Developments in Ki67 and other biomarkers for treatment decision making in breast cancer

A. Sheri & M. Dowsett*

Department of Academic Biochemistry, Royal Marsden Hospital, London SW3 6JJ, UK

Estrogen receptor (ER) and HER2 are well established as predictive markers for treatment benefit, although methodological deficiencies can still affect their predictive accuracy. The shift towards earlier diagnosis poses a challenge in identifying those low-risk patients who may safely avoid adjuvant chemotherapy for early breast cancer. Therefore, recent research has focused on developing biomarkers to quantify residual risk on adjuvant endocrine therapy. For widespread adoption into clinical practice, these must be validated in well-designed clinical trials and provide additional information to current standards using reproducible and cost-effective methodologies. Furthermore, evidence from preoperative studies indicates that on- or post-treatment biomarkers can be more predictive than at baseline. In particular, Ki67 has recently emerged as an intermediate marker of long-term outcome. The power of Ki67 to predict treatment benefit from endocrine therapy has facilitated the design of studies where Ki67 is the primary endpoint. This has also led to investigations into the predictive power of Ki67 to determine benefit from signal transduction inhibitors and chemotherapy in several recent and ongoing trials.

Key words: adjuvant therapy, biomarkers, breast cancer, IHC4, Ki67

introduction

In recent decades, the widespread adoption of the use of adjuvant therapy for breast cancer has led to a substantial decline in breast cancer mortality [1]. Additionally, the introduction of screening programs has led to the increasing diagnosis of early-stage disease with an inherently better prognosis. This challenges clinicians to identify patients appropriately in whom adjuvant chemotherapy is warranted. Optimal clinical decision making incorporates the use of prognostic factors which identify risk independently of treatment and also predictive makers which identify sensitivity or resistance to a particular therapy. Several biomarkers such as estrogen receptor, (ER), progesterone receptor (PgR) and HER2 are established in breast cancer and routinely measured at baseline. Their role and that of other less established biomarkers are discussed in relation to treatment decision making.

The administration of neoadjuvant therapy before surgery, as well as facilitating breast conserving surgery, allows an in vivo assessment of the primary tumours sensitivity to systemic therapy. The absence of invasive tumour cells in the excision specimen following neoadjuvant chemotherapy, described as pathological complete response (pCR), has been validated as an intermediate marker of long-term outcome with those patients whose tumours undergo a pCR having an excellent long-term outcome [2, 3]. The neoadjuvant trial design, where the pCR rate is often compared between treatment arms, has been favoured as a method of testing the activity of new agents in clinical development, requiring fewer patients and less time than large-scale adjuvant studies. However, failure to achieve a pCR following neoadjuvant chemotherapy, particularly in ER-positive (pos) cancers is associated with a heterogeneous outcome. Furthermore, a pCR in response to neoadjuvant endocrine therapy is uncommon and extensive research has been undertaken to establish biomarkers which predict long-term outcome based on the on- or post-neoadjuvant therapy characteristics of residual disease. This has the potential not only to improve prediction of long-term outcome but also to rationalise and accelerate the development of new therapies for high-risk patients.

baseline biomarkers

estrogen and progesterone receptor

For over two decades, the expression of ER has been the definitive biomarker for benefit from endocrine therapy. With the exception of the small minority of ER-negative (neg) tumours positive for the PgR, ER-neg tumours do not derive benefit from endocrine therapy [4–6]. The most recent Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) overview of trials evaluating 5 years of adjuvant tamoxifen demonstrates a reduction in breast cancer mortality in ER-pos disease of around a third throughout the first 15 years [6].

Given the importance of ER and PgR expression on treatment decision making and that reports have suggested up to 20% of immunohistochemical ER and PgR testing worldwide may be inaccurate, recent American Society of Clinical Oncology/College of American Pathologists (ASCO/
CAP) guidelines focus on standardised testing of ER and PgR in breast cancer [7]. It is recommended that ER and PgR assays may be considered positive if there are at least 1% positive tumour nuclei in the presence of expected reactivity in controls. Furthermore, recommendations are made for external quality assurance procedures requiring laboratories to undergo proficiency testing.

Quantitative expression of ER has been investigated as a predictive marker of endocrine therapy benefit. Studies in advanced disease from the 1970s have correlated a greater benefit from endocrine therapy with higher concentrations of ER [8]. In the adjuvant setting, the most recent EBCTCG meta-analysis reported no apparent benefit from tamoxifen for tumours classified as ER poor (<10 fmol/mg), but a substantial benefit in marginally ER-pos disease (10–19 fmol/mg), measured using a ligand-binding assay. Highly ER-pos disease (≥200 fmol/mg) was associated with an even greater benefit with a rate ratio for breast cancer mortality with tamoxifen of 0.53 compared with 0.67 in marginally ER-pos disease [6]. These results are also consistent with the observation that higher mRNA expression of ESR1 correlates with greater benefit from tamoxifen in the NSABP B-14 trial [9]. The expression of ER has also been investigated as a marker of benefit from chemotherapy. Lower rates of pCR are observed in ER-pos cancers following neoadjuvant chemotherapy and some studies have reported a smaller proportional benefit from adjuvant chemotherapy in ER-pos compared with ER-neg tumours. However, this is not borne out in meta-analyses and the recent EBCTCG overview reported a similar proportional benefit for adjuvant chemotherapy in ER-pos and ER-neg tumours. The potential benefit from chemotherapy in ER-pos disease therefore depends on the absolute risk without chemotherapy on appropriate endocrine therapy.

It is important to note that ER-pos, PgR-neg cancers are associated with a worse prognosis. However, analyses suggest that they derive a similar proportional benefit from adjuvant tamoxifen or aromatase inhibitors compared with ER-pos, PgR-pos tumours [6, 10, 11]. Therefore, although PgR expression does not predict proportional benefit from endocrine therapy, it may be useful in determining residual risk and therefore may be an aid in adjuvant chemotherapy treatment decisions.

HER2
Amplification of the HER2 gene on chromosome 17q21 is present in around 15% of breast cancers and before the clinical use of trastuzumab was a strong predictor of poor outcome. HER2 testing is now considered a standard as part of the management of breast cancer. ASCO/CAP guidelines define HER2-amplified cases as those which demonstrate staining of 3+ by immunohistochemistry (IHC), a fluorescent in situ hybridisation (FISH) result of more than six HER2 gene copies per nucleus or a FISH HER2:CEP17 ratio that is >2.2 [12]. An equivocal result is defined as an IHC result of 2+, a FISH result of 1.8–2.2 or 4–6 HER2 gene copies per nucleus. It should be noted however, that patients with a HER2:CEP17 ratio of ≥2.0 were eligible for treatment with trastuzumab in the adjuvant trials of trastuzumab and so current evidence does not support excluding these patients from trastuzumab therapy. A recent study has reported that the degree of HER2 staining was not correlated with long-term outcome following chemotherapy or benefit from trastuzumab [13].

HER2 amplification does appear to be associated with chemotherapy sensitivity, with HER2 status reported to independently predict pCR following neoadjuvant chemotherapy [14]. Studies in the adjuvant setting have suggested that the benefit of anthracycline chemotherapy may be confined to those patients with HER2-amplified tumours, although a recent meta-analysis did not support this [15]. The 2011 St Gallen consensus guidelines do not recommend a specific chemotherapy regime for HER2-amplified cancers although the majority favoured the use of anthracyclines and taxanes [16].

Whilst many HER2-positive cancers do appear chemo-sensitive, recent evidence in the neoadjuvant setting suggests that in some patients anti-HER2 therapy alone may be sufficient. The NeoSphere study was a four-arm study testing the addition of pertuzumab to trastuzumab, docetaxel or trastuzumab and docetaxel compared with trastuzumab and docetaxel. pCRs were observed in over 16% of tumours with the combination of trastuzumab and pertuzumab alone [17]. Biomarkers to identify these patients are still lacking. Recent analyses reported that higher HER2 membrane staining determined using an H-score correlated with sensitivity to the addition of pertuzumab to docetaxel and trastuzumab. However the implications of this on the long-term outcome are unknown [18].

baseline Ki67 and prognosis
A fundamental hallmark of cancer cells involves their ability to sustain chronic proliferation [19, 20]. The most widely practiced measurement of proliferation involves immunohistochemical detection of the nuclear non-histone protein Ki67. Its precise function remains ill-defined although it is thought to be involved in ribosomal RNA synthesis [21, 22]. The observation that Ki67 is detected only in proliferating cells and absent in quiescent cells led to its adoption as a measure of the proportion of cells proliferating in a tumour. Ki67 expression is commonly assessed using the mindbomb E3 ubiquitin protein ligase 1 antibody (MIB1) and reported as a percentage of cells Ki67 positive.

Ki67 has been reported to correlate with other biomarkers in breast cancer such as grade and ER expression, with ERPositive cancers typically exhibiting lower levels of proliferation [23, 24]. Ki67 has also been used to identify luminal class with a cut-off level of 13.25% proposed to distinguish poorer prognosis luminal B cancers from luminal A [25]. However, this cut-off only achieved a concordance of 75% with luminal status and a lack of between laboratory standards may limit application as a surrogate marker.

Numerous studies have investigated the potential role of Ki67 as a prognostic marker. In a meta-analysis of 40 studies involving over 11 000 patients, baseline Ki67 was found to have a modest prognostic value in multivariable analysis, which was more evident in lymph node-negative patients [26]. Another
meta-analysis of 46 studies including over 12,000 patients found that Ki67 positivity (using cut-offs defined by individual authors) was associated with a higher risk of relapse and a worse survival in patients with early breast cancer [27]. Standardised methodologies for measurement and cut-off points for Ki67 are lacking which has limited the evaluation and application of this biomarker in clinical practice. As a result, the ASCO Tumour Marker Guidelines Committee determined that evidence supporting the clinical utility of Ki67 was insufficient to recommend routine use for prognostic purposes in patients with newly diagnosed breast cancer [28]. In 2011, the International Ki67 in Breast Cancer Working Group published recommendations for Ki67 assessment in breast cancer [29]. These guidelines aim to minimise pre-analytical and analytical variables in Ki67 assessment and harmonise scoring methodology and data handling.

baseline Ki67 and prediction of adjuvant therapy benefit

Other studies have focused on the role of Ki67 in predicting response to adjuvant therapy. Specific questions investigated have been whether baseline Ki67 predicts benefit from adjuvant chemotherapy or can even predict benefit from a specific agent.

Despite studies suggesting that high Ki67 is associated with a poorer prognosis, high Ki67 has been associated with a good response to neoadjuvant chemotherapy. Although in multivariable analyses, not all studies have shown Ki67 to be an independent predictor of pCR [30]. Furthermore, Ki67 alone has not been shown to predict the benefit of adjuvant cyclophosphamide, methotrexate and fluorouracil (CMF) chemotherapy to adjuvant endocrine therapy in lymph node-negative patients [31]. Other studies have examined the power of baseline Ki67 to predict benefit from specific regimens in patients treated with adjuvant chemotherapy reporting a trend towards benefit from the addition of taxanes to anthracycline-based chemotherapy in Ki67 high versus Ki67 low cancers which warrants further investigation [32, 33].

Studies of neoadjuvant endocrine therapy have not reported a clear association with baseline Ki67 and response to therapy [34]. However, a higher Ki67 has been associated with a shorter time to treatment failure in patients with advanced breast cancer treated with an aromatase inhibitor [35]. Additionally in a central analysis of the BIG1-98 trial, a higher Ki67 was associated with a trend towards a greater proportional benefit from letrozole versus tamoxifen [36].

identifying residual risk in ER-pos breast cancer treated with endocrine therapy and prediction of chemotherapy benefit

With the advent of screening, a large number of women are currently diagnosed with small ER-pos breast cancers, many of which have a generally good prognosis. Accurate determination of residual risk in patients treated with endocrine therapy allows low-risk patients to safely be spared the toxicity of chemotherapy. Several multigene signatures have been developed which have been shown to be prognostic and predict chemotherapy benefit. However, these are costly and may not necessarily inform adjuvant therapy decisions more than more established markers. It is recognised that ER, PgR, HER2 and Ki67 measured using immunohistochemistry independently provide some prognostic and sometimes predictive information. The IHC4 incorporates these established markers along with nodal status, tumour size, grade and age into a prognostic score for ER-pos breast cancer patients receiving endocrine treatment. In an analysis of the arimidex, tamoxifen, alone or in combination (ATAC) trial and subsequently validated in a separate cohort, the IHC4 was highly prognostic. Furthermore, the prognostic information provided by the IHC4 was similar to that provided by the 21-gene recurrence score (RS), which is weighted to measure ER, HER2 and proliferation-associated genes [37]. Similarly, Mammostrat is a tool measuring five immunohistochemical markers but unlike with the IHC4, these are not routinely measured in clinical practice (P53, HTF9C, NDRG1, and SLCA5 and CEACAM5). Mammostrat scores have been shown to be prognostic in ER-pos breast cancer and predict the degree of benefit in the NSABP B20 trial [38, 39]. No comparison has been made with the RS. As with the RS, an intermediate group is identified in whom the degree of benefit is uncertain. Incorporation of standard clinico-pathological variables such as tumour size and nodal involvement may allow more accurate determination of prognosis and therefore absolute benefit from chemotherapy.

on and post-treatment biomarkers

Ki67 and preoperative endocrine therapy

Ki67 has been studied extensively as a biomarker to predict long-term outcome and to assess potential therapeutic efficacy in the neoadjuvant setting (see Table 1 for the summary of endocrine studies) [40–51]. On- or post-treatment measurements of Ki67 in residual disease appear to integrate information on both intrinsic tumour biology and responsiveness to therapy and are more predictive of long-term outcome following neoadjuvant endocrine therapy than pre-treatment measures.

In the IMPACT trial, higher expression of Ki67 after just 2 weeks of endocrine therapy was found to be associated with a statistically significant lower recurrence-free survival (RFS) where as higher Ki67 at baseline was not [42]. The ongoing POETIC trial is prospectively testing the hypothesis that Ki67 measured after 2 weeks of endocrine therapy can predict long-term outcome. Similarly, post-treatment Ki67 has been incorporated into the preoperative endocrine prognostic index (PEPI) score along with ER expression and the post-treatment measures of residual disease burden, pathological T and N stage [52]. Moreover, differences in Ki67 suppression during preoperative endocrine therapy have been shown to predict differential treatment effects on long-term outcome in the adjuvant setting. The greater suppression of Ki67 with letrozole versus tamoxifen in the PO24 study and anastrozole over the combination with tamoxifen or tamoxifen alone in IMPACT mirrored the results of the BIG 1-98 and ATAC trials, respectively [53, 54]. More recently, the American College of Surgeons Oncology Group Z1031 study demonstrated the equivalence of the aromatase inhibitors exemestane,
anastrozole and letrozole on Ki67 suppression mirroring disease-free survival (DFS) results of the adjuvant MA.27 trial [46, 55].

An exception to this trend is the study of Tibolone effects on Mammary carcinoma tissue (STEM) trial, where the proliferative effects of tibolone were compared against placebo. No significant differences were observed at 14 days in Ki67 expression [51]. These results were not consistent with the increase in breast cancer recurrence observed in ER-pos early breast cancer patients in the Livial Intervention following breast cancer: efficacy, recurrence and tolerability end-points (LIBERATE) study. This may relate to the time point assessed (14 days) which may in some cases be too early to identify the proliferative effects of a hormone replacement therapy or the early acquired escape from the anti-proliferative effects of endocrine therapy. In the IMPACT study, ~15% of patients with suppression of Ki67 at 2 weeks demonstrated a rebound effect with higher levels measured at 12 weeks. Therefore, although failure to suppress Ki67 at 2 weeks is likely to identify patients with an adverse prognosis, suppression at 2 weeks may not necessarily always indicate a good prognosis. This recovery in some patients by 12 weeks may, however, be exploited in assessing the ability of new agents to prevent this Ki67 recovery.

### Ki67 and signal transduction inhibitors

The predictive power of Ki67 in the neoadjuvant endocrine setting has led to the extension of its use as a potential biomarker for the efficacy of several signal transduction inhibitors.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Primary Endpoint</th>
<th>Biomarker endpoints</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith et al. [40], Dowsett et al. [42, 45]</td>
<td>Double blind, randomised, phase 3. 12 weeks anastrozole 1mg, tamoxifen20mg or combination od in non metastatic ER-pos postmenopausal breast cancer.</td>
<td>Objective response (OR) rate at 12 weeks</td>
<td>Reduction in Ki67 at 2 and 12 weeks. Ki67 expression at 2 weeks.</td>
<td>No significant differences in OR. Greater suppression of Ki67 at 2 and 12 weeks with anastrozole compared to tamoxifen (p= 0.004 and p= 0.001). No difference between tamoxifen and combination. Higher Ki67 at 2 weeks associated with lower RFS p= 0.004.</td>
</tr>
<tr>
<td>Eiermann et al. [44], Ellis et al. [45]</td>
<td>Double blind randomised phase 2b/. 16 weeks letrozole 2.5 mg or tamoxifen 20mg od in postmenopausal ER-pos locally advanced breast cancer.</td>
<td>OR at 16 weeks</td>
<td>Reduction in Ki67 at 16 weeks</td>
<td>Greater OR rate in letrozole compared to tamoxifen group p &lt; 0.001. Greater reduction in Ki67 with letrozole compared to tamoxifen p = 0.0009. No differences in Ki67 suppression or PPEI score.</td>
</tr>
<tr>
<td>Kuter et al. [48]</td>
<td>Open label, randomized phase 2. Fulvestrant 500mg (day 0, 14, 28 every 28 days) vs 250mg (day 0, 28 every 28 days) in postmenopausal ER-pos locally advanced breast cancer.</td>
<td>Change in Ki67 from baseline to week 4.</td>
<td>Ki67 ER and PgR expression.</td>
<td>Reduction in Ki67 in ER-pos tumours p = 0.0043. No effect in ER-neg or placebo groups. No significant effect on apoptosis.</td>
</tr>
<tr>
<td>Dowsett et al. [50]</td>
<td>Double blind, randomized, placebo controlled phase 2. Postmenopausal stage I or II primary breast cancer. Raloxifene 60mg od, 300mg bd or placebo.</td>
<td>Change in Ki67 at 14 days.</td>
<td>Ki67 Apoptosis ER and PgR expression</td>
<td>In ER-pos tumours: Reduction in Ki67 with raloxifene 60mg vs placebo p= 0.015. Reduction in Ki67 raloxifene 600mg vs placebo p= 0.064. No significant effect on apoptosis.</td>
</tr>
</tbody>
</table>
inhibitors in neoadjuvant and short-term pre-surgical ‘window of opportunity’ studies (see Table 2) [56–63]. However, the ability of Ki67 to predict benefit from agents whose main mechanism of action may not necessarily always be directly anti-proliferative is less certain.

### Table 2. Preoperative studies of signalling agents assessing change in Ki67 as an endpoint.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Biomarker endpoints</th>
<th>Clinical endpoints</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith et al. [56]</td>
<td>Postmenopausal patients with stage I-IIb ER or PgR -pos breast cancer randomized to anastrozole + gefitinib 250mg od for 16 weeks, placebo for 2 weeks followed by gefitinib for 14 weeks or placebo for 16 weeks.</td>
<td>Change in Ki67 at 2 and 16 weeks.</td>
<td>OR rate</td>
<td>No clinical or biological effect of the addition of neoadjuvant gefitinib to anastrozole.</td>
</tr>
<tr>
<td>Guix et al. [57]</td>
<td>Preoperative window of opportunity study. Primary breast cancer patients with stage I-IIIa primary breast cancer treated with erlotinib 150mg od 6-10 days.</td>
<td>Change in Ki67 Apoptosis P-EGFR, P-HER2 P-MAPK, P-AKT P-S6 S118 P-ERα</td>
<td>n/a</td>
<td>Significant inhibition of Ki67 in ER-pos tumours only p= 0.0006. Reduction in P-MAPK, P-AKT, P56 and S118 P-ERα in ER + tumours.</td>
</tr>
<tr>
<td>Mohsin et al. [58]</td>
<td>HER2 + breast cancers &gt; 4cm. NCT00133796: Neoadjuvant trastuzumab 4mg/m2 then weekly at 2mg/m2 for 3 weeks then addition of docetaxel100mg/m2 every 3 weeks for 4 cycles. NCT00206427: Neoadjuvant lapatinib 1500mg od for 6 weeks followed by docetaxel 100mg/m2 every 3 weeks for 4 cycles.</td>
<td>Change in Ki67, Apoptosis P-AKT P-MAPK PTEN, PIK3CA mutation</td>
<td>Clinical response</td>
<td>Lapatinib alone: Reduction in Ki67 with associated reduction in P-MAPK. No alteration in apoptosis index. Higher rate of pCR associated with low PTEN p= 0.007. No association with PIK3CA mutation. Trastuzumab : Significant increase in apoptosis index at week 1 p = 0.004. No alteration in Ki67. Non significant trend towards lower incidence of pCR in low PTEN tumours. Non significant trend towards lower incidence of pCR with PIK3CA mutation. Significant decrease in Ki67 in lapatinib arm vs placebo . Significant increase in Ki67 observed in placebo arm p= 0.008.</td>
</tr>
<tr>
<td>Dave et al. [59]</td>
<td>HER2 + breast cancers &gt; 4cm. NCT00133796: Neoadjuvant trastuzumab 4mg/m2 then weekly at 2mg/m2 for 3 weeks then addition of docetaxel100mg/m2 every 3 weeks for 4 cycles. NCT00206427: Neoadjuvant lapatinib 1500mg od for 6 weeks followed by docetaxel 100mg/m2 every 3 weeks for 4 cycles.</td>
<td>Change in Ki67, Apoptosis P-AKT P-MAPK PTEN, PIK3CA mutation</td>
<td>Clinical response</td>
<td>Lapatinib alone: Reduction in Ki67 with associated reduction in P-MAPK. No alteration in apoptosis index. Higher rate of pCR associated with low PTEN p= 0.007. No association with PIK3CA mutation. Trastuzumab : Significant increase in apoptosis index at week 1 p = 0.004. No alteration in Ki67. Non significant trend towards lower incidence of pCR in low PTEN tumours. Non significant trend towards lower incidence of pCR with PIK3CA mutation. Significant decrease in Ki67 in lapatinib arm vs placebo . Significant increase in Ki67 observed in placebo arm p= 0.008.</td>
</tr>
<tr>
<td>DeCensi et al. [60]</td>
<td>HER2 + breast cancers &gt; 4cm. NCT00133796: Neoadjuvant trastuzumab 4mg/m2 then weekly at 2mg/m2 for 3 weeks then addition of docetaxel100mg/m2 every 3 weeks for 4 cycles. NCT00206427: Neoadjuvant lapatinib 1500mg od for 6 weeks followed by docetaxel 100mg/m2 every 3 weeks for 4 cycles.</td>
<td>Change in Ki67, Apoptosis P-AKT P-MAPK PTEN, PIK3CA mutation</td>
<td>Clinical response</td>
<td>Lapatinib alone: Reduction in Ki67 with associated reduction in P-MAPK. No alteration in apoptosis index. Higher rate of pCR associated with low PTEN p= 0.007. No association with PIK3CA mutation. Trastuzumab : Significant increase in apoptosis index at week 1 p = 0.004. No alteration in Ki67. Non significant trend towards lower incidence of pCR in low PTEN tumours. Non significant trend towards lower incidence of pCR with PIK3CA mutation. Significant decrease in Ki67 in lapatinib arm vs placebo . Significant increase in Ki67 observed in placebo arm p= 0.008.</td>
</tr>
<tr>
<td>Martin and Davies et al. [61]</td>
<td>Phase 2 randomised placebo controlled window of opportunity study. Postmenopausal patients with stage I-II primary breast cancer. Celecoxib 400mg bd.</td>
<td>Change in Ki67, ER, PgR, HER2, PTEN</td>
<td>Radiological response</td>
<td>Non-statistically significant trend toward reduction in Ki67. No alteration in apoptosis.</td>
</tr>
<tr>
<td>Baselga et al. [63]</td>
<td>Phase II neoadjuvant study of everolimus 10mg od/ placebo plus letrozole 2.5mg od.</td>
<td>Change in Ki67, PIK3CA mutation P-S6 Cyclin D1 PR</td>
<td>Clinical response.</td>
<td>Greater mean reduction in Ki67 with everolimus + letrozole vs letrozole alone p = 0.0002.</td>
</tr>
</tbody>
</table>

Ki67 and other biomarkers to predict long-term outcome following neo-adjuvant chemotherapy post-treatment Ki67
High Ki67 in residual disease following neoadjuvant chemotherapy correlates with poor long-term outcome
One of the largest series published is from the Royal Marsden where in a cohort of 284 patients, post-therapy Ki67 was highly prognostic, with those patients with an excision Ki67 in the highest tertile, having a 5-year relapse-free survival of just 27% and overall survival (OS) of 39% compared with 77% and 93%, respectively, in the lowest Ki67 tertile. In an analysis of 103 matched pre- and post-treatment samples, reduction in Ki67 from pre-treatment to excision was also correlated with long-term outcome although less so than the excision reading.

Other studies have reported partially conflicting results regarding the prognostic significance of change in Ki67 with neoadjuvant chemotherapy (see Table 3) [64, 67–70]. Time of measurement after a course of chemotherapy may be a critical variable in interpreting these results. Whilst, differences in pCR rates are commonly used to assess the efficacy of chemotherapy or targeted therapy combinations in the neoadjuvant setting, no studies have yet reported that Ki67 measured in residual disease or change in Ki67 following neoadjuvant chemotherapy can predict the differential treatment effects of agents on long-term outcome.

### on-treatment markers

Fewer studies have examined the role of on- rather than post-treatment biomarkers of response to neoadjuvant chemotherapy. Clinical response following two cycles of chemotherapy has not been shown to reliably predict pathological response [71]. Clinical studies also indicate that apoptosis can be detected within 24–48 h of commencing chemotherapy with some studies correlating these changes with the pathological response. However, these changes have not been correlated with long-term outcome [72–74]. A marker of homologous recombination competence, RAD51, measured 24 h after the first dose of chemotherapy has also been investigated as a marker of chemotherapy sensitivity, with 33% of cancers with low RAD51 scores achieving a pCR in one neoadjuvant study [75]. Additionally, a small 33 patient study from the Karolinska institute reported that a change in Ki67 of >25% after just one cycle of cyclophosphamide, epirubicin and 5-fluorouracil chemotherapy significantly correlated with a decreased risk of disease recurrence, $P = 0.033$ [76].

### RCB and tumour cellularity

In 2003, Miller and Payne published a five-point grading system based on the changes in tumour cellularity in the excised tumour compared with the diagnostic core biopsy to predict long-term outcome following neoadjuvant chemotherapy. To account for potential heterogeneity in tumour cellularity, at least three pre-treatment core biopsies were taken from different areas of the tumour. Grade of response was significantly correlated with DFS ($P = 0.02$) and OS ($P = 0.01$) [77]. The RCB extended this concept of measuring tumour cellularity in residual post-chemotherapy disease, incorporating the dimensions of residual tumour bed and nodal involvement into a prognostic index. This score classifies patients into one of three risk categories, with patients with minimal residual disease (class I) having the same prognosis as those with a pCR [78]. A pathological response index (PRI) which includes the presence of vascular invasion

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Biomarker endpoints</th>
<th>Clinical endpoints</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jones et al. [64]</td>
<td>Retrospective analysis of single centre cohort of patients treated with neoadjuvant chemotherapy.</td>
<td>Change in Ki67 Pre and Post Ki67 ER, PgR, HER2</td>
<td>RFS</td>
<td>Excision Ki67 significantly associated with RFS and OS in multivariate analysis. Change in Ki67 (combined prognostic index) correlated with RFS and OS.</td>
</tr>
<tr>
<td>Colleoni et al. [67]</td>
<td>Retrospective analysis of single centre cohort of patients treated with neoadjuvant chemotherapy.</td>
<td>pCR ER, PgR, HER2, Ki67</td>
<td>DFS</td>
<td>No correlation between modification of Ki67 and clinical response.</td>
</tr>
<tr>
<td>Billgren et al. [68]</td>
<td>Single centre study of patients treated with 3–4 cycles of cyclophosphamide, epirubicin and 5-fluorouracil chemotherapy.</td>
<td>Ki67 pre and before 2nd course of chemotherapy.</td>
<td>OR rate.</td>
<td>Decrease of more than 25% in Ki67 after 1st course of chemotherapy correlated with decreased risk of disease recurrence $p = 0.033$. Decrease in Ki67 after 1st course added prognostic information in multivariable analysis.</td>
</tr>
<tr>
<td>Bottini et al. [69]</td>
<td>Retrospective single institution study of patients treated with neoadjuvant chemotherapy in phase 2 studies of CMF + concurrent tamoxifen in ER-pos disease or single agent epirubicin.</td>
<td>Change in and Ki67 pre and post. ER and PgR HER2, BCL2.</td>
<td>RFS</td>
<td>Ki67 at surgery rather than baseline better predictor of RFS. Reduction in Ki67 not correlated with RFS.</td>
</tr>
<tr>
<td>Burcombe et al. [70]</td>
<td>Patients treated with neoadjuvant fluorouracil, epirubicin, cyclophosphamide (FEC) chemotherapy.</td>
<td>Pre, post and change in Ki67. Apoptosis pCR</td>
<td>OR</td>
<td>No association between clinical and pathological response and change in Ki67 at 21 days.</td>
</tr>
</tbody>
</table>
and evidence of chemotherapy-related changes along with reduction in the tumour size and the presence of positive apical lymph node metastases was able to differentiate RCB class II patients into good and poor prognostic sub-groups [79].

Meaningful reporting of residual disease following neoadjuvant therapy, particularly in ER-pos breast cancers, is likely to not only improve prognostication in these patients, but also will potentially further inform interpretation of neoadjuvant trials. Furthermore, if high-risk patients are clearly identified on the basis of post-neoadjuvant therapy characteristics, this would also facilitate the design of post-neoadjuvant adjuvant studies tailored to the molecular profile of high-risk residual disease.

circulating markers

The potential to profile tumour biomarkers using less invasive methods is appealing and has attracted substantial interest in recent years. Measurements of circulating tumour cells (CTCs) have been reported to correlate with the outcome in metastatic disease and the response to chemotherapy and endocrine therapy [80, 81]. However, with no data to support the measurement of CTCs in improving progression-free survival or quality of life, the ASCO guidelines do not currently recommend the measurement of CTCs in the management of breast cancer [28].

In colorectal cancer, the detection of tumour-derived DNA in plasma following surgery has been reported to be predictive of disease relapse [82]. Studies in metastatic breast cancer have also examined assays of tumour phenotype with detection of PIK3CA mutations and determination of HER2 status reported [83, 84]. These results highlight the potential for patients to be stratified for targeted therapies without the need for further biopsies and require further investigation.

conclusions

Recent years have seen advances in the molecular understanding of the biology of breast cancer with the development of several prognostic and predictive multigene signatures. Before clinical application, these require appropriate validation against the current standards and demonstration of reproducible methodologies. The established biomarkers such as ER and HER2 remain powerful predictors of response to therapy. Whilst in many instances insufficient to be used in isolation, the integration of several markers using well-validated methodologies may allow more accurate risk estimation and therefore aid adjuvant chemotherapy decision making.

The development of on- or post-treatment biomarkers of response has the potential to improve prognostication even further. Moreover, if intermediate markers such as Ki67 were shown to have general applicability in predicting the long-term outcome, the impact on trial design could be substantial. Current adjuvant studies require large numbers of patients and often many years before results become apparent. Reliable prediction of drug efficacy in the preoperative setting therefore has the potential to accelerate the development of novel treatment strategies.

acknowledgements

We are grateful for funding from the Royal Marsden NIHR Biomedical Research Centre.

disclosures

AS has no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. MD has acted as an advisor for AstraZeneca, Roche, GSK, Sanofi and Ipsen.

references

15. Di Leo A, Basellet C, Bartlett JM et al. HER2 and TOP2A as predictive markers for anthracycline-containing chemotherapy regimens as adjuvant treatment of


