Possible Roles of Ki67 and other Biomarkers for Treatment Decision Making in Breast Cancer

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Abstract

Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors have revolutionized the treatment landscape for hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (MBC). However, response rates and overall survival vary significantly among patients. This abstract summarizes the impact of Ki-67 proliferation index and progesterone receptor (PR) status as predictive biomarkers for clinical benefit from CDK4/6 inhibitor therapy in MBC. Ki-67, a marker of cellular proliferation, reflects tumor growth rate and is inversely correlated with PR expression in HR+ breast cancer. Higher Ki-67 levels generally indicate a more aggressive tumor phenotype and poorer prognosis. Preclinical and clinical data suggest that patients with high Ki-67 expression may derive less benefit from CDK4/6 inhibitors. This is potentially due to the dependence of CDK4/6 inhibition on effectively suppressing cell cycle progression, which is less impactful in tumors with intrinsically high proliferative rates. Studies analyzing the impact of Ki-67 on progression-free survival (PFS) and overall survival (OS) in MBC patients receiving CDK4/6 inhibitors show variable results, with some demonstrating a significant association between high Ki-67 and reduced benefit, while others report less conclusive findings. The heterogeneity of study populations, including varying CDK4/6 inhibitor regimens and inclusion criteria, likely contributes to this inconsistency. PR status, a key component of HR status, also influences the response to CDK4/6 inhibitors. While CDK4/6 inhibitors primarily target the estrogen receptor (ER) pathway, PR expression is often correlated with ER status and may modulate the sensitivity to endocrine therapy, impacting the effectiveness of combined CDK4/6 inhibitor and endocrine therapy regimens. Patients with PR-negative tumors may exhibit different responses compared to their PR-positive counterparts, potentially owing to divergent downstream signaling pathways involved in cell cycle regulation and tumor growth. Evidence suggests a potential interaction between Ki-67 and PR status, with the impact of Ki-67 on treatment response potentially being more pronounced in PR-positive patients. Further research is crucial to clarify the interplay between Ki-67 and PR status in predicting the efficacy of CDK4/6 inhibitors. Standardized methodologies for Ki-67 assessment and larger, well-designed clinical trials are needed to definitively establish the clinical utility of these biomarkers in guiding treatment decisions and

personalizing therapy for MBC patients. Ultimately, incorporating these biomarkers into clinical practice could optimize the selection of patients who are most likely to benefit from CDK4/6 inhibitors, improving treatment outcomes and reducing unnecessary exposure to potential toxicities.

Keywords: Ki67, metastatic breast cancer

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Introduction

Metastatic breast cancer (MBC) is diagnosed at the time of presentation, i.e., de novo metastatic disease, in 6% to 10% of patients [1]. Bone is the most common site of distant metastasis in breast cancer; other common sites include lymph nodes, lung, liver, and, less frequently, brain. MBC is considered incurable, though there are reports of long-term durable remissions in around 2% of patients with MBC treated with anthracycline therapy and in a small subset of oligometastatic patients [1].

A) Therapeutic goals: the primary goals of systemic treatment for MBC are prolongation of survival, alleviation of symptoms, and maintenance or improvement in quality of life, while balancing the toxicity associated with treatment. The median survival for MBC varies widely based on subtype of tumor, sites of metastatic involvement, and burden of metastatic disease, and some patients experience long-term survival [2, 3].

B) Diagnostic workup Once metastatic disease is suspected, careful evaluation of the primary tumor disease history, current symptoms, and existing comorbid diseases is essential. A review of the initial presentation should include disease stage, histology, HR and HER2 status, and treatment modalities used [4].
Knowledge of the initial tumor histology may yield clues about the sites of disease as well as its biology. For instance, infiltrating ductal carcinoma most commonly involves the bone, lungs, pleura, liver, and brain. Infiltrating lobular carcinoma frequently recurs in unusual sites such as the bone marrow, meninges, peritoneum, and retroperitoneal structures, such as the ureters [5].

1. Biopsy of metastatic lesion A biopsy of the metastatic site is required to confirm histology and receptor status. Discordance in the receptor status from diagnosis to recurrence is common, and treatment is tailored to the receptor status of the relapsed disease [6]. Pathologic confirmation is also essential in patients with atypical presentations, including single-lesion metastasis, unusual metastatic sites, and long disease-free interval (DFI). Solitary lesions should always be biopsied and may represent a benign process or a distinct tumor type. In rare cases, when biopsy of metastatic disease is infeasible or the results are uninterpretable, treatment is

guided by the primary tumor histology/receptor subtype, though biopsy should be reattempted at the time of progression.

• Molecular subtypes of breast cancer according to estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor status (HER2) [7]. • IHC evaluation for programmed cell death ligand 1 (PD-L1) expression, among those with triple-negative cancers as this guides initial therapy selection [8]. • Somatic testing for genetic alterations for those in whom hormone receptor positivity is confirmed, we assess tumor PIK3CA status, either at initial diagnosis of metastatic disease, or at the time of progression on first-line therapy. We assess this using the companion diagnostic test approved by the US Food and Drug Administration to select patients for possible second-line treatment with the alpha isoform-specific phosphoinositide 3-kinase inhibitor alpelisib [9]. The diagnostic test is approved for use on tumor tissue specimens and circulating tumor DNA for the detection of 11 PIK3CA (activating mutations) [10]. • Germline testing for BRCA1/2 pathogenic variants. Germline testing for breast cancer susceptibility gene 1 or 2 (BRCA1 or BRCA2) is also recommended for all patients with metastatic breast cancer in view of therapeutic options (poly [ADP-ribose] polymerase [PARP] inhibitors) [11, 12].

2. Laboratory and radiographic studies in addition to a comprehensive physical examination, basic laboratory evaluation should include a complete blood count with differential, renal and liver function tests including alkaline phosphatase, and serum calcium. Alkaline phosphatase is correlated with bone metastasis in patients with breast cancer but is neither sensitive nor specific [13]. In addition, the serum tumor markers CA 15-3, CA 27-29, and CEA are elevated in approximately 70% of patients with MBC and can be helpful in monitoring response to therapy; however, they are rarely used alone to determine disease response [14]. Additionally, we perform computed tomography (CT) of the chest, abdomen, and pelvis and nuclear medicine bone scan to determine the extent of disease. Positron emission tomography/CT may be obtained, alternatively [15]. Further imaging may be appropriate to evaluate specific signs or symptoms, as well. For example, for those with signs or symptoms suggestive of central nervous system involvement, we obtain dedicated imaging such as contrast-enhanced magnetic resonance imaging of the brain and/or spinal cord [15].

C) Prediction of response: the following are predictors of treatment response: • Hormone receptor status and HER2 overexpression are the most important predictors of treatment response in patients with MBC [16]. • Presence of specific genetic alterations predicts response to certain targeted agents (e.g., PARP inhibitors in germline BRCA1/2 mutation carriers, or tumor TRK fusions for entrectinib or larotrectinib) [17, 18]. • In regards to response to chemotherapy, consistent predictors of poor response are progression with prior chemotherapy for advanced disease, relapse within 12 months of completing adjuvant chemotherapy, poor performance status, and multiple metastatic disease sites [19]. • Patients with visceral metastases (especially if rapidly progressing) generally have an aggressive

phenotype, while patients with soft tissue and bone metastases, or bone metastases only, have a more indolent phenotype [20].

D) Treatment modality of metastatic breast cancer Hormone receptor-positive, HER2-negative disease:
In general, endocrine therapy (with cyclin-dependent kinase [CDK] 4/6 inhibition) is beneficial for these patients, with fewer side effects compared with chemotherapy. Therefore, these options should be used as initial treatment for most patients with hormone receptor-positive disease as follows:
• Selection of endocrine therapy and accompanying targeted agents

• Selection of chemotherapy • Special considerations for patients with germline breast cancer susceptibility gene 1/2 (BRCA1/2) mutations

i) Chemotherapy treatment First-line chemotherapy: Patients who require first-line chemotherapy due to imminent organ failure, or who did not have access to a CDK4/6 inhibitor in the first-line setting [21]. Sequential single agents versus combination chemotherapy: In general, single-agent chemotherapy, used in sequence, is preferable to combination chemotherapy, since the single-agent chemotherapy is reasonably likely to induce palliation with fewer side effects, and no studies have demonstrated an overall survival (OS) benefit for the combination chemotherapy as long as both drugs are available in sequence [22]. Combination chemotherapy (rather than single-agent, sequential therapy) is most appropriate when the higher chance of response is assessed to be more important than the potential for higher treatment toxicity, due to concerns about impending organ dysfunction from existing or rapidly progressing disease burden. However, both clinicians and patients should know there are no prospective data that show that combination chemotherapy improves OS compared with single-agent, sequential cytotoxic chemotherapy [23]. Available drugs for single-agent chemotherapy include anthracyclines, taxanes, capecitabine, eribulin, vinorelbine, platinums, and other agents. Rechallenge with anthracyclines or taxanes is feasible in patients with a DFI ≥12 months. If available, the use of liposomal anthracyclines or protein-bound paclitaxel may be considered for the rechallenge [24].

Second-line treatment: Patients who require first-line chemotherapy due to imminent organ failure, or who did not have access to a CDK4/6 inhibitor in the first-line setting, it is clinically acceptable to use ET plus a CDK4/6 inhibitor as a subsequent therapy in cases of progressive disease or intolerable toxicity. Maintenance ET (single agent) following chemotherapy may be an option in clinically stable patients based on the judgment of the treating physician. The selection of chemotherapy versus further ET should be based on the extent and aggressiveness of the disease [25, 21].

ii) Endocrine therapy Preferred first-line therapy: An aromatase inhibitor plus cyclin-dependent kinase 4/6 inhibitors. The preferred initial regimen is a cyclin-dependent kinase (CDK)4/6 inhibitor with an aromatase inhibitor (AI). Premenopausal women must have ovarian suppression/ablation when on AIs [26].

Alternative front-line options: Acceptable alternatives to AIs plus CDK 4/6 inhibitors are discussed below. Ovarian suppression or ablation is added for premenopausal women. • Fulvestrant monotherapy: Fulvestrant is an alternative option, but is less preferable than front-line AI and CDK 4/6 inhibition. Fulvestrant is an estrogen receptor antagonist that blocks ER dimerization and DNA binding, increases ER turnover, and inhibits nuclear uptake of the receptor. Fulvestrant is administered as an intramuscular injection (500 mg loading dose on days 1, 14, and 29 of the first month, then maintenance dosing monthly at day 28, ±3 days). Fulvestrant monotherapy has never been compared with the combination of an AI and a CDK 4/6 inhibitor [27].

• Fulvestrant plus a CDK 4/6 inhibitor: Fulvestrant plus a CDK 4/6 inhibitor may be an option for treatment-naive patients who do not tolerate AI-based therapy as well as those with prior lines of endocrine therapy [28].

• Fulvestrant plus anastrozole: The combination of fulvestrant plus anastrozole is an acceptable alternative to the AI/CDK 4/6 inhibitor combination for the patient who presents with de novo metastatic breast cancer (and is therefore ET naïve) [29].

• Aromatase inhibitor monotherapy: For patients who have not received an AI in the adjuvant setting and are unlikely to tolerate a CDK 4/6 inhibitor, AI monotherapy is an appropriate alternative. Although fulvestrant as a single-agent has shown better activity than aromatase inhibition, some patients may prefer oral therapy to intramuscular injection. The efficacy of AIs as a first-line treatment for advanced or metastatic breast cancer and their OS superiority to tamoxifen in postmenopausal women were shown in a 2006 meta-analysis of 23 randomized trials (n = 8504 patients). Treatment with an AI resulted in an improvement in OS compared with tamoxifen (HR 0.89, 95% CI 0.80-0.99) and with other ETs (HR 0.87, 95% CI 0.82-0.93) [30, 31].

Second-line treatment: After progression on ET plus CDK4/6 inhibitor therapy, obtaining next-generation sequencing on prior or current tumor samples is necessary and should be performed before first progression for determination of PIK3CA and ER1 (or ESR1 if further AI therapy is being considered) as well as gBRCA1/2m status to guide treatment [15]. Also, although there are little data on the use of CDK4/6 inhibitors after progression on CDK4/6 inhibitors, rechallenge may be possible after a treatment-free interval of 12 months based on evidence regarding rechallenge with other therapies. In this setting, the small, randomized, phase II MAINTAIN trial in patients whose cancer had previously progressed on any CDK4/6 inhibitor and any ET showed that continuing a CDK4/6 inhibitor (ribociclib) after progression on a CDK4/6 inhibitor (87% palbociclib) and changing the endocrine agent (fulvestrant/exemestane) is more effective than changing the endocrine agent alone [32].

Selection of second-line therapy (chemotherapy versus further endocrine-based therapy) should be based on disease aggressiveness, extent and organ function, and consideration of the associated toxicity profile [15].

Alpelisib + fulvestrant is a treatment option for patients with PIK3CA-mutant tumors (in exons 7, 9, or 20), prior exposure to an AI (±CDK4/6 inhibitors), and appropriate hemoglobin A1c [33].

Everolimus + exemestane is an option since it significantly prolongs PFS. Tamoxifen or fulvestrant can also be combined with everolimus. If everolimus is used, stomatitis prophylaxis must be used [34]. PARP inhibitor monotherapy (olaparib or talazoparib) should be considered for patients with germline pathogenic BRCA1/2 mutations and as an option for those patients with somatic pathogenic or likely pathogenic BRCA1/2 or germline PALB2 mutations [35].

At least two lines of endocrine-based therapy are preferred before changing to chemotherapy in the absence of endocrine-refractory disease and/or imminent organ failure. In patients with imminent organ failure, chemotherapy is the preferred option.

A proposed schema of management of advanced ER+ disease is given in Figure 1 [36]. (Figure 1 would be inserted here).

Choosing between endocrine therapy and chemotherapy: Since endocrine therapy (ET; alone or in combination with targeted agents) is generally less toxic than chemotherapy, with comparable outcomes, it is preferable for most patients with hormone receptor-positive disease to begin treatment with ET [37]. For the minority of patients who have extensive visceral metastases, chemotherapy may be considered an appropriate alternative to ET plus targeted agents; however, there are no data suggesting a survival benefit to this approach [38].

Considerations for those who received adjuvant endocrine therapy: Women who progress ≥12 months from the end of adjuvant endocrine therapy (ET) and patients who present with de novo metastatic breast cancer are offered first-line ET or ET in combination with a cyclin-dependent kinase (CDK) 4/6 inhibitor (first-line therapy). Those who progress on or within 12 months of completing adjuvant ET are eligible for subsequent-line ET in combination with a CDK 4/6 inhibitor (subsequent therapy) [36].

Beyond second-line treatment

i) Chemotherapy: Sequential single-agent chemotherapy is generally preferred over combination strategies. In patients where a rapid response is needed due to imminent organ failure, combination chemotherapy is preferred.

ii) ii) Antibody drug conjugates

iii) • Sacituzumab govitecan: Sacituzumab govitecan is an anti-Trop-2 antibody drug conjugate for patients with hormone receptor-positive, HER2-negative cancers after prior treatment including ET, a cyclin-dependent kinase (CDK) 4/6 inhibitor, and at least two lines of chemotherapy (including a taxane in either neo/adjuvant or advanced disease setting) for advanced breast cancer [24].

iv) • Fam-trastuzumab deruxtecan: For patients with tumors that are either HER2-immunohistochemistry 1+, or 2+, and in situ hybridization negative, who have received at

least one prior line of chemotherapy for metastatic disease and, if the tumor is hormone receptor-positive, are refractory to ET, fam-trastuzumab deruxtecan is an appropriate option [39, 40].

iii) Endocrine therapy: For patients who are asymptomatic with slowly progressive disease, continuation of ET is reasonable, with one of the options below:

● Tamoxifen plus abemaciclib: For patients without prior treatment with a CDK 4/6 inhibitor, the combination of tamoxifen plus abemaciclib has shown efficacy and tolerability [41].

● Tamoxifen monotherapy: Although we prefer other options over tamoxifen for initial lines of ET, it may be an option in the later-line setting, recognizing that response rates are low. In the front-line setting, tamoxifen has yielded lower response rates relative to aromatase inhibitors (AIs), but similar OS; however, comparisons are not available for tamoxifen versus the combination of AIs and CDK 4/6 inhibitors, which is a more typical front-line regimen [42].

● Abemaciclib monotherapy: While CDK 4/6 inhibitors have been shown to combine effectively with ET, they also possess single-agent activity. The CDK 4/6 inhibitor abemaciclib is US Food and Drug Administration approved for use as monotherapy for women with progressive disease after ET and chemotherapy [43].

ADDITIONAL CONSIDERATIONS

Local treatments for the primary or metastatic sites: Although systemic therapy is the mainstay of treatment for metastatic breast cancer, local management of both the primary breast cancer, as well as metastasis-specific local treatment (i.e., metastasectomy, radiation therapy, surgery, etc.) may palliate symptoms and prevent cancer-related complications [44].

Osteoclast inhibitors: Patients with bone metastases should be treated with osteoclast inhibitors (bisphosphonates or receptor activator of nuclear kappa-B [RANK] ligand inhibition), as these agents have been shown to reduce the risk of skeletal-related events such as fractures, the need for surgery or radiation to bone, spinal cord compression, and hypercalcemia of malignancy [45].

Definition of treatment failure: Monitoring for treatment failure by taking into account serial changes in symptoms, physical findings, or tumor markers, as well as evidence of disease progression based on serial imaging. Criteria to define treatment failure include any of the following:
• Clinical deterioration during treatment (i.e., increasing disease-related symptoms, intolerable treatment toxicities, declining performance status)

• Evidence of new metastases

• Increasing size of previously documented metastatic lesions

The primary role of Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 is to standardize the reporting of results on clinical trials (Table 2). RECIST primarily applies to imaging of metastatic

disease, and it encompasses two of the three reasons for treatment failure. According to RECIST, disease progression on imaging is defined as any of the following:

● A 20 percent or more increase in the sum of measurable target lesions compared with the smallest sum previously recorded

● The appearance of any new lesions

Worsening of existing nontarget lesions, for example, bone metastases [46].

Monitoring therapy: Careful assessment of the response to therapy will assist in decisions for duration of treatment and in selection of subsequent treatments. However, the best approach for monitoring patients with MBC is not well established. Symptom relief without measurable disease response and achievement of stable disease as compared with disease progression may be clinically important [47].

a) History and examination: If symptom palliation is the main objective, clinical history alone may suffice to determine the success of therapy. Physical examination may allow response quantitation if disease is easily accessible (e.g., chest wall nodules, palpable lymphadenopathy) and serial changes in tumor markers or radiographic studies are essential in establishing the response to therapy [47].

b) Tumor markers: Serial assay of serum tumor markers (e.g., cancer antigen [CA] 15-3 and CA 27.29, both products of the mucin 1 [MUC1] gene, and carcinoembryonic antigen [CEA]) can aid in response assessment, particularly if disease sites are not assessable by usual criteria. Judicious use of serial tumor marker measurements may decrease the need for periodic radiographic evaluation [48]. Elevated tumor markers may occasionally be spurious. Thus, for patients without clear clinical or radiographic signs of progression, a rise in tumor markers alone should not dictate a change in treatment, although more frequent monitoring may be appropriate, in some instances. Up to 20 percent of patients successfully treated with systemic therapy may experience a transient increase (marker "flare") during the first one or two months after treatment initiation, presumably due to release of antigen by cytolysis [49]. Patients with abnormal liver function may also have falsely elevated marker levels because they are cleared by the liver [49]. CA 15-3 levels may be aberrantly elevated in patients with vitamin B12 deficiency and megaloblastic anemia, as well as in patients with thalassemia or sickle cell disease [50, 51].

d) Radiographic studies: Serial plain radiographs, computed tomography (CT) scan, or magnetic resonance imaging (MRI) can permit assessment of tumor response. A reasonable frequency of routine monitoring is every two to four months, although scans may be obtained earlier if there are clinical signs or symptoms of progression. Periodic scintigraphic bone scans, while helpful, may also be misleading. Technetium (Tc99) phosphonate accumulates in areas of osteoblastic activity rather than in cancer cells. In a patient experiencing a response to therapy, a "scintigraphic healing flare" may appear as early as two months and persist for as long as 12 months after initiating therapy [24]. Integrated positron emission tomography (PET)/CT is popular as a whole-body examination in monitoring response to therapy in MBC, as it has demonstrated high sensitivity and specificity in

detecting metastatic disease and can reliably assess response to therapy. There is also some evidence that metabolic changes in bone metastases in response to systemic therapy (i.e., a change in standardized uptake value) can predict response duration or time to progression [24].

e) Approach not routinely used: Circulating tumor cells: Detection of circulating tumor cells (CTCs) in blood samples of patients with MBC (≥5 CTCs per 7.5 mL of blood) has been shown to be a predictor of PFS and OS. However, the role of CTCs in the monitoring of patients remains controversial [52].

PROGNOSIS

Clinical factors that predict the rate of progression and survival include the interval between initial diagnosis and relapse, the number of metastatic sites, the presence or absence of visceral involvement, performance status, and biologic markers. Median survival for patients with MBC appears to have improved over time, a trend which has been attributed to the availability of new, more effective agents, including taxanes, aromatase inhibitors, cyclin-dependent kinase 4/6 inhibitors, and trastuzumab, pertuzumab, and other human epidermal growth factor receptor 2 (HER2) targeted agents [53].

Ki67 and other biomarkers for treatment decision making in breast cancer

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 [54]. 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 tumors sensitivity to systemic therapy. The absence of invasive tumor 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 tumors undergo a pCR having an excellent long-term outcome [55, 56]. 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) tumors positive for the PgR, ER-neg tumors do not derive benefit from endocrine therapy [57–59]. 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 [59].

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 [60]. It is recommended that ER and PgR assays may be considered positive if there are at least 1% positive tumor 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 [61]. In the adjuvant setting, the most recent EBCTCG meta-analysis reported no apparent benefit from tamoxifen for tumors 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 [59]. 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 [62].

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 tumors. 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 tumors. 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 tumors [59, 63, 64]. 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 [65]. 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 [66].

HER2 amplification does appear to be associated with chemotherapy sensitivity, with HER2 status reported to independently predict pCR following neoadjuvant chemotherapy [67]. Studies in the adjuvant setting have suggested that the benefit of anthracycline chemotherapy may be confined to those patients with HER2-amplified tumors, although a recent meta-analysis did not support this [68]. The 2011 St Gallen consensus guidelines do not recommend a specific chemotherapy regime for HER2-amplified cancers although the majority favored the use of anthracyclines and taxanes [69].

Whilst many HER2-positive cancers do appear chemosensitive, 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 tumors with the combination of trastuzumab and pertuzumab alone [70]. 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 [71].

baseline Ki67 and prognosis

A fundamental hallmark of cancer cells involves their ability to sustain chronic proliferation [72, 73]. 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 [74, 75]. 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 tumor. 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 ER-positive cancers typically exhibiting lower levels of proliferation [76, 77]. 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 [78]. 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 [79]. 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 [80]. 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 tumor 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 [81]. In 2011, the International Ki67 in Breast Cancer Working Group published recommendations for Ki67 assessment in breast cancer [82]. 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 poor 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 [83]. 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 [84]. Other studies have examined the power of baseline Ki67 to predict benefit from specific regimes 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 [85, 86].

Studies of neoadjuvant endocrine therapy have not reported a clear association with baseline Ki67 and response to therapy [87]. 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 [88]. 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 [89].

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, tumor 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 [90]. 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 [91, 92]. 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 tumor 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) [93–104]. 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) whereas higher Ki67 at baseline was not [105]. 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 [106]. 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 [107, 108]. 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 [109, 110].

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 [110]. 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 in neoadjuvant and short-term pre-surgical 'window of opportunity' studies (see Table 2) [111–118]. 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.

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 [119–121]. 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) [119, 122–125]. 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 [126]. 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 [127–129]. 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 [130]. 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-fluoruracil chemotherapy significantly correlated with a decreased risk of disease recurrence, P = 0.033 [131].

RCB and tumor cellularity

In 2003, Miller and Payne published a five-point grading system based on the changes in tumor cellularity in the excised tumor compared with the diagnostic core biopsy to predict long-term outcome following neoadjuvant chemotherapy. To account for potential heterogeneity in tumor cellularity, at least three pre-treatment core biopsies were taken from different areas of the tumor. Grade of response was significantly correlated with DFS (P = 0.02) and OS (P = 0.01) [132]. The RCB extended this concept of measuring tumor cellularity in residual post-chemotherapy disease, incorporating the dimensions of residual tumor 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 [133]. A pathological response index (PRI) which includes the presence of vascular invasion and evidence of chemotherapy-related changes along with reduction in the tumor 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 [134].

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 tumor biomarkers using less invasive methods is appealing and has attracted substantial interest in recent years. Measurements of circulating tumor cells (CTCs) have been reported to correlate with the outcome in metastatic disease and the response to chemotherapy and endocrine therapy [135, 136]. 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 [81].

In colorectal cancer, the detection of tumor-derived DNA in plasma following surgery has been reported to be predictive of disease relapse [137]. Studies in metastatic breast cancer have also examined assays of tumor phenotype with detection of PIK3Ca mutations and determination of HER2 status reported [138, 139]. 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.

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