−2·2	8·7	
Unknown	7/79 (8·9%)	5/84 (6·0%)	2·4	1·8	
(E) Nodal status (trend χ^2_1=0·9; 2p=0·3; NS)					
N0/N–	461/3865 (11·9%)	541/3869 (14·0%)	−40·5	233·1	0·84 (SE 0·06)
N1–3	520/2442 (21·3%)	543/2418 (22·5%)	−10·0	243·4	0·96 (SE 0·06)
N4+	612/1234 (49·6%)	647/1233 (52·5%)	−23·1	273·4	0·92 (SE 0·06)
Other/unknown	416/1402 (29·7%)	459/1406 (32·6%)	−31·9	196·8	0·85 (SE 0·07)
(F) ER status (χ^2_1=0·1; 2p=0·8; NS)					
ER-poor	120/4488 (26·8%)	1287/4518 (28·5%)	−43·7	564·6	0·93 (SE 0·04)
ER+	569/3279 (17·4%)	610/3257 (18·7%)	−26·5	267·0	0·91 (SE 0·06)
ER unknown	239/1176 (20·3%)	293/1151 (25·5%)	−35·2	115·2	0·74 (SE 0·08)
Subsets of ER+					
ER 10–99 fmol/mg	247/1072 (23·0%)	279/1094 (25·5%)	−21·2	108·3	0·82 (SE 0·09)
ER ≥100 fmol/mg	86/450 (19·1%)	116/450 (25·8%)	−15·4	42·0	0·69 (SE 0·13)
ER+, age <55 years	426/2359 (18·1%)	461/2345 (19·7%)	−22·9	202·3	0·89 (SE 0·07)
ER+, 55–69 years	134/846 (15·8%)	140/847 (16·5%)	−3·6	61·1	0·94 (SE 0·12)
ER+, poorly differentiated	131/868 (15·1%)	130/793 (16·4%)	−4·1	52·7	
ER+, moderately/well differentiated	125/952 (13·1%)	136/1047 (13·0%)	−1·8	58·3	
Total	**2009/8943 (22·5%)**	**2190/8926 (24·5%)**	**−105·4**	**946·8**	**0·895 (SE 0·031)**
					2p=0·0006

99% or <> 95% CI

Global heterogeneity: χ^2_6=9·9; p=0·1

0·5 1·0 1·5

Anthracycline better CMF better

Treatment effect 2p=0·0006

Figure 4: **Subgroup analyses of breast cancer mortality (mortality with recurrence, by log-rank subtraction) for any anthracycline-based regimen versus standard CMF (or near-standard CMF)**

A=doxorubicin (Adriamycin). E=epirubicin. Dose/cycle (and cumulative dosage) is given after the drug name in mg/m²; A_{60}/E_{90} means 60 mg/m² of doxorubicin or 90 mg/m² of epirubicin. iv=intravenous. NS=not significant. ER=oestrogen receptor. IHC=immunohistochemistry. *First four subgroups are as in the forest plots (webappendix pp 27–32) that give details of each trial's cytotoxic regimens.

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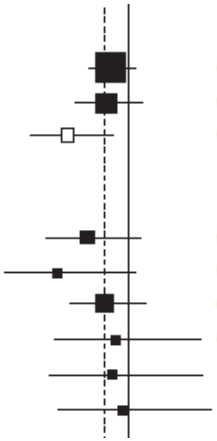


Value of molecular subtyping and prediction to effect of chemotherapy

- Classification often dependent of method used
- Despite differences in gene lists, outcome similar
- Most signatures discriminate based on ER-status and proliferative activity
- Prognostic value restricted to ER-positive tumors
- Subclassification ER-positive breast cancer in luminal A and luminal B is arbitrary, based on proliferation
- Expression signatures are complementary to standard clinico-pathological parameters

Weigelt et al., Nature Reviews Clinical Oncology (2011)

Predictive profiles fail

- Even the best arrays unable to give a sufficient signal at low expression of very relevant genes
- Subtle, non detectable changes in level of expression can make the difference
- Expression profiling unable to pick up resistance mechanisms if such a mechanism is only present in a proportion of the tumors
- Tumors are heterogeneous, RNA bulk analysis will not help

Borst & Wessels, Cell Cycle (2011) 9: 4836

Integration of tumor features is essential

- Adequate morphological diagnosis
- Robust and reliable IHC-panel
  - ER, PR, HER2, (Ki-67)
- Gene signature has additional value for a substantial subgroup
- Requires for all disciplines sufficient volume and expertise

The clinical issue.
Think step by step
(the most difficult one)

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)

My conlusions (for debate)

- Is every luminal A a luminal A? No, there are some high risk cancers between them
- What makes luminal B a luminal B? The proliferation/propensity to disseminate: you need extra information because you do not see it sufficiently on standard pathology/IHC (Ki 67: to much differences in quality, to many ‘in betweens’, not proven to be chemopredictive).

The clinical issue.
Think step by step
(the most difficult one)

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)

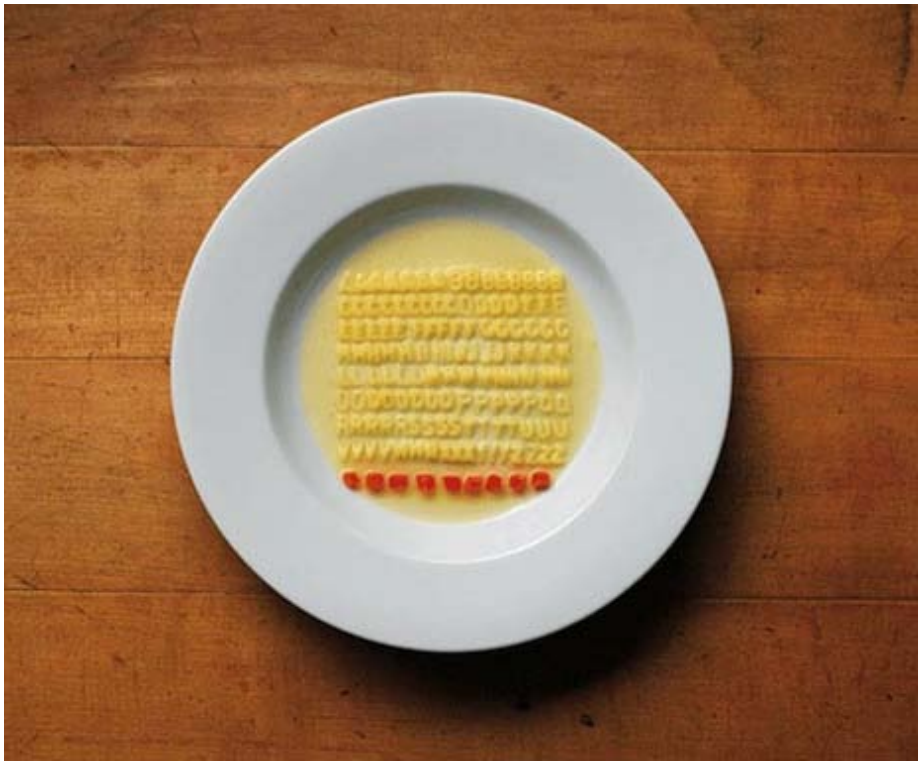
My conlusions (for debate)

- What is the effect of chemotherapy: different for luminal A or B?

  Actually not proven: depends on prognosis & prediction

  (and now, I'm sorry, the circle is round again)

Did you get some order out of chaos?



日本乳がん情報ネットワーク

Japan Comprehensive Cancer Network, Breast (JCCNB)

Thanks to the patients and all those who provided me with the presented information,
and for inviting me, your attention & discussion

Intermediate Clinico/Pathological Risk

What to do?

- Treat all patients with chemotherapy?
- Or be more selective, and treat those patients who benifit

  (and thus minimizing the risk of losing lives by foregoing chemotherapy)

70-gene assay (MammaPrint)

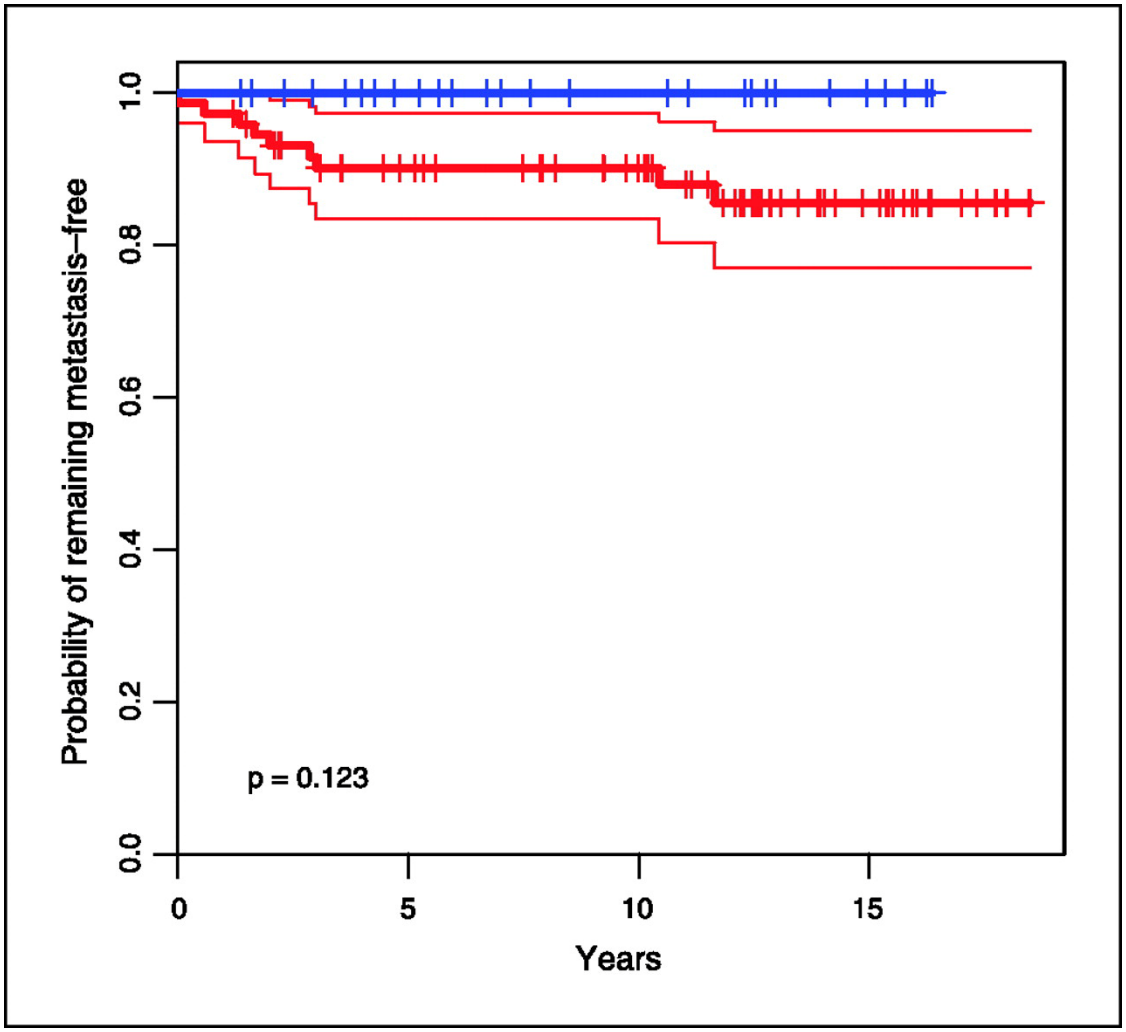
- Is not just another prognostic factor
- Is designed from the beginning to tell you the metastatic potential of an individual breast cancer

70-gene MammaPrint

- Functions of many genes are identified and are all related to the process of dissemination including proliferation

Validation 4: N = 100

Wittner et al., Clin Cancer Res 14: 2988, 2008



MGH series, Boston;

Time to metastasis

Aims RASTER study

- Feasibility of using 70-gene signature in community-based settings
- Effect of 70-gene signature on adjuvant systemic treatment (AST) decisions
  - AST decision at that time based on restrictive Dutch National Guideline 2004, 70GS result and doctors' and patients' preferences
- Outcome after 5 and 10 years of follow-up

Current aim RASTER study

- Outcome after 5 years of follow-up

- What would the risk estimation of the RASTER cohort be with currently used risk estimation tools to guide AST decisions

  – Adjuvant!Online

Inclusion criteria

- Female
- cT1-4N0M0 invasive breast cancer
- Age < 61 years, amended to < 55 years (after 242 patients had been enrolled)
- Operable, unilateral tumor
- No history of previous malignancy, except for basal cell carcinoma or cervical carcinoma in situ
- No neoadjuvant systemic therapy

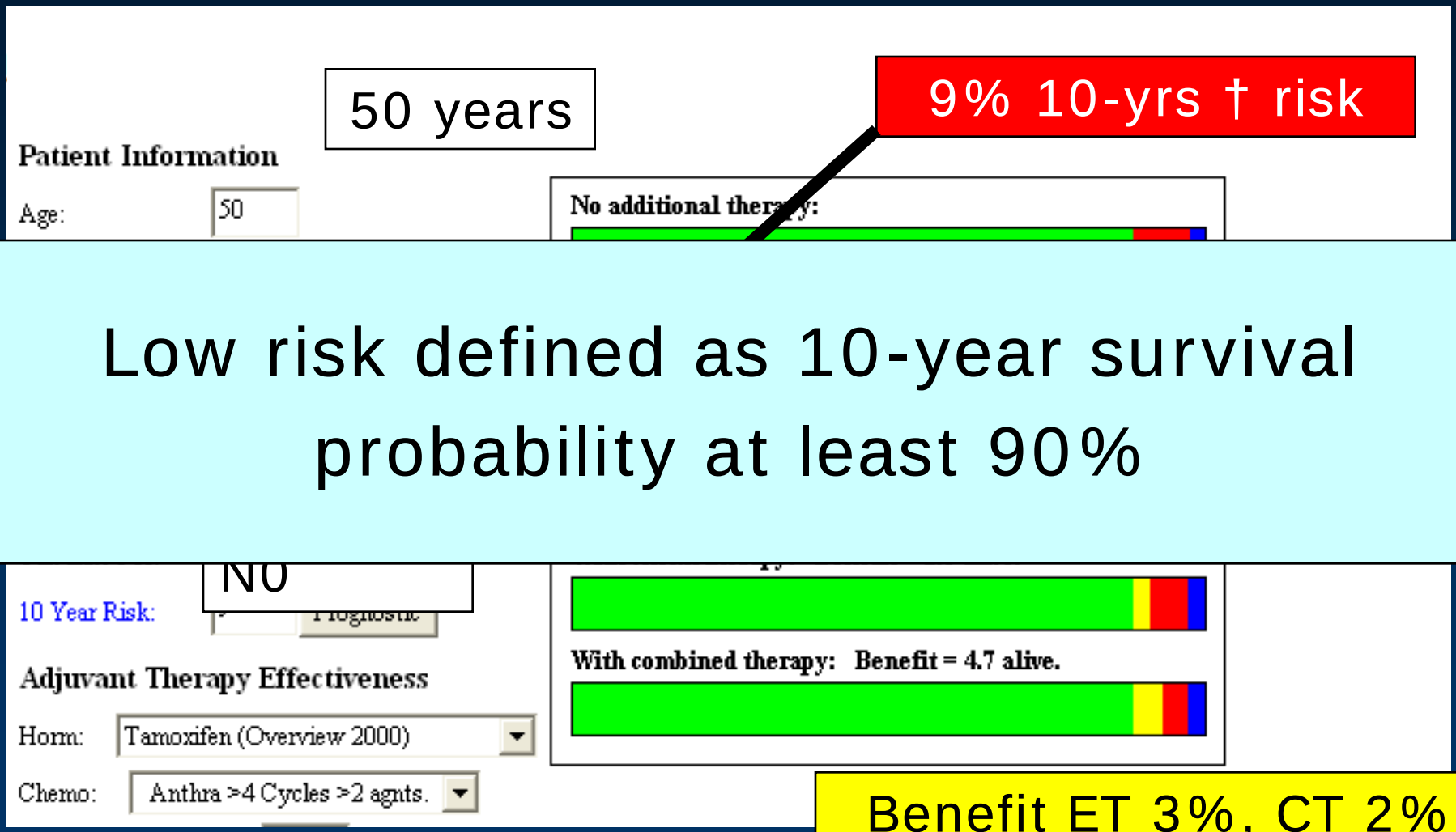
Dutch guideline 2004

RASTER enrollment 2004-2006

High risk

- N+
- N0; ≤35 years
  - except for tumor ≤ 1 cm grade I
- N0; > 35 years:
  - Larger than 1 cm grade III
  - Larger than 2 cm grade II
  - Larger than 3 cm any grade

Adjuvant Online version 8.0



Low risk defined as 10-year survival probability at least 90%

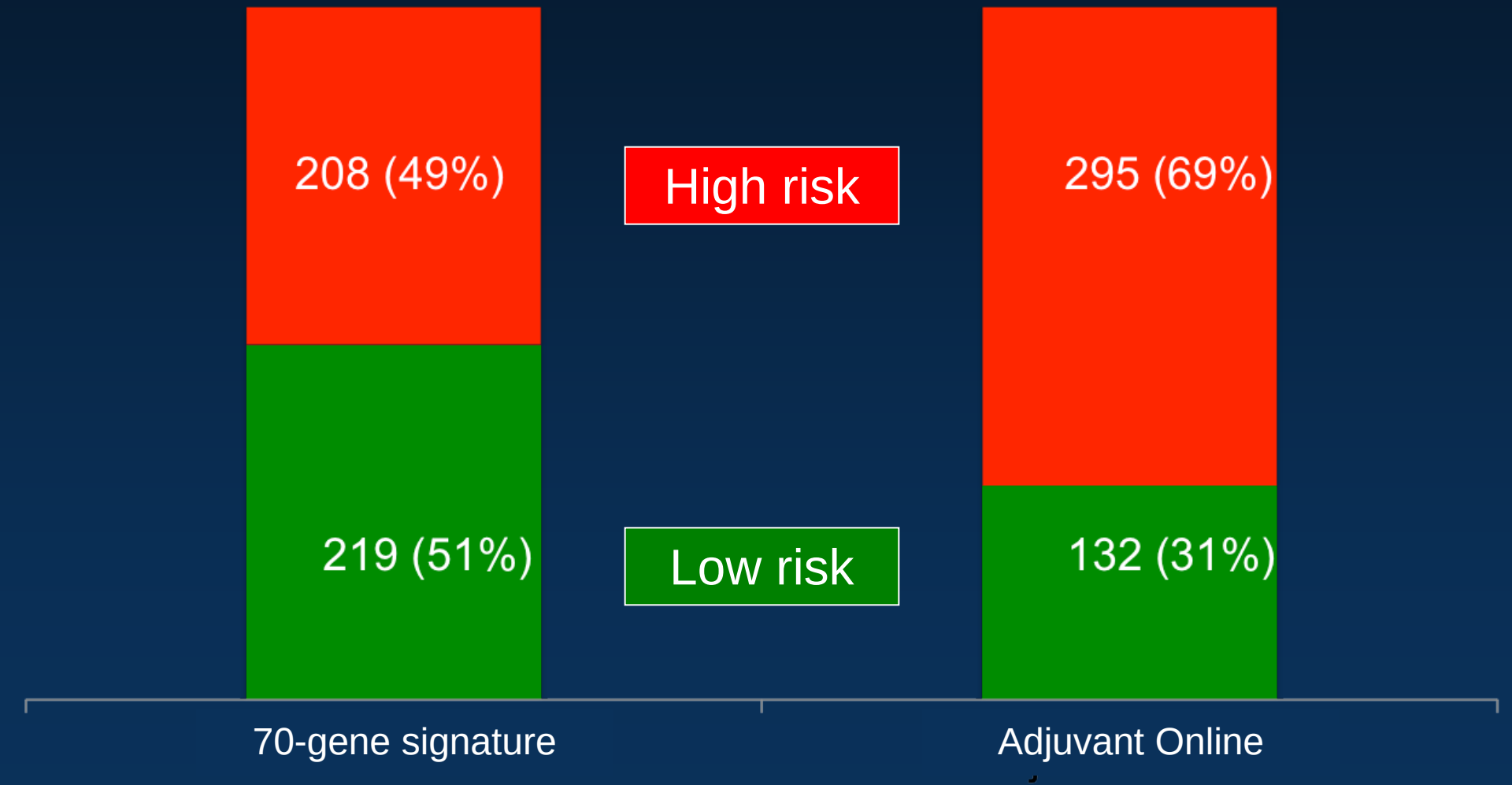
Benefit ET 3%, CT 2%

www.adjuvantonline.com

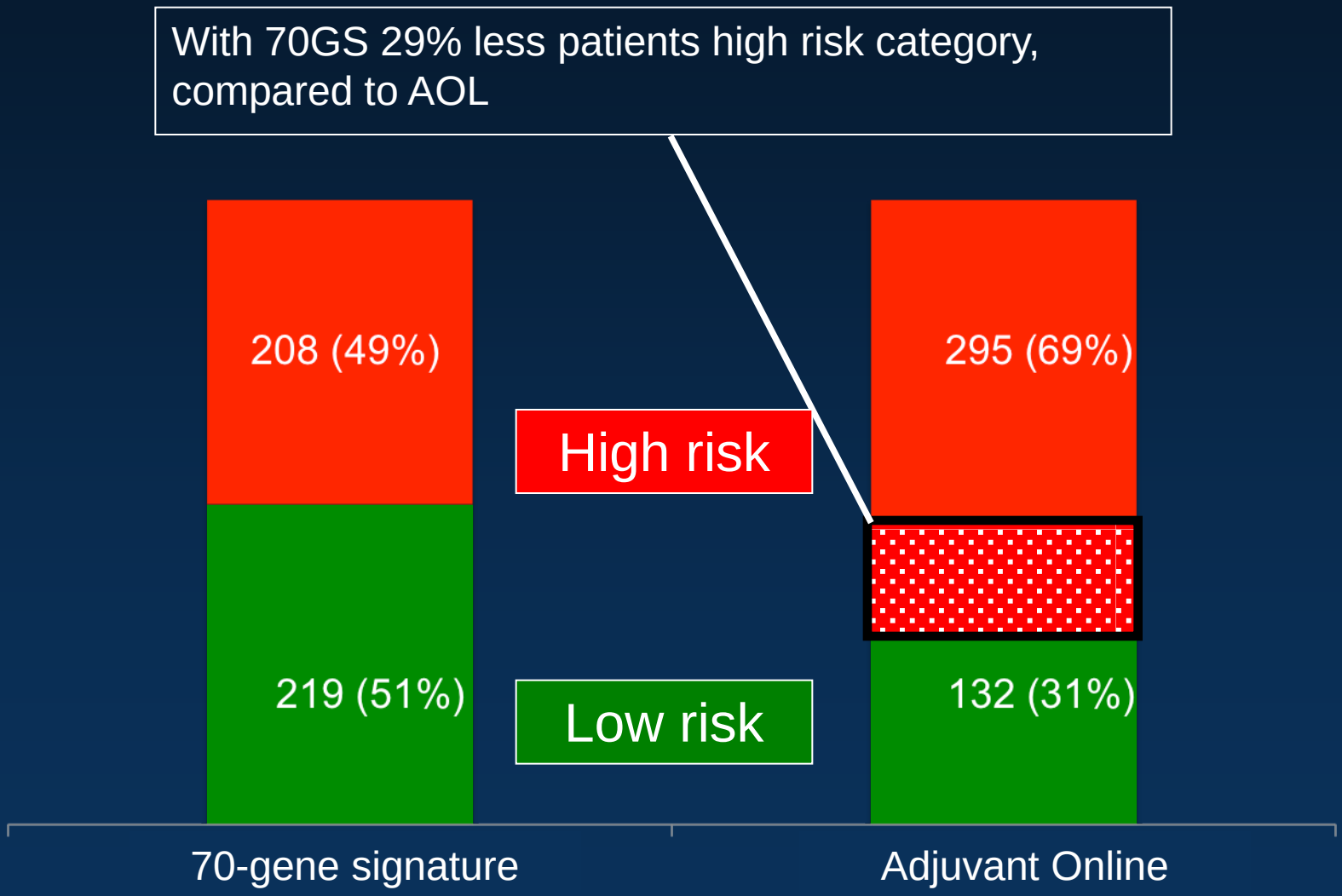
Results

- 427 patients tested between 2004-2006
- Median FUP time 61.6 months
- 33 DDFS events
  - DDFS event = distant recurrence, death (any cause), 2nd primary other than breast
- 11 deaths
- 9 breast cancer specific deaths

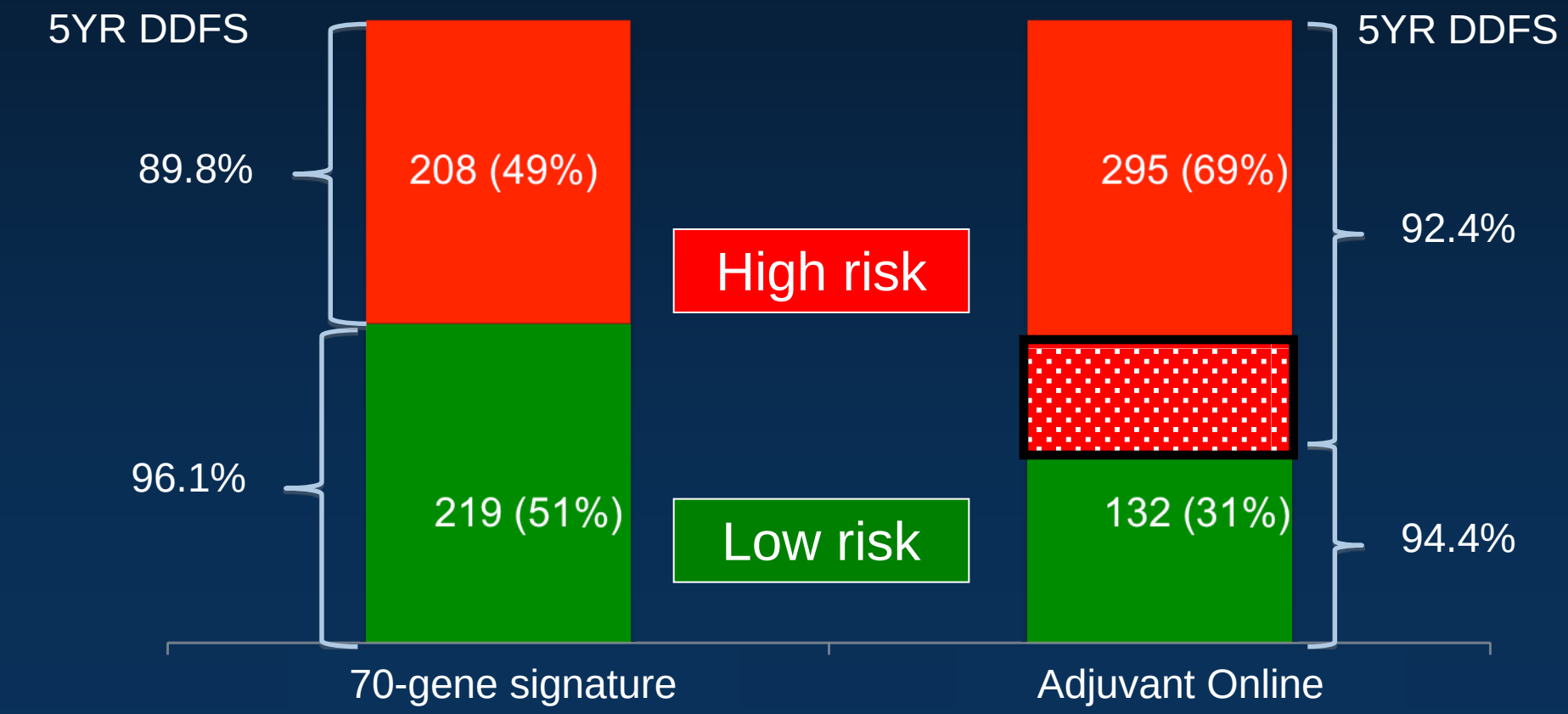
Proportion of patients labeled as high risk



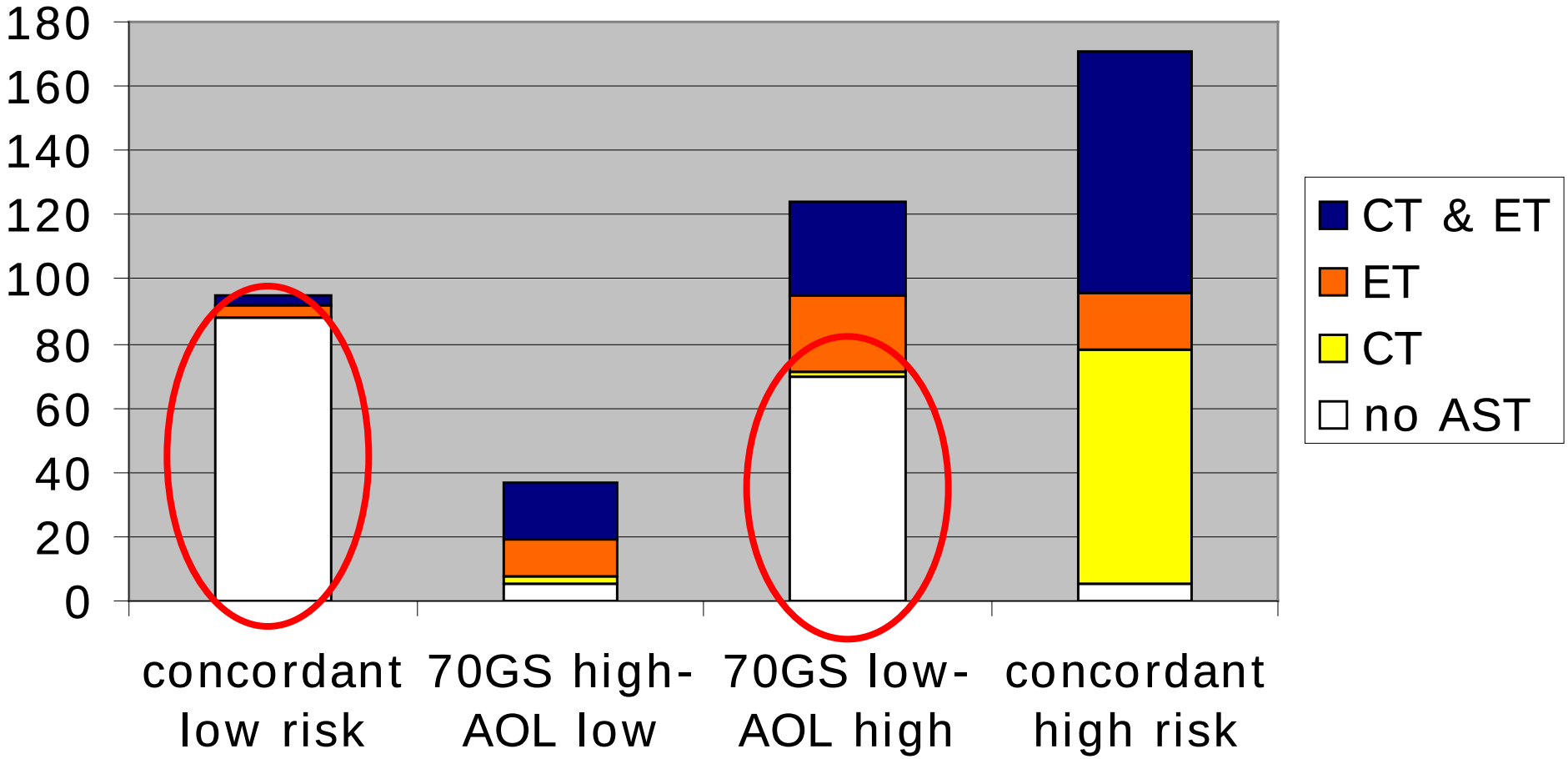
With 70GS 29% less patients high risk



5-year DDFS of 427 patients according to 70GS or AOL



70GS-AOL risk groups and AST



Patient characteristics discordant group

n=94 patients no AST or ET only

Age 45-55 years	75%
pT1 (< 2 cm)	80%
Grade II	82%
IDC / ILC	72 / 20%
ER pos	98%
PgR pos	78%
HER neg	90%

Conclusions

- AOL high risk and 70GS low risk patients who did not receive adjuvant systemic therapy or hormonal therapy only had an excellent 5-year DDFS (97.8%)
- This percentage is unlikely to drop below 90% at 10 years of follow-up
- Of this patient group at least 80% had an ER positive, HER2 negative, grade II tumor of 1 to 2 cm in size
- The percentage of high risk patients could be reduced by almost 30% when 70GS risk estimation was used

Acknowledgements

All participating patients

máxima medisch centrum gewoon goed

Diakonessenhuis

Spaarne ZIEKENHUIS SPECIALISTEN IN MENSENWERK

Reinier de Graaf Groep

nij smellinghe ziekenhuis · drachten
Met aandacht. Dat voelt beter.

gelre ziekenhuizen

Medisch Spectrum Twente

Rijnstate

olvg onze lieve vrouwe gasthuis

atrium medisch centrum parkstad

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Ziekenhuis Amstelland

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Rijnland ziekenhuis

agendia decoding cancer.

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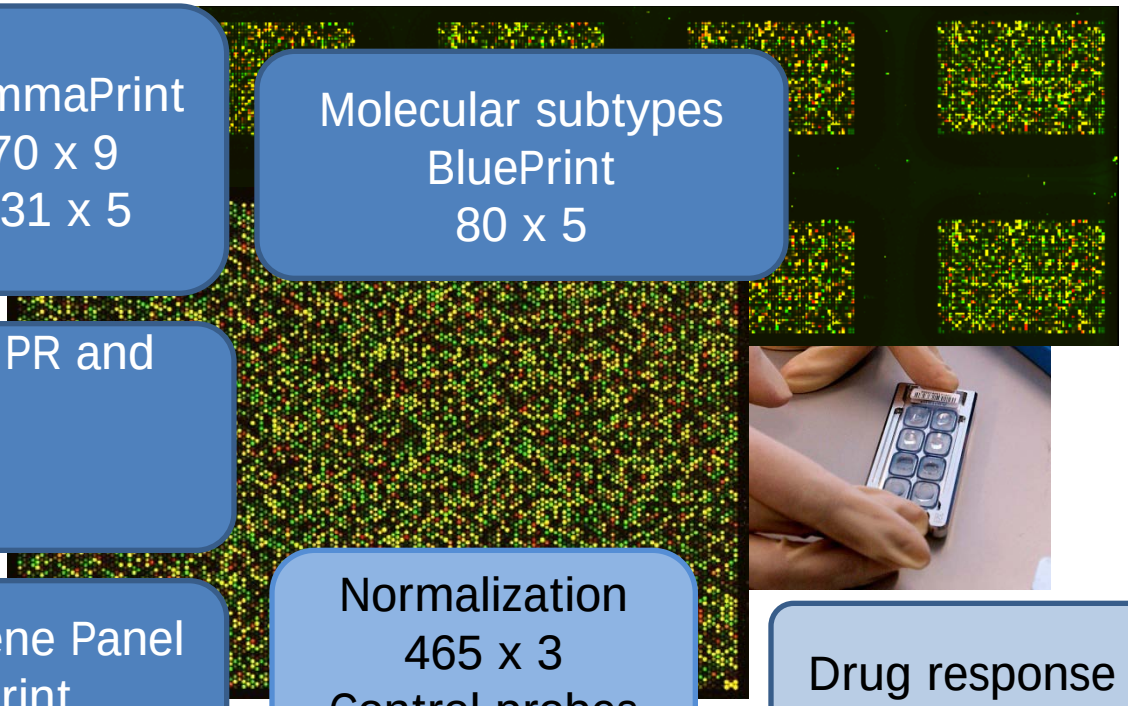
NKI-AVL

Multiple answers from a single array



MammaPrint
70 x 9
231 x 5

Molecular subtypes
BluePrint
80 x 5

mRNA readout ER, PR and HER2
TargetPrint
3 x 5

Research Gene Panel
TheraPrint
56 x 3

Normalization
465 x 3
Control probes
536

Drug response profile

Drug response profile