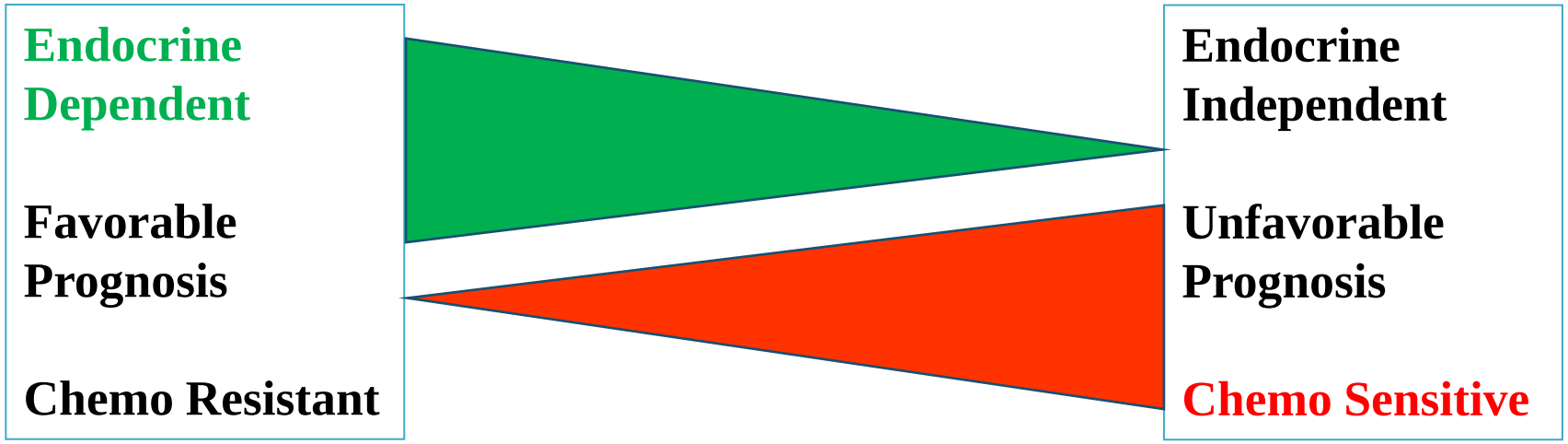
Prediction of 10-year distant recurrence (DR) using the Prosigna® (PAM50) test in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5-year of endocrine therapy (ET) alone.

Anne-Vibeke Lænkholm[1], Maj-Britt Jensen[2], Jens Ole Eriksen[1], Torben Kibøl[1], Birgitte Bruun Rasmussen[3], Ann S. Knoop[4], Sean Ferree[5], Taryn Haffner[5], Carl Schaper[5], Bent Ejlertsen[2,4]

1. Department of Pathology, Region Zealand
2. The Danish Breast Cancer Cooperative Group, DBCG Secretariat, Rigshospitalet
3. Department of Pathology, Herlev Hospital
4. Department of Oncology, Rigshospitalet
5. NanoString Technologies, Inc., Seattle, WA

Breast Cancer Intrinsic Subtypes



Lum A | **Lum B** | **HER2-enriched** | **Basal-like**

Background PAM50/ROR

- 50-gene test developed to identify the intrinsic breast cancer subtypes (luminal A, luminal B, HER2-enriched, Basal-like)
- Subtypes used to generate a Risk of Recurrence (ROR) Score
  - Including
    - Tumor size
    - Proliferation (18 genes)
    - Number of positive lymph nodes
- Designed to be performed in local pathology laboratories on FFPE tissue.

Purpose:

- The purpose of this study was to validate that, within the intended population, NanoString's PAM50 assay can identify:

  A substantial number of node negative and positive women treated with 5 years of endocrine therapy who have a risk of distant recurrence at 10 years of <10% and, therefore, may be spared over-treatment with the addition of adjuvant chemotherapy to endocrine therapy.

Validation Study:

Gnant M et al. Identifying clinically relevant prognostic subgroups of postmenopausal women with node-positive hormone receptor-positive early-stage breast cancer treated with endocrine therapy: a combined analysis of ABCSG-8 and ATAC using the PAM50 risk of recurrence score and intrinsic subtype.

Ann Oncol. 2015 Aug;26(8):1685-91. doi: 10.1093/annonc/mdv215.

Objectives:

- Demonstrate that the PAM50 ROR score provides additional prognostic information for time to distant recurrence (TDR) over and above standard clinical variables in patients with each of 1, 2 and 3 positive nodes, both
  - (a) as a continuous measure (ROR score)

    and
  - (b) using pre-defined risk groups

- Demonstrate that the PAM50 ROR score provides additional prognostic information for time to distant recurrence (TDR) over and above standard clinical variables for all patients regardless of nodal status.

Primary end point:

Time to Distant Recurrence (TDR)

TDR: Interval from diagnosis until distant recurrence, or death due to breast cancer.

DBCG 99C Protocol
ER+ (≥10%) postmenopausal breast cancer patients
Endocrine treatment: 5 years

High Risk:

Tumor > 2 cm

or

N+

or

ductal grade II-III

Treatment:

Tamoxifen (TAM) + Aromatase inhibitor (AI)

AI + TAM

TAM

AI

The DBCG 99C cohort study consisted of N=6,529 patients, of which N=2,971 met the selection criteria for the current study:

Inclusion year 2000-2003

Number of positive nodes between 0 and 3

Median F/U: 9.25 years

Ejlertsen B et al. Acta Oncol. 2014 Feb;53(2):174-85.

Flow Chart

DBCG 99C Study Cohort
N = 6,259

Did not meet selection criteria (N = 3558)

Patients Available for testing
N = 2,971

Not included in analysis (N = 231)

- Insufficient Tumor Tissue (N = 9)
- No tumor block (N = 137)
- No tumor tissue (N = 53)
- DCIS (N = 7)
- No description in the pathology system (N = 24)
- Block could not be cut (N = 1)

Patients Analyzed
N = 2,740

Excluded from passing results (N = 14)

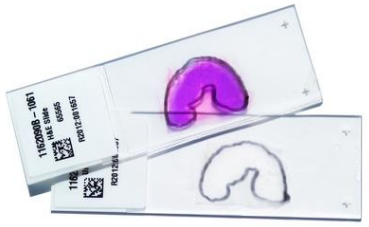
- Insufficient RNA quality (N = 5)
- Failed the Prosigna geomean QC metric (N = 7)
- Failed due to high background (N = 2)

Patients with Passing Results
N = 2,726

Excluded from analysis (N = 4)

- Data entry error (N = 3)
- Node-negative patient (N = 1)

Patients Included in Analysis
N = 2,722



Measured Tumor Surface Area (H&E stained slide)	Number of Unstained Slides to Use*
4 – 19 mm^2	6
20 – 99 mm^2	3
More than 100 mm^2	1

Prediction of 10-year distant recurrence
Patient characteristics:

Characteristic	Study Population
Age (yr)	N (%)
50-59	1013 (37%)
60-69	1142 (42%)
≥70	567 (21%)
Tumor Size (mm)	
≤ 10	260 (10%)
11-20	1166 (43%)
21-30	913 (34%)
>30	383 (14%)
Number of Pos Nodes	
0	1256 (46%)
1	809 (30%)
2	426 (16%)
3	231 (8%)
Histological Subtype	
Ductal	2277 (84%)
Lobular	351 (13%)
Other	94 (3%)
Histological Grade	
1	644 (24%)
2	1452 (53%)
3	358 (13%)
Not graded	268 (10%)

Molecular Intrinsic subtype	N(%)
Luminal A	1.515 (55.7%)
Luminal B	977 (35.9%)
HER2 Enriched	203 (7.5%)
Basallike	27 (1%)

Prediction of 10-year distant recurrence Results:

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Description of intrinsic subtypes by number of positive nodes

Intrinsic Subtype*	N [%]		Prob of 10-yr DR [95% CI]	
	Node Negative	Node-Positive (1-3 Nodes)	Node-Negative	Node-Positive (1-3 Nodes)
Luminal A	632 [50.3%]	883 [60.2%]	6.3% [4.4-8.6]	8.7% [6.7-10.9]
Luminal B	502 [40.0%]	475 [32.4%]	14.1% [10.9-17.7]	22.2 [18.1-26.7]
Luminal A/B P-value	-	-	<0.0001	<0.0001

Cumulative incidence by risk group and nodal status

Nodal Status	Risk Category	10-Year DR [95% CI]	P values	
			Any Diff.	Diff. from Int
Node-Negative	High (497)	18.5 [14.9-22.4]	<0.0001	<0.0001
	Intermediate (389)	7.3 [4.8-10.5]		
	Low (370)	4.9 [2.8-7.8]		0.1543
Node-Positive (1-3 nodes)**	High (1103)	21.9 [18.9-25.1]	<0.0001	NA
	Low (363)	4.8 [3.1-6.9]		

Validation of prediction of distant recurrence (DR)
in a subgroup of node positive patients:

N: 1466

Follow up – 9.25 years

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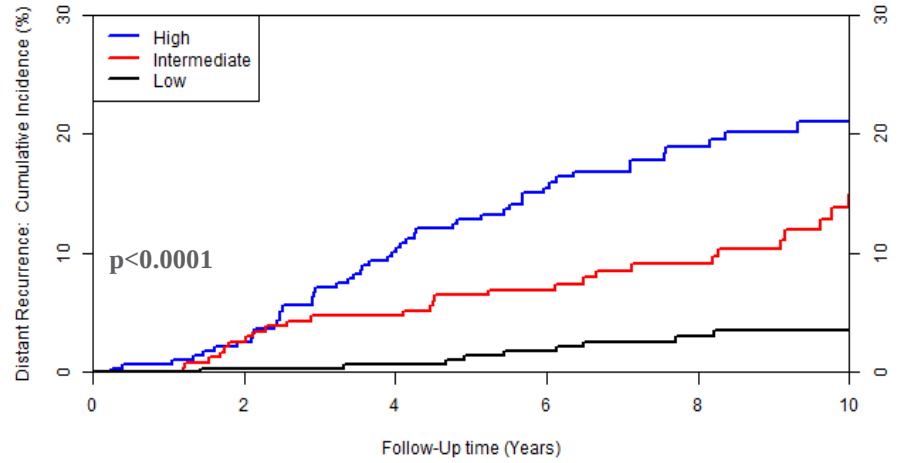
DR incidence by ROR-based risk groups for N+ patients

Nodal Status	Risk Category	N	10-Year DR [95% CI]	P values	
				Any Diff.	Diff. from Int
All Patients (1-3 Positive Nodes)	High	703	22.4% [19.1-25.9]	<0.0001	<0.0001
	Intermediate	400	11.8% [8.4-15.9]		-
	Low	363	3.8% [2.0-6.3]		0.0007
1-Positive Node	High	274	21.0% [15.9-26.6]	<0.0001	0.0202
	Int	237	14.9% [9.9-20.9]		-
	Low	298	3.6% [1.7-6.5]		0.0001
2-Positive Nodes	High	224	20.7% [15.2-26.8]	0.0007	0.0034
	Int	137	8.7% [4.4-15.0]		-
	Low	65	4.6% [1.2-11.8]		0.4165
3-Positive Nodes	High	205	26.1% [19.5-33.0]	0.0086	0.0086
	Int	26	0% [NA]		-
	Low	N/A	-		-

37% of patients with 1 and 15% with 2 positive nodes who have an excellent prognosis

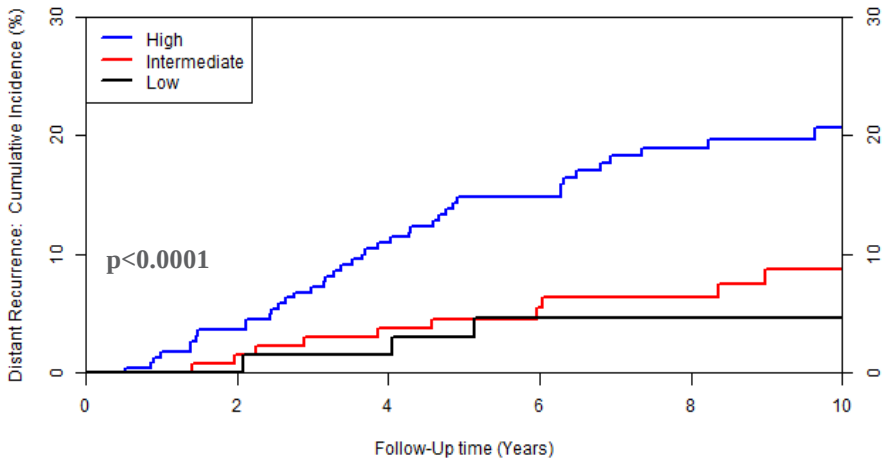
Validation of prediction of distant recurrence (DR) in a subgroup of node positive patients:

Cumulative DR incidence for patients with 1 positive node



Risk Group:		Cumulative Incidence: Distant Recurrence				
High	(n=274)	2.6	10.2	15.5	19.0	21.0
Intermediate	(n=237)	2.6	4.7	6.9	9.1	14.9
Low	(n=298)	0.3	0.7	1.8	3.1	3.6

Cumulative DR incidence for patients with 2 positive nodes



Risk Group:		Cumulative Incidence: Distant Recurrence				
High	(n=224)	3.6	11.0	14.8	18.9	20.7
Intermediate	(n=137)	1.5	3.8	5.5	6.4	8.7
Low	(n= 65)	0.0	1.5	4.6	4.6	4.6

In multivariate analysis (Cox proportional hazards regression model and Fine and Gray´s model), the PAM50 derived ROR score provided prognostic information in addition to and beyond established clinical factors for predicting distant recurrence: p< 0.0001 (1 positive node), p< 0.0002 (2 positive nodes)

In Conclusion

- Prosigna (PAM50) accurately discriminate postmenopausal patients with ER positive breast cancer according to risk of recurrence.
- a proportion of postmenopausal patients in a real-world setting could be spared chemotherapy (CT) following appropriate endocrine therapy.
- Prosigna can identify at least 37% of patients with 1 and 15% with 2 positive nodes who have an excellent prognosis and, in conjunction with other clinicopathological factors, may be spared adjuvant CT in a real world setting.

DBCG: Arbejdsgruppe genprofil

- Formål: At mindske andelen af patienter der anbefales kemoterapi
  - Aktuelt modtager ca. 95% af danske brystkræftpatienter systemisk adjuverende behandling og 57% får kemoterapi (= protokolallokerede patienter, DBCG kvalitetsindikatorrapport 2014).
  - Den relative risikoreduktion ved kemoterapi er ca. 30%
  - Genekspressionsprofiler kan identificere patienter med en så god prognose at behandling med kemoterapi synes unødig.
  - Status: Man afventer HER2 resultater fra DBCG99C studiet med henblik på at foretage endelig beregning af hvor mange patienter der kan skånes for kemoterapi-
  - DBCG arbejdsgruppe:
    - Medicinsk udvalg: Ann Knoop, Bent Ejlertsen
    - Patologiudvalg: Eva Balslev, Maj-Lis Møller Talman, Trine Tramm, Anne-Vibeke Lænkholm

Tak til

patologiafdelingerne
for hjælp
med indsamling af materialet

Background
Perou, Sørlie et al, Nature 2000
Molecular portraits of human breast tumours

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Intrinsic gene subset: 476 genes

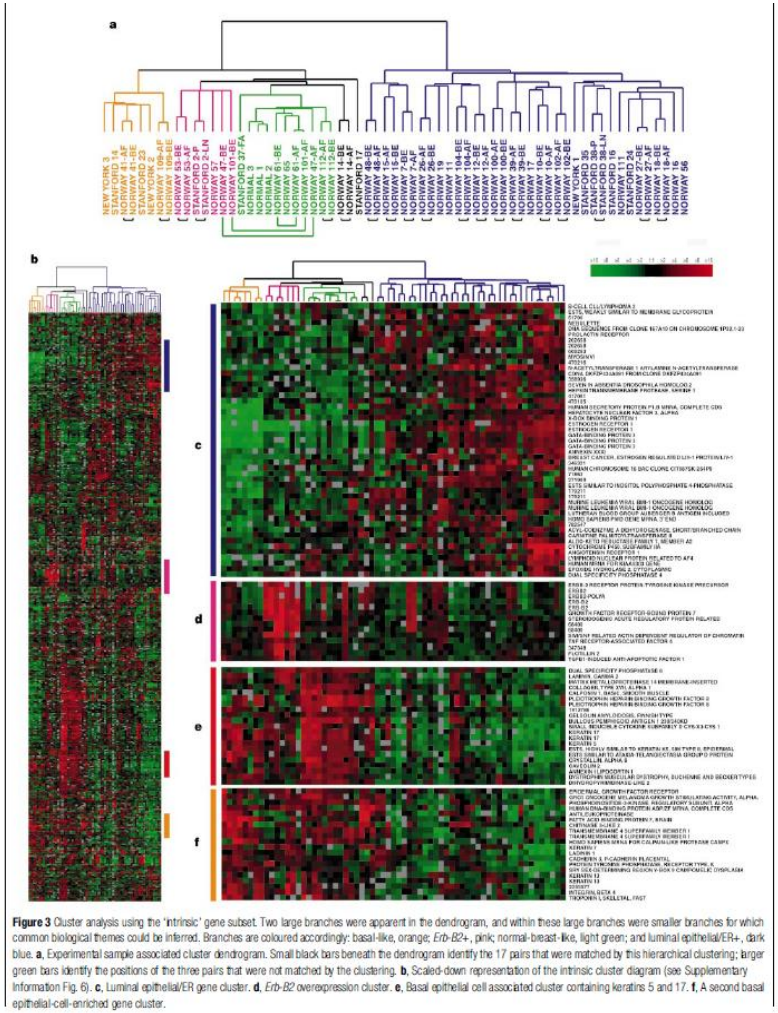

Figure 3 Cluster analysis using the 'intrinsic' gene subset. Two large branches were apparent in the dendrogram, and within these large branches were smaller branches for which common biological themes could be inferred. Branches are coloured accordingly: basal-like, orange; *Erb-B2+*, pink; normal-breast-like, light green; and luminal epithelial/ER+, dark blue. **a**, Experimental sample associated cluster dendrogram. Small black bars beneath the dendrogram identify the 17 pairs that were matched by this hierarchical clustering; larger green bars identify the positions of the three pairs that were not matched by the clustering. **b**, Scaled-down representation of the intrinsic cluster diagram (see Supplementary Information Fig. 6). **c**, Luminal epithelial/ER gene cluster. **d**, *Erb-B2* overexpression cluster. **e**, Basal epithelial cell associated cluster containing keratins 5 and 17. **f**, A second basal epithelial-cell-enriched gene cluster.

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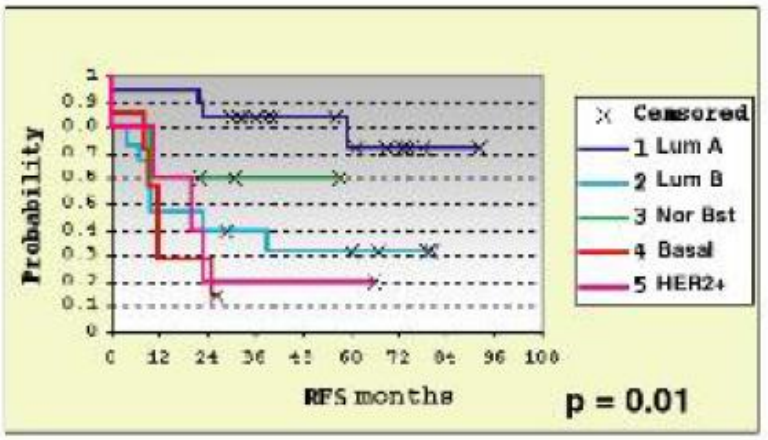
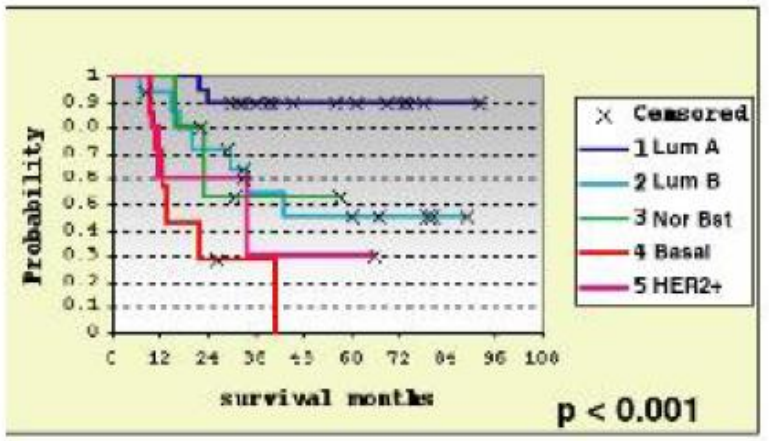
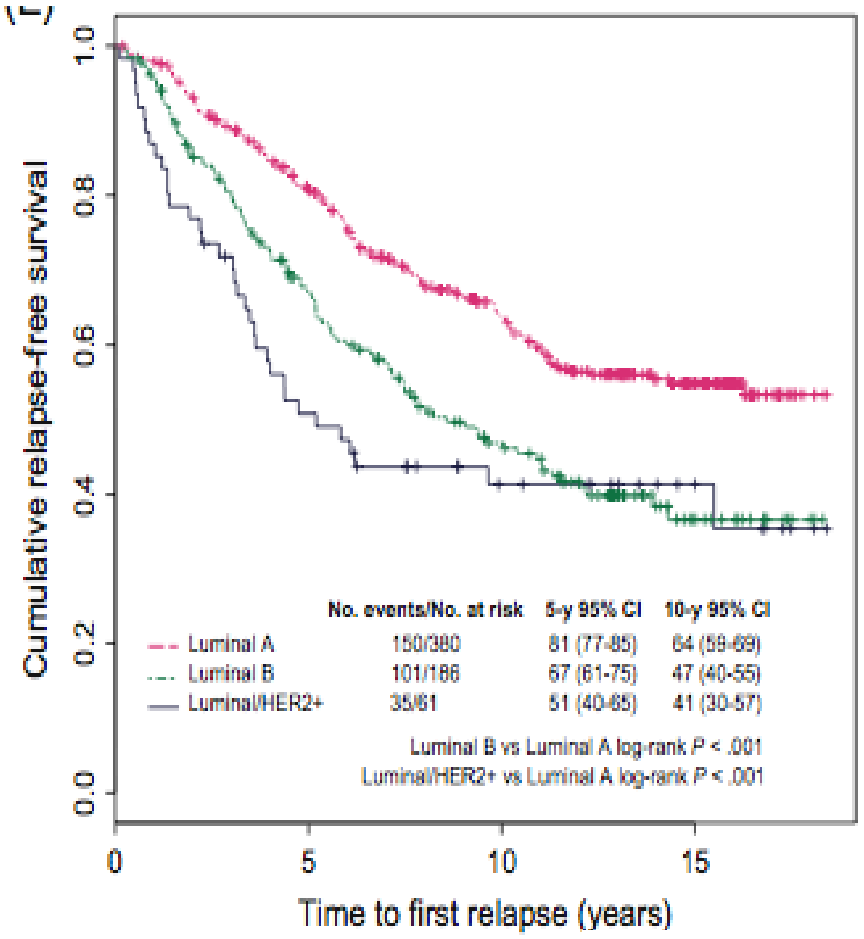


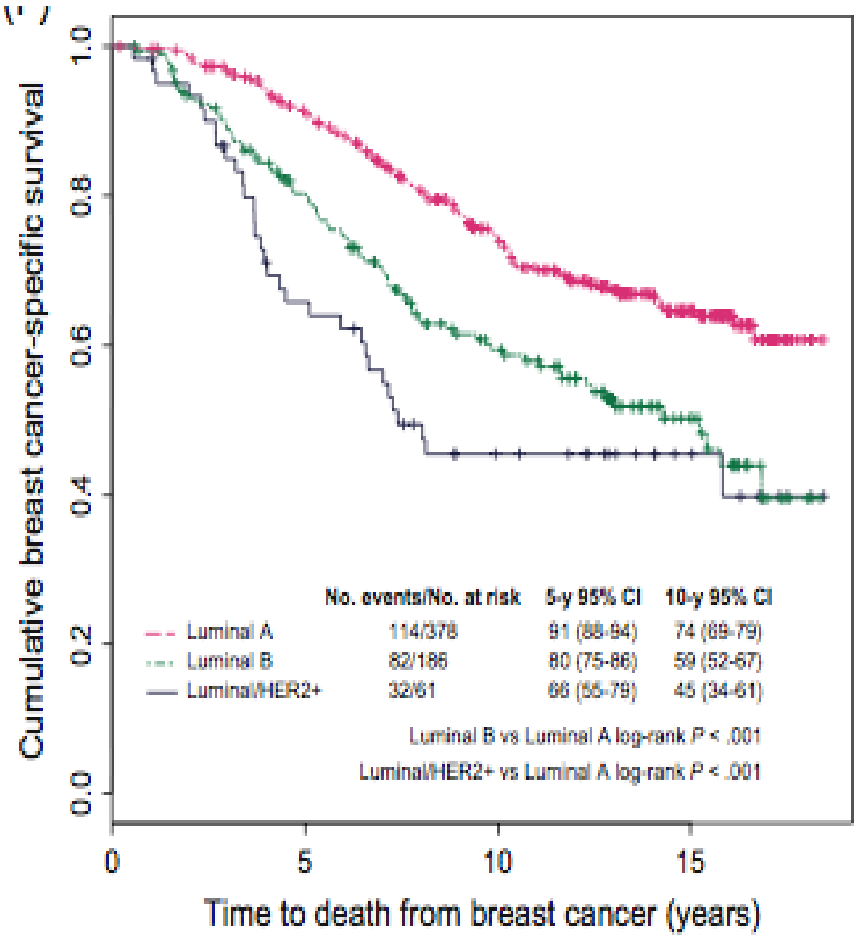


Perou & Sorlie et al, Nature 2000

Background

- Intrinsic subtypes are proven prognostic in many settings with specific interest for the luminal A versus Luminal B subtype in ER positive postmenopausal breast cancer patients.





J Natl Cancer Inst 2009;101:736–750

St Gallen International Expert Consensus 2015
Annals of Oncology 00: 1–14, 2015
doi:10.1093/annonc/mdv221

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Table 2. Treatment-oriented classification of subgroups of breast cancer

Clinical grouping	Notes
Triple-negative	Negative ER, PgR, and HER2
Hormone receptor-negative and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-negative luminal disease as a spectrum:	ER and/or PgR positive ≥1%[a]
High receptor, low proliferation, low tumor burden (luminal A-like)	Multiparameter molecular marker 'favorable prognosis' if available. High ER/PgR and clearly low Ki-67[b]. Low or absent nodal involvement (N 0–3), smaller T size (T1 T2).
Intermediate	Multiparameter molecular marker 'intermediate' if available[c]. Uncertainty persists about degree of risk and responsiveness to endocrine and cytotoxic therapies.
Low receptor, high proliferation, high tumor burden (luminal B-like)	Multiparameter molecular marker 'unfavorable prognosis' if available. Lower ER/PgR with clearly high Ki-67[b]. More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).

[a]ER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.
[b]Ki-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low.
[c]Not all multiparameter molecular marker tests report an intermediate score.

DOI: 10.1093/jnci/djr393
Advance Access publication on September 29, 2011.

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.

Correspondence to: Mitch Dowsett, BSc, PhD, Department of Biochemistry, Royal Marsden Hospital and Breakthrough Breast Cancer Centre, Fulham Rd, London SW3 6JJ, UK (e-mail: mitch.dowsett@icr.ac.uk).

Uncontrolled proliferation is a hallmark of cancer. In breast cancer, immunohistochemical assessment of the proportion of cells staining for the nuclear antigen Ki67 has become the most widely used method for comparing proliferation between tumor samples. Potential uses include prognosis, prediction of relative responsiveness or resistance to chemotherapy or endocrine therapy, estimation of residual risk in patients on standard therapy and as a dynamic biomarker of treatment efficacy in samples taken before, during, and after neoadjuvant therapy, particularly neoadjuvant endocrine therapy. Increasingly, Ki67 is measured in these scenarios for clinical research, including as a primary efficacy endpoint for clinical trials, and sometimes for clinical management. At present, the enormous variation in analytical practice markedly limits the value of Ki67 in each of these contexts. On March 12, 2010, an international panel of investigators with substantial expertise in the assessment of Ki67 and in the development of biomarker guidelines was convened in London by the cochairs of the Breast International Group and North American Breast Cancer Group Biomarker Working Party to consider evidence for potential applications. Comprehensive recommendations on preanalytical and analytical assessment, and interpretation and scoring of Ki67 were formulated based on current evidence. These recommendations are geared toward achieving a harmonized methodology, create greater between-laboratory and between-study comparability, and allow earlier valid applications of this marker in clinical practice.

J Natl Cancer Inst 2011;103:1656–1664

IHC surrogat markører for brystkræft

(Prat, Pineda et al. 2015)

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Table 1
Distribution of the PAM50 intrinsic subtypes within the pathology-based groups.[a]

IHC-based group	References	N	PAM50 intrinsic subtype distribution			
			Luminal A	Luminal B	HER2-enriched	Basal-like
HR+/HER2−	[10,14,16–22]	4295	60.3%	31.9%	6.6%	1.2%
Luminal A	[10,14,17,21]	637	62.2%	27.0%	10.2%	0.6%
Luminal B	[10,14,17,21]	317	34.1%	51.1%	11.0%	3.8%
HER2+	[6,23–26]	831	17.6%	26.8%	44.6%	11.0%
HER2+/HR+	[25,26]	182	33.0%	46.2%	18.7%	2.2%
HER2+/HR−	[25,26]	168	19.0%	4.2%	66.1%	10.7%
TNBC	[12–15]	868	1.6%	3.2%	9.1%	86.1%

[a] The data has been obtained from the different publications. Several studies have performed a standardized version of the PAM50 assay (RT-qPCR-based or nCounter-based) from formalin-fixed paraffin-embedded tumour tissues [10,14,17,19–22], while others have performed the microarray-based version of the PAM50 assay [6,16,18,23–26].

Digital Image analysis outperforms manual biomarker assessment in breast cancer - but still a substantial proportion of misclassification.

Ki67 Scoring method	SI for PAM50 Luminal B vs. A	SP for PAM50 Luminal B vs. A	Proportion misclassified
DIA Invasive margin			
Cutoff ≥20%	84 %	78 %	20 %
Cutoff ≥20.2 %*	82 %	79 %	20 %
DIA Hot spot			
Cutoff ≥20%	90 %	65 %	24 %
Cutoff ≥25.2 %*	86 %	77 %	19 %
DIA Average			
Cutoff ≥20%	60 %	90 %	31 %
Cutoff ≥ 15.5%*	80 %	83 %	19 %
Manual			
Cutoff ≥20%	75 %	70 %	30 %
Cutoff ≥22.5 %*	74 %	75 %	29 %

SI:Sensitivity **SP:Specificity**

P1-01-06 SABCS 2015, Stålhammer G et al. Karolinska University Hospital

Aktuelt kommercielt tilgængelige genomiske tests

Table 1. Different genomic tests that are currently available to refine prognosis of patients with ER-positive HER2-negative primary breast cancer

	Oncotype Dx	MammaPrint	PAM50 ROR	EndoPredict	Breast Cancer Index (BCI)	Genomic grade
Has it been retrospectively validated on prospective phase III trials?	B-20 ATAC S-8814	x	ATAC ABCSG8	ABCSG6 ABCSG8 GEICAM 9906	ATAC Stockholm trial	BIG 1–98
Can it predict early recurrence (0–5 years)?	√	√	√	√	√	√
Can it predict late recurrence (after 5 years)?	x	?	√ (superior to Oncotype Dx)	√	√ (superior to Oncotype Dx)	?
Can it be tested on FFPE tissue?	√	√[a]	√	√	√	√
Can the test be decentralized with established reproducibility data?	x	x	√	√	x	x
Is it subjected to a randomized prospective trial to demonstrate clinical utility?	TailorX RxPonder	MINDACT	x	x	x	ASTER70s

[a]Test subjected to prospective validation is on frozen tissue.
FFPE, formalin-fixed paraffin-embedded.

Annals of Oncology 00: 1–3, 2015
doi:10.1093/annonc/mdv248

PAM50 gene list

UBE2T	RRM2	KIF2C	SFRP1	MDM2
BIRC5	UBE2C	ACTR3B	KRT14	NAT1
NUF2				
CDC6				
CCNB1				
TYMS				
MYBL2				
CEP55	CCNE1	MIA	CXXC5	GRB7
MELK	CDC20	KRT17	MLPH	TMEM45B
NDC80	**MKI67**	FOXC1	BCL2	**ERBB2**

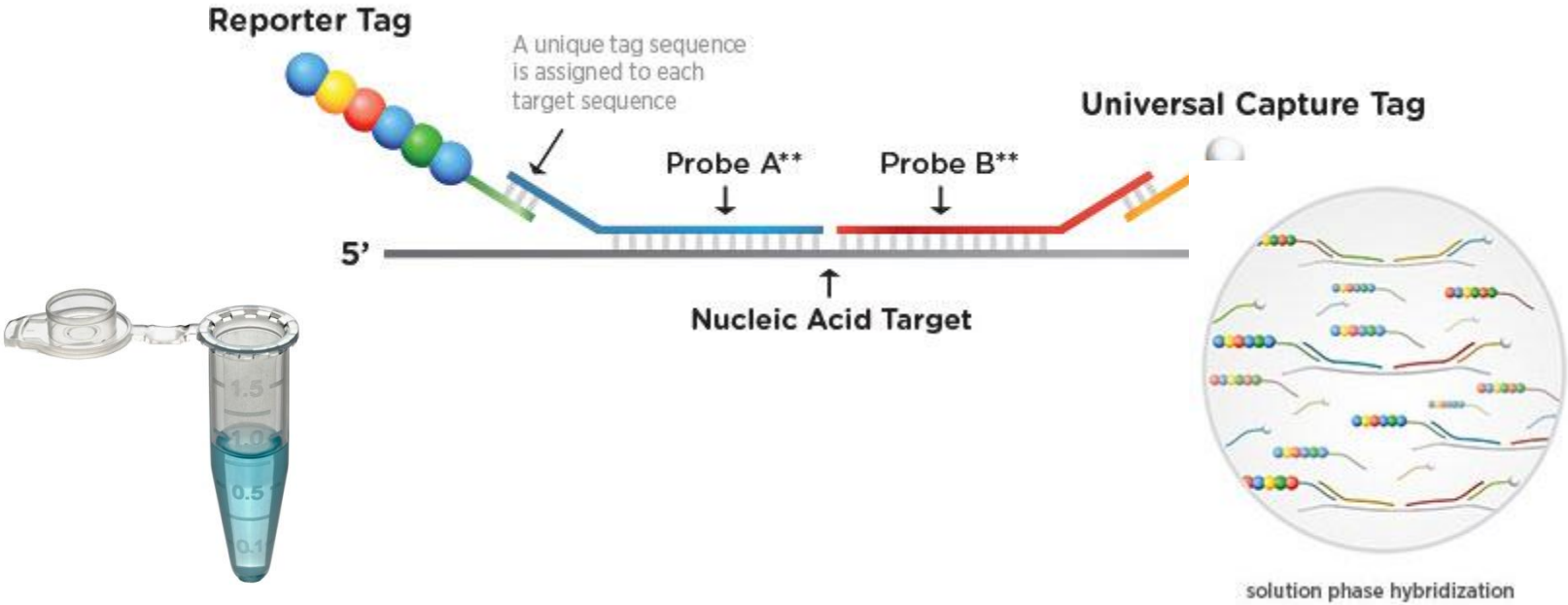
Eighteen genes that constitute the proliferation score in ROR

ANLN	KNTC2
CCNE1	MELK
CDC20	MKI67
CDC6	ORC6L
CDCA1	PTTG1
CENPF	RRM2
CEP55	TYMS
EXO1	UBE2C
KIF2C	UBE2T

Plus 8 household genes

PAM50/ROR:
Nanostring Technology

- Direct multiplexed measurement of gene expression with color-coded probe pairs
- two ~50 base probes per mRNA that hybridize in solution
- The Reporter Probe carries the signal, the Capture Probe allows the complex to be immobilized for data collection
- No amplification



Nature Biotechnology vol 26 march 2008

Risk of Recurrence (ROR)

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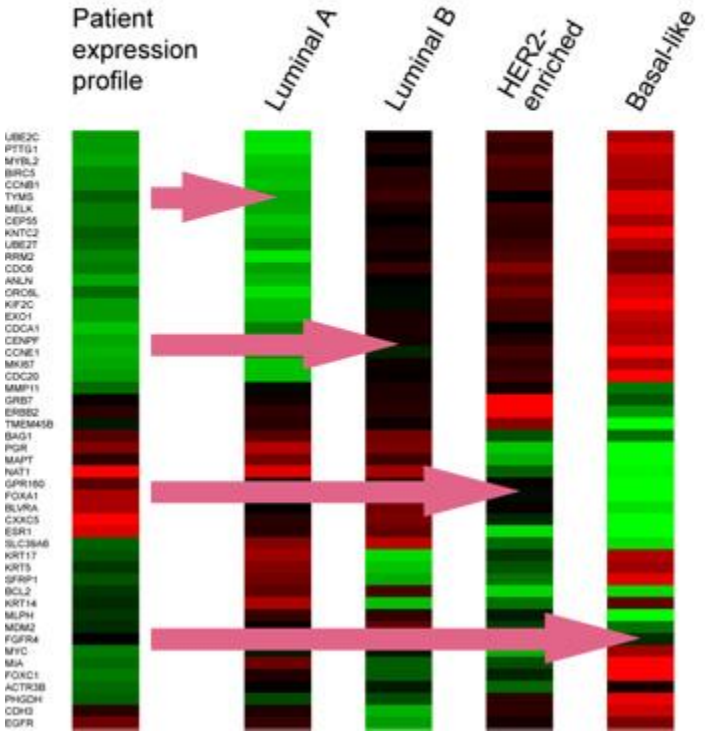
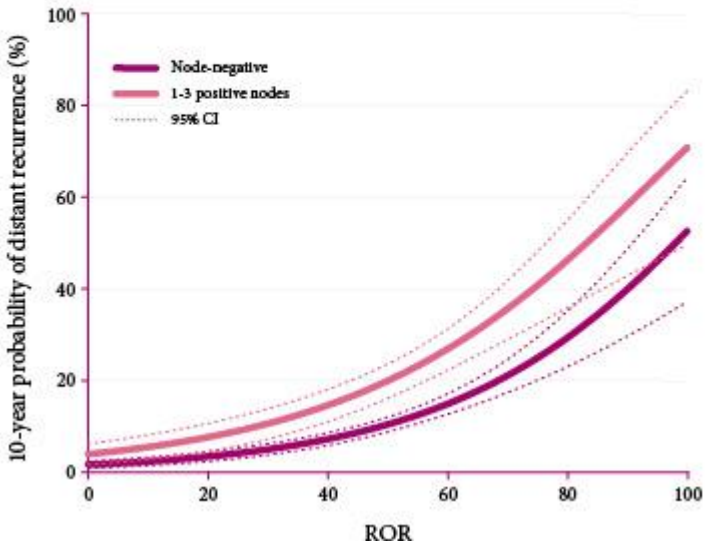
Risk of Recurrence (ROR) algorithm

Compare patient profile to intrinsic subtypes



10-year predicted recurrence probability estimated within nodal status group[1]



Event Distribution

Event Type	TR Analysis	Local Recurrence Reclassification	TDR Analysis
Distant Recurrence	**239**	**15**	**254**
Death (Breast Cancer)	**52**	0	**52**
Local Recurrence	**48**		
Secondary Carcinoma	123	1	124
Contralateral Breast Cancer	63	1	64
Death (Other)	210	4	214
Censored	1987	27	2014
Total	2,722	48	2,722

For the TDR analysis, there were a total of 306 patients (254 + 52) who had a distant recurrence. For the TR analysis, there were a total of 339 patients (239 + 52 + 48) who had a recurrence event.

TDR: Time to distant recurrence
TR: Time to recurrence (local or distant)

Results:
Distribution of intrinsic subtype by number of positive lymph node

Intrinsic Subtype	Number of Positive Nodes				All
	0	1	2	3	
LuminalA	632 (50.3%)	509 (62.9%)	250 (58.7%)	124 (53.7%)	1,515 (55.7%)
LuminalB	502 (40.0%)	248 (30.7%)	142 (33.3%)	85 (36.8%)	977 (35.9%)
Her2Enriched	105 (8.4%)	45 (5.6%)	32 (7.5%)	21 (9.1%)	203 (7.5%)
BasalLike	17 (1.4%)	7 (0.9%)	2 (0.5%)	1 (0.4%)	27 (1.0%)