

Protocol for the Examination of Specimens from Patients with Invasive Carcinoma of the Breast

Protocol applies to all invasive carcinomas of the breast of any type, with or without ductal carcinoma in situ (DCIS). Includes invasive and microinvasive carcinomas. This protocol does not apply to DCIS without invasive carcinoma, Paget disease of the nipple without invasive carcinoma, encapsulated or solid papillary carcinoma without invasion, phyllodes tumor, lymphoma, or sarcoma.

Based on:

AJCC Cancer Staging Manual, Eighth Edition pTNM Requirements
CAP Cancer Protocols: Breast.Invasive_4.7.0.0, Breast.Invasive.Bx_1.1.1.1
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Revision History:

1st Edition – March 2017
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Summary of Changes:

This protocol is revised to the 8th edition of the AJCC Cancer Staging Manual and the current version of the CAP Cancer Protocols Breast.Invasive_4.7.0.0 and Breast.Invasive.Bx_1.1.1.1.

This is a major revision to the protocol with extensive changes throughout the document.

Procedures Covered in this Protocol:

- ✚ Biopsies
- ✚ Excisions less than total mastectomy with or without axillary contents
 - Localized excision
 - Excisions without localization
- ✚ Additional excision performed after the definitive resection (re-excision of surgical margins)
 - Complete
 - Select
- ✚ Total mastectomy with or without axillary contents
 - Simple mastectomy
 - Skin-sparing mastectomy
 - Nipple-sparing mastectomy
 - Modified radical mastectomy
 - Radical mastectomy

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Structured Macroscopic Report
Invasive Carcinoma of the Breast

Procedure

☐ Excision (*less than total mastectomy*)
☐ Mastectomy (*including modified radical, simple, nipple-sparing, and skin-sparing mastectomy*)
☐ Other (*specify*): _____
☐ Not specified

Specimen Laterality

☐ Right
☐ Left
☐ Not specified

How Specimen Received

☐ Fresh
 + ☐ Intraoperative consultation (*frozen section, touch preparation, etc.*)
 + ☐ Tissue collected for ancillary diagnostic studies, (*specify*): _____
☐ In fixative
 ☐ 10% neutral buffered formalin
 ☐ Other fixative (*specify*): _____
 + Cold ischemic time: _____
 + Fixation time (*specify if for entire specimen or specific components*): _____
☐ Other transport media (*specify*): _____

Specimen Orientation

+ ☐ Specimen received oriented
 + ☐ Oriented by surgeon (*specify*): _____
 + ☐ Oriented based on anatomic landmarks (*i.e., presence of axillary contents / lymph nodes*)
+ ☐ Not oriented

Specimen Integrity

+ ☐ Intact
+ ☐ Disrupted / fragmented
+ ☐ Other (*specify*): _____

Ink Key

+ ☐ Ink applied (*provide anatomic site and color applied*): _____

Overall Dimensions (cm) (g)

Mastectomy / Excision

Oriented dimensions:

___ (*superior to inferior*) x ___ (*medial to lateral*) x ___ (*anterior to posterior*) cm

Unoriented but orientation can be acquired and reasonably presumed based on anatomic landmarks dimensions:

___ (*presumed superior to inferior*) x ___ (*presumed medial to lateral*) x ___ (*presumed anterior to posterior*) cm

Unoriented and unable to orient based on anatomic landmarks:

___ x ___ x ___ cm

___ g

Specimen Anatomical Components and Size (cm)

____ Skin (if applicable): ____ x ____ cm, include location (anterior, medial, lateral, etc.) _____
____ Nipple and areola (if applicable): ____ x ____ cm
____ Axilla (if applicable): ____ x ____ x ____ cm
____ Muscle
 + ____ Small, scattered straps
 + ____ Significant muscle resected with the specimen: ____ x ____ x ____ cm
____ Chest wall: ____ x ____ x ____ cm

Macroscopic Tumor

____ Not identified
____ Previous biopsy site (include with or without presence of macroscopic tumor)
____ Present

Macroscopic Tumor Size

____ Greatest dimension of largest tumor: ____ cm
 + Additional dimensions: ____ x ____ cm
 + ____ Location:
 + ____ Upper outer quadrant
 + ____ Lower outer quadrant
 + ____ Upper inner quadrant
 + ____ Lower inner quadrant
 + ____ Central
 + ____ Nipple
 + ____ Clock position (specify): ____ o'clock
 + ____ Other (specify): _____
 + ____ Not specified

+ ____ Additional tumor(s):
 + ____ Number of additional tumors: ____
 + ____ Size of additional tumor(s): ____ x ____ x ____ cm (if more than one give range)
 + ____ Location: (if multiple additional tumors, check all locations involved by additional tumors)
 + ____ Upper outer quadrant
 + ____ Lower outer quadrant
 + ____ Upper inner quadrant
 + ____ Lower inner quadrant
 + ____ Central
 + ____ Nipple
 + ____ Clock position (specify): ____ o'clock
 + ____ Other (specify): _____
 + ____ Not specified

____ No residual tumor
 + ____ In the absence of macroscopic residual tumor give previous biopsy site measurement in three dimensions: ____ x ____ x ____ cm
____ Indeterminate (explain): _____

+Macroscopic Appearance of the Largest Tumor

+ ____ Color: _____
+ ____ Borders (circumscribed, ill-defined): _____
+ ____ Consistency (indurated, soft, fibrous): _____
+ ____ Presence or absence of previous biopsy (hemorrhage, fat necrosis): _____
 + ____ Number of biopsy sites identified: ____
 + ____ Presence or absence of radiographic biopsy marker
 + ____ Number of radiographic biopsy markers recovered: ____
 + ____ Presence or absence of radioactive seed

Macroscopic Appearance of Additional, Smaller tumors

- + ☐ Similar to the largest
- + ☐ Other (*specify*): _____
- + ☐ Presence or absence of previous biopsy in smaller tumors (*specify including location and presence or absence of biopsy marker*): _____

Macroscopic Extent of Tumor (*involvement of adjacent structures*)

Skin

- ☐ Direct extension of tumor into skin, without ulceration
- ☐ Direct extension of tumor causing skin ulceration
- ☐ Satellite skin nodules / possible tumor nodules are present (*i.e., not contiguous with the primary tumor in the breast*) (*classified as T4b*)

Nipple

- ☐ Peau d'orange
- ☐ Scaly
- ☐ Erythema
- ☐ Ulceration

Skeletal Muscle

- ☐ Skeletal muscle is not involved by tumor
- ☐ Skeletal muscle is involved by tumor
 - ☐ Depth of skeletal muscle involvement: ____ cm
- ☐ Tumor invades through skeletal muscle and into the chest wall (*classified as T4a*)

Note: Invasion into pectoralis muscle is not considered chest wall invasion, and cancers are not classified as T4a unless there is invasion deeper than this muscle.

Macroscopic Margin Involvement

- ☐ Cannot be assessed
- ☐ Margin(s) macroscopically uninvolved by tumor
 - Distance of tumor to closest margin: ____ cm
 - Specify margin, if possible: _____
 - + ☐ Distance from other margins (*specify cm distance including greater than if appropriate*):
 - + ☐ Anterior: ____ cm
 - + ☐ Posterior: ____ cm
 - + ☐ Superior: ____ cm
 - + ☐ Inferior: ____ cm
 - + ☐ Medial: ____ cm
 - + ☐ Lateral: ____ cm
 - + ☐ Other (*specify margin*): ____ cm
- ☐ Margin(s) macroscopically involved by tumor
 - Specify margin(s), if possible: _____
- ☐ Indeterminate (*explain*): _____

+Macroscopic Appearance of Uninvolved Tissue / Organ(s)

- + ☐ Skin
 - + ☐ Peau d'orange
 - + ☐ Other skin lesion(s)
- + ☐ Breast parenchyma
 - + ☐ Fibro-fatty ratio

Lymph Node Examination

- ☐ No lymph nodes submitted or macroscopically identified
- ☐ Total number of lymph nodes sectioned and macroscopically examined:
- Number of sentinel nodes macroscopically examined (*if applicable*):
- Number of lymph nodes macroscopically suspected to be involved by tumor:
- Size of largest macroscopic metastatic deposit (*mm*): mm
- Size of lymph nodes in three dimensions: x x cm or range in size: - cm
- + ☐ Clip or other marker (*previously biopsied lymph node*)

Extranodal Extension

- ☐ No macroscopic extranodal extension
- ☐ Macroscopic extranodal extension

+ ☐ **Tissue Submitted for Research** (*specify, if possible*): _____

(+) Data elements preceded by this symbol are optional elements which may be clinically important but are not yet validated or regularly used in patient management.

Recommended Block Allocation Key

Excisional / Lumpectomy Specimens Serial Sequential Sampling

A1:	Slice / level 1, anterior margin, perpendicular section
A2:	Slice / level 2 to include medial, lateral, superior, and inferior margins
A3:	Slice / level 3 to include medial, lateral, superior, and inferior margins
A4-A5:	Slice / level 4 to include medial, lateral, superior, and inferior margins
A6-A7:	Slice / level 5 to include tumor with medial, lateral, superior, and inferior margins
A8-A9:	Slice / level 6 to include tumor and core biopsy site with medial, lateral, superior, and inferior margins
A10-A11:	Slice / level 7 to include tumor and core biopsy site with medial, lateral, superior, and inferior margins
A12:	Slice / level 8 to include medial, lateral, superior, and inferior margins
A13:	Slice / level 9 to include medial, lateral, superior, and inferior margins
A14:	Slice / level 10 to posterior margin, perpendicular section

Non-Sequential Sampling

A1:	Slice / level 1, superior margin, perpendicular section
A2:	Slice / level 5 to include tumor with medial and posterior margins
A3:	Slice / level 6 to include tumor and core biopsy site with medial and posterior margins
A4:	Slice / level 7 to include tumor and core biopsy site with medial and posterior margins
A5:	Slice / level 10 separate nodular lesion with anterior margin
A6:	Lateral margin
A7:	Anterior margin
A8:	Slice / level 12, inferior margin, perpendicular section

Mastectomy (macroscopic tumor present)

A1:	Nipple (or deep surface of nipple for nipple sparing simple mastectomy)
A2:	Tumor to include core biopsy site, with / without clip
A3-A4:	Tumor
A5:	Closest deep margin
A6:	Closest anterior margin
A7:	Random, upper outer quadrant
A8:	Random, lower outer quadrant
A9:	Random, upper inner quadrant
A10:	Random, lower inner quadrant
A11:	Lymph nodes (intramammary, etc.), entirely submitted*

Mastectomy (no macroscopic tumor present)

A1:	Nipple (or under surface of nipple for nipple sparing simple mastectomy)
A2-A4:	Core biopsy site to include adjacent breast tissue
A3:A5:	Closest deep margin
A6:	Closest anterior margin
A7:	Random, upper outer quadrant
A8:	Random, lower outer quadrant
A9:	Random, upper inner quadrant
A10:	Random, lower inner quadrant
A11:	Lymph nodes (intramammary, etc.), entirely submitted*

Neoadjuvant Mastectomy (no macroscopic tumor present)

A1:	Nipple (or deep surface of nipple for nipple sparing simple mastectomy)
A2-A14:	Tumor bed entirely submitted as per diagram, with anterior margin in A4, A5, A7, and A8, and possible residual tumor in A6 and A11
A15-A16:	Posterior margin
A17:	Random, upper outer quadrant
A18:	Random, lower outer quadrant
A19:	Random, upper inner quadrant
A20:	Random, lower inner quadrant
A21:	Lymph nodes (inframammary, etc.), entirely submitted*

*Note: All lymph nodes identified must be entirely submitted.

Specimen Handling / Dissection Guidelines

There are currently guidelines for reporting results of ER, PgR, and HER2 testing in breast carcinomas, published by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), which require recording specific pre-analytic and analytic variables that can affect test results. Compliance with cold ischemia time (less than one hour) and fixation time (between 6 and 72 hours) is required for the validity of immunohistochemical testing and is relevant to patient treatment. The cold ischemic time and time placed in formalin should be recorded and included in the report.

Breast Biopsy

■ **Core Needle Biopsy (CNB) (radiographic, ultrasound guided, MRI)**

CNBs performed for detection of microcalcifications should be radiographed to confirm that the calcifications have been sampled. CNBs may be submitted by clinician / radiologist previously radiographed and separated into cores with and without calcifications. However, when they are submitted in one container with no designation as to which cores may have calcifications present, the tissue maybe radiographed in pathology, a using a cabinet x-ray system for example, to identify which cores have calcifications and designate those in a particular block or blocks.

- Measure the cores individually (length x diameter), or range in size when multiple (greater than 7).
- Place cores individually in the appropriate number of cassettes to avoid overlapping of specimens (consider using lens paper or biopsy wraps).

■ **Excisional Biopsy / Lumpectomy (excision, segmental resection, lumpectomy, quadrantectomy, and segmental or partial mastectomy, with or without axillary contents)**

Incisional biopsies (open biopsies) performed by surgeons to establish a diagnosis will be handled in this protocol as an excisional biopsy. The nipple is usually not included with these specimens.

- Weigh specimen (g).
- Indicate specimen orientation as provided by surgeon.
- Measure specimen (cm) in planes of dimension (anterior – posterior / medial – lateral superior – inferior).
- Document if present:
 - Skin – two-dimension measurement (cm) and record location. Describe appearance (retraction, etc.) and document any lesion or scars.
 - Localization wire / needle – indicate the site of insertion. **(Figure 1)**



Figure 1: Photograph of wire localized lumpectomy specimen with suture markings prior to inking.
Photograph courtesy of Mayo Clinic, Rochester, MN.

- Document the presence and location of any defects to margins.

A gross photograph may be helpful for later documentation. If the final surgical margin is compromised, this should be explicitly documented in the macroscopic description.

- Differentially ink the margins per institutional protocol to maintain orientation. **(Figure 2)**

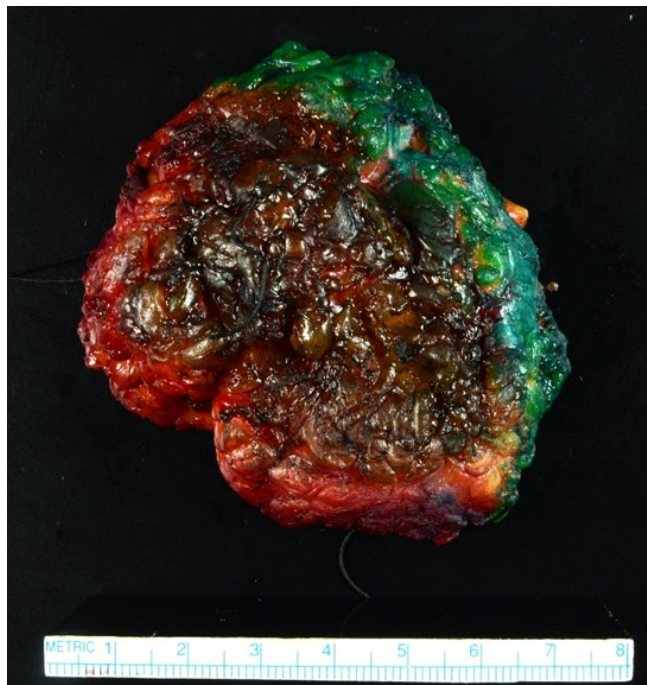


Figure 2: Differential ink application, lumpectomy specimen.
Photograph courtesy of Mayo Clinic, Rochester, MN.

- Serially section the specimen parallel to the short axis into thin slices (aim for 0.3 – 0.5 cm thick) maintaining orientation.
 - Indicate how the specimen was sectioned (i.e., medial to lateral, superior to inferior, etc.), the number of slices, and the average thickness (or range of thickness) of slices to aid the pathologist in determining the size of the lesion.
- If possible, the sectioned specimen should be radiographed and photographed. A specimen radiograph helps confirm the adequacy of the excision by verifying that the correct localized or previously biopsied area was removed as well as to identify where in the specimen the radiographic marker is located to aid in sectioning (*refer to imaging considerations, page 24*).

The radiograph and / or photograph (or other diagram method) is useful for mapping out sections (refer to imaging considerations).

- Describe the cut surface.

Macroscopic Tumor Present

- Document location and size (cm) of macroscopic tumor.
- The size of the tumor is an important prognostic factor. Note the pT classifications based on tumor size:
 - No residual tumor (pTX or pT0)
 - Tumor $\leq$ to 2.0 cm (pT1)
 - Tumor >2.0 cm but $\leq$ 5.0 cm (pT2)
 - Tumor >5.0 cm (pT3)
- Note if tumor is well-circumscribed or ill-defined, and describe macroscopic features (color, texture etc.) (*refer to macroscopic features of tumor*).
- Document the distance (cm) of tumor to all six margins.
- Note core biopsy changes associated with tumor.
- Note radiographic marker(s), describe shape, and indicate relationship to tumor and which slice the marker is identified in.
- If specimen contains more than one tumor:
 - Document number and size (cm) of each additional tumor. Provide all information for each individual mass as noted above.
 - Note the pT classification will be based on the size of the largest tumor.
 - Document the distance between each additional tumor.
- Describe the remaining tissue, documenting size, macroscopic features, and location of any additional unbiopsied lesions.

No Macroscopic Tumor Present

- Document the size (cm) and location of the area of radiographic concern. If the area of radiographic concern is not macroscopically identified, the sectioned specimen should be radiographed (*see imaging considerations*).
- Describe the changes associated with the area of prior biopsy when present (hemorrhage, fat necrosis).
- Describe the radiographic marker (shape) when identified.
- Document the distance (cm) of the prior biopsy site / area of radiographic concern to all six margins.
- Describe the remaining tissue, documenting size, macroscopic features, and location of any additional unbiopsied lesions.

■ Special Considerations When Submitting Excisional Breast Specimens

Serial Sequential Sampling (SSS)

With this method of sampling the entire specimen is submitted sequentially for microscopic examination such that the location of each block can be determined by means of dictation and / or directly on the specimen radiograph, specimen photograph, or other method deemed appropriate. When using SSS, indicate the anatomic direction in which the specimen was submitted and note the tissue blocks that contain tumor / area of radiographic concern and closest margin(s) (specify margins). When multiple, different colored inks are used to demonstrate individual margins; sections may contain more than one margin. Additionally, note tissue blocks that contain additional unbiopsied lesions. (**Figure 3**)

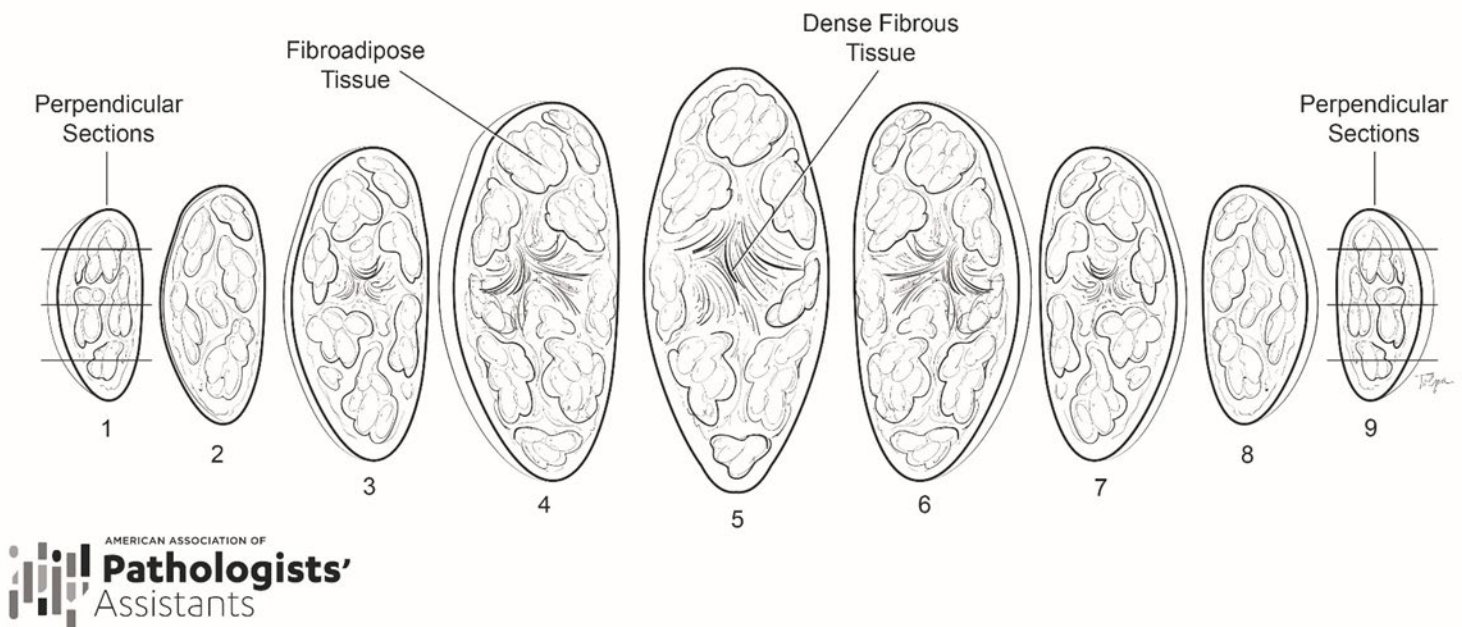


Figure 3: Excisional Biopsy - Serial Sequential Sectioning with Number Designation

When serially sectioning an excisional specimen, number each slice and record which slice(s) the tumor, biopsy site, or clip is located.

Non-sequential Sampling (NSS)

With this method of specimen sampling, representative sections of the tumor / area of radiographic concern are submitted in a serial sequential fashion along with perpendicular sections of the closest margin(s) when possible. When multiple, different colored inks are used to demonstrate individual margins; sections may contain more than one margin. Also, if residual tumor is small or not identified, consideration should be given to submitting the entire area of radiographic concern. Any newly identified unbiopsied lesions are sampled along with closest margin when possible. Remaining margins will be sampled with perpendicular sections. The orientation and location of each block can be mapped by means of dictation and / or directly on the specimen radiograph, specimen photograph, or other method deemed appropriate.

Specimen Radiograph Following Sectioning

The sectioned lumpectomy specimen can be laid out, numbered and radiographed. (**Figure 4**) The specimen radiograph can aid in the identification of the radiographic marker as well as detect calcifications. Utilization of specimen radiography can guide the selection of tissue to submit for histologic examination and minimize the number of sections submitted.

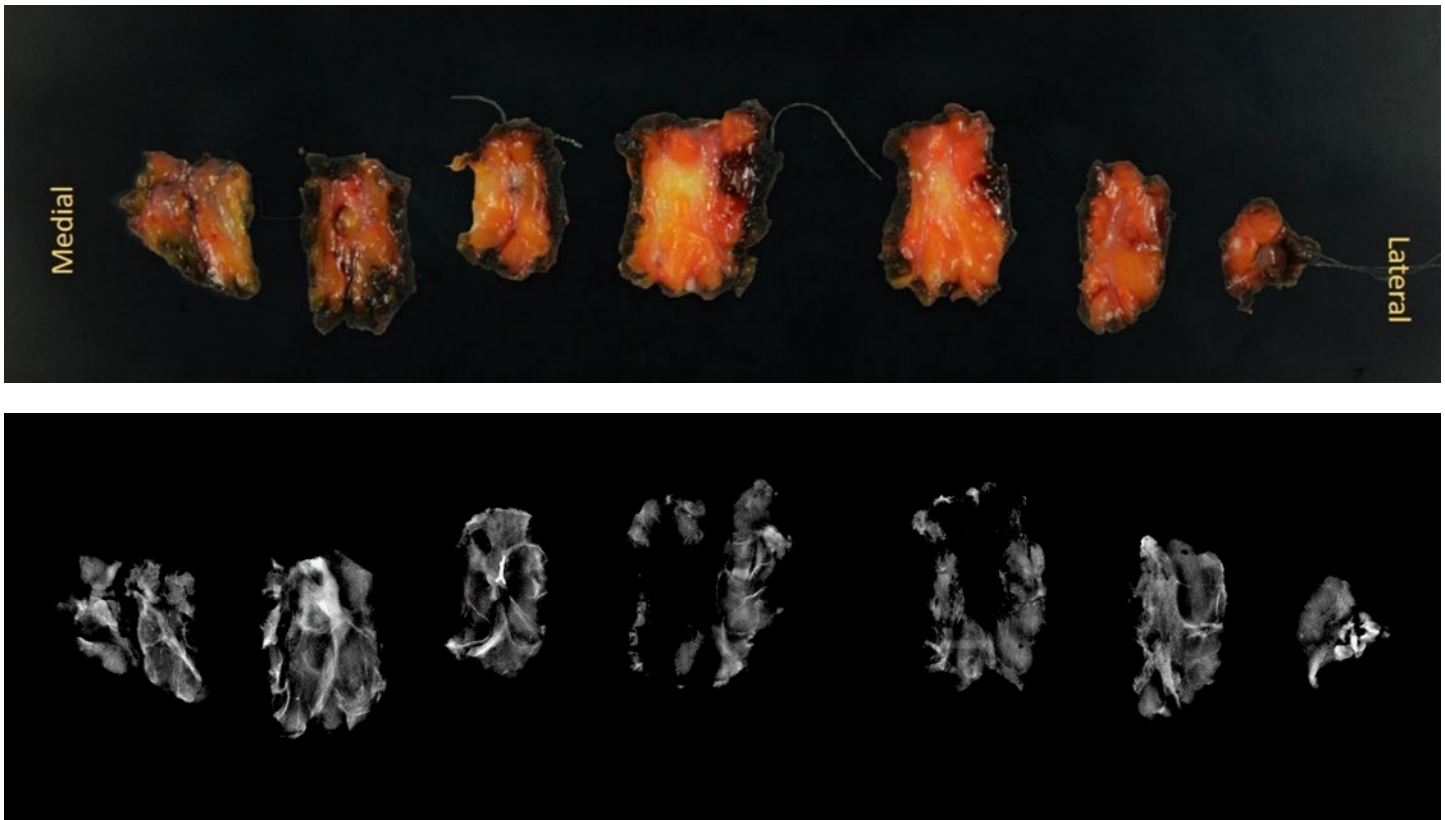


Figure 4: Sectioned lumpectomy with corresponding radiographic image.
Photograph and radiograph courtesy of Mayo Clinic, Rochester, MN.

- Submit Sections:
 - When practical the entire specimen should be submitted for microscopic examination using the SSS method.
 - For larger specimens, when it is not practical to submit the entire specimen, submit representative sections using the NSS method.
 - Serial sequential sections of tumor / area of radiographic concern with closest margin(s).
 - If more than one tumor / area of radiographic concern:
 - Each tumor / area or radiographic concern is sampled.
 - Include closest margin(s) whenever possible.
 - Sample tissue in between each lesion.
 - One perpendicular section of each remaining margin.
 - All additional unbiopsied macroscopic lesions and closest margin.
 - Representative section of uninvolved fibrous areas if indicated.

■ Re-excisions

Complete Re-excisions (six margin excision)

- Weigh (g), measure (cm), orient, ink, and section the specimen as described above.
- When possible, radiograph and photograph the sectioned specimen for mapping sections.
- Describe the cut surface:
 - Document size of biopsy cavity in three dimensions (cm), including anatomic planes of orientation.

- Describe the macroscopic features including the presence or absence of residual tumor.
 - Note size (cm) of residual tumor if present and distance (cm) to all six margins.
 - In the absence of residual tumor give measurement (cm) of margin clearance to all six margins.
- Describe uninvolved parenchyma / cut surfaces.
- Submit sections:
 - When practical, the entire specimen should be submitted.
 - For larger specimens submit representative sections maintaining specimen orientation.
 - Submit all tissue for any margin in which the prior resection was positive or had a clearance of 0.1 cm or less.
 - Representative perpendicular section of negative margins giving preference to fibrous areas or areas of narrow clearance.

Select re-excision (less than six margin re-excision)

- Weigh (g), measure (cm), and orient the specimen as described above.
- Measure and describe the previous resection / lumpectomy surface.
- Ink the new margin, and if more than one margin is given, differentially ink the margins according to your institution's protocol.
 - If the previous resection / lumpectomy surface is marked or macroscopically evident, this surface can also be inked, but be clear to state the color used represents the old resection surface and is not a true margin.
- Serially section the specimen parallel to the short axis into thin slices (aim for 0.3 – 0.5 cm thickness).
- When possible, radiograph and photograph the sectioned specimen for mapping sections.
- Describe the cut surface.
 - Describe the old resection surface.
 - Describe the macroscopic features including the presence or absence of residual tumor.
 - Note size (cm) of residual tumor if present and distance (cm) to all six margins.
 - In the absence of residual tumor give measurement (cm) of margin clearance to all margins.
- Describe the uninvolved specimen.
- Submit sections:
 - When practical, the entire specimen should be submitted for microscopic examination using the SSS method.
 - For larger specimens use the NSS method maintaining specimen orientation.
 - Submit all tissue for any margin in which the prior resection was positive or had a clearance of 0.1 cm or less.
 - Representative perpendicular sections of negative margins giving preference to fibrous areas or areas of narrow clearance.
 - For other thicker areas, non-sequential sections at regular intervals, with preference given to fibrous areas, are acceptable.

■ Mastectomy

Includes skin-sparing and nipple-sparing mastectomy, with or without axillary contents, simple mastectomy, and modified radical mastectomy. Mastectomy specimens for DCIS and / or biopsy proven cancers are routinely accompanied by sentinel lymph node biopsy. For a detailed discussion on handling sentinel lymph nodes or axillary contents, refer to lymph node sampling and Appendix III.

- Weigh (g) specimen.
- Measure specimen (cm) in planes of dimension (anterior – posterior / medial – lateral / superior – inferior).
- Indicate specimen orientation as provided by the surgeon.
- Document presence of additional structures if present, and measure (cm) in two dimensions:
 - Skin ellipse
 - Nipple / areola.
 - Axilla
 - Pectoralis muscle
 - Other structures when present
- Describe skin ellipse (color, retraction, ulceration overlying tumor, direct tumor involvement).
 - Note that direct tumor involvement of the dermis or epidermis with skin ulceration or the presence satellite skin foci of tumor that are not contiguous with the main tumor in the breast are classified as pT4b.
- Describe nipple (inverted, everted, retracted, and / or ulcerated).
- Document the presence and location of any defects to margins.

A gross photograph may be helpful for later documentation. If final surgical margin is compromised, this should be explicitly documented in the macroscopic description.

- Differentially ink the margins per institutional protocol.
- Serially section the specimen as thin as possible while preserving deep margin to skin surface. Ability to thinly section will be dependent upon each specimen.
- A specimen radiograph may be useful for identifying the radiographic marker when there is no grossly visible tumor, or the biopsy was performed several months prior to mastectomy and there are no gross changes of recent biopsy. A specimen radiograph may also be useful for detection of calcifications that were not originally sampled. Utilization of specimen radiography can guide in the mapping out and selection of tissue submitted for histologic examination, as well as minimize the number of sections submitted (*refer to imaging considerations*).

Macroscopic Tumor Present

- Document location and size (cm) of macroscopic tumor.
 - The size of the tumor is an important prognostic factor. Note the tumor size and the effect on pT classification:
 - No residual tumor (pTX or pT0)
 - Tumor ≤ 2.0 cm (pT1)
 - Tumor >2.0 cm but ≤ 5.0 cm (pT2)
 - Tumor >5.0 cm (pT3)
- Note if tumor is well-circumscribed or ill-defined, and describe macroscopic features (color, texture, etc.) (*refer to macroscopic features of tumor*).
- Document distance (cm) of tumor to the anterior margin (or overlying skin) and posterior margin.
- Note the radiographic marker(s), describe shape, and indicate relationship to tumor (*see radiographic markers*).

- Describe the remaining tissue, documenting size, unexpected or remarkable macroscopic features, and location of any unbiopsied lesions.
- If mastectomy contains more than one tumor / area of radiographic concern:
 - Document number and size of each tumor / area of radiographic concern.
 - Note the T classification will be based on the size of the largest tumor.
 - Document macroscopic features, and distance to the anterior margin (or overlying skin) and posterior margin for each tumor / area of radiographic concern.
 - Note any associated radiographic biopsy markers, shape, and relationship to tumor / area of radiographic concern.
 - Document the distance between each additional tumor / area of radiographic concern.

No Macroscopic Tumor Present

- Identify and measure the area of radiographic concern.
- If radiographic area of concern and marker are not macroscopically identified, specimen should be radiographed (*see imaging considerations*).
- Describe the changes associated with the area of prior biopsy when present (hemorrhage, fat necrosis).
- Describe the radiographic marker (shape) when identified.
- Document measurement of the area of radiographic concern to the anterior margin (or overlying skin) and posterior margin.
- Describe the remaining tissue, documenting size, macroscopic features, and location of any additional unbiopsied lesions.

Post Lumpectomy Mastectomy

- Document the size of biopsy cavity (cm) in three dimensions.
- Describe the gross features including the presence or absence of residual tumor.
 - Note size (cm) of residual tumor if present and distance (cm) to margins.
 - In the absence of residual tumor give measurement (cm) of margin clearance.
- Describe the remaining tissue, documenting size, macroscopic features, and location of any additional unbiopsied lesions.
- Submit sections:
 - Several sections of tumor, one of which to include prior biopsy site.
 - In the absence of macroscopic tumor submit the entire area of radiographic concern.
 - In post lumpectomy specimens sample the cavity surface to adjacent breast tissue giving preference to fibrous areas.
 - Include margins when both can be demonstrated on one slide.
 - Closest posterior (deep) and anterior margins.
 - Include separate section of pectoralis muscle if present but not close to tumor / prior biopsy site.
 - Nipple - perpendicular sections.
 - For nipple sparing simple mastectomy: shave, serially section, and entirely submit the nipple margin.
 - If there is a clinical history of Paget's disease see section on nipple for Paget's disease (**Figure 6, page 19**).
 - Additional macroscopically evident unbiopsied lesion(s).
 - Random quadrant sections (1 block / quadrant), focusing on fibrous tissue.

■ **Neoadjuvant Mastectomy (Figure 5)**

If patient has undergone neoadjuvant therapy, which is systemic treatment prior to surgical resection, the pre-treated tumor may be difficult to identify based of response to treatment. The tumor bed refers to the location of the prior invasive carcinoma (see macroscopic appearance).

- Weigh (g) specimen, measure (cm), orient, ink, and section as described above.
- A specimen radiograph may be especially useful in a previously treated mastectomy for identifying the radiographic marker and the tumor bed when there is no gross visible tumor (*see imaging considerations*). Utilization of specimen radiography can guide the mapping out and selection of tissue submitted for histologic examination, as well as minimize the number of sections submitted (*refer to imaging considerations*).
- Document location and size (cm) of macroscopic tumor or tumor bed.
 - Site of tumor / tumor bed should be marked with a clip and may be the only means of identifying the tumor bed depending on response to treatment.
 - Size and location of tumor prior to treatment should be available. The tumor bed can be difficult to macroscopically identify and usually consists of an ill-defined fibrotic area.
 - Post-neoadjuvant tumors are staged based on the largest single focus of residual viable tumor (if present) and are given a “y” designation before the pT which denotes prior treatment. Note the pT classifications based on tumor size:
 - No residual tumor (ypTX or ypT0)
 - Tumor ≤2.0 cm (ypT1)
 - Tumor >2.0 cm but ≤5.0 cm (ypT2)
 - Tumor >5.0 cm (ypT3)

Macroscopic Tumor Present

- Note if residual tumor is well-circumscribed or ill-defined, and describe gross features (color, texture, etc.) (*see macroscopic appearance*).
- Document distance (cm) of residual tumor to the anterior margin (or overlying skin) and posterior margin.
- Note the radiographic marker(s), describe shape, and indicate relationship to tumor / tumor bed.
- If mastectomy contains more than one focus of residual tumor, document number, size (cm) of each tumor, and relationship to margins.
- Describe the remaining tissue, documenting size (cm), macroscopic features and location of any additional lesions not previously biopsied.

No Macroscopic Tumor Present

- Identify and measure (cm) the area of the tumor bed, and document distance to margins.
- If the tumor bed and marker are not macroscopically identified, the specimen should be radiographed (*see imaging considerations*).
- Describe the changes associated with the area of treatment.
- Describe the radiographic marker (shape) when identified.
- Describe the remaining tissue, documenting size (cm), macroscopic features, and location of any additional lesions not previously biopsied.
- Submit sections:
 - One block per centimeter of pre-treatment tumor size to include any areas of macroscopic residual tumor.
 - If no residual tumor is found microscopically, additional sampling may be necessary.
 - If residual tumor is still large (>5 cm), then at least 5 representative sections from the largest cross-sectional area should be considered.
 - Closest posterior and anterior margins.
 - Include separate section of pectoralis muscle if present but not close to tumor / tumor bed.
 - Nipple, perpendicular section.
 - For nipple sparing simple mastectomy: shave, serially section, and entirely submit the nipple margin.

- Additional macroscopically evident lesion(s) not previously biopsied.
- Random quadrant sections (1 block / quadrant), focusing on fibrous tissue.

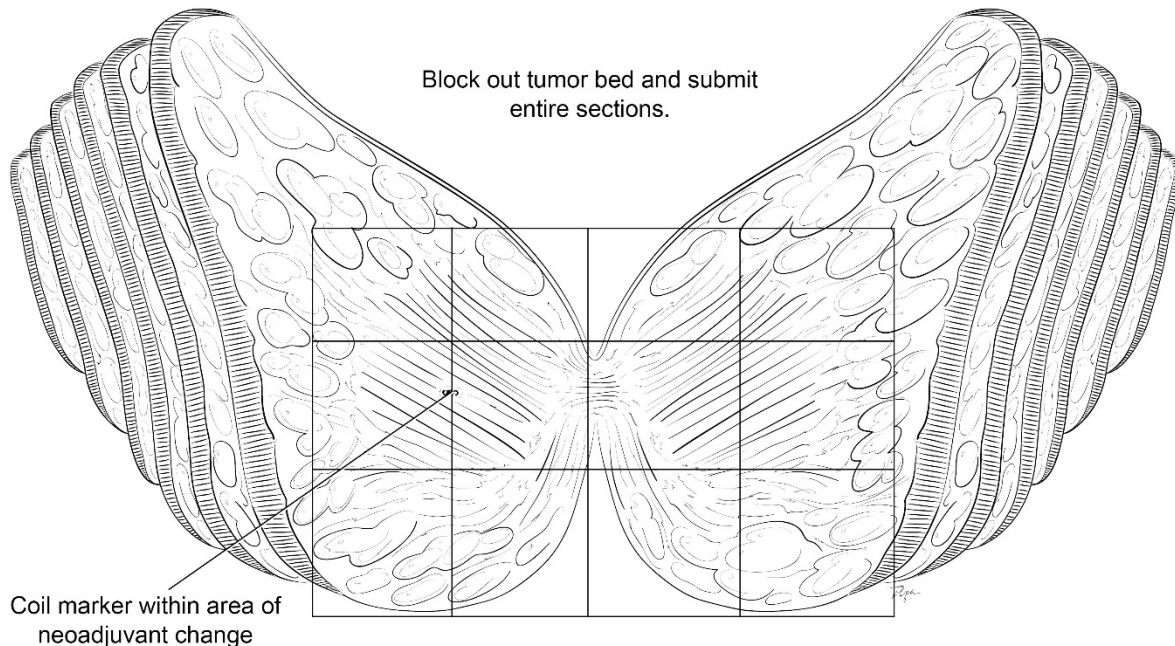


Figure 5: Tumor treated with Neoadjuvant Chemotherapy
Posterior Portion of Mastectomy

This illustration demonstrates a method to block out tissue that has undergone a full treatment response to neoadjuvant chemotherapy.

■ Nipple Resection

When an amputated nipple is received, a single perpendicular section should be sampled to examine the nipple ducts. Make note if nipple is inverted or everted and if any gross abnormalities are appreciated. Additional sections should be taken if gross abnormalities are seen or there is a history of Paget's disease.

An inverted nipple on a mastectomy specimen may indicate underlying tumor. In this case take a deeper perpendicular section to show relationship from the possible lesion or tumor to the nipple skin surface.

If the nipple is everted, amputate the nipple parallel to the skin. Take a second deeper section in the same plane to demonstrate more ducts and submit *en face*. Serially section the nipple and submit all slices.

In the usual nipple examination, one perpendicular section is taken which only examines one to four of the main nipple ducts. If the nipple is macroscopically abnormal or there is a history of Paget disease of the nipple, additional sections may be taken. (**Figure 6**)

- Document the size of the nipple (i.e., the raised area where the ducts emerge and not the pigmented areola).
- Document the size of the areola.
- State if the nipple is everted, inverted, or retracted.
- Describe the surface (irregular, crusted).
 - A crusted surface may indicate Paget disease of the nipple.
- The nipple is amputated in a plane parallel to the skin surface.
- A second, deeper section is taken in the same plane. This section will demonstrate all the major ducts as they approach the nipple.
- The more superficial section is serially sectioned perpendicular to the skin surface and all these slices are submitted. This section will demonstrate all of the nipple ducts as they empty onto the skin surface.

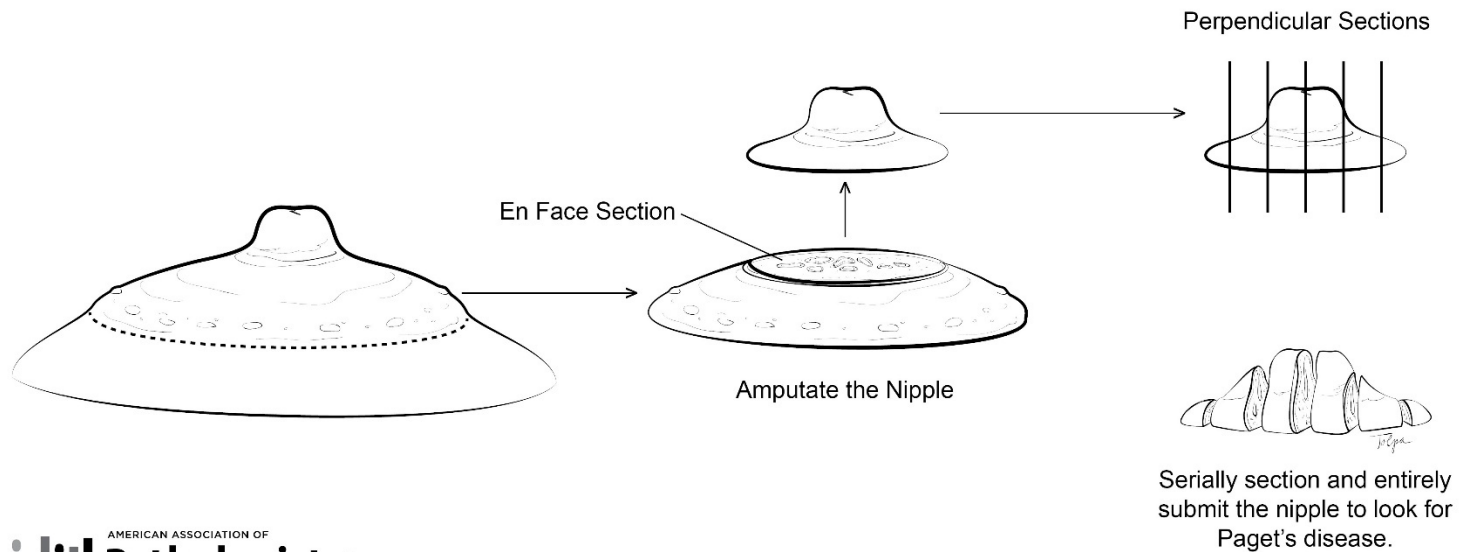


Figure 6: Paget's Breast - Nipple Sectioning

■ Lymph Node Sampling

Sentinel Lymph Node Biopsy

For sentinel lymph node biopsies (SLNB) received for frozen section examination refer to Appendix III. SLNBs are identified by the surgeon and are only considered sentinel lymph nodes if less than six nodes are removed. For cases in which the node characterization is based only on the SLN the (sn) modifier is used for staging.

- Sentinel lymph nodes are often submitted in an aggregate of adipose tissue which could contain more than one lymph node.
- Dissect out lymph nodes and report the number identified.
- Document three-dimensional measurement (cm) if one lymph node is present or give a range if more than one lymph node is present.
- Serially section lymph nodes larger than 3 mm along the long axis at 2 mm intervals.
 - Sectioning at 2 mm intervals is adequate to detect all macrometastases (≥ 2.0 mm) which are known to have prognostic importance.
- Describe the cut surface of the identified lymph nodes and the macroscopic appearance of tumor metastasis including the size and location within the node.

- Indicate if extracapsular extension is identified.
 - Include another point regarding treated lymph nodes, and if there are biopsy clips or changes to describe.
- Submit sections.
 - Lymph nodes must be submitted in such a way that every node can be evaluated and counted separately.
 - Submit small lymph nodes (0.2 – 0.3 cm) in toto.
 - Macroscopically negative, larger lymph nodes (>0.3 cm), submit the entire lymph node in a sequential manner.
 - Macroscopically positive, larger lymph nodes (>0.3 cm), submit representative sections to include any areas where extranodal tumor extension is macroscopically seen or cannot be excluded.

*Lymph nodes with treatment response are usually fibrotic or may show small tumor deposits within an area of fibrosis. A lymph node with complete response should not be reported as “positive for carcinoma.”

Axillary Lymph Nodes (non-sentinel lymph nodes)

- Non-sentinel lymph nodes may be submitted as separate specimens accompanying lumpectomies / excisional biopsies and mastectomies.
- Removed by en bloc resection of axillary tissue.
 - When received attached to mastectomy, this is referred to as a modified radical mastectomy.
 - Whether submitted separately or as part of a modified radical mastectomy, measure (cm) the axillary tissue in three dimensions.
 - If the axillary dissection is oriented into levels, maintain orientation.
 - Level I: low-axilla - lateral to the lateral border of the pectoralis minor muscle.
 - Level II: mid-axilla - between the medial and lateral borders of the pectoralis minor muscle and the interpectoral (Rotter's) lymph nodes.
 - Level III: apical axilla or infraclavicular nodes – medial to the medial margin of the pectoralis minor muscle and inferior to the clavicle.
 - Levels I and II are typically removed in the axillary dissection. Level III nodes are only removed if considered intraoperatively suspicious by the surgeon.
 - There are additional pN categories for Level III lymph nodes so these must be specifically identified.
- Dissect out lymph nodes and report the number identified and measurements (cm) (three dimensions if one lymph node is present, range if more than one lymph node is present).
- Serially section larger lymph nodes along the long axis at 2 mm intervals.
- Describe the cut surface of the identified lymph nodes and the macroscopic appearance of tumor metastasis including the size and location within the node.
 - Indicate if extracapsular extension is identified.
- Submit sections.
 - Lymph nodes must be submitted in such a way that every node can be evaluated and counted separately.
 - Submit small lymph nodes (0.2 – 0.3 cm) in toto.
 - Macroscopically negative, larger lymph nodes (>0.3 cm), submit the entire lymph node in a sequential manner.
 - Macroscopically positive, larger lymph nodes (>0.3 cm), submit representative sections to include any areas where extranodal tumor extension is macroscopically seen or cannot be excluded.

Invasive Carcinoma of the Breast

Introduction

Invasive breast cancers can be classified into at least 21 distinct histological subtypes and four distinct molecular groups which differ in risk factors, presentation, response to treatment, and outcomes. The distinct histological subtypes are based on a wide range of criteria including cell type, architectural features, pattern of spread, and type of secretion. Approximately 80% of invasive breast cancers are histologically categorized as mammary carcinoma “no special type,” also known as “ductal” carcinoma, or invasive ductal carcinoma (IDC). The most common special histologic subtype of breast carcinoma is invasive lobular carcinoma (ILC) which accounts for approximately 10-15% of all breast carcinomas. The four molecular subtypes of breast cancer are determined by presence or absence of specific hormone receptors (estrogen ER and progesterone PR) and gene expression (HER2/neu). The four molecular subtypes are:

Luminal A-Like	(ER+ and/or PR+, HER2/neu-, Ki-67 < 14%)
Luminal B-Like	(ER+ and/or PR+, HER2/neu + or -, Ki-67 > 14%)
HER2/neu-Like	(ER/PR-, HER2/neu+)
Basal-Like	(ER/PR-, HER2/neu-).

Luminal A-like is the most common molecular subtype accounting for approximately 50% of all invasive breast cancers. Luminal A-like breast cancers are generally slower growing and less aggressive as compared to the other subtypes. They are typically responsive to endocrine therapy and generally have a good overall prognosis. Luminal B-like breast cancers account for approximately 20% of all invasive breast cancers and tend to be higher grade as compared to Luminal A-like cancers. Luminal B-Like breast cancers have been more recently defined by higher Ki-67 staining, which indicates a higher rate of proliferation in these cancers. HER2/neu-like breast cancers account for approximately 15% of invasive breast cancers. These breast cancers are generally higher grade and often lymph node positive but have a very good prognosis as a result of targeted immunotherapy with trastuzumab (Herceptin). Basal-like breast cancers, also known as triple negative breast cancers account for approximately 15% of all invasive breast cancers. These tumors are typically higher grade and are negative for ER/PR and HER2/neu. Because these tumors are ER/PR negative they do not respond to endocrine based therapy. Basal-like breast cancers tend to have an overall poor prognosis and occur at twice the rate in African American women as compared to white women in the US. Additionally, this group of cancers is also more common in premenopausal women and those with a BRCA1 gene mutation. Identification of these molecular subtypes based on biomarkers has factored into selected therapy and prognosis for these breast cancer patients. Although the TNM staging system still has value predicting a patient's outcome, clinicians must take into account these other factors when approaching breast cancer patients.

Multigene expression assays like Oncotype DX and MammaPrint, which are in widespread use, add an additional layer of complexity to breast cancer treatment, prognosis, and predictive outcomes beyond that of the TNM system. These assays which incorporate additional biomarkers may even provide additional prognostic information beyond the current gold standards, ER/PR and HER2/neu. One example of how these multigene expression assays may affect the future of breast cancer staging and treatment came from two reports presented at the 2015 European Society for Medical Oncology, which confirmed that Oncotype DX identified a group of breast cancer patients who could forgo chemotherapy and still have an excellent prognosis.

Biomarkers and multigene expression assays have become extremely important in the clinical management of breast cancer patients. Most assays used to detect specific genes and biomarkers are RNA based, however some (ER, PR, HER2/neu, and Ki-67) still use immunohistochemistry (IHC) staining on formalin fixed, paraffin-embedded tissue. Therefore, accurate results of these biomarkers are very important for patient care. Research has shown that pre-analytical variables can have

negative effects on detecting these clinically significant biomarkers, chief among them is delayed fixation time, or the cold ischemic time.

Cold ischemic time is the time between tissue removal and fixation of the specimen. Studies have demonstrated adverse consequences of prolonged cold ischemic times (greater than one hour) which can result in reduced expression of ER, PR, and HER2/neu. HER2/neu detection by FISH (fluorescence in-situ hybridization) has been shown to be particularly vulnerable to extended cold ischemic time resulting in preferential loss of HER2 signal creating the potential for false negative results, which may directly impact patient care. The American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) Guidelines recommend that the cold ischemic time for breast specimens be less than one hour, and that the cold ischemic time be documented in the specimen requisition slip, final pathology report, or in both.

The TNM staging system for breast cancer was first established in 1959, providing standardized nomenclature and a systematic approach to assess the risk of distant spread and death relative to local therapy, which in the beginning amounted to aggressive surgery (radical mastectomy) and radiation. Since then, the American Joint Committee on Cancer (AJCC) has made revisions to the TNM staging system for invasive breast cancer to reflect the advances made in adjuvant chemotherapy and its effects on tumor progression and distant spread. These changes have resulted in a more conservative surgical approach to breast cancer that from a purely pathology-based perspective has resulted in increased excisional biopsies / lumpectomies and simple mastectomies over radical mastectomy, and sentinel lymph node biopsy of clinically negative axilla over axillary dissection.

Epidemiology

Breast cancer is the most common cancer in females (accounting for 24% of all female cancers) both in the developed and less developed world, and the leading cause of female cancer related death worldwide. In 2018, nearly 2 million new breast cancer cases were diagnosed. Globally, breast cancer now represents 1 in 4 of all cancers in females. Since 2008, worldwide breast cancer incidence has increased by more than 20 percent. Mortality has increased by 14 percent.

In the United States breast cancer is the most common cancer among women after skin cancer and the second leading cause of cancer related deaths in women after lung cancer. In 2020, there will be an estimated 276,480 new cases of invasive breast cancer diagnosed in women and 2,670 cases diagnosed in men. About 5-10% of breast cancers can be linked to known inherited gene mutations. Mutations in the *BRCA1* and *BRCA2* genes are the most common of these mutations. On average, women with a *BRCA1* mutation have up to a 72% lifetime risk of developing breast cancer; for *BRCA2* mutation, the risk is 69%. Breast cancer that is positive for the *BRCA1* or *BRCA2* mutations tend to develop more often in women under the age of 45. In men, *BRCA2* mutations are associated with a lifetime breast cancer risk of about 6.8%; *BRCA1* mutations are a less frequent cause of breast cancer in men.

Incidence in the United States

About 1 in 8 U.S. women (about 12%) will develop invasive breast cancer over the course of her lifetime. A man's lifetime risk of breast cancer is about 1 in 883. The incidence rate of breast cancer in 2019 was 127.8 per 100,000.

Breast cancer is the most commonly diagnosed cancer among African American women. The incidence rate in African American women from 2012-2016 was 124 per 100,000 as compared to 131.5 per 100,000 in white women. In women under 45 years of age, breast cancer is more common in the African American population than the Caucasian population.

A woman's risk of breast cancer nearly doubles if she has a first-degree relative (mother, sister, daughter) who has been diagnosed with breast cancer.

Morbidity and Mortality in the United States

In 2020 about 42,170 women and 520 men in the U.S. are expected to die from breast cancer. The overall death rate in 2019 was 19.9 per 100,000. In African American women the death rate is higher than the national average at 28.1 per 100,000, vs Caucasian women at 20.1 per 100,000.

Overall, African American women are more likely to die of breast cancer. For Asian, Hispanic, and Native-American women, the risk of developing and dying from breast cancer is lower. Ashkenazi Jewish women have a higher risk of breast cancer because of a higher rate of *BRCA* mutations.

The 5- and 10-year relative survival rates for women with invasive breast cancer are 90% and 83%, respectively. The overall 5-year relative survival rate is 99% for localized disease, 85% for regional disease, and 27% for distant-stage disease.

Since 1975, the breast cancer 5-year relative survival rate has increased significantly for both African American and Caucasian women. While a substantial gap remains, especially for late-stage diagnoses, the racial disparity seems to be narrowing. In the most recent period, the 5-year relative survival rate was 83% for African American women and 92% for Caucasian women.

Death rates have been steady in women under 50 years of age since 2007 but have continued to drop in women over 50. The overall death rate from breast cancer decreased by 1.3% per year from 2013 to 2017. These decreases are thought to be the result of treatment advances and earlier detection through screening.

Clinical Presentation

Clinical presentation of breast cancer varies and can be assessed through physical examination and / or through screening mammography or other imaging modality (ultrasound, magnetic resonance imaging). Lesions or masses detected through physical exam or imaging could also represent benign breast disease, so evaluation by biopsy (core biopsy) or fine needle aspiration are indicative to establish a definitive diagnosis.

■ **Findings on Physical Examination**

- New lump / mass / swelling in the breast or axilla
- Thickening or swelling in all or part of the breast
- Breast skin irritation or dimpling
- The nipple or skin appears red, scaly / flaky, itchy, or thickened
- Pulling in of the nipple or pain
- Nipple discharge other than breast milk, including blood
- Any change in the size or the shape of the breast
- Pain in any area of the nipple or breast

■ **Radiological Findings**

- The classic finding is a spiculated mass with or without calcifications.
- Well-circumscribed mass
- Architectural distortion
- Microcalcifications without an obvious mass

Imaging Considerations

■ **Microcalcifications (MC)**

Microcalcifications are tiny deposits of calcium in the soft tissue of the breast. They can appear in multiple patterns throughout the breast and can sometimes be an early and the only presenting sign of breast cancer. Approximately 90% of DCIS appears as MCs, 40% of which progress to an invasive breast cancer. Approximately 30% of early invasive breast cancers have fine, granular MCs detectable on mammography. Around 40% of breast cancers present with MCs and frequently are the only mammographic features indicating the presence of a tumor. The role, pathophysiology, and chemistry of calcifications in breast cancer are poorly understood. However, there is evidence that calcifications may have a role in the proliferation of cancer cells (including gene upregulation) and their invasion into the surrounding tissues.

There are two types of microcalcifications composition. Type I MCs are calcium oxalate crystal and are usually found in benign cystic breast lesions but are rarely associated with malignant breast lesions. Calcium oxalate crystals are birefringent, not clearly visible on routine histologic sections. Therefore, it is recommended to examine all breast biopsies under polarized light. Type II MCs are calcium phosphate crystals (mainly calcium hydroxyapatite) and are found in both benign and malignant lesions, most often in proliferative lesions. Calcium phosphate crystals are non-birefringent.

■ **BI-RADS (Breast Imaging Reporting and Data System)**

There are multiple mammographic patterns throughout the breast which can be classified as benign, intermediate, or highly suspicious for malignancy. These patterns of calcifications can be categorized with the help of BI-RADS. BI-RADS is a classification system that was proposed by the American College of Radiology (ACR) in 1986 with the original report released in 1993. It was implemented to standardize risk assessment and quality control of mammography and to provide uniformity in the reports for non-radiologists. The latest edition is from 2013 and includes six classifications for lesions. BI-RADS lexicon includes multiple category descriptions for breast composition or density, masses (to include shape, margins, and density), calcifications, asymmetries, associated features, and location of lesion.

Both the shape and distribution of calcifications factor into the classifications of calcifications. Benign calcifications can be eggshell or rim-like, coarse and popcorn-like, linear configuration (vascular calcifications), large and rod-like, milk of calcium, or lucent-centered. Amorphous calcifications are of intermediate concern and are often difficult to pick up on computed radiography. Calcifications that are highly suspicious for malignancy are fine, linear, branching, casting, or pleomorphic.

The distribution of calcifications can be varied. Calcifications can be found in loose groups or clusters are more likely to represent benign conditions whereas calcifications found in compact groups or clusters are more likely to be malignant. Linear or segmental calcifications are typically suspicious calcifications arranged in a line or branching pattern, typically along a duct. Regional calcifications are seen in a large volume but not necessarily conforming to a duct. These are more likely to be benign. Calcifications that are diffuse or scattered are seen all over the breast and may be bilateral. These are typically benign.

BI-RADS Categories

BI-RADS 0: incomplete evaluation with further imaging required including additional mammographic views including spot compression or magnification and / or ultrasound.

BI-RADS 1: negative examination meaning there are no masses, suspicious calcifications, or areas of architectural distortion; there can be no description of a finding in the report if categorized as BI-RADS 1.

BI-RADS 2: benign findings; this includes secretory calcifications, simple cysts, fat-containing lesions, calcified fibroadenomas, implants, and intramammary lymph nodes. These include eggshell, popcorn, lucent-centered, dermal, vascular calcifications, milk of calcium, and scattered calcifications and do not need biopsy or follow-up.

BI-RADS 3: probably benign but of intermediate concern and should be closely monitored with shortened interval follow-up to determine stability; risk of malignancy is below 2%; has very strict classifications: a non-palpable, circumscribed mass on a baseline mammogram; a focal asymmetry, which becomes less dense on spot compression images; or a solitary group of punctate calcifications.

BI-RADS 4: suspicious abnormality with pleomorphic and casting-type calcifications where a biopsy is recommended; these abnormalities can represent the chance of being malignant (in percent); subdivided into A, B, and C.

- BI-RADS 4A: Low probability of malignancy ranging from 2%-10%
- BI-RADS 4B: Intermediate chance of malignancy ranging from 10% to 50%
- BI-RADS 4C: High probability of malignancy ranging from 50% to 95%

BI-RADS 5: highly suggestive of malignancy, more than 95% and a biopsy is recommended. If something is placed in this classification and pathology comes back benign, the recommendation is still surgical consultation because the pathology is discordant with radiographic findings.

BI-RADS 6: recently added, pathology proven malignancy.

■ Cabinet X-ray Systems

Core biopsies

Core biopsies for calcifications may be submitted by clinicians previously separated into cores with and without calcifications. However, when they are submitted in one container with no designation as to which cores may have calcifications present, a cabinet x-ray system can be used to locate which have calcifications and designate those in a particular block. This can aid the pathologist in multiple ways:

- Documents that the specimen obtained by the radiologist and received in pathology does have calcifications.
- Separating the cores containing calcifications into a specific block may allow for a different targeted cutting protocol in histology as well as for re-cuts if calcifications are not seen on the initial slides.
- If the blocks come divided by the clinician or if the tissue is all submitted in one block and calcifications are not seen on the slides, the processed blocks can be examined by radiograph to see if there are calcifications present deeper in the block.

Lumpectomies

Lumpectomies will typically come with a needle localization wire and a radiographic marker, another type of localizing device and a radiographic marker, just a radiographic marker or without any type of marker. **(Figure 7)** Specimen radiography is important to assess the adequacy of the excision. A cabinet x-ray in pathology can be utilized to verify that the correct localized or previously biopsied area was removed as well as to identify where in the specimen the radiographic marker is located to aid in sectioning. The specimen should not be compressed or should be minimally compressed as it can compromise the ability to assess the distance of the DCIS from the surgical margins.



Figure 7: Lumpectomy with needle localization wire and radiographic marker.
Photograph courtesy of Mayo Clinic, Rochester, MN.

Once a lumpectomy has been serially sectioned, the slices can be laid out and numbered and another x-ray obtained if assistance is needed identifying the radiographic marker within the area of concern. Areas of calcification can be identified and submitted for sections. Utilization of radiography can guide the selection of tissue to submit for histologic examination and minimize the number of sections submitted.

Mastectomies (Figure 8)

Mastectomies (especially neoadjuvant specimens) can be difficult cases without the aid of cabinet x-ray. There are multiple ways that the imaging can be utilized:

- Using the radiographic marker to orient the specimen if no orientation is provided but there is a known location for the marker.
- Identifying the radiographic marker / area of interest when there is no visible tumor (DCIS only or neoadjuvant).
- Mapping out a DCIS specimen using biopsy markers or calcification(s).
 - Sections can be concentrated on the areas surrounding the radiographic marker or areas of calcification.
- If a mass can be visualized radiographically, dimensions of the mass can be obtained from the radiographic image.

A metal probe or forceps can be utilized in the radiograph to help narrow down an area of interest for locating a radiographic marker.

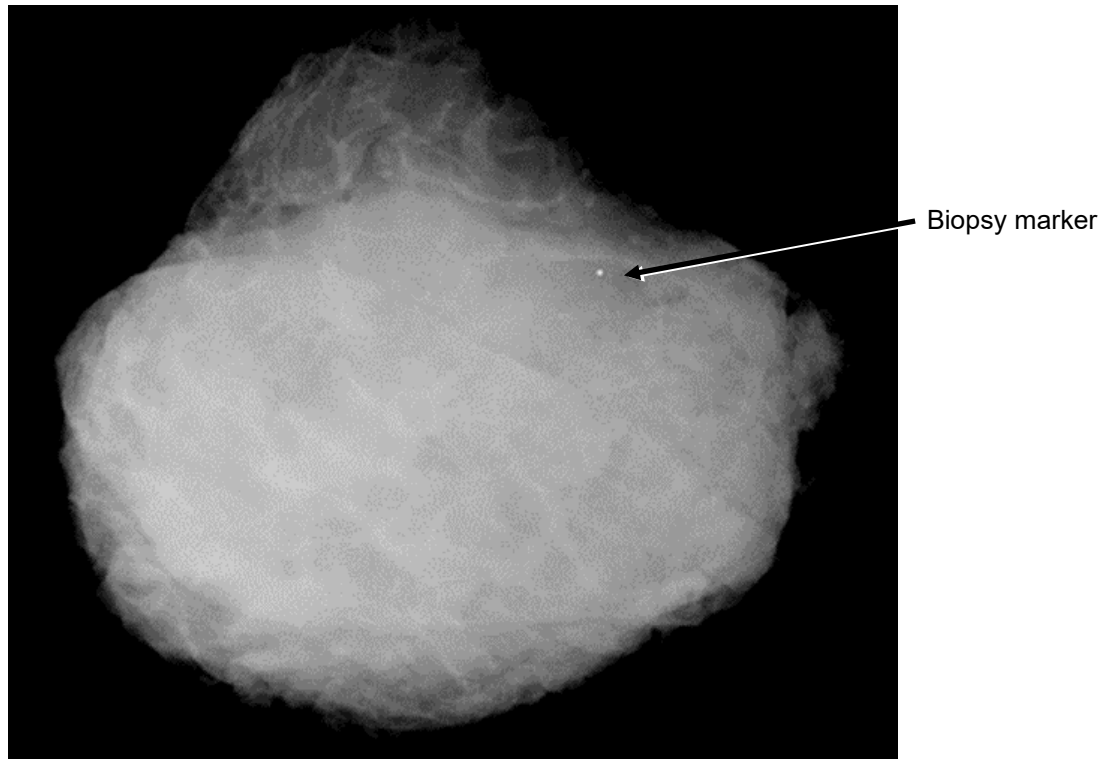


Figure 8: Radiograph of a mastectomy specimen with radiographic biopsy marker.
Radiograph courtesy of Mayo Clinic, Rochester, MN.

Pertinent Anatomy

Breast

The breast or mammary gland is a modified sweat gland that consists of glandular tissue, fibrous tissue, and adipose tissue, which is covered by skin and subcutaneous adipose tissue and lies on the pectorals muscle. The glandular element consists of a series of ducts (minor and major) and secretory lobules (15-20 / breast) which are divided into segments. The lobules are composed of cells that produce and secrete breast milk (lactation). The lobules connect to a series of ducts that are drained by a lactiferous duct which opens to the nipple. The lobule and the distal end of the ductal system form terminal ductal-lobular units.

Nipple and Areola

The nipple is a cylindrical or conical prominence located in the center of the areola and contains the openings to the lactiferous ducts. The areola is the pigmented area surrounding the nipple composed of numerous sebaceous glands.

Chest Wall

The pectoralis muscle is not considered part of the chest wall, and therefore involvement of the pectoralis muscle alone is not considered chest wall involvement (pT4). The chest wall is composed of the ribs, intercostal muscles, and serratus anterior muscle, and tumor invasion into these structures is classified as pT4.

Axillary Tail

The axillary tail (also called the tail of Spence) is the lateral extension of breast tissue from the upper outer quadrant which extends into the axilla through an opening in the deep fascia called the foramen of Langer.

Axillary lymph nodes

Most (75-90%) of the lymphatic drainage of the breast is to the ipsilateral axilla. Axillary lymph nodes include all nodes along the axillary vein and its tributaries and interpectoral nodes (Rotter's nodes). The apex of the axilla lies below the mid-clavicle. Axillary nodes are divided into levels:

- Level I - low-axilla
- Level II - mid-axilla
- Level III - apical axilla or infraclavicular nodes

Level I axillary nodes are lateral to the lateral border of the pectoralis minor muscle. Level II axillary nodes are located between the medial and lateral borders of the pectoralis minor muscle and the interpectoral (Rotter's) lymph nodes. Level III axillary nodes are medial to the medial margin of the pectoralis minor muscle and inferior to the clavicle. Metastatic involvement of Level III nodes is associated with a worse prognosis. In the "N" portion of Pathologic and Clinical TNM classification, Level III nodes are designated infraclavicular nodes and designated are pN3 if positive / involved. These lymph nodes should be separately identified by the surgeon, and separately submitted for histologic examination.

Regional Lymph Nodes

The lymphatic drainage of the mammary gland originates in the terminal lobular units and flows through intramammary lymph node to the subareolar plexus. From the subareolar plexus lymphatic drainage occurs through three main routes:

- Axillary (ipsilateral): interpectoral (Rotter's) nodes and lymph nodes along the axillary vein and its tributaries.
- Internal mammary (ipsilateral): lymph nodes in the intercostal spaces along the edge of the sternum in the endothoracic fascia.
- Retro-mammary route.
- Supraclavicular: lymph nodes in the supraclavicular fossa, a triangle defined by the omohyoid muscle and tendon (lateral and superior border), the internal jugular vein (medial border), and the clavicle and subclavian vein (lower border). Adjacent lymph nodes outside of this triangle are considered lower cervical nodes (M1).

Internal mammary lymph nodes

The internal mammary lymphatic trunks are located along the intercostal spaces and run along the sternal edge in the endothoracic fascia.

Correlation of Specific Surgical Procedure with Anatomy

Excision specimens are breast resections without the intent of removing the entire breast and are more commonly referred to as "lumpectomies." The nipple is usually not included with excisions. These specimens are routinely oriented by the surgeon in order for the pathologist to identify specific margins (e.g., superior, inferior, medial, lateral, anterior, or deep). The identification of individual margins is usually done by placement of sutures or clips on the outer surface of excisional specimens. Identification of margins allows for measurement of margin clearance, as well as allows a surgeon the ability to re-excise residual tumor at a specific margin when positive. All identifiable margins should be evaluated for involvement by carcinoma both macroscopically and microscopically.

Total Mastectomy specimens are breast resections with the intent of removal of all breast tissue, with or without nipple and areola and axillary lymph nodes.

Simple mastectomy specimen consists of a total mastectomy without removal of axillary lymph nodes. These specimens are routinely received oriented with a suture on the axillary tail (tail of Spence) which is the lateral extension of breast tissue to the upper outer quadrant.

Skin sparing mastectomy specimen consists of a total mastectomy with removal of the nipple and only a narrow surrounding rim of skin. These specimens are routinely received oriented with a suture on the axillary tail.

Nipple sparing mastectomy specimen consists of a total mastectomy without removal of skin or nipple. These specimens are routinely received oriented with a suture on the axillary tail.

Modified radical mastectomy specimen consists of a total mastectomy with an axillary lymph node dissection (ALND). The ALND may be attached to the mastectomy or received as a separate part. A small portion of pectoralis muscle is sometimes removed.

Radical mastectomy specimen consists of a total mastectomy with removal of the pectoralis major and pectoralis minor muscles as well as an ALND.

Macroscopic Features of Tumor

Most invasive breast cancers (IBC) can be visualized and palpated as a distinct mass and encompass a variety macroscopic features. Typically, the tumor edge is poorly defined with an irregular or stellate nodular appearance lacking circumscription. IBCs are quite often firm or hard (indurated) on palpation and are gray-white with a gritty cut surface. Often the core biopsy site and radiographic marker are present within or immediately adjacent to the tumor. (**Figure 9**)



Figure 9: Residual invasive carcinoma with central biopsy site.
Photograph courtesy of Rachel Larsen, PA(ASCP)^{CM}, Mayo Clinic, Rochester, MN.

Additionally, these specific histologic subtypes of IBC such as invasive lobular carcinoma (ILC) and mucinous carcinoma also have unique macroscopic appearances. ILCs have poorly delineated tumor edges and are difficult to visualize macroscopically due to the diffuse growth pattern of the lesion. These tumors often resemble native tissue but tend to be firmer to palpation compared to surrounding tissue, making it difficult to determine the size of the lesion.

Mucinous carcinoma (MC), also known as colloid carcinoma or mucinous adenocarcinoma, displays well-circumscribed or lobulated tumor edges with pushing margins. The cut surface of these tumors has gelatinous and glistening features.

Lastly, the macroscopic appearance of neoadjuvant treated tumors with a complete / near complete response may not be visually apparent, often with only a scarred area present. In specimens with less response, the tumor may appear soft and be difficult to palpate.

Treatment / Therapy Guidelines

Breast conservation therapy (BCT) is the therapeutic approach for treating breast cancer that combines breast conservative surgery (BCS: i.e., excisional biopsy / lumpectomy) and radiation therapy. The overall survival rates for patients undergoing BCT for early-stage breast cancer are similar to those patients who received total mastectomy, given adequate surgical margin status and radiation. The primary goals of BCT are to provide overall survival rates equivalent to total mastectomy, a cosmetically acceptable outcome, and adequate surgical excision (i.e., negative surgical margins). Achieving adequate margin status is challenging and dependent on many variables (non-palpable disease, extent of disease) and can result in close or positive margins, resulting in high re-excision rates which vary from 20 - 70%. A second surgical procedure has many negative consequences including increased post-operative risks for the patient, increased healthcare costs, and delays to additional treatment (radiation, adjuvant therapy).

Metastatic Sites

Breast cancer tumor cells spread to distant sites via lymphatics and / or blood vessels. The four most common metastatic sites for breast cancer are bone, lung, liver, and brain, but breast cancers can metastasize to any location within the body. Imaging modalities used to detect metastatic breast cancer include positron emission tomography (PET), magnetic resonance imaging (MRI), computed tomography (CT), and bone scans. Definitive diagnosis is confirmed by a tissue biopsy. IHC stains of breast markers such as GATA3, mucicarmine, ER and PR are used for metastatic breast cancer confirmation.

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APPENDIX I TNM Criteria

Definition of Primary Tumor (pT) – Pathological T

pT Category	pT Criteria
pT not assigned	(Cannot be determined based on available pathological information)
pT0	No evidence of primary tumor#
pTis (DCIS)*	Ductal carcinoma <i>in situ</i> #
pTis (Paget)	Paget disease of the nipple NOT associated with invasive carcinoma and/or carcinoma <i>in situ</i> (DCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted.
pT1	Tumor ≤20 mm in greatest dimension
pT1mi	Tumor ≤1 mm in greatest dimension
pT1a	Tumor >1 mm but ≤5 mm in greatest dimension (round any measurement 1.0-1.9 mm to 2 mm).
pT1b	Tumor >5 mm but ≤10 mm in greatest dimension
pT1c	Tumor >10 mm but ≤20 mm in greatest dimension
pT2	Tumor >20 mm but ≤50 mm in greatest dimension
pT3	Tumor >50 mm in greatest dimension
pT4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or macroscopic nodules); invasion of the dermis alone does not qualify as T4
pT4a	Extension to chest wall; invasion or adherence to pectoralis muscle in the absence of invasion of chest wall structures (i.e., ribs, intercostal muscles, serratus anterior muscle) does not qualify as T4
pT4b	Ulceration and/or ipsilateral macroscopic satellite nodules and/or edema (including peau d'orange) of the skin that does not meet the criteria for inflammatory carcinoma
pT4c	Both T4a and T4b are present
pT4d	Inflammatory carcinoma; inflammatory carcinoma requires the presence of clinical findings of erythema and edema involving at least one-third or more of the skin of the breast.

For the purposes of this checklist, these categories should only be used in the setting of preoperative (neoadjuvant) therapy for which a previously diagnosed invasive carcinoma is no longer present after treatment. Patients with pathological complete response (absence of residual invasive carcinoma in both the breast and lymph nodes) should be categorized as ypT0N0 or ypTisN0, not ypTX.

* Note: Lobular carcinoma in situ (LCIS) is a benign entity and is removed from TNM staging in the AJCC Cancer Staging Manual, 8th Edition.

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Definition of Regional Lymph Nodes – Pathological (pN)

pN Category	pN Criteria
pN not assigned	(No nodes submitted or found)
pN not assigned	(Cannot be determined based on available pathological information)
pN0	No regional lymph node metastasis identified or ITCs only#
pN0 (i+)	ITCs only (malignant cell clusters no larger than 0.2 mm) in regional lymph node(s)
pN0 (mol+)	Positive molecular findings by reverse transcriptase polymerase chain reaction (RT-PCR); no ITCs detected
pN1	Micrometastases; or metastases in 1 to 3 axillary lymph nodes; and/or clinically negative internal mammary nodes with micrometastases or macrometastases by sentinel lymph node biopsy

pN1mi	Micrometastases (approximately 200 cells, larger than 0.2 mm, but none larger than 2.0 mm)
pN1a	Metastases in 1 to 3 axillary lymph nodes, at least one metastasis larger than 2.0 mm##
pN1b	Metastases in ipsilateral internal mammary sentinel nodes, excluding ITCs
pN1c	pN1a and pN1b combined
pN2	Metastases in 4 to 9 axillary lymph nodes; or positive ipsilateral internal mammary lymph nodes by imaging in the absence of axillary lymph node metastases
pN2a	Metastases in 4 to 9 axillary lymph nodes (at least one tumor deposit greater than 2.0 mm)##
pN2b	Metastases in clinically detected internal mammary lymph nodes with or without microscopic confirmation; with pathologically negative axillary nodes
pN3	Metastases in 10 or more axillary lymph nodes; or in infraclavicular (Level III axillary) lymph nodes; or positive ipsilateral internal mammary lymph nodes by imaging in the presence of one or more positive Level I, II axillary lymph nodes; or in more than three axillary lymph nodes and micrometastases or macrometastases by sentinel lymph node biopsy in clinically negative ipsilateral internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastases in 10 or more axillary lymph nodes (at least one tumor deposit larger than 2.0 mm)##; or metastases to the infraclavicular (Level III axillary lymph) nodes
pN3b	pN1a or pN2a in the presence of cN2b (positive internal mammary nodes by imaging); or pN2a in the presence of pN1b
pN3c	Metastases in ipsilateral supraclavicular lymph nodes

Isolated tumor cells (ITCs) are defined as small clusters of cells not greater than 0.2 mm or single tumor cells, or a cluster of fewer than 200 cells in a single histologic cross-section. ITCs may be detected by routine histology or by immunohistochemical (IHC) methods. Nodes containing only ITCs are excluded from the total positive node count when determining the N category but should be included in the total number of nodes evaluated.

Approximately 1000 tumor cells are contained in a 3-dimensional 0.2-mm cluster. Thus, if more than 200 individual tumor cells are identified as single dispersed tumor cells or as a nearly confluent elliptical or spherical focus in a single histologic section of a lymph node, there is a high probability that more than 1000 cells are present in the lymph node. In these situations, the node should be classified as containing a micrometastasis (pN1mi). Cells in different lymph node cross-sections or longitudinal sections or levels of the block are not added together; the 200 cells must be in a single node profile even if the node has been thinly sectioned into multiple slices. It is recognized that there is substantial overlap between the upper limit of the ITC and the lower limit of the micrometastasis categories due to inherent limitations in pathologic nodal evaluation and detection of minimal tumor burden in lymph nodes. Thus, the threshold of 200 cells in a single cross-section is a guideline to help pathologists distinguish between these 2 categories. The pathologist should use judgment regarding whether it is likely that the cluster of cells represents a true micrometastasis or is simply a small group of isolated tumor cells.

Note: (sn) and (f) suffixes should be added to the N category to denote confirmation of metastasis by sentinel node biopsy or FNA / core needle biopsy respectively, with NO further resection of nodes.

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Definition of Distant Metastasis (pM) (required only if confirmed pathologically)

pM Category	pM Criteria
Not applicable	pM cannot be determined from the submitted specimens(s)
pM1	Histologically proven metastases larger than 0.2 mm

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TNM Descriptors:

For identification of special cases of pTNM classifications the “m” suffix and “y”, “r”, and “a” prefixes are used. Although they do not affect the stage grouping, they indicate cases needing separate analysis.

“m” suffix: indicates the presence of multiple primary tumors in a single site and is recorded in parentheses: pT(m)NM.

“y” prefix: indicates those cases in which classification is performed during or following initial multimodality therapy. The cTNM or pTNM category is identified by a “y” prefix. The ycTNM or ypTNM categorizes the extent of tumor actually present at the time of that examination. The “y” categorization is not an estimate of tumor prior to multimodality therapy.

“r” prefix: indicates a recurrent tumor when staged after a disease-free interval and is identified by the “r” prefix: rTNM.

“a” prefix: designates the stage determined after autopsy: aTNM.

Residual Tumor (R) Category

The absence or presence of residual tumor at the primary tumor site after treatment with surgery and / or with neoadjuvant therapy is denoted by the symbol R. The R categories for the primary tumor site are as follows:

R Category	R Definition
RX	Presence of residual tumor cannot be assessed
R0	No residual tumor
R1	Microscopic residual tumor
R2	Macroscopic residual tumor at the primary cancer site or regional nodal sites

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The presence of residual tumor may indicate the effect of therapy, influence further therapy, and be a strong predictor of prognosis. In addition, the presence or absence of disease at the margin of resection may be a predictor of the risk of recurrent cancer.

Note: The R category is not incorporated into TNM staging itself. However, the absence or presence of residual tumor and status of the margins may be recorded in the medical record and cancer registry.

APPENDIX II **Ancillary Studies**

Over the last decade there have been fundamental changes to the understanding of the biology of breast cancer. Breast cancers are now thought of as a single group of neoplastic diseases arising from breast epithelial tissue with different molecular characteristics as identified by specific gene expression / amplification, immunohistochemistry, and next-generation sequencing. These molecular characteristics have been shown to have predictability in terms of prognosis, recurrence, spread, and response to treatment, and have become as important if not more important than the traditional TNM staging system. This has called into question the relevance of the current TNM classification for breast cancer. Biomarkers (ER, PR and HER2/neu) and genetic profiling (i.e., Oncotype Dx) have become integral components in directing patient management and predicting a patient's overall prognosis. As a result, the 8th edition of the AJCC incorporated the biomarkers into a second tier of prognostic modifiers. The AJCC recognized the importance of the anatomic TNM staging system for breast cancer especially in parts of the world where early diagnosis and use of biomarker and gene profiling are not the standard of care. In low to middle income nations, a large percentage of cases are diagnosed in later stages (stage III - IV), with many patients dying of metastatic disease. As a result, the TNM classification remains important for prognosis and predictability in those settings.

Molecular Diagnostics

It is recommended by CAP and NCCN that hormone receptor (ER/PgR) and HER2 testing be done on all primary invasive breast carcinomas and on recurrent or metastatic tumors. Repeat testing on a subsequent resection specimen should be considered if the core biopsy is triple-negative: ER, PR and HER2 are all negative, particularly when the results are discordant with the histopathologic findings. The largest invasive focus should be tested when multiple invasive foci are present. However, it is recommended to test smaller invasive carcinomas if they are of a different histologic type or higher grade than the tested focus, which emphasizes the importance of handling the entire specimen to meet recommended cold ischemia and fixation times in the event of unexpected findings. Other biomarker tests (e.g., Ki-67 or multigene expression assays) are optional and are not currently recommended for all carcinomas.

There are currently guidelines for reporting results of ER, PgR, and HER2 testing in breast carcinomas, published by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), which require recording specific pre-analytic and analytic variables that can affect test results, such as cold ischemia time (less than one hour recommended) and fixation time (between 6 and 72 hours recommended). These important handling times are used to determine if the specimen meets requirements specified in latest version of the ASCO/CAP guidelines for cold ischemia time and fixation time. Other variables include fixative type, tissue treatment that may alter immunoreactivity, status of internal and external controls, adequacy of sample for evaluation, primary antibody clone, and the test regulatory status (FDA cleared vs. laboratory-developed).

Estrogen Receptor (ER) / Progesterone Receptor (PgR)

It is recommended by CAP and NCCN that hormone receptor testing be done on all primary invasive breast carcinomas and on recurrent or metastatic tumors to predict response to hormonal therapy. Even though receptor status is only a weak prognostic factor, studies have shown a substantial survival benefit from endocrine therapy among patients with ER-positive tumors. Though extremely rare, true ER-negative, PgR-positive carcinomas are also considered eligible for hormonal therapy.

ER/PgR testing is performed on formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry (IHC). Only nuclear staining is considered positive. Expression as low as 1% positive staining has been associated with clinical response, so the guidelines recommend classifying all cases with at least 1% positive cells as receptor positive. For patients with low ER expression (1% to 10% weakly positive cells), the decision on endocrine therapy should be based on an analysis of its

risks and potential benefits. Use of single-gene expression assays for ER/PgR are not recommended for routine use.

Human Epidermal Growth Factor Receptor 2 (HER2/*ERBB2*)

HER2 is a membrane protein and oncogene, which is overexpressed, and gene amplified in 20% of invasive breast cancers and 50% of ductal carcinoma in-situ cases. HER2 positive (overexpressed) breast cancers are typically high grade, poorly differentiated, have high cell proliferation rates, can be associated ER/PR negative or positive cancers, and is both a prognostic and predictive factor. HER2 status is primarily evaluated to determine patient eligibility for anti-HER2 therapy. HER2 positive carcinomas are eligible for treatment with trastuzumab (Herceptin), which may improve progression-free survival. HER2 status may also identify patients who have a greater benefit from anthracycline-based adjuvant therapy and determine eligibility for clinical trials. HER2 is performed on formalin-fixed, paraffin-embedded tissue sections by assessing protein expression on the membrane of tumor cells using immunohistochemistry (IHC) or by assessing the number of HER2 gene copies using in situ hybridization (ISH), when IHC is equivocal.

Ki-67

Ki-67 is a nuclear protein found in all phases of the cell cycle and is a marker of cell proliferation. Ki-67 testing is performed on formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry (IHC), most often using the monoclonal antibody MIB-1. The percentage of Ki-67 positive tumor cells is often used to stratify patients into good and poor prognostic groups, but there is a lack of consensus on scoring, definition of low versus high expression, an appropriate cut point for positivity, or which part of the tumor should be scored (e.g., leading edge, hot spots, overall average). There is also insufficient data on the effects of pre-analytic variables on Ki-67 staining. For these reasons, routine testing of breast cancers for Ki-67 expression is not currently recommended by either ASCO or the National Comprehensive Cancer Network (NCCN).

Multiparameter Gene Expression / Protein Expression Assay

Multigene molecular profiling / panels in breast cancer test for levels of expression of a large number of genes in breast cancer tissue. Many multigene panels provide valuable prognostic information about tumor biology in terms of tumor aggressiveness and likely response to treatment. Examples of multigene molecular profiling assays available for breast cancer are: Oncotype Dx, MammaPrint, PAM50 (Prosigna), Breast Cancer Index, and EndoPredict.

Oncotype Dx is based on genomic science which is the study of the interaction of certain genes in the body and their role in disease. Oncotype Dx looks at 21 genes within breast cancer tissue and how those genes influence tumor biology including growth and spread. In the 8th edition of AJCC, the Oncotype Dx score has been incorporated into the Pathological Prognostic Staging tables to stage node-negative breast cancer patients.

Multigene assays detect gene expression patterns by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) or by hybridization of labeled nucleic acids derived from the tumor to a number of small, immobilized, synthetic DNA strands (microarrays). Some of the assays have been optimized for use on formalin-fixed tissue, while others require frozen tissue.

At the discretion of the pathologist, the results of proprietary assays may be incorporated into pathology reports to make the information more accessible for patient care. Some of the genes listed below may be included in the proprietary assays or can be tested separately when indicated.

BRCA1/2

Inherited mutations in the *BRCA* gene are responsible for 5-10% of breast cancers. *BRCA2* mutation predisposes to ER positive breast carcinoma 50% of the time, while *BRCA1* mutation is more commonly associated with ER negative tumors. In the metastatic setting, genetic testing may have therapeutic implications. NCCN recommends assessing for germline *BRCA1/2* mutations in all patients with recurrent or metastatic breast cancer to direct treatment, as there are 2 FDA-approved treatments for germline *BRCA1/2* mutations for all subtypes of breast cancer. *BRCA* status may also

influence eligibility for accelerated partial breast irradiation (APBI). *BRCA* testing is performed on FFPE via next-generation sequencing (NGS).

PIK3CA

NCCN recommends testing ER/PgR-positive, HER2-negative breast cancers for *PIK3CA* mutations to determine eligibility for an FDA-approved treatment regimen. *PIK3CA* mutation testing is evaluated on FFPE via polymerase chain reaction (PCR)-based NGS.

PD-L1

Programmed death-ligand 1 (PD-L1) is a biomarker that may predict the response to anti-programmed death 1 (PD-1)/PD-L1 immunotherapy. NCCN recommends testing tumor-infiltrating immune cells on triple-negative invasive breast cancer to identify candidates for an FDA approved treatment regimen. Appropriate test results include reporting the anti PD-L1 antibody clone used, the type of IHC platform, the scoring algorithm and the final PD-L1 score. PD-L1 status may also be used for clinical trial eligibility. PD-L1 is tested on FFPE via IHC.

***NTRK* gene fusions**

Though only seen in a few rare types of cancer, *NTRK* 1/2/3 are tyrosine receptor kinases that may be rearranged and cause dysregulation and inappropriate signaling. NCCN recommends testing for *NTRK* in invasive breast cancer patients of all subtypes that have no satisfactory alternative treatments or with tumors that have progressed following treatment. *NTRK* fusions can be detected on FFPE via IHC, FISH, DNA-based NGS or RNA-based NGS.

MSI/MMR

NCCN recommends MSI/MMR testing in invasive breast cancer patients with unresectable or metastatic tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. Pembrolizumab is FDA approved for treatment in microsatellite-high (MSI-H) or mismatch repair deficient (dMMR) tumors.

MMR testing is performed on formalin-fixed, paraffin-embedded tissue via immunohistochemistry (IHC). MSI testing is performed on formalin-fixed, paraffin-embedded tissue via polymerase-chain reaction (PCR) or next-generation sequencing (NGS). An advantage of using MMR IHC for testing is that the results will help identify the specific protein / gene that may be affected. If MMR IHC testing has not been done but MSI has and shows an MSI-H phenotype, MMR IHC testing is recommended because this information will help identify the gene that is most likely to have a germline (or somatic) mutation.

Biomarker-directed therapy and clinical trials

Of the biomarker-directed therapies for invasive breast cancer, 29 are FDA-approved in at least one setting and 28 have NCCN guidelines in at least one setting. Treatments targeting tumors with mutations that include *BRCA*1/2 are approved in various settings (adjuvant, metastatic). HER2, ER, and PgR are the most frequent gene inclusion criteria for invasive breast carcinoma clinical trials.

Specimen Handling for Molecular Studies

Improper fixation and handling of the tumor tissue can lead to failure to obtain reliable results with PCR / sequencing-based assays, IHC, and FISH.

A cold ischemia time of 1 hour or less is recommended to meet the most current molecular testing needs for cancer patient specimens. It is recommended actual cold ischemia times or, at a minimum, deviations from the 1-hour recommendation are recorded in the pathology report.

10% neutral buffered formalin is the preferred fixative. The macroscopic description should provide the fixative used, and the length of fixation time.

Molecular tests, including solid tumor next generation sequencing (NGS), can be performed on FFPE tissue sections and fresh frozen tissue. Contact your reference laboratory for specific specimen requirements. For those tests that have multiple testing modalities, it is assumed that only one of these testing modalities would be used per case unless one test yields equivocal results.

The following tissues are suboptimal specimens for molecular testing:

- *Tissue treated with acid (e.g., decalcification agents, fixation in Bouin's), as this may degrade DNA.*
- *Tissues fixed with heavy metals (e.g., Zenker's, B5, B+, zinc formalin) – the metals may inhibit the enzymes used in PCR.*
- *Tissues that are under or over-fixed in formalin. Fixation for at least 6 hours and less than 72 hours in 10% neutral buffered formalin is recommended. Prolonged fixation, particularly in unbuffered formalin, degrades DNA.*
- *Low tumor content, as defined by the molecular diagnostics laboratory. The cutoff for acceptable tumor content depends on the method used by the laboratory. Samples with tumor content below the recommended cutoff may be falsely negative and should be reported as indeterminate if no mutations are detected.*

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APPENDIX III

Frozen section considerations for invasive breast cancer

■ **Sentinel lymph node (SLN) biopsy (SLNB)**

The sentinel lymph node (SLN) is the first lymph node to drain from a tumor site. The importance of the sentinel lymph node biopsy is based on an ordered dissemination of tumor cells from the peritumoral lymphatics to the SLN, and then to other regional lymph nodes, and eventually distant metastatic sites. SLNB is widely performed for axillary staging in patients with breast cancer and can reduce postoperative side effects resulting from an axillary lymph node dissection (ALND).

The SLN node is identified at time of surgery via injection of one or two tracers into breast skin or parenchyma either at the tumor site or under the areolar plexus. These tracers drain through lymphatic channels to the SLNs, which are subsequently identified and removed. Numerous types of tracers, dyes, and radioisotopes are used by surgeons to identify the SLN. Currently, the gold standard for SLN biopsy is lymphoscintigraphy with technetium (Tc-99m). Generally, Tc-99m is injected into the ipsilateral subareolar plexus. Blue dye tracers are also utilized for SLNB, usually in combination with a radioisotope. The two most common blue dye stains utilized for SLNB are methylene blue and isosulfan blue dye.

Frozen section examination (FSE) is not performed on all SLNBs, but when utilized FSE can provide valuable information at the time of surgery. There are several advantages for FSE on SLNs. First, FSE of SLNs allows for single-stage surgery with immediate ALND when metastatic disease is identified in the SLN. Second, FSE can also provide immediate axillary lymph node status in patients for whom imaging findings and preoperative pathology are discordant. Finally, FSE of SLN can be performed for patients who desire immediate reconstruction following mastectomy, avoiding an additional surgical procedure. FSE of SLN is however limited and is less accurate than permanent section examination. False-negative rates of FSE for breast cancer range from 9 - 59%. In the last decade there has been a reduction in the number of SLNBs submitted for FSE as a result of the ACOSOG Z11 (American College of Surgeons Oncology Group) trial. ACOSOG Z11 demonstrated that select axillary lymph node negative patients with one or two positive SLNs with no ALND performed the same in terms of survival and local / regional recurrence as those who received ALND. The results of this study have been reproduced and have changed clinical and surgical management of these patients.

■ **Intraoperative Examination of Lumpectomy (Excisional Biopsy) Margins**

One of the main issues with breast conservation therapy is local recurrence, and one of the major risk factors for local recurrence is surgical margin positivity. Intraoperative evaluation of surgical margins has been shown to be useful when margins are positive, as re-excision can be performed at the time of initial surgery, in turn reducing the need for a second procedure and the associated patient risks and expense, as well as providing for a better cosmetic result. There are several methods for evaluating margins on BCS specimens: macroscopic evaluation of margins, imprint cytology, and frozen section examination.

Macroscopic Evaluation of Margins

Macroscopic evaluation of lumpectomy margins involves the prompt examination of the fresh lumpectomy specimen immediately following its removal. After the specimen has been inked and sectioned, macroscopic examination of the tumor and its relation to the margins are determined and reported to the surgeon. In the absence of macroscopic tumor, the previous biopsy site is evaluated along with its relationship to closest margins.

Imprint Cytology (IC)

Imprint Cytology (IC) has been shown to be effective and allows for complete and rapid evaluation of the entire margin. Most studies which have evaluated the usefulness of IC in

evaluating lumpectomy margins used Diff-Quick, Rapid Papanicolaou, or hematoxylin and eosin stain. Imprints of each margin are made prior to inking. Because IC allows for evaluation of the entire margin it could be more effective than FSE which is more selective. Multiple studies have demonstrated that IC is generally sensitive (38% - 100%) and specific (69% - 100%). False negative rates have been associated with ductal carcinoma in-situ (DCIS), invasive lobular carcinoma, and lobular carcinoma in-situ. False positives have been associated with atypical ductal cells and sclerosing adenosis.

Frozen section examination (FSE)

Frozen section examination (FSE) of breast lumpectomy margins is not a standard procedure in most surgical pathology departments. FSE of lumpectomy margins are not practical for a variety of reasons including added cost, labor intensive, technical difficulties freezing and cutting adipose tissue using standard freezing techniques, and extension of the length of surgery. FSE may compromise the permanent section due to freezing artifact and the potential for sectioning through diagnostic tissue. Additionally, FSE of lumpectomy margins should be avoided for DCIS.

With that said, FSE of lumpectomy margins has been shown to substantially reduce re-excision rates in BCS. In a 2012 study published by Jorns *et al*, researchers were able to demonstrate that the use of FSE of lumpectomy margins reduced re-excision rates by 34%.

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APPENDIX IV Sample Narrative Macroscopic Description

Excisional Biopsy / Lumpectomy with Macroscopic Tumor

Submitted *[fresh / in formalin]* in one container for gross and microscopic examination, labeled with the patient's name, [], MR number, [], and "*[specimen designation]*" is a ____ gram, ____ cm superficial to deep x ____ cm medial to lateral x ____ cm superior to inferior, oriented, irregular portion of red-tan to yellow-tan fibrofatty tissue with three attached sutures and an inserted localization wire. The suture orientation is as follows: *[provide orientation (example – two short - superior, two long - inferior, one long - deep)]*. The localization wire enters the specimen from superficial aspect, and measures ____ cm in length. The surgical margins are inked as follows: superior - blue, inferior - green, lateral - yellow, medial - violet, superficial - orange, and deep - black. The specimen is serially sectioned from superficial to deep *[or other appropriate axis, per tumor configuration (along longest axis)]* into *[#]* slices. Cut surface reveals a ____ x ____ x ____ cm indurated, ill-defined, *[color]* tumor located in levels *[____ to / and ____]*. The tumor is located ____ cm from the superior margin, ____ cm from the inferior margin, ____ cm from the lateral margin, ____ cm from the medial margin, ____ cm from the superficial margin, and greater than ____ cm from the deep margin. A hemorrhagic area with foci of chalky yellow-white fat necrosis consistent with previous core biopsy site is identified adjacent to the tumor in level *[#]*. A ____ cm *[marker description]* cylindrical metallic radiographic marker is identified. The remaining cut surface reveals predominantly yellow lobulated adipose tissue with minimal interspersed white fibrous tissue. No additional lesions are identified.

Cold Ischemic Time: _____

Fixation Time: _____

ASCO/CAP Guidelines Met:

Representative / Entirely submitted sections are submitted for microscopic examination to include the entire tumor as designated:

- A1: Slice / level 1, anterior margin, perpendicular section
- A2: Slice / level 2 to include medial, lateral, superior, and inferior margins
- A3: Slice / level 3 to include medial, lateral, superior, and inferior margins
- A4-A5: Slice / level 4 to include medial, lateral, superior, and inferior margins
- A6-A7: Slice / level 5 to include tumor with medial, lateral, superior, and inferior margins
- A8-A9: Slice / level 6 to include tumor and core biopsy site with medial, lateral, superior, and inferior margins
- A10-A11: Slice / level 7 to include tumor and core biopsy site with medial, lateral, superior, and inferior margins
- A12: Slice / level 8 to include medial, lateral, superior, and inferior margins
- A13: Slice / level 9 to include medial, lateral, superior, and inferior margins
- A14: Slice / level 10 to posterior margin, perpendicular section.

Simple Mastectomy with Macroscopic Tumor

Submitted *[fresh / in formalin]* in one container for gross and microscopic examination, labeled with the patient's name, [], MR number, [], and "*[specimen designation]*" is a ____ gram, ____ cm superior to inferior x ____ cm medial to lateral x ____ cm anterior to posterior oriented simple mastectomy with a suture indicating _____. The specimen is partially surfaced by a ____ x ____ cm ellipse of *[color]* skin with a central nipple areolar complex measuring ____ x ____ x ____ cm. The anterior margin is inked blue, and the posterior margin, black. The specimen is serially sectioned. Located in the *[upper / lower] [inner / outer]* quadrant is a ____ x ____ x ____ cm area of hemorrhage and chalky yellow-white fat necrosis consistent with the site of recent core biopsy. Adjacent to the core biopsy site is a ____ x ____ x ____ cm indurated, gray-tan focus of *[induration /*

probable residual tumor], which is located ____ cm from the posterior margin and ____ cm from the anterior margin. The core biopsy site is located ____ cm from the posterior margin and ____ cm from the anterior margin, and a *[marker description]* metallic radiographic marker is identified. The remaining cut surface reveals moderate amounts of focally dense gray-white fibrous tissue with interspersed yellow lobulated adipose tissue. No additional lesions are identified. *[No gross obvious lymph nodes OR # of lymph nodes ranging in size from ____ to ____ cm in greatest dimension]*.

Cold Ischemic Time: _____

Fixation Time: _____

ASCO/CAP Guidelines Met:

Representative sections are submitted as follows:

- A1: Nipple, perpendicular section
- A2-A5: Core biopsy to include area of induration (possible tumor) in A4; with deep margin in A4 and A5
- A6: Closest anterior margin
- A7: Grossly uninvolved section upper outer quadrant
- A8: Grossly uninvolved section lower outer quadrant
- A9: Grossly uninvolved section upper inner quadrant
- A10: Grossly uninvolved section lower inner quadrant
- A11 -A__: Lymph nodes (if applicable)

Nipple Sparing Simple Mastectomy with Two Macroscopic Tumors

Submitted *[fresh / in formalin]* in one container for gross and microscopic examination, labeled with the patient's name, [], MR number, [], and "*[specimen designation]*" is a ____ gram, ____ cm superior to inferior x ____ cm medial to lateral x ____ cm anterior to posterior oriented nipple sparing simple mastectomy. There are two attached sutures which orient the specimen as follows: two long - axillary tail, two short - under surface of nipple *[or other orientation]*. No skin or nipple are present on the specimen. The anterior margin is inked blue and the posterior margin black. The cut surface of the specimen reveals *[#]* distinct tumors which will be designated T1 and T2. T1 measures ____ x ____ x ____ cm and is located in the upper *[outer / inner]* quadrant *[or other location]*. T1 is indurated with an ill-defined stellate gray-white cut surface and is located ____ cm from the posterior margin and ____ cm from the anterior margin. A definitive previous core biopsy site *[is / is not]* identified, and a biopsy marker *[is / is not]* macroscopically seen. T2 measures ____ x ____ x ____ cm and is located centrally ____ cm from T1. T2 is indurated with an ill-defined stellate gray-white cut surface and is located ____ cm from the posterior margin and ____ cm from the anterior margin. A *[marker description]* radiographic marker is identified within the tumor. The breast tissue between T1 and T2 two is markedly firm, focally gritty, and nodular, gray-tan. The total measurement of T1, T2 and in-between tissue measures ____ cm in greatest dimension. The remaining cut surface reveals *[minimal / moderate / extensive]* amounts of dense gray-tan fibrous tissue which is focally nodular throughout with interspersed tan-yellow adipose tissue. No additional lesions are identified. Sectioning through the axillary tail reveals *[no gross obvious lymph nodes OR # of lymph nodes ranging in size from ____ to ____ cm in greatest dimension]*.

Cold Ischemic Time: _____

Fixation Time: _____

ASCO/CAP Guidelines Met:

Representative sections are submitted as follows:

- A1: Undersurface of nipple
- A2-A4: T1 to include superficial and deep margins, sequentially submitted from lateral to medial
- A5-A6: T2 to include deep and superficial margins, sequentially submitted from lateral to medial

A7-A8: Sections in-between T1 and T2
A9: Random section upper outer quadrant
A10: Random section lower outer quadrant
A11: Random section upper inner quadrant
A12: Random section lower inner quadrant
A13 -A__: Lymph nodes (if applicable)

Modified Radical Mastectomy

Submitted *[fresh / in formalin]* in one container for gross and microscopic examination, labeled with the patient's name, [], MR number, [], and "*[specimen designation]*" is a ____ gram, ____ cm superior to inferior x ____ cm medial to lateral x ____ cm anterior to posterior, oriented, modified radical mastectomy. An attached suture designates the axillary tail. The axillary tail measures ____ x ____ x ____ cm. The specimen is partially surfaced by a ____ x ____ cm ellipse of *[color]* skin with a central nipple areolar complex measuring ____ x ____ x ____ cm. The anterior margin is inked blue, and the posterior margin black. The specimen is serially sectioned and cut surface reveals a ____ x ____ x ____ cm tumor in the upper outer quadrant. The tumor has an indurated, stellate, gray-white cut surface and is located ____ cm from the posterior margin and ____ cm from the anterior margin. A definitive previous core biopsy site *[is / is not]* identified, and a biopsy marker *[is / is not]* macroscopically seen. The remaining cut surface reveals *[minimal / moderate / extensive]* amounts of dense gray-tan fibrous tissue which is focally nodular throughout with interspersed tan-yellow adipose tissue. No additional lesions are identified. Sectioning through the axillary tail reveals *[no gross obvious lymph nodes OR # of lymph nodes ranging in size from ____ to ____ cm in greatest dimension]*.

Cold Ischemic Time: _____
Fixation Time: _____
ASCO/CAP Guidelines Met:

Representative sections are submitted as follows:

A1: Nipple
A2-A4: Tumor to include superficial and deep margins, sequentially submitted from lateral to medial
A5: Random section upper outer quadrant
A6: Random section lower outer quadrant
A7: Random section upper inner quadrant
A8: Random section lower inner quadrant
A9 -A__: Lymph nodes (if applicable)

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