Title: Standardization of pathologic evaluation and reporting of post-neoadjuvant specimens in clinical trials of breast cancer: Recommendations from an international working group

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Abstract

Neoadjuvant systemic therapy is being used increasingly in the treatment of early-stage breast cancer. Response, in the form of pathological complete response (pCR), is a validated and evaluable surrogate endpoint of survival after neoadjuvant therapy. Thus, pCR has become a primary endpoint 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 additional information that should be 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 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 post-neoadjuvant-therapy, and retesting of markers post-treatment.

If there has been a pCR, this must be clearly stated, and the presence or 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 or 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; other methods can be included as per trial protocols and regional preference. Post-treatment tumor staging using the Tumor-Node-Metastasis system should be included.

The group concluded that 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.

INTRODUCTION

Neoadjuvant systemic therapy (NAST) is being increasingly used in the treatment of early-stage breast cancer. Response, in the form of pathological complete response (pCR), is being put forward as an evaluable endpoint 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 pCR or residual disease. This not only alters the endpoint, 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 post-NAST surgical specimen is essential.

Several classification systems have been developed for the assessment of pathologic response to NAST; 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 [OS], event-free survival [EFS], and/or distant relapse-free survival [DRFS]) **(6, 10, 12-16)**. However, different staging systems yield different estimates of future risk **(17)**. The Residual Cancer Burden (RCB) 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 (R-P-CB), which combines RCB with post-treatment Ki67 index **(20)**. There are also combined clinical and pathological systems that take into account pre-treatment information such as clinical stage as well as post-treatment pathology findings, for example the "clinical-pathologic stage - estrogen/grade" (CPS-EG) staging system **(21)**. These approaches also show promise as future means to predict outcome by combining additional clinical or biological information with RCB or American Joint Committee on Cancer (AJCC) 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 post-neoadjuvant specimen.

Frequently, NAST 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 multi-center NAST 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 multi-center 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 pCR rate fell by almost 10% among pathologists at 9 centers after they were trained on how to use the RCB tool (Laura Esserman, personal communication, August 2, 2013). In a French multi-center study

which used the Chevallier system **(30)**, the pCR rate in one arm of the study fell from 16% to 8% following central pathology review of slides **(31)**.

Lastly, the definition of pCR has not been uniform, making reporting and interpretation of data challenging **(5, 32)**. The frequency of use of different definitions of pCR in major neoadjuvant clinical trials is illustrated in **Figure 1**. These different definitions of pCR can change the apparent survival benefit associated with pCR, 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 pCR in the breast, and so the definition of pCR 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 pCR **(32, 33)**. The U.S. Food and Drug Administration (FDA)-led meta-analysis of 12 neoadjuvant randomized trials with long-term follow-up undertaken by the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) found similar EFS and OS in patients without residual invasive carcinoma regardless of the presence/absence of residual DCIS **(2)**. But in a different statistical approach, a pooled analysis of the seven prospective NAST clinical trials by the German and Austrian Breast Groups demonstrated significantly worse event-free survival (EFS) for patients with ypTisypN0 compared with patients who were ypT0ypN0. There was no significant difference in overall survival (OS) **(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 **(33) (Figure 2)**. It is conceivable that an internationally uniform procedure for handling and reporting on post-NAST specimens would eventually resolve this issue.

Overall, the FDA-supported pooled analysis was not able to validate differences in pCR rate as a surrogate endpoint for difference in EFS from these neoadjuvant clinical trials. But it did point to substantial improvements in survival in individuals with pCR and supported standardization of the definition of pCR, proposing it should be defined as either ypT0/is ypN0 or ypT0 ypN0 in future trials **(2)**.

MATERIALS AND METHODS

Given the lack of consensus regarding the pathological assessment of post-NAST 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 (SOPs) described below. Members represented an array of disciplines and countries.

First, to gauge existing variability in approaches to post-NAST pathologic assessment, we collected SOPs 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 FDA meta-analysis noted above, or otherwise were major trials (e.g., above 1 000 patients). SOPs were requested of 48 trials, both from the leaders of the trials themselves (trial SOPs) and, where leaders responded, the sites involved in those trials (site-specific SOPs). Information from the SOPs was abstracted into categories of “extent of sampling”, “quantification/ size/ grading/ cellularity”, “lymph node evaluation”, “re-testing of markers”, and “other information”. The abstracted information was then compared and contrasted.

The working group convened on seven teleconferences (plus 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 post-NAST breast cancer specimens. The working group has also written a companion paper intended for a more multi-disciplinary audience, explaining how a standardized approach would benefit the entire medical team and summarizing the more detailed recommendations provided below **(insert reference, when available)**.

RESULTS

SOPs were collected from 28 trials and 25 sites (Supplement #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 H&E, 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 post-NAST specimens, within practicable limits. Further details are provided in Supplement #1.

RECOMMENDATIONS

The working group's practical suggestions are detailed below.

1. Pre-treatment assessments

A. Initial diagnosis on core biopsy of the breast

Percutaneous image-guided core needle biopsy (CNB) is strongly recommended, and must be adequate for an unequivocal diagnosis of invasive breast carcinoma. Caution must be used if imaging or CNB 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 CNB or surgery for accurate diagnosis, rather than NAST, 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 NAST (e.g., Ki67, multi-gene assays), should be evaluated on the CNB.

Several systems for grading tumor response to treatment require comparison of cellularity with the pre-treatment biopsy, such as the Miller-Payne, Pinder, Sinn, and Sataloff systems **(7, 13, 15, 41)**. Inclusion of an estimate of tumor cellularity in the CNB 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 **(42)**. 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 prior to freezing or immersion into a dedicated solution. If using OCT freezing media, one tissue core can be embedded per block. In some cases, formalin-fixed cores can be re-embedded as a research block after reporting. Some trials

also require "on-treatment" research core biopsies at subsequent time points (for example, after the first cycle or at mid-course) as well.

B. Evaluation of the axilla before treatment

Routine axillary ultrasound is recommended to assess the axillary lymph nodes, with fine needle aspiration (FNA) or CNB of morphologically abnormal lymph nodes. Thus, sentinel lymph node biopsy (SLNB) prior to neoadjuvant treatment should be limited to cases where the pre-therapeutic lymph node status is required for systemic or local treatment decisions **(43)**. Pre-treatment SLNB precludes assessment of nodal response to NAST, and invalidates AJCC yp Stage and calculation of the RCB score if an excised SLN was originally positive.

2. Evaluation of the surgical specimen post-neoadjuvant systemic therapy

A. Clinical information required for pathologic evaluation

It is important that the multi-disciplinary team (e.g., surgeons, radiologists, and pathologists) communicate as a team for patient care**; this is covered in detail in the companion multi-disciplinary paper (insert ref when available)**. At a bare minimum, the request form must clearly indicate NAST has been given, along with the location and pre-treatment size of the tumor(s). A suggested template requisition form that can be sent with the specimen is included in Supplement #2.

B. Specimen handling

Priorities for evaluation of the surgical specimen are different after NAST, 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.

1. Complete submission of small lumpectomy specimens.

Many institutional SOPs thinly slice and submit small specimens in their entirety (for example, < 5 cm in greatest diameter in Yale University's SOP, < 30g 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. In cases processed this way, sampling is adequate.

Unfortunately, this approach does not allow for tissue collection for research. Thinning the sections further and submitting the trim for research can overcome this. Alternatively, small cylinders of tissue can be taken with a punch biopsy tool. Depending on the type of processing used for the research tissue, histology can still be evaluated if deemed clinically necessary, such

as H&E stained sections of research blocks. A previous international working group has addressed the collection of research tissue in the neoadjuvant setting in detail **(42)**.

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.

2. Sampling of large lumpectomy/ mastectomy specimens (partial submission).

Targeted representative sections can be taken from specimens too large to be submitted in their entirety, 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 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 pre-treatment area of involvement (PAI) in the discussion below. Degree of sampling is then determined by the pre-treatment 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 PAI. Block(s) representing the full face of the PAI should be taken of every 1 cm slice containing PAI, or, for very large tumors, five representative blocks of a cross-section of PAI per 1-2 cm of pre-treatment size, up to a total maximum of about 25 blocks. This should be sufficient to determine the presence of pCR. For assessment of cellularity of very large tumor beds, 5 representative blocks are sufficient to represent the largest cross-section of residual tumor bed and calculate the RCB **(44)**.

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.

Table 2 details the suggested number of blocks of the PAI that should be submitted for post-neoadjuvant specimens. 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 additional 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 **(45)**.

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 **Section 2.B.1** above. If only FFPE tissue is needed, additional blocks can be submitted from a second pass for research from areas that had residual tumor on microscopy.

3. Multiple lesions in lumpectomy or mastectomy

Same as **Section 2** above for each lesion, plus blocks of tissue in between the lesions to ensure that they are truly separate and to evaluate the presence of other intervening disease, such as DCIS.

C. Microscopic reporting

Prognostic and predictive factors traditionally evaluated in surgical specimens following primary surgery are all relevant in the NAST 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.

1. Histologic tumor type and grade

The method for determination of **histologic tumor type** and **tumor grade** is identical to non-neoadjuvant specimens, although it is not clear whether these add prognostic information to the pre-treatment results. Tumors with a typical appearance of no special type before treatment may have a lobular growth pattern following neoadjuvant chemotherapy **(46)**. Treatment can cause nuclear hyperchromasia and pleomorphism; however, the findings should be compared to the pre-treatment biopsy before assuming they are treatment-related. The mitotic rate may be reduced by treatment; this finding is associated with a better prognosis (DFS and OS) **(47)** and lower risk of developing distant metastases **(48)**. 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 post-NAST hormone receptor and HER2 assessment.

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

2. Size and extent

Tumor size/extent is often more difficult to assess after NAST. There are two main patterns of tumor response following NAST – 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**.

3. Cellularity

In addition to its effect on tumor size, NAST 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 way to assess response **(49)**. Comparison of pre- and post-treatment cellularity is the key element of several systems for grading response **(7, 13, 15, 41)**. If a formal classification system for grading of response is used, this should be noted in the report. Since tumor cellularity is often heterogeneous, the pre-treatment core biopsy may not be representative of the entire tumor. Similarly, changes in tumor cellularity induced by NAST can be heterogeneous and therefore 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.

The RCB system does not require pre-treatment 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 RCB, 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 those 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 on the RCB website **(44)** 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 RCB.

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

4. Lymphovascular invasion

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

5. 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)**.

D. Evaluation of the axilla after treatment

Several studies have shown that post-treatment nodal status is an important determinant of DFS and OS, regardless of response within the breast **(32, 35-40)**. Currently, lymph node staging in patients who have received NAST is usually performed by either SLNB or axillary lymph node dissection (ALND). The accuracy of SLNB for staging post-NAST is still under investigation, especially in patients with clinically positive nodes pre-treatment **(43, 50)**. The paradigm in surgical management of the axilla is evolving, and is the subject of ongoing investigation **(43, 50)**. This is reflected in the use of the phrase *"sampled regional lymph nodes"* by the FDA in its proposed definition of pCR **(34)**.

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

Some studies have indicated a lower number of lymph nodes identified at ALND after NAST, whilst others have found no significant difference following careful pathological evaluation **(51-53)**. Pathologists evaluating ALND 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. Post-NAST 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, not just the size of the largest tumor cluster. Clearly separate smaller foci in a node are not included in the maximum size measurement. Since

micrometastases and isolated tumor cells (ITC's) found post-NAST are predictors of worse survival, specimens with nodal micrometastases or ITC's should not be designated as pCR **(40, 54)**. Our suggested approach to assessing ITC's 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 post-NAST **(55)**. 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 **(56)**. Previously involved nodes may also look completely normal after treatment. The latter scenario can cause concern when there was histologically-proven metastasis pre-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 re-examined to ensure all nodes have been retrieved, and the patient re-examined, before assuming there has been complete response. Clipping the involved node pre-treatment can also be of value in determining nodal response.

In some centers, SLNs are assessed by molecular assays (e.g., OSNA) without any morphological evaluation. This does not allow assessment of response in the node; moreover, OSNA is usually not calibrated to detect ITC's **(57)**. Therefore we do not recommend the use of these techniques in the neoadjuvant setting.

E. Pathologic complete response (pCR)

Our group agrees with the following core principle of the definition of pCR as proposed by the FDA: *"Pathological complete response (pCR) is defined as the absence of residual invasive cancer on…. evaluation of the complete resected breast specimen and all sampled*

417 *regional lymph nodes following completion of neoadjuvant systemic therapy"* **(34)**. However, we
418 advocate that the presence of invasive tumor cells is considered residual disease regardless of
419 method of detection – i.e., H&E or immunohistochemistry – although the latter is not routinely
420 recommended. The alternative definition, requiring absence of both DCIS and invasive
421 carcinoma in the breast, can also be used. The definition of pCR chosen should be agreed
422 between pathologists and clinicians within individual institutions, and clearly stated in the report.
423 If the patient is enrolled in a clinical trial, the definition of pCR prescribed by the trial SOP
424 should be included as part of the report with an explanatory note. Regardless of which definition
425 is used, the presence/ absence and extent of residual DCIS should be reported as detailed in our
426 recommended pro forma **(Table 3)**.

427 Microscopically, the tumor bed may be identified as a focal area of loose, oedematous
428 reactive stroma with a variable inflammatory cell infiltrate that may include collections of lipid
429 or haemosiderin laden macrophages, lymphocytes, and plasma cells. Background breast lobules
430 often appear hyalinised and atrophic with a perilobular lymphocytic infiltrate.

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

437 ***F. Retesting of markers in the post-neoadjuvant therapy specimen***

438 Reassessment of hormone receptor and HER2 status in residual cancer after NAST is
439 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 prior to NAST. 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 4.**

Finally, in some centers, assessment of Ki67 labeling index is performed before and after NAST. Post-treatment Ki67 index has been shown to correlate with long-term outcome after both neoadjuvant endocrine **(58)** and chemotherapy **(59, 60)**, although its routine use in clinical practice has not yet been formally recommended due to lack of standardization in its assessment **(61-63)**. Proliferation is commonly reduced by NAST, so, in addition to Ki67, results of multi-gene assays that include proliferation genes may also change if assessed before and after treatment **(64)**.

G. Minimum data set to be reported by pathologists

A suggested pro forma for reporting NAST specimens is presented in **Table 3,** with minimum data set items highlighted. The U.S. National Cancer Institute BOLD Task force has also recommended standardized data elements for collection in preoperative breast cancer clinical trials **(65)**.

Conclusion

Post-NAST histopathological changes are complex, and careful systematic review of the specimen is required for accurate diagnosis and follow-up treatment. For pCR 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, 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:

- Pathologic 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 RCB 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 (AJCC)/ Union for International Cancer Control (UICC) Staging definitions when evaluating tumor size after neoadjuvant chemotherapy.

Supplementary information is available at *Modern Pathology*'s website.

Supplement #1: MS Word document. This file describes our collection and review of SOPs for the histologic assessment of post-neoadjuvant breast cancer specimens that we collected from various institutions/trials.

Supplement #2: MS Word document. This file is a suggested template for a requisition form that can be provided with the specimen when it is sent to the pathologist.

Disclosure/Conflict of Interest:

Dr. Symmans filed Residual Cancer Burden (RCB) as intellectual property (Nuvera Biosciences), patenting the RCB equation. (The RCB 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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REFERENCES

1. Esserman LJ, Woodcock J. Accelerating identification and regulatory approval of investigational cancer drugs. JAMA 2011;306:2608-9.

2. Cortazar P, Zhang L, Untch M*, et al.* Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164-72.

3. Mamounas EP, Anderson SJ, Dignam JJ*, et al.* Predictors of locoregional recurrence after neoadjuvant chemotherapy: results from combined analysis of National Surgical Adjuvant Breast and Bowel Project B-18 and B-27. J Clin Oncol 2012;30:3960-6.

4. Apple SK, Suthar F. How do we measure a residual tumor size in histopathology (the gold standard) after neoadjuvant chemotherapy? Breast 2006;15:370-6.

5. Kuroi K, Toi M, Tsuda H, Kurosumi M, Akiyama F. Issues in the assessment of the pathologic effect of primary systemic therapy for breast cancer. Breast Cancer 2006;13:38-48.

6. Marchio C, Sapino A. The pathologic complete response open question in primary therapy. J Natl Cancer Inst Monogr 2011;2011:86-90.

7. Pinder SE, Provenzano E, Earl H, Ellis IO. Laboratory handling and histology reporting of breast specimens from patients who have received neoadjuvant chemotherapy. Histopathology 2007;50:409-17.

8. Sahoo S, Lester SC. Pathology of breast carcinomas after neoadjuvant chemotherapy: an overview with recommendations on specimen processing and reporting. Arch Pathol Lab Med 2009;133:633-42.

9. Fan F. Evaluation and Reporting of Breast Cancer after Neoadjuvant Chemotherapy. Open Pathol J 2009;3:58-63.

10. Symmans WF, Peintinger F, Hatzis C*, et al.* Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414-22.

11. Chollet P, Abrial C, Durando X*, et al.* A new prognostic classification after primary chemotherapy for breast cancer: residual disease in breast and nodes (RDBN). Cancer J 2008;14:128-32.

12. Chollet P, Amat S, Belembaogo E*, et al.* Is Nottingham prognostic index useful after induction chemotherapy in operable breast cancer? Br J Cancer 2003;89:1185-91.

13. Sataloff DM, Mason BA, Prestipino AJ*, et al.* Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of outcome. J Am Coll Surg 1995;180:297-306.

14. Penault-Llorca F, Abrial C, Raoelfils I*, et al.* Comparison of the prognostic significance of Chevallier and Sataloff's pathologic classifications after neoadjuvant chemotherapy of operable breast cancer. Human Pathol 2008;39:1221-8.

15. Ogston KN, Miller ID, Payne S*, et al.* A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. Breast 2003;12:320-7.

16. Romero A, Garcia-Saenz JA, Fuentes-Ferrer M*, et al.* Correlation between response to neoadjuvant chemotherapy and survival in locally advanced breast cancer patients. Ann Oncol 2013;24:655-61.

17. Corben AD, Abi-Raad R, Popa I*, et al.* Pathologic Response and Long-Term Follow-up in Breast Cancer Patients Treated With Neoadjuvant Chemotherapy: A Comparison Between Classifications and Their Practical Application. Arch Pathol Lab Med 2013;137:1074-82.

18. Peintinger F, Hatzis C, Morkowski J*, et al.* Inter-pathologist agreement of residual cancer burden (RCB) assessment in breast cancer after treatment with preoperative chemotherapy [abstract #576]. J Clin Oncol 2008;26(Supplement):576.

19. Symmans WF, Wei C, Gould R*, et al.* Long-term prognostic value of residual cancer burden (RCB) classification following neoadjuvant chemotherapy [abstract S6-02]. San Antonio Breast Cancer Symposium: San Antonio, TX, USA;2013.

20. Sheri A, A'Hern R, Jones RL*, et al.* Integration of Ki67 with residual cancer burden (RCB) compared to Ki67 or RCB alone to predict long-term term outcome following neoadjuvant chemotherapy [abstract #535]. J Clin Oncol 2013;31:535.

21. Mittendorf EA, Jeruss JS, Tucker SL*, et al.* Validation of a novel staging system for disease-specific survival in patients with breast cancer treated with neoadjuvant chemotherapy. J Clin Oncol 2011;29:1956-62.

22. RCPA. Royal College of Pathologists of Australasia Invasive Breast Cancer Structured Reporting Protocol. 2012 [cited; November 2012, 2nd Edition (Version 2.0).

23. Lambein K, Van de Vijver K, Faverly D, Colpaert C. Belgian guidelines for laboratory handling and pathology reporting of breast carcinoma after neoadjuvant therapy. Belg J Med Oncol 2011;5:144-53.

24. AGO (Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe) http://www.ago-online.de/de/fuer-mediziner/leitlinien/mamma/en/

25. NHSBSP. Pathology Reporting Of Breast Disease: A Joint Document Incorporating the Third Edition of the NHS Breast Screening Programme's Guidelines for Pathology Reporting in Breast Cancer Screening and the Second Edition of The Royal College of Pathologists' Minimum Dataset for Breast Cancer Histopathology. NHSBSP Publication

No 58. NHS Cancer Screening Programmes jointly with The Royal College of Pathologists; 2005.

26. Integraal Kankercentrum Nederland. Beoordeling na neoadjuvante chemo- of endocriene therapie http://www.oncoline.nl/breastcancer. 2012

27. CAP. Protocol for the Examination of Specimens From Patients With Invasive Carcinoma of the Breast. 2012. http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2012/BreastInvasive_12protocol_3100.pdf

28. Leyland-Jones BR, Ambrosone CB, Bartlett J*, et al.* Recommendations for collection and handling of specimens from group breast cancer clinical trials. J Clin Oncol 2008;26:5638-44.

29. Provenzano E, Vallier AL, Champ R*, et al.* A central review of histopathology reports after breast cancer neoadjuvant chemotherapy in the neo-tango trial. Br J Cancer 2013;108:866-72.

30. Chevallier B, Roche H, Olivier JP, Chollet P, Hurteloup P. Inflammatory breast cancer. Pilot study of intensive induction chemotherapy (FEC-HD) results in a high histologic response rate. Am J Clin Oncol 1993;16:223-8.

31. Dieras V, Fumoleau P, Romieu G, *et al.* Randomized parallel study of doxorubicin plus paclitaxel and doxorubicin plus cyclophosphamide as neoadjuvant treatment of patients with breast cancer. J Clin Oncol 2004;22:4958-65.

32. von Minckwitz G, Untch M, Blohmer JU, *et al.* Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796-804.

33. Mazouni C, Peintinger F, Wan-Kau S, *et al.* Residual ductal carcinoma in situ in patients with complete eradication of invasive breast cancer after neoadjuvant chemotherapy does not adversely affect patient outcome. J Clin Oncol 2007;25:2650-5.

34. U.S. Food and Drug Administration. Guidance for Industry: Pathological Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval. October 2014. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM305501.pdf.

35. Rouzier R, Extra JM, Klijanienko J, *et al.* Incidence and prognostic significance of complete axillary downstaging after primary chemotherapy in breast cancer patients with T1 to T3 tumors and cytologically proven axillary metastatic lymph nodes. J Clin Oncol 2002;20:1304-10.

36. McCready DR, Hortobagyi GN, Kau SW*, et al.* The prognostic significance of lymph node metastases after preoperative chemotherapy for locally advanced breast cancer. Arch Surg 1989;124:21-5.

37. Rastogi P, Anderson SJ, Bear HD*, et al.* Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 2008;26:778-85.

38. Buchholz TA, Tucker SL, Masullo L*, et al.* Predictors of local-regional recurrence after neoadjuvant chemotherapy and mastectomy without radiation. J Clin Oncol 2002;20:17-23.

39. Hennessy BT, Hortobagyi GN, Rouzier R*, et al.* Outcome after pathologic complete eradication of cytologically proven breast cancer axillary node metastases following primary chemotherapy. J Clin Oncol 2005;23:9304-11.

40. Klauber-DeMore N, Ollila DW, Moore DT*, et al.* Size of residual lymph node metastasis after neoadjuvant chemotherapy in locally advanced breast cancer patients is prognostic. Ann Surg Oncol 2006;13:685-91.

41. Sinn HP, Schmid H, Junkermann H*, et al.* [Histologic regression of breast cancer after primary (neoadjuvant) chemotherapy]. Geburtshilfe und Frauenheilkunde 1994;54:552-8.

42. Loi S, Symmans WF, Bartlett JM*, et al.* Proposals for uniform collection of biospecimens from neoadjuvant breast cancer clinical trials: timing and specimen types. Lancet Oncol 2011;12:1162-8.

43. Kuehn T, Bauerfeind I, Fehm T*, et al.* Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. Lancet Oncol 2013;14:609-18.

44. Residual Cancer Burden calculator and associated documents [Guide for Measuring Cancer Cellularity, Examples of Gross & Microscopic Evaluation, Pathology Protocol for Macroscopic and Microscopic Assessment of RCB] http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

45. Ibarra JA. The Value of Combined Large Format Histopathology Technique to Assess the Surgically Removed Breast Tissue following Neoadjuvant Chemotherapy: A Single Institution Study of 40 Cases. Int J Breast Cancer 2012;2012:361707.

46. Carder P. Typing breast cancer following primary chemotherapy. Histopathology 1999;35:584-5.

47. Penault-Llorca F, Abrial C, Raoelfils I*, et al.* Changes and predictive and prognostic value of the mitotic index, Ki-67, cyclin D1, and cyclo-oxygenase-2 in 710 operable breast cancer patients treated with neoadjuvant chemotherapy. Oncologist 2008;13:1235-45.

48. Diaz J, Stead L, Shapiro N*, et al.* Mitotic counts in breast cancer after neoadjuvant systemic chemotherapy and development of metastatic disease. Breast Cancer Res Treat 2013;138:91-7.

49. Rajan R, Poniecka A, Smith TL*, et al.* Change in tumor cellularity of breast carcinoma after neoadjuvant chemotherapy as a variable in the pathologic assessment of response. Cancer 2004;100:1365-73.

50. Boughey JC, Suman VJ, Mittendorf EA*, et al.* Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. JAMA 2013;310:1455-61.

51. Belanger J, Soucy G, Sideris L*, et al.* Neoadjuvant chemotherapy in invasive breast cancer results in a lower axillary lymph node count. J Am Coll Surg 2008;206:704-8.

52. Neuman H, Carey LA, Ollila DW*, et al.* Axillary lymph node count is lower after neoadjuvant chemotherapy. Am J Surg 2006;191:827-9.

53. Boughey JC, Donohue JH, Jakub JW, Lohse CM, Degnim AC. Number of lymph nodes identified at axillary dissection: effect of neoadjuvant chemotherapy and other factors. Cancer 2010;116:3322-9.

54. Fisher ER, Wang J, Bryant J*, et al.* Pathobiology of preoperative chemotherapy: findings from the National Surgical Adjuvant Breast and Bowel (NSABP) protocol B-18. Cancer 2002;95:681-95.

55. Newman LA, Pernick NL, Adsay V*, et al.* Histopathologic evidence of tumor regression in the axillary lymph nodes of patients treated with preoperative chemotherapy correlates with breast cancer outcome. Ann Surg Oncol 2003;10:734-9.

56. Donnelly J, Parham DM, Hickish T, Chan HY, Skene AI. Axillary lymph node scarring and the association with tumour response following neoadjuvant chemoendocrine therapy for breast cancer. Breast 2001;10:61-6.

57. Feldman S, Krishnamurthy S, Gillanders W*, et al.* A novel automated assay for the rapid identification of metastatic breast carcinoma in sentinel lymph nodes. Cancer 2011;117:2599-607.

58. Ellis MJ, Tao Y, Luo J*, et al.* Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst 2008;100:1380-8.

59. Jones RL, Salter J, A'Hern R*, et al.* The prognostic significance of Ki67 before and after neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat 2009;116:53-68.

60. von Minckwitz G, Schmitt W, Loibl S, *et al.* Ki67 measured after neoadjuvant chemotherapy for primary breast cancer. Clin Cancer Res 2013;19:4521-31.

61. Harris L, Fritsche H, Mennel R, *et al.* American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.

62. Polley MC, Leung S, McShane LM, *et al.* An International Ki67 Reproducibility Study. J Natl Cancer Inst 2013;105:1897-906.

63. Dowsett M, Nielsen TO, A'Hern R, *et al.* Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011;103:1656-64.

64. Earl HM, Chin S, Dunning M, *et al.* Neo-tAnGo science: A translational study of PAM 50 sub-typing in sequential fresh tissue samples during neoadjuvant chemotherapy [abstract #1015]. J Clin Oncol 2013;31.

65. National Cancer Institute. Breast Cancer Steering Committee. Investigator Resources. NCI BOLD Task Force CDEs. http://www.cancer.gov/aboutnci/organization/ccct/steering-committees/breast-cancer/.

66. Edge SB, Byrd DR, Compton CC, *et al.* (eds). American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 7th edition. Springer, 2009.

67. Greene FL, Page DL, Fleming ID*, et al.* (eds). American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 6th edition Springer-Verlag, 2002.

68. Carey LA, Metzger R, Dees EC*, et al.* American Joint Committee on Cancer tumor-node-metastasis stage after neoadjuvant chemotherapy and breast cancer outcome. J Natl Cancer Inst 2005;97:1137-42.

69. Rabban JT, Glidden D, Kwan ML, Chen YY. Pure and predominantly pure intralymphatic breast carcinoma after neoadjuvant chemotherapy: an unusual and adverse pattern of residual disease. Am J Surg Pathol 2009;33:256-63.

70. Colleoni M, Rotmensz N, Maisonneuve P*, et al.* Prognostic role of the extent of peritumoral vascular invasion in operable breast cancer. Ann Oncol 2007;18:1632-40.

71. Lakhani SR, I.O. E, Schnitt SJ, Tan PH, van de Vijver MJ. WHO Classification of Tumours of the Breast, Fourth Edition, 2012.

72. Loya A, Guray M, Hennessy BT*, et al.* Prognostic significance of occult axillary lymph node metastases after chemotherapy-induced pathologic complete response of cytologically proven axillary lymph node metastases from breast cancer. Cancer 2009;115:1605-12.

73. Chen S, Chen CM, Yu KD, Zhou RJ, Shao ZM. Prognostic value of a positive-to-negative change in hormone receptor status after neoadjuvant chemotherapy in patients with hormone receptor-positive breast cancer. Ann Surg Oncol 2012;19:3002-11.

74. Hirata T, Shimizu C, Yonemori K*, et al.* Change in the hormone receptor status following administration of neoadjuvant chemotherapy and its impact on the long-term outcome in patients with primary breast cancer. Br J Cancer 2009;101:1529-36.

75. Tacca O, Penault-Llorca F, Abrial C*, et al.* Changes in and prognostic value of hormone receptor status in a series of operable breast cancer patients treated with neoadjuvant chemotherapy. Oncologist 2007;12:636-43.

76. Mittendorf EA, Wu Y, Scaltriti M*, et al.* Loss of HER2 amplification following trastuzumab-based neoadjuvant systemic therapy and survival outcomes. Clin Cancer Res 2009;15:7381-8.

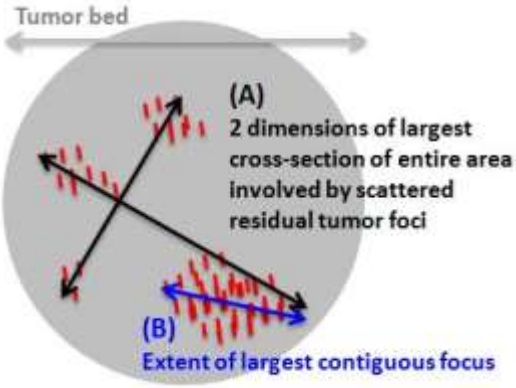
77. Jabbour MN, Massad CY, Boulos FI. Variability in hormone and growth factor receptor expression in primary versus recurrent, metastatic, and post-neoadjuvant breast carcinoma. Breast Cancer Res Treat 2012;135:29-37.

78. Zhang N, Moran MS, Huo Q, Haffty BG, Yang Q. The hormonal receptor status in breast cancer can be altered by neoadjuvant chemotherapy: a meta-analysis. Cancer Invest 2011;29:594-8.

79. Valent A, Penault-Llorca F, Cayre A, Kroemer G. Change in HER2 (ERBB2) gene status after taxane-based chemotherapy for breast cancer: polyploidization can lead to diagnostic pitfalls with potential impact for clinical management. Cancer Genet 2013;206:37-41.

80. von Minckwitz G, Darb-Esfahani S, Loibl S, *et al.* Responsiveness of adjacent ductal carcinoma in situ and changes in HER2 status after neoadjuvant chemotherapy/trastuzumab treatment in early breast cancer--results from the GeparQuattro study (GBG 40). Breast Cancer Res Treat 2012;132:863-70.

854 **Table 1: Controversial scenarios in reporting breast cancer post-neoadjuvant systemic**
855 **therapy**
856

Scenario	Current evidence/ guidelines	Suggested approach
Residual tumor present as scattered foci (common)	• Tumor size 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 as to how size is measured in this setting: **(A)** Residual tumor bed size in two dimensions (used to calculate the RCB 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 AJCC: 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 pre-treatment 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. • When there are scattered islands of tumor cells, measurement (B) as described by AJCC 7th edition **(66)** may result in significant underestimation of tumor extent. There is also currently no data on the relationship of measurement (B) to outcome. • Lesion size including the cell clusters and intervening fibrous tissue (A), which is congruent with the earlier, 6th edition of AJCC **(67)**, correlates with survival **(68)** and may be more relevant, for example for comparison with radiology. • In our opinion, the combination of residual tumor cellularity and measurement (A) is the better indicator of response. Perhaps residual tumor bed size and cellularity could be included in future AJCC recommendations in the neoadjuvant setting. • When required to report AJCC 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. • If there are multiple tumors present on pre-treatment 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.
Presence of lymphovascular invasion (LVI) in the absence of an identifiable residual invasive tumor mass	• There is insufficient data on the independent prognostic significance of the presence of LVI or extensive LVI in neoadjuvant specimens. • One small study found that such intra-lymphatic tumor carries adverse prognostic significance, even in the absence of residual stromal invasion **(69)**. However, most of the patients also had residual disease in the lymph nodes and multivariate analysis was not possible.	• **Residual LVI should NOT be classed as pCR** – make a statement in the pathology report that residual tumor is present in the form of intravascular disease. • Ensure tumor bed has been accurately localized and adequately sampled to exclude residual invasive disease. • Ensure truly LVI, not DCIS or retraction artifact. This may be difficult; immunohistochemistry (IHC) may be helpful. • Measurement is optional. If a limited area is

(rare)		involved, a measurement in mm can be given. Alternatively, LVI can be quantified as focal or extensive with "extensive" defined as one or more foci in more than one block **(70)**. • Whilst it was agreed residual LVI should not be regarded as pCR, in the absence of adequate data the working group felt it was not appropriate to give definite reporting recommendations. Advice on how to score LVI only in some of the current grading systems is given below: • RCB: Measure extent of LVI in two dimensions and estimate cellularity as per invasive disease. • Chevallier: Class 3 • Sataloff: T-A • Pinder: Partial response to therapy (i) minimal residual disease/ near total effect (<10% of tumor remaining)

Presence of ITC's in lymph nodes
 	
(common)
	• AJCC TNM recommends ITC's post-chemotherapy be called node negative ($ypN0_{itc}$) but not regarded as pCR **(66)**.
	• WHO recommends ITC's post-chemotherapy be called node positive **(71)**.
	• Findings include:
	  • DFS and OS worsened with increasing number of nodes and deposit size. Size of largest metastasis was strongest predictor of OS in multivariate analysis. Micrometastatic disease < 2mm, including ITC's, was predictive of worse outcome **(40)**.
	  • No difference in RFS and OS between groups when size of the largest residual metastatic deposit was classified as ≤0.1cm, 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 IHC staining for cytokeratins **(72)**.
		• **Any residual disease in the lymph node, including micrometastases and ITC's, should NOT be classified as pCR.**
		• If no associated fibrosis, treat as in adjuvant setting and call node negative.
		• 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.
		• Additional levels and / or IHC are not routinely required. However, IHC may be useful if suspicious cells are identified on H&E, and levels can be used to clarify the size of a deposit if ITC's/ micrometastasis are present on the initial section.

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858 AJCC: American Joint Committee on Cancer; DFS: Disease-free survival; H&E: hematoxylin and eosin; IHC:
859 Immunohistochemistry; ITC's: Isolated tumor cells; LVI: Lymphovascular invasion; OS: Overall survival; pCR:
860 Pathologic complete response; RCB: Residual Cancer Burden; RFS: Relapse-free survival

Table 2: Recommended extent of sampling of the post-neoadjuvant breast cancer specimen

Largest pretreatment size of tumor determined by clinical evaluation/ imaging	Approximate suggested number of blocks on first pass	Additional blocks if the first blocks do not contain residual invasive disease	Maximum number of blocks for documentation of pCR (5 x pretreatment size)
1 cm	2	3	5x1= 5
2 cm	4	6	5x2=10
3 cm	9	6	5x3= 15
4 cm	12	8	5x4= 20
5 cm	15	10	5x5= 25
> 5 cm			
6 cm	15	10	25
8 cm	20	5	25
> 10 cm	25	0	25

Example: 8 cm pretreatment size: 5x4=20 blocks on initial sectioning (5 blocks taken every 2 cm of pre-treatment size). If no residual tumor identified on initial blocks, go back and submit an additional 5 blocks, indicating the location on the block diagram. Total of 25 blocks.

Example: 6 cm pretreatment size: 5x3=15 blocks on initial sectioning (5 blocks taken every 2 cm of pre-treatment size). If no residual tumor identified on initial blocks, go back and submit an additional 10 blocks. Total of 25 blocks.

Table 3: Suggested pro forma for reporting breast cancer specimens post-neoadjuvant systemic therapy in clinical trials *(Italicized items = Suggested minimum data set specific to post-neoadjuvant specimens, IN ADDITION to minimum required for other types of specimens)*

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

Type of procedure
Breast - (Wide local excision +/- localization, mastectomy, other)
Lymph nodes – (SLN, axillary dissection, other LN e.g., internal mammary)

Laterality – (left, right, not specified)

MACROSCOPY

Residual macroscopic tumor identified– yes/ no

If residual macroscopic tumor:
Site of tumor – (UOQ, LOQ, UIQ, LIQ, central)
Unifocal versus multi-focal
If multi-focal, 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 (….)
Post-treatment 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 SLN/ axillary LN
Number of SLN/ axillary LN with metastases
Size of largest metastasis
Evidence of treatment response in the metastases – present/ absent
Number of LN with evidence of treatment response (fibrosis or a 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:
1. pCR
2. Residual invasive carcinoma, no definite response
3. 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:
1. Metastasis present, no response
2. Metastasis present, evidence of response
3. No residual metastasis but evidence of previous metastasis with response
4. No metastasis or fibrosis (true negative)

ypTN stage

Repeat marker testing:
ER/ PR/ HER2 if initial biopsy was negative or equivocal
(Ki67)

List of abbreviations: CIS: carcinoma in situ component DCIS: Ductal carcinoma in situ; LN: Lymph node; SLN: Sentinel lymph node; UOQ: Upper outer quadrant; LOQ: Lower outer quadrant; UIQ: Upper inner quadrant; LIQ: Lower inner quadrant

926 **Table 4: Retesting of hormone receptors and HER2 post-neoadjuvant therapy**
927

Recommendation	Clinical setting	Comments
• Routine reassessment not currently recommended	• Positive ER/ PR/ HER2 result on pre-treatment core biopsy	• No change in marker expression in the majority of patients **(73, 74)** • Uncertainty whether conversion should inform the choice of future adjuvant therapies (i.e., to stop or start a targeted treatment) **(73-75)** • 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 **(76)**.
• Reassessment must be performed	• Retesting of markers is required as part of a clinical trial protocol • Biomarker status not known	• Re-assessment 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 pre-treatment core biopsy • Insufficient invasive tumor for accurate assessment or DCIS only on pre-treatment core biopsy[1] • Retesting requested by clinicians • Biopsy performed in another institution • Heterogeneous tumor or multiple tumors with different morphology on resection • No response to therapy	• Two different meta-analyses of published studies have reported a mean prevalence of discordant results pre- and post-NAST of 13% and 18% for ER, 32% and 26% and for PR, and 9% and 6% for HER2 **(77, 78)**. • Causes of discordance include technical artifacts, misinterpretation of test results, intratumoral heterogeneity of marker expression, and changes induced by the intervening therapies **(79)** (e.g., inclusion of trastuzumab in neoadjuvant treatment may increase the rate of negative conversion for HER2) **(76, 80)**. • 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 IHC and in situ hybridization.

928 DCIS: ductal carcinoma in situ; ER: estrogen receptor; IHC: immunohistochemistry; NAST: neoadjuvant systemic
929 therapy; PR: progesterone receptor.

930 [1] Pre-treatment core biopsy should be adequate for unequivocal diagnosis of invasive carcinoma and assessment of
931 key prognostic and predictive markers as this forms the only tumor sample if there is a complete pathological
932 response. If this is not the case, repeat core biopsy should be performed or primary surgery considered.
933

FIGURE LEGENDS

Figure 1: Use of different definitions[1] of pCR in major neoadjuvant breast cancer clinical trials.

Trials included in graphic above: **1st bar:** GeparDuo, GeparTrio, GeparQuattro, GeparQuinto, GeparSixto, GeparSepto, NEOCENT **2nd 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), NEO-ZOTAC, NOAH, REMAGUS 02, S0800, TECHNO **3rd 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 **4th bar:** EORTC-10994

[1] Definition used in the primary endpoint or, where pCR was not the primary endpoint, in the secondary endpoint.

Figure 2: Survival curves for different definitions of pCR

Graphs showing impact of different definitions of pathological complete response on survival. **A.** Data from the German Breast Group (GBG) and AGO-B trials pooled analysis showing reduced disease-free survival (DFS) for patients with ypTisypN0 versus ypT0ypN0. Patients who had residual nodal disease despite absence of invasive cancer in the breast (ypT0/isypN+) had the poorest DFS **(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, ypT0 pN0 and ypT0/is ypN0 were more strongly associated with improved EFS and OS than ypT0/is alone. Moreover, ypT0 ypN0 and ypT0/is ypN0 were similar in their associations to EFS and OS. **D.** MD Anderson study showing 5- and 10-year OS (left) and DFS (right) rates were identical for

the patients with pCR versus pCR+DCIS **(33)**. **E.** Categories of reduction in cellularity in the Miller-Payne system correlate with disease-free survival **(15)**. **F.** Residual Cancer Burden (RCB) score is an independent variable that predicts likelihood of relapse. Minimal residual disease (RCB-I) carries the same prognosis as pCR **(10)**.

Figures 2A and 2B reprint permission caption:

von Minckwitz G et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012 May 20; 30: 1796-804.

Figure 2C reprint permission caption:

Figures 2D reprint permission caption:

Mazouni C et al. Residual ductal carcinoma in situ in patients with complete eradication of invasive breast cancer after neoadjuvant chemotherapy does not adversely affect patient outcome. J Clin Oncol 2007 Jul 1; 25: 2650-5.

Figure 2E reprint permission caption:

prognostic significance and survival. Pages 320-7. Copyright 2003, with permission from Elsevier.

Figure 2F reprint permission caption:

Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414-22 .

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.

Figure 4: Extensive lymphovascular space invasion post-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

Figure 5: Tumor bed present at the resection margin

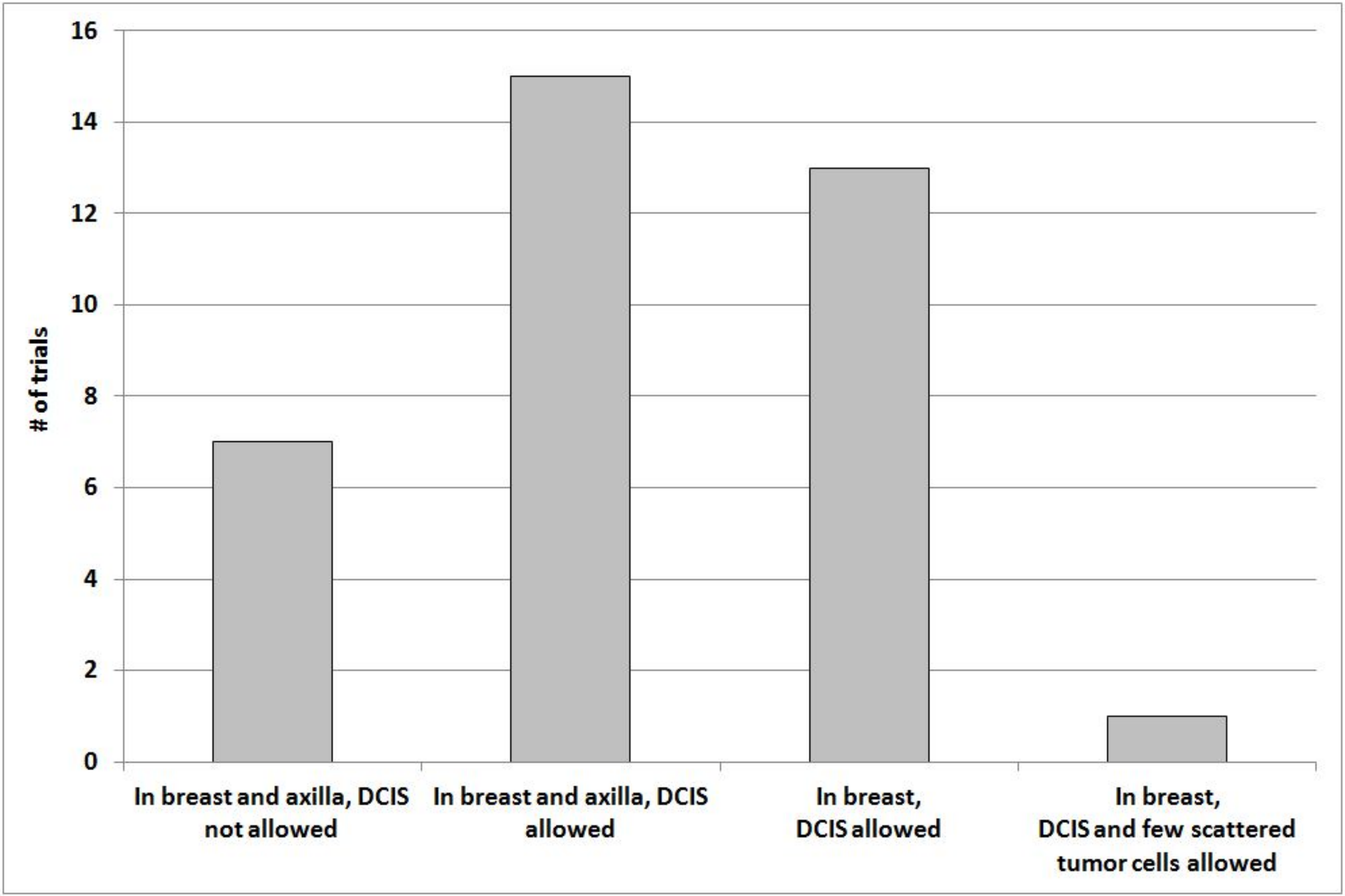
Courtesy of Frédérique Penault-Llorca

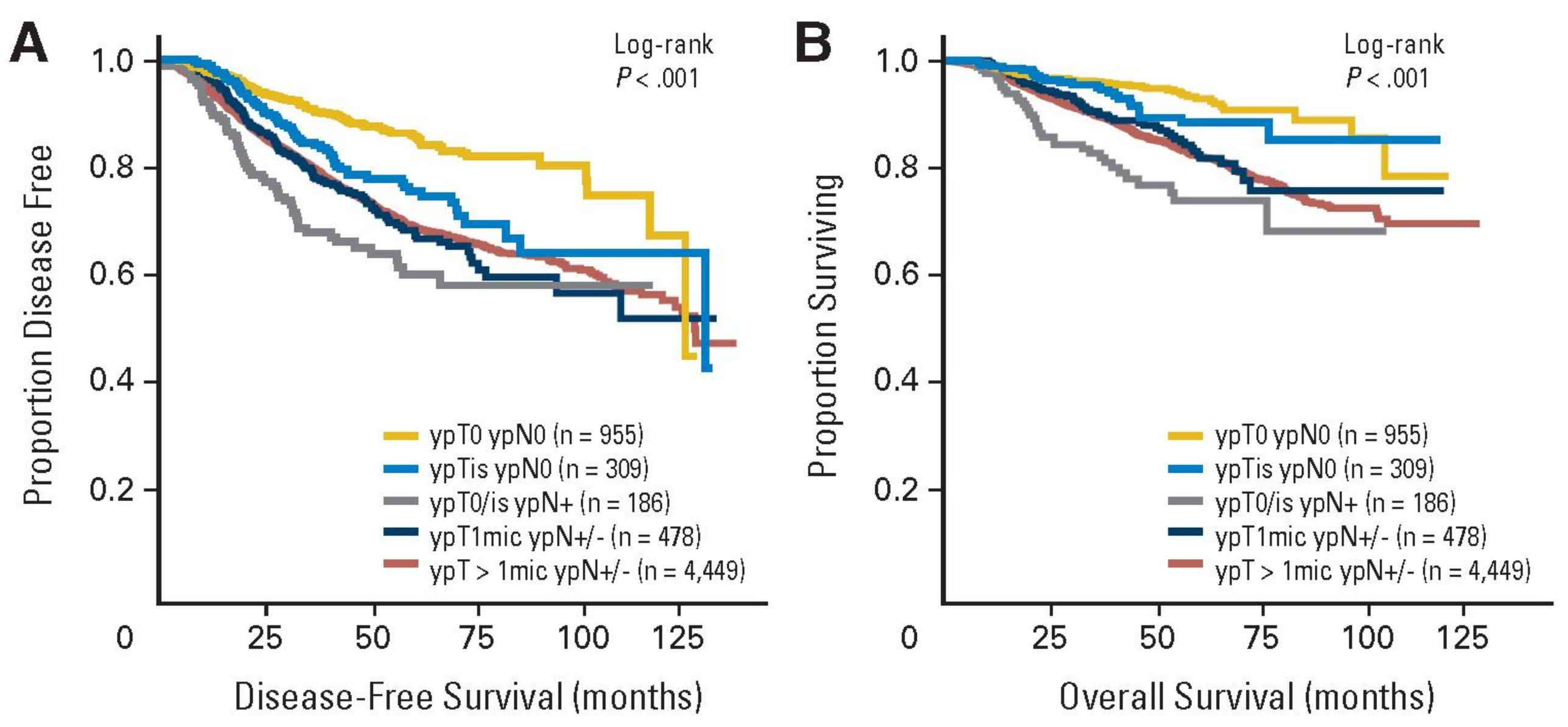
Figure 6: Lymph node showing zonal areas of fibrosis post-chemotherapy indicative of metastasis with response to therapy

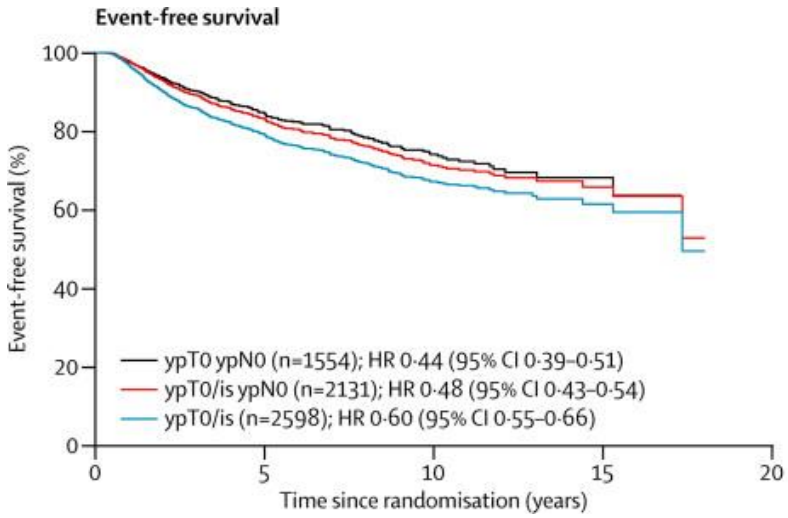
Courtesy of Elena Provenzano

A. Low-power image of lymph node showing zonal fibrosis indicating site of metastasis.

B. On higher magnification, residual islands of tumor cells are present in a setting of reactive fibrosis with haemosiderin-laden macrophages, consistent with chemotherapy effect.


16
14
12
10
8
6
4
2
0
# of trials
In breast and axilla, DCIS not allowed
In breast and axilla, DCIS allowed
In breast, DCIS allowed
In breast, DCIS and few scattered tumor cells allowed


A
Log-rank
P < .001
Proportion Disease Free
1.0
0.8
0.6
0.4
0.2
0
25
50
75
100
125
Disease-Free Survival (months)
ypT0 ypN0 (n = 955)
ypTis ypN0 (n = 309)
ypT0/is ypN+ (n = 186)
ypT1mic ypN+/- (n = 478)
ypT > 1mic ypN+/- (n = 4,449)
B
Log-rank
P < .001
Proportion Surviving
1.0
0.8
0.6
0.4
0.2
0
25
50
75
100
125
Overall Survival (months)
ypT0 ypN0 (n = 955)
ypTis ypN0 (n = 309)
ypT0/is ypN+ (n = 186)
ypT1mic ypN+/- (n = 478)
ypT > 1mic ypN+/- (n = 4,449)



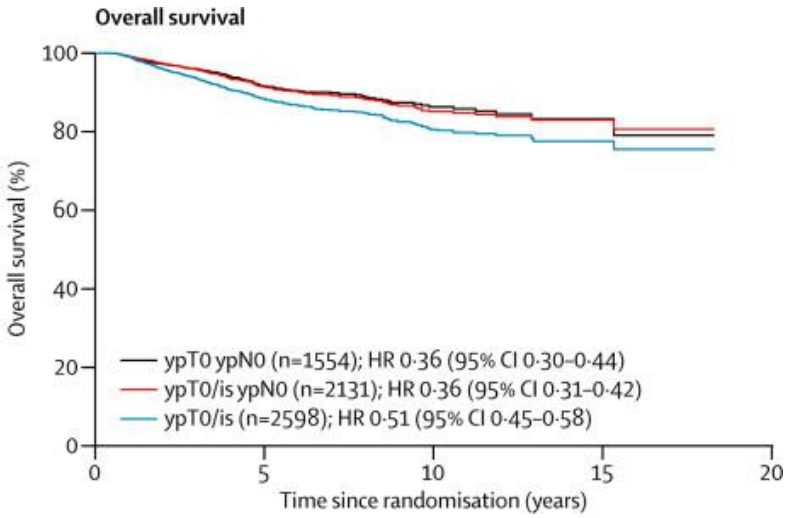


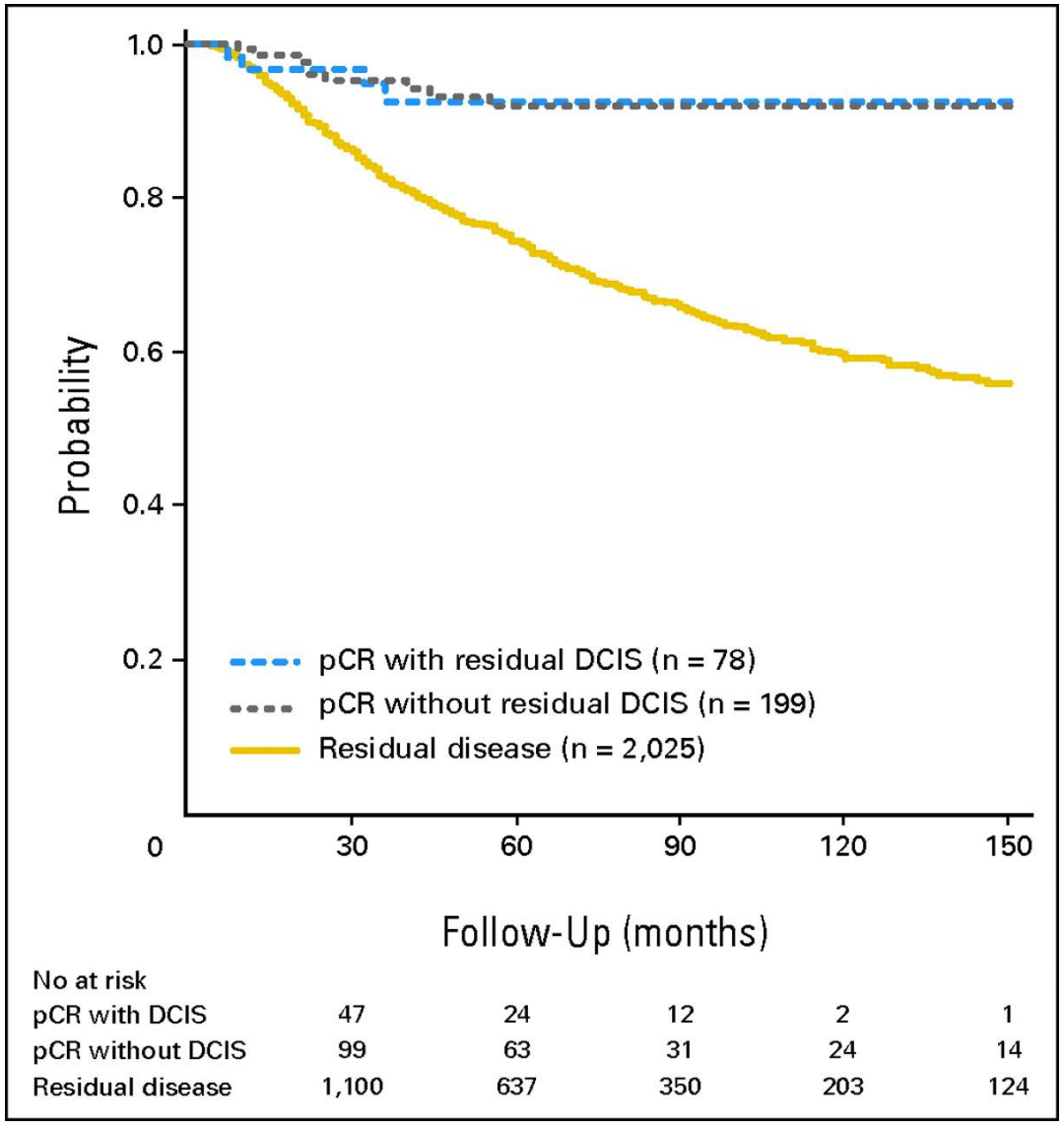
Figure 3 Associations between three definitions of pathological complete response and event-free survival and overall survival We compared event-free survival and overall survival between patients who did and did not achieve a pathological complete respon...

Patricia Cortazar , Lijun Zhang , Michael Untch , Keyur Mehta , Joseph P Costantino , Norman Wolmark , Hervé Bonn...

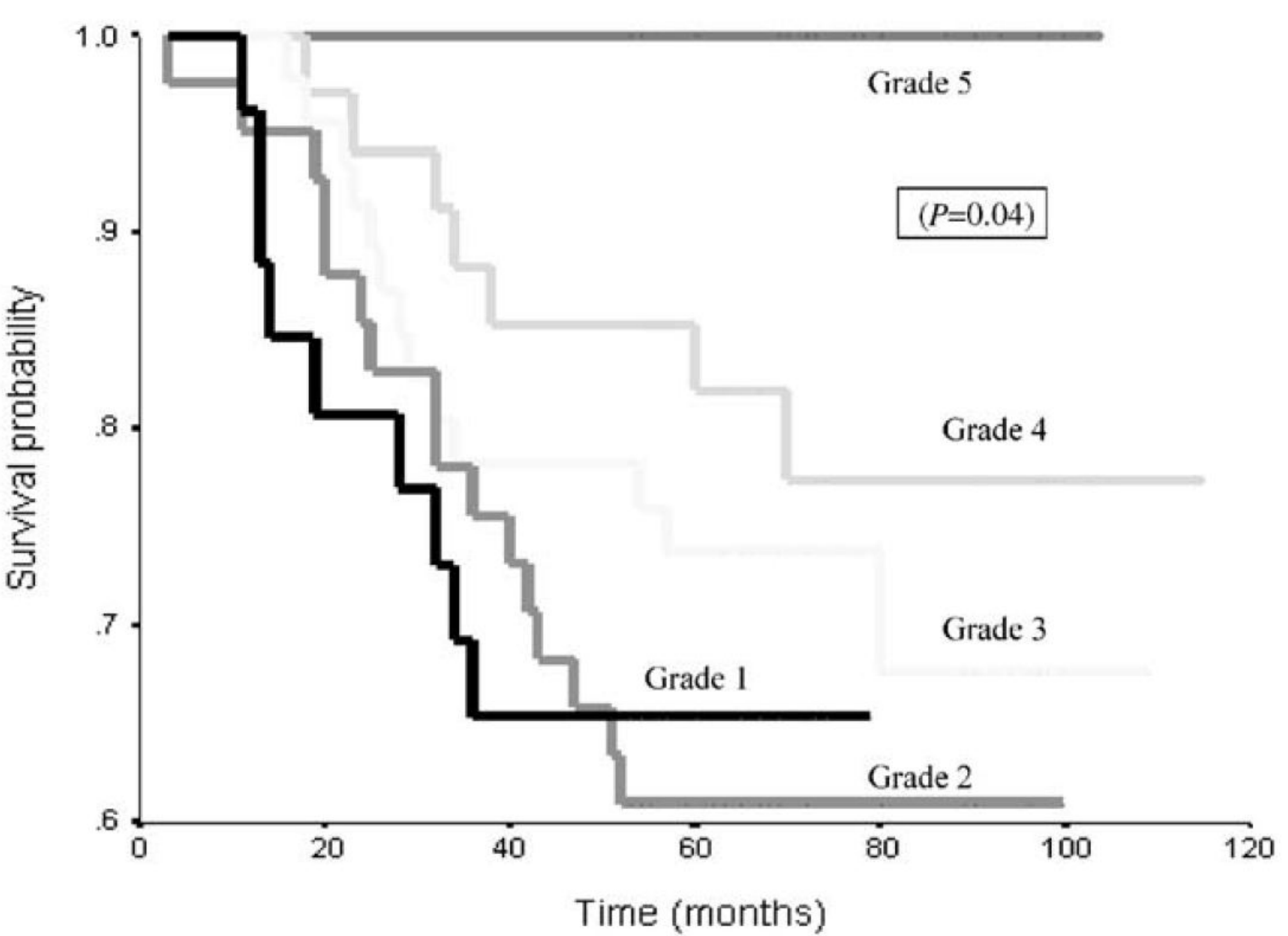
Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis

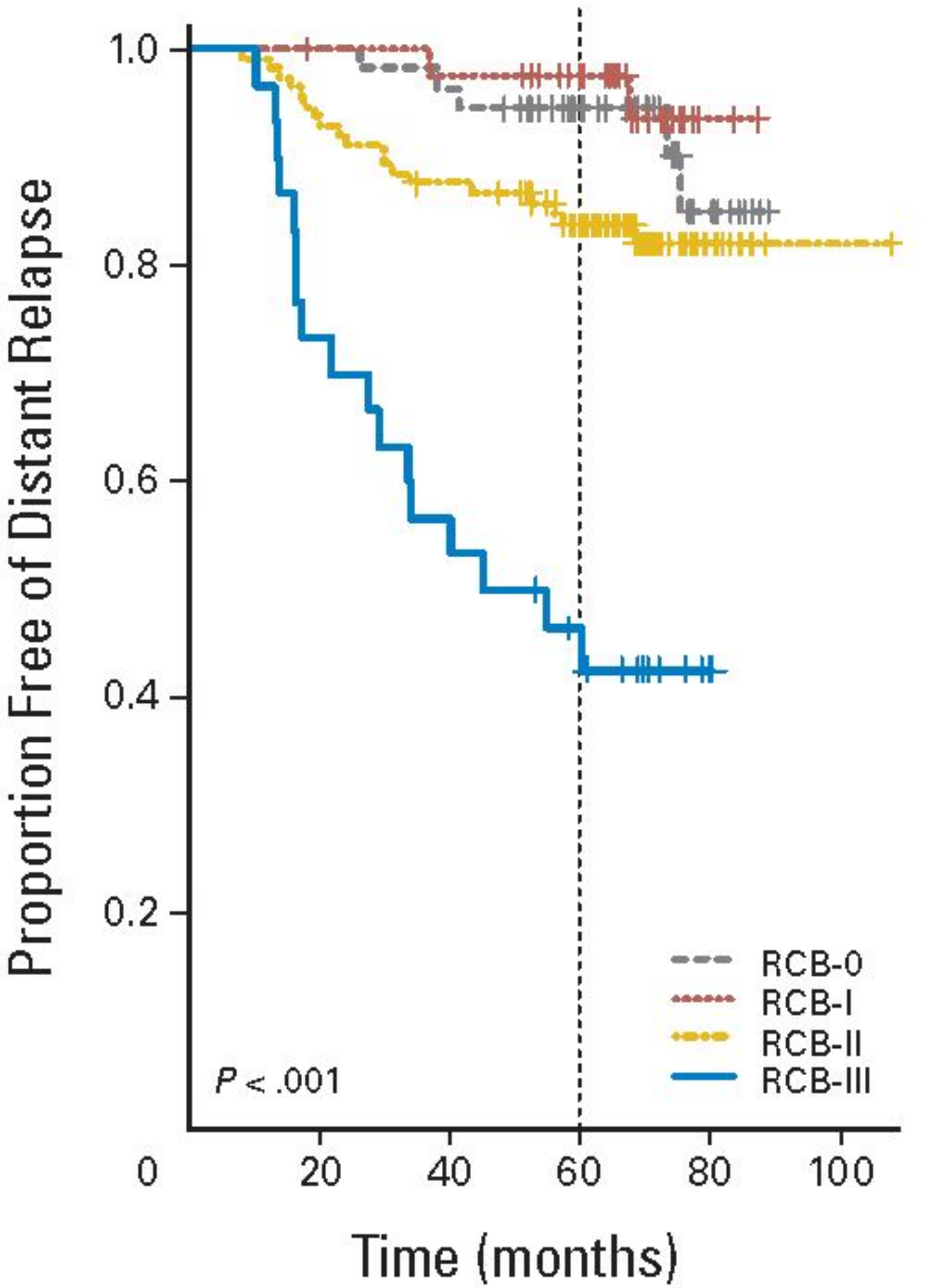
The Lancet, Volume 384, Issue 9938, 2014, 164 - 172

http://dx.doi.org/10.1016/S0140-6736(13)62422-8



No at risk					
pCR with DCIS	47	24	12	2	1
pCR without DCIS	99	63	31	24	14
Residual disease	1,100	637	350	203	124


1.0
.9
.8
.7
.6
Survival probability
Grade 5
(P=0.04)
Grade 4
Grade 3
Grade 1
Grade 2
0
20
40
60
80
100
120
Time (months)


Proportion Free of Distant Relapse
1.0
0.8
0.6
0.4
0.2
0
20
40
60
80
100
Time (months)
RCB-0
RCB-I
RCB-II
RCB-III
P < .001

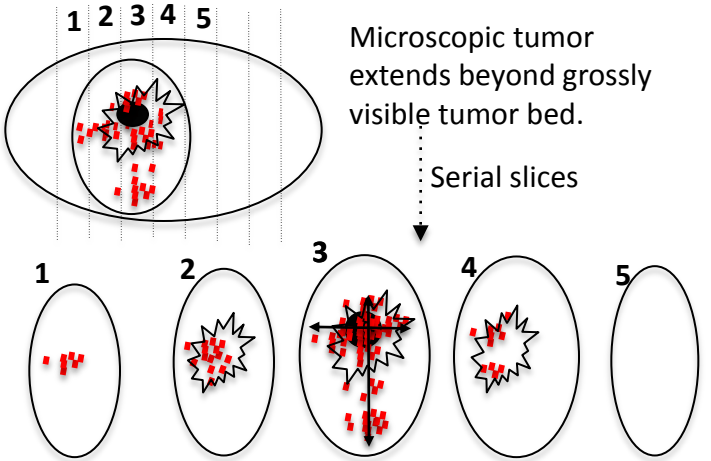
No residual tumor grossly

- No residual tumor
  - Tumor bed with clip identified
  - Tumor bed indistinct Clip identified
- Microscopic residual disease
  - Tumor bed with clip identified Microscopic residual disease

Residual tumor grossly

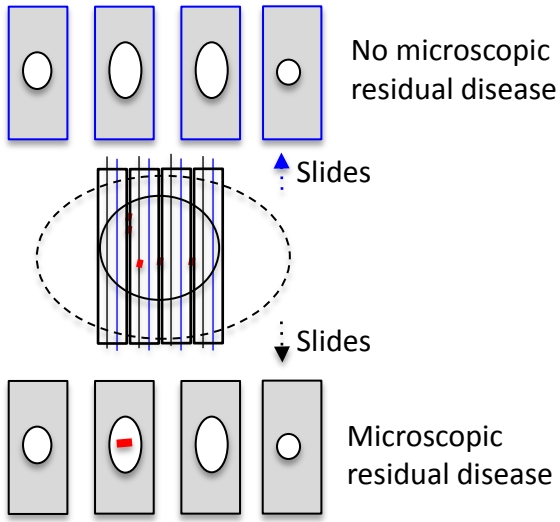
- Gross size confirmed
- Microscopic residual disease beyond grossly visible tumor

Systematic sampling is appropriate

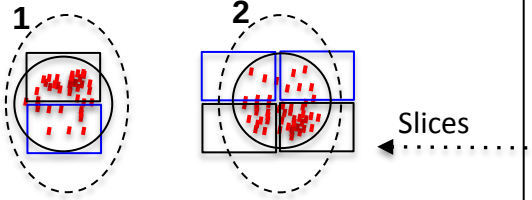


Mapping of the specimen
Correlation with pre-treatment size/ imaging
Largest cross-section of tumor bed and estimate of cellularity in slice 3

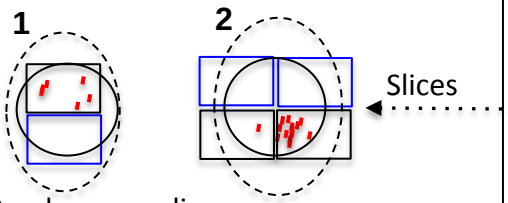
Sampling has limits



Random sampling is a problem

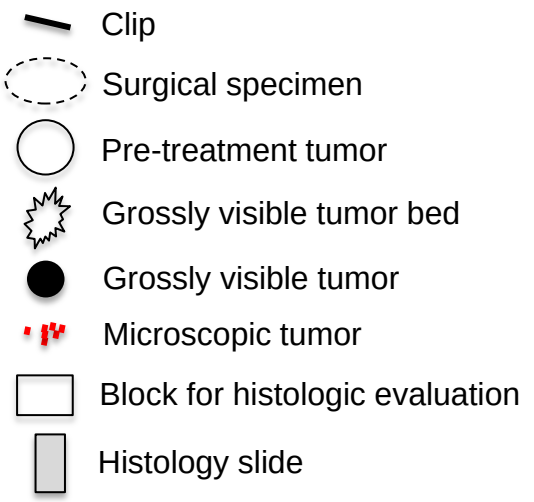


Black and blue rectangles represent 3 random blocks of tumor, each with very different estimates of cellularity.

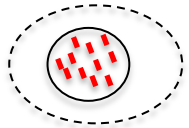


Random sampling:
Blue blocks = complete response
Black blocks = residual disease

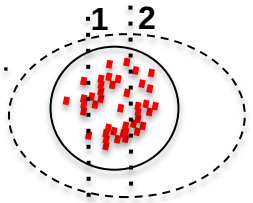
Inter-observer variability and discrepancies among guidelines regarding size



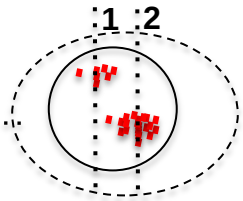
Patterns of residual disease



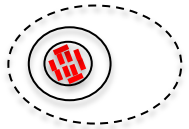
Size unchanged
Cellularity decreased



Size changed or unchanged
Cellularity decreased, heterogeneous



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



Size decreased
Cellularity similar
"Concentric shrinking"