The Breast 22 (2013) S88–S91

Contents lists available at SciVerse ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Characterization and clinical impact of residual disease after neoadjuvant chemotherapy

Giuseppe Viale*

University of Milan School of Medicine, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy

Keywords:
Neoadjuvant chemotherapy
Pathology of residual breast cancer
Tumour markers
Immunohistochemistry
Breast cancer

A B S T R A C T

One of the most important lessons learned from trials of neoadjuvant chemotherapy (NACT) is that achievement of pathological complete response (pCR) is a powerful prognostic predictor of long-term outcome, with significantly better disease-free and overall survival for patients achieving pCR, as compared with patients having residual tumour after NACT. The pathologists' role in the neoadjuvant setting is: (i) to ensure an accurate assessment of pCR, and (ii) to evaluate burden and biological characteristics of residual tumour if pCR has not been achieved. A conversion of receptor status from the core biopsy to the post-NACT surgical specimen may cause uncertainty in the choice of the post-surgical systemic treatment for the patients. It is therefore imperative to ensure accuracy in the assessment of ER, PgR and HER2, and to double check any apparent conversion by re-staining the previous core biopsy and the residual tumour in the same run, thus minimizing the technical artifacts, and to use both immunohistochemical and in situ hybridization assays to evaluate HER2 status. It is essential that protocols for evaluation of tumour response and for assessment of prognostic/predictive parameters of residual disease after NACT be eventually harmonized.

Introduction

Neoadjuvant chemotherapy (NACT) was initially devised to offer patients with inoperable locally advanced or inflammatory breast cancer the chance of downstaging their tumours to a point that they would become operable. Similarly, downstaging of tumours in patients candidate for mastectomy could allow performance of breast-conserving surgery. In terms of long-term outcome, it has been shown that NACT regimens achieve the same survival results as the more conventional adjuvant systemic chemotherapy [1–5].

More recently, the neoadjuvant setting has been viewed as an exciting opportunity for *in vivo* assessment of tumour response to different systemic interventions (chemotherapy with or without targeted therapies, and endocrine therapies), including newly developed drugs [6]. This setting also is an ideal scenario for evaluating biological markers of responsiveness or resistance to the treatments, and to study intermediate end-points.

One of the most important lessons learned from NACT trials is that achievement of pathological complete response (pCR) is a powerful prognostic predictor of long-term outcome, with significantly better disease-free and overall survival for patients achieving pCR, as compared with patients having residual tumour after NACT [7,8]. The likelihood of achieving pCR after NACT, however, is correlated with several morphological and biological characteristics of the primary tumour, including tumour grade [9], hormone receptor status and the proliferative fraction [10]. Indeed, high-grade tumours, with low/absent hormone receptor expression and high proliferative fraction are most likely to show a complete response to NACT [11], while HER2-positive breast cancer are exquisitely responsive to neoadjuvant treatments including HER2-targeted agents, especially when a dual blockade of the receptor is achieved with different drugs [12,13]. Using the modern molecular classification of breast cancer, it has been documented that Luminal A tumours are least likely to achieve pCR after NACT, Luminal B have an intermediate response, whereas HER2-enriched and Basal-like breast cancer show the highest likelihood of pCR [14].

It is surprising, however, that despite the unanimous consensus that pCR after NACT is the most important prognostic information for long-term outcome of the disease, there is not a general agreement on the actual definition of pCR. Indeed, both in the clinical trials and in the daily practice, a number of different definitions of pCR are used, including absence of invasive cancer in the breast only or both in the breast and in axillary lymph nodes, and absence of invasive and in situ cancer in the breast only, or both in the breast and in axillary lymph nodes. Use of different definitions for pCR may well affect the interpretation of the results of

* Tel.: +39 0257489419; fax: +39 257489417.
E-mail address: giuseppe.viale@ieo.it.

0960-9776/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.breast.2013.07.016

neoadjuvant studies, and make the prognostic implications of pCR in the clinical setting quite ambiguous.

Most recently, the Food and Drug Administration (FDA) of the United States of America has endorsed the proposal of defining pCR as the absence of any residual invasive cancer on evaluation of the surgically resected breast specimen and all sampled ipsilateral lymph nodes following completion of neoadjuvant systemic therapy. The rationale for the endorsement of this definition is that neither ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) are expected to regress with chemotherapy, and that persistence of DCIS or LCIS is not believed by most to have prognostic significance [15]. On the contrary, involvement of regional lymph nodes, either at presentation or after neoadjuvant therapy is associated with a worse long-term outcome [16,17].

Patients not achieving pCR may be totally unresponsive to the neoadjuvant interventions (showing tumour progression), or exhibit variable degrees of response, from stabilization of the disease to a partial shrinking of the tumour, to a near-pCR. Quite understandably, if there is discordance about the definition of pCR, even greater differences are expected in the definition of partial response, and in the alleged prognostic implications of these different degrees of response.

To try and homogenize the current differences in the definitions of pCR and partial responses, in the evaluation of the surgical specimens after NACT, and in the assessment of the burden and the biological features of residual tumour, with the derived implications on the long-term outcome, the Breast International Group (BIG) and the North American Breast Cancer Group (NABCG) have jointly appointed an International Working Group for the characterization of residual disease, to address all these open questions and to issue recommendations especially for defining the minimum, essential set of components for the histopathological evaluation of residual disease in breast cancer.

The role of pathologists in the assessment of tumour response to NACT

In short, the pathologists' role in this setting could be summarized in the achievement of two main tasks: (i) ensure an accurate assessment of pCR, and (ii) evaluate burden and biological characteristics of residual tumour if pCR has not been achieved.

An accurate pathologic assessment is the gold standard for determining a complete tumour response. Indeed, a clinically complete response does not imply a pCR, because between 30% and 50% of patients with a complete clinical response actually have residual cancer in the surgical specimen; on the other hand, some 20% of patients with clinical residual disease, actually have a pCR [18,19]. To ensure an accurate assessment of pathologic response of breast cancer after NACT, it is imperative that the pathologist evaluating the surgical specimen be aware that the patient has received NACT. In an ideal word, this information would be always available and provided with the clinical history of the patients, but in the daily life it is often not provided, at least in a timely fashion, i.e. prior to the pathologic examination of the surgical specimen. Pathologists should be aware that this relevant clinical information may be missing, and be warned about a possible previous NACT by some clues, like a long interval between a prior core biopsy and surgery, or the lack of obvious cancer in the surgical specimens. If this is the case, the evaluation and sampling of the surgical specimen should be postponed, until more information is obtained (most commonly with a call to the treating physician). Once the prior NACT has been ascertained, it is important to get the radiology reports for assessing the location of the primary tumour (sometimes there were multiple foci) and for evaluating the degree of radiological response.

Gross examination and sampling of the surgical specimen

Here the main task is the identification of the tumour bed. This may be very challenging in case of a substantial or complete response to the NACT. The tumour bed appears as an irregular area of rubbery fibrous tissue, with occasional small pseudocystic spaces and haemorragic spots. Within, or at the periphery of the tumour bed, residual tumour nodules may be visible or not. To facilitate the identification of the tumour bed, prior clinical and radiological information should be used, and of course a clip left in the tumour at the time of the core biopsy may prove extremely useful. Specimen radiographs may be needed to help identifying the clip and to ensure complete excision of the tumour bed whenever its precise location and extent is not readily apparent. Once the tumour bed has been indentified, measurements have to be taken of the size of the surgical specimen, of the tumour bed, of any visible tumour nodule, together with the distance of the tumour bed from all margins [20,21].

The rule of thumb for sampling post-NACT surgical specimens is to embed the entire tumour bed, and any visible residual tumour nodules. If the specimen is not big, and the tumour bed has not been clearly identified, all the specimen should be submitted for histological examination. If the specimen is large (e.g., mastectomy) and the tumour bed is also large, then there is wide heterogeneity in the sampling protocols, both in clinical studies and in the daily practice. One option is to start with a preliminary sampling of a limited number of blocks (say 1 block per cm), and if residual tumour is not found then submit all the tumour bed. If the tumour bed is not clearly recognized, it may be useful to start sampling the tumour bed area immediately adjacent to the clip, or be guided by radiological abnormalities discovered by X-rays of slices of the specimen. Needless to say, all suspicious nodules of residual tumour, within or around the tumour bed should be extensively sampled.

Microscopic examination

At the microscope, the tumour bed is characterized by extensive hyalinized stroma, with oedematous areas, inflammatory infiltration with patchy aggregates of lymphocytes, macrophages, foamy histiocytes, sometimes multinuclear giant cells and hemosiderin deposition [20–22].

Residual cancer may maintain the same morphology as prior to NACT, but often there are impressive morphological changes of the tumour cells, with bizarre shape and contour, and enlarged nuclei with clumped chromatin. Large foci or small nests of invasive tumour may be present, or scattered individual tumour cells may be encountered within the tumour bed. The identification of these individual tumour cells may be facilitated by use of immunohistochemical stains for cytokeratins. In case of substantial response, residual tumour may only be seen within vascular spaces (tumour emboli), and it may be sometime difficult to distinguish these tumour emboli from small DCIS showing retraction artifacts.

If residual invasive tumour is identified, its size and grade should be estimated, as well as the distance from the surgical margins. There are several different systems to evaluate the residual tumour burden (discussed in the next section). Similarly, there is no consensus about how to grade residual cancer, and whether tumour grade after NACT has the same prognostic value as for tumours not previously treated with systemic chemotherapy. Indeed, when using the classic histopathologic grading system of Elston and Ellis [23], it remains to be elucidated if the architectural features (tubule formation), the nuclear characteristics, and the mitotic index do actually reflect the intrinsic biological characteristics of the tumour cells, or they are merely reflecting the effects of

the chemotherapy, thus hampering the actual prognostic value of the histological grade.

Finally, the histopathological examination of residual cancer must include the re-evaluation of the biological markers (oestrogen and progesterone receptors, HER2 and Ki-67, as a marker of cell proliferation), that have been previously assessed in the core biopsy. Again, in the literature there are discordant data about the actual rate of "conversion" from positive to negative and vice versa of hormone receptors and HER2, with the majority of the studies reporting changes in 10%–20% for oestrogen receptor (ER) status, an higher rate for progesterone receptors (PgR), and some 10% rate for HER2 [24–29]. There may be several plausible explanations for the discordant receptor status between core biopsy and surgical specimens after NACT, including technical reasons (with false-positive or false-negative assessments in the core biopsy or in the surgical specimen), intratumoral heterogeneity in the expression of these markers with a clonal selection induced by the treatment, or changes in the expression of the markers induced by the chemotherapy (with or without HER2-targeted treatments).

A conversion of receptor status may cause uncertainty in the choice of the post-surgical systemic treatment for the patients. It is therefore imperative to ensure accuracy in the assessment of ER, PgR and HER2, and to double check any apparent conversion by re-staining the previous core biopsy and the residual tumour in the same run, thus minimizing the technical artifacts, and to use both immunohistochemical and in situ hybridization assays to evaluate HER2 status.

Changes in the Ki-67 labelling index between core biopsy and surgical specimen may be informative of the degree of response of the tumour to the administered therapy, and also have prognostic significance [21].

Examination of lymph nodes after NACT

It may be very difficult to identify axillary lymph nodes after NACT due to atrophy and fibrosis. If the patient has undergone completion axillary dissection (for a positive sentinel lymph node biopsy prior or after NACT, or a clinically positive axilla with cytologic confirmation of the metastasis), it may be useful clearing the axillary fat to facilitate the identification of the lymph nodes. All the fibrotic areas in the fat and around the vessels should be sampled and submitted for histology.

Histologically, the nodes may show treatment effects (fibrotic areas and hyaline scars, aggregates of foamy histiocytes) with or without metastatic tumour. Even in the lymph nodes, residual cancer may only be present as individual tumour cells (isolated tumour cells) scattered within the fibro-inflammatory nodal stroma [17,30]. The clinical implications of isolated tumour cells and micrometastases in the regional lymph nodes after NACT, and especially if they are in a background of treatment effects may be different from those in patients without NACT.

Staging of residual tumour

As anticipated, there are several different systems to stage residual breast cancer after primary systemic therapy. The most commonly used in the clinical trials and in the clinical practice are the AJCC/TNM, the Miller-Payne and the Residual Cancer Burden (RCB) systems.

In the AJCC/TNM system [31], the prefix "y" identifies the post-NACT evaluation, but it uses the very same pT and pN classes as for tumours without previous NACT. Accordingly, if multiple foci of residual invasive tumour are present, the ypT class will be determined by the extent that encompasses all these foci. The cellularity of residual tumour is not taken into account, and therefore this system may lead to an upstaging of the residual tumour.

The Miller-Payne system [32] classifies residual tumour into 5 grades, based on the relative reduction of tumour cellularity from the prior core biopsy to the surgical specimen after NACT. In grade 1, there is no reduction in overall cellularity (no response), whereas in grade 5 there is disappearance of invasive tumour (in situ cancer may be present). Despite lymph node status is not included in this system, the grades have been reported to correlate with disease-free and overall survival.

Finally, the RCB system [33] takes into account the size of primary tumour bed size, the residual cancer cellularity, the percentage of cancer that is DCIS, the number of positive lymph nodes, and the size of the largest lymph node metastasis. All these parameters are combined into 4 possible scores (from RCB 0 -meaning pCR- to RCB III-meaning chemoresistance), and the scores do correlate with long-term survival. Although the system requires the use of a formula, a web-based calculation script is freely available to calculate the score (http://www.mdanderson.org/breastcancer_RCB).

Epilogue

Neoadjuvant systemic treatments will be used more and more often both in clinical research and in the daily practice. Assessing tumour response to the different systemic treatments will become a major responsibility for the pathologists. Harmonization of protocols for macroscopic and microscopic evaluation of tumour response and for assessment of prognostic/predictive markers is urgently needed.

Conflict of interest statement

None to declare.

References

[1] Hortobagyi GN, Ames FC, Buzdar AU, Kau SW, McNeese MD, Paulus D, et al. Management of stage III primary breast cancer with primary chemotherapy, surgery, and radiation therapy. Cancer 1988;62:2507–16.
[2] Valagussa P, Zambetti M, Bonadonna G, Zucali R, Mezzanotte G, Veronesi U. Prognostic factors in locally advanced noninflammatory breast cancer. Long-term results following primary chemotherapy. Breast Cancer Res Treat 1990;15:137–47.
[3] 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.
[4] Schwartz GF, Hortobagyi GN, Masood S, Palazzo J, Holland R, Page D. Proceedings of the consensus conference on neoadjuvant chemotherapy in carcinoma of the breast, April 26–28, 2003, Philadelphia, PA. Hum Pathol 2004;35:781–4.
[5] Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. J Natl Cancer Inst 2005;97:188–94.
[6] Kaufmann M, von Minckwitz G, Bear HD, Buzdar A, McGale P, Bonnefoi H, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: new perspectives 2006. Ann Oncol 2007;18:1927–34.
[7] Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumour and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol 1999;17:460–9.
[8] Chollet P, Amat S, Cure H, de Latour M, Le Bouedec G, Mouret-Reynier MA, et al. Prognostic significance of a complete pathological response after induction chemotherapy in operable breast cancer. Br J Cancer 2002;86:1041–6.
[9] Schneeweiss A, Katretchko J, Sinn HP, Unnebrink K, Rudlowski C, Geberth M, et al. Only grading has independent impact on breast cancer survival after adjustment for pathological response to preoperative chemotherapy. Anticancer Drugs 2004;15:127–35.
[10] Colleoni M, Viale G, Zahrieh D, Pruneri G, Gentilini O, Veronesi P, et al. Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment. Clin Cancer Res 2004;10:6622–8.
[11] Rouzier R, Pusztai L, Delaloge S, Gonzalez-Angulo AM, Andre F, Hess KR, et al. Nomograms to predict pathologic complete response and metastasis-free survival after preoperative chemotherapy for breast cancer. J Clin Oncol 2005;23:8331–9.

[12] Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012;18:633–40.
[13] Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25–32.
[14] Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005;11:5678–85.
[15] Mazouni C, Peintinger F, Wan-Kau S, Andre F, Gonzalez-Angulo AM, Symmans WF, 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.
[16] Curé H, Amat S, Penault-Llorca F, le Bouëdec G, Ferrière JP, Mouret-Reynier MA, et al. Prognostic value of residual node involvement in operable breast cancer after induction chemotherapy. Breast Cancer Res Treat 2002;76:37–45.
[17] Newman LA, Pernick NL, Adsay V, Carolin KA, Philip PA, Sipierski S, et al. Histopathologic evidence of tumour regression in the axillary lymph nodes of patients treated with preoperative chemotherapy correlates with breast cancer outcome. Ann Surg Oncol 2003;10:734–9.
[18] Fisher ER, Wang J, Bryant J, Fisher B, Mamounas E, Wolmark N. Pathobiology of preoperative chemotherapy: findings from the National Surgical Adjuvant Breast and Bowel (NSABP) protocol B-18. Cancer 2002;95:681–95.
[19] Fan F. Evaluation and reporting of breast cancer after neoadjuvant chemotherapy. Open Pathol J 2009;3:58–63.
[20] 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.
[21] 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.
[22] Carder P. Typing breast cancer following primary chemotherapy. Histopathology 1999;35:584–5.
[23] Ellis IO, Galea M, Broughton N, Locker A, Blamey RW, Elston CW. Pathological prognostic factors in breast cancer. II. Histological type. Relationship with survival in a large study with long-term follow-up. Histopathology 1992;20:479–89.
[24] Bottini A, Berruti A, Bersiga A, Brunelli A, Brizzi MP, Marco BD, et al. Effect of neoadjuvant chemotherapy on Ki67 labelling index, c-erbB-2 expression and steroid hormone receptor status in human breast tumours. Anticancer Res 1996;16:3105–10.
[25] Lee SH, Chung MA, Quddus MR, Steinhoff MM, Cady B. The effect of neoadjuvant chemotherapy on estrogen and progesterone receptor expression and hormone receptor status in breast cancer. Am J Surg 2003;186:348–50.
[26] Arens N, Bleyl U, Hildenbrand R. HER2/neu, p53, Ki67, and hormone receptors do not change during neoadjuvant chemotherapy in breast cancer. Virchows Arch 2005;446:489–96.
[27] Burcombe RJ, Makris A, Richman PI, Daley FM, Noble S, Pittam M, et al. Evaluation of ER, PgR, HER-2 and Ki-67 as predictors of response to neoadjuvant anthracycline chemotherapy for operable breast cancer. Br J Cancer 2005;92:147–55.
[28] Quddus RM, Sung JC, Zhang C, Pasqueriello T, Eklund M, Steinhoff MM. HER-2/neu expression in locally advanced breast carcinomas: pre- and post-neoadjuvant chemotherapy. Breast Cancer 2005;12:294–8.
[29] Tacca O, Penault-Llorca F, Abrial C, Mouret-Reynier MA, Raoelfils I, Durando X, 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.
[30] 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.
[31] Sobin L, Gospogdarowicz M, Wittekind C, editors. TNM classification of malignant tumours. 7th ed. Geneva: Wiley-Blackwell; 2009.
[32] Ogston KN, Miller ID, Payne S, Hutcheon AW, Sarkar TK, Smith I, et al. A new histological grading system to assess response of breast carcinomas to primary chemotherapy: prognostic significance and survival. Breast 2003;12:320–7.
[33] Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414–22.