PATHOLOGISTS
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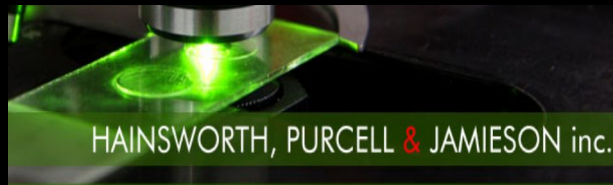
Key to Diagnostic Excellence

How responsive are you?

Assessing response to neoadjuvant therapy

by Dr Craig Jamieson




STAR WARS
THE CLONE WARS

What we know:

- Tumours of different types, grades and stages respond differently to treatment.
- Tumours with different hormonal profiles respond differently to treatment.
- Systemic therapy before or after surgery gives identical results for locoregional control and metastasis-free survival.
- Patients who achieve a good clinical response have improved long-term disease-free survival (DFS) and overall survival (OS).
- More women are being treated with chemotherapy or hormonal agents before surgery for earlier-stage operable breast carcinoma.

Mauri D, et al. J Natl Cancer Inst. 2005;97:188– 194.

Fisher ER, et al. Cancer. 2002;95(4):681–695.

van der Hage JA, et al. J Clin Oncol. 2001;19(22):4224–4237.

What we know:

Major advantages of neoadjuvant therapy (NAT):

- Response / non-response of cancers can be determined in individual patients.
- Response can be used as an early indication of outcome.
- Pathologic complete response (pCR)(no residual invasive carcinoma) = excellent prognosis.
- Tumour response is a short-term endpoint for clinical trials.
    - Next endpoint is recurrence or death.
    - If a patient does well post-surgery:
      ?due to the effectiveness of the systemic treatment or surgery.
- Increased eligibility for breast conservation.
- Research linking tumour response to tissue samples is facilitated.
    - Allows for tissue samples to be collected before, during, and after treatment.
    - Tumour samples can be paired with information about tumour.
    - Allows for genetic analysis of the tumours at all 3 stages .

How do we assess response to neoadjuvant therapy (NAT)?

- **Clinical assessment:** tumour size, nodal status.
- **Histological assessment:** complete / partial pathological response.
- **Radiological assessment:** tumour size, nodal status, distant spread.

What the pathologists know:

- Pathologists play a key role in the evaluation of pathologic response.
- The pathologic examination of these specimens can be quite challenging.
- We can‘t do it alone.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JUDGMENT DAY

What the pathologists need to know:

- How best to dissect, assess and report on the post-treatment breast specimen.
- Presentation of the lesion before treatment (palpable mass or radiologic lesion, skin changes such as edema, erythema, fixation to chest wall).
- Size and position of the invasive carcinoma before treatment.
- Prior diagnostic procedure: core needle biopsy or incisional biopsy—ideally, this specimen should be available for comparison with the posttreatment carcinoma.
- Presence of a clip and/or calcifications in the tumour.
    - It may be impossible to reliably identify the tumour bed in cases with pCR.
    - Post-removal x-ray for areas of calcification Should be considered.
- Prior evaluation of lymph nodes (fine-needle aspiration, core biopsy, or sentinel lymph node biopsy) and the results.
- Type of neoadjuvant therapy given.
- Clinical/radiologic response of the carcinoma to the treatment (complete, partial, minimal).
- Orientation of the specimen (via sutures / ink / clips)

Arch Pathol Lab Med. 2009;133:633–642

Sampling the tumour bed: how much is enough?

- Consensus has not been reached.
- If tumour is found:
  - Residual tumour bed is small, sample the entire area.
  - Residual tumour is still large, 1 block per centimeter.
- No identifiable bed:
  - Sample all areas of fibrosis / areas suspicious for tumour bed.

What do we need to be aware of when reporting?

- Tumour size.
- Tumour cellularity.
- Histologic appearance and tumour grade.
- Tumour associated lymphocytes.
- Receptor status / IHC profile.
- Response in lymph nodes.

Assessment of tumour size:

- Most reduce in size post-NAT
- Assessed by palpation + gross examination +- radiology
- May prove difficult as tumours and stroma soften with tumour regression and the lesion may disappear entirely.

Assessment of tumour cellularity post-NAT:

- Typically less cellular after treatment (even when there is not a marked decrease in size).
- Loss of cellularity correlates with clinical response and prognosis.
- Estimates of cellularity are more difficult when there has been a marked response:
  - Islands of highly cellular carcinoma may interspersed within a large, difficult-to-delineate tumour bed.
  - A change in cellularity is easier to determine when pretreatment and post treatment specimens are compared.

Histologic appearance and tumour grade:

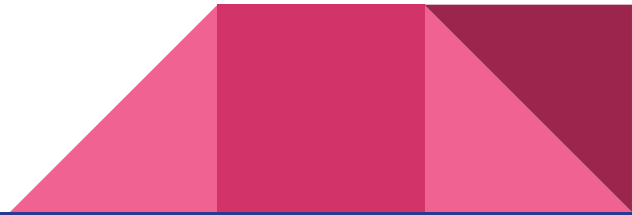
- Most carcinomas do not change in appearance after treatment, except for the loss of cellularity.
- Occasionally may appear to be higher grade.
- Rarely may appear to be of lower grade.

True change in grade or treatment effect?

- A change in tumour grade can only be assessed by comparing the post-treatment tumour to the pretreatment biopsy.
- Post-NAT change in the grade has not been clearly correlated with clinical outcome.
- In some tumours, the in situ carcinoma and tumour emboli in vascular spaces (LVI) are relatively resistant to treatment when compared to carcinoma invading the stroma.

Systems for evaluating response to NAT:

- Criteria have not been standardized.
- AJCC / NSABP B-18 / RCB / Miller-Payne / Sataloff / Chevallier / RDBN
- All systems have:
  - Pathologic complete response (pCR).
  - Little or no response.
  - Categories of partial response in different systems range from 1 to 4 or expressed by a continuous variable.
- pCR = absence of invasive carcinoma in the breast.
- Residual ductal carcinoma in situ may be present - does not alter survival.
- In systems that include lymph nodes:
  - Nodes must also be free of carcinoma for a pCR.



Systems for evaluating response to NAT:

- **AJCC System (Sixth Edition)**
  - Post-NAT T and N category indicated by the prefix “y.”
  - Post-NAT AJCC stage retains prognostic information .
  - Relies on tumour size and lymph node status → can be difficult to evaluate after NAT.
  - When nests of residual tumours post-NAT: yT = the distance over which the nests extend.
  - does not include changes in cellularity or lymphovascular invasion (LVI).
  - The post-NAT “y” AJCC stage, by itself, does not give an indication of the response to treatment.

- **NSABP B-18 System**
  - Three categories of response:
    - pCR.
    - Partial response (sparse invasive tumour).
    - No response.
  - Metastases to lymph nodes were analyzed separately.

Carey et al


MAX PAYNE

Systems for evaluating response to NAT:

- **The Miller-Payne Grading**
  - 5 grades based on a comparison of tumour cellularity before and after treatment .
  - Showed that a grade-4 response (almost a pCR) had a worse prognosis than a pCR (grade 5)
  - Does not include the response in lymph nodes or the presence of LVI.

Carey et al

Miller-Payne Grading

Grade	Description
1	No change or some alteration to individual malignant cells but no reduction in the overall cellularity
2	A minor loss of tumor cells but overall cellularity still high; up to 30% loss
3	Between an estimated 30% to 90% reduction in tumor cells
4	A marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain; more than 90% loss of tumor cells
5	No malignant cells identifiable in sections from the site of tumor; only vascular fibro-elastic stroma remains often containing macrophages. However, DCIS may be present

Systems for evaluating response to NAT:

- **Residual Disease in Breast and Nodes (RDBN):**
  - Level 1: pathological complete response in breast and nodes +- carcinoma in situ
  - Levels 2–4:
    - 0.2 (residual breast tumour size in cm) + the index for the involved nodes (0 for no positive nodes, 1 for 1–4 nodes, 2 for 5–7 nodes, 3 for ≥8 nodes) + the Scarff-Bloom-Richardson grade (1, 2, or 3).
  - Takes into account tumour size, lymph node stage, and histological grade.
- Studies have shown that MPG and RDBN are independent predictors of distant disease-free survival and local recurrence-free survival.
- Survival post-NAT correlated with the pathological reaction rather than with the molecular subtype of breast cancer.

Zhao Y, et al

Response in lymph nodes:

- Most important prognostic factor in patients who receive NAT.
- The response in the breast and lymph nodes generally similar.
- Not all of a patient’s lymph node metastases will respond equally to chemotherapy.
- pCR in both breast and axillary lymph nodes = significantly improved OS and DFS.
- Prognostic significance of pathologic response in lymph nodes > response in the breast.

Response in lymph nodes:

- Evaluation may not be possible in certain patients:
  - If a positive lymph node was removed before therapy (SLNB), and the lymph nodes after therapy are not involved by metastases, response to therapy cannot be evaluated with certainty.
    - Some suggest evaluation abnormal nodes with FNA rather than biopsy if NAT is planned.
  - ALND not always submitted (sentinel LN -ve)
- After NAT, lymph nodes are difficult to recognize because of atrophy and fibrosis.
  - ALND requires careful search for lymph nodes
  - Nodes thinly sectioned and completely submitted.
  - Fibrotic areas in the axillary fat and tissue around the vessels should be submitted

Response in lymph nodes:

- Microscopy:
  - Hyaline stromal scars, mucin pools, or aggregates of histiocytes without any viable tumour cells.
  - Metastasis can resolve without a scar

- 3 groups:
  - Positive nodes +- disease regression (partial or no response)
  - Negative nodes with evidence of treatment-induced change but no viable tumour (complete response).
  - Negative nodes without treatment effect (negative / ? complete response).

Response in lymph nodes:

- NSABP B-18 (9 years of follow-up)
  - No NAT before surgery:
    - negative nodes = micrometastases (<2 mm) >> macrometastases.
  - NAT then surgery:
    - Negative nodes >> minimetastases (<1 mm) = micrometastases (<2 mm) = macrometastases
    - Micrometastases in lymph nodes in patients who receive NAT probably represent macrometastases that have partially responded to chemotherapy.

Tumour associated lymphocytes:

- Breast carcinomas with significant numbers tumour associated lymphocytes (TAL) respond well to NAT with a significantly increased pCR rate compared to patients with poorly infiltrated tumours (~30% vs 10%).
- Independent predictive value.
- > 60% infiltration by lymphocytes = lymphocyte predominant breast cancer (LPBCs).

Ali HR, et al, Annals of Oncology 2014, 25:8: 1536–43 Denkert C, et al. J Clin Oncol 2010; 28:105-13 Wang K, et al Oncotarget. 2016;7(28):44288-98.

How do we measure TAL’s?

- H&E.
- Computational image analysis (ARTemis trial).
- Does it matter where the TALs are situated?
    - Intratumoural lymphocytes - intraepithelial mononuclear cells within tumour cell nests or in direct contact with tumour cells.
    - Stromal lymphocytes - the percentage of tumour stroma area that contains a lymphocytic infiltrate without direct contact to tumour cells.
- Answer: yet to be determined, but probably no.

Receptor status:

- In general, receptor status remain the same before and after treatment.
- Where they differ consider:
  - laboratory error/discrepancy in testing, interpretation of the stains
  - tumour sampling (eg, there may be little tumour to evaluate either before or after treatment)
  - tumour heterogeneity.
  - directly from specific types of neoadjuvant therapy.
    - Progesterone receptor loss after treatment with aromatase inhibitors, but not with tamoxifen.
    - HER2/*neu* expression diminished after treatment with trastuzumab.
    - ? due to downregulation of receptor or selection of tumour cells not expressing receptor?
- Tacca et al:
  - 23% of patients who received NAT had a change in hormone receptor status on repeat immunohistochemical studies.
  - 42% who were initially receptor negative became receptor positive —> correlated with better overall survival in these patients vs those who remained unchanged.

Ki-67:

- In general, patients with low Ki-67 scores have a better prognosis and disease-free interval than those with intermediate to high scores.
- Changes in Ki-67 have been suggested as a means to measure response to therapy
  - particularly with hormonal therapy where inhibition of proliferation is the primary goal
  - changes in proliferation have not yet been linked to survival.
- Ki-67 is an independent predictor of a pCR
- A higher Ki-67 value (≥25%) is a significant predictive factor for the response to NAT (especially in ER- HER2+ breast cancers).

Kwan K, et al Yerushalmi R, et al Niikura N, et al Amadori D, et al

Immunohistochemistry and molecular subtypes:

A number of studies have shown good correlation between IHC & molecular subtypes:

- Luminal A: (ER+/PR+, HER2−, Ki-67 <14%)
- Luminal B/HER2− (ER+/PR+, HER2−, Ki-67 ≥14%)
- Luminal B/HER2+ (ER+/PR+, HER2+)
- HER2-enriched (ER−, PR−, HER2+)
- Triple-negative (ER−, PR−, HER2−)

Triple-negative subtypes were more likely to achieve a pathological complete response than those with other molecular subtypes of breast cancer.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Genomic assays:

- Certain gene expression profiles are associated with overall survival.
- Testing most valuable for ER+ HER2- patients.
- Certain assays can be used to predict response to NAT (EndoPredict, Oncotype DX).
- T1 and T2, ER/PR +, HER2- and lymph node negative tumours with any of the following multigene panel results are placed into the same prognostic category as T1a-T1b N0 M0 tumours:
  - Oncotype Dx® recurrence score less than 11 (Level 1 Evidence).
  - Breast Cancer Index in the low risk range (Level 2 Evidence).
  - EndoPredict® low risk score (Level 2 Evidence).
  - Mammaprint® low risk score (Level 2 Evidence).
  - PAM 50® risk of recurrence score in the low range (Level 2 Evidence).

Soliman HH, et al

The final report:

Breast Specimen:

1. Presence and size of tumour bed:
    a. Size and extent of residual tumour.
    b. Two-dimensional measurements of the largest area of invasive cancer.
    c. Number of foci or number of blocks with foci of invasion.
2. Average cancer cellularity of the residual tumour bed.
3. Appearance of the residual tumour and grade, if applicable: compare to pretreatment carcinoma, if possible .
4. Viability (necrosis, mitotic figures).
5. Proliferation index by Ki-67.
6. Lymphovascular invasion.
7. Presence and extent of ductal carcinoma in situ.
8. Margins with respect to tumour bed, invasive, and in situ carcinoma.
9. A comment on the overall response to treatment.

The final report:

Lymph Nodes:

1. Number of lymph nodes.
2. Number of lymph nodes with metastases.
3. Size of the largest metastasis.
4. Presence of extranodal extension.
5. Number of metastases with evidence of treatment response.
6. Number of lymph nodes with evidence of treatment response but without tumour cells (ie, fibrosis, necrosis, aggregates of histiocytes).

Conclusions:

- NAT is being offered more commonly to patients with earlier-stage breast cancer.
- Likely to become the standard of care for patients receiving systemic therapy.
- Pathologic evaluation of tumour response is the gold standard.
    - clinical and radiologic responses do not correlate well with residual tumour after treatment.
- Pathologists will continue to play an important role:
    - in providing this information to optimize the knowledge gained by this approach to breast cancer therapy.
    - in standardizing the existing classification schemes and in developing new schemes for ongoing trials.

Ref

Pathology of Breast Carcinomas After Neoadjuvant Chemotherapy: An Overview With Recommendations on Specimen Processing and Reporting

Sunati Sahoo, MD; Susan C. Lester, MD, PhD

Evaluation of the pathological response and prognosis following neoadjuvant chemotherapy in molecular subtypes of breast cancer

Yue Zhao, Xiaoqiu Dong, Rongguo Li, Xiao Ma, Jian Song, Yingjie Li, Dongwei Zhang

I’ll be back ……..

Genomic assays:

- The basal-like and erbB2+ subgroups associated with the highest rates of pCR (both ~45%)
- Luminal tumours had a pCR rate of 6%.
- No pCR observed among the normal-like cancers
- Molecular class is not independent of conventional cliniocopathologic predictors of response (eg ER, Ki-67 and nuclear grade).
- None of the 61 genes associated with pCR in the basal-like group were associated with pCR in the erbB2+ group, suggesting that the molecular mechanisms of chemotherapy sensitivity may vary between these two estrogen receptor negative subtypes.

Rouzier R, et al Hatzis C, Pusztai L, Valero V, et al.