Pathologic Response Following Neoadjuvant Chemotherapy As An Outcome For Clinical And Translational Research.

W. Fraser Symmans, M.D.
UT M.D. Anderson Cancer Center

Keypoints:

1. The definition of pathologic complete response (pCR) should be the absence of any residual invasive cancer in the breast and absence of any metastatic cells in the regional lymph nodes.

2. Neoadjuvant chemotherapy trials provide a valid clinical model in which to test for further improvements in adjuvant treatment.

3. Residual disease encompasses a broad spectrum of actual responses, and so evidence-based stratification of this group can further refine the prognostic utility of pathologic response following chemotherapy.

4. Efforts to standardize gross description, sampling and section codes, and reporting of residual disease after neoadjuvant chemotherapy should be practical and support evidence-based systems that evaluate pathologic response as a clinical outcome.
   a. If minimal residual disease has identical prognosis as pCR, do we really need to exhaustively sample the residual tumor bed to find a last remaining cancer cell?

5. Successful adoption of point 4 would improve the knowledge that is gleaned from clinical trials, help to identify at-risk patients for additional post-surgical systemic therapy (currently in clinical trials), and provide meaningful information in the usual pathology report.

1. Background

A central tenet of neoadjuvant clinical trials is that tumor response, as a surrogate endpoint, should be strongly correlated with long-term patient survival.\textsuperscript{1,2} Pathologic complete response (pCR) is associated with long-term survival, and has been adopted as the primary endpoint for neoadjuvant trials.\textsuperscript{3-12} While it is generally held that a definition of pCR should include patients without residual invasive carcinoma in the breast (pT0), the presence of nodal metastasis, minimal residual cellularity, and residual \textit{in situ} carcinoma are not consistently defined as pCR or residual disease (RD).\textsuperscript{12-15} When there is no residual invasive cancer in the breast, the number of involved axillary lymph nodes is inversely related to survival.\textsuperscript{16} Conversely, patients who convert to node-negative status after treatment have excellent survival, even if there is residual disease in the breast.\textsuperscript{17} Consequently, the combination of tumor size and nodal status after neoadjuvant treatment is prognostic.\textsuperscript{18}

Alternatively, the Miller and Payne classification ignores tumor size and nodal status altogether, and estimates only the decrease in cancer cellularity after treatment.\textsuperscript{10} However, the reduction in cellularity is often greatest when the residual tumor is small, suggesting a relationship between residual size and cellularity.\textsuperscript{19} Other systems, such as those reported by Honkoop, Chevallier and Sataloff, incorporate pathologic evidence of treatment response (using different criteria) to stratify patients with residual disease.\textsuperscript{3-5} While microscopic residual disease, altered cytologic appearance, and estimated tumor volume <1 cm\textsuperscript{3} also indicate good response, these tend to be descriptive parameters and are also difficult to apply to tumor beds with dispersed microscopic foci of carcinoma.\textsuperscript{3,6,9,20} Finally, there is no evidence that residual \textit{in situ} carcinoma alone increases risk of future distant relapse.\textsuperscript{12,21,22} Therefore, the definition of pathologic complete response (pCR) should be the absence of any residual invasive cancer in the breast and absence of any metastatic cells in the regional lymph nodes.
2. Improvements in Pathologic Complete Response Translates To Predicts Improved Survival

Patients who achieve pathologic complete response (pCR) from neoadjuvant (pre-operative) systemic therapy have excellent 5-year overall survival that is independent of treatment regimen or tumor phenotype. Furthermore, Table 1 illustrates how improvement in pCR observed in a neoadjuvant trial anticipated a survival difference in a phase III adjuvant trial for three recent treatment advances: addition of a taxane to anthracycline-based chemotherapy (white), more frequent paclitaxel dosing schedule (gray), and the addition of trastuzumab (Herceptin) to sequential anthracycline-taxane chemotherapy (blue). Therefore, neoadjuvant chemotherapy trials provide a valid clinical model in which to test for further improvements in adjuvant treatment.

3. The Prognostic Importance of Residual Cancer Burden (RCB) After Neoadjuvant Treatment.

Residual disease (RD) after neoadjuvant treatment includes a broad range of actual responses from near-pCR to frank resistance. We developed a method to measure RD by combining histopathologic components of residual disease (cellularity, overall diameter, number and extent of nodal involvement) into a numerical index of residual cancer burden (RCB). Minimal RD (RCB-I) in 17% of patients carried the same prognosis as pCR, even in hormone receptor-negative breast cancers (Figure 1A). Furthermore, extensive RD (RCB-III) in 13% of patients was associated with poor prognosis (Figure 1). Even for ER-positive breast cancer, patients with RCB-III had a 5-year distant relapse rate of 40% despite ongoing treatment with adjuvant hormonal treatment (Figure 1B). This identifies an important subset of patients with either combined insensitivity to chemotherapy and hormonal therapy, or with residual disease (after surgery) that is too extensive to be controlled by hormonal therapy alone, and illustrates how identification of the subset of receptor-positive patients who might correctly be spared (denied) adjuvant chemotherapy despite consensus treatment recommendations will require very careful selection based on the tumor’s predicted chemosensitivity and the predicted endocrine sensitivity. In summary, RCB incorporates the information from pCR, represents the extent of residual disease, more strongly predicts distant relapse-free survival, and can define clinically relevant subsets with near-pCR (RCB-I) or resistance (RCB-III).
4. Detailed Pathology Methods For Using Residual Cancer Burden

Residual cancer burden (RCB) is estimated from routine pathologic sections of the primary breast tumor site and the regional lymph nodes after the completion of neoadjuvant therapy. Six variables are included in a calculation formula. The calculated RCB index value can also be categorized as one of four RCB classes. The calculation formula and detailed description can be found at a dedicated website http://www.mdanderson.org/breastcancer_RCB.

Relevant information can be included within a pathology report (diagnoses or comment) without need for reporting calculated RCB index results. An example of relevant information from a report would be:

- Residual invasive carcinoma with chemotherapy effect.
- Residual carcinoma measures 2.4 x 1.8 cm and contains approximately 10% cancer cellularity.
- Residual intraductal carcinoma, solid type with necrosis, comprising 5% of the residual carcinoma.
- Metastatic carcinoma involving three of fourteen axillary lymph nodes (3/14).
- The largest metastasis measures 4 mm in greatest dimension.

From the results above, one could calculate RCB using these results: \(d_1 = 24\) mm, \(d_2 = 18\) mm, \(\%CA = 10\%\), \(\%CIS = 5\%\), \(LN = 3\), \(d_{met} = 4\) mm.

**Primary Tumor Bed:** In general terms, pathologic evaluation of the primary tumor bed in the breast requires that the pathologist make three judgments about the primary tumor bed:

i. Identify the cross-sectional dimensions of the residual tumor bed \((d_1 \text{ and } d_2)\),
ii. Estimate of the proportion of that residual tumor bed area that is involved by cancer (%CA), and
iii. Estimate the proportion of the cancer that is *in situ* component (%CIS).

**Defining the Tumor Bed.**

In cases of multicentric disease, the RCB measurements are from the largest residual tumor bed. In cases where the extent of residual cancer under the microscope does not correlate with the gross measurement of the residual tumor bed, the tumor bed dimensions can be revised according to the microscopic findings (Figure 1).

![Diagram](image)

**Figure 2.** Diagrams to illustrate how gross residual tumor bed dimensions are first estimated from the gross findings (pink area) but may be revised after review of the slides from the gross tumor bed area according to the extent of residual cancer (blue).

In these diagrams, the macroscopic tumor bed dimensions in examples A, C, D also define the final dimensions of the residual tumor bed after microscopic review. However, the macroscopic tumor bed dimensions in example B overestimate the extent of residual cancer, and so the dimensions of the residual tumor bed ($d_1$ and $d_2$) would be revised after microscopic evaluation of the extent of residual cancer in the corresponding slides from the gross tumor bed. In a different example (E), microscopic residual cancer extends beyond the confines of the macroscopic tumor bed. Again, the dimensions of the residual tumor bed ($d_1$ and $d_2$) would be revised after microscopic evaluation of the recognizable extent of residual cancer beyond the macroscopic tumor bed.

This approach accounts for differences in the concentration and distribution of residual cancer within a tumor bed. In the illustration above, the estimated %CA in example A would be high (in a small area), whereas the estimated %CA for examples C and D would be lower (in a larger area). In examples C and D, the estimated %CA would likely be similar, even though the distribution of cancer within the residual tumor bed is different in those two examples.

**Estimating Cellularity within the Tumor Bed**

The proportion of cancer (%CA) and the proportion of in *situ* component (%CIS) are estimated from microscopic evaluation of the slides from the residual tumor bed area. The most effective way to obtain this
information is to measure and submit for histology the largest cross-sectional area of residual tumor bed, and to designate in the report which slides represent the cross section of tumor bed. After reviewing those slides, the pathologist can estimate the average cellularity in the tumor bed on each slide in order to estimate the overall average cellularity of the tumor bed area (illustrated below).

The key is to simply:

i. Define the gross tumor bed as the largest cross-sectional area
ii. Submit sections representing that tumor bed area as individual slides
iii. Review those slides to estimate the %CA and %CIS within the residual tumor bed

![Illustrated summary of the pathology protocol to record the dimensions, section, and evaluate the primary tumor bed after neoadjuvant chemotherapy.](image)

Figure 3. Illustrated summary of the pathology protocol to record the dimensions, section, and evaluate the primary tumor bed after neoadjuvant chemotherapy.

A practical way to estimate %CA in a slide is to encircle with ink dots the tumor bed on each slide from the grossly defined residual tumor bed (e.g. slides A1-A5 in the example above). Then use the microscope to estimate the cellularity in each microscopic field across the area of tumor bed. In each microscopic field, %CA can be estimated by comparing the proportion of residual tumor bed area containing cancer (invasive or in situ). Estimate an average of the readings for %CA in the cross-sectional area. The same can be done for in situ component (%CIS). Estimates are to the nearest 10%, but include 0%, 1%, and 5% for areas with low cellularity. The average cellularity within the tumor bed from each slide across the tumor bed can then be estimated (illustrated above). The website contains computer-generated diagrams of % cellularity per area to assist pathologists to estimate accurately the cellularity of a microscopic field. Those diagrams are appended at the end of this document.

**Regional Lymph Nodes:** Pathologic evaluation of the primary tumor bed in the breast requires that the pathologist make two judgments:

i. Count the number of positive lymph nodes ($LN$),
ii. Measure the diameter of the largest nodal metastasis ($d_{met}$).
5. Special Circumstances With Relevance For Assessment of RCB

**Inoperable or Progressive Disease**

The RCB index cannot be accurately calculated for patients whose disease remains inoperable at the completion of the neoadjuvant treatment course (e.g. requiring subsequent additional treatments before surgical resection is possible), or those who experience disease progression and so do not undergo surgical resection at the completion of the neoadjuvant treatment course. For those patients, RCB is assigned as extensive, i.e. RCB-III.

**Internal Mammary Lymph Node Metastasis**

There were no examples of internal mammary nodal metastasis in the published study that evaluated the prognostic value of RCB. However, it is reasonable to include internal mammary nodes with the other regional (axillary) nodes in the assessment of RCB.

**Pre-treatment Sentinel Lymph Node Biopsy**

Surgical excision of a positive sentinel lymph node before the neoadjuvant treatment would invalidate the accuracy of measuring RCB after the treatment to assess response. If all sentinel lymph nodes were negative before treatment began, this would not affect the assessment of RCB after treatment ended.

6. Validation of Pathologic Response and Survival Risk

Independent validation is eventually required of any prognostic or predictive tool. The dichotomous pCR versus residual disease has been extensively validated, but other systems have only recently been validated for prognostic utility.

The Chevallier and Sataloff systems have recently been validated and compared in a retrospective study of 710 subjects.\textsuperscript{37,38} It should be noted that Chevallier classes 1 and 2 are both included in the current definition of pCR (see section 1), and that combined classes 1 and 2 have significantly better prognosis than combined classes 3 and 4 (residual disease).\textsuperscript{38} The authors also reported that class 4 had worse survival than other classes combined (DFS, \(p = 0.01\); OS, \(p = 0.07\)). Sataloff class T-A (cancer cells absent or comprising < 5% of the residual tumor bed area) was significantly different from more extensive residual disease in the breast (DFS, \(p = 0.005\); OS, \(p = 0.006\)).\textsuperscript{38} There was no difference in DFS or OS between partial response classes T-B (> 50% therapeutic effect) and class T-C (< 50% therapeutic effect in the primary tumor), but the authors reported that class T-D (no therapeutic effect in the primary tumor) had worse survival than other classes combined (DFS, \(p = 0.01\); OS, \(p = 0.07\)).\textsuperscript{38} Although this system has four classes of nodal status, only node-negative versus node-positive status had prognostic import, and this held in the subset with class T-A.\textsuperscript{38} It is not reasonable to compare the Kaplan Meyer survival plots from this study with those from the RCB studies because the treatments were very different, as well as other cohort differences. Nevertheless, at some time it might be advantageous for a future study to directly compare different systems in a single cohort of patients who received a current standard therapy.

A study of 45 cases of locally advanced or inflammatory breast cancers determined that RCB score was significantly prognostic in univariate (HR 1.57; 95% CI 1.04 to 2.38; \(p = 0.018\)) and in multivariate (HR 1.59; 95% CI 1.04 to 2.43; \(p = 0.033\)) Cox regression analyses of event-free survival, whereas pCR was not.\textsuperscript{39} Recently, the prognostic utility of RCB categories has been demonstrated in two other study cohorts (323 patients at MDACC who received T/FAC (Hatzis \textit{et al} ASCO, 2008) and 200 patients in the iSPY trial who received AC/docetaxel or AC/paclitaxel (Esserman \textit{et al} ASCO, 2009), each with 3 years median follow-up.\textsuperscript{40,41} Additional validation cohorts and longer follow-up from these studies is desirable.

7. Breast Cancer Phenotype

The data are currently limited, but the evaluation of all 564 cases from MDACC shows that RCB (as a measure of pathologic response) is prognostic for each of the three main phenotypic groups of breast cancer (HER2+, ER+/HER2-, and ER-/HER2-).\textsuperscript{40} The survival curves for ER+/HER2- breast cancers are similar to those for ER+ (Figure 1). Of note, approximately 20% of ER+/HER2- subjects with RCB-III after chemotherapy still have an approximate 30-40% risk of distant relapse within 5 years, despite ongoing endocrine therapy. Also, the curves for ER-/HER2- breast cancer are similar to those of ER- breast cancer (Figure 1). Of note, the prognosis of RCB-I is the same as for pCR, and the patients with RCB-III have a very high probability of early distant relapse. The analysis of the iSPY trial showed a similar result.
Although RCB after chemotherapy is also prognostic in HER2+ breast cancer, the new standard of HER2-targeted therapy with chemotherapy now renders that result obsolete. There are only limited data for RCB after concurrent chemotherapy and trastuzumab for HER2-positive breast cancer, in which the frequency of pCR or RCB-I was 70%, irrespective of ER-status. However, this study is small in number and there is currently insufficient follow up of those patients to evaluate the prognostic relevance of RCB following trastuzumab.

8. Reproducibility of Residual Cancer Burden Assessments

We have preliminary evidence that more detailed assessments of response (e.g. RCB) can be reproducible among pathologists. In a pilot study we asked 3 pathologists to evaluate RCB in 100 cases from the original study cohort with residual disease (in situ, invasive, or nodal), and included the RCB assessments as a fourth reading. Correlation of 100 RCB measurements was high among these 4 pathologists (Pearson coefficient 0.92-0.96, Spearman coefficient 0.94-0.96). Perhaps more importantly, the survival analyses using the results from each pathologist all showed strong prognostic differences between the classes of RCB.

9. Future Directions

It is likely that neoadjuvant studies will become more common, and thereafter, trials of additional post-surgical treatments for patients who have a significant amount of residual disease at the completion of neoadjuvant treatment. An example of such a trial opened at M.D. Anderson Cancer Center in 2009. In that trial, patients with RCB-III, or RCB-II if ER-negative, after neoadjuvant chemotherapy are randomized to receive post-surgical ixabepilone, versus placebo. This approach contrasts with trials that include any residual disease by concentrating on those at greater residual risk of relapse.

Response measurements should be improved if better methods can be developed to more accurately measure pre-treatment tumor burden. When we combined the cellularity from the core biopsy with the radiologists’ measurement of the pre-treatment dimensions we found no significant improvement in the prognosis (beyond RCB alone), except for the subset of RCB-II patients. This suggests that improvements would be likely, but are probably currently hampered by limitations to the accuracy of information about the extent of tumor when using standard breast imaging of mammography and ultrasound.

There is interest in the imaging professions to develop methods to study tumor response in vivo, and also to develop better methods to direct post-treatment surgical planning.

There is current controversy about whether sentinel lymph node sampling should be performed to stage the axilla before neoadjuvant treatment or after the treatment has been completed. Is it more useful prognostic information to know what the patient began with, or what was left after the chemotherapy? At MDACC we prefer post-treatment SLN evaluation because it reduces the number of surgeries and we believe it has stronger prognostic value, but there are surgical concerns in the other camp that question the accuracy of sampling at that time. We do note that RCB measurements might be unreliable if a positive SLN was excised prior to the chemotherapy. The issue remains controversial and will likely be the subject of a prospective randomized trial.

10. Summary of Key Points For Pathologic Assessment of the Pathologic Resection Specimen

1. **GROSS.** Identify the probable tumor bed and describe this macroscopic finding
   a. Report the measurements of the largest gross dimensions (prefer 3 dimensions, but minimum is 2 dimensions).
   b. Submit the largest cross-sectional area for histology and specifically describe those blocks in the Section Code
      i. Try to indicate how they are oriented by photography, radiography, photocopy, or intelligent description (e.g. “sections B1 – B7 cross section of tumor bed in rows from antero-superior to posteroinferior”).
      ii. If additional sections are from surrounding tissues, then describe those as well
      iii. Five representative sections from a big, obvious tumor bed should be sufficient

2. **MICROSCOPY.** Review the slides that correspond to the tumor bed (+/- surrounding tissues)
   a. Estimate the extent of spread of residual cancer relative to the gross tumor bed
i. If similar to the gross description, then keep the original measurements.

ii. If obviously different, then revise the dimensions of the tumor bed based on the microscopic review of the tumor bed.

iii. Suggestion: dotting the perimeter of cancer in each slide can be helpful to reconstruct the tumor extent across multiple slides (see point 1-b-i).

b. Using the microscope, make visual snapshots of cancer cellularity as you go from field to field across the defined tumor bed from one end to the opposite (e.g. left to right, then top to bottom) to estimate the:

i. Average cancer cellularity (%) across the entire tumor bed. This is all cancer, whether invasive or in situ.

ii. Average percent of the cancer within the tumor bed that is in situ.

iii. Cellularity estimates are to the nearest 10%, with additional selections of 1% and 5% for very low cellularity. For reference there are images of computer-generated examples linked to our website http://www.mdanderson.org/breastcancer_RCB.

iv. The usual misunderstanding is to only make estimates in foci of the tumor bed that contain lots of cancer. The estimated cancer cellularity should represent the average across the entire residual tumor bed area.

3. REPORTING. There are many different formats for the diagnosis in pathology reports, and for making additional comments in the report. Furthermore, many pathologists would prefer not to report calculated indices such as residual cancer burden. However, it is nearly impossible for a clinician or clinical investigator to calculate RCB from usual pathology reports, if they wish to do so. We now encounter this situation when patients participate in some clinical trials. One option is to provide the relevant data in the text of the diagnosis or comment, or in a synoptic report. An illustrative example is as follows:

a. Residual carcinoma involves a tumor bed that measures 37 x 25 mm in greatest dimensions and contains approximately 20% average cancer cellularity by area, of which 10% is carcinoma in situ.


c. The largest metastasis measures 4 mm in greatest dimension.

A clinician or investigator can readily obtain RCB from this information, as below:

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

<table>
<thead>
<tr>
<th>1) Primary Tumor Bed</th>
<th>2) Lymph Nodes</th>
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<tr>
<td>Primary Tumor Bed Area:</td>
<td>Number of Positive Lymph Nodes:</td>
</tr>
<tr>
<td>Overall Cancer Cellularity (as percentage of area):</td>
<td>Diameter of Largest Metastasis:</td>
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<tr>
<td>Percentage of Cancer That Is in situ Disease:</td>
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<table>
<thead>
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<td>4 (mm)</td>
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<td>10 (%)</td>
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**Reset** | **Calculate**

Residual Cancer Burden: 3.329
Residual Cancer Burden Class: RCB-III
REFERENCES


