Guy's and St Thomas' NHS
NHS Foundation Trust

KING'S College LONDON

Breast Pathology and OPTIMA

Sarah E Pinder

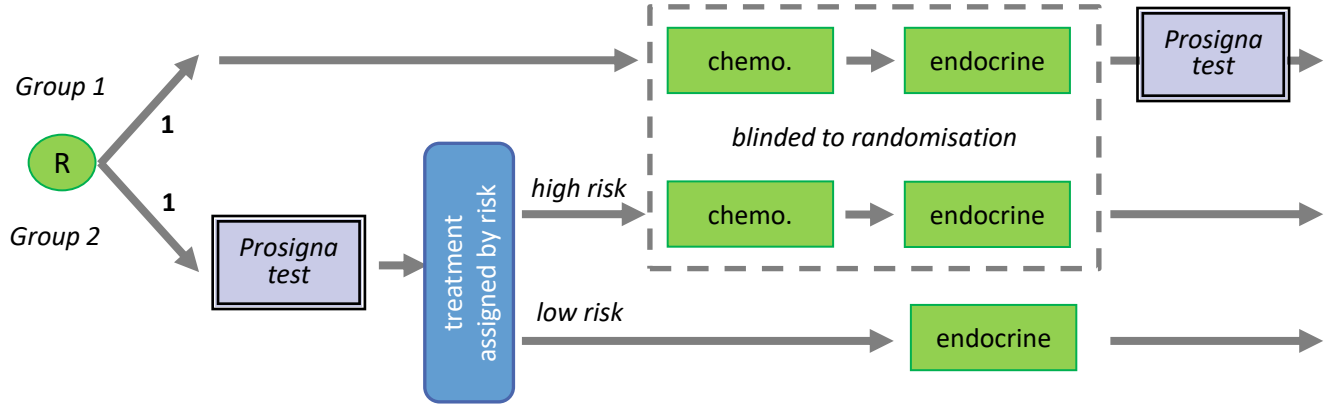
Learning objectives

- To understand the pathology aspects of OPTIMA eligibility criteria
- Sample selection

TRIAL

Eligible: Age ≥ 40
Adequate surgery
ER +ve, HER2 –ve
pN1-2 OR pN0 & T≥30mm

Exclusion: advanced stage = pN3-4/ IM+



Sample size = 4500 patients (+ OPTIMA prelim)
Recruitment period = 4 years

1° Outcomes = Non-inferiority of IDFS (Δ=-3%)
Cost-effectiveness of test-directed chemotherapy

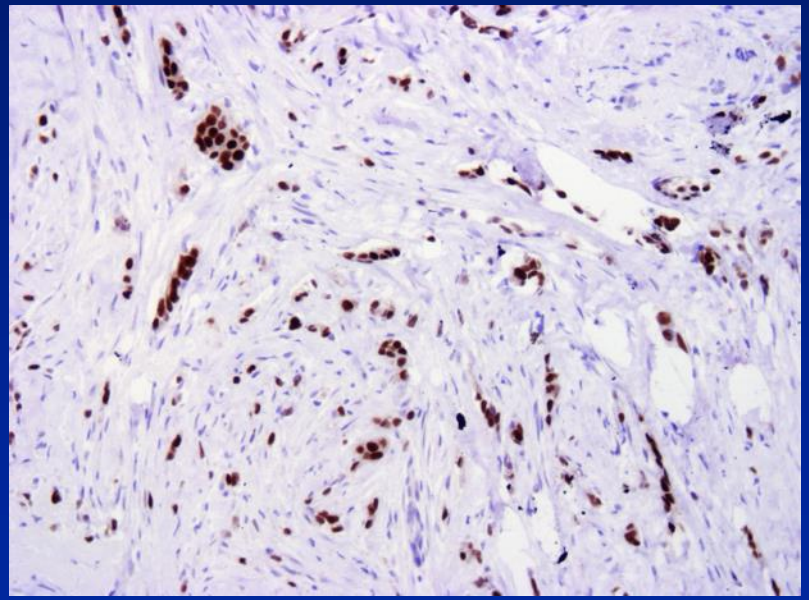
Invasive Carcinoma of the Breast
Histopathology Minimum Data Set (some of)

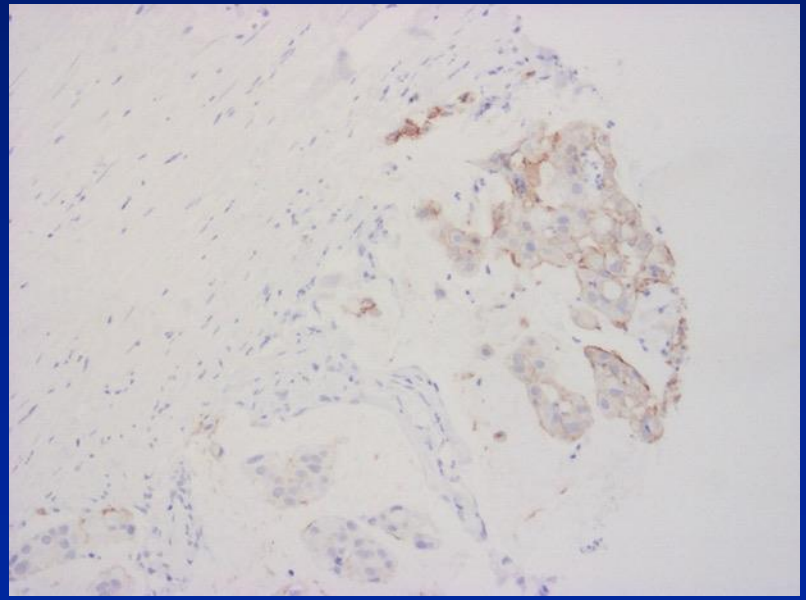
- Invasive tumour size
- Whole tumour size (includes DCIS if beyond invasive)
- Extent (localised vs multiple foci)
- Histological type
- Histological grade
- Lymph node stage
- Lympho-vascular invasion
- Excision margins
- Oestrogen receptor status
- HER2 status

https://www.rcpath.org/uploads/assets/7763be1c-d330-40e8-95d08f955752792a/G148_BreastDataset-hires-Jun16.pdf

Pathology Inclusion Criteria

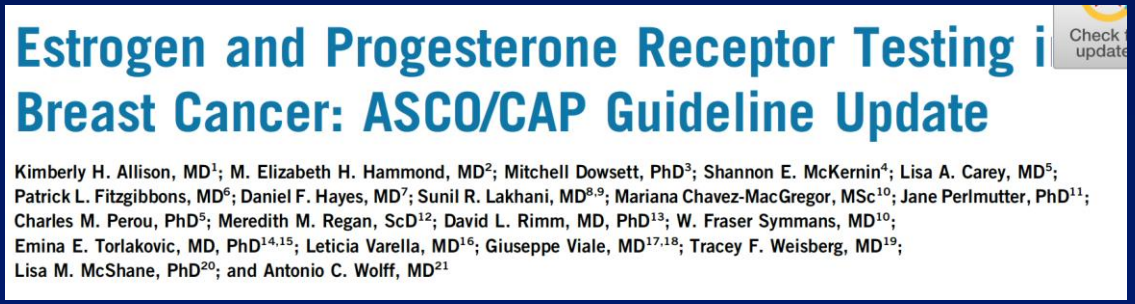
- ER +ve and HER2 –ve (core biopsy)
- pN1-2 OR pN0 & T≥30mm (excision)





Optima Inclusion Criteria – ER positive

- ER positive (>10% of tumour cells stained positive) as determined by referring site in a laboratory meeting national external quality assurance standards, and in accordance with national or ASCO-CAP guidelines



Estrogen and Progesterone Receptor Testing i
Breast Cancer: ASCO/CAP Guideline Update

Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; and Antonio C. Wolff, MD[21]

J Clin Oncol 2020;38:1346-1366

- 2010 guideline: 1-100% tumour nuclei positive should be interpreted as ER positive
- Limited data on ET benefit for patients with cancer with 1-10% positivity
- New category = ER low positive

ER Scoring

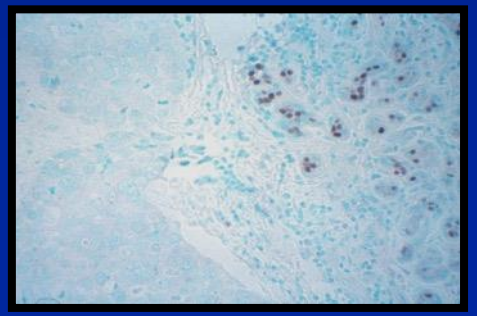
"Allred" Score

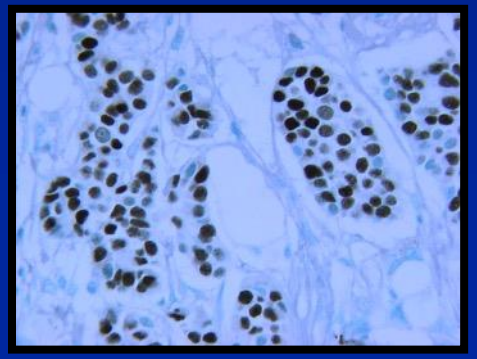
Proportion	Intensity
0 = None	0 = No staining
1 = < 1 %	1 = Weak
2 = 1 - 10 %	2 = Moderate
3 = 11 - 30 %	3 = Strong
4 = 34 - 66 %	
5 = 67 - 100 %	

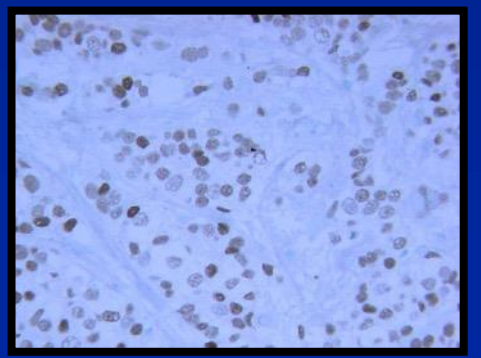
Add; Range 0 - 8

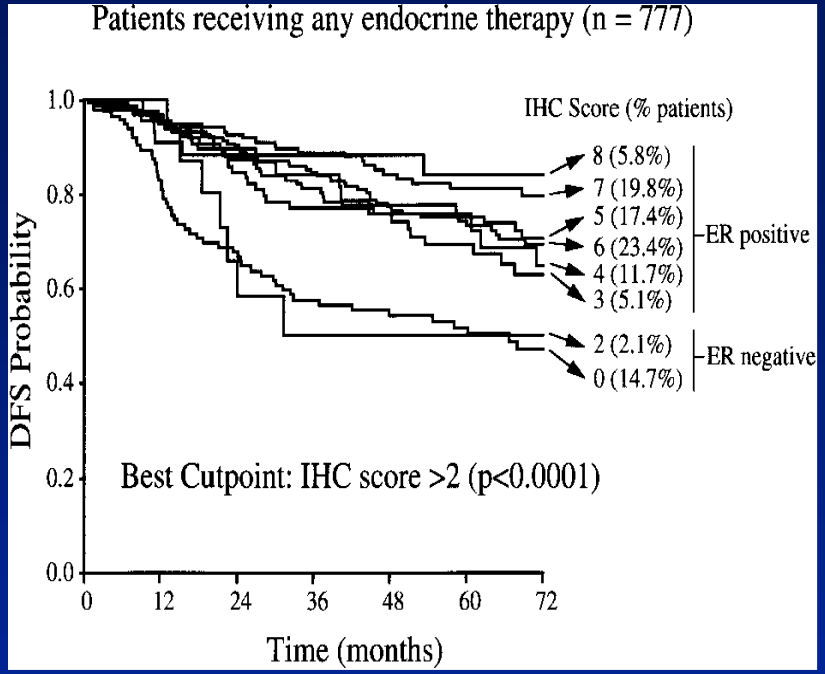
"H" Score

	% cells		Total
0 x	% negative	=	0
1 x	% weak	=	? +
2 x	% moderate	=	? +
3 x	% strong	=	?
	100%		0 - 300









Harvey et al. J Clin Oncol 1999; 17:1474-1481

- >10% of tumour cells (eligible in Optima)
- 1-10% interpreted as ER low positive (not eligible)

- Allred score 3 or more interpreted as positive (but must be <10% of cells) so not eligible

- 1-10%, weak intensity = Allred 3
- 1-10%, moderate intensity = Allred 4
- 1-10%, strong = Allred score 5

Optima Inclusion Criteria – ER positive

Where ER status is reported by Allred (or Quick) Score or by H-Score, tumours with high scores meet the ER-positive definition, but % staining component is required to determine eligibility for intermediate-score tumours

	Eligible (ER staining >10%)	Eligibility determined by %staining component of the score	Ineligible (ER staining ≤10%)
Allred (or Quick) Score	6, 7, or 8	4 or 5	3 or less
H-Score	>30	10-30	<10

Updated UK guidelines (in press):

- **For ER, staining in 1-10% of tumour cells should be reported as ER low positive**

pISSN 1598-2998, eISSN 2005-9256
https://doi.org/10.4143/crt.2021.890
Cancer Res Treat. 2021 Nov 17 [Epub ahead of print]

Original Article

Effect of Estrogen Receptor Expression Level and Hormonal Therapy on Prognosis of Early Breast Cancer

Kyung-Hwak Yoon[1], Yeshong Park[1], Eunyoung Kang[1], Eun-Kyu Kim[1], Jee Hyun Kim[2], Se Hyun Kim[2], Koung Jin Suh[2], Sun Mi Kim[3], Mijung Jang[3], Bo La Yun[3], So Yeon Park[4], Hee-Chul Shin[1]

- 2162 breast cancer patients
- 1654 (76.5%) ER high, 54 (2.5%) ER low, 454 (21.0%) ER neg
- ER low - associated with smaller size, higher grade, HER2 positivity, PgR negativity, higher Ki-67
- Recurrence rate highest in ER neg and inversely proportional to ER expression
- RFS not affected by hormonal therapy in the ER low group (p=0.418)

ESMO Open. 2021;6(6):100289

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN CANCER HORIZONS

ORIGINAL RESEARCH

The prognostic and predictive impact of low estrogen receptor expression in early breast cancer: a systematic review and meta-analysis

N.-M. Paakkola[1†], A. Karakatsanis[2†], D. Mauri[3], T. Foukakis[4] & A. Valachis[5*]

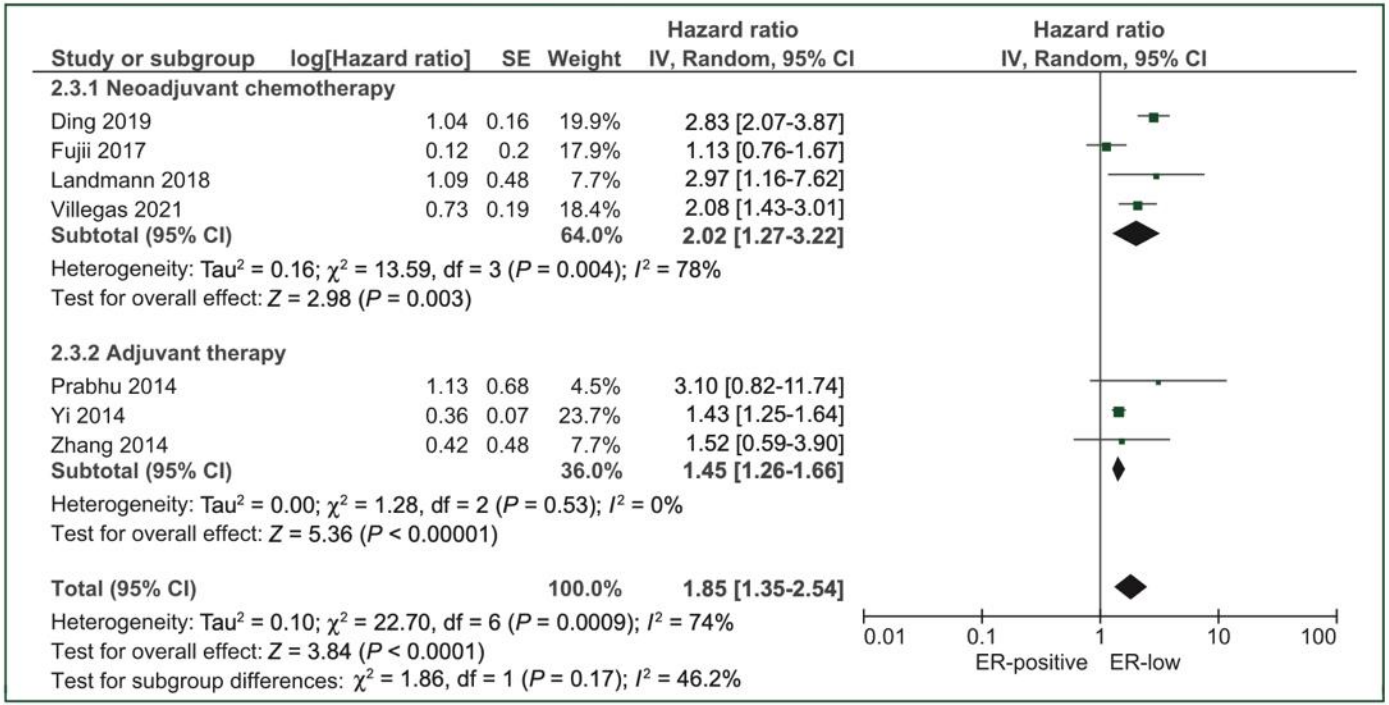


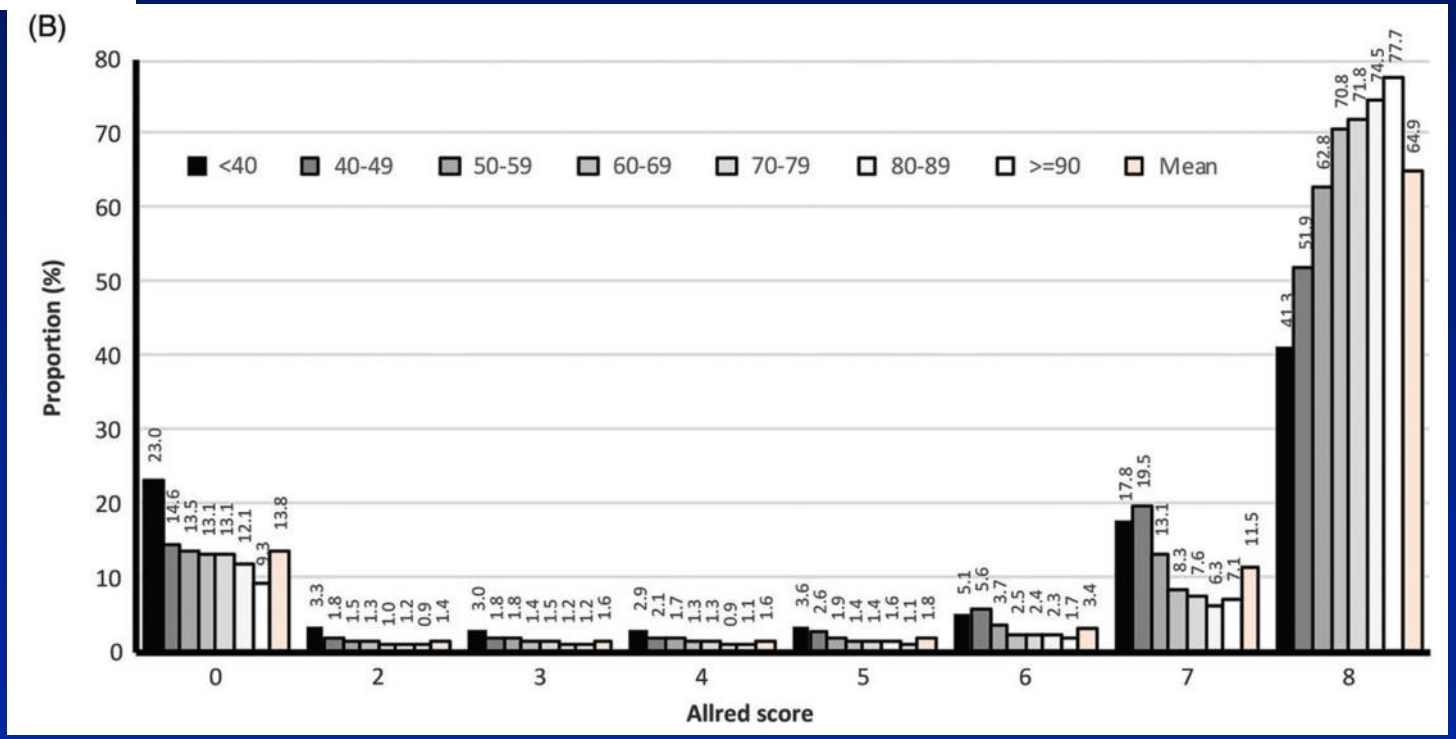
Figure 2. Pooled hazard ratio for disease-free survival between patients with ER-low and ER-positive breast cancer.
CI, confidence interval; df, degrees of freedom; ER, estrogen receptor; SE, standard error.

The Journal of Pathology: Clinical Research
J Pathol Clin Res October 2018; 4: 262–273
Published online in Wiley Online Library
(wileyonlinelibrary.com). DOI: 10.1002/cjp2.112

ORIGINAL ARTICLE

Breast cancer biomarkers in clinical testing: analysis of a UK national external quality assessment scheme for immunocytochemistry and in situ hybridisation database containing results from 199 300 patients

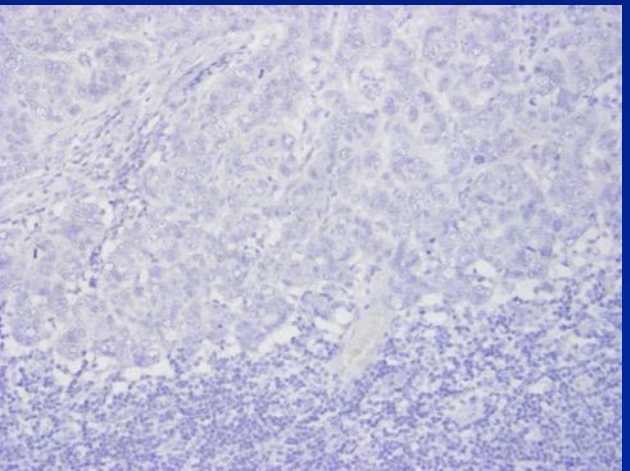
Andrew Dodson[1,2*], Suzanne Parry[3], Merdol Ibrahim[3], John MS Bartlett[4], Sarah Pinder[5], Mitch Dowsett[1,2] and Keith Miller[3]

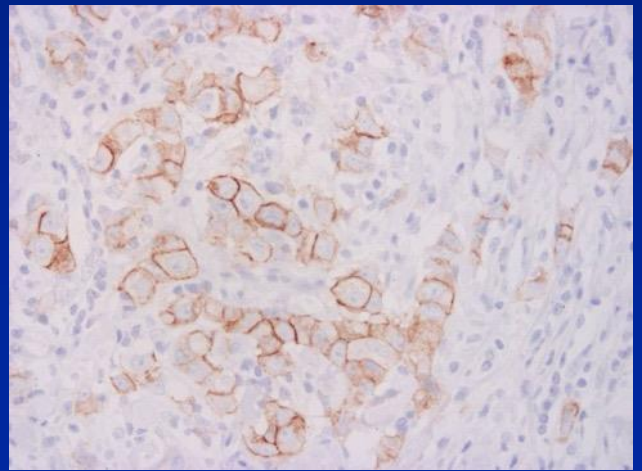


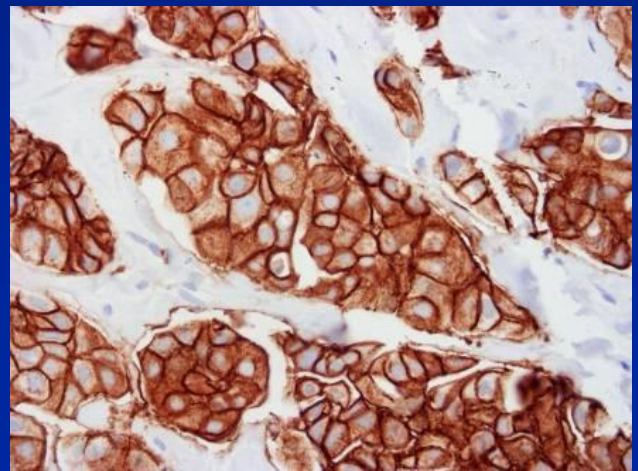
82.7% ER positive for the whole analysable population (n = 78 465 / 94 887)

Inclusion Criteria – HER2 negative

- HER2 negative (IHC 0, 1+ or ISH negative/non-amplified) as determined by referring site in a laboratory meeting national external quality assurance standards, and in accordance with national or ASCO-CAP guidelines







Repeat receptor testing on excision

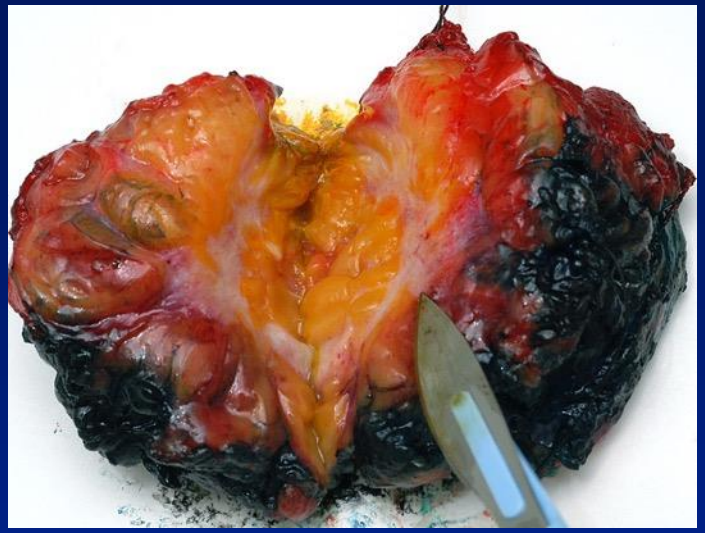
- Insufficient for reliable result
- Carcinoma is morphologically heterogeneous in the resection and this heterogeneity was not present in the core biopsy
- A repeat on concurrent metastatic nodal disease if morphologically distinct from the primary breast tumour
- In setting of OPTIMA, receptors to be tested on separate synchronous primary carcinomas, bilateral lesions

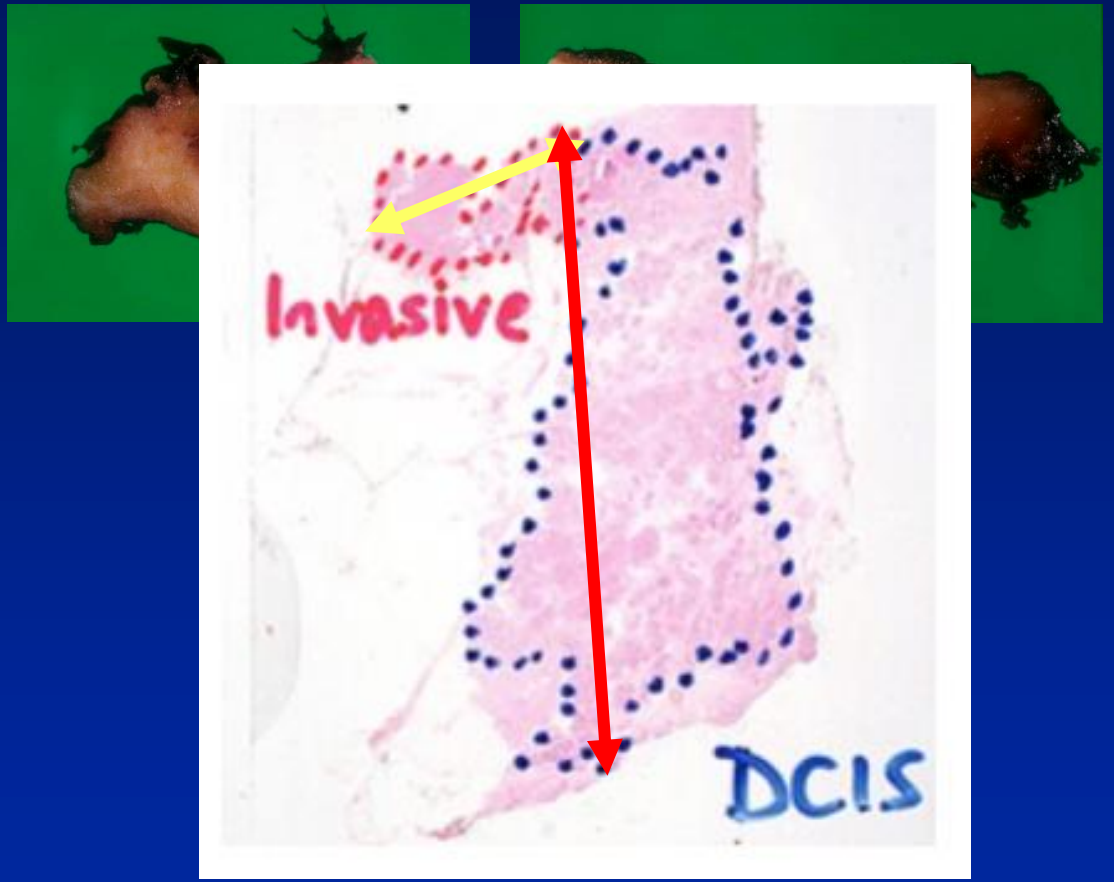
Optima Inclusion Criteria

Tumour size and axillary lymph node status

One of following must apply:

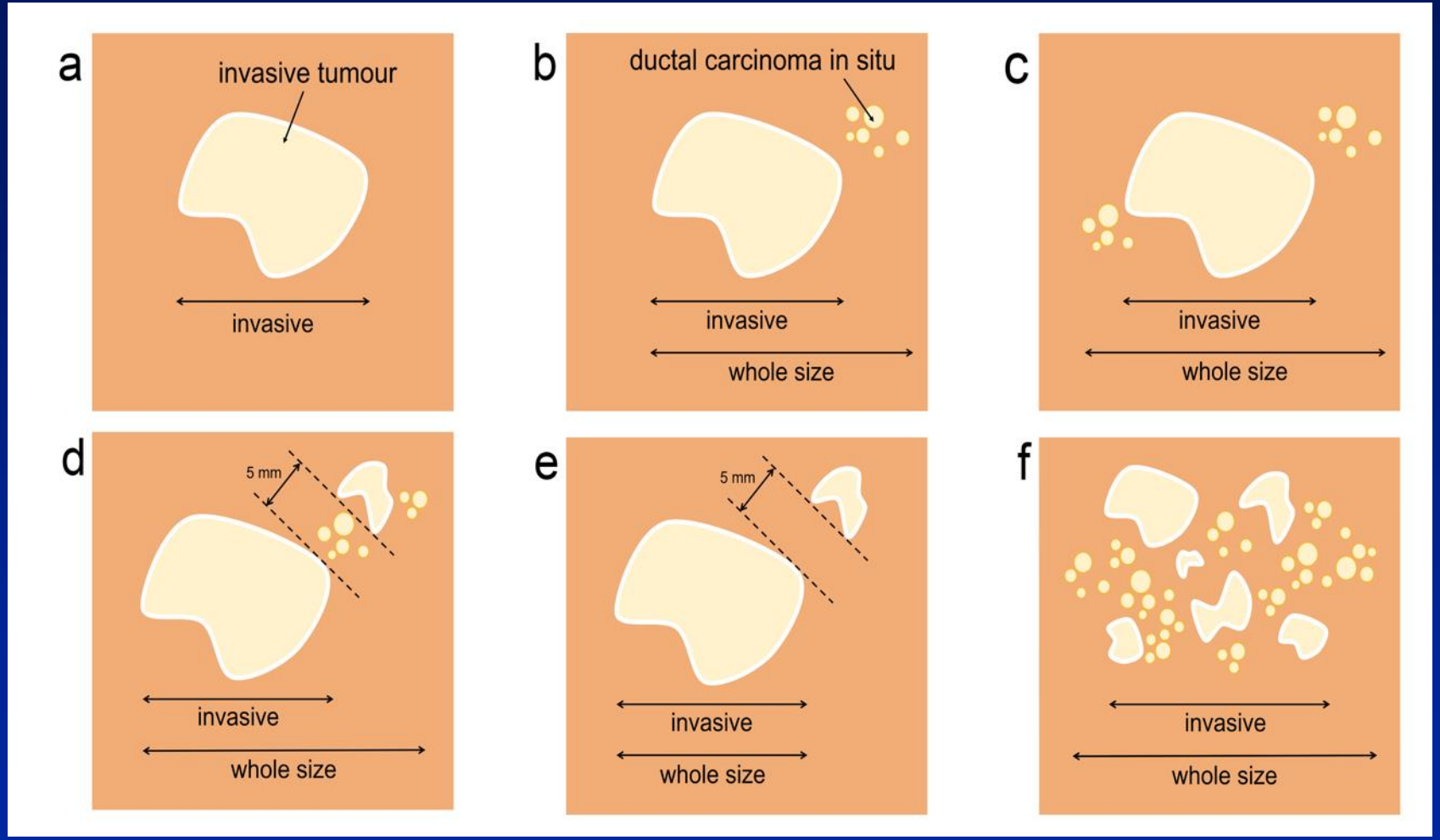
- **4-9 lymph nodes involved AND any invasive tumour size**
- **1-3 nodes involved, with at least 1 node containing a macrometastasis (i.e. deposit >2mm diameter) AND any invasive tumour size**
- **1-3 lymph nodes involved with micrometastases only (i.e. deposit >0.2-2mm diameter) AND invasive tumour size ≥ 20mm**
- **node negative AND invasive tumour size ≥ 30mm**



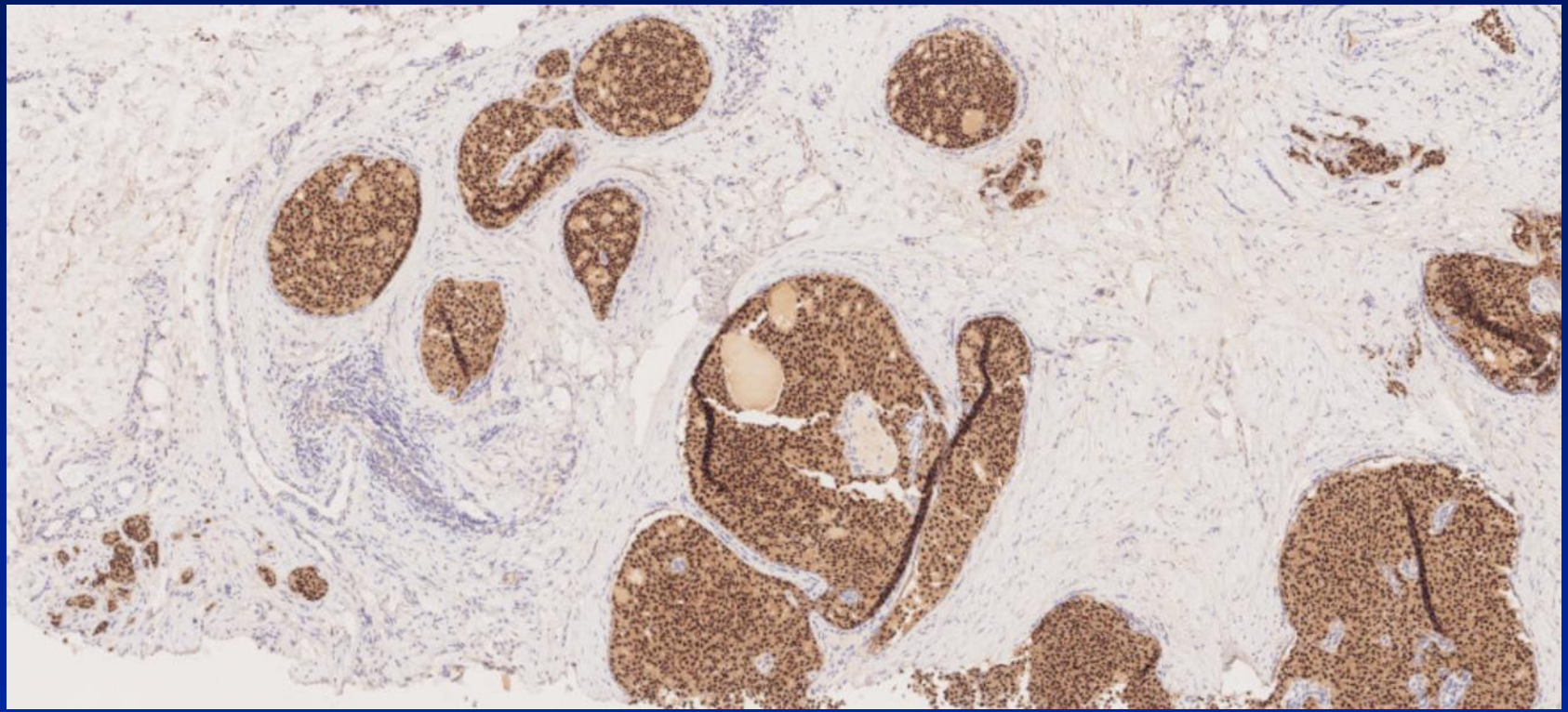


Invasive size

Whole tumour size


a
invasive tumour
invasive
b
ductal carcinoma in situ
invasive
whole size
c
invasive
whole size
d
5 mm
invasive
whole size
e
5 mm
invasive
whole size
f
invasive
whole size

ER



Inclusion

Multiple ipsilateral cancers

Permitted provided at least one tumour fulfils the tumour size & axillary lymph node entry criteria, and none meet any of exclusion criteria (i.e. none are HER2 positive or ER <10%)

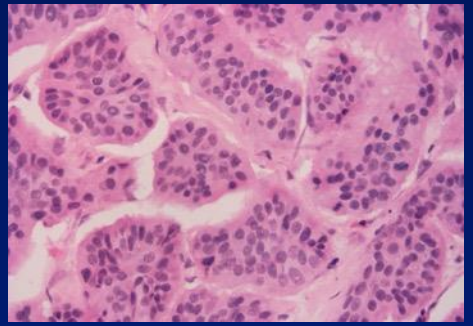
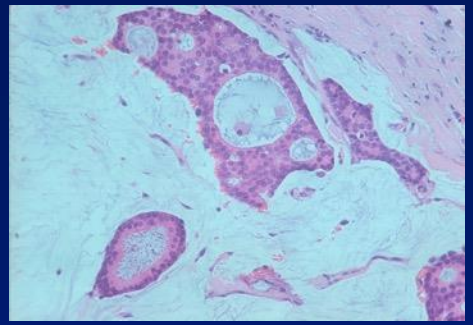
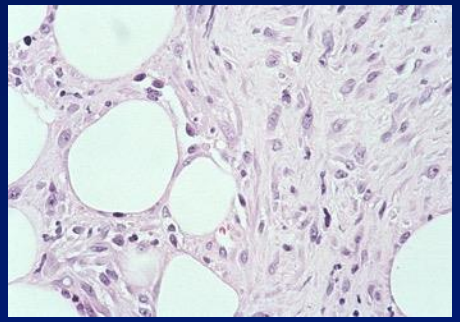
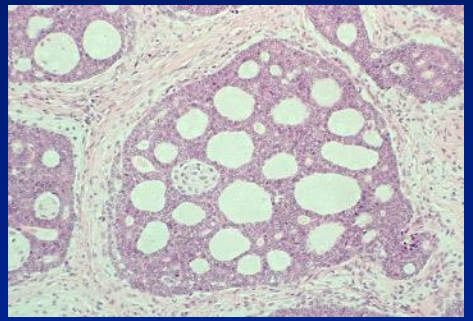
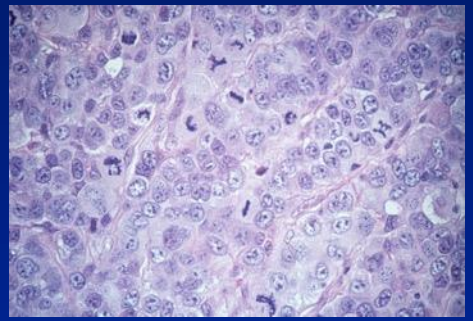
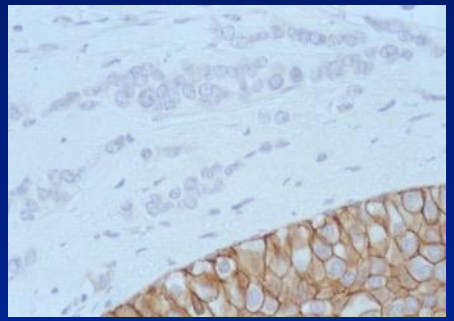
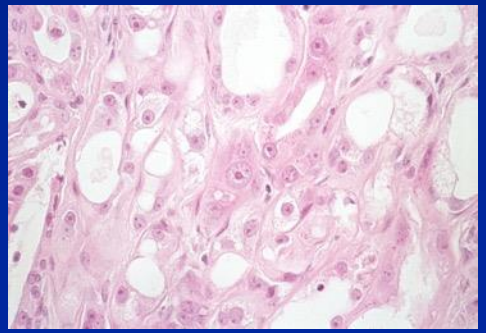
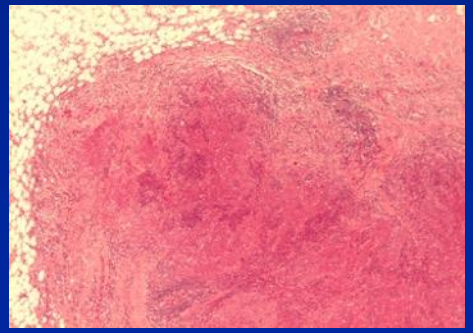
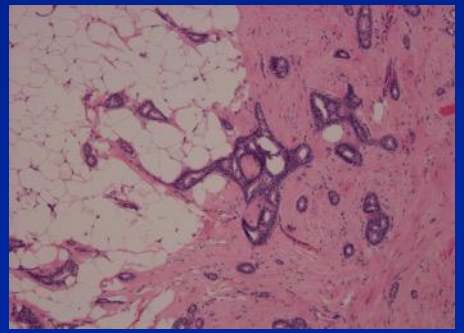
Samples

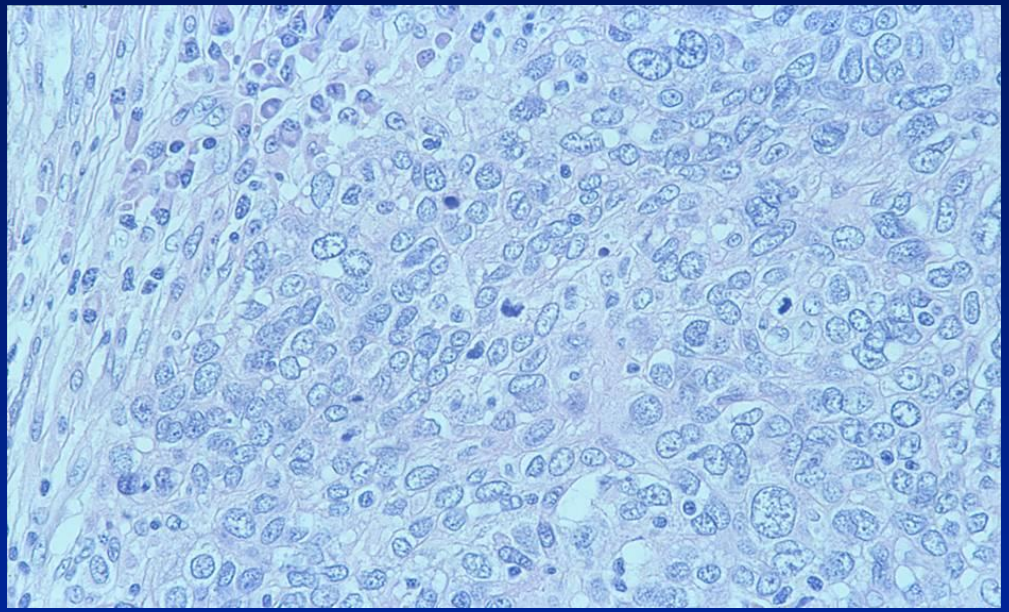
Multiple ipsilateral cancers

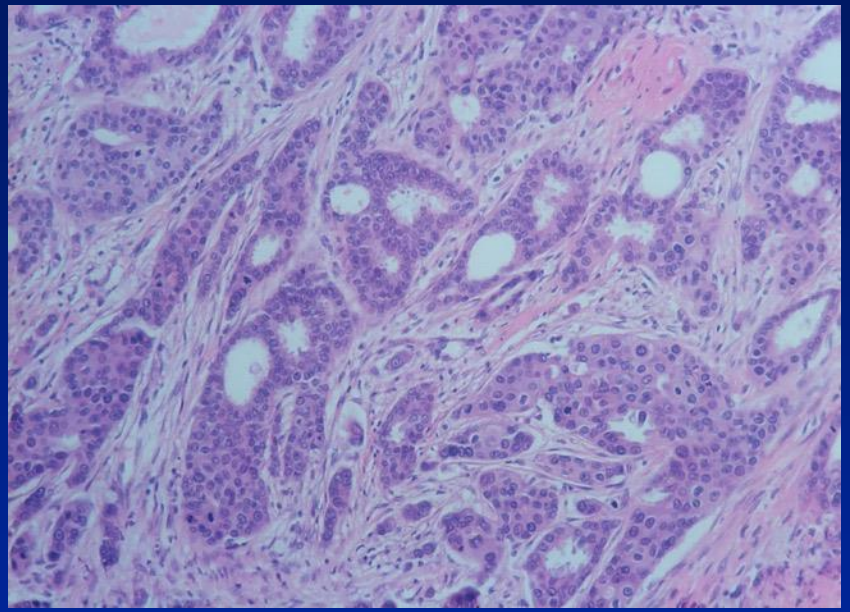
Blocks from >1 lesion should be submitted for Prosigna testing when the lesions are:

considered to be clinically significant by the referring site
AND
interpreted as synchronous primary cancers (based either on site of lesions, or differing morphology (type/grade))

For patients randomised to test-directed treatment, clinical management based on higher Prosigna score





Macroscopic Description

A. Left breast wide local excision - stitch at 12, nipple

Pot A labelled with patient's details and 'left breast wide local excision'

Specimen weighs 28.8g and represents an ellipse of skin measuring 55 x 20mm. The skin appears unremarkable. The underlying fibrofatty tissue measures 52mm medial to lateral, 30mm superior to inferior and 28mm anterior to posterior. The nipple suture marks the medial side. Specimen is inked according to the lab protocol and sliced from medial to lateral into 7 slices. A well defined tumour measuring 22mm AP, 18mm SI and 24mm ML in slices 3 to 6. The tumour abuts the superior margin and lies 1mm from inferior and more than 3mm from deep margin. The medial and lateral margins are well clear.

A1 - Slice 1, medial cruciate
A2 - Slice 2
A3 - Slice 3
A4+A5 - Slice 4
A6-A8 - Slice 5 to 7

B. Left revised superior margin

Pot B labelled 'left breast superior margin'

Specimen weighs 13.5g and represents fibrofatty tissue measuring 60 x 45 x 10mm. The cavity site is inked black and the true margin is inked yellow. Entirely sampled in 7 blocks.

Macroscopic Description

A) Specimen labelled: Right breast
Via tissue banking.
Mastectomy weighs 92.3g. Measures in the fixed state 90mm ML, 70mm SI and 25mm AP.
Skin 90 x 45mm. Bears normal nipple.
Sliced medial to lateral into 8 slices. Deep inked black.
Nipple in slice 5.

There is a firm ill-defined mass in slices 4-5, measures 35mm ML and 25mm SI, corresponding to the upper central 12 o'clock position. It is possible slices other side of it could be involved due to the ill-defined nature, and if so, the final size measurement is subject to microscopic assessment.

A1 nipple
A2 IUQ slice 2
A3 ILQ slice 2
A4 slice 3 medial to disease area
A5 slice 4, tumour, with deep = black
A6 slice 5 tumour
A7 slice 6 tumour
A8 slice 7 ? skin tumour
A9 OUQ slice 8 and lateral to the tumour
A10 OLQ slice 8. TR.

B) Specimen labelled: Right SLNB
Two blue nodes.
B1 one node
B2 one node. AE.

Serially sliced from medial to lateral into 6 slices (very large WLE specimen resembling a mastectomy).

Two lesions are present described as follows:

Lesion 1:
This is the medially located tumour, positioned towards the posterior aspect of the specimen. Present in slice 3. Measures 15mm ML in that slice, 12mm SI and 8mm AP. It is well clear of all margins (>10mm).
B1 medial margin cruciate slice 1 (please note orange = medial)
B2 slice 2, medial to Lesion 1
B3-B4 slice 3, composite Lesion 1 with superior and inferior margins
B5 deep margin
B6 slice 4 lateral to Lesion 1
B7 slice 4 tissue between both lesions (medial to Lesion 2 below)

Lesion 2:
This is the laterally located tumour, positioned towards the anterior aspect of the specimen. Present in slice 5. Measures 15mm ML, 9mm SI and 8mm AP.
B8 slice 5, Lesion 2 with superior margin
B9 slice 5 inferior margin
B10 slice 5 deep margin
B11 slice 6 lateral margin cruciate.
TR.

Distance between both lesions is 40mm.

LESION 1 (MEDIAL TUMOUR)
IN SITU CARCINOMA: Present, DCIS
DCIS grade: Intermediate grade
DCIS growth patterns: Cribriform
Comedo necrosis present: Absent
Size of pure DCIS: Not applicable
Paget's disease of nipple: Not assessable
Microinvasion: Not applicable

INVASIVE CARCINOMA: Present
Tumour extent: Multiple invasive foci
Size of invasive carcinoma: 14mm (B3, superior to inferior)
Whole tumour size (DCIS + invasive): 14mm
Histological Grade (components): 1 (121)
Tumour type: Tubular
Lympho-vascular invasion: Absent

Tumour is best present microscopically in blocks numbered: B3

LESION 2 (LATERAL TUMOUR)
IN SITU CARCINOMA: Present, DCIS
DCIS grade: Intermediate
DCIS growth patterns: Cribriform
Comedo necrosis present: Absent
Size of pure DCIS: Not applicable
Paget's disease of nipple: Not assessable
Microinvasion: Not applicable

INVASIVE CARCINOMA: Present
Tumour extent: Multiple invasive foci
Size of invasive carcinoma: 19mm (B8)
Whole tumour size (DCIS + invasive): 19mm
Histological Grade (components): 1 (221)
Tumour type: NST
Lympho-vascular invasion: Absent

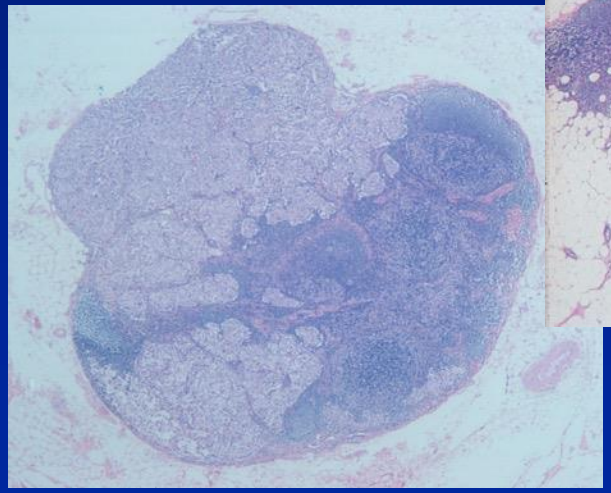
Tumour is best present microscopically in blocks numbered: B8

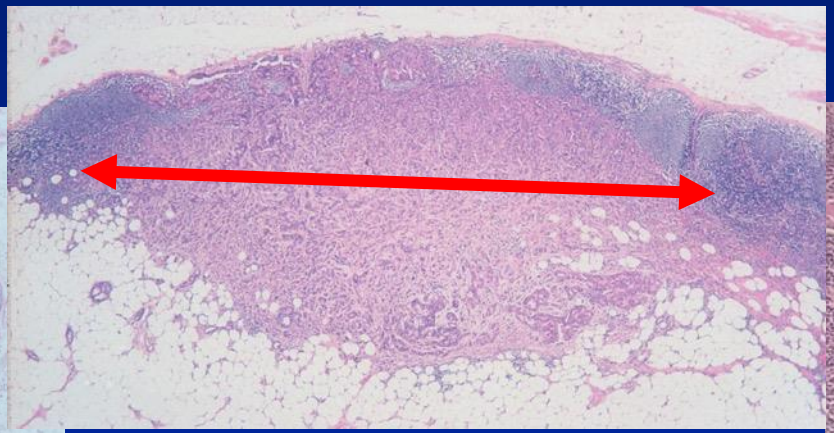
Issues

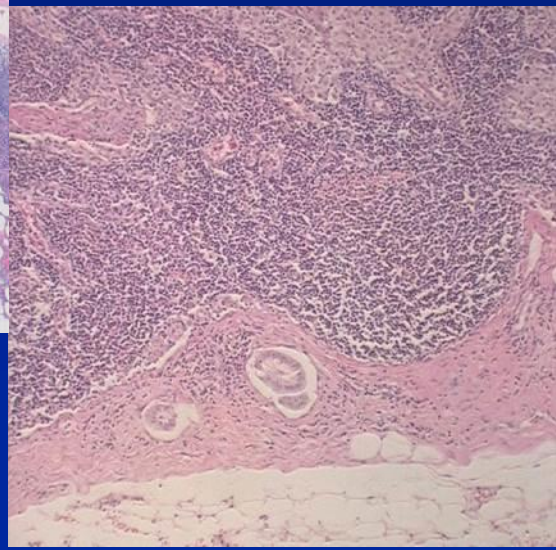
- **Important for pathologist to be clear where tissue has been sampled, particularly if several tumours, either in macroscopic description, block key or microscopy section of report – the latter most useful and easiest for research nurses and for trials office, multigene assays or other molecular tests……**

Axillary LN Metastasis Reporting

Macrometastasis	> or = 2mm	LN Positive
Micrometastasis	<2mm >0.2mm	
Isolated Tumour Cells	<0.2mm	LN Negative







Lymph Node Stage

Axillary nodes present: No ☐ Yes ☐
Total present: ..... **Total positive:.....**
Extracapsular spread: **Present ☐** **Not identified ☐**
Extent of extracapsular spread (mm)
For single node positive: **Macrometastasis ☐** **Micrometastasis ☐**
For node negative: **No metastasis ☐** **ITCs ☐**

Other nodes present: **No ☐** **Yes ☐** **Site: .....**
Total present: ..... **Total positive:.....**
For single node positive: **Macrometastasis ☐** **Micrometastasis ☐**
For node negative: No metastasis ☐ ITCs ☐

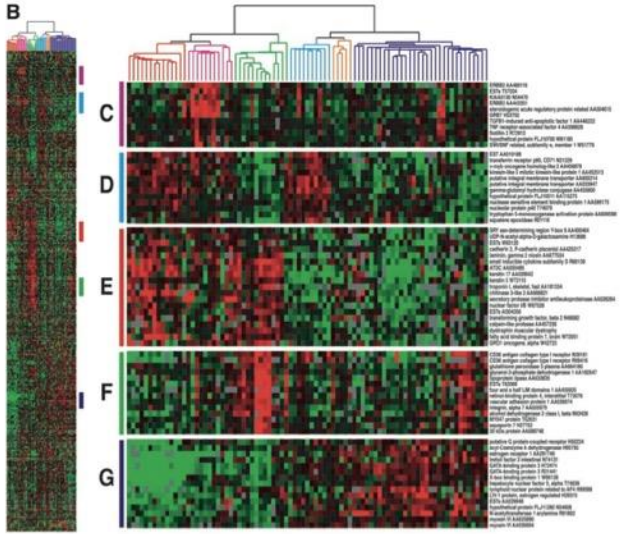
Summary lymph node stage:
1 = Node negative ☐. 2 = 1–3 nodes positive ☐ 3 = 4 or more nodes positive ☐

Comments about TNM stage

- Intramammary (not internal mammary) nodes considered as axillary for staging
- Tumour deposits in axillary fat considered to be nodes

Exclusion Criteria

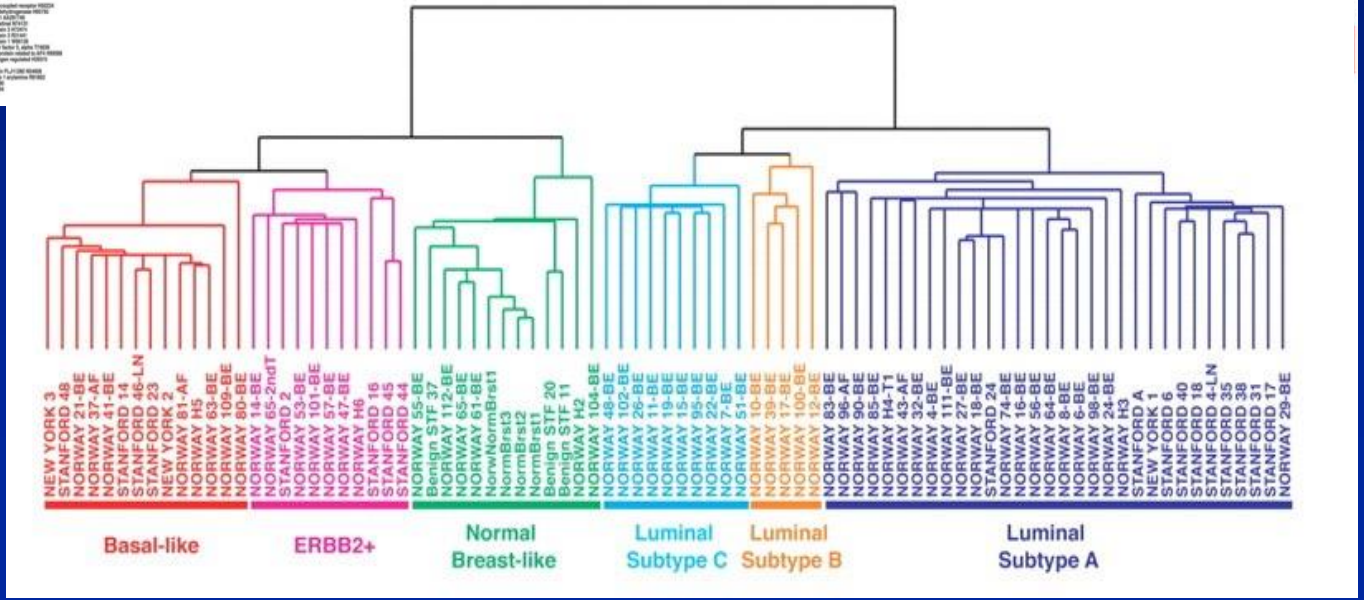
- **≥10 involved axillary lymph nodes (with either macrometastases and/ or micrometastases) or evidence for internal mammary lymph node involvement.**
- **ER negative/low OR HER2 positive/amplified tumour (determined by the referring site)**
- **Metastatic disease**
  [Formal staging according to local protocol is recommended for patients where there is a clinical suspicion of metastatic disease or for stage III disease (i.e. tumour >50mm diameter with any nodal involvement OR any tumour size with 4 or more involved nodes)]



Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications

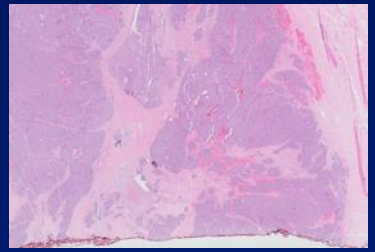
Therese Sørlie[a,b,c], Charles M. Perou[a,d], Robert Tibshirani[e], Turid Aas[f], Stephanie Geisler[g], Hilde Johnsen[b], Trevor Hastie[e], Michael B. Eisen[h], Matt van de Rijn[i], Stefanie S. Jeffrey[j], Thor Thorsen[k], Hanne Quist[l], John C. Matese[c], Patrick O. Brown[m], David Botstein[c], Per Eystein Lønning[g], and Anne-Lise Børresen-Dale[b,n]

85 cDNA microarrays from 78 cancers

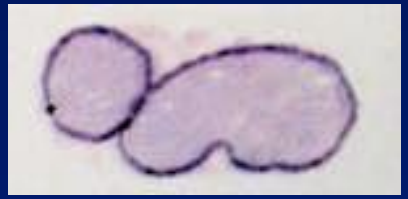


Prosigna test

- Prosigna (PAM50) is qRT-PCR expression assay
- 50 genes used for intrinsic subtype classification, 8 housekeeping genes for signal normalisation, 6 positive controls and 8 negative controls
- Assay provides subtyping information and additionally a numerical “Risk of Recurrence” (ROR) score
- PAM50 commercialised by NanoString Technologies (subsequently Veracyte Inc) as Prosigna
- Prosigna Score or ROR PT includes parameters derived from expression of the PAM50 proliferation-related genes and tumour size



H&E stain to identify tumour area and cellularity

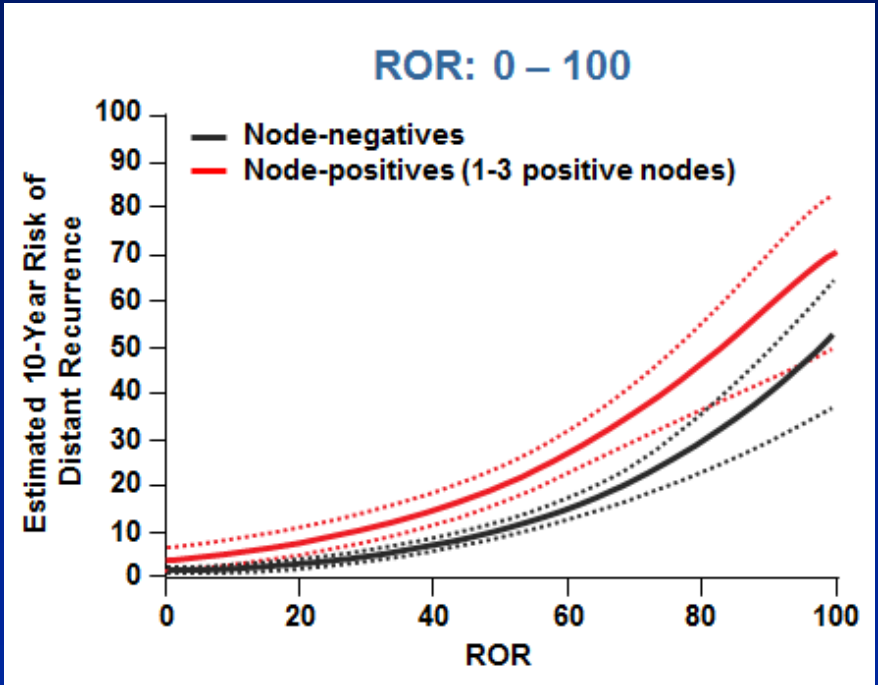


Tumour area transposed to unstained slides and macrodissected

RNA extracted

Specimen Attribute	Requirement
Tissue input	Invasive carcinoma
Tissue input format	Macro-dissected 10-micron-thick slide-mounted tissue sections
Minimum tumor size	4 mm^2 tumor area
Minimum tumor cellularity	10% within tumor area
Minimum RNA amount	125 ng (12.5 ng/ml)
Slides required	Area: > 100mm^2; 20mm^2 – 100mm^2; 4mm^2 – 20mm^2 — No. Slides: 1; 3; 6

Estimate 10-Year Risk of Distant Recurrence With Endocrine Therapy Alone



- Prosigna Score (ROR score) based on similarity of gene expression profile to intrinsic subtypes, proliferation score and tumour size
- Prosigna score from 0 to 100

Most common problems reported by HSL-AD

- Blocks with no invasive tumour present
- Suboptimal fixation/processing
- Excess of intermixed invasive and *in situ* carcinoma – if possible and if another block is available, please send alternative block
- Lymph nodes submitted – not appropriate for Prosigna

Summary - Samples for OPTIMA

- Unifocal tumour - a representative tumour block (ideally well-fixed and without too much admixed DCIS)
- If pre-operative endocrine treatment - pre-treatment core biopsy block
- NOT involved lymph nodes
- Patients with multiple ipsilateral tumours - blocks from >1, if:
  - lesions considered to be clinically significant by referring site AND
  - interpreted as synchronous primaries - based either on site (i.e. in different quadrants) or differing morphology (histological type or grade)
  - ER and HER2 required on different lesions if considered synchronous primaries

If not sure if need more than one block or any other questions – just ask the OPTIMA team……..