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Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group

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Neoadjuvant systemic therapy is being used increasingly in the treatment of early-stage breast cancer. Response, in the form of pathological complete response, is a validated and evaluable surrogate end point of survival after neoadjuvant therapy. Thus, pathological complete response has become a primary end point for clinical trials. However, there is a current lack of uniformity in the definition of pathological complete response. A review of standard operating procedures used by 28 major neoadjuvant breast cancer trials and/or 25 sites involved in such trials identified marked variability in specimen handling and histologic reporting. An international working group was convened to develop practical recommendations for the pathologic assessment of residual disease in neoadjuvant clinical trials of breast cancer and information expected from pathology reports. Systematic sampling of areas identified by informed mapping of the specimen and close correlation with radiological findings is preferable to overly exhaustive sampling, and permits taking tissue samples for

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translational research. Controversial areas are discussed, including measurement of lesion size, reporting of lymphovascular space invasion and the presence of isolated tumor cells in lymph nodes after neoadjuvant therapy, and retesting of markers after treatment. If there has been a pathological complete response, this must be clearly stated, and the presence/absence of residual ductal carcinoma *in situ* must be described. When there is residual invasive carcinoma, a comment must be made as to the presence/absence of chemotherapy effect in the breast and lymph nodes. The Residual Cancer Burden is the preferred method for quantifying residual disease in neoadjuvant clinical trials in breast cancer; other methods can be included per trial protocols and regional preference. Posttreatment tumor staging using the Tumor–Node–Metastasis system should be included. These recommendations for standardized pathological evaluation and reporting of neoadjuvant breast cancer specimens should improve prognostication for individual patients and allow comparison of treatment outcomes within and across clinical trials.
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Neoadjuvant systemic therapy is being increasingly used in the treatment of early-stage breast cancer. Response, in the form of pathological complete response, is being put forward as an evaluable end point for determining the efficacy of new agents in neoadjuvant clinical trials[1] and is an excellent prognostic indicator.[2] Data are also emerging on the frequency of regional recurrence based on the presence of residual disease in both breast and lymph nodes.[3] However, accurate evaluation of the original tumor bed depends on correct localization and sampling of the tumor bed. Therefore, gross pathologic methods are the single greatest determinant for accurate definition of pathological complete response or residual disease. This not only alters the end point, but could increasingly affect decisions regarding the need for further local–regional or systemic therapy, if based on the extent of residual disease.[3] Therefore, a standard approach to the evaluation of the postneoadjuvant systemic therapy surgical specimen is essential.

Several classification systems have been developed for the assessment of pathologic response to neoadjuvant systemic therapy; these have been reviewed elsewhere.[4–11] Although, collectively, they have their advantages and disadvantages, most have been validated as correlating with outcome (overall survival, event-free survival, and/or distant relapse-free survival).[6,10,12–16] However, different staging systems yield different estimates of future risk.[17] The Residual Cancer Burden is an online tool for the quantification of residual disease that is simple to apply, reproducible, and has been clinically validated with long-term follow-up data.[10,18,19]

Moreover, novel classification systems are continually being developed, for example, those that incorporate biomarkers in addition to traditional histologic prognostic variables, such as the Residual Proliferative Cancer Burden that combines Residual Cancer Burden with posttreatment Ki67 index.[20] There are also combined clinical and pathological systems that take into account pretreatment information such as clinical stage as well as posttreatment pathology findings, for example, the 'clinical-pathologic stage–estrogen/grade' staging system.[21] These approaches also show promise as future means to predict outcome by combining additional clinical or biological information with Residual Cancer Burden or American Joint Committee on Cancer stage after treatment.

National guidelines have been developed for histopathologic assessment of breast cancer specimens in individual countries/regions, including Australasia,[22] Belgium,[23] Germany,[24] the United Kingdom (now being updated),[25] The Netherlands,[26] and the United States.[27] These vary in their approach to evaluating the postneoadjuvant specimen.

Frequently, neoadjuvant systemic therapy will be administered in the setting of a clinical trial. Pathologists must be involved at an early stage in trial development so that specimen handling, reporting, and tissue collection is specified.[28] Currently, in many multicenter neoadjuvant systemic therapy trials, the surgical specimens are reported by the treating hospital without even minimum guidelines for specimen handling or centralized review to ensure validity and reproducibility of results. A central review of histopathology reports within the neo-tAnGo trial, a UK-based multicenter randomized neoadjuvant chemotherapy trial in early breast cancer, revealed huge variation in handling and reporting of neoadjuvant specimens between centers.[29] In the I-SPY 1 trial, the pathological complete response rate fell by almost 10% among pathologists at 9 centers after they were trained on how to use the Residual Cancer Burden tool (Laura Esserman, personal communication, 2 August 2013). In a French multicenter study that used the Chevallier system,[30] the pathological complete response rate in one arm of the study fell from 16 to 8% following central pathology review of slides.[31]

Finally, the definition of pathological complete response has not been uniform, making reporting and interpretation of data challenging.[5,32] The frequency of use of different definitions of pathological complete response in major neoadjuvant clinical trials is illustrated in Figure 1. These different definitions of pathological complete response can change the apparent survival benefit associated with pathological complete response,

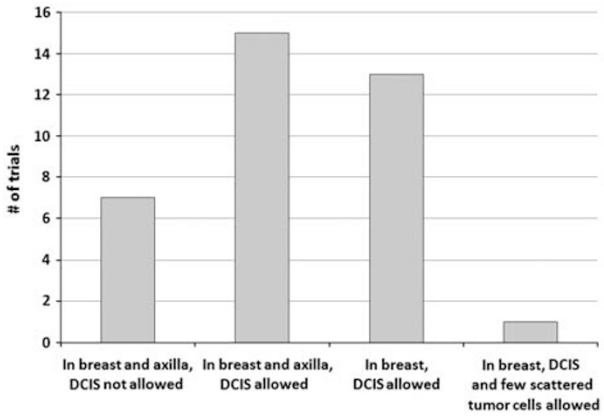


Figure 1 Use of different definitions[a] of pathological complete response in major neoadjuvant breast cancer clinical trials. Trials included in graphic above: first bar: GeparDuo, GeparTrio, GeparQuattro, GeparQuinto, GeparSixto, GeparSepto, NEOCENT; second bar: ABCSG 32, ACOSOG-Z1031, ACOSOG-Z1071, ARTemis, CHERLOB, CNIO-BR-01 2010, I-SPY 2, MDACC 2012-0167, neo-tAnG0, neo-TN (used Neoadjuvant Response Index), NEOZOTAC, NOAH, REMAGUS 02, S0800, TECHNO; third bar: ACOSOG-Z1041, AGO1, CALGB-40601, CALGB-40603, ECTO, GEICAM/2006-14, Neo-ALTTO, Neo-Sphere, NSABP-B-18, NSABP-B-27, NSABP-B-40, NSABP-B-41, S0012; fourth bar: EORTC-10994. [a]Definition used in the primary end point or, where pathological complete response was not the primary end point, in the secondary end point.

depending upon which definition is used. (Figure 2).[2,10,15,32,33] There is general consensus that residual disease in the axillary lymph nodes indicates a worse prognosis, even when there has been a pathological complete response in the breast, and hence the definition of pathological complete response should include absence of disease in both the breast and axillary lymph nodes.[2,3,17,32,34–40]

A more contentious issue is whether the presence of residual ductal carcinoma *in situ* (DCIS) in the absence of residual invasive disease should be included or excluded from pathological complete response.[32,33] The US Food and Drug Administration-led pooled analysis of 12 neoadjuvant randomized trials with long-term follow-up undertaken by the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) found similar event-free survival and overall survival in patients without residual invasive carcinoma regardless of the presence/absence of residual DCIS.[2] However, in a different statistical approach, a pooled analysis of the seven prospective neoadjuvant systemic therapy clinical trials by the German and Austrian Breast Groups demonstrated significantly worse event-free survival for patients with ypTisypN0 compared with patients who were ypT0ypN0. There was no significant difference in overall survival[32] (Figure 2). An analysis of a smaller cohort of patients treated at the MD Anderson Cancer Center, however, showed no difference in survival between patients with ypT0ypN0 and ypTisypN0 (Figure 2).[33] It is conceivable that an internationally uniform procedure for handling and reporting on postneoadjuvant systemic therapy specimens would eventually resolve this issue.

Overall, the US Food and Drug Administration-supported pooled analysis was not able to validate differences in pathological complete response rate as a surrogate end point for difference in event-free survival from the neoadjuvant clinical trials included in the analysis. However, it did point to substantial improvements in survival in individuals with pathological complete response and supported standardization of the definition of pathological complete response, proposing it should be defined as either ypT0/isypN0 or ypT0ypN0 in future trials.[2]

Materials and methods

Given the lack of consensus regarding the pathological assessment of postneoadjuvant systemic therapy breast cancer specimens in clinical trials, an international working group of pathologists, radiologists, surgeons, medical and radiation oncologists, and gynecologists was convened by the BIG-NABCG collaboration. Members were nominated by BIG-NABCG leadership and the working group co-chairs, as well as by sites responding to the collection of standard operating procedures described below. Members represented an array of disciplines and countries.

First, to gauge existing variability in approaches to postneoadjuvant systemic therapy pathologic assessment, we collected standard operating procedures from neoadjuvant breast cancer trials and from sites participating in such trials. ClinicalTrials.gov was searched for mainly academic, phase II or III neoadjuvant trials activated since 2005, with a planned recruitment of at least 100 patients. Earlier trials were included if they were one of the trials included in the US Food and Drug Administration pooled analysis noted above, or otherwise were major trials (e.g., >1000 patients). Standard operating procedures were requested of 48 trials, both from the leaders of the trials themselves (trial standard operating procedures) and, where leaders responded, the sites involved in those trials (site-specific standard operating procedures). Information from the standard operating procedures was abstracted into categories of 'extent of sampling,' 'quantification/size/grading/cellularity,' 'lymph node evaluation,' 'retesting of markers,' and 'other information.' The abstracted information was then compared and contrasted.

The working group convened on seven teleconferences and three smaller planning calls, exchanged emails, and went through several rounds of comments, resulting in the development of practical recommendations for a minimum, essential set of components that should be included in the pathologic evaluation and reporting of postneoadjuvant systemic therapy breast cancer specimens. The working group has also written a companion paper intended for a

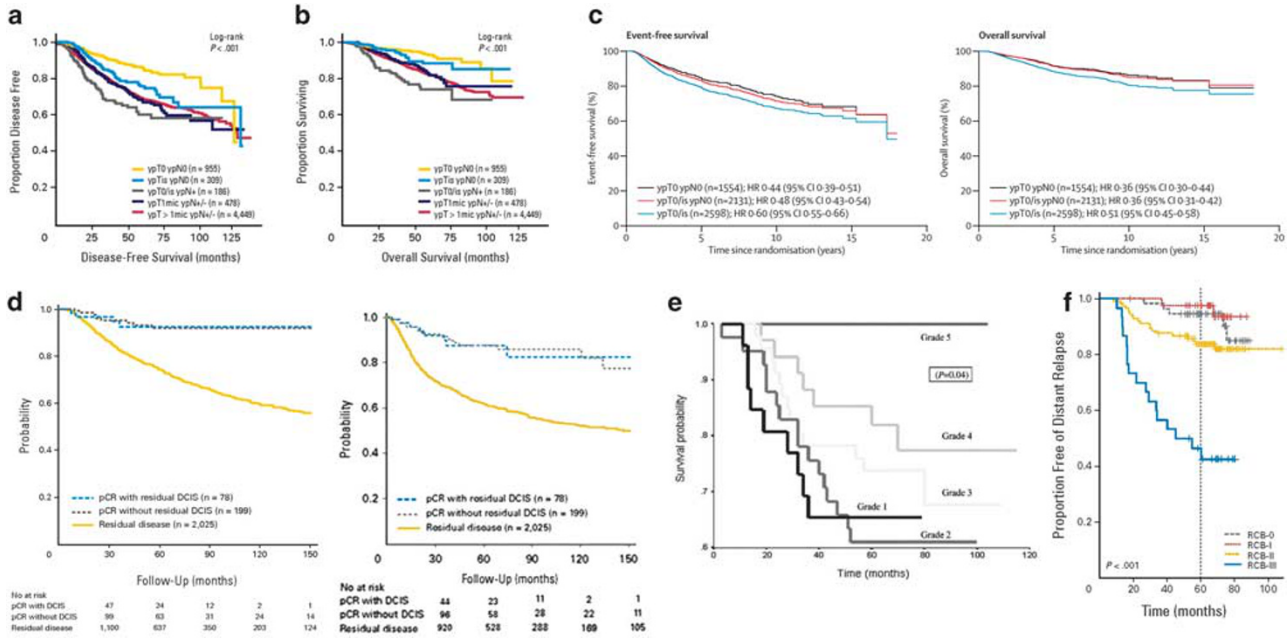


Figure 2 Survival curves showing impact of different definitions of pathological complete response on survival. (**a**) Data from the German Breast Group and AGO-B trials pooled analysis showing reduced disease-free survival for patients with ypTisypN0 *vs* ypT0ypN0. Patients who had residual nodal disease despite absence of invasive cancer in the breast (ypT0/isypN+) had the poorest disease-free survival.[32] (**b**) Results from the same study showing no significant difference in overall survival between ypT0ypN0 and ypTisypN0. The ypT0/isypN+ has a significantly worse overall survival compared with ypT0/isypN0. (**c**) In the CTNeoBC pooled analysis,[2] ypT0pN0 and ypT0/isypN0 were more strongly associated with improved event-free survival and overall survival than ypT0/is alone. Moreover, ypT0ypN0 and ypT0/isypN0 were similar in their associations to event-free survival and overall survival. (**d**) MD Anderson study showing 5- and 10-year overall survival (left) and disease-free survival (right) rates were identical for the patients with pathological complete response *vs* pathological complete response+DCIS.[33] (**e**) Categories of reduction in cellularity in the Miller–Payne system correlate with disease-free survival.[15] (**f**) Residual Cancer Burden score is an independent variable that predicts likelihood of relapse. Minimal residual disease (RCB-I) carries the same prognosis as pathological complete response.[10] (**a** and **b**) Reprinted with permission. © 2012 American Society of Clinical Oncology. All rights reserved. von Minckwitz *et al*.[32] (**c**) Reprinted from Cortazar *et al*.[2] © 2014 with permission from Elsevier. (**d**) Reprinted with permission. © 2007 American Society of Clinical Oncology. All rights reserved. Mazouni *et al*.[33] (**e**) Reprinted from Ogston *et al*.[15] © 2003, with permission from Elsevier. (**f**) Reprinted with permission. © 2007 American Society of Clinical Oncology. All rights reserved. Symmans *et al*.[10]

more multidisciplinary audience, explaining how a standardized approach would benefit the entire medical team and summarizing the more detailed recommendations provided below.[41]

Results

Standard operating procedures were collected from 28 trials and 25 sites (Supplementary Information 1). Substantial variability of practice was found in all stages of histological evaluation of both breast and nodal neoadjuvant specimens: extent of sampling (ranging from 4 to 40 blocks, depending on presence/absence of a macroscopic identifiable lesion and on tumor size), thickness of primary-tumor sectioning (ranging from 2 to 10 mm), the routine performance of immunohistochemical staining when no tumor was identified on hematoxylin and eosin, how amount of residual tumor was measured and documented, and whether or not a formal system was used to grade response and, if so, which system was used. For small specimens, most sites submitted the entire specimen. Only 6 of 20 sites that discussed retesting of markers in their response noted they retested markers routinely. Of note, several sites emphasized a need for standardization of the pathologic assessment of postneoadjuvant systemic therapy specimens, within practicable limits. Further details are provided in Supplementary Information 1.

Recommendations

The working group's practical suggestions are detailed below.

Pretreatment Assessments

Initial diagnosis on core biopsy of the breast

Percutaneous image-guided core needle biopsy is strongly recommended, and must be adequate for an unequivocal diagnosis of invasive breast carcinoma. Caution must be used if imaging or core needle biopsy findings suggest that a significant portion of the lesion may represent *in situ* disease, or if there is only a limited amount of invasive carcinoma represented in the core. In these cases, repeat core needle

biopsy or surgery for accurate diagnosis, rather than neoadjuvant systemic therapy, may be indicated. Histologic type, tumor grade, estrogen receptor (ER), progesterone receptor (PR), and HER2 status, as well as any other parameters used to select for neoadjuvant systemic therapy (e.g., Ki67, multigene assays) should be evaluated on the core needle biopsy.

Several systems for grading tumor response to treatment require comparison of cellularity with the pretreatment biopsy, such as the Miller–Payne, Pinder, Sinn, and Sataloff systems.[7,13,15,42] Inclusion of an estimate of tumor cellularity in the core needle biopsy is of value if these systems will be used to grade response in the excision specimen.

Consideration should be given to dedicated baseline cores for research, either at the time of diagnostic biopsy or as a separate designated biopsy procedure.[43] Research cores should be in addition to those required for diagnosis and should be preserved in order to best meet the research need. Touch preparations or frozen sections can be used to confirm the presence of malignant cells in the dedicated research cores before freezing or immersion into a dedicated solution. If using Optimum Cutting Temperature freezing media, one tissue core can be embedded per block. In some cases, formalin-fixed cores can be reembedded as a research block after reporting. Some trials also require 'on-treatment' research core biopsies at subsequent time points (e.g., after the first cycle or at mid-course).

Evaluation of the axilla before treatment

Routine axillary ultrasound is recommended to assess the axillary lymph nodes, with fine needle aspiration or core needle biopsy of morphologically abnormal lymph nodes. Thus, sentinel lymph node biopsy before neoadjuvant treatment should be limited to cases where the pretherapeutic lymph node status is required for systemic or local treatment decisions.[44] Pretreatment sentinel lymph node biopsy precludes assessment of nodal response to neoadjuvant systemic therapy, and invalidates American Joint Committee on Cancer yp stage and calculation of the Residual Cancer Burden score if an excised sentinel lymph node was originally positive.

Evaluation of the Surgical Specimen After Neoadjuvant Systemic Therapy

Clinical information required for pathologic evaluation

It is important that the multidisciplinary team (e.g., surgeons, radiologists, and pathologists) communicate as a team for patient care; *this is covered in detail in our companion multi-disciplinary paper.*[41] At a bare minimum, the request form must clearly indicate neoadjuvant systemic therapy has been given, along with the location and pretreatment size of the tumor(s). A suggested template requisition form that can be sent with the specimen is included in Supplementary Information 2.

Specimen handling

Priorities for evaluation of the surgical specimen are different after neoadjuvant systemic therapy, with emphasis on informed and accurate evaluation of tumor response to treatment. In general, one should apply the principles within national and institutional guidelines for standardization of processing and reporting of breast specimens, such as those noted above. Ideally, specimens should be sliced when fresh to identify the markers of the original tumor bed and to ensure formalin penetration.

Residual tumor is usually less well defined and softer than untreated tumor, making it more difficult to detect grossly. Therefore, careful mapping and more extensive sampling is required for histopathologic study. *It is strongly recommended that an image of the sliced specimen be recorded (radiograph, photograph, photocopy, or drawing) and then used as a map for the sections taken, so that the histopathologic findings of any residual disease in the breast can be more easily understood.* For example, the sections taken can be drawn on a printed image of the sliced specimen and then scanned into the pathology database for viewing at the time of histopathologic study. More precise imaging of the gross specimen and correlation with the histopathologic sections will decrease the number of sections taken from the breast, and increase the efficiency and accuracy of pathologic assessment. This can save time and money while enabling consistent and careful pathologic interpretation. The recommendations below will attempt to supplement existing national guidelines for specific situations encountered in the neoadjuvant setting; however, the pathologist should use sound clinical judgment on a case-by-case basis.

Sampling of small lumpectomy specimens. Many institutional standard operating procedures call for thinly slicing and submitting small specimens in their entirety (e.g., < 5 cm in greatest diameter in Yale University's standard operating procedure, and < 30 g in the Dutch national guideline[26]) in a manner that allows reconstruction of the specimen at the time of microscopic evaluation through accurate description or with the help of a diagram. Unfortunately, this approach does not allow for tissue collection for research. Clinical judgment should be applied in this setting. If there is obvious gross residual tumor, then a research sample can be taken without compromising accurate histological assessment. In cases where the macroscopic findings are nonspecific, or there is clinical doubt about the location of the tumor bed, then consideration should be given to submitting the entire specimen. Research samples may still be taken by thinning the blocks and submitting the trim, or, alternatively, small cylinders of tissue can be taken with a punch biopsy tool.

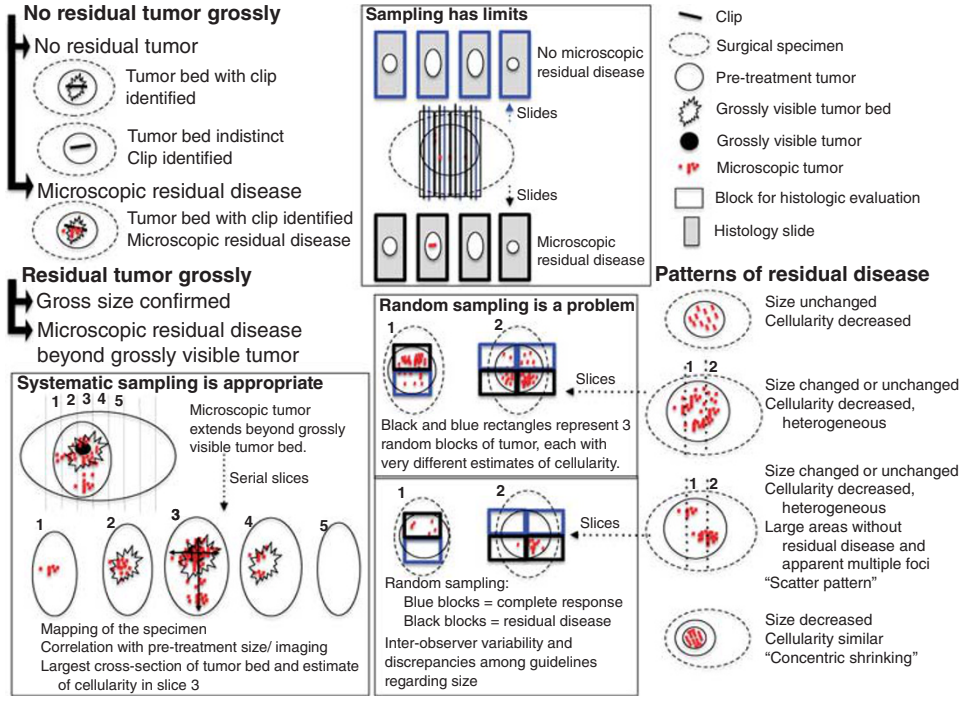


Figure 3 Problems related to sampling for histologic evaluation. Gross residual tumor may or may not be present after neoadjuvant therapy (top left). Even when the tumor bed is entirely submitted, histologic evaluation has limits (top center). The blue and black slides represent different levels obtained from the same block. The blue slides show a complete response. The black slides show minimal residual microscopic disease. Partial response shows various patterns and the decrease in cellularity is often heterogeneous (right). In these cases, random sampling of tumor can lead to very different estimates of tumor cellularity (bottom center). Random sampling with the blue blocks would conclude a complete response. Random sampling with the black blocks would document residual disease. Often, the microscopic tumor extends beyond a grossly visible tumor bed (bottom left). The largest cross-section of tumor bed is sampled for an estimate of tumor cellularity.

Depending on the type of processing used for the research tissue, histology can still be evaluated if deemed clinically necessary, such as hematoxylin and eosin-stained sections of research blocks. A previous international working group has addressed the collection of research tissue in the neoadjuvant setting in detail.[43]

It is important to document that these small resections have adequately excised the lesion. The tumor bed/clip must be identified. Tumor bed extending to the margins should be documented.

Sampling of large lumpectomy/mastectomy specimens (partial submission). Targeted representative sections can be taken from larger specimens, but it is essential to carefully and accurately represent the tumor bed in a manner that can be retrospectively mapped to the gross and/or radiologic findings. This enables more accurate estimation of the extent of residual disease. Correlation with clinical and imaging findings is imperative to ensure the correct area is sampled. Sampling should include grossly visible tumor bed and/or the location of any marker clips and immediately adjacent tissue to encompass the area suspected of involvement by carcinoma before treatment (Figure 3). This area to be sampled is referred to as the pretreatment area of involvement in the discussion below. Degree of sampling is then determined by the pretreatment size in addition to any visible tumor bed or grossly visible residual disease.

Ideally, the specimen is sliced to reveal the largest cross-section of the pretreatment area of involvement. Block(s) representing the full face of the pretreatment area of involvement should be taken of every 1 cm slice containing pretreatment area of involvement, or, for very large tumors, five representative blocks of a cross-section of pretreatment area of involvement per 1–2 cm of pretreatment size, up to a total maximum of ~25 blocks. In the absence of trial-based evidence as to the degree of sampling required, the committee felt this to be a pragmatic approach that should be sufficient to determine the presence of pathological complete response. The US Food and Drug Administration, in their guidance, have recommended taking 'a minimum of one block per cm of pre-treatment tumor size, or at least 10 blocks in total, whichever is greater'.[34] The extent of sampling should be guided by good clinical judgment on a case-by-case basis—informed, directed sampling is more important than blindly taking a prescribed number of blocks. For assessment of cellularity of very large tumor beds, five representative blocks are sufficient to represent the largest

cross-section of residual tumor bed and calculate the Residual Cancer Burden.[45]

Precise description must be used to allow reconstruction of the specimen during histologic evaluation for accurate measurements and cellularity estimates. We strongly recommend visual images, such as photographs, specimen radiographs, or sketched diagrams, with annotations to indicate the sites where tissue sections were taken for histopathologic evaluation.

If no residual disease is seen on initial sections, or if the distribution of the disease does not correspond to the initial gross impression, then a second pass may be needed to submit further blocks. Additional blocks, including sections documenting margins, should be obtained as with non-neoadjuvant specimens.

Laboratories with access to large tissue cassettes are encouraged to utilize this technique as a superior method for mapping the residual tumor bed. Large cassettes enable sampling of a bigger area with fewer blocks, with the entire lesion often captured on a single slide. This simplifies reconstruction of the extent of residual disease, measurement of lesion size, and examination of margins.[46]

In cases where the above cutoffs would not result in submission of the entire tumor bed, remaining tissue can be sampled for research. Areas with grossly visible tumor can easily be sampled. Cases where the above cutoffs result in submission of the entire tumor bed can be sampled for research as described in the section 'Sampling of small lumpectomy specimens' under 'Specimen handling' above. If only formalin-fixed, paraffin-embedded tissue is needed, additional blocks can be submitted from a second pass for research from areas that had residual tumor on microscopy.

Multiple lesions in lumpectomy or mastectomy. In specimens containing multiple lesions, each lesion should be handled as a single lesion as described under 'Sampling of large lumpectomy/mastectomy specimens (partial submission)' above, with the addition of blocks of tissue taken from in between the lesions to ensure that they are truly separate and to evaluate the presence of other intervening disease, such as DCIS.

Microscopic reporting

Prognostic and predictive factors traditionally evaluated in surgical specimens following primary surgery are all relevant in the neoadjuvant systemic therapy setting. Although some familiar prognostic information may be altered by treatment (e.g., tumor grade and histological type) or may be less reliable (lymph node and margin status), much can be gained from the opportunity to evaluate response to treatment.

Histologic tumor type and grade. The method for determination of histologic tumor type and tumor grade is identical to that used for non-neoadjuvant specimens, although it is not clear whether these add prognostic information to the pretreatment results. Tumors with a typical appearance of no special type before treatment may have a lobular growth pattern following neoadjuvant chemotherapy.[47] Treatment can cause nuclear hyperchromasia and pleomorphism; however, the findings should be compared with the pretreatment biopsy before assuming they are treatment-related. The mitotic rate may be reduced by treatment; this finding is associated with a better prognosis (disease-free survival and overall survival)[48] and lower risk of developing distant metastases.[49] Clonal heterogeneity within the tumor may be reflected by variable response to therapy, and by areas with different morphology and grade. A comment regarding the presence of such heterogeneity should be made in the report, and is important when choosing blocks for postneoadjuvant systemic therapy hormone receptor and HER2 assessment.

If multiple, morphologically distinct tumors are present that are clearly separated by adipose tissue, they should be reported as separate lesions. However, it should be noted that the largest residual primary tumor is used for determination of both Residual Cancer Burden and yp stage. Note that ypT stage is defined by the largest contiguous focus of invasive cancer, whereas Residual Cancer Burden uses the two dimensions of the largest residual area of residual invasive cancer (i.e., that does not need to be contiguous) in the tumor bed.

Size and extent. Tumor size/extent is often more difficult to assess after neoadjuvant systemic therapy. There are two main patterns of tumor response following neoadjuvant systemic therapy—concentric shrinking and the scatter pattern (Figure 3). Measurement of lesion size in this latter scenario may be difficult. Our suggested approach is described in Table 1.

Cellularity. In addition to its effect on tumor size, neoadjuvant systemic therapy often has a profound effect on tumor cellularity. Tumor size may not decrease, but overall cellularity may be markedly reduced (Figure 3), making residual tumor cellularity an important factor in assessing response.[50] Comparison of pre- and post-treatment cellularity is the key element of several systems for grading response.[7,13,15,42] If a formal classification system for grading of response is used, this should be noted in the report. As tumor cellularity is often heterogeneous, the pretreatment core biopsy may not be representative of the entire tumor. Similarly, changes in tumor cellularity induced by neoadjuvant systemic therapy can be heterogeneous and therefore more extensive sampling may be needed to accurately assess cellularity. The descriptions of these scoring systems do not explicitly state how to deal with this heterogeneity, and it can be tempting only to assess the most cellular areas of the tumor.

Table 1 Controversial scenarios in reporting breast cancer after neoadjuvant systemic therapy

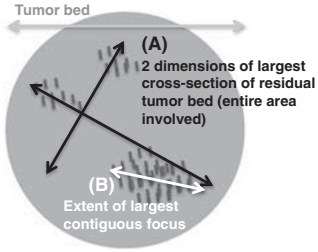
Scenario	*Current evidence/guidelines*	*Suggested approach*
Residual tumor present as scattered foci (common)	• Tumor size is often more difficult to assess after neoadjuvant treatment. • Residual carcinoma may be present as multiple, small foci scattered over a (ill-defined) tumor bed. • There are two main options to measure size in this setting: (**A**) Residual tumor bed size in two dimensions (used to calculate the Residual Cancer Burden score): the extent of the area involved by all islands of residual invasive tumor cells and intervening stroma. This does not include tumor bed beyond the area containing residual invasive tumor cells. (**B**) Tumor size according to American Joint Committee on Cancer staging 7th edition[67]: if the residual tumor consists of microscopic nests in a fibrotic stroma, ypT should be based on the largest contiguous area of invasive carcinoma, with an indication that multiple foci are present ('m').  	• If there is a single lesion present on pretreatment imaging, then regard residual disease as a single tumor, especially if tumor cells are present within a reactive stromal background consistent with a solitary tumor bed (Opinion). • When there are scattered islands of tumor cells, measurement (B) (diagram at left) as described by American Joint Committee on Cancer staging 7th edition[67] may result in significant underestimation of tumor extent. There are also currently no data on the relationship of measurement (B) to outcome (Opinion). • Lesion size including the cell clusters and intervening fibrous tissue (A) (diagram at left), which is congruent with the earlier, 6th edition of American Joint Committee on Cancer staging,[68] correlates with survival[69] and may be more relevant, for example for comparison with radiology (Limited Evidence). • In our opinion, the combination of residual tumor cellularity and measurement (A) is the better indicator of response (Opinion). • When required to report American Joint Committee on Cancer 7th edition stage, both measurements (A) and (B) should be given in the pathology report, with an explanation of how the final size and stage designation was made (Published Guideline). • If there are multiple tumors present on pretreatment imaging or tumor foci are separated by normal breast tissue, then regard as multiple lesions and measure independently as distinct tumor foci. Dimensions from the largest tumor deposit should be used for ypTNM staging (Published Guideline).
Presence of lymphovascular invasion in the absence of an identifiable residual invasive tumor mass (rare)	• There are insufficient data on the independent prognostic significance of the presence of lymphovascular invasion or extensive lymphovascular invasion in neoadjuvant specimens. • One small study found that such intralymphatic tumor carries adverse prognostic significance, even in the absence of residual stromal invasion.[70] However, most of the patients also had residual disease in the lymph nodes and multivariate analysis was not possible.	• **Residual lymphovascular invasion should NOT be classed as pathological complete response**—make a statement in the pathology report that residual tumor is present in the form of intravascular disease (Opinion). • Ensure tumor bed has been accurately localized and adequately sampled to exclude residual invasive disease (Opinion). • Ensure truly lymphovascular invasion, not DCIS or retraction artifact. This may be difficult; immunohistochemistry may be helpful (Opinion). • Measurement is optional. If a limited area is involved, a measurement in mm can be given. Alternatively, lymphovascular invasion can be quantified as focal or extensive with 'extensive' defined as one or more foci in more than one block[71] (Opinion). • Although it was agreed residual lymphovascular invasion should not be regarded as pathological complete response, in the absence of adequate data the working group felt it was not appropriate to give definite reporting recommendations (Opinion).
Presence of isolated tumor cells in lymph nodes (common)	• American Joint Committee on Cancer TNM recommends isolated tumor cells after chemotherapy be called node negative ($ypN0_{itc}$) but not regarded as pathological complete response.[67]	• **Any residual disease in the lymph node, including micrometastases and isolated tumor cells, should NOT be classified as pathological complete response (Limited Evidence).**

Table 1 (Continued)

Scenario	*Current evidence/guidelines*	*Suggested approach*

	• World Health Organization recommends isolated tumor cells after chemotherapy be called node positive.[72]
	• Findings include:
	  • Disease-free survival and overall survival worsened with increasing number of nodes and deposit size. Size of largest metastasis was strongest predictor of overall survival in multivariate analysis. Micrometastatic disease < 2 mm, including isolated tumor cells, was predictive of worse outcome.[40]
	  • No difference in relapse-free survival and overall survival between groups when size of the largest residual metastatic deposit was classified as ≤ 0.1 cm, 0.1–1 cm, and ≥ 1 cm in patients with proven axillary nodal disease before neoadjuvant chemotherapy.[39]
	  • No change in prognosis with occult metastases identified by immunohistochemical staining for cytokeratins.[73]
		• If no associated fibrosis, treat as in adjuvant setting and call node negative (Opinion).
		• If associated fibrosis present, the likelihood is this represents previous micro- or macrometastasis with response. A comment should be included regarding the presence of chemotherapy effect, and the size of the entire area, including tumor cells and intervening stroma, should be measured, rather than the size of the largest cell cluster (Limited Evidence).
		• Additional levels and/or immunohistochemistry are not routinely required. However, immunohistochemistry may be useful if suspicious cells are identified on hematoxylin and eosin, and levels can be used to clarify the size of a deposit if isolated tumor cells/micrometastasis are present on the initial section (Limited Evidence).

Limited Evidence = consensus opinion of committee based on limited evidence; Opinion = consensus opinion of committee in the absence of reliable evidence.

The Residual Cancer Burden system does not require pretreatment cellularity, but proposes standardized sampling of the specimen with assessment of the average cellularity across the largest two-dimensional area of residual tumor bed. For Residual Cancer Burden, the tumor bed area is defined by the two largest dimensions of gross tumor bed defined by macroscopic examination with or without accompanying specimen radiography, but can be later revised after these corresponding slides have been reviewed under the microscope. Hence, the importance of accurate block description and advisability of an illustrative map to determine how the slides map to the gross tumor bed (described above). The online cellularity standard provided in the Residual Cancer Burden website[45] and the images in the publication for the Miller–Payne score are useful aids for pathologists in estimating cellularity.[15] The presence *or absence* of residual DCIS, and the percentage of residual tumor present as *in situ* disease, should also be documented as per the Residual Cancer Burden.

We advocate submitting the largest cross-section of the residual tumor bed with the relevant sections noted in the pathology report.

Lymphovascular invasion. The presence or absence of lymphovascular invasion should be documented (Figure 4). There are insufficient data on the independent prognostic significance of lymphovascular invasion in neoadjuvant specimens. See Table 1 for suggested approaches to assessing and reporting lymphovascular invasion.

Margins. In cases with variable response leading to multiple, small foci of residual disease in a subtle tumor bed, carcinoma may extend beyond an apparently negative margin. Tumor bed extending to the margins, and which margin is involved, should be documented (Figure 5).

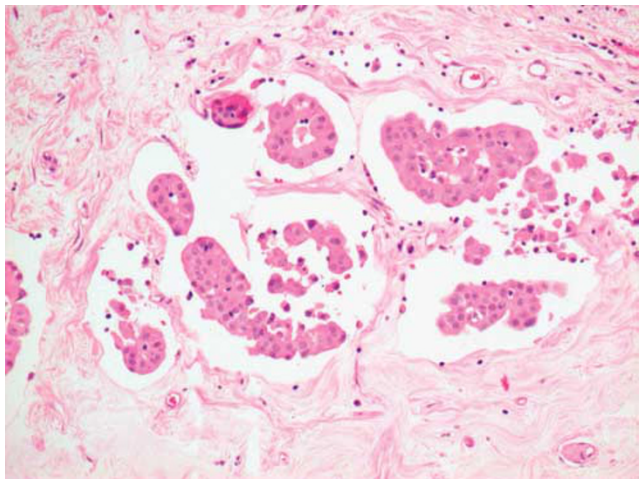


Figure 4 Extensive lymphovascular space invasion after chemotherapy. In this case, an invasive tumor focus was not identified despite extensive sampling. The axillary nodes were positive for residual metastatic carcinoma (courtesy of Elena Provenzano).

Evaluation of the axilla after treatment

Several studies have shown that posttreatment nodal status is an important determinant of disease-free survival and overall survival, regardless of response within the breast.[32,35–40] Currently, lymph node staging in patients who have received neoadjuvant systemic therapy is usually performed by either

sentinel lymph node biopsy or axillary lymph node dissection. The accuracy of sentinel lymph node biopsy for staging postneoadjuvant systemic therapy is still under investigation, especially in patients with clinically positive nodes before treatment.[44,51] The paradigm in surgical management of the axilla is evolving,[34] and is the subject of ongoing investigation.[44,51] This is reflected in the use of the phrase '*sampled regional lymph nodes*' by the US Food and Drug Administration in its proposed definition of pathological complete response.[34]

The procedure for evaluating sentinel lymph nodes and axillary lymph nodes should be the same as for non-neoadjuvant specimens. All surgically removed lymph nodes should be sectioned at 2 mm intervals and entirely submitted for histologic evaluation. Some special considerations apply, however.

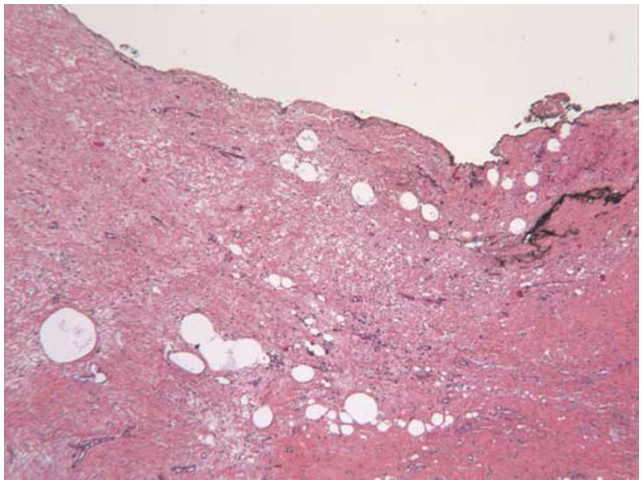


Figure 5 Tumor bed present at the resection margin (courtesy of Frédérique Penault-Llorca).

Some studies have indicated a lower number of lymph nodes identified at axillary lymph node dissection after neoadjuvant systemic therapy, whereas others have found no significant difference following careful pathological evaluation.[52–54] Pathologists evaluating axillary lymph node dissection tissue should subject any tissue that may represent lymph node for microscopic evaluation.

The size of the largest metastatic deposit should be measured microscopically and the presence or absence of any extranodal extension documented. Postneoadjuvant systemic therapy tumor cells are often present as scattered single cells within an area of reactive stromal changes or lymphoid tissue. When measuring the size of the metastasis in this context, the size of the area that is even partly involved by metastatic tumor should be measured, and not just the size of the largest tumor cluster. Clearly separate smaller foci in a node are not included in the maximum size measurement. As micrometastases and isolated tumor cells found after neoadjuvant systemic therapy are predictors of worse survival, specimens with nodal micrometastases or isolated tumor cells should not be designated as having pathological complete response.[40,55] Our suggested approach to assessing isolated tumor cells in this context is provided in Table 1.

The presence of treatment effect in the lymph nodes in the form of fibrosis (Figure 6), mucin pools, or large aggregates of foamy histiocytes identifies a subset of patients with an outcome intermediate between that of completely node negative and node positive after neoadjuvant systemic therapy.[56] However, small fibrous scars in lymph nodes can also be seen in patients without treatment, and in patients who have had a previous biopsy it can be impossible to reliably distinguish biopsy site changes from regressed metastasis.[57] Previously involved nodes

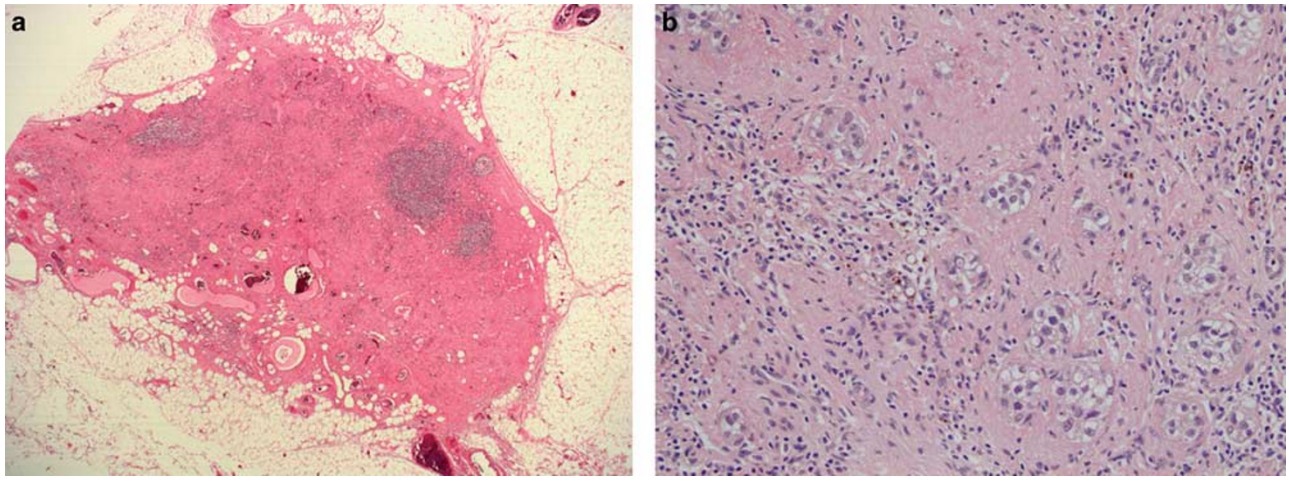


Figure 6 Lymph node showing zonal areas of fibrosis after chemotherapy indicative of metastasis with response to therapy (courtesy of Elena Provenzano). (**a**) Low-power image of lymph node showing zonal fibrosis indicating site of metastasis. (**b**) On higher magnification of a different node, residual islands of tumor cells are present in a setting of reactive fibrosis with hemosiderin-laden macrophages, consistent with chemotherapy effect.

may also look completely normal after treatment. The latter scenario can cause concern when there was histologically proven metastasis before treatment, but evidence of a positive node cannot be found in the final surgical specimen. In this setting, the specimen (including axillary tail, if a mastectomy) should be carefully reexamined to ensure all nodes have been retrieved, and the patient reexamined, before assuming there has been complete response. Clipping the involved node before treatment can also be of value in determining nodal response.

In some centers, sentinel lymph nodes are assessed by molecular assays (e.g., one-step nucleic acid amplification) without any morphological evaluation. This does not allow assessment of response in the node; moreover, one-step nucleic acid amplification is usually not calibrated to detect isolated tumor cells.[58] Therefore, we do not recommend the use of these techniques in the neoadjuvant setting.

Pathological complete response

Our group agrees with the following core principle of the definition of pathological complete response as proposed by the US Food and Drug Administration: '*Pathological complete response is defined as the absence of residual invasive cancer on…. evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy.*'[34] However, we advocate that the presence of invasive tumor cells is considered residual disease regardless of the method of detection—that is, hematoxylin and eosin or immunohistochemistry—although the latter is not routinely recommended. The alternative definition, requiring absence of both DCIS and invasive carcinoma in the breast, can also be used. The definition of pathological complete response chosen should be agreed between pathologists and clinicians within individual institutions, and clearly stated in the report. If the patient is enrolled in a clinical trial, the definition of pathological complete response prescribed by the trial standard operating procedure should be included as part of the report with an explanatory note. Regardless of which definition is used, the presence/absence and extent of residual DCIS should be reported as detailed in our recommended template (Table 2).

Microscopically, the tumor bed may be identified as a focal area of loose, edematous reactive stroma with a variable inflammatory cell infiltrate that may include collections of lipid or hemosiderin-laden macrophages, lymphocytes, and plasma cells. Background breast lobules often appear hyalinized and atrophic with a perilobular lymphocytic infiltrate.

We would like to stress the following. Accurate, reproducible documentation of pathological complete response requires adequate sampling of the correct area of the breast. Overly exhaustive sampling and histologic evaluation of the entire tumor bed are generally not required and are far less valuable than intelligent mapping of the correct locations within the specimen. Therefore, correlation of clinical and imaging information and markers of the tumor site with gross pathology of the specimen are indispensible.

Retesting of markers in the postneoadjuvant therapy specimen

Reassessment of hormone receptor and HER2 status in residual cancer after neoadjuvant systemic therapy is variable between individual centers, with no consensus regarding if and when retesting of markers is advisable. The clinical utility of reassessing marker status in the surgical specimen may depend on the results from the core biopsies taken before neoadjuvant systemic therapy. If retesting is performed, it may be done on either the residual primary tumor or residual nodal disease if the latter contains a better representation of residual tumor cells. Our recommendations are provided in Table 3.

Finally, in some centers, assessment of Ki67 labeling index is performed before and after neoadjuvant systemic therapy. Posttreatment Ki67 index has been shown to correlate with long-term outcome after both neoadjuvant endocrine[59] and chemotherapy,[60,61] although its routine use in clinical practice has not yet been formally recommended because of lack of standardization in its assessment.[62–64] Proliferation is commonly reduced by neoadjuvant systemic therapy and hence, in addition to Ki67, results of multigene assays that include proliferation genes may also change if assessed before and after treatment.[65]

Minimum data set to be reported by pathologists

A suggested summary template for reporting neoadjuvant systemic therapy specimens is presented in Table 2, with minimum data set items highlighted. The US National Cancer Institute's Breast Oncology Local Disease (BOLD) Task Force has also recommended standardized data elements for collection in preoperative breast cancer clinical trials.[66]

Conclusion

Postneoadjuvant systemic therapy histopathological changes are complex, and careful systematic review of the specimen is required for accurate diagnosis and follow-up treatment. For pathological complete response to be used as an indicator of response to novel therapies, it is essential to have a standardized way in which residual disease is measured and reported. We designed the recommendations specifically for the clinical trial setting; however, they can be optionally incorporated into routine practice because, in our opinion, standardization is most effective when uniformly applied. Hopefully, such standardization will improve our knowledge and ability to compare outcomes, promote the submission of specimens for translational research,

Table 2 Suggested template for reporting breast cancer specimens after neoadjuvant systemic therapy in clinical trials[a]

Pretreatment
Pretreatment core biopsy findings (where available)
Histological tumor type
Pretreatment histological grade
(Pretreatment cellularity)
Presence/absence of DCIS
Hormone receptor and HER2 status
(Ki67, multigene assays)
Type of neoadjuvant treatment: chemotherapy, hormone therapy, radiotherapy, chemo+radiotherapy

Type of procedure
Breast: (wide local excision ± localization, mastectomy, other)
Lymph nodes: (sentinel lymph nodes, axillary dissection, other lymph nodes, eg, internal mammary)

Laterality: (left, right, not specified)

Macroscopy
Residual macroscopic tumor identified: yes/ no
If residual macroscopic tumor:
Site of tumor (upper outer, lower outer, upper inner, lower inner, central)
Unifocal *vs* multifocal
If multifocal, number of foci
Size of macroscopic lesion(s): _ x _ x _ mm
If no residual macroscopic tumor:
Area of fibrosis present: yes/ no
Site of fibrosis
Size of fibrosis: _ x _ x _ mm
Radiological marker identified: yes/no/not present

Microscopy
Size/extent of residual tumor: _ mm
Largest cross-section of residual tumor bed (entire area involved) _ x _ mm represented in cassettes (….)
Posttreatment histological grade
Residual cellularity:__%
DCIS: present/absent
Total lesion size including DCIS: _ x _ mm
Percentage of residual cellularity that is CIS:__%
Lymphovascular space invasion: present/absent/indeterminate/extensive
In the absence of residual tumor, is the previous tumor site identified (clip site/area of fibrosis): yes/no

Margin status
Invasive carcinoma: present/absent; distance to closest margin
DCIS: present/absent; distance to closest margin
Tumor bed: present/absent

Lymph node status
Number of sentinel/axillary lymph nodes
Number of sentinel/axillary lymph nodes with metastases
Size of largest metastasis
Evidence of treatment response in the metastases: present/absent
Number of lymph nodes with evidence of treatment response (e.g., fibrosis or histiocytic infiltrate) but no tumor cells
Presence (extent) of extracapsular extension

Final classification of chemotherapy response
Grade of response and classification system used
If no formal grading system used, minimum comment regarding response as below:
Breast:
Pathological Complete Response
Residual invasive carcinoma, no definite response
Residual invasive carcinoma with probable or definite response to chemotherapy
*If there is more than one tumor with variable response between lesions, then the poorest level of response should be taken as the overall classification.
Lymph nodes:
Metastasis present, no response
Metastasis present, evidence of response
No residual metastasis but evidence of previous metastasis with response
No metastasis or fibrosis (true negative)
ypTN stage
Repeat marker testing:
ER/PR/HER2 if initial biopsy was negative or equivocal
(Ki67)

Abbreviations: CIS, carcinoma *in situ*; DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PR, progesterone receptor.
[a]**Bold items** indicate suggested minimum data set specific to postneoadjuvant specimens, IN ADDITION to minimum required for other types of specimens.

Table 3 Retesting of hormone receptors and HER2 after neoadjuvant therapy

Recommendation	*Clinical setting*	*Comments*
• Routine reassessment not currently recommended	• Positive ER/PR/HER2 result on pretreatment core biopsy	• No change in marker expression in the majority of patients.[74,75] • Uncertainty whether conversion should inform the choice of future adjuvant therapies (i.e., to stop or start a targeted treatment).[74–76] • Unknown independent prognostic value of marker conversion for disease-free and overall survival. However, loss of HER2 amplification following neoadjuvant trastuzumab has been associated with worse outcome.[77]
• Reassessment must be performed	• Retesting of markers is required as part of a clinical trial protocol • Biomarker status not known	• Reassessment of ER/PR and HER2 in residual invasive disease should be included in clinical trial protocols to gather high-quality data to clarify the significance of change of receptor status on outcome.
• Reassessment should be considered	• Negative or equivocal result on pretreatment core biopsy • Insufficient invasive tumor for accurate assessment or DCIS only on pretreatment core biopsy[a] • Retesting requested by clinicians • Biopsy performed in another institution • Heterogeneous tumor or multiple tumors with different morphologies on resection • No response to therapy	• Two different meta-analyses of published studies have reported a mean prevalence of discordant results pre- and post-neoadjuvant therapy of 13 and 18% for ER, 32 and 26% for PR, and 9 and 6% for HER2.[78,79] • Causes of discordance include technical artifacts, misinterpretation of test results, intratumoral heterogeneity of marker expression, and changes induced by the intervening therapies[80] (eg, inclusion of trastuzumab in neoadjuvant treatment may increase the rate of negative conversion for HER2).[77,81] • Retesting may give a positive result in a small percentage of patients. • Positive result would indicate eligibility for targeted therapy. • If HER2 status reassessed and found to be discordant, retesting should be performed with both immunohistochemistry and *in situ* hybridization.

Abbreviations: DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PR, progesterone receptor.
[a]Pretreatment core biopsy should be adequate for unequivocal diagnosis of invasive carcinoma and assessment of key prognostic and predictive markers as this forms the only tumor sample if there is a complete pathological response. If this is not the case, repeat core biopsy should be performed or primary surgery considered.

and facilitate the more timely introduction of new agents.

The recommendation of this committee is that pathologic reports of residual disease after neoadjuvant chemotherapy and/or targeted therapy in clinical trials should include the following information:

- Pathological Complete Response or Residual Disease. This should separately describe whether there was residual invasive cancer in the breast, *in situ* cancer in the breast, and the pathologic status of the regional lymph nodes.
- Residual Cancer Burden as the preferred method for more detailed quantification of residual disease. The report should provide the final residual tumor dimensions, cellularity of cancer in the final tumor bed area and the proportion of *in situ* component within that cancer, and the number of positive nodes and the size of the largest metastasis, as well as the Residual Cancer Burden score and class.
- ypTN Stage. The report should separately report the ypT and ypN stages and the pathologist should use the most current edition of the American Joint Committee on Cancer/Union for International Cancer Control staging definitions when evaluating tumor size after neoadjuvant chemotherapy.

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Disclosure/conflict of interest

Dr Symmans filed Residual Cancer Burden as intellectual property (Nuvera Biosciences), patenting the Residual Cancer Burden equation. (The Residual Cancer Burden equation is freely available on the worldwide web.) Dr Symmans reports current stock in Nuvera Biosciences and past stock in Amgen.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)