Clinical Science

Neoadjuvant Chemotherapy for Breast Cancer: Moving Beyond Pathological Complete Response in the Molecular Age

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Abstract
This review focuses on neoadjuvant chemotherapy for breast cancer which introduces practical issues for pathologists, including predicting response, optimising specimen handling, size measurement and assessment of residual disease, and recent advances in management of the axilla. The role of neoadjuvant chemotherapy in breast cancer is increasing, and it has become standard of care for high risk Human Epidermal Growth Factor Receptor 2 positive and triple negative breast cancers. The benefits of the neoadjuvant approach extend beyond pathological complete response to tumour downstaging permitting conservative surgical options in the breast and axilla, and assessment of response provides valuable prognostic information to enable escalation and de-escalation of adjuvant therapy to optimise oncological outcomes. Hence histopathologists play a vital role in patient management in the neoadjuvant setting. Optimal patient selection for neoadjuvant chemotherapy requires consideration of pretreatment histopathological and molecular tumour characteristics. Post chemotherapy, tumour staging can be challenging, and changes in criteria for measurement of primary tumour and metastases in the 7th and 8th editions of the TNM have led to confusion amongst pathologists. This review offers practical guidance on specimen handling and measurement of lesion size. Moving forwards more detailed information on degree of response will be required for adjuvant therapy decision making, and the Residual Cancer Burden is emerging as the preferred method for quantifying residual disease not just within clinical trials but in routine practice. Recent advances in management of the axilla are discussed, including the significance of minimal residual disease in the form of isolated tumour cells and micrometastases which portend a worse prognosis in the neoadjuvant setting.
Conclusion. Neoadjuvant chemotherapy now forms part of routine breast cancer management, and detailed histopathological assessment and an understanding of the importance of molecular tumour biology is essential for clinical decision making.

Key Words: Breast Cancer ▪ Neoadjuvant Therapy ▪ Staging ▪ Grading Response.

Introduction

Neoadjuvant chemotherapy (NACT) has evolved from treatment of locally advanced breast cancer to routine management of biologically aggressive disease, particularly oestrogen receptor negative (ER-) and/ or human epidermal growth factor 2 positive (HER2+) cancers. The neoadjuvant approach shows similar survival outcomes to adjuvant therapy, but offers potential advantages in both standard clinical care and clinical trial settings (1). Firstly, response to neoadjuvant therapy with complete eradication of disease or a reduction of tumour volume enables less aggressive surgical options, with

the potential for breast conservation surgery (BCS) in patients that would have required mastectomy pre-treatment (2). There is also a growing body of evidence to support the role of sentinel node biopsy (SLNB) following NACT in both node negative and node positive patients, leading to avoidance of axillary clearance (ALND) following a complete response in the axilla (3-5). Interestingly, in our own multidisciplinary meetings, it is now the surgeons rather than the oncologists driving decisions regarding NACT. Tumour downstaging to enable conservative procedures can reduce surgical morbidity without compromising oncological out-

comes, however NACT is not the correct approach for all cases and careful patient selection based on clinical features and histological and molecular tumour subtypes is essential to optimise results.

Perhaps even more importantly, assessment of response to NACT provides valuable prognostic information that is increasingly used to guide further adjuvant therapy (6). Complete pathological response (pCR) shows an association with survival outcomes across all molecular subtypes, although this is strongest for ER- and/or HER2+ disease (1, 7). As a result, pCR has been approved by the U.S. Food and Drug Administration (FDA) as a surrogate outcome to survival for neoadjuvant clinical trials in high risk breast cancer (8). The neoadjuvant context provides faster results in smaller cohorts of patients, and alongside novel adaptive trial designs such as ISPY provides exciting potential to screen new agents resulting in more rapid introduction of effective drugs into clinical practice (9, 10). Furthermore, patients who experience a pCR may not benefit from further adjuvant therapy, and there are trials looking at de-escalation of adjuvant therapy in complete responders (11). Hence accurate identification of pCR is vital for ongoing patient management, and requires careful and methodical histological assessment beginning with gross specimen handling.

At the other end of the spectrum, patients who show a limited response to NACT have a poor prognosis. Recent trials, including the KATHER-INE and CREATE-X trials, have shown improved survival outcomes with additional adjuvant therapy in incomplete responders with HER2+ and triple negative breast cancer (TNBC) respectively (12, 13). However, non-pCR encompasses a wide variation of response from almost complete response with minimal residual disease (MRD), to minimal or absent response with significant residual tumour. Some series have shown similar survival outcomes for patients with MRD to those who undergo a pCR, however the impact of residual disease volume on survival outcomes varies by molecular tumour subtype (14, 15). Assessment of the degree of response beyond pCR will form an integral part of patient care moving forwards.

Tumour staging post NACT also shows a strong association with survival outcomes (16, 17). Measuring residual tumour size can be challenging, particularly when there has been patchy response across the tumour bed. Definitions of size measurements used for staging in both the breast and axillary lymph nodes have evolved across the 6th, 7th and 8th editions of the TNM, leading to confusion amongst pathologists (18-20). Accurate staging is essential not only in determining patient prognosis, but to generate reliable population based data from cancer registries around the world.

Hence, the pathologist plays a key role in determining optimal patient care in the neoadjuvant setting. This review will focus on some of the key practical issues for pathologists, including predictors of response, optimising specimen handling, size measurement and assessment of residual disease, and recent advances in management of the axilla.

Predictors of Response to NACT

Response to NACT, including the likelihood of achieving pCR and its association with prognosis, is strongly linked to tumour biology (1, 21-25). This has important implications for clinical decisions regarding whether to give neoadjuvant versus adjuvant therapy, particularly if the goal is tumour downsizing to enable conservation. Breast cancer is generally divided into 3 broad molecular groups; luminal (ER+/HER2-), HER2+ and TNBC (25). HER2+ and TNBC show the greatest response to NACT, but even these tumour types contain subgroups with different behaviour.

NACT response of HER2+ breast cancers largely depends on ER status. ER+/HER2+ cancers given standard chemotherapy without HER2 targeted agents show a pCR rate of 18%, rising to 31% with the addition of trastuzumab (1). In contrast, ER-/HER2+ tumours have a much higher pCR rate of 30% without trastuzumab and 50% with trastuzumab; the addition of pertuzumab gives pCR rates as high as 80% (26). The association between pCR and survival outcomes is also much stronger for ER-/HER2+ cancers (HR 0.29; 95% CI 0.17-0.50) without trastuzumab and HR 0.08(95% CI 0.03-

0.22) with trastuzumab) than for ER+/HER2+ cancers where it does not reach significance (HR 0.57(95% CI 0.31-1.04) without trastuzumab and HR 0.56 (95% CI 0.23-1.37) with trastuzumab). ER+/HER2+ tumours also show a different pattern of recurrence with late relapses, in comparison to ER-/HER2+ disease where the majority of relapses occur within the first 5 years after diagnosis (27).

Similar differences are seen when clinically defined HER2+ tumours are classified as HER2-Enriched or luminal subtypes by gene expression profiling (28). Within the NOAH trial only 55% of tumours were HER2-Enriched, with 21% luminal, 7% basal and 18% normal-like. The pCR rate was significantly higher in HER2-Enriched compared with luminal HER2+ tumours (53% versus 29% respectively), and there was a larger improvement in event free survival with the addition of trastuzumab indicating greater benefit from HER2 pathway blockade (29). These findings have been confirmed in a meta-analysis of 16 neoadjuvant trials which showed a significant association with HER2-Enriched subtype and pCR in both ER+ and ER- disease (30). Recent reviews suggest intrinsic subtype as defined by PAM50 is a valuable adjunct to clinical receptor status in making decisions about NACT (27, 31). Studies have also suggested a relationship between higher HER2 protein expression, gene copy number >10 and HER2:CEP17 ratio >4.5 and improved pCR rates following NACT with trastuzumab (32-34). Cancers that are HER2 3+ on immunohistochemistry show higher pCR rates than those that are 2+ with *HER2* gene amplification on FISH (35). Presence of intratumoural heterogeneity for HER2, more commonly found in association with equivocal cases and polysomy/ co-amplification of the *HER2* and *CEP17* probe sites, is also associated with lower pCR rates and poorer survival outcomes; in one series 10% of cases showed HER2 heterogeneity of which none went on to pCR (31). Newer drug conjugates which use the HER2 receptor to enter cells and have a bystander effect, such as trastuzumab-deruxtecan, may prove to be an effective treatment option in these difficult cases. Approximately one third of apocrine carcinomas are HER2+; a recent

study found androgen receptor (AR) positivity was associated with improved response to NACT with trastuzumab, and better survival outcomes in ER- disease (36). Other tumour features that have been associated with response to NACT in HER2+ disease include higher levels of tumour infiltrating lymphocytes (TILs), and presence of *PIK3CA* alterations has been associated with lower pCR rates and poorer survival (31).

TNBC form an even more heterogeneous group, perhaps unsurprising given they encompass several histological subtypes including salivary type and metaplastic carcinomas. Overall, TNBC show a pCR rate of 34% with a very strong association between pCR and survival outcomes (HR 0.16; 95% CI 0.11-0.25) (1). Modern chemotherapy regimens with inclusion of platinum agents have increased the pCR rate to over 50% (37). Gene expression analysis identified six different subtypes of TNBC which was revised to four subgroups; two basal-like, a mesenchymal, and a luminal AR group (38). The luminal AR group has high expression of genes related to AR signalling, and a response pattern similar to ER+ cancers with a relatively low pCR rate (29%) but better survival outcomes than other TNBC subtypes (39). The basal-like 1 group has a signature enriched for genes involved in proliferation and DNA damage repair and shows the highest pCR rate (49%) with intermediate survival outcomes, whilst the basal-like 2 group driven by growth factor receptor signalling has a low pCR rate (18%) and poor survival.

The original 6 types included an immune modulatory group with a pCR rate of 30% and a relatively good prognosis; this signature is now believed to reflect infiltration with TILs which is associated with chemotherapy response and improved outcomes in TNBC (38, 40). A recent meta-analysis confirmed the relationship between increasing levels of TILs with pCR, disease free survival (DFS) and overall survival (OS) in TNBC (41).

Metaplastic carcinoma is a subtype of TNBC associated with poor response to NACT and adverse survival outcomes. This reflects the difference in molecular profile compared to NST TNBC, with lower levels of genomic instability and a higher

rate of EGFR and PI3K and Wnt signalling abnormalities (42, 43). In one single institution series of 18 patients, 7 showed no response or progressed whilst on treatment, and only 2 had a pCR (44). In another single institution series, there were 29 cases of metaplastic carcinoma that received NACT with a pCR rate of 17% (45). Interestingly, 4 of the 5 cases that had a pCR were matrix-producing metaplastic carcinomas with a pCR rate of 24% for this subtype, although pCR or tumour type were not associated with survival. There are several special types of TNBC associated with good prognosis, including adenoid cystic carcinoma, secretory carcinoma, the recently described tall cell carcinoma with reversed polarity (TCCRP), and low grade adenosquamous and fibromatosis-like variants of metaplastic carcinoma, where systemic therapy is not indicated (Figure 1). These tumours do not have the genomic instability typical of NST type TNBC, with adenoid cystic and secretory carcinomas characterised by translocations of *MYB-NFIB* and *ETV6-NTRK3* genes respectively, and TCCRP with mutations in the *IDH2* gene (46). The important thing is to recognise these cancers on core biopsy to prevent the patient from receiving unnecessary NACT. If the diagnosis is uncertain then primary surgery should be recommended.

Luminal, or ER+, breast cancers are generally associated with low pCR rates of 0-16% (1). In the intrinsic subtype classification, they are divided into luminal A with low proliferation and high expression of ER signalling genes, and luminal B cancers with high proliferation and/ or HER2 positivity (47, 48). Low grade ER+ tumours with low proliferation have a very low pCR rate (2-7%) but retain an excellent prognosis due to their response to endocrine therapy, and do not derive any additional benefit from chemotherapy (1, 48-52). Many invasive lobular cancers fall into the luminal A or low risk subtypes on gene expression profiling, and several studies have shown poor response to NACT with lower pCR rates than grade and ER matched ductal NST cancers, as well lower rates of tumour downstaging and BCS (53-57). In one study, lobular histological type predicted absence of response to NACT (58).
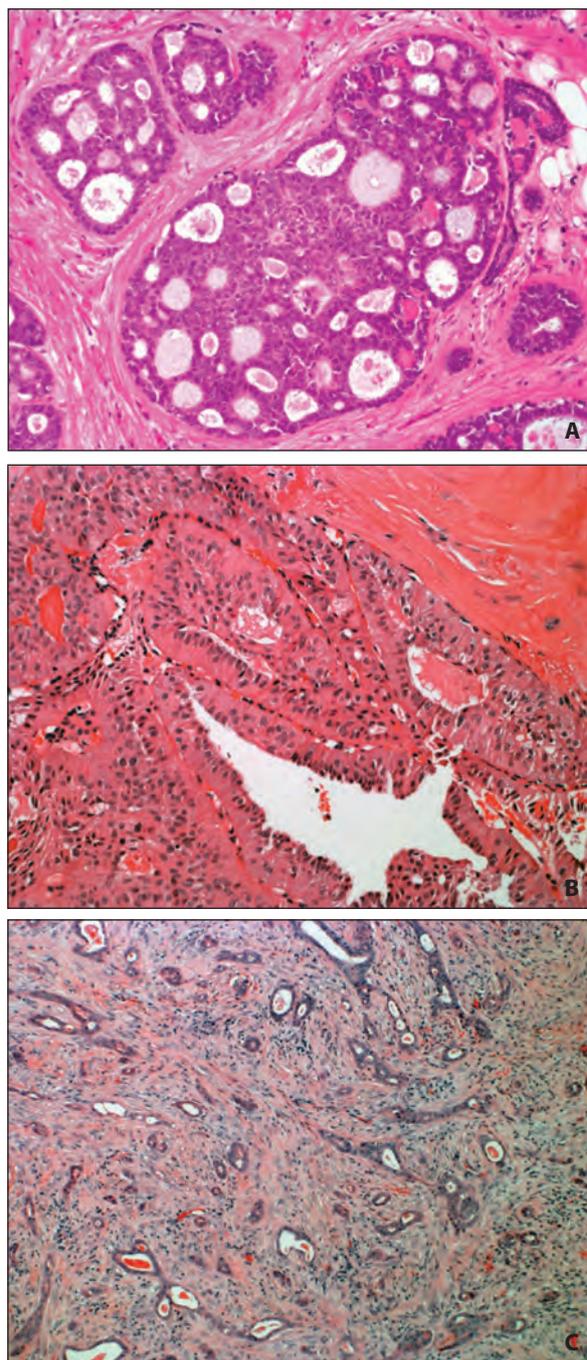

Figure 1. Special types of triple negative breast cancer associated with good prognosis: A) Classical adenoid cystic carcinoma (× 20); B) Tall cell carcinoma with reversed polarity (×10); C) Low grade adenosquamous carcinoma (×20).

However, there is a subset of ER+/HER2- breast cancers with a worse prognosis in which chemotherapy is indicated; features associated with in-

creased responsiveness to NACT include grade 3, PR negativity and a high Ki67 labelling index (55). In the Cortazar analysis, grade 3 ER+ tumours had a pCR rate of 16%, with pCR showing a significant association with improved OS with a HR of 0.29 (95% CI 0.13-0.65) (1). High Ki67 has been shown to predict pCR in ER+/HER2- cancers, however there are difficulties interpreting the literature due to differences in methodology and variation in cut points (59-61). The most recent ASCO-CAP guidelines recognise a Low Positive ER group with nuclear staining in 1-10% of cells, representing less than 5% of ER+ cancers (62); many of these tumours have a basal-like gene expression profile (63, 64). In one trial, 18% of ER+/HER2- cancers were of basal intrinsic subtype, and these tumours had a pCR rate of 32% (65). This reinforces data from HER2+ cancers that intrinsic subtype provides additional information regarding benefit of NACT.

Specimen Handling

Surgical excisions post NACT are becoming increasingly common, and represent the most complex breast specimens handled by histopathology laboratories. Methodical detailed gross specimen handling is essential for accurate determination of pCR, assessment of response and tumour staging. For this to occur, communication between pathologists and the multidisciplinary team, with provision of adequate clinical information on pathology request forms is vital (66). At a minimum, the clinical notes need to state neoadjuvant therapy has been given and it's nature, with a clear description of the number of tumour foci and their location within the breast; a schematic diagram indicating the site of tumour/s is very helpful. Where available, details of tumour size on pre-treatment imaging should also be provided, as sampling should include the area of the original pre-treatment tumour bed, which may extend beyond macroscopically detectable residual disease.

Basic principles of specimen handling also apply in the neoadjuvant setting. Where national guidelines exist these should be followed. Good fixation is vital for subsequent histological interpretation, and specimens should be sliced when fresh if possible to ensure formalin penetration. When delays are likely, one option is to instruct surgeons on how to slice larger specimens such as mastectomies to aid fixation without compromising subsequent pathological evaluation.

Residual tumour is often more ill-defined and softer post NACT, especially if there has been a good response to treatment, making it more challenging to detect on gross assessment. Textural changes may be found on palpation, even if there is no visible tumour bed. Placement of fiducial marker clips at the time of diagnosis is extremely helpful in localising the tumour bed when there is no gross residual lesion, and is recommended even in patients planned for mastectomy to aid localisation of the tumour bed (67). Gel foam or larger metallic clips may be seen on slicing; gel foam clips appear as a cyst filled with gelatinous substance (68). Alternatively, the markers can be identified on x-ray of the specimen slices. Where the tumour was associated with malignant calcification this can also be identified on specimen x-ray, although calcifications can increase or decrease with NACT, and the presence of residual calcification does not show a good correlation with pathological tumour response (69).

As residual tumour is harder to delineate macroscopically, it is typically necessary to take more sections than in the adjuvant setting. Blocks should include any gross residual disease and/ or marker clips, and adjacent uninvolved tissue to encompass the extent of the tumour on pre-treatment imaging (67, 70). For small wide local excision (WLE) specimens it is prudent to submit the entire specimen for histological evaluation. For larger WLE or mastectomy specimens, close clinical-pathological correlation guided by the imaging findings to localise the site of the tumour bed is preferable to exhaustive blind sampling. There is some guidance on the number of blocks required for diagnosis of pCR and assessment of response. The US FDA have recommended taking one block for every cm of tumour size on pre-treatment imaging, or at least 10 tumour blocks, whichever is greater (8). In guid-

ance published in 2015, the international Residual Disease Working Group advised taking blocks representing the full face of the pre-treatment tumour area from every 1-2 cm slice of the specimen, up to a maximum of 25 blocks (70). To determine the Residual Cancer Burden (RCB), described below, five sections representing the maximum cross section of the tumour bed is sufficient to estimate residual tumour cellularity (71). If clip site or tumour bed changes are not present in the initial sections, it may be necessary to review the specimen and take further blocks. Additional routine blocks, such as those for assessment of margins, should also be taken as per the adjuvant setting.

Precise description of where blocks have been taken is essential for reconstruction of the specimen from the glass slides to enable size and cellularity estimates. A visual annotation of the position of blocks on sketched diagrams, photographs of specimen slices, or copies of specimen radiographs is the best way to do this, and is invaluable in subsequent reporting of the microscopic findings. Where available, large tissue cassettes or 'megablocks' are helpful for measurement of lesion size and assessment of margins.

Defining pCR

The ultimate goal of NACT is the attainment of pCR, i.e. the complete eradication of invasive disease. The broadest definition of pCR is the absence of residual invasive disease in the breast and axilla (ypT0 ypN0 and ypTis ypN0 – the y prefix indicating post NACT). The overall rate of pCR decreases according to the stringency of definition used; in a pooled analysis the rate of pCR was 22% for no invasive tumour in breast only, 18% for no invasive tumour in breast and axilla, and only 13% for no invasive tumour or DCIS in the breast and no disease in the axilla (1). Early clinical trials considered pCR in the breast only, however up to 4% of patients who have a pCR in the breast will have residual disease in the axilla (72). Residual disease in the axilla, including the presence of isolated tumour cells (ITCs) and micrometastases, is associated with worse survival outcomes independent of tumour response in the breast. Several series have shown number of involved nodes and size of largest metastasis post NACT to be the strongest determinants of overall survival (72-74). Hence, currently accepted definitions of pCR require absence of residual disease in the axilla also. Importantly, whilst ITCs are staged as ypN0(i+), their presence indicates treatment resistant residual disease and is not regarded as pCR (19).

Whether the presence of residual DCIS should be considered pCR is controversial. A pooled analysis found no difference in survival outcome with residual DCIS alone (1), however in a cumulative analysis of their trials the German Breast Group found residual DCIS was associated with worse DFS but not OS (7). This may be due to increased local recurrence risk with incompletely excised DCIS, although a differential response in DCIS and invasive components has been reported in HER2+ disease (75). The histopathology report should include a comment on the presence of residual DCIS in the breast regardless of the definition of pCR used, along with measurement of its extent and proximity to margins as per the minimum dataset in the adjuvant setting.

A rare but challenging scenario with respect to staging is the presence of lymphovascular invasion (LVI) in the absence of a residual invasive tumour focus. Firstly, ensure that the tumour bed has been adequately sampled and invasive tumour has not been missed. An alternative possibility is invasive disease or DCIS with retraction artefact; immunostaining for a lymphatic marker such as D2-40 (podoplanin) may be helpful in distinguishing the two (Figure 2) (70). When presence of residual LVI alone is confirmed, although this is strictly staged as ypN0, it should not be regarded as pCR, similar to the scenario with ITCs above. If the area of LVI is localised, the LVI itself can be measured and cellularity assessed to quantify residual disease and calculate the RCB. This pattern of residual disease has been associated with poor survival outcomes in small series (76, 77), although one slightly larger study suggested that pre and post treatment nodal involvement are also important (78).
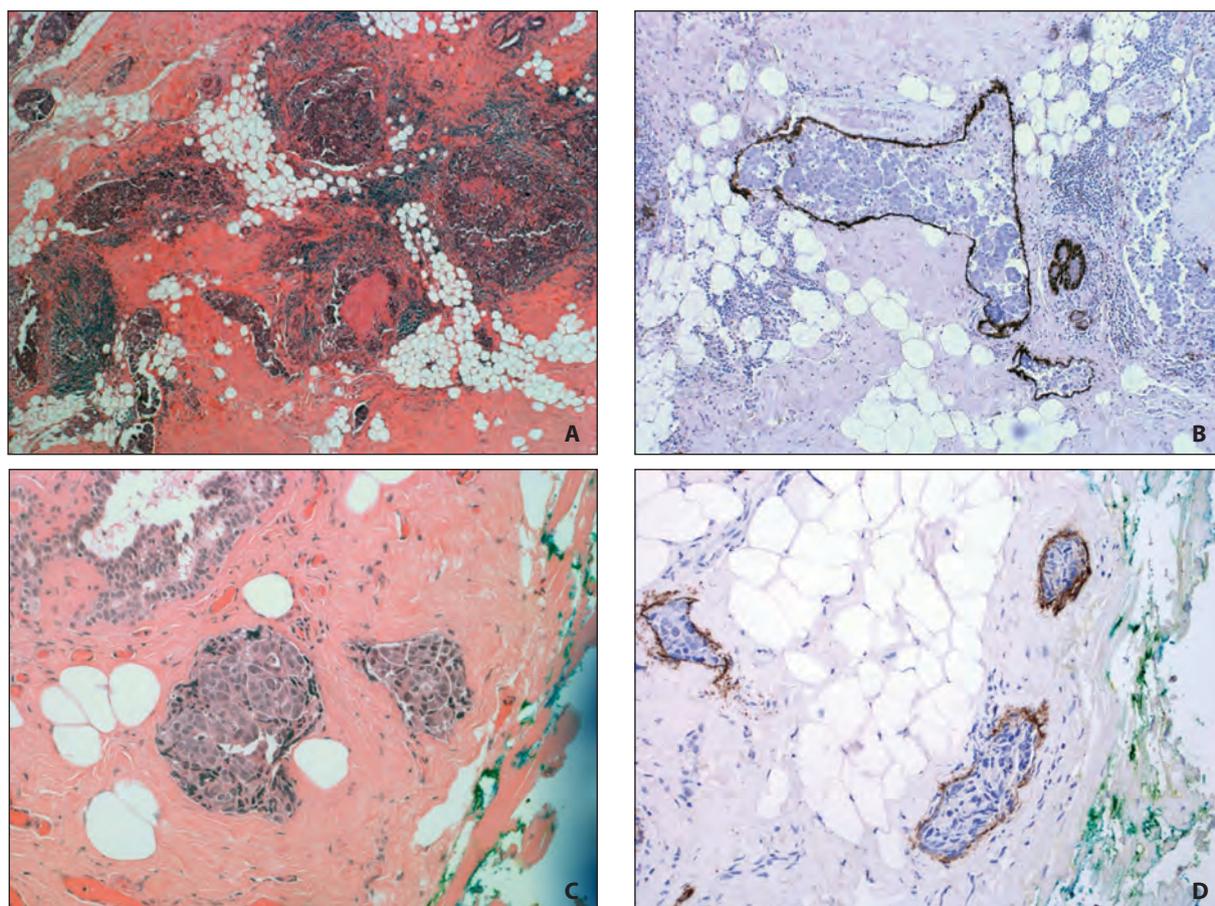
Figure 2. Residual invasive carcinoma predominantly in the form of LVI (A); D2-40 immunohistochemical staining distinguishing tumour in lymphatic spaces from invasive disease (B); Tumour adjacent to a margin confirmed as LVI on D2-40 staining (C+D).

TNM Staging

Traditional staging systems, such as the TNM and Nottingham Prognostic Index (NPI), retain prognostic significance following NACT (16, 79). Pathological TNM staging post NACT is given a y prefix. There have been modifications to how primary invasive tumour and metastases are measured for staging purposes in the 7th and 8th editions (18, 19), which are summarised in Table 1. Although only currently applied following primary surgery, there is emerging evidence that the AJCC prognostic stage incorporating grade and receptor status introduced in the 8th edition is also predictive of outcome post NACT and may provide better discrimination of prognostic groups (17, 80). Future stag-

ing systems incorporating both molecular tumour characteristics and tumour response are required.

There are two main patterns of response seen on serial imaging in patients receiving NACT (81, 82). The first is concentric shrinking, where there is a single tumour mass that progressively decreases in size. Measurement of tumour size in this situation is relatively straight forward as there is a single invasive tumour focus. Tumour bed changes may extend beyond the invasive carcinoma, however it is the maximum residual invasive cancer size that is measured; surrounding stroma without invasive tumour is excluded (Figure 3) (66, 70, 71).

The second pattern is the scatter or Swiss cheese pattern, where there is a patchy response with scattered foci of residual enhancement across the tumour bed. This pattern is a reflection of in-

Table 1. Definitions Used for Primary Tumour and Metastasis Measurement in Residual Cancer Burden and Subsequent Editions of the TNM Staging System

| Staging system	Size measurement breast	Size measurement nodal metastases
Residual Cancer Burden	Maximum size residual invasive disease in two dimensions. Scattered foci measured as a single lesion including areas of intervening fibrosis.	Maximum dimension metastatic focus including associated fibrosis. ITCs regarded as positive.
AJCC/ UICC 6th edition	Maximum size residual invasive disease in one dimension. Scattered foci measured as a single lesion including areas of intervening fibrosis.	Maximum dimension metastatic focus including associated fibrosis. ITCs regarded as negative.
AJCC/ UICC 7th edition	Measurement largest contiguous tumour focus, with use of (m) classifier if multiple deposits present across the tumour bed	Maximum dimension size of metastatic focus including associated fibrosis. ITCs regarded as negative.
AJCC/ UICC 8th edition	Measurement largest contiguous tumour focus, with use of (m) classifier if multiple deposits present across the tumour bed	Maximum dimension of largest contiguous tumour cell deposit excluding associated fibrosis. ITCs regarded as negative.

ITCs=Isolated tumour cells.
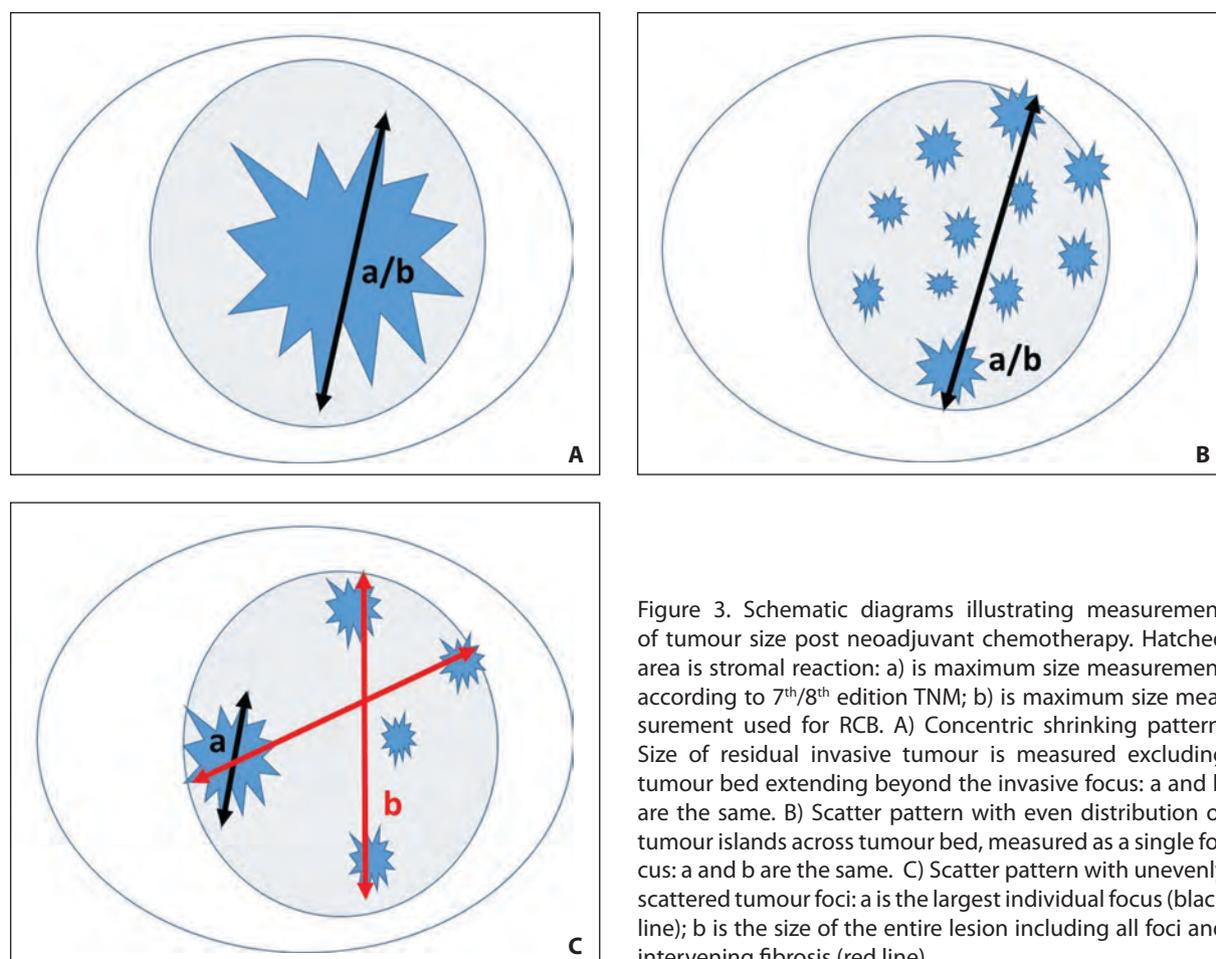
Figure 3. Schematic diagrams illustrating measurement of tumour size post neoadjuvant chemotherapy. Hatched area is stromal reaction: a) is maximum size measurement according to 7th/8th edition TNM; b) is maximum size measurement used for RCB. A) Concentric shrinking pattern. Size of residual invasive tumour is measured excluding tumour bed extending beyond the invasive focus: a and b are the same. B) Scatter pattern with even distribution of tumour islands across tumour bed, measured as a single focus: a and b are the same. C) Scatter pattern with unevenly scattered tumour foci: a is the largest individual focus (black line); b is the size of the entire lesion including all foci and intervening fibrosis (red line).

tratumoural heterogeneity leading to a differential response to NACT. At the histological level, this is seen as separate nests and islands of tumour cells dispersed within an ill-defined background of reactive fibrous stroma (Figure 4).
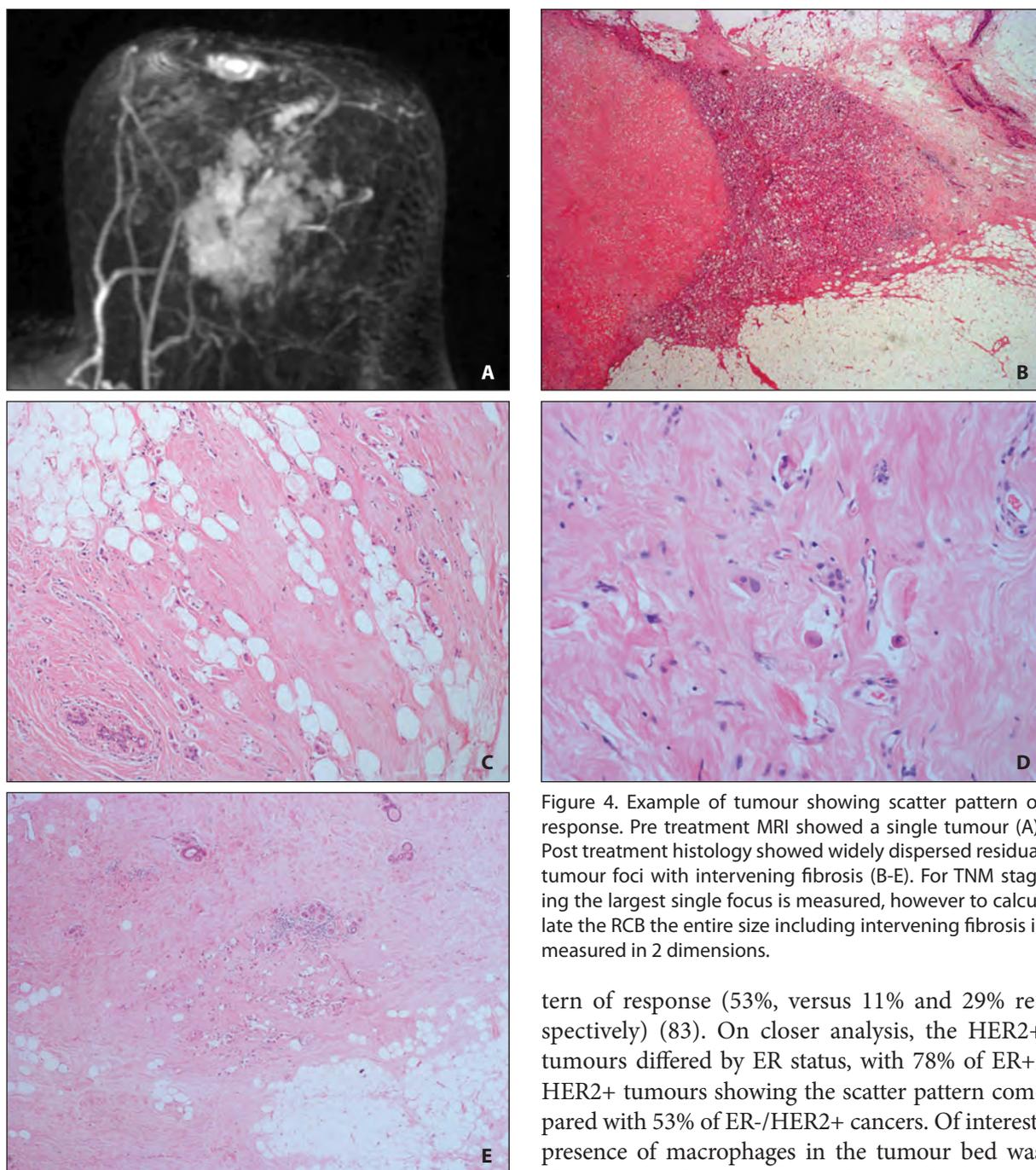
Figure 4. Example of tumour showing scatter pattern of response. Pre treatment MRI showed a single tumour (A). Post treatment histology showed widely dispersed residual tumour foci with intervening fibrosis (B-E). For TNM staging the largest single focus is measured, however to calculate the RCB the entire size including intervening fibrosis is measured in 2 dimensions.

Perhaps unsurprisingly, the pattern of response has been shown to correlate with molecular subtype. In one series looking at histological findings, TNBC was more likely to show the concentric shrinking pattern, whilst ER+/HER2- and HER2+ tumours more commonly showed the scatter pattern of response (53%, versus 11% and 29% respectively) (83). On closer analysis, the HER2+ tumours differed by ER status, with 78% of ER+/HER2+ tumours showing the scatter pattern compared with 53% of ER-/HER2+ cancers. Of interest, presence of macrophages in the tumour bed was also associated with TNBC, whereas elastosis and myxoid change was more common in ER+/HER2- cancers. In contrast, the study of Ballesio et al looked solely at MRI patterns of response and found that ER-/HER2+ showed a concentric pattern, whilst TNBC showed a multinodular pattern (82).

The scatter pattern has been associated with a higher locoregional recurrence (LRR) rate post

breast conservation surgery and increased risk of positive margins. Standard definitions of clear margins as 'tumour at ink' are likely to be inadequate in this context, and if residual invasive tumour lies in close proximity to the margin with transection of the tumour bed consideration should be given to re excision (2). The MD Anderson group identified four features associated with increased risk of LRR post NACT; clinical nodal stage 2/3, residual invasive tumour size >2 cm, scatter/ multifocal pattern of residual disease and presence of LVI (84). A recent study found no difference in LRR rates between a margin <1 mm and wider margins of excision, although numbers were too small for meaningful subset analyses (85). When assessing margin status in BCS specimens post NACT, it is important to comment on the presence of tumour bed at the inked margin, however this is not an indication for further surgery in the absence of invasive tumour or DCIS. When the clip site/ tumour bed is located centrally within the specimen and has been well sampled, then excision is most likely adequate even if tumour bed extends to margins. However, if the clip site/ tumour bed lies at the edge of the specimen this should be noted in the histopathology report, and multidisciplinary discussion is needed to determine if the tumour bed has been accurately targeted and adequately sampled.

Accurate determination of lesion size can be particularly challenging with the scatter pattern, and this is further complicated as the TNM and various national and international guidelines differ in their approach to what is measured (70, 86). The original approach was applied in TNM 6th edition (20). Where there is a single lesion present on pre-treatment imaging and the tumour cells are present within a reactive stromal tumour bed, then the residual disease is treated as a single tumour with the maximum extent being the area involved by all the residual islands of tumour cells including intervening stroma; i.e. residual islands of tumour cells, although separated, are treated as a single lesion and measured together (Figure 3, Table 1). As above, tumour bed beyond the residual invasive foci is not included. This is the tumour size measurement used to calculate the RCB, and adopted

by the U.K. Royal College of Pathologists (87, 88), and has been shown to correlate with survival (16).

The method of size measurement was amended in 7th edition TNM, whereby if the residual tumour consists of multiple nests in a fibrotic stroma, the largest contiguous focus of invasive carcinoma is measured and used for ypT staging, with the 'm' modifier to indicate multiple tumour foci are present (18, 19). So in simple terms, the largest single tumour focus is measured and this is used for TNM staging; other foci and the associated stromal background are NOT included. Confusion arises in what precisely is meant by a 'contiguous focus', and an element of practical judgement is required. My approach is to look at the way the residual disease is distributed across the tumour bed; if there are discrete foci situated some distance from each other, I regard them as separate foci and measure them individually. If the tumour foci are distributed relatively evenly across the tumour bed, then I measure it as a single large focus (Figure 3). For this situation, a more detailed descriptive report including both measurements is often best; for example 'Residual invasive carcinoma is present as scattered islands of cells extending across a tumour bed 52 mm by 36 mm, the largest single focus measuring 16 mm in maximum dimension'. This scenario would have a T classification of ypT1b(m). When there were multiple tumours present on pre-treatment imaging, the residual tumour foci are separated by intervening normal breast tissue, or are morphologically distinct with different grade and/ or histological subtype, then they should be regarded as distinct tumour foci and measured independently. Response should be assessed separately for each focus.

Evaluation of nodal metastases can also be more complex post NACT. The number of positive nodes, the size of the largest metastatic deposit measured microscopically and the presence of extracapsular extension should be reported. The presence of fibrosis or evidence of regressed metastatic disease should be documented; metastasis with complete regression has an intermediate prognosis to a true negative axilla, and an estimate of the number of positive nodes pre-treatment will

influence decisions to give adjuvant regional radiotherapy (70). If a node was clipped pre-treatment, then presence of the clip site should be documented and specific comment made as to presence of residual disease and treatment effect in the clipped node. As with the primary tumour, there is a lack of agreement in how to measure disease in this setting that generates confusion amongst pathologists. In the 6th and 7th edition TNM the approach was to measure the size of the entire area involved by metastatic tumour including intervening fibrosis (18, 20); as in the breast, this is the distance between tumour cells, and fibrous tissue extending beyond metastatic tumour cells is excluded. This is also the maximum metastasis size measurement used for calculating the RCB, and has been

associated with survival (71, 72, 74). The 8th edition TNM has changed the method of measuring metastases to the size of the largest contiguous focus in the node not including tumour associated fibrosis (19). According to the definition in adjuvant disease, a contiguous focus is tumour cells directly in contact with one another without intervening lymphocytes. When there has been good response to NACT, residual metastatic disease is often present as scattered single cells within a reactive fibrous background and this is now defined as ITCs under the 8th edition (Figure 5). This could potentially downstage nodal involvement in a significant number of patients, and again an element of clinical judgement is required. My personal approach, as with the primary tumour, is to look at
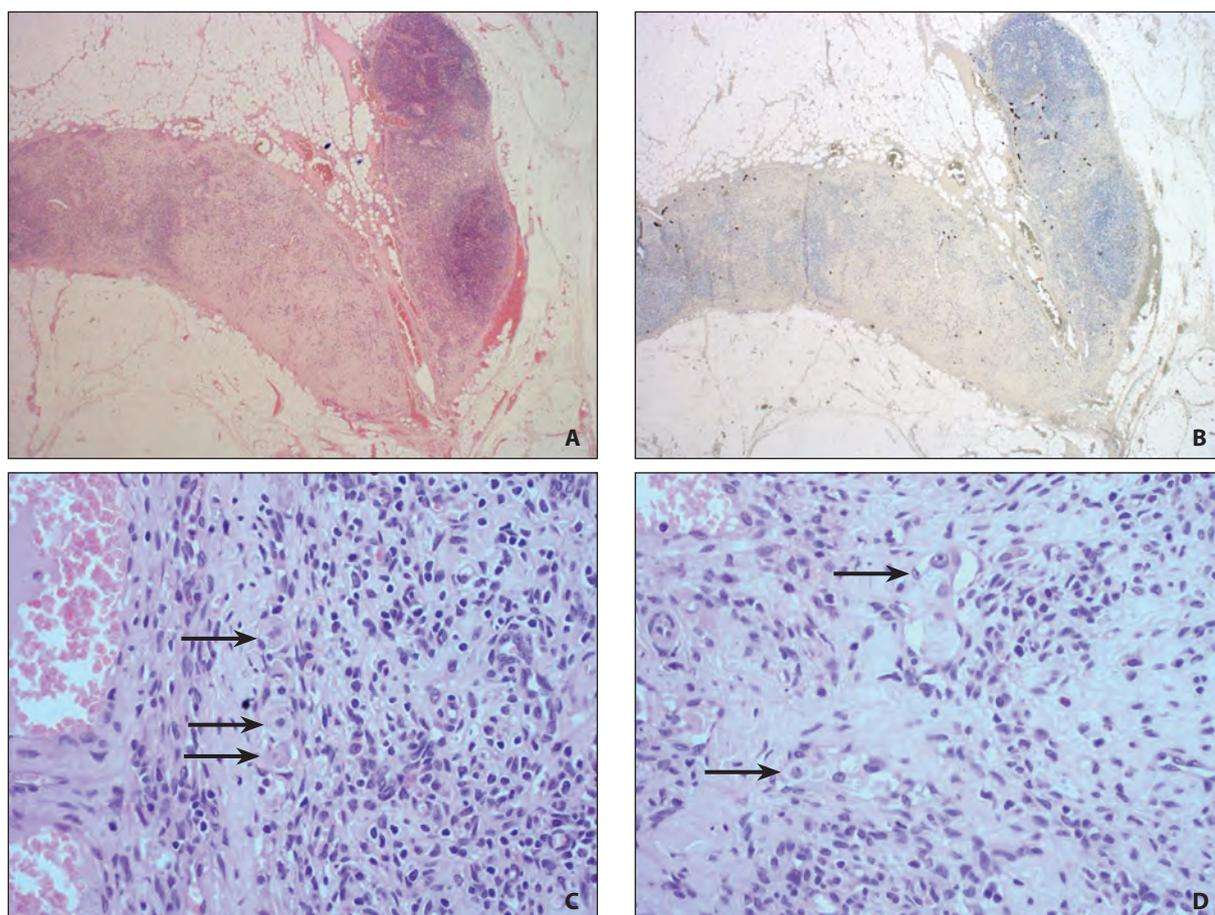

Figure 5. Lymph node post chemotherapy showing an area of fibrosis containing scattered single cells and small clusters, classified as isolated tumour cells in the 8th edition TNM: A) Low power H&E showing area of fibrosis; B) Low power cytokeratin stain highlighting distribution of residual tumour cells; C-D) Higher power images of residual tumour cells (bold arrows) on H&E (×40 magnification).
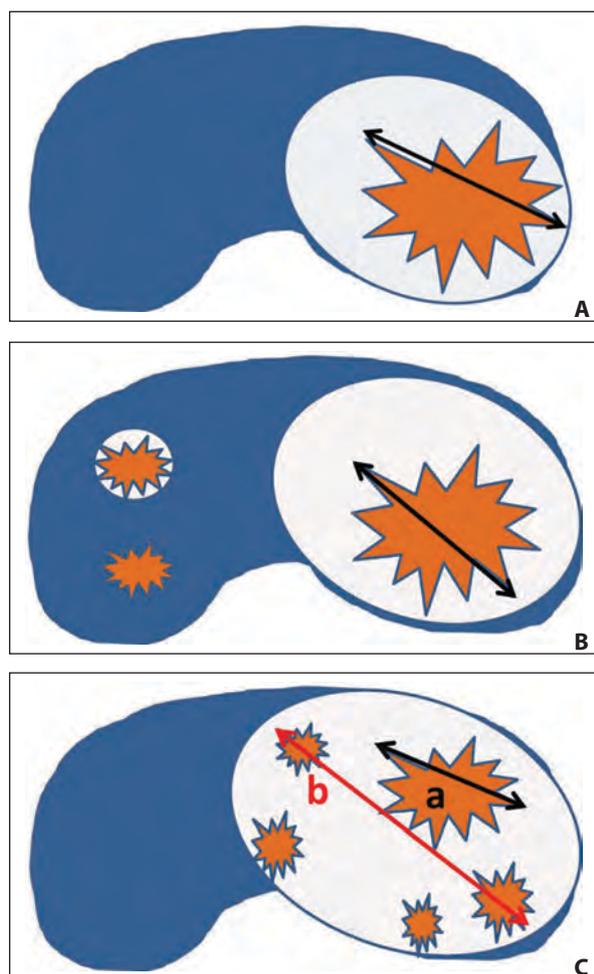
Figure 6. Schematic diagram illustrating measurement of metastases post neoadjuvant chemotherapy. Hatched area represents associated fibrosis. A) Single metastatic focus within area of fibrosis. Measure extent of tumour only, not background fibrosis extending beyond tumour. B) If multiple separate foci of tumour, with or without fibrosis, them measure the largest single focus. C) Scattered metastatic foci in a single area of fibrosis: a) size of largest single contiguous cluster of cells excluding background fibrosis used for TNM staging (black line); b) maximum size including all foci and intervening fibrosis (red line) used to calculate the RCB.

the pattern of spread of the tumour cells across the metastatic deposit; if they form discrete foci a distance apart then these should be measured individually, however if they are evenly dispersed cells/foci across the area of fibrosis I measure the entire lesion as a single deposit (Figure 6).

The interpretation of ITCs is a particular source of controversy in the neoadjuvant setting (70).

ITCs are handled the same way as in the adjuvant setting in the TNM Staging System and are classed as node negative [ypN0(i+)] (19), whereas in the UK reporting guidelines nodes containing ITCs should be counted as positive (87). Regardless of whether they are considered positive or negative, ITCs post NACT represent tumour cells that have persisted despite systemic therapy and have different significance to the adjuvant setting and it is agreed they should not be regarded as axillary pCR. There is considerable evidence that the presence of any residual tumour cells in the axillary lymph nodes following NACT, even in the form of ITCs, is associated with worse prognosis (72-74). In a recent series examining a US National Cancer Database (NCD) cohort, ITCs were associated with poorer survival outcomes with 83% 5 year OS compared with 89% for ypN0; this was present in patients that were cN0 and cN1 pre-treatment (66% and 81% increase in mortality respectively), with the greatest impact on TNBC (89).

Assessing Response

Whilst early clinical trials showed a drop in proportion of cases classified as pCR following central histology review compared with local reports (90), our own experience with the ARTEMIS trial showed excellent agreement between source laboratory reports and central review with respect to pCR (91). However, in an audit of local pathology reports as part of the trial, only 45% of reports included an assessment of tumour response in the breast, dropping to 30% for response in the axillary lymph nodes (92). A similar review of external pathology reports is being undertaken as part of the UK multicentre PARTNER trial, and whilst most reports now include a general comment on presence or absence of response in both the primary tumour and axillary nodes, the majority still do not incorporate formal grading of response (unpublished data).

There are two main approaches to assessment of residual disease post NACT. The first examines actual response by comparing tumour cellularity before and after treatment. Response to NACT is

often accompanied by a reduction in tumour cellularity, and this is associated with improved survival outcomes. Comparison between pre and post treatment cellularity forms the basis for several grading systems of response, including the Chevallier, Sataloff, Miller-Payne and Pinder systems (93-96). The second approach is quantification of residual disease post NACT by looking at invasive tumour size and cellularity, the main example of which is the RCB proposed by Symmans et al. (71). Newer systems such as the Neo-Bioscore have been developed that incorporate tumour molecular profile and biomarkers such as Ki67, although these are currently not in widespread use (60, 97, 98). The different systems have advantages and disadvantages and at present there is no one universally agreed system; readers are referred to review articles comparing the different systems (99-101). The important thing for pathologists is to work closely with their oncology colleagues to agree which system to use.

Residual Cancer Burden

The RCB is presently the most widely used system and will be described in more detail; it has been well validated, is simple and reproducible (91, 102, 103), and shows a strong association with survival outcomes across all molecular subtypes. As a result the RCB has been incorporated in the soon to be released International Collaboration on Cancer Reporting (ICCR) minimum dataset for breast pathology reporting post NACT. The RCB website provides detailed instructions on how to assess the RCB score, including macroscopic specimen handling, a visual guide to estimating the percentage of residual tumour cells, and an online calculator that provides both the numerical RCB score and RCB class (88).

The RCB incorporates four variables; maximum invasive tumour size measured in two dimensions, average residual invasive tumour cellularity, number of positive lymph nodes and size of largest metastasis. There are several important things to note when making measurements for the RCB. The website refers to primary tumour

bed area; 'tumour bed area' refers to the size of the residual invasive cancer, i.e. the greatest distance between invasive tumour cell foci (Figure 3). Background stromal changes such as reactive fibrosis or DCIS that extends outside the limit of the invasive tumour are not included. It is not necessary to measure the area of stromal change, just the dimensions of the residual invasive disease. Second, the invasive tumour dimension for the RCB includes intervening background stroma, i.e. include fibrosis between invasive tumour cell foci. If there are scattered islands of tumour cells across the tumour bed you measure the total size across all the islands as a single lesion, unless there are multiple separate primary tumours. This is different to the size measurement for TNM staging from the 7th edition onwards, described above (Table 1, Figure 3). Similarly, when evaluating cellularity, the entire tumour area including intervening fields with no tumour should be assessed to calculate the average, not just fields that contain tumour cells.

There are similar caveats in evaluating the nodal disease. The total nodal count includes all nodes that contain tumour cells including nodes with ITCs only, although these are not regarded as positive nodes for TNM staging. As with invasive tumour, the size of the largest metastasis is the greatest distance between tumour cells within a lymph node including background reactive fibrosis between metastatic tumour cell islands, but not fibrosis that extends outside the metastasis. Again, this is different to how metastases are measured according to the 8th edition of the TNM (Table 1, Figure 6)

These values are combined in an algorithm available online that calculates a continuous numerical score, and places residual tumour in 3 classes with class I representing MRD and class III extensive residual disease. Although class I correlates with excellent response and class III with poor response, this system is not strictly a measure of response as cellularity in this case is absolute cellularity post treatment rather than the change in cellularity. Indeed low cellularity post-treatment does not necessarily equate with response as some cancers, e.g. lobular cancers, are hypocellu-

lar to begin with. Cellularity is heavily weighted in the algorithm, so small tumours with a high cellularity will often end up as RCB II, whereas larger tumours with low cellularity can still be RCB I.

As mentioned, both RCB class and the RCB score as a continuous variable show an association with survival outcomes across all molecular subtypes, although the nature of the relationship varies by subtypes (14). Early data suggested for TNBC, patients that achieve RCB I have an excellent prognosis similar to that of pCR. A more recent multicentre pooled analysis with larger numbers has shown a linear relationship between RCB and BCSS, with a small but significant difference between pCR and RCB I (15). In contrast, for ER-/HER2+ patients the curve has a slightly different shape with a steeper rise at low levels of residual disease that plateaus out across higher RCB scores suggesting even small volumes of residual disease has an adverse prognosis for this subtype. ER+/HER2- cancers had the opposite profile with the curve rising slowly across low volumes of residual disease and a steeper rise beginning in RCB II. The relationship between residual disease and survival in ER+/HER2- cancers has been a source of controversy, with these tumours having a relatively favourable prognosis despite low pCR rates and a poorer correlation between residual disease and survival outcomes; this data confirms the prognostic relevance of RCB for this subtype, and molecular type-specific RCB class cut-offs could improve clinical accuracy. This highlights the importance in considering molecular subtype when assessing residual disease, and the future need for a combined system including anatomical residual disease extent and tumour biological characteristics.

Whilst ypAJCC and RCB staging both provide a quantitative assessment of residual disease and show an association with survival outcomes, an analysis of cases from the I-SPY-1 trial showed a discrepancy in classification in up to one third of cases using 7th edition TNM (104). Of 55 discrepant cases, 36 had a higher RCB class, and 19 had a higher ypAJCC stage. The source of discrepancy was weighting of lymph node involvement and tumour cellularity in the RCB. For example, a small

tumour with high cellularity will be low AJCC stage but RCB class II, and conversely a large tumour with low cellularity will have a higher stage but a relatively low RCB score. For discrepant cases, if residual disease was RCB or ypAJCC stage 3 there was a poor outcome suggesting the two systems are complementary.

Management of the Axilla

In patients that are axillary node negative pre-treatment, the safety and accuracy of SLNB post NACT has now been established in several large series with identification (IR) and false negative rates (FNR) comparable to the adjuvant setting. A meta-analysis found IR of 93-97%, a FNR of 6% and axillary recurrence rates of 2% (105). In patients with proven positive axillary lymph nodes pre-NACT surgical management of the axilla is still subject to debate. Early series showed huge variation in results, with one meta-analysis finding an IR of 68-100% with a pooled FNR of 11%, although the FNR was as high as 33% in individual studies (106). However, the reliability of SLNB in node positive patients has now been examined in several prospective clinical trials, and with better patient selection and the evolution of targeted axillary sampling techniques is yielding more promising results.

Early evidence came from the NSABP-B27 trial, where a subset of 428 patients underwent SLNB followed by ALND; the SLN was positive in 36%, and in 56% was the only positive node (107). The FNR was 12% for patients that were cN1-2, and in patients with breast pCR this fell to 2%. Subsequently, the ACOSOG Z1071 trial specifically addressed the question of post NACT SLNB in patients with biopsy proven axillary metastases with no prior axillary surgery (108). Patients underwent SLNB followed by completion ALND; the overall nodal pCR rate was 41%, rising to 49% in TNBC and 65% for HER2+ disease, and in 21% residual nodal disease was confined to the SLN. The overall FNR was 12.6% which fell short of the study target of 10%, however if dual mapping with blue dye and radioisotope was used the FNR fell

to 11%, and if 3 or more nodes were sampled the FNR was only 9% compared with 21% for 2 nodes and 31% if one node was removed. The SN FNAC study looked at SLNB in node positive patients, with immunohistochemistry (IHC) undertaken on all negative nodes; the FNR was 13%, which fell to 8% when nodes with ITCs were regarded as positive (109). A subset analysis of Z1071 utilizing IHC found a similar FNR of 9%.

The Europe-based SENTINA study had a more complex design including both cN0 and cN1 disease. Patients that were cN1 proceeded directly to post NACT SLNB, with an IR of 80% and a FNR of 14%. cN0 patients had a pre chemotherapy SLNB, and if positive had a second attempt at SLNB post NACT with ALND (110). The second line SLNB had an IR of 60% and a FNR of 52%, showing repeat SLNB has a poor success rate.

Within Z1071, a substudy of 170 patients examined the role of clipping the biopsied node and identifying the clip at the time of SLNB. The clip was present in a SLN in 76% of cases with a FNR of 7%, however in the remaining 24% where the clip was in a non-SLN the FNR was 19% (111). In 41% the clipped node was the only positive node. This has led to the evolution of targeted axillary sampling techniques, where the biopsied node is clipped or otherwise labelled and localised at the time of surgery and/ or at least 3 SLN are removed following dual localisation, achieving acceptably low FNR.

In a series of 12 patients, Caudle et al. clipped the positive biopsied node then placed a radioactive 125I seed before surgery to localise the clipped node; five underwent SLN, which included the clipped node in 4 patients (112). A total of 9 patients including the SLN group had an ALND; 4 had residual metastatic disease and the clipped node was positive in all cases. In a larger follow up series, removing the clipped node gave a FNR of 4%, which fell to 2% when combined with SLNB (113). In a similar approach, a Dutch group used 125I seed to label the positive node at the time of diagnosis, termed the MARI procedure (Marking the Axillary lymph node with Radioactive I) then removed the labelled node alone with a FNR of 7% (114).

The Mayo Clinic group reported 38 SLNB after clipping the biopsied node; 25 had a 125I seed placed in the clipped node, 9 had no preoperative localisation of the clip, and 4 had no documentation (115). In the 25 patients with the 125I seed, the labelled node was successfully identified preoperatively in 20; in the remaining 14 where the seed was not localised or localisation was not attempted, the clipped node was found in 11. Overall, the IR for the clip was 78% with a FNR of 3%. The same group had trialled using HydroMARK gel clips to mark the biopsied node but found these were no longer visible following NACT, however in the Spanish ILINA trial placement of Hydro-MARK clips with intraoperative US successfully localised the clipped node in 96% of cases, with a 4% FNR when combined with SLNB; in all cases the clipped node was positive except one false negative case where both clipped node and SLN were negative (116).

In a separate single institution series of 630 cN1 patients without clipping of the positive node, 91% converted to cN0 post NACT and proceeded to SLNB (117, 118). Three or more SLNs were mapped in 93% of cases, regarded as adequate mapping, with 7% having less than 3 nodes identified and complete failure in 2%. Unsuccessful mapping was associated with high body mass index and presence of LVI. In patients with successful mapping, 41% had nodal pCR and were able to avoid ALND, by molecular subtype 20% of HR+/HER2-, 44% of TNBC, 55% of HR+/HER2+ and 78% of HR-/HER2+. Of note, 43% of patients with unsuccessful mapping also achieved an axillary pCR. Other predictors for avoiding ALND included ductal or apocrine histological subtype (44% and 50% versus 17% for lobular cancers), grade 3 cancers (54% versus 24% for grade 2 and 14% for grade 1) and absence of LVI (78% versus 22%). Grade 3, molecular subtype and presence of LVI remained significant predictors of ALND on multivariate analysis. This supports the conclusion from an earlier study that clipping the biopsied node is not required if there is thorough SLN technique with dual labelling and removal of 3 or more nodes at the time of SLNB (119).

The ISPY-2 trial group have published guidance on surgical management of the axilla for use in clinical trials that is generalizable to routine practice (3). For cN0 patients, SLN with removal of at least 2 nodes is advised. For proven node positive patients, the biopsied node should be marked at the time of diagnosis with SLN or ALND after completion of NACT. Where SLN is performed, dual tracer mapping of the SLN is required with identification and removal of the clipped node. If the node was not clipped, a minimum of 2 SLNs must be removed. If the SLN is positive ALND is advised but not mandated; however, if RCB calculation is part of the trial then completion ALND is needed to determine the RCB score. In multidisciplinary UK guidelines, in patients with a positive axilla SLN may be considered post NACT but dual mapping with removal of four nodes is advised (120). If any residual disease including ITCs is identified then ALND is recommended.

Of note, a recent review of the US NCD has shown an increase in adoption of SLNB for cN+ patients post NACT from 32% in 2012 to 49% in 2015, with SLNB more frequent in younger patients, TNBC or HER2+ disease, and following BCS (5). Of concern, follow up ALND was not performed for 37% of patients with ITCs (21% in 2012 increasing to 49% in 2015), 24% with micrometastases (19% in 2012 to 31% in 2015) and 13% with ypN1 macrometastases. This is despite clinical guidelines recommending ALND for any residual nodal disease including ITCs post NACT due to a lack of clinical evidence on safety of omission of ALND. Studies post NACT show higher FNR with additional non-SLN positivity in 17% of cases with ITCs, 64% with micrometastases and 62% with macrometastases (121). There is evidence showing worse DFS for ypN0(i+) and ypN1(mic) (1.9 and 2.2 times increased mortality respectively); this was true for both cN0 and cN1 disease, with the greatest impact of low volume residual nodal disease in TNBC and HER2+ cancers (89). Using NCD data, Almahariq et al. showed inferior survival outcomes in ypN1 patients that underwent SLNB alone with regional nodal irradiation, with 71% 5 year OS compared with 77%

in those that had ALND (122). There is still limited data on LRR rates in patients who achieve axillary pCR post NACT. The study of Pitilin et al. found 17 LRR in 602 patients after 34 months of follow up, 3 in patients that were ypN0; of interest none of the 9 patients with ITCs had a LRR (123).

Results of two ongoing clinical trials are awaited. The NSABP B-51/RTOG 1304 trial is looking at the oncological safety of SLNB alone in node positive patients that revert to node negative post NACT, and is randomising patients to regional nodal irradiation versus no further axillary therapy. In contrast, the Alliance 11202 trial will examine the group of women with positive SLNB post NACT and randomise them to nodal radiotherapy versus ALND. Of note, both trials regard women with ITCs as node negative so will not provide direct evidence as to the need for further axillary therapy in this important subset of patients.

A rare clinical scenario is presentation with axillary nodal disease and no identifiable primary breast tumour. A recent study looking at 28 women with occult primary breast cancer found a pCR rate of 80%, 93% in those with cN1 disease, suggesting that SLN alone post NACT may be an option for these patients (124). Interestingly, looking at the molecular subtypes the pCR rate was 50% in ER+/HER2- tumours, 88% for TNBC and 100% for HER2+ disease, higher than for women with an identifiable breast primary in most series. One proposed theory is this represents a subset of tumours that invoke a strong immune response with regression of the primary disease, and immune therapies may be a future treatment option for these patients.

Conclusion

In conclusion, NACT is now routine breast cancer management. Assessment of response is becoming increasingly important in adjuvant therapy decisions, and more than ever the pathologist plays a vital role in patient care. Management of the axilla remains controversial but there is growing evidence supporting the safety of SLNB in previously node positive patients, however even minimal re-

sidual nodal involvement in the form of ITCs and micrometastases has adverse prognostic significance and clinical evidence for the safety of omitting ALND in these patients is currently lacking.

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