Breast Pathology Post-Neoadjuvant Chemotherapy

Megan Troxell, MD/PhD
Stanford Pathology
Society of Surgical Oncology–American Society for Radiation Oncology–American Society of Clinical Oncology Consensus Guideline on Margins for Breast-Conserving Surgery With Whole-Breast Irradiation in Ductal Carcinoma In Situ


2 mm
Objectives

• Develop a framework for gross analysis of post-chemotherapy breast specimens.
• Recognize histologic features of tumor bed and post-chemo carcinoma, including pitfalls
• Understand reporting schemes, utility and clinical impact of residual tumor burden
Neoadjuvant chemotherapy

- Equivalent long term outcome whether chemotherapy before or after surgery
- Neoadjuvant:
  - Can assess response/non-response
    - Degree of response: **prognostic for survival**
  - Response is short term endpoint for clinical trials
    - Tissue collection for research before/during/after
    - Downsize tumor for breast conserving surgery
- Chemo most efficacious in Her2+ or triple negative tumors, (vs ER+)
- Now standard of care for locally advanced breast cancer
More extensive diagrams
• Prognostic information from degree of pathologic response?
• Propose RCB as continuous variable:
  – primary tumor dimensions,
  – residual cellularity of tumor bed
  – axillary nodal burden
Initial diagnostic pathology

**Breast**
- Adequate core biopsy essential
  - Caution if limited tumor or extensive DCIS
- ER, PR, Her2 & other markers
  - % tumor cellularity for some studies
- Clip placement essential!

**Axilla**
- Status impacts local/systemic therapy
- Routine axillary ultrasound
- FNA or core bx for clinical, radiologic abnormal nodes
  - Clip!? Recommended by NCCN’15
- Up-front surgical SLN bx not recommended
  - Invalidates ypN & RCB
  - Precludes assessment of nodal response

**Hint:** core is often ~6 months ago if neoadjuvant Rx
Steps to evaluate post-chemo breast

1. Recognize its post-chemo
2. Identify gross tumor/tumor bed & document size
3. Judiciously sample (map)
4. Assess residual size (largest contiguous and span), cellularity & standard parameters
5. Evaluate lymph nodes
6. Report as per local custom/mandates
Essential clinical info with specimen

• “At an absolute minimum, the specimen must be clearly marked as post-NAST; pre-NAST location and size of the tumor must be indicated.” (Bossuyt)

<table>
<thead>
<tr>
<th>Table 1. Essential information to be provided to the pathologist with the surgical specimen removed after neoadjuvant systemic therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential and critical information to be provided or made available to the pathologist</td>
</tr>
<tr>
<td>This information is very important to maintain a high quality of histopathological evaluation and to minimize turnaround time. A suggested requisition form is provided in the supplementary Material S2, available at Annals of Oncology online.</td>
</tr>
<tr>
<td>Comment</td>
</tr>
<tr>
<td>1 Clearly marked as post-NAST specimen.</td>
</tr>
<tr>
<td>2 Is this part of a clinical trial? Does the trial protocol recommend a grading system for response?</td>
</tr>
<tr>
<td>3 Results of previous core biopsies, especially if they were carried out in another hospital.</td>
</tr>
<tr>
<td>In daily clinical routine, this information is often not passed along to the pathologist. The pathologist should be informed of any previous therapy (hormonal therapy, chemotherapy, radiation therapy, and/or other therapy) for the cancer. Important to follow trial protocol. Pathologist needs to know this information in advance of the surgery in order to follow protocol for description, processing, and reporting of the specimen. If this is a drug registration trial, the pathologist should be blinded to the treatment arm, or arrange for independent blinded secondary review of the case by another colleague. Core biopsy results: histologic type, grade, ER/PR, HER2 (and Ki67). Lab reference number. Ideally, slides should be available for review.</td>
</tr>
</tbody>
</table>

Was the axillary node clipped??

& five other elements
Patterns of residual CA

- Size unchanged
  - Cellularity decreased

- Size changed or unchanged
  - Cellularity decreased, heterogeneous

- Size changed or unchanged
  - Cellularity decreased, heterogeneous
  - Large areas without residual disease and apparent multiple foci
  - “Scatter pattern”

- Size decreased
  - Cellularity similar
  - “Concentric shrinking”

Research samples only if grossly obvious residual invasive cancer. (Bossuyt)
Specimen sampling

• CAP: “Special attention to finding and evaluating the tumor bed is necessary for these specimens.”

• Proenzano: “It is strongly recommended that an image of the sliced specimen be recorded (radiograph, photograph, photocopy, or drawing) and then used as a map for the sections taken, so that the histopathologic findings of any residual disease in the breast can be more easily understood.”

• Bossuyt: “Overly exhaustive sampling and histologic evaluation of the entire tumor bed are not required and not as efficient or informative as informed mapping of the specimen.”
Specimen sampling

• Small specimens: submit entirely, mapped
  – Document if tumor bed at specimen edge (esp if residual scattered ca)

• Large specimens: guided by pretreatment size & location, mapped
  – Provenzano: full face of pretreatment area every 1 cm
    • If very large, 5 blocks per every 1-2 cm, up to 25 blocks
    • Good clinical judgment on a case-by-case basis
  – RCB: Submit the largest cross-sectional area for histology
  – FDA: at least one block per cm of pre-treatment tumor size, or at least 10 blocks in total (greater of)
  – Sahoo ‘09: ~1 section/cm of original tumor size

• Multiple pretreatment lesions: as above & sample between lesions
  – Intervening invasive CA or DCIS?
  – Largest is used for RCB and ypT stage
Localizing post-chemo

Once you have the slides, document at least one of:

1) Tumor/lesion compatible with findings on prior biopsy
   -- If abundant tumor, I don’t mandate section of biopsy site
   -- Caution: second ‘occult tumor’

2) Tumor bed

3) Biopsy site or clip
   -- Rarely, minimal histologic evidence
   -- But clips can migrate, or displace during sectioning....
Grossly: fibrous rubbery area; here with residual tumor

Sahoo & Lester. Surgical Pathology Clinics 2012:5;749–74
Mastectomy: clips at 12:00 (or nipple bed)
Stitch axillary tail
Mastectomy: clips at 12:00 (or nipple bed)
Stitch axillary tail
Need to know location, size of pre-chemo cancer(s)
Any additional lesions on imaging

Section nipple, ink, slice, photo or Xray in order, then map sections taken
Pre-chemo cores heterogeneity, focal geographic necrosis (not shown)

Post-chemo
Poor response
Pre-chemo core

Post-chemo tumor bed: DCIS (below)
Safety pins mark lesions ID’ed fresh
Mapped with specimen Xray

Also see calcifications
Lumpectomy with 3 clips!
What is this in the specimen?


Gilcrease. AJSP 2016; 40:1375–1379
What is this in the specimen?

Savi SCOUT

Titanium Encapsulated Radioactive Seed


Mango. AJR 2016; 207:W1–W4

Gilcrease. AJSP 2016; 40:1375–1379
New wire-loc alternatives

- Savi SCOUT
- Radioactive seeds
- Intraoperative US
- Magseed

Transection of Radioactive Seeds in Breast Specimens

Gilcrease. AJSP 2016; 40:1375–1379

Michael Z. Gilcrease, MD, PhD,* Basak E. Dogan, MD,† Dalliah M. Black, MD,‡ Alejandro Contreras, MD,* Mark J. Dryden, MD,† and Sandra M. Jimenez, CHP, CLSO§

• I\(^{125}\) localized w/ gamma probe
• Mayo: 0/2000
• MD Anderson: 2/1400
• Case 1
  – Specimen radiograph shows parts of Ti cap in 2 slices!!
• Case 2: large ossified mass
  – Seed cut with bone saw
  – Titanium cap not damaged
  – Why the &^%$###!! was localization used?
• If immobilized with forceps, seeds can severed with scalpel blade
• “Use forceps with finesse;” no scissors
• Added recommendations:
  – Know where seed is; use same gamma probe as is used in OR
  – “as blade approaches the location of the seed, slow and careful slicing of the tissue”
  – Dedicated grossing bench; do not discard anything until seed retrieved
Histologic tumor bed

- Fibrosis +/- elastosis
- Prominent vessels
- Fewer to absent normal epithelial structures
- Inflammation
  - histiocytes, lymphs, giant cells
- Hemosiderin
- Calcification

Find:
1) Tumor
2) Tumor bed
3) Bx site or clip
Tumor bed: vessels, fibrosis, paucity of normal
Histiocyte rich tumor bed
Normal breast

Edge of tumor bed; same specimen
Edge of tumor bed

Biopsy site in tumor bed
Left: parenchymal atrophy post-chemo
Below: LCIS unperturbed by chemo
Lack of ‘tumor bed’ stromal change
Tumor Bed

......
History of G3 IDC, triple negative

Find:
1) Tumor
2) Tumor bed
3) Bx site or clip
History of G3 IDC, triple negative, prior

Find:
1) Tumor
2) Tumor bed
3) Bx site or clip

Several foci like this post-chemo
Prior axillary LN core biopsy
Biopsy site

Find:
1) Tumor
2) Tumor bed
3) Bx site or clip
Changes in carcinoma histology

- Histiocytoid appearance
- Cytoplasmic vacuolization, eosinophilia
- Nuclear hyperchromasia, pleomorphism
  - Multinucleation
- Decreased mitotic activity
- Lobular-like growth pattern
- Retraction artifact
- Heterogeneity (selection by chemo?)
  - Expect cellularity to vary across tumor

Sahoo, Provenzano
Tumor bed & DCIS
Tumor bed & DCIS
Tumor bed & DCIS
Atypia vs. DCIS?
Grading post chemo

• Often increased nuclear pleomorphism
• Often decreased mitotic rate
• Should still be graded
  – Nottingham/mSBR/Elston-Ellis
  – Tubules, nuclei, mitosis
Treated carcinoma

• Metaplasia due to chemotherapy?

• Squamous elements relatively resistant to chemotherapy?
Different morphologies across tumor
Heterogeneity post-chemo

Pre-chemo core
Residual histiocytoid IDC & DCIS
Heterogeneous tumor vs. histiocytes?
Residual invasive carcinoma vs histiocytes?
Residual invasive carcinoma vs histiocytes?
Multiple tumors: different chemo-sensitivity

**Left:**
Core bx IDC3, met to LN
ER+, PR- Her2+

**Right:**
Post-chemo tumor bed with no residual IDC
Same patient

Left:
Core bx ILC, E-cad negative

Right:
Post-chemo residual ILC
Chemo response: 
Her2+ > Triple Neg >> ER+Her2-
Post-chemo reporting: CAP (AJCC R)

In the Breast
• No known presurgical therapy
• No definite response to presurgical therapy in the invasive carcinoma
• Probable or definite response to presurgical therapy in the invasive carcinoma
• No residual invasive carcinoma is present in the breast after presurgical therapy

In the Lymph Nodes
• No known presurgical therapy
• No lymph nodes removed
• No definite response to presurgical therapy in metastatic carcinoma
• Probable or definite response to presurgical therapy in metastatic carcinoma
• No lymph node metastases. Fibrous scarring, possibly related to prior lymph node metastases with pathologic complete response
• No lymph node metastases and no prominent fibrous scarring in the nodes
<table>
<thead>
<tr>
<th>System</th>
<th>Score in breast</th>
<th>Correlate w/ core?</th>
<th>LN included?</th>
<th># categories partial</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-18</td>
<td>Any treatment effect on invasive</td>
<td>No</td>
<td>Yes, size met</td>
<td>1</td>
</tr>
<tr>
<td>Chevallier</td>
<td>Presence of invasive with sclerosis, fibrosis</td>
<td>No</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Sataloff</td>
<td>Presence of invasive</td>
<td>No</td>
<td>Yes +/- - TE</td>
<td>2</td>
</tr>
<tr>
<td>Sataloff</td>
<td>Presence of treatment effect</td>
<td>No</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>UICC</td>
<td>Product of 2 dimensions</td>
<td>No</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>Miller-Payne</td>
<td>Presence of invasive, cellularity</td>
<td>Yes</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>AJCC (y)</td>
<td>Size of invasive</td>
<td>No</td>
<td>Yes, #</td>
<td>Up to 4</td>
</tr>
<tr>
<td>MNPI</td>
<td>Size of invasive, grade</td>
<td>No</td>
<td>Yes, #</td>
<td>3</td>
</tr>
<tr>
<td>Pinder</td>
<td>% tumor remaining in breast</td>
<td>Yes</td>
<td>Yes, evidence of response</td>
<td>3</td>
</tr>
<tr>
<td>RCB</td>
<td>size in 2-dimensions, cellularity</td>
<td>No</td>
<td>Yes, # &amp; size</td>
<td>4</td>
</tr>
</tbody>
</table>
• Prognostic information from degree of pathologic response?
• Propose RCB as continuous variable: primary tumor dimensions, residual cellularity of tumor bed, and axillary nodal burden
  – Cohort of 382 patients; 2 different neoadjuvant chemo regimens
  – Validation cohort of 141
Residual Cancer Burden (RCB)

- Tumor size, 2 dimensions
- % residual invasive cellularity
- Number of residual involved lymph node
- Size of largest metastasis

**RCB index:**

\[ RCB = 1.4 \left( f_{inv} d_{prim} \right)^{0.17} + \left[ 4 \left( 1 - 0.75^{LN} \right) d_{met} \right]^{0.17} \]
Illustrative examples of how residual tumor bed would be defined.
Illustrative examples of how residual tumor bed would be defined.

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

**RCB**
- Largest area of residual invasive cancer (A, span)
- Does not need to be contiguous
- Two dimensions
- Favored by Provenzano

**AJCC 7th (ypT)**
- Largest **contiguous** focus of invasive cancer (B)
  - Use ‘m’ for scattered foci
- One dimension
Residual tumor % cellularity

Symmans W F et al. JCO 2007;25:4414-4422
Residual Cancer Burden (RCB)

Purists include all cancer cellularity, subtract DCIS (I just score invasive)

See website for instructions, helpful diagrams
### Post-chemo synopsis: example

<table>
<thead>
<tr>
<th>Focality of residual invasion:</th>
<th>Present as scattered single cells/small clusters. Present as multiple residual foci/masses. Present as single contiguous residual mass/focus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span of residual invasion:</td>
<td>______ cm x ______ cm</td>
</tr>
<tr>
<td>Size of largest contiguous focus:</td>
<td>______ cm</td>
</tr>
<tr>
<td>Average cellularity of invasive carcinoma in tumor bed:</td>
<td>______ %</td>
</tr>
<tr>
<td>Changes consistent with treatment effect:</td>
<td>Present Indeterminate No definite</td>
</tr>
</tbody>
</table>
Residual Cancer Burden Calculator

*Values must be entered into all fields for the calculation results to be accurate.

1. **Primary Tumor Bed**
   - Primary Tumor Bed Area: 11 mm x 14 mm
   - Overall Cancer Cellularity (as percentage of area): 25%
   - Percentage of Cancer That Is in situ Disease: 5%

2. **Lymph Nodes**
   - Number of Positive Lymph Nodes: 1
   - Diameter of Largest Metastasis: 8 mm

---

Instructions and several helpful downloads (diagrams)

http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3
Residual LVI

- Should not be considered pCR
- Ensure ID & adequate sampling of tumor bed
  - Most patients have other residual disease (LN)
- Confirm LVI, not DCIS, not retraction artifact (IHC)
- Don’t include in residual invasive cancer size
- LVI at margin: separately comment
- Lack of data for current reporting recommendations

- LVI per Rosen/CAP:
  - Outside the border of the invasive carcinoma
  - Tumor emboli do not conform to the contours of space; invasive with retraction has exactly the same shape.
  - Endothelial cell nuclei should be seen lining the space
  - Lymphatics often adjacent to blood vessels

- ‘Extensive LVI’
  - Provenzano: one or more foci in more than one block
  - Stanford: 3 foci
  - NCCN: not defined
  - Colleoni:
    - focal: one focus of in one tumor block only
    - Moderate: more than one focus in one block
    - Extensive: one or more foci in more than one tumor block
LVI with ‘satellite’
D2-40 expression by breast myoepithelium: potential pitfalls in distinguishing intralymphatic carcinoma from in situ carcinoma

Joseph T. Rabban MD, MPH*, Yunn-Yi Chen MD, PhD

Human Pathology. 2008;39:175-83

Endothelial markers
CD31, CD34, ERG

Lymphatic markers
LYVE-1, D2-40 (podoplanin)
Tumor in lymphatics relatively resistant to chemotherapy (as is DCIS)
• Retrospective study
  – Predominanty AC-4 cycles
  – 14% no residual cancer cells (pCR)
  – 10% DCIS only (pCR)
  – 4% pure IL tumor
  – 3.4% predominantly IL
  – 69% residual IDC

• IL 3-fold higher risk of death
Lymphovascular invasion is an independent predictor of survival in breast cancer after neoadjuvant chemotherapy

Ying L. Liu¹ • Anurag Saraf² • Shing M. Lee³ • Xiaobo Zhong³ • Hanina Hibshoosh⁴ • Kevin Kalinsky⁵ • Eileen P. Connolly² Breast Cancer Res Treat 2016; 157:555–64

• LVI was associated with poorer PFS and OS
  – independent of post-surgical stage/nodal status
  – No pathology review!

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No event</th>
<th>Event</th>
<th>HR (95 % CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Total patients</td>
<td>110</td>
<td>66</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td>LVI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>75</td>
<td>82</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>47</td>
<td>39</td>
<td>53</td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone positive/HER2 negative</td>
<td>51</td>
<td>71</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>43</td>
<td>74</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>TNBC</td>
<td>16</td>
<td>44</td>
<td>20</td>
<td>56</td>
</tr>
</tbody>
</table>

HR Hazard ratio, CI confidence interval, LVI lymphovascular invasion, TNBC triple-negative breast cancer
Axilla post-chemo: controversial

- **Pre-chemo clinical LN neg**
  - Post-chemo Clinical LN neg
    - SLN neg
      - No ALND
- **Pre-chemo LN positive**
  - Post-chemo Clinical LN neg
    - SLN & clip neg
      - No ALND???
  - Post-chemo Clinical LN positive
    - SLN or clip pos
      - ALND???
Axillary Lymph Nodes

• Up to 40% of patients convert to node-negative
• May be more difficult to ID post chemo
  – If so, submit fibrotic & perivascular areas in axilla
• Handle nodes with standard protocols
  – 2mm gross sections
• Report with standard protocols
  – #, sizes, ECE
  – AND treatment effect/fibrosis
SLN: fibrosis, no residual CA
SLN: fibrosis,
Exclude residual tumor cells
Post-chemo SLN: Granulomas?
Post-chemo SLN
Germinal center?
Post-chemo SLN Germinal center?
SLN FS

Both foci cancer?
SLN FS

How about now?
SLN permanents

Right: CA
Left: clip
SLN frozen section
SLN Permanents

Top: tumor & fibrosis
Bottom: clip
“In our opinion, it is best to exercise caution....and maintain a low threshold for deferral of the final diagnosis to permanent sections in order to avoid false-positive & unnecessary ALND.”
LN reporting post chemo

AJCC (ypN)
• Isolated tumor cells reported as node negative (ypN0itc)
• But not regarded as pCR

WHO
• Isolated tumor cells node positive

Provezano (opinion)
• Any residual disease in LN (mi, itc) should NOT be classified as pCR
• If no associated fibrosis, report as in adjuvant setting (ypN0itc)
• If fibrosis, likely macro- or micromet with response
  – Describe in comment
  – Measure entire area, including tumor cells & intervening stroma
Different pCR definitions

Provenzano, Mod Pathol

Residual LVI?

ypTis vs. ypT0:
--German study
Lower DFS but same overall survival
--MD Anderson, no difference
<table>
<thead>
<tr>
<th></th>
<th>pCR</th>
<th>NOT pCR</th>
<th>Insufficient evidence</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>X</td>
<td></td>
<td></td>
<td>Definitions vary for DCIS</td>
</tr>
<tr>
<td>LCIS</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVI</td>
<td>X</td>
<td>X</td>
<td></td>
<td>LVI w/o LN disease very rare</td>
</tr>
<tr>
<td>LN: macro- &amp; micro-met</td>
<td>X</td>
<td></td>
<td></td>
<td>Residual CA in LN worse prognosis irrespective of breast</td>
</tr>
<tr>
<td>LN: isolated tumor cells</td>
<td>X</td>
<td></td>
<td></td>
<td>Mi &amp; itc difference significance than adjuvant setting</td>
</tr>
</tbody>
</table>

From Bossuyt
Retesting of biomarkers

Proenzano
• Routine retest not recommended
  – Positive ER/PR/Her2 core
• Consider retest
  – Negative or equivocal result on core
  – Outside biopsy/markers
  – Heterogeneous or multiple tumors
  – No response to therapy

• Discordance post-chemo
  – ER: ~15%
  – PR: ~30%
  – Her2: 6-9%

• Due to:
  – Technical failures
  – Intratumoral heterogeneity
  – Changes due to therapy
  (selection)

• Working group: 6/20 retest routinely
Left: Core biopsy
Her2+

Right: Tumor bed with fibrosis, lymphs, histiocytes, no tumor
Steps to evaluate post-chemo breast

1. Recognize its post-chemo
2. Identify gross tumor/tumor bed & document size
3. Judiciously sample (map)
4. Assess residual size (largest contiguous and span), cellularity & standard parameters
5. Evaluate lymph nodes
6. Report as per local custom/mandates
Selected references

- Lakhani et al eds. WHO Classification of Tumours of the Breast. 2012
- Symmans WF et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy.JCO 2007;25:4414-4422
- See also on-slide references
End