Neoadjuvant Chemotherapy in Breast Cancer: Practical Aspects in Specimen Handling and Recent Advances

ELENA PROVENZANO
ADDENBROOKES HOSPITAL
CAMBRIDGE, U.K.

Neoadjuvant Chemotherapy

- Indications:
- Management of locally advanced invasive breast cancers including inflammatory breast cancer
- 'Down-staging' of large inoperable cancers to permit surgical resection
- Routine management of women with high risk disease who would require adjuvant chemotherapy based on biological tumour characteristics and clinical-radiological findings

Neoadjuvant Therapy

- Ability to monitor treatment response and tailor subsequent locoregional and systemic therapy -> more individualised patient care
  - Patients with pCR may not benefit from further regional therapy such as adjuvant radiotherapy
  - Patients with poor response can be identified and entered into trials of novel targeted agents
- Evaluation of treatment response to new agents using pathological complete response (pCR) as a surrogate marker of outcome
  - Neoadjuvant studies smaller, cheaper, faster results

Specimen Handling

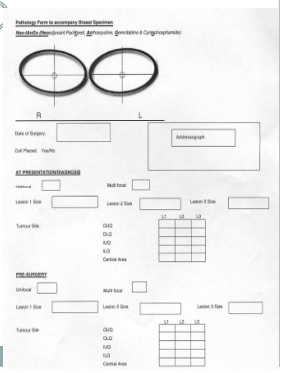
- Thorough macroscopic (gross) assessment of the specimen critical for accurate classification of pCR
- A multidisciplinary approach with adequate clinical information and access to imaging results is essential
- Close clinical/ radiological correlation to map the precise location of the tumour bed is preferable to exhaustive blind sampling
- Placement of a marker clip at the time of diagnosis is very helpful in the event of an excellent response to treatment

Specimen Handling

Minimum information required:

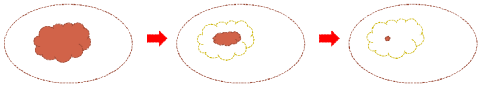
- Clear indication neoadjuvant Rx has been given and it's nature
- Location of tumour/s within the breast – diagram best
- Pre treatment size on imaging

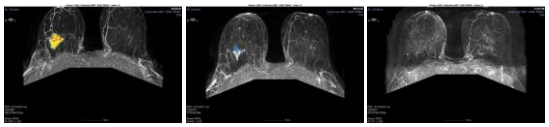
- Is the patient on a clinical trial – may be requirement for tissue banking as part of protocol



Patterns of tumour response

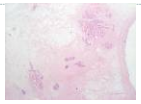
A. Concentric shrinking

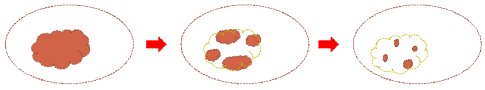


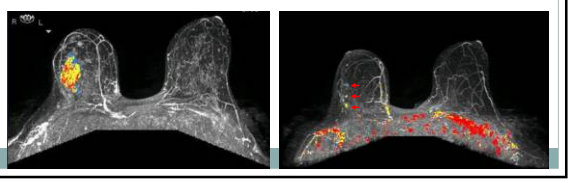


Patterns of tumour response

B. Scatter pattern







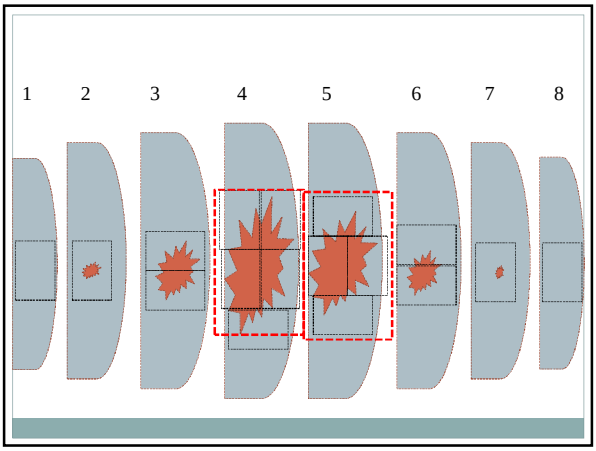
Specimen Handling

- Specimen should be sent fresh to the histopathology laboratory as quickly as possible for slicing to aid fixation – good fixation is critical for accurate assessment.
- Wide local excisions
  - Ink and slice as per local protocol
  - Thorough sampling of the specimen including sections to assess margins
- Mastectomies
  - Ink and slice as per local protocol
  - If macroscopic residual tumour sample as per usual including area of pre treatment tumour bed
  - If good response there may only be a vague area of fibrosis or no gross lesion at all – intelligent mapping of the tumour bed with radiological correlation better than exhaustive sampling

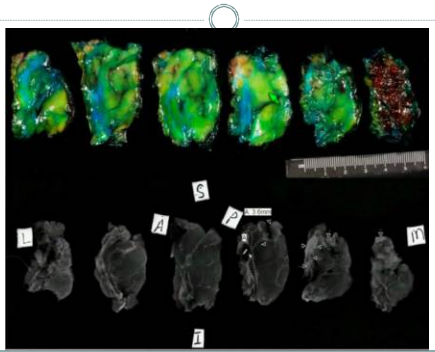
Specimen Handling

- BIG-NABCG Residual Disease Working Group
- Systematic sampling of areas identified by intelligent mapping and close clinical-pathological correlation is more important than overly exhaustive sampling
- Specimen divided into 1-2 cm thick slices
- Full face section of tumour bed taken from each slice up to a maximum of 25 blocks should be sufficient to document pCR
- Five blocks representing the maximum full face dimension of the tumour bed adequate for assessment of cellularity to calculate the RCB
- Additional blocks required if tumour bed not identified
- Large tissue cassettes can be very useful and make assessment of cellularity and lesion size easier

Provenzano et al., Mod Pathol 2015;28(9): 1085-201.

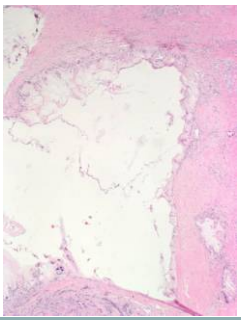


Specimen Handling

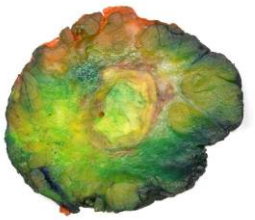


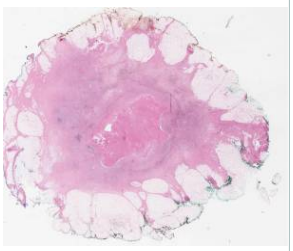
Courtesy of WF Symmans

Specimen handling – clip site



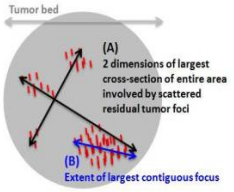
Specimen handling





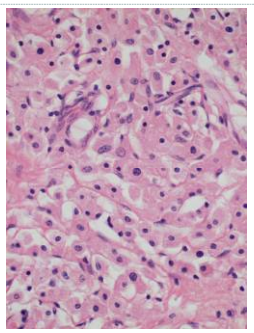
Measuring size post NAST

- Tumour size often more difficult to assess after neoadjuvant treatment.
- If there is a single lesion present on pre-treatment imaging, then treat residual disease as a single tumour, especially if tumour cells are present within a reactive stromal background consistent with a solitary tumour bed.
- The combination of size and residual tumour cellularity is the best indicator of response.
- 7th edition AJCC – largest contiguous area of tumour cells (B)



Microscopic findings

- Chemotherapy effect can alter traditional prognostic factors:
- Change in grade
  - Increased nuclear pleomorphism
  - Reduced mitotic activity
- Altered growth pattern with lobular appearance in previous NST carcinomas
- Histiocytoid cell morphology

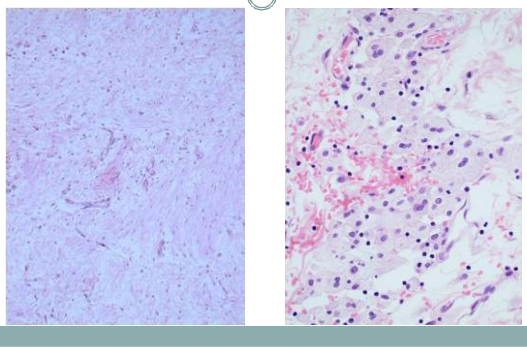


Microscopic findings

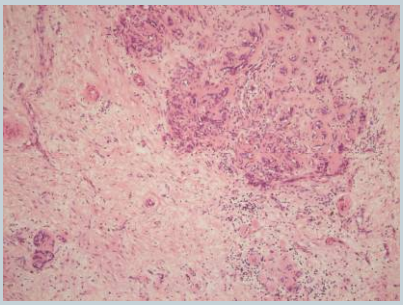
Background changes:

- Oedematous reactive stroma
- Central fibrous scar – commonly seen after neoadjuvant hormonal therapy
- Collections of foamy histiocytes or haemosiderin laden macrophages
- Chronic inflammatory cell infiltrate
- Sclerosis and atrophy of benign lobular units
- Reactive nuclear atypia within normal lobules

Stromal Changes



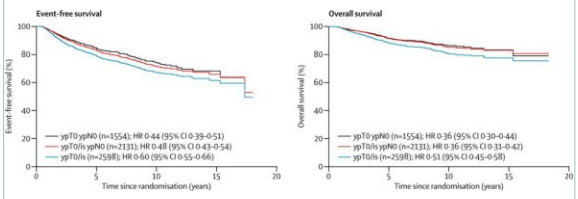
Atrophy, sclerosis and lymphoid infiltrate



Various Definitions of pCR

- No residual invasive carcinoma OR DCIS in the breast and negative axillary lymph nodes
  = ypT0 ypN0
- No residual invasive carcinoma or DCIS only in the breast and negative axillary lymph nodes
  = ypT0/is ypN0
- No residual invasive carcinoma OR DCIS in the breast irrespective of axillary lymph node status
  = ypT0 ypNx

Various Definitions of pCR

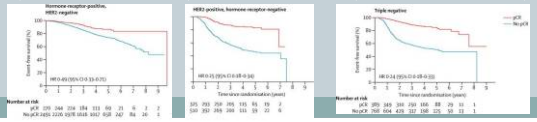


Cortazar et. al. The Lancet, Volume 384, Issue 9938, 2014, 164 – 172.

pCR by tumour subtype

- HR +ve tumours have low pCR rates BUT better survival
- HER2+ and triple negative cancers more likely to achieve pCR, but overall prognosis is worse with poor DFS and OS if no pCR

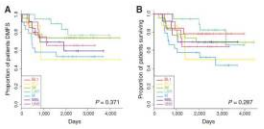
	pCR	3y DFS
HR+/HER2-	2-8%	91-96%
HR+/HER2+	8-33%	82-90%
HR-/HER2+	33-52%	33-68%
HR-/HER2-	24-38%	65-67%



pCR by tumour subtype

- Masuda et al., Clin Ca Res 2013;19(19):5533-40
- 7 subtypes of triple negative breast cancer
- Different rates of pCR between subtypes
- No difference in OS – LAR group had low pCR rate but best survival at 3 years

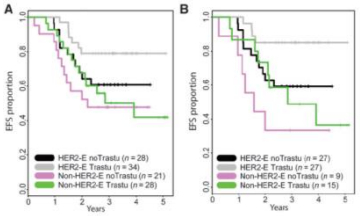
	pCR	Non-pCR	pCR rate
BL1	11	10	0.52
BL2	0	8	0.00
M	8	18	0.31
IM	8	19	0.30
MSL	3	10	0.23
LAR	2	18	0.10
UNS	5	10	0.33



pCR by tumour subtype

- Retrospective analysis of NOAH study looking at PAM50 subtypes
- Only 55% of HER2+ tumours HER2-E subtype; 21% luminal, 7% basal-like, 18% normal-like

Å Better pCR rates in HER2-E vs luminal HER2+ tumours (53% v 29%) with larger improvement in EFS with addition of Trastuzumab



Prat et al., Clin Cancer Res 2014;20(2):511-21.

Grading response

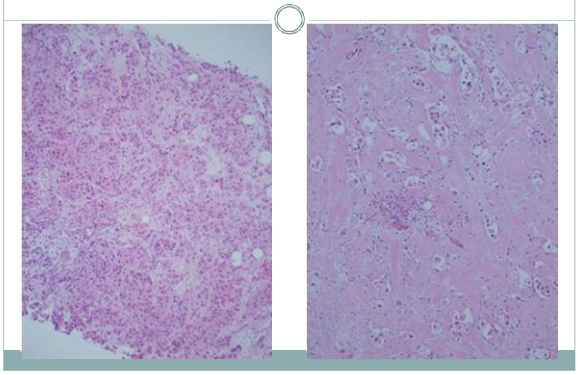
- Several grading systems in the literature
- Variables include:
- Residual tumour size
- Residual tumour cellularity
- Change from pre-treatment cellularity
- Number of lymph nodes involved
- Size of metastasis in lymph nodes
- Features of response in lymph nodes
- Biomarkers – ER, Ki67

Grading response

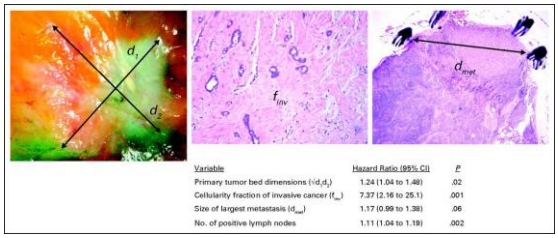
- Pinder et al., Histopathology 2006
- 1 = Complete pathological response, either no residual carcinoma or DCIS only
- 2 = Partial response to therapy,
- i minimal residual disease (<10% remaining)
- ii evidence of response but 10-50% remaining
- iii >50% tumour cellularity with features of response evident
- iv no evidence of response to therapy

- Axillary lymph nodes
- 1 = No evidence of metastatic disease or changes in the lymph nodes
- 2 = Metastatic tumour not detected but evidence of response eg. fibrosis
- 3 = Metastatic disease present but evidence of response
- 4 = Metastatic disease present with no evidence of response

Reduced cellularity post chemotherapy



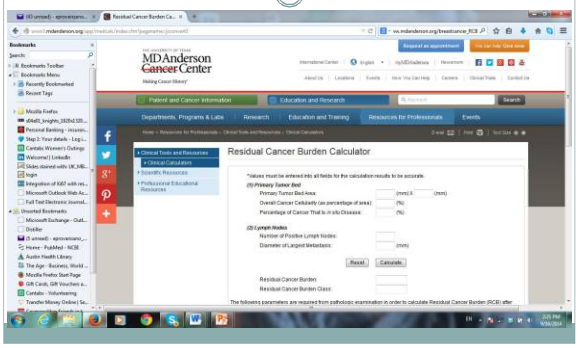
Residual Cancer Burden



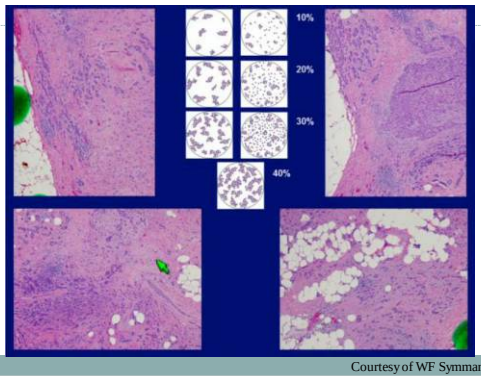
Variable	Hazard Ratio (95% CI)	P
Primary tumor bed dimensions ($\sqrt{d_1 d_2}$)	1.24 (1.04 to 1.48)	.02
Cellularity fraction of invasive cancer (f_{inv})	7.37 (2.16 to 25.1)	.001
Size of largest metastasis (d_{invT})	1.17 (0.99 to 1.38)	.06
No. of positive lymph nodes	1.11 (1.04 to 1.19)	.002

Symmans W F et al. JCO 2007;25:4414-4422

Residual Cancer Burden

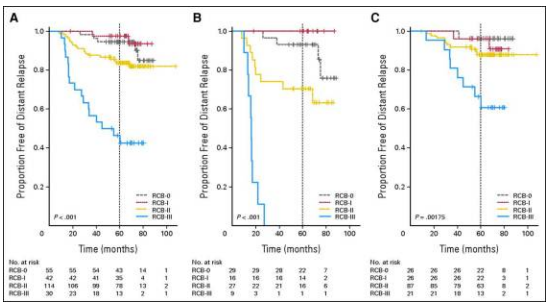


Residual Cancer Burden



Courtesy of WF Symmans

Residual Cancer Burden

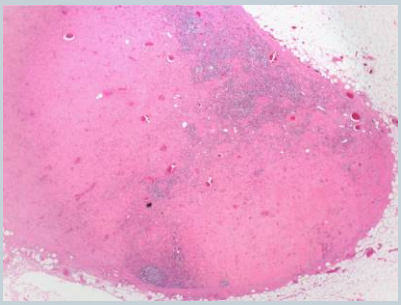


Symmans W F et al. JCO 2007;25:4414-4422

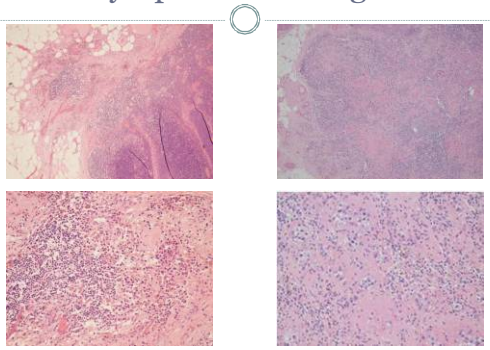
Assessment of axillary lymph nodes

- All nodes received should be embedded in their entirety
- Areas of myxoid change, fibrosis or collections of foamy macrophages suggestive of previous metastasis with response – intermediate prognosis compared with 'true' node negativity
- Low threshold for doing immunohistochemistry if suspicious of metastatic disease, especially if fibrosis HOWEVER no change in prognosis with occult metastasis identified by IHC alone (Loya et al., Cancer, 2009;115:1605-12)

Lymph node changes

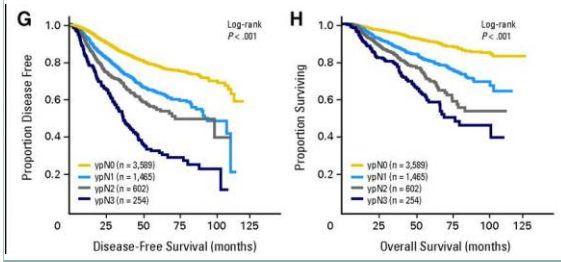


Lymph node changes



Significance of nodal response

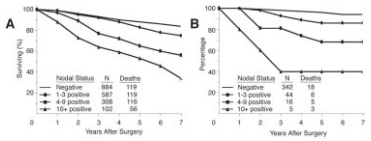
- Nodal status post chemotherapy is one of the strongest predictors of outcome



von Minckwitz G et al. JCO 2012;30:1796-1804

Significance of nodal response

- Around 5-6% of patients have residual disease in axilla despite pCR in the breast
- Residual nodal disease an independent predictor of poor prognosis, even in presence of pCR in the breast



No pCR pCR

ITC's post chemotherapy

- Presence of isolated tumour cells in lymph nodes
- TNM – call ypN0(i+) BUT not pCR
- WHO – call node positive i.e. NOT pCR
- NHS BSP – any residual tumour including ITCs call node positive

- do NOT regard as pCR
- often background fibrosis indicating previous macrometastatic disease with regression – nodal equivalent of minimal residual disease
- measure size of entire deposit including intervening fibrosis

ypN post chemotherapy

- New definition in 8th edition TNM – to be introduced beginning 2018
- Size of the largest contiguous focus of residual tumour in the node
- Any treatment associated fibrosis should NOT be included
- A description of number of foci present and total distance over which they extend may be helpful for clinicians to determine extent of residual disease
- This is NOT the measurement of size of metastasis used in the RCB, which is the largest deposit INCLUDING associated treatment related fibrosis

SLNB Pre Chemo: Pros and Cons

Pros:

- Accurate staging before treatment – may guide decisions regarding need for chemo/ radio Rx
- More experience with SLN in this setting
- Low axillary recurrence rates

Cons:

- Need for two operations
- May delay therapy
- Unnecessary ALND in patients who have axillary pCR (20-40%)
- Loss of information regarding chemotherapy response in axillary lymph nodes
- Can't calculate RCB if SLN +ve

SLNB Post Chemo: Pros and Cons

Pros:

- Large series suggest comparable accuracy to SLN in adjuvant setting
- No delays to commencement of therapy
- Single procedure
- Information regarding nodal response – may be better predictor of survival
- Avoid unnecessary ALND in patients who have pCR in axilla

Cons:

- Limited data regarding axillary recurrence rates
- Variable false negative rate if node positive before Rx – up to 30% in some series
- Learning curve for procedure

ACOSOG Z1071

- Eligibility – invasive breast cancer T0-4 N1-2 M0, proven axillary metastasis (Bx/ FNA), no prior axillary surgery
- Axillary US pre and post Rx – clip biopsied node
- 585 women cN1 with at least 2 SLNs and completion axillary clearance
- Nodal pCR rate 41% - 21% ER+/ HER2-, 49% TN, 65% HER2+
- 20.6% residual nodal disease confined to SLN
- Overall FNR 12.6% (10.8% if dual mapping vs 20.8% single mapping only)
- FNR 9.1% if 3 or more SLNs examined compared with 21% if 2 nodes examined and 31% if only a single node examined

Boughey et al., JAMA 2013;310(14):1455-61.

ACOSOG Z1071

- Conclusion – 'changes in approach and patient selection that result in greater sensitivity would be necessary to support use of SLN as an alternative to ALND in this population' -> avoid SLN if clinically evident residual nodal disease or poor response to therapy

Boughey et al., JAMA 2013;310(14):1455-61.

Targeted Axillary Dissection

- ACOSOG Z1071
- Follow up paper looking at patients with clip in biopsied node (n=170)
- 29 patients clip not located at time of axillary surgery
- 107 (76%) clip in SLN – FNR 6.8%
- 34 (24%) clip in non-SLN – FNR 19%
- 41% of cases clipped node was only positive node
- Correlation with overall LN status 93%

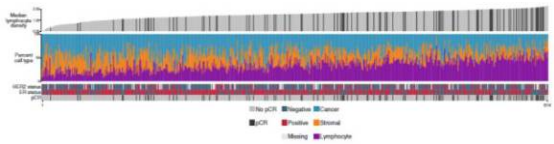
Boughey et al. Ann Surg 2016; 263(4):802-7.

SLNB - summary

- Loss of valuable information regarding axillary response with upfront SLNB
- Good evidence for accuracy in patients who are clinically N0 pre Rx
- Conflicting evidence regarding accuracy in node positive pts pre Rx
  - unacceptably high FNR in some series
- Role for clipping biopsied node

Immune response

- Increased levels of TILs pre Rx associated with pCR
- Highest lymphocyte density in HER2+ tumours
- Highest stromal cell density in ER+ HER2- tumours



Ali et al., Breast Cancer Research 2016;18:21.

Immune response

- Garcia-Martinez et al., Breast Ca Res 2014
- Higher levels of TILs post NACT associated with worse DFS
- High CD68 associated with worse DFS and OS
- ER+ cancers – high CD8 associated with worse survival
- Decrease in CD4 and CD68 and increase in CD8 post Rx
- Decrease in TILs in tumours with pCR

Immune response (TNBC)

- Dieci et al., Ann Onc 2014
- High levels of TILs associated with small residual tumour size and improved MFS (84% v 36%) and OS
- Majority of cases showed an increase in TILs post NACT compared with pre treatment biopsy
- Miyashita et al., Breast Can Res 2015;17:124.
- Increased CD8+ lyms post NACT associated with smaller residual tumours and better RFS and BCSS
- High CD8:FOXP3 ratio associated with RCB group and better RFS and BCSS
- Majority of tumours showed an increase in CD8 post Rx, and high rate of change associated with better survival