BREAST CANCER

Adjuvant Chemotherapy of Breast Cancer: Improving Patient Selection and Understanding Benefits and Risks

CHAIR
Angelo Di Leo, MD
Istituto Toscano Tumori
Prato, Italy

SPEAKERS
Joseph Sparano, MD
Albert Einstein College of Medicine, Montefiore Medical Center
Bronx, NY

Erica Mayer, MD, MPH
Dana-Farber Cancer Institute
Boston, MA
Adjuvant Chemotherapy: Which Patient? What Regimen?

Natalie Turner, MBBS, Laura Biganzoli, MD, PhD, Luca Malorni, MD, PhD, Ilenia Migliaccio, MD, PhD, Erica Moretti, MD, Marta Pestrin, MD, PhD, Giuseppina Sanna, MD, Olimpia Siclari, MD, and Angelo Di Leo, MD, PhD

OVERVIEW

In the past, treatment decisions regarding adjuvant chemotherapy in early breast cancer (EBC) were made solely based on clinico-pathologic factors. However, with increased awareness of the importance of underlying tumor biology, we are now able to use genomic analyses to determine molecular breast cancer subtype and thus identify patients with tumors that are chemotherapy resistant and unlikely to benefit from the addition of chemotherapy. Although genomics has allowed some patients to avoid chemotherapy—specifically those with luminal A–like breast cancer—these assays do not indicate which regimen is most appropriate. For this, consideration must be given to the combination of underlying tumor biology, tumor stage, and patient characteristics, such as age and tolerability of side effects.

Adjuvant chemotherapy improves outcomes in EBC. However, as our understanding of breast cancer biology increases, and the choice of chemotherapy regimen broadens, two major questions are raised concerning adjuvant chemotherapy treatment decisions, namely: should my patient receive chemotherapy? And if so, which is the best regimen?

WHICH PATIENT?

Decisions regarding chemotherapy have previously been made based solely on clinicopathologic factors, such as tumor stage and node status. However, in recent years there is increasing acknowledgment of the importance of tumor biology in terms of both prognosis and variable response to chemotherapy. In particular, as was initially demonstrated in pioneering work from the International Breast Cancer Study Group (IBCSG) and Cancer and Leukemia Group B (CALGB), some hormone receptor–positive (HR+) breast cancers do not benefit from adjuvant chemotherapy, are relatively chemotherapy resistant, and have an otherwise excellent prognosis with endocrine therapy alone. As such, we recommend avoidance of adjuvant chemotherapy for node-negative, luminal A–like EBC. In the setting of nodal involvement, although the proportional benefit of chemotherapy is low, the role of genomics is that it allows us to identify a subset of breast cancers, namely luminal A–like tumors, where proportional benefit from adjuvant chemotherapy is minimal independent of disease stage. As such, we recommend avoidance of adjuvant chemotherapy for node-negative, luminal A–like EBC. In the setting of nodal involvement, although the proportional benefit of chemotherapy is low, the
absolute benefit is uncertain, hence the addition of chemotherapy to endocrine therapy may be discussed with the patient in this setting.

Pending Issues for Selecting Which Patients Should Receive Adjuvant Chemotherapy

Immunohistochemical (IHC) definition of luminal breast cancers. The use of molecular classifiers in routine clinical practice may be limited by their relative cost and complexity. Alternatively, luminal A and B tumors may be defined by IHC determination of estrogen receptor (ER), progesterone receptor (PgR), HER2, and Ki-67. Various criteria have been suggested, though, as yet, there is no consensus on the best definition to employ.\(^\text{10-12}\) In one approach, distinction between receptor (PgR), HER2, and Ki-67. Various criteria have been suggested, though, as yet, there is no consensus on the best definition to employ.\(^\text{10-12}\) In one approach, distinction between luminal A and HER2-negative-luminal B tumors relies on the semi-quantitative expression of Ki-67, with a putative cut point of 14%.\(^\text{11,13}\) This 14% cutoff, first identified by Cheang et al, was able to distinguish molecular-defined luminal A and luminal B subtypes with a sensitivity and a specificity of 77% and 78%, respectively.\(^\text{11}\) Furthermore, in a large cohort of patients with EBC, IHC-defined luminal B tumors (either HER2 positive or Ki-67 $\geq$ 14%) were associated with a worse outcome compared with IHC-defined luminal A tumors.\(^\text{11}\) Yet the accuracy of this method compared with molecular subtype determination by genomics has been shown to be less than 80%, indicating that further refinements and validation are needed.

We recommend that, where genomic assays are available without additional costs to patients, this approach be used to determine tumor subtype. However, where issues of cost and/or access to genomic assays exist, IHC can be utilized as the first step in breast cancer subtype determination, with a genomic assay reserved for cases that cannot be clearly categorized as luminal A or B using IHC. It is critical that, if relying on IHC assessment, quality assurance checks are made in collaboration with your pathologist, to avoid common problems related to IHC assessment, such as inaccurate fixation time, and variable quality of antibodies, which can lead to false-positive and -negative results.

Late relapses in luminal breast cancers. The majority of luminal A tumors have an excellent prognosis with endocrine therapy alone; however, luminal cancers, particularly luminal A, harbor risk of late relapse after disease-free intervals of five to 15 or more years. Luminal A and B breast cancers are extremely molecularly heterogeneous. A recent integrated analysis of copy number changes and gene expression profiling of nearly 2,000 EBCs dissected luminal cancers in different prognostic subgroups, some with particularly unfavorable outcomes, such as the IntClust2 group.\(^\text{14}\) The molecular determinants of late relapses are still under investigation. In the future, should it be possible to identify patients at higher risk of late relapse, it is anticipated that these patients would benefit from extended endocrine therapy, rather than the addition of adjuvant chemotherapy.

Defining treatment for patients with intermediate-risk disease. The approach to luminal A tumors with nodal involvement is uncertain. Classically nodal involvement implied the need for adjuvant chemotherapy; however, the generally good outcomes with luminal A disease with endocrine therapy alone, combined with relative chemoresistance, has raised the possibility that tumors should preferentially be treated based on biology, rather than stage. Just as a tumor with aggressive biologic characteristics will often be treated with chemotherapy despite small size or no nodal involvement, it may be that chemotherapy provides no additional benefit over endocrine therapy in less-aggressive breast cancers. This clinical question is currently being investigated in the RxPONDER trial (NCT01272037), in which patients with low-risk ER+ breast cancer, defined by RS of 25 or less, but with involvement of one to three axillary nodes are randomly assigned to endocrine therapy alone or chemotherapy plus endocrine therapy. Results from this trial should help to answer whether favorable tumor biology is more important than unfavorable tumor stage when making adjuvant therapy decisions.

Two current prospective trials should help refine the approach to these borderline or intermediate-risk patients. The TAILORx trial (NCT00310180) randomly assigned patients with intermediate risk on RS to receive treatment with endocrine therapy alone or chemotherapy plus endocrine therapy. The MINDACT trial (NCT00433589) randomly assigned patients with EBC with 3 or fewer positive nodes and discordant recurrence risk assessment based on the 70-gene signature or clinical assessment to chemotherapy or not using the result of one or the other. Until results from these trials become available, we recommend treatment of intermediate-risk disease based on standard clinicopathologic risk factors. Chemotherapy could be avoided in patients with small node-negative tumors, while features such as nodal involvement, large T size, histology, high prolifera-

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**KEY POINTS**

- Identification of breast cancer molecular subtypes and use of genomics has revolutionized the approach to the selection of patients requiring adjuvant chemotherapy.
- A subgroup of hormone receptor-positive breast cancers (luminal A-like tumors) derive little, if any, benefit from the addition of chemotherapy to endocrine therapy and can therefore be spared chemotherapy-associated toxicities.
- Pending issues regarding the selection of patients for adjuvant chemotherapy include the need for an accurate immunohistochemical (IHC) surrogate for defining molecular subtypes, how to best approach patients with intermediate relapse risk on current assays, and the need to ideally incorporate peripheral markers of micrometastases into relapse risk assessment.
- Genomics cannot provide guidance on the selection of specific adjuvant chemotherapy regimens, and thus the choice of what regimen to utilize should be made based on both tumor biology and patient characteristics.
tion, or lymphovascular invasion would favor chemotherapy. Additional patient factors to be considered include age at diagnosis, performance status, and presence or absence of comorbidities.

Not all patients with high-risk disease benefit from chemotherapy. From retrospective data from the NSABP B-20 trial used in the validation of the RS, a clear advantage for chemotherapy in patients with disease classified as high risk for recurrence was seen. However, after 10 years’ median follow-up, approximately 60% of patients in this high-risk category who only received tamoxifen remained disease free. Thus, a significant proportion of patients with high-risk disease do not relapse despite not receiving chemotherapy. Current risk prediction tools are therefore over-sensitive, and refinements that allow more accurate stratification of high-risk disease may allow some patients to avoid chemotherapy and the associated toxicities. However, until such refinements are made or new tools are defined, we recommend treatment of high-risk disease with adjuvant chemotherapy, acknowledging that some patients, who are currently not identifiable, are not receiving benefit.

Considerations for the Future
All currently utilized prognostic factors for EBC are derived from the excised primary tumor. Yet there is now increasing awareness of the role of peripheral factors—such as markers of micrometastatic disease—in predicting tumor behavior, disease course, and patient outcomes. Assessment of these peripheral factors should enable a more holistic approach to determining the risk of relapse in EBC.

Detection of micrometastases may be possible through analysis of disseminated tumors cells (DTC; e.g., on bone marrow assessment), circulating tumor cells (CTC), cell-free DNA (cf-DNA), plasma microRNAs, or metabolomics. A large number of studies showed that the presence of DTC in bone marrow has prognostic effect for patients with EBC, suggesting that DTC could be used as a marker for disease recurrence. Similarly, CTC assessment may predict poor survival after neoadjuvant treatment or primary breast cancer surgery, whereas cf-DNA concentration and miRNA have been associated with EBC disease relapse. These novel techniques may thus have utility in predicting disease recurrence, improving early detection of relapse, and enhancing patient outcomes; although in each case, further validation in larger cohorts is needed before routine use in the EBC setting. In the future, these tools could be combined with tumor biologic and genomic characteristics to provide a more complete picture of a patient’s relapse risk and thus help inform treatment decisions regarding the need for adjuvant chemotherapy.

WHAT REGIMEN?
Gene expression tests, such as the RS, are extremely promising in identifying who should benefit from chemotherapy but not informative on the type of chemotherapy to be given. For this, consideration must be given to the combination of tumor subtype, tumor stage, and patient characteristics.

Endocrine-Responsive Breast Cancer
Luminal A. As discussed above, the general recommendation is avoidance of chemotherapy, particularly in node-negative disease and instead to treat solely with endocrine therapy. One of the challenges in the implementation of genomic assays into adjuvant chemotherapy treatment decisions relates to the fact that, regardless of the specific assay employed, gene expression is initially reported as a continuous variable, with arbitrary cut-off points, then assigned to separate the lower-risk tumors from the higher-risk tumors. These cut-off points have been carefully calculated to maximize the accuracy of the assay to discriminate between better and poorer outcomes. Nonetheless, use of arbitrary cut-off points when evaluating a continuous variable invariably leads to some tumors residing close to that cut-off point, where interpretation of relapse risk for these borderline cases is difficult.

Luminal B. As luminal B tumors are characterized by high proliferation rates, increased aggressiveness, and increased risk of relapse, chemotherapy in addition to endocrine therapy is recommended. However, there are no data to determine whether chemotherapy should be a standard adjuvant regimen as would be used for HR- disease, or if less intensive chemotherapy is as effective. Choice of approach should therefore be made based on clinical assessment of relapse risk.

HER2+ Breast Cancer
The standard adjuvant treatment of HER2+ EBC is trastuzumab plus chemotherapy, preferably using an anthracycline-based regimen. However, only a small percentage of patients receiving adjuvant anthracyclines actually benefit, yet all are exposed to the associated toxicities. In particular, the risk of cardiotoxicity is increased with sequential trastuzumab therapy. Thus the major consideration in HER2+ EBC is whether or not to include an anthracycline in the treatment regimen.

The observed increased sensitivity of HER2+ tumors to anthracyclines may be related to coexpression of TOP2A. In the BCIRG-006 trial, both trastuzumab-containing arms (AC plus docetaxel/trastuzumab [ACTH] and docetaxel/carboplatin/trastuzumab [TCH]) were superior to the non-trastuzumab arm of AC plus docetaxel (ACT), with the study not powered to detect a difference between ACTH and TCH. Treatment response based on TOP2A status was also assessed, with benefit from anthracyclines evident in patients with TOP2A amplification but not with TOP2A-
Triple-Negative Breast Cancer (TNBC)

TNBC with good prognosis. TNBC is typically associated with poor prognosis; however, emerging molecular, clinical, and pathologic data indicate that the TNBC group is heterogeneous. In particular, histologic special types of breast cancer—some of which are known to have excellent prognosis—are classified as TNBC. Of the 18 different histologic special types of breast cancer, at least four rare types—medullary, apocrine, metaplastic, and adenoid cystic carcinomas—are predominantly negative for ER, PgR, and HER2. Metaplastic carcinomas show worse prognosis compared with TNBC of no special type. Conversely, adenoid cystic, and medullary carcinomas, despite the high proliferation index of the latter, have been consistently reported to have excellent prognoses. However, data on the role of adjuvant chemotherapy for special types of breast cancer are lacking, mainly because of their low frequencies.

We recommend that, in the case of a good prognosis TNBC, the diagnosis first be confirmed by a pathologist with experience in rare tumor subtypes. Then, in the absence of nodal involvement, adjuvant chemotherapy may be avoided. However, for node-positive disease, even of a good prognosis subtype, adjuvant chemotherapy is recommended, as the safety of not treating with chemotherapy in this setting is unknown because of an absence of data.

Other TNBC. Excluding the rare, good prognosis types, TNBC should be treated with chemotherapy, usually a sequential anthracycline/taxane regimen, especially in patients with node-positive disease. There is no data comparing a less intensive chemotherapy regimen with standard anthracycline plus taxane for lower-risk (pT1, pN0) TNBC; although consideration may be given to regimens such as TC or CMF, particularly if there was specific concern regarding treatment toxicities.

With TNBC and BRCA-associated breast cancers sharing many biologic characteristics, the use of platinum has been considered in TNBC. Although excellent responses to cisplatin have been seen in patients with triple-negative, BRCA1-associated breast cancer, with pathologic complete response (pCR) rates of over 80%, pCR rates with cisplatin for sporadic TNBC are considerably lower, around 20%. Thus, platinum-based regimens are not recommended over standard adjuvant regimens in sporadic TNBC outside of a clinical trial.

Chemotherapy in the Very Young Patient

Breast cancer in young patients (younger than age 35) is typically characterized by aggressive disease, including higher incidence of hormone-insensitive, undifferentiated, and HER2+ tumors, and may be associated with unique biologic features compared with older women, while young age itself is an independent risk factor for poor prognosis.

Although luminal A-like EBC occurs less commonly in younger women than older women, these tumors can be treated with endocrine therapy alone (usually combined estrogen blockade), with excellent outcomes expected. However, because young age itself is an poor prognostic factor, for luminal B, HER2+, or TNBC, we recommend use of adjuvant polychemotherapy with an anthracycline plus taxane-based regimen, even for node-negative disease.

Importantly, with all adjuvant breast cancer chemotherapy regimens being at least moderately gonadotoxic, young patients with EBC should have early referral to a reproductive specialist.

Chemotherapy in the Older Patient

When considering adjuvant chemotherapy in older patients (age 65 or older) with breast cancer, the potential benefits from effective treatment must be balanced against risk of toxicities, functional decline, and decreased quality of life. Older women with biologically aggressive EBC may gain as much benefit from adjuvant chemotherapy as younger women; however, this specifically applies to fit elderly patients, with few, if any, comorbidities. Even in this select group, increased toxicities from chemotherapy may be seen. The utility of adjuvant chemotherapy in older patients who are less fit is unknown.

Determining which patients meet the definition of “fit” is also problematic because of a lack of suitable frailty screening tools. The comprehensive geriatric assessment remains the gold standard for detecting functional deficits, although its integration into oncology practice is challenging.

Ideally older patients should undergo some form of geriatric assessment to determine their fitness. We recommend consideration of adjuvant chemotherapy for fit older patients with node-positive, HR- EBC, whereas chemotherapy may also be considered for HR+, luminal-B disease and node-negative, HR- disease. Based on superior outcomes with polychemotherapy, combination treatment is preferable to single agent therapy. Conversely, in patients who are frail and less fit, adjuvant chemotherapy outside of a clinical trial setting is typically not recommended.

CONCLUSION

The selection of both patients and regimens for adjuvant chemotherapy in EBC is an evolving field. Ongoing research
should ideally be directed at better defining which patients may avoid chemotherapy, which patients require chemotherapy because of the presence of peripheral markers of micrometastases, and which specific regimen is most effective and safe based on the underlying tumor subtype and individual patient characteristics.

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Early and Late Long-Term Effects of Adjuvant Chemotherapy

Erica L. Mayer, MD, MPH

OVERVIEW

Adjuvant chemotherapy continues to play an important role in breast cancer management. Exposure to chemotherapy can lead to a variety of early and late long-term toxicities, including ovarian failure (with resultant infertility and sexual dysfunction), bone loss, weight gain, neurotoxicity, neurocognitive changes, cardiac toxicity and secondary malignancy. Although chemotherapy effects may vary in medical severity, all effects have the potential to lead to a decrease in quality of life and a decrement on overall health status. Improved understanding of the etiology and management of chemotherapy-related toxicity may allow optimization of patient selection for treatment and ameliorate the concerns of patients who are considering embarking on a chemotherapy program. This article presents an overview of relevant early and late long-term toxicities, with a focus on recent advances and clinical management.

With the advent of genomic testing, rates of use of adjuvant chemotherapy for breast cancer have been in decline, although chemotherapy continues to play a significant role in the adjuvant therapy of patients with triple-negative and HER2-positive disease. Administration of chemotherapy can reduce risk of breast cancer recurrence and is generally considered tolerable with the use of contemporary supportive care, however, exposure to typical regimens may lead to both early and late toxicities. Given the estimated 3 million breast cancer survivors in the United States, many women are at risk of chemotherapy-related effects. Improved understanding of the etiology and management of chemotherapy-related toxicity may allow optimization of patient selection for chemotherapy and ameliorate the concerns of patients who are considering embarking on a chemotherapy program.

OVARIAN FAILURE: PREMATURE MENOPAUSE, INFERTILITY, SEXUAL DYSFUNCTION

Chemotherapy-induced premature ovarian failure is a noteworthy toxicity for premenopausal women receiving adjuvant chemotherapy. Although the majority of women may experience temporary amenorrhea, in many, ovarian function will return in the months following completion of treatment. However, a subset will experience permanent chemotherapy-induced ovarian failure, with the risk of permanent menopause increasing with age and modulated by chemotherapy type and duration. An implied additional cost of infertility exists for patients who sustain permanent amenorrhea. For patients with hormone receptor–positive cancers, infertility can also occur secondary to natural ovarian aging during long-term endocrine therapy. Even for patients who regain menses after chemotherapy, there may be an increased likelihood of premature menopause (under age 45) in patients who receive chemotherapy at a younger age. Concerns over risks of infertility can be substantial for young patients with breast cancer and may influence treatment selection. Pretherapy referral to a fertility specialist for a discussion of fertility preservation may help alleviate concerns and is endorsed by ASCO guidelines. Assisted reproductive technologies may be considered for patients facing infertility after treatment, although there are theoretical concerns about ovarian stimulation in the setting of hormone receptor–positive cancer. Specialty consultation to discuss newer techniques, including ovarian stimulation with aromatase inhibitors, is recommended. Available data to date do not suggest adverse outcomes in patients who become pregnant after a breast cancer diagnosis, regardless of hormone receptor status of the tumor.

One of the most prominent early effects of ovarian failure can be frequent and disruptive hot flashes, which may be accentuated by concomitant use of adjuvant endocrine therapy. Multiple management techniques have been evaluated; commonly used strategies include behavior modification, antidepressants, clonidine, gabapentin, vitamin E, and acupuncture. Data to date do not conclusively suggest inferior outcomes after concurrent use of CYP2D6-inhibiting antidepressants and tamoxifen; however, recent prescribing trends have supported a shift toward greater use of weak CYP2D6-inhibiting antidepressants in breast cancer survivors. Atrophic vaginitis is another common and significant side effect which can lead to dysuria, frequent urinary tract infections, pruritus, and dyspareunia. Nonhormone lubricants (both
water-based and silicone) are typically recommended, particularly in a patient with a history of hormone receptor–positive breast cancer. Topical vaginal estrogen therapy may be a more effective approach; although estrogen levels have been noted to rise with local estrogen delivery, no signal of increased risk of cancer recurrence has been noted. Additional recommended lifestyle interventions include increased sexual activity and Kegel exercises to improve pelvic muscle tone.

Sexual dysfunction is a common sequelae of treatment-related ovarian failure, and can be manifest by decreased libido, dyspareunia, and difficulty with orgasm; it is estimated to affect up to 50% of all breast cancer survivors, particularly those who experience treatment-related ovarian failure. The etiology of sexual dysfunction is multifactorial, likely reflecting not only reduced levels of estrogen, but also the psychologic trauma of cancer diagnosis and treatment for a young woman. Specific interventions may include vaginal lubricants, avoidance of concomitant medications which decrease libido, and counseling with a sexual health specialist.

**WEIGHT GAIN**

It has long been recognized that women who receive adjuvant chemotherapy for breast cancer gain weight, on average about 10 lb. Reasons for weight gain are likely multifactorial, including changes in activity level, menopausal status, endocrine manipulation, diet, metabolism, and mood. Results of observational studies describing the effect of weight gain after a breast cancer diagnosis on future disease recurrence have been variable, although analyses from the Nurses’ Health Study and other studies have suggested weight gain may increase risk of disease recurrence. Diet and exercise interventions have demonstrated success in preventing weight gain or stimulating weight loss and current guidelines for exercise training in early cancer survivors have been published. All efforts should be made to encourage breast cancer survivors to “avoid inactivity” and pursue a more active lifestyle.

**BONE LOSS**

Loss of bone density is a common occurrence in both pre- and postmenopausal breast cancer survivors. Proposed etiologies include premature menopause as well as the effect of breast cancer therapies, specifically aromatase inhibitors, on circulating estrogen levels. The development of decreased bone density may have significant health consequences. In an analysis from the Women’s Health Initiative, increased fracture risk was seen in postmenopausal breast cancer survivors compared with participants without breast cancer. A variety of guidelines for bone health screening and management of osteopenia and osteoporosis have been published. In general, it is recommended to screen at-risk individuals for osteoporosis by dual-energy x-ray absorptiometry every 1 to 2 years, and consider initiation of bisphosphonate therapy for scores defining osteoporosis. Other maneuvers, including pursuing weight-bearing exercise and adequate calcium and vitamin D, are generally recommended for breast cancer survivors.

**NEUROPATHY**

Peripheral sensory or motor neuropathy can occur after exposure to microtubule inhibitors such as taxanes. Sensory neuropathy can be characterized by both paresthesias and pain, which can significantly affect a patient’s quality of life. Rates and severity of taxane-related neuropathy vary and reflect agent selection, dose, schedule, and comorbidities. Supportive management during treatment typically includes dose modification and treatment delay; supplemental preventive agents including glutathione, acetyl-L-carnitine, and alpha-lipoic acid are under investigation. Although some patients will experience gradual improvement in neuropathy, many are left with residual and potentially disabling symptoms. Multiple management techniques have been evaluated, including use of gabapentin or venlafaxine, for which there is supportive data from a randomized trial. CALGB 170601, a randomized phase III trial of duloxetine for painful chemotherapy-induced neuropathy, demonstrated reductions in pain scores with duloxetine compared with placebo. Investigation of additional nonpharmacologic modalities is ongoing.

Ultimately, best management of peripheral neuropathy would be avoidance of the toxicity through identification of individuals at greatest risk. One path of investigation has suggested higher paclitaxel acute pain syndrome (P-APS) scores with first dose of chemotherapy may correlate with peripheral neuropathy. Pharmacogenomic analysis has identified single nucleotide polymorphisms (SNPs) associated with the development of moderate to severe peripheral neuropathy after paclitaxel exposure. Reassuringly, the development of neuropathy does not appear to be correlated with chemotherapy efficacy, therefore supporting the safety of further development of predictive biomarkers for neurotoxicity, including SNPs or P-APS scores, which may allow modification of adjuvant chemotherapy selection to reduce the risk of permanent disabling neuropathy.

**KEY POINTS**

- Adjuvant chemotherapy can lead to early and late long-term side effects for breast cancer survivors.
- Given the number of breast cancer survivors, many women are at risk of experiencing toxicity.
- Effects of chemotherapy vary in severity, but can often negatively affect quality of life and overall health status.
- Management of long-term chemotherapy-related toxicity involves screening for symptoms, use of supportive medication, and referral for specialty consultation as needed.
- Ongoing research is evaluating both the etiology of toxicity as well as effective interventions.
CARDIAC DYSFUNCTION
The most common chemotherapy-related cardiotoxicity observed in breast cancer survivors is left ventricular dysfunction.41 Although cyclophosphamide and taxanes have been associated with a variety of cardiac complications, the most common agents implicated in adjuvant therapy after chemotherapy are anthracyclines and trastuzumab.

Anthracycline-mediated cardiotoxicity can occur in an acute or subacute fashion, however, the majority of cases are late-onset, occurring at least 1 year after completion of therapy.42 Late-onset cardiotoxicity tends to be irreversible, is related to cumulative anthracycline dose, and may reflect a variety of intracellular mechanisms including free radical formation.43 Although absolute rates of anthracycline-related cardiac dysfunction in clinical trials generally have been low, analysis of nontrial older populations through the Surveillance, Epidemiology, and End Results (SEER) database suggests the incidence may be higher, with a rate of CHF of 38.4% in patients with breast cancer ages 66 to 70 treated with adjuvant anthracycline-based therapy compared with 29% in those who did not get chemotherapy.44 Exposure to trastuzumab, the highly effective monoclonal antibody for HER2-positive breast cancer, may lead to cardiac dysfunction following a different pattern than anthracycline-mediated toxicity: typically occurring during time of medication administration, and generally reversible with a hold in therapy and use of cardiac medication.45 It has been proposed that the differential patterns of cardiotoxicity between the two agents reflect divergent mechanisms of action, with anthracyclines causing permanent cardiomyocyte apoptosis and necrosis, and trastuzumab leading to temporary cellular changes.41 Rates of asymptomatic or severe cardiac dysfunction in the major trials of adjuvant trastuzumab have varied from a maximum of 4.1% with a standard anthracycline-containing regimen,46 to 0.6% to 1.87% in regimens without concurrent chemotherapy and trastuzumab or without anthracyclines.47–49 Rates of asymptomatic drop in cardiac function are higher, in the range of 17% to 19% after anthracycline exposure in the major adjuvant trials.36,37 Reassuringly, long-term follow-up from the adjuvant trastuzumab trials have suggested late cardiotoxicity events related to trastuzumab appear to be rare.50,51 Routine evaluation of cardiac function is recommended for patients receiving ongoing trastuzumab; however, following completion of therapy, further cardiac evaluation is symptom-driven only.

Prechemotherapy identification of individuals at risk of cardiac toxicity would improve selection of chemotherapy regimens. Clinical risk factors predictive of cardiotoxicity have included older age, pre-existing hypertension, low baseline left ventricular ejection fraction, and elevated body mass index. Long-term follow-up data from NSABP B-31 have been used to construct a Cardiac Risk Score, incorporating age and left ventricular ejection fraction (LVEF), to predict risk of a cardiac event.51 Novel imaging, including echocardiographic techniques with greater sensitivity to discern subclinical cardiac dysfunction, and potentially concurrent use of cardiac biomarkers, may have even more promise as predictive tools.52 There is no clear role for routine screening for left ventricular dysfunction in breast cancer survivors in the absence of clinical symptoms, however, for survivors with suspected cardiac toxicity, imaging with echocardiography and referral to cardiology are strongly recommended.

NEUROCOGNITIVE DYSFUNCTION
Neurocognitive changes after chemotherapy exposure, termed “chemo-brain,” are a source of serious concern and anxiety for breast cancer survivors.53,54 It is estimated up to 75% of women who receive chemotherapy will report a change in cognitive function in the 2 years after treatment.55 Patients typically describe problems with attention, memory, and concentration.

Studies to date have been complicated by small sample size, diverse definitions of cognitive impairment, and variable pre-exposure assessments of cognitive function. Additionally, studies evaluating cognitive dysfunction during chemotherapy present divergent results from those using a postchemotherapy time-point. Early cross-sectional studies demonstrated increased cognitive impairment in patients with breast cancer during and after chemotherapy when compared with healthy matched controls.56,57 Subsequently, prospective longitudinal studies have suggested evidence of postchemotherapy change in cognitive dysfunction, at least in a subset of patients.54 A recent meta-analysis has analyzed 17 studies evaluating cognitive functioning in the post-chemotherapy period, confirming small deficits in verbal ability and visuospatial control in patients at least 6 months

### Table 1. Summary of Complications with Adjuvant Chemotherapy

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<thead>
<tr>
<th>Toxicity</th>
<th>Role for Screening/Recommended Interventions</th>
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<tbody>
<tr>
<td>Ovarian failure</td>
<td>Behavioral/medical management hot flashes, reproductive endocrinology consultation for fertility counseling</td>
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<tr>
<td>Sexual dysfunction</td>
<td>Non-hormonal lubricants, counseling</td>
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<tr>
<td>Weight gain</td>
<td>Exercise and diet interventions</td>
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<tr>
<td>Neuropathy</td>
<td>Consider venlafaxine, duloxetine for painful symptoms, glutamate, vitamin B6</td>
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<tr>
<td>Cardiotoxicity</td>
<td>Echocardiogram for symptoms, Referral to cardiology as needed</td>
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<tr>
<td>Cognitive dysfunction</td>
<td>Referral to neuropsychologist, cognitive behavioral therapy</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Screening bone density examination, Use of calcium, vitamin D, bisphosphonates</td>
</tr>
<tr>
<td>Secondary hematologic malignancies</td>
<td>Evaluation of peripheral blood counts in setting of symptoms</td>
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out from chemotherapy compared with testing prechemotherapy or in healthy participants. Studies evaluating pre- and post chemotherapy imaging have also attempted to investigate anatomic correlates of cognitive deficits; small prospective trials using MRI have suggested both structural changes and changes in brain activation after chemotherapy exposure. It has been proposed, however, that the etiology of cognitive dysfunction is likely multifactorial; although exposure to chemotherapy may contribute, other factors are likely relevant as well, including other modalities of treatment (surgery, radiotherapy, endocrine therapy), supportive care medications, menopausal symptoms, anxiety, depression, fatigue, or other comorbid conditions. An International Cognition and Cancer Task Force has suggested a subgroup of patients may be especially sensitive to neurocognitive effects of chemotherapy, including those of advanced age or lower cognitive reserve. Furthermore, genetic polymorphisms in susceptibility genes, including apolipoprotein E (APOE) and catechol-O-methyltransferase (COMT) may identify individuals with vulnerability to cognitive dysfunction after chemotherapy. Variability in age and polymorphism distribution may explain the variability in results in some of the prospective cognitive studies to date. There is interest in whether prophylactic or therapeutic interventions, including the psychostimulant modafinil, fluoxetine, or structured cognitive behavioral therapy, may improve symptoms. Further work is necessary to confirm if individuals at particularly high risk of cognitive change can be identified in advance to protect from exposures or treat with preventative medication, while providing reassurance to others who may be unlikely to experience this toxicity.

SECONDARY MALIGNANCY
One of the rarest yet most feared long-term toxicities of adjuvant chemotherapy is hematologic malignancy, specifically myelodysplasia (MDS) or acute myeloid leukemia (AML). The etiology of this complication is thought to reflect exposure to topoisomerase II–targeted agents (anthracyclines) or alkylating agents (cyclophosphamide), which are frequently included in adjuvant chemotherapy regimens. Topoisomerase II–related myeloid malignancies may present within 5 years of exposure, may not be preceded by MDS, and may have abnormal cytogenetics of 11q23. Alkylator-related malignancies present after a longer duration of time, may be preceded by MDS, and may have cytogenetic abnormalities of chromosomes 5 and 7. The risk of secondary malignancy appears to reflect increased cumulative dose exposure, although most series report population rates of less than 1%. Concurrent use of growth factors to support “dose-dense” scheduling, or radiotherapy, may contribute a slight additional risk of myeloid malignancy, although these topics remain under investigation.

Older patients are thought to be at increased risk of myeloid complications, although they have typically been excluded from analysis of younger cohorts who participate in clinical trials. A SEER-Medicare population-based analysis of over 64,000 older patients with breast cancer (median age 76) suggested a small but significant increase in the risk of developing AML after chemotherapy, with a hazard ratio of 1.53 (p = 0.005), although absolute risks at 10 years remain small (1.8% with chemotherapy vs. 1.2% without chemotherapy). Analysis of over 21,000 patients in the National Comprehensive Cancer Network (NCCN) suggested a 10-year rate of MDS/AML of 0.27%, with a slight additional increase in those who had received adjuvant chemotherapy.

Methods to reduce exposure to potentially bone marrow–toxic chemotherapy may help reduce the risk of secondary myeloid malignancies. Adjuvant regimens which replace anthracycline with a taxane have demonstrated slightly decreased rates of secondary myeloid malignancies, and could be considered in a situation where risk of myeloid malignancy is a significant concern. Specific predictors of future MDS/AML are not known, however, continued use of genomic tools to better identify chemotherapy-sensitive tumors would help improve the risk/benefit ratio for this toxicity by reducing chemotherapy exposure in patients unlikely to receive anticancer benefit.

CONCLUSION
Despite the gains in reduction of cancer recurrence with administration of adjuvant chemotherapy, breast cancer survivors may be exposed to risks of both short- and long-term toxicities after chemotherapy. Although chemotherapy effects may vary in medical severity, all effects have the potential to lead to a decrease in quality of life and a decrement on overall health status. Improved management of the effects of chemotherapy will require better understanding of management strategies, and larger prospective trials evaluating a variety of interventions are underway. Additionally, for the first time, the 2013 NCCN Guidelines will include guidelines for screening breast cancer survivors for many of the common short- and long-term effects from therapy. After decades of recognition of many of the detrimental effects of treatment, it is hoped that future clinical and research activities will definitively reduce adverse therapy-related outcomes for breast cancer survivors.

Disclosures of Potential Conflicts of Interest

The author(s) indicated no potential conflicts of interest.
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Translating Genomic Research into Clinical Practice: Promise and Pitfalls

Joseph A. Sparano, MD, Harry Ostrer, MD, and Paraic A. Kenny, PhD

OVERVIEW

Breast cancer is a heterogeneous disease associated with variable clinical outcomes despite standard local therapy for the primary tumor and systemic adjuvant therapy to prevent distant recurrence. Management decisions are typically made using classical prognostic and predictive clinicopathologic factors, and more recently gene expression profiling assays are commonly used in practice. Recent advances in genomic sequencing—often referred to collectively as next-generation sequencing (NGS)—have facilitated more in-depth evaluation of the cancer genome than could be afforded by the initial generation of gene expression studies, including DNA single nucleotide variants, small insertions and deletions, structural alterations, and copy number alterations (CNAs). In addition, this information has been integrated with other molecular profiling methods of processes that affect gene transcription (e.g., epigenetic, microRNA) and protein expression—the ultimate readout of the genetic code. Although NGS has provided new insights on the classification of breast cancer and identified potential predictive biomarkers and novel targets, there are formidable logistical and scientific obstacles that must be addressed before the promise of this technology is fully realized.

GENOMICS

Genomics is defined as the study of all of the nucleotide sequences in an organism. The original sequencing methods relied on Sanger sequencing using synthesis with DNA polymerase and termination with dideoxynucleotides described in 1975, then modified in 1977 to be more rapid and accurate. These methods were used to sequence the first human genomes that were reported in 2001. Over the past decade, newer methods allow sequencing to be done more quickly, accurately, and cheaply, which are often referred to as NGS. Such assays are now being used in the clinic not only for research but also commercially for clinical use. Techniques are also available that allow high-throughput evaluation of the epigenome, microRNAs, and proteins and analytic approaches that integrate information from multiple profiling methods. This review will address how NGS is being used for discovery-based research and its potential clinical application in breast cancer.

FIRST GENERATION: GENE EXPRESSION PROFILING

Until recently genomic profiling focused on the evaluation of gene expression or the translation of information encoded in genomic DNA into an RNA transcript. RNA transcripts include mRNAs that are translated into proteins and various other RNAs (e.g., transfer RNA, ribosomal RNA, microRNA, noncoding RNA) that have important biologic functions. Perou et al first identified “intrinsic” breast cancer subtypes by evaluating variation in gene expression patterns using hierarchical clustering in a set of 65 breast cancers from 42 individuals using complementary DNA microarrays representing 8,102 human genes. The subtypes were recapitulated in other datasets and shown to be clinically relevant with distinct clinical outcomes. It was hypothesized that these subtypes had distinctive gene expression profiles because they originated from different cell types, including luminal epithelial cells (the cells that line the duct and give rise to the majority of breast cancers) and basal epithelial cells of the normal mammary gland (characterized by expression of cytokeratins 5/6 and 17), hence the terms “luminal” and “basal” subtypes. More recent data indicate that the basal tumors arise from luminal progenitor cells rather than the basal myoepithelial cells of the mammary gland. The intrinsic gene panel was subsequently reduced to a panel of 50 genes (called the PAM50) detectable by qRT-PCR, with 10 genes selected for each centroid used to define four intrinsic subtypes, including luminal A, luminal B, basal, and HER2-enriched (plus a “normal” subtype, which reflects an inadequate biopsy specimen containing predominantly normal breast tissue). The PAM50 and several multiparameter gene expression assays have been approved for clinical use, including some that have been recommended by expert panels for clinical decision making.
**NEXT GENERATION: BEYOND GENE EXPRESSION**

NGS relies on the use of high-throughput, massively parallel sequencing and bioinformatic approaches to analyze massive datasets, including a variety of approaches for genome sequencing and gene expression. When applied to RNA (RNA-Seq), NGS not only provides absolute expression levels (as the precise number of transcripts can be counted) but also allows the identification of alternatively spliced isoforms, mutant transcripts, and novel transcripts arising from fusion genes. Terms commonly used in NGS studies are summarized in Table 1, and the processes typically used are illustrated in Fig. 1.

The technology platforms for NGS are developing at a rapid rate, which is inexorably leading to faster, cheaper, and more accurate sequencing data. Currently, the field is dominated by machines from Roche, Illumina (HiSeq2000), and ABI (SOLiD). Each of these platforms is capable of performing whole genome sequencing, whole exome sequencing, RNA-Seq, and methylation analysis on a time scale between approximately 10 hours (Roche) and 11 days (Illumina). These platforms are being supplemented by smaller machines from the same manufacturers that may be run more quickly but handle smaller amounts of sequencing (i.e., not sufficient for whole genome analysis). Of the possible approaches, whole genome sequencing that sequences all of the DNA base pairs in the genome is the most intensive. Whole exome sequencing, in which libraries are generated from the transcribed exons of the genome (encoding proteins, microRNAs, and other RNAs), offers a less intensive approach, which still likely captures the majority of the interpretable information. RNA-Seq analysis sequences cDNA copies of the sample RNA component, providing detailed information on gene expression levels, splicing, and mutations. DNA methylation can also be analyzed using Methyl-Seq, providing detailed coverage of the distribution of methylated CpG islands throughout the genome, which play key roles in controlling gene expression.

Although the precise technical details of conducting these assays vary between platforms, the principles are quite similar in each case. Short, single-stranded DNA sequences are generated from the starting material, which can be genomic DNA (whole genome), hybridization-captured DNA exons (exome sequencing), cDNA (RNA-Seq) or bisulfite-treated DNA (Methyl-Seq). Small DNA adapters are ligated to each strand of the library, and the samples are then amplified to provide enough representations of each individual strand for sequencing. Amplification can be performed on beads (Illumina and SOLiD) or on a glass slide (Illumina). Each strand can then be sequenced by sequential addition of the four DNA bases (A, C, G, and T).

In all cases, the output of the experiment is an extremely large amount of raw DNA sequence reads (of between 60 and 400 bp, depending on the platform), which must be processed further to be interpreted. This computationally intensive step requires mapping of each individual read to the correct location on the reference genome. A variety of commercial and open-source software packages are available to perform this function and subsequent steps of the analysis. In the case of cancer, the goal is usually to identify sequence variants between the tumor sample and the reference genome or nontumor DNA from the same patient, if available. Comparing normal and tumor DNA from the same individual is particularly advantageous, as it excludes the influence of the large number of single nucleotide polymorphisms, which differ between individuals in a species. Sequence alterations—which can include point mutations, insertions, deletions, or translocations—can be identified using the analysis packages. Comparison of differences in mutation patterns, amplification/deletion, and translocation in individual tumor samples may be visually represented by Circos plots.

Because tumors have a high mutation rate, any sequencing experiment will identify a large number of sequence variations—only some of which make a contribution to the disease pathology. Distinguishing causative (driver) from random (passenger) mutations can be challenging for variants and genes that have not been previously reported as playing a role in cancer. For such novel mutations, some insight can be gained from software tools such as SIFT, which can predict whether a particular change in a protein sequence is likely to have a function-altering effect. Both the sequencing technology platforms and the relevant analysis strategies have been described in detail elsewhere.

Whole genome and exome sequencing and the analysis of the data generated are important research tools, but the sheer volume of data generated makes it difficult to efficiently deploy these approaches in a routine clinical setting. Instead, it is likely that targeted resequencing of small portions of the genome, known to be frequently altered in cancer is likely to prove more useful. Approaches such as AmpliSeq (Life Technologies), which uses ultra-high multiplex PCR to amplify all of the exons of 400 cancer-relevant genes from small amounts of starting material, will generate clinically actionable data in the near future.
Matched DNA and RNA were extracted from each specimen with either lymph node–positive or ER-negative disease did. Patients who did not receive adjuvant chemotherapy, whereas patients with ER-positive and/or lymph node–negative disease were typically somatically acquired—some of which have been defıned in at least 40 genes in breast cancer, gene “hills” that are mutated at a low frequency.15 Driver mutations of breast cancer is characterized by a handful of commonly mutated gene “mountains” and a much larger number of gene “hills” that are mutated at a low frequency.15 Driver mutations have been defined in at least 40 genes in breast cancer, and somatic genomic rearrangements resulting in oncogenic fusion transcripts are also common—some of which have been postulated to play a key role in disease progression.16–18 The results of selected NGS studies in breast cancer are summarized in Table 2 and described below, which includes studies correlating genomics with clinical outcomes for the purpose of classification or prediction, with specific breast cancer phenotypes for target discovery, or with genomics integrated with epigenomic, microRNA, and proteomic data to gain a deeper understanding of how specific genes may be regulated and influence protein expression.

NGS IN BREAST CANCER

All cancers carry somatic mutations in their genome that are dominated by point mutations, amplifications, translocations, and complex rearrangements.14 The genomic landscape of breast cancer is characterized by a handful of commonly mutated gene “mountains” and a much larger number of gene “hills” that are mutated at a low frequency.15 Driver mutations have been defined in at least 40 genes in breast cancer, and somatic genomic rearrangements resulting in oncogenic fusion transcripts are also common—some of which have been postulated to play a key role in disease progression.16–18 The results of selected NGS studies in breast cancer are summarized in Table 2 and described below, which includes studies correlating genomics with clinical outcomes for the purpose of classification or prediction, with specific breast cancer phenotypes for target discovery, or with genomics integrated with epigenomic, microRNA, and proteomic data to gain a deeper understanding of how specific genes may be regulated and influence protein expression.

USE OF NGS FOR DISEASE CLASSIFICATION: METABRIC

The Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) study was a joint effort by research teams from Canada and the United Kingdom to genomically classify breast tumors. Approximately 2,000 clinically annotated, fresh-frozen breast cancer specimens from patients with operable breast cancer were assembled from tumor banks in the United Kingdom and Canada; this included 997 tumors analyzed in a discovery group and 995 in a validation group.18 Nearly all patients with estrogen receptor (ER)-positive and/or lymph node–negative disease did not receive adjuvant chemotherapy, whereas patients with either lymph node–positive or ER-negative disease did. Matched DNA and RNA were extracted from each specimen and subject to copy number and genotype analysis on the Affymetrix SNP 6.0 platform, transcriptional profiling on the Illumina HT-12 v3 platform, and TP53 mutations by Sanger sequencing in a cohort of 820 patients. This allowed evaluation of somatically acquired CNAs, germline copy number variants, and whether genomic variants acted in cis (impacts its own expression) or in trans (impacts expression of other genes in genome, defined as outside at least a contiguous three megabase window).

There were several important observations in this study. First, a number of known driver mutations (e.g., ZNF704, PTEN, MYC, CCND1, MDM2, ERBB2, CCNE1) and putative driver mutations (e.g., MDM1, MDM4, CDK3, CDK4, CAMK1D, PI4KB, NCOR1) were identified. There were also important deletions identified, including known deletions (e.g., PTEN) and novel deletions (e.g., PPP2R2A, MTAP, and MAP2K4). PPP2R2A deletions were noted in luminal B cancers and have likewise been reported in clear cell and ovarian and endometrioid cancers. MTAP is often codeleted with CDKN2A and CDKN2B tumor suppressor genes in a variety of cancers. MAP2K4 mutations were noted in ER-positive tumors and felt to be consistent with a tumor suppressor gene. A deletion event on chromosome 5 was also noted in basallike tumors. Second, the effect of specific genes acting in trans by influencing expression of distant sites of the genome identified known aberration hot spots that could be grouped into pathway modules (e.g., ERBB2, MYC) and novel loci, including T-cell receptor loci on chromosome 7 (TRG) and 14 (TRA). These upregulate mRNAs and are highly enriched from T-cell activation and proliferation, dendritic cell presentation, and leukocyte activation, thereby indicating an adaptive immune response associated with tumor-infiltrating lymphocytes. Third, joint clustering of copy number and gene expression data revealed that 10 integrative clusters characterized by well-defined copy number aberrations that split many of the intrinsic subtypes were associated with variable clinical outcomes and were

TABLE 1. Glossary of Terms Commonly Used in Next-Generation Sequencing Studies (Listed Alphabetically)

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circos plot</td>
<td>A circular ideogram figure that visually represents differences in genomic structure between individual specimens</td>
</tr>
<tr>
<td>Codon</td>
<td>A series of three adjacent bases in one polynucleotide chain of a DNA or RNA molecule, which codes for a specific amino acid</td>
</tr>
<tr>
<td>Copy number alterations</td>
<td>Alterations in gene copy number, which are typically somatically acquired</td>
</tr>
<tr>
<td>Copy number variants</td>
<td>Germ-line variation in the number of copies of a particular gene, which varies between individuals</td>
</tr>
<tr>
<td>Exome</td>
<td>The part of the genome formed by exons; the exome of the human genome consists of roughly 180,000 exons constituting approximately 1% of the total genome or about 30 megabases of DNA</td>
</tr>
<tr>
<td>Exon</td>
<td>Any nucleotide sequence encoded by a gene that remains present within the final mature RNA product of that gene after introns have been removed by RNA splicing; these include exons that are translated into protein and the untranslated region flanking them, as well as exons encoding microRNAs and noncoding RNAs.</td>
</tr>
<tr>
<td>Indel</td>
<td>Mutation resulting in a co-localized insertion or deletion and a net gain or loss in nucleotides</td>
</tr>
<tr>
<td>Intron</td>
<td>Any nucleotide sequence within DNA or RNA that is not encoded into protein; introns are removed by RNA splicing while the final mature RNA product of a gene is being generated</td>
</tr>
<tr>
<td>Library</td>
<td>A collection of DNA fragments prepared from a more complex sample</td>
</tr>
<tr>
<td>RNA-Seq</td>
<td>Use of high-throughput sequencing technologies to sequence cDNA to get information about a sample’s RNA content</td>
</tr>
<tr>
<td>Single nucleotide variant</td>
<td>DNA sequence variation when a single nucleotide (A, C, T, G) differs between members of a biological species</td>
</tr>
</tbody>
</table>
consistent in discovery and validation sets. This indicates heterogeneity even within “intrinsic subtypes” revealed by integration of information regarding somatic CNAs, including subgroups of ER-positive tumors that were high risk (characterized by 11q13/14 cis-acting alterations), favorable risk (characterized by a paucity of copy number and cis-acting alterations), and intermediate risk (characterized by 17q23/20q and 8p12 cis-acting subgroups). In addition, this report demonstrated for the first time that genomic copy number loss at T-cell receptor loci drives a trans-acting immune response in an otherwise genomically quiescent subgroup of ER-positive and ER-negative tumors associated with a good prognosis, whereas copy number loss at 5q in a group of basal-like cancers drives a trans-acting transcriptional control of genomic and chromosomal instability associated with a poor prognosis.

**USE OF NGS TO IDENTIFY PREDICTORS OF SENSITIVITY AND RESISTANCE TO ENDOCRINE THERAPY**

Aromatase inhibitors (AIs) are commonly used as adjuvant endocrine therapy in postmenopausal women with ER-
positive disease. Response to preoperative AI therapy has been shown to be a short-term surrogate reflecting favorable prognosis with AI therapy alone. Response may be assessed by an algorithm that reflects the extent of residual disease after a 16-week course of therapy and Ki67 expression, or Ki67 expression after a 16-week course of therapy and Ki67 expression, or Ki67 expression.20 Ellis et al performed whole genome analysis of 46 samples and exome sequencing in 31 additional samples from postmenopausal women with ER-positive breast cancers treated with preoperative AI therapy, including 29 samples with Ki67 levels above 10% after a 16-week course of AI therapy (and thus considered to have AI-resistant disease) and 48 with Ki67 levels of 10% or less (indicating AI-sensitive disease).21 There were several important observations from this study. First, the background mutation rate was about twofold higher for AI-resistant tumors than for sensitive tumors. Second, 18 significantly mutated genes were identified, including genes previously identified in breast cancer (e.g., PIK3CA, TP53, GATA3, CDH1, RB1, MLL2, MAP3K1, CDKN1B) and novel genes not previously observed, including five previously seen in hematopoietic cancers (e.g., RUNX1, CBFB, MYH9, MLL3, and SF3B1). Third, certain mutations were associated with specific subtypes, including an association between MAP3K1 mutations and luminal A tumors and TP53 mutations with luminal B tumors. Fourth, GATA3 mutations correlated with AI-induced suppression of proliferation and hence sensitivity to AI therapy.

### INTEGRATING GENOMIC, EPIGENOMIC, AND PROTEIN DATA: THE CANCER GENOME ATLAS (TCGA)

TCGA included tumor and germ-line DNA samples obtained from 825 patients, which included evaluation by Affymetrix 6.0 SNP arrays (733 patients) and Agilent mRNA expression microarrays (547 patients), plus Illumina Infinium DNA methylation chips (802 patients), miRNA sequencing (697 patients), whole exome sequencing (507 patients), and reverse-phase protein arrays (403 patients).22 This included 466 tumors evaluated for gene expression, methylation, miRNA, and exome sequencing, and 348 who also had protein arrays. Correlation was not performed with clinical outcomes because of the short follow-up time (median 17 months) and resultant small number of events. Nearly all genes previously implicated in breast cancer were identified (PIK3CA, PTEN, AKT1, TP53, GATA3, CDH1, RB1, MLL2, MAP3K1, CDKN1B), plus a number of novel genes recently identified in other studies (TXB1, RUNX1, CBFB, AFF2, PIK3R1, PTEN, NF1, SF3B1, CCND3). The overall mutation rate was lowest in the luminal A subtype and highest in the basal-like and HER2-enriched intrinsic subtypes. The most commonly mutated genes included TP53 (37%), PIK3CA (36%), GATA3 (11%), MAP3K1 (8%), MLL3 (7%), and CHD1 (7%), with 17 other mutations occurring in 1% to 4% (Fig. 2A). The distribution of mutations varied by subtype (Fig. 2B), with PIK3CA mutations occurring more commonly in luminal A/B and HER2-enriched intrinsic subtypes. The most commonly mutated genes included TP53 (37%), PIK3CA (36%), GATA3 (11%), MAP3K1 (8%), MLL3 (7%), and CHD1 (7%), with 17 other mutations occurring in 1% to 4% (Fig. 2A). The distribution of mutations varied by subtype (Fig. 2B), with PIK3CA mutations occurring more commonly in luminal A/B and HER2-enriched than in basal subtypes (45%/29% and 39% vs. 9%), and TP53 mutations dominating in basal (80%) and HER2-enriched subtype (72%) compared with luminal B (29%) and luminal A (12%) subtypes. This pattern is similar to other reports, with PIK3CA and TP53 mutations predominating and consistently varying in frequency by subtype and with other mutations relatively uncommon. The types of mutations also differed by intrinsic subtypes, including differences in TP53 mutations between basal-like (nonsense and frame shift) and luminal tumors (missense). Approximately 9% of 507 cases evaluated revealed germ-line predisposing

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**TABLE 2. Summary of Selected Next-Generation Sequencing Studies in Breast Cancer**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. Patients</th>
<th>Patient Population</th>
<th>Clinical Outcomes</th>
<th>Profiling Methods</th>
<th>Key Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curtis et al22</td>
<td>1,992</td>
<td>All types</td>
<td>Yes</td>
<td>CNV</td>
<td>10 subtypes identified that correlate with clinical outcomes</td>
</tr>
<tr>
<td>Ellis et al25</td>
<td>77</td>
<td>ER-positive</td>
<td>Yes</td>
<td>Whole genome sequencing</td>
<td>18 significantly mutated genes identified</td>
</tr>
<tr>
<td>TCGA26</td>
<td>825</td>
<td>All types</td>
<td>No</td>
<td>DNA copy number and CNA</td>
<td>TP53 and PIK3CA mutations most common, with others substantially less common</td>
</tr>
<tr>
<td>Shah et al28</td>
<td>104</td>
<td>Triple-negative</td>
<td>No</td>
<td>RNA-Seq</td>
<td>Wide and continuous spectrum of genomic evolution</td>
</tr>
</tbody>
</table>

**Abbreviations:** CNV, copy number variants; CNA, copy number alterations; ER, estrogen receptor; TCGA, The Cancer Genome Atlas.
variants (e.g., ATM, BRCA1, BRCA2, BRIP1, CHEK2, NBN, PTEN, RAD51C, TP53). Similar to other reports, copy number changes correlated with some intrinsic subtypes, including loss of 5q and gain of 10p in basal-like cancers and gain of 1q and/or 16q loss in luminal tumors.

A unique feature of TCGA was the comprehensive nature of the molecular profiling, which included evaluation of miRNA, methylation, and proteins. Clustering analysis revealed seven subtypes by miRNA that did not correlate with mutational status or PAM50, with the exception of two miRNA groups that showed overlap with basal-like subtype and TP53 mutations. Five distinct DNA methylation groups were identified, including at the extremes a hypermethylated phenotype enriched for luminal B subtype and a
hypomethylated phenotype that overlapped with basal-like tumors enriched for TP53 mutations. Protein analysis identified seven subtypes that were highly concordant with the mRNA intrinsic subtypes, especially basal-like and HER2-enriched subtypes. A multiplatform data matrix analysis revealed that the information content from copy number aberrations, miRNAs, and methylation is captured at the level of gene expression and protein expression and activity. TCRA permitted a detailed analysis of each of the intrinsic mRNA-defined subtypes. Luminal tumors exhibited the most heterogeneous gene expression, mutational spectrum, and copy number changes and were associated with several characteristics. First, although PIK3CA mutations were common in luminal tumors, markers typical of an activated PI3K pathway (e.g., pAKT, pS6, p4EBP1 protein) were not present. In contrast, PIK3CA mutations were usually associated with pathway activation in basal and HER2-enriched tumors. Second, luminal tumors also were frequently associated with MAP3K1 and MAP2K4 mutations—two contiguous steps in the p38-JNK pathway—whereas they occurred infrequently in other subtypes. Third, in comparison to luminal B tumors, luminal A tumors were associated with higher frequency of intact TP53 and Rb1 tumor suppressor genes. HER2-enriched tumors showed high aneuploidy, somatic mutation rate, and DNA amplification of other potential targets (e.g., FGFR, EGFR, CDK4, cyclin D1). The basal subtype showed a high degree of TP53 mutations and high PI3K pathway activity despite a low PI3K mutation rate (because of PTEN and INPP4B loss and/or amplification of PIK3CA). Similar to serous ovarian carcinoma, DNA repair deficits (ATM mutations, BRCA1 and 2 inactivation, RB1 loss, and cycle E activation), genomic instability, and increased activity of the HIF1-alpha/ARNT, MYC and FOXM1 pathways were also common.

**GENOTYPIC ANALYSIS OF PHENOTYPICALLY DEFINED TRIPLE-NEGATIVE BREAST CANCER**

The METABRIC and TCGA studies focused on using NGS to sub-classify intrinsic breast cancer subtypes. However, phenotypic classification based on patterns of ER, progesterone receptor, and HER2/neu expression is typically used in clinical practice for clinical decision making. Triple-negative breast cancer (TNBC) is a phenotypic subset that accounts for approximately 15% of all breast cancers, occurs more commonly in younger women and black or Hispanic women, and is characterized by a higher risk of relapse, earlier time to relapse, proclivity for recurrence in visceral organs and the central nervous system, and absence of specific targeted therapy. Shah et al described an analysis of 104 patients with TNBC subjected to RNA-Seq and deep resequencing measurements of allelic abundance for more than 2,400 somatic mutations. Approximately 20% of tumors had potentially clinically actionable somatic aberrations, including BRAF V600E, high-level EGFR amplifications, and ERBB2 and ERBB3 mutations. The distribution of somatic mutation abundance varied in a continuous distribution and was unrelated to CNA or tumor cellularity. In another report, Banerji et al identified a recurrent MAGI3-AKT3 fusion in TNBC that led to constitutive activation of AKT kinase that was abolished by a competitive AKT small-molecule inhibitor.

**CLINICAL TRIALS INTEGRATING HIGH-THROUGHPUT GENOMIC ANALYSIS INTO CLINICAL CARE**

Andre et al tested the ability of array comparative genomic hybridization (CGH) and Sanger sequencing to provide improved therapeutic direction in the SAFIR1 trial. Patients with metastatic breast cancer underwent biopsy of metastatic sites for genomic analysis, followed by genotype-directed therapy after progression on standard therapy. At the time of their initial preliminary report, of the 423 patients who

**TABLE 3. Summary of Key Findings from Next-Generation Sequencing Studies in Breast Cancer**

<table>
<thead>
<tr>
<th>Disease Classification, Prognosis, and Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer is a heterogeneous disease with variable genomic complexity.</td>
</tr>
<tr>
<td>Gene expression classifies disease into “intrinsic” subtypes (luminal A, luminal B, HER2-enriched, basal).</td>
</tr>
<tr>
<td>Evaluation of somatically acquired CNAs and germ-line CNVs permit further sub-classification.</td>
</tr>
<tr>
<td>Basal-like breast cancers are genotypically similar to serous ovarian carcinoma.</td>
</tr>
<tr>
<td>Some mutations are predictive of response to aromatase inhibitors (GATA3).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target Discovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic mutations are common and dominated by point mutations, duplications, translocations, and rearrangements.</td>
</tr>
<tr>
<td>Rearrangements commonly result in fusion genes, some of which produce oncogenic proteins.</td>
</tr>
<tr>
<td>Most common currently targetable mutations are in the PI3K-mTOR-AKT pathway.</td>
</tr>
<tr>
<td>Not all PIK3CA mutations lead to pathway activation (especially in luminal disease).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Promise</th>
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</thead>
<tbody>
<tr>
<td>Next-generation sequencing is available for clinical use due to improved methodology and declining costs.</td>
</tr>
<tr>
<td>Refined disease classification may assist in guiding standard therapy.</td>
</tr>
<tr>
<td>Up to approximately 20% of genomic alterations may be potentially actionable targets.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Pitfalls</th>
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</thead>
<tbody>
<tr>
<td>Few breast cancers are “addicted” to driver alterations.</td>
</tr>
<tr>
<td>Inactivation of tumor suppressor genes (e.g., TP53, RB1) are difficult to therapeutically target.</td>
</tr>
<tr>
<td>Preliminary clinical trials show that fewer than about 30% of screened patients receive a genomically directed therapy.</td>
</tr>
<tr>
<td>Logistical barriers include need for representative biopsy and current dearth of available targeted agents.</td>
</tr>
</tbody>
</table>

| Scientific barriers include tumor heterogeneity, intrinsic and acquired resistance, and need for combinatorial therapy. |

Abbreviations: can, copy number alterations; CNV, copy number variants.
CONCLUSION

Key points about the knowledge gained thus far from applying NGS to clinical breast cancer specimens are summarized in Table 3. Potentially promising applications of NGS include target discovery, refined and more accurate disease classification, and improved therapeutic direction. However, there remain significant pitfalls including logistical obstacles to having an effective drug available to target every oncogenic alteration, and a regulatory and cancer care delivery system that would allow matching the right patient with the right drug, as exemplified by the SAFIR1 trial. In addition to the logistical obstacles, other pitfalls include a dearth of tumors that are "addicted" to oncogenic mutations, innate tumor heterogeneity at presentation resulting in the need for combinatorial therapy directed at multiple aberrant pathways, and the rapid emergence of resistance to therapy even when appropriately applied.

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Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked "L" indicate leadership positions. Relationships marked "I" are those held by an immediate family member; those marked "B" are held by the author and an immediate family member. Relationships marked "U" are uncompensated.


References


BREAST CANCER

Beyond Trastuzumab and Lapatinib: New Options for HER2-Positive Breast Cancer

CHAIR
David Cameron, FRCP
The University of Edinburgh
Edinburgh, United Kingdom

SPEAKERS
Ian Krop, MD, PhD
Dana-Farber Cancer Institute
Boston, MA

Martine Piccart-Gebhart, MD, PhD
Jules Bordet Institute
Brussels, Belgium
Beyond Trastuzumab and Lapatinib: New Options for HER2-Positive Breast Cancer

Dimitrios Zardavas, MD, David Cameron, MD, Ian Krop, MD, PhD, and Martine Piccart, MD, PhD

OVERVIEW

HER2-positive breast cancer (BC) constitutes a molecular subtype of the disease with an aggressive biologic behavior. Trastuzumab revolutionized the treatment of this disease, changing its natural history. Lapatinib is active in the metastatic setting, approved for patients who were pretreated with trastuzumab. However, resistance to anti-HER2 agents is a major clinical issue, occurring in both early-stage and advanced disease, and new treatment options are clearly needed. An abundance of HER2-targeted agents are being clinically developed: monoclonal antibodies, small molecule inhibitors, and antibody drug conjugates (ADC). Combining HER2-targeted agents in regimens of dual HER2 blockade has already reached clinical practice in the metastatic setting, confirming the preclinical efficacy of enhanced HER2 inhibition. Promising results have been generated in the neoadjuvant setting, and large randomized trials are seeking evidence for dual HER2 blockade in the adjuvant setting. ADC represent another hope for improved treatment outcomes of HER2-positive BC, as exemplified by the positive results of clinical trials employing trastuzumab-DM1 (trastuzumab emtansine, T-DM1). Moreover, an understanding of the molecular mechanisms mediating resistance to HER2 blockade has opened new therapeutic avenues, with several targeted agents entering clinical trials. This paper presents the clinical data of the HER2-targeted agents under development, as well as an overview of the biologic rationale for the development of agents aimed at circumventing anti-HER2 resistance.

Epidermal growth factor receptor 2 (EGFR2; HER2/neu, ErbB2), a type II transmembrane receptor, constitutes a well-studied oncogene, mediating a plethora of oncogenic effects, through interlinked activation of PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways. Amplification of the gene and/or protein overexpression is detected in approximately 20% of patients with BC and confers an aggressive biologic phenotype, resulting in poor clinical outcome. The development of trastuzumab, a humanized monoclonal antibody targeting domain IV of HER2, represents the first significant milestone in the development of anti-HER2-targeted agents. Trastuzumab is the mainstay of HER2 blockade in all stages of HER2-positive disease, changing its natural history. Lapatinib constitutes another important development in the arena of HER2-blocking agents, being a dual EGFR/HER2 reversible tyrosine kinase inhibitor (TKI), currently approved in combination with either capecitabine or letrozole for patients with HER2-positive metastatic BC (MBC).

Despite the antitumor activity of those two agents, a subset of patients with early stage HER2-positive BC unfortunately relapses; in the metastatic setting, primary or secondary resistance develops inevitably. This clinical reality mandates new treatment options, with a plethora of HER2-targeted agents showing different mechanisms of action (Table 1) currently under clinical development (Tables 2 and 3). One promising approach is dual HER2-blockade. Combined administration of different HER2-targeted agents shows heightened antitumor activity, based on the complementarity of their mechanisms of action, leading to more complete HER2 blockade. This strategy has already reached clinical practice in the metastatic setting and has demonstrated higher rates of pathologic complete response (pCR) in the neoadjuvant setting (Fig. 1). A second approach is the development of anti-HER2 ADC, as exemplified by the development of trastuzumab-DM1. A third approach, which is earlier in clinical development, derives from the elucidation of the molecular mechanisms mediating resistance to HER2 blockade. This strategy combines HER2 blockade with a compound blocking a molecular mediator of anti-HER2 resistance, with the goal of “(re)sensitizing” HER2-positive BC cells to HER2 inhibition (Table 4).

TRASTUZUMAB AND LAPATINIB

Trastuzumab and lapatinib exhibit complementary mechanisms of HER2 blockade (Table 1), and preclinical studies have shown a synergy between these two agents: increased...
In the metastatic setting, the combination of trastuzumab and lapatinib was assessed in the phase III EGF104900 study, which randomly selected patients who were pretreated with trastuzumab to receive lapatinib alone or lapatinib combined with trastuzumab. The dual HER2 blockade resulted in a significant prolongation of median progression-free survival (mPFS; 11.1 week vs. 8.1 week, \( p = 0.011 \)) and of overall survival (OS; 14 months vs. 9.5 months, \( p = 0.026 \)), despite significant cross-over (52%).6 The toxicity of the lapatinib/trastuzumab regimen was acceptable, with increased rates of grade 1 and 2 diarrhea (\( p = 0.03 \)) and a higher frequency of 20% or greater reduction in the left ventricular ejection fraction (LVEF; 5.4 vs. 2.1%), as compared to lapatinib monotherapy.

In the neoadjuvant setting, Neo-Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization trial (Neo-ALTTO) was a phase III study that randomly selected 455 patients with HER2-positive operable BC to receive lapatinib, trastuzumab, or their combination for a total of 6 weeks. After this period, the assigned HER2-blocking strategy was combined with paclitaxel for further 12 weeks. The dual HER2-blockade resulted in a significant improvement in the pCR rate, outperforming the trastuzumab and lapatinib arms (pCR 51.3% vs. 29.5% vs. 24.7%, respectively; \( p = 0.0001 \) for the trastuzumab-lapatinib arm versus trastuzumab alone).7 The pCR rates were more pronounced in the patients with hormone receptor–negative disease. No major cardiac dysfunction was noted, and the lapatinib-containing arms were associated with more grade 3 and 4 adverse events (AEs) and higher rates of treatment discontinuation.

Similar results were reported by the Chemotherapy, Herceptin, and Lapatinib in Operable Breast Cancer study (CHER-LOB).8 In this phase II study, 121 patients with operable HER2-positive BC were allocated to receive neoadjuvant taxane-anthracycline chemotherapy combined with lapatinib (Arm A), trastuzumab (Arm B), or lapatinib plus trastuzumab (Arm C). The lapatinib/trastuzumab combination was associated with higher pCR rates (48%) than the lapatinib (pCR 28%) and trastuzumab arms (pCR 32%). A third neoadjuvant study—the Translational Breast Cancer Research Consortium (TBCRC 006)—assessed the lapatinib/trastuzumab combination in a chemotherapy-free regimen administered for 12 weeks (plus hormone therapy for patients with hormone receptor–positive BC).9 In this phase II study, 64 patients diagnosed with HER2-positive BC with a median tumor size of 6 cm achieved pCR in 28% overall; once again hormone receptor negativity was associated with increased pCR rates (42% vs. 21%) for patients with estrogen receptor (ER)–negative and ER-positive BC, respectively.

The combined administration of trastuzumab and lapatinib is currently under clinical investigation in the adjuvant setting. The ALTTO study (NCT00490139)—a phase III study with a completed accrual exceeding 8,300 patients—compared the activity of lapatinib monotherapy, trastuzumab monotherapy, trastuzumab followed by lapatinib, and trastuzumab concomitantly with lapatinib in patients with early-stage HER2-positive BC. The primary endpoint of
TABLE 2. Selected Ongoing Trials with T-DM1 and Pertuzumab in HER2-Positive Breast Cancer

<table>
<thead>
<tr>
<th>Trial (NCT Identifier)</th>
<th>Phase</th>
<th>Setting</th>
<th>Treatment</th>
<th>Primary Endpoint</th>
<th>Secondary Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00951665</td>
<td>I</td>
<td>Trastuzumab pretreated</td>
<td>T-DM1 + pertuzumab + paclitaxel</td>
<td>AEs, DLTs, PK</td>
<td>ORR, PFS, CBR, duration of response</td>
</tr>
<tr>
<td>NCT0067534I</td>
<td>II</td>
<td>Trastuzumab naive</td>
<td>T-DM1 versus trastuzumab + docetaxel</td>
<td>PFS</td>
<td>OS, ORR, CBR, duration of OR, TTSP</td>
</tr>
<tr>
<td>NCT01745965</td>
<td>II</td>
<td>Neoadjuvant</td>
<td>T-DM1 versus T-DM1 + hormone therapy versus trastuzumab + hormone therapy</td>
<td>pCR</td>
<td>OS, cardiac safety, toxicity, HRQL</td>
</tr>
<tr>
<td>TH3RESA (NCT0141997)</td>
<td>III</td>
<td>Trastuzumab retreated</td>
<td>T-DM1 versus physician’s choice</td>
<td>PFS, OS</td>
<td>ORR, duration of OR, AEs, HRQL, PK of T-DM1</td>
</tr>
<tr>
<td>NCT01745965</td>
<td>II</td>
<td>Neoadjuvant</td>
<td>T-DM1 versus T-DM1 + HT versus trastuzumab + HT</td>
<td>pCR</td>
<td>OS, toxicity, safety, HRQL</td>
</tr>
<tr>
<td>NCT01772472</td>
<td>III</td>
<td>Adjuvant</td>
<td>T-DM1 versus trastuzumab</td>
<td>IDFS</td>
<td>DFS, OS, DRFI, safety, PRO</td>
</tr>
</tbody>
</table>

Table 2: Pertuzumab

| NCT00934856            | I     | Trastuzumab pretreated | Pertuzumab + T-DM1 + docetaxel | DLTs, AEs | PFS, ORR, CBR, duration of OR, TTF, PK |
| NCT01565083            | II    | Trastuzumab naive      | Pertuzumab + trastuzumab + vinorelbine | ORR | TTR, duration of OR, PFS, TTP, OS, cardiotoxicity, HRQL |
| NCT01491737            | II    | Trastuzumab naive      | Pertuzumab + Trastuzumab + Al versus trastuzumab + Al | PFS | OS, ORR, CBR, duration of OR, TTR, safety, HRQL |
| PHEREXA (NCT01026142)  | II    | Trastuzumab pretreated | Trastuzumab + capecitabine + pertuzumab versus trastuzumab + capecitabine | PFS | TTP, TTF, ORR, CBR, duration of OR, safety |
| PERUSE (NCT01572038)   | III   | Trastuzumab naive      | Pertuzumab + trastuzumab + taxane | Safety | PFS, ORR, CBR, duration of OR, TTR, HRQL |
| APHINITY (NCT01358877) | III   | Adjuvant               | Chemotherapy + trastuzumab + pertuzumab versus chemotherapy + trastuzumab | IDFS | DFS, OS, RFI, DRFI, cardiac safety, safety, HRQL |

Abbreviations: NCT, National Clinical Trial; T-DM1, trastuzumab-DHICA; AE, adverse event; DLT, dose-limiting toxicity; PK, pharmacokinetics; ORR, overall response rate; PFS, progression-free survival; CBR, clinical benefit rate; OS, overall survival; TTD, time to deterioration; HRQL, health-related quality; HT, hormonal therapy; IDFS, invasive disease-free survival; pCR, pathologic complete response; DFS, disease-free survival; DRFI, distance recurrence-free interval; PRO, patient-reported outcome; AI, aromatase inhibitor; TTF, time to treatment failure; RFI, recurrence-free interval.

TABLE 3. Selected Ongoing Clinical Trials with HER-Family Small Molecule Inhibitors

<table>
<thead>
<tr>
<th>Trial (NCT Identifier)</th>
<th>Phase</th>
<th>Setting</th>
<th>Treatment</th>
<th>Primary Endpoint</th>
<th>Secondary Endpoints</th>
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<tr>
<td>Afatinib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NCT00950742</td>
<td>I</td>
<td>Trastuzumab pretreated</td>
<td>Afatinib + trastuzumab</td>
<td>MTD</td>
<td>AE, PK, ORR, PFS</td>
</tr>
<tr>
<td>LUX-Breast 1 (NCT01125566)</td>
<td>III</td>
<td>Trastuzumab pretreated</td>
<td>Afatinib + vinorelbine versus Trastuzumab + Vinorelbine</td>
<td>PFS</td>
<td>OS, TTD, HRQL, Safety, PK</td>
</tr>
<tr>
<td>LUX-Breast 2 (NCT01277725)</td>
<td>II</td>
<td>Trastuzumab pretreated</td>
<td>Afatinib</td>
<td>ORR</td>
<td>Duration of OR, PFS, safety</td>
</tr>
<tr>
<td>LUX-Breast 3 (NCT01441596)</td>
<td>II</td>
<td>Patients with CNS mets trastuzumab and/or lapatinib pretreated</td>
<td>Afatinib versus afatinib + vinorelbine versus investigator’s choice of treatment</td>
<td>Patient benefit at 12 wk</td>
<td>PFS, OS</td>
</tr>
<tr>
<td>DAFNE (NCT01594177)</td>
<td>III</td>
<td>Neoadjuvant</td>
<td>Afatinib + trastuzumab + NAC</td>
<td>pCR</td>
<td>AE, CR, TR</td>
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<tr>
<td>Neratinib</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NCT01423123</td>
<td>I</td>
<td>Trastuzumab pretreated</td>
<td>Neratinib + trastuzumab + paclitaxel</td>
<td>Safety, DLTs</td>
<td>ORS, PFI</td>
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<tr>
<td>NCT01008150</td>
<td>II</td>
<td>Neoadjuvant</td>
<td>Trastuzumab + NAC versus neratinib + NAC versus neratinib + trastuzumab + NAC</td>
<td>pCR</td>
<td>CCR, RFI, OS, toxicity</td>
</tr>
<tr>
<td>NCT01494662</td>
<td>II</td>
<td>Patients with CNS mets</td>
<td>Neratinib</td>
<td>ORR</td>
<td>PFS, OS, CNS response, safety, association of CTC and OS</td>
</tr>
<tr>
<td>NEFERTT (NCT00915018)</td>
<td>II</td>
<td>Trastuzumab naive</td>
<td>Neratinib + paclitaxel versus trastuzumab + paclitaxel</td>
<td>PFS</td>
<td>OS, ORR, duration of OR, CBR, AE, HRQL, time to CNS mets</td>
</tr>
</tbody>
</table>

Abbreviations: NCT, National Clinical Trial; MTD, maximum tolerated dose; AE, adverse event; PK, pharmacokinetics; ORR, overall response rate; CBR, clinical benefit rate; PFS, progression-free survival; OS, overall survival; TTD, time to deterioration; HRQL, health-related quality; OR, objective response; CNS, central nervous system; mets, metastases; NAC, neoadjuvant chemotherapy; pCR, pathologic complete response; CR, conservation rate; TR, translational research; CCR, clinical complete response; DLT, dose-limiting toxicity; PFI, progression-free interval; RFI, recurrence-free interval; CTC, circulating tumor cell.
FIG 1. pCR rates in trials assessing dual HER2 blockade.
Abbreviations: pCR, pathologic complete response; Anthra, anthracycline; C, carboplatin; D, docetaxel; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; L, lapatinib; P, pertuzumab; pac, paclitaxel; T, trastuzumab; tax, taxane.

TABLE 4. Selected Ongoing Trials with Targeted Agents in HER2 Breast Cancer

<table>
<thead>
<tr>
<th>Trial (NCT Identifier)</th>
<th>Phase</th>
<th>Setting</th>
<th>Treatment</th>
<th>Primary Endpoint</th>
<th>Secondary Endpoints</th>
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</thead>
<tbody>
<tr>
<td>Everolimus</td>
<td>II</td>
<td>CNS Mets</td>
<td>Everolimus + trastuzumab + vinorelbine</td>
<td>CNS RR</td>
<td>ORR, toxicity</td>
</tr>
<tr>
<td>BOLERO-3 (NCT01007942)</td>
<td>III</td>
<td>Trastuzumab pretreated</td>
<td>Everolimus + trastuzumab vinorelbine versus trastuzumab + vinorelbine</td>
<td>PFS</td>
<td>OS, ORR, PRO, CBR</td>
</tr>
<tr>
<td>BOLERO-1 (NCT00876395)</td>
<td>III</td>
<td>Trastuzumab naive</td>
<td>Everolimus + trastuzumab + paclitaxel versus trastuzumab + paclitaxel</td>
<td>PFS</td>
<td>OS, ORR, AEs, TTOR, OS, CBR, PK</td>
</tr>
<tr>
<td>Other PI3K-Blocking agents</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NCT01285466</td>
<td>I</td>
<td>Trastuzumab pretreated</td>
<td>BEZ235 or BKM120 + paclitaxel + trastuzumab</td>
<td>DLTs</td>
<td>AEs, ORR, PK</td>
</tr>
<tr>
<td>NCT01042925</td>
<td>I/II</td>
<td>Trastuzumab pretreated</td>
<td>XL147 + trastuzumab + (paclitaxel)</td>
<td>MTD, OTR</td>
<td>PFS, PK, PD</td>
</tr>
<tr>
<td>NCT01132664</td>
<td>I</td>
<td>Trastuzumab pretreated</td>
<td>BKM120 + trastuzumab</td>
<td>AES, DLT</td>
<td>ORR</td>
</tr>
<tr>
<td>PIKH2 (NCT01589861)</td>
<td>I/II</td>
<td>Trastuzumab pretreated</td>
<td>(with PI3K activation)</td>
<td>MTD, ORR</td>
<td>Safety, CBR, PFS, PK</td>
</tr>
<tr>
<td>IGF Blocking agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00684983</td>
<td>II</td>
<td>Trastuzumab pretreated</td>
<td>Cixutumumab + lapatinib + capecitabine versus lapatinib + capecitabine</td>
<td>PFS</td>
<td>OS, TTF, ORR, HRQL, TR</td>
</tr>
<tr>
<td>SRC Blocking Agents</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01306942</td>
<td>I/II</td>
<td>Trastuzumab pretreated</td>
<td>Dasatinib + trastuzumab + paclitaxel</td>
<td>MTD</td>
<td>Safety, CBR, PFS, RD</td>
</tr>
<tr>
<td>HER3 Blocking Agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01512199</td>
<td>I/II</td>
<td>Trastuzumab naive</td>
<td>U3-1287 + trastuzumab + paclitaxel</td>
<td>MTD, PFS</td>
<td>PK, DCR</td>
</tr>
</tbody>
</table>

Abbreviations: NCT, National Clinical Trial; CNS, central nervous system; mets, metastases; RR, response rate; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; PRO, patient-reported outcome; CBR, clinical benefit rate; AE, adverse event; TTOR, time to overall response; PK, pharmacokinetics; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; OTR, objective tumor response; PD, pharmacodynamic; TTF, time to treatment failure; HRQL, health-related quality; TR, translational research; RD, response duration; DCR, disease control rate.
this study is the comparison of disease-free survival (DFS) between the trastuzumab-alone and the combination arms. Importantly, the first interim analysis of the ALTTO study resulted in the closure of the lapatinib-alone arm, since it was deemed unlikely to cross the boundary of noninferiority.10

PERTUZUMAB
Pertuzumab is a humanized monoclonal antibody, binding to the dimerization domain II of HER2, thus blocking ligand-induced HER2/HER3 heterodimerization.11 This dimerization represents an important molecular event driving malignant progression of HER2-positive BC cells through its potent ability to activate the PI3K/Akt/mTOR signaling pathway. Preclinical evidence showed a synergistic effect of pertuzumab combined with trastuzumab, so that clinical trials assessing this dual HER2 blockade strategy followed.12

Several phase I studies have been conducted in the metastatic setting, assessing pertuzumab either as monotherapy or in combination with cytotoxic agents. A small phase II trial assessed the combination of pertuzumab and trastuzumab in 11 patients who were pretreated with trastuzumab and had had HER2-positive MBC.13 An overall response rate (ORR) of 18% and a clinical benefit rate (CBR) of 44.5% were reported; however, the accrual to this study was prematurely terminated because of cardiac toxicities. Six patients developed an LVEF decrease, with two exceeding a reduction of 15%, and one of them developed symptomatic congestive heart failure. Another phase II study assessed the combination of pertuzumab plus continued trastuzumab therapy in 66 patients with HER2-positive MBC progressing on trastuzumab-based therapy, showing promising antitumor activity: ORR reached 24.2% and CBR was 50%, with five patients achieving a complete response (CR; 7.6%) and 11 patients achieving a partial response (PR; 16.7%).14 The cardiac safety profile was favorable in this trial in comparison to the smaller one, with no patient withdrawing from the study as a result of cardiac toxicity. The cardiac safety of pertuzumab was proved by a pooled analysis study of 598 patients who received pertuzumab either as monotherapy or in combination with other compounds, showing that pertuzumab did not result in a notable increase in cardiac-related side effects.15

Clinical Evaluation of Pertuzumab and Trastuzumab (CLEOPATRA; NCT00567190) is a randomized, phase III clinical trial that assigned 808 previously untreated patients with HER2-positive MBC to receive either docetaxel plus trastuzumab or the same regimen combined with pertuzumab.16 The dual HER2 blockade resulted in a significant prolongation of independently assessed progression-free survival (PFS), the trial’s primary endpoint, as compared with the standard of care (mPFS 18.5 vs. 12.4 months, HR 0.65; 95% CI, 0.51 to 0.75, p < 0.001). An interim analysis of OS showed a trend favoring the pertuzumab-containing arm, which did not cross the stringent predefined p value (HR 0.64; 95% CI, 0.47 to 0.88, p = 0.005), with few reported OS events. A second OS analysis performed with 69% of planned events for the final analysis showed a statistical significance favoring the pertuzumab-containing arm (mOS not reached compared with 37.6 months in the placebo arm; HR 0.66; 95% CI, 0.52–0.84, p = 0.0008).17 ORR reached 69.3% in the control group and 80.2% in the pertuzumab group. Of note, only 10% of the patients enrolled in CLEOPATRA had received trastuzumab as part of a (neo)adjuvant regimen. However, exploratory analysis revealed no difference in efficacy of the pertuzumab-containing arm according to prior trastuzumab exposure. In terms of toxicity, diarrhea, rash, mucosal inflammation, febrile neutropenia, and dry skin of any grade were reported more frequently in the pertuzumab-containing arm. The cardiac safety profiles between the two arms were comparable, with left ventricular systolic dysfunction grade 3 and greater reported in 2.8% of the patients in the control group and in 1.2% of the patients in the pertuzumab group. On the basis of these results, pertuzumab is approved for use in the first-line setting of HER2-positive MBC, in combination with trastuzumab and docetaxel.

Pertuzumab has been also assessed in combination with trastuzumab in the neoadjuvant setting. The phase II study Neoadjuvant Study of Pertuzumab and Herceptin in Early Regimen Evaluation (NeoSphere; NCT00545688) randomly selected 417 patients to receive four cycles (12 weeks) of one of the following four preoperative regimens: trastuzumab plus pertuzumab plus docetaxel; trastuzumab plus docetaxel; pertuzumab plus docetaxel; or trastuzumab plus pertuzumab.18 The pCR rates, the primary endpoint of this trial, were 45.8% for the triplet, 29% for the trastuzumab plus docetaxel, 24% for pertuzumab plus docetaxel, and 16.8% for the chemotherapy-free pertuzumab plus trastuzumab combination. In all arms of the study, the rates of pCR were higher in the subgroup of women with ER-negative disease, with a pCR rate of 27.3% for women receiving the chemotherapy-free combination of pertuzumab plus trastuzumab. Importantly, this suggests that a proportion of women with HER2-positive disease could be cured without cytotoxic chemotherapy. TRYPHAENA (NCT00976989) is another phase II study that has been reported in the neoadjuvant setting. It randomly selected 225 patients to receive one of the three following arms: pertuzumab plus trastuzumab given concurrently with an anthracycline-taxane regimen (5-fluorouracil, epirubicin, cyclophosphamide [FEC] followed by docetaxel) or the dual HER2-combination given sequentially to FEC, but concurrently with docetaxel, or this anti-HER2 doublet given concurrently with carboplatin and docetaxel.19 The primary endpoint of this trial was cardiac safety, which was comparable across all three tested arms. pCR was 61.6% for arm 1, 57% for arm 2, and 66.2% in arm 3, with ER negativity associated with higher pCR rates (79.4%, 65.0%, and 83.8% for arms 1, 2, and 3, respectively).

The aforementioned clinical efficacy data led to the development of a phase III prospective multicenter clinical trial assessing the efficacy of pertuzumab in the adjuvant setting: Adjuvant Pertuzumab and Herceptin in Initial Therapy of Breast Cancer, BIG 4–11 (APHINITY; NCT01358877) is an ongoing international collaborative study, aiming to ran-
domly selected approximately 4,800 patients with early-stage HER2-positive BC to receive either trastuzumab or trastuzumab with pertuzumab in conjunction with cytotoxic chemotherapy.

**HER FAMILY SMALL MOLECULE INHIBITORS**

Neratinib is an oral, irreversible pan-HER TKI of EGFR/HER1, HER2, and HER4 under clinical development. A phase II study assessed neratinib in patients with HER2-positive MBC who had either previously received trastuzumab (66 patients) or not (70 patients).

Neratinib showed substantial antitumor activity with the 16-week PFS rates being 59% for patients who had been pretreated with trastuzumab and 78% for patients who were trastuzumab-naive and median PFS reaching 22.3 and 39.6 weeks, respectively. The most common AEs were diarrhea, which was the most frequent grade 3 to 4 AE, nausea, vomiting, and fatigue. No neratinib-related cardiotoxicity of grade 3 or 4 was reported. Neratinib has also been evaluated in combination with trastuzumab in a phase I/II study of 45 patients with trastuzumab-pretreated HER2-positive MBC.

Promising antitumor activity was noted, with a 16-week PFS rate of 47% and a mPFS of 19 weeks. No new toxicity signals were observed. Preliminary results of a phase I study assessing a triplet of weekly paclitaxel with neratinib and trastuzumab in women with heavily pretreated HER2-positive MBC have been presented; out of 10 patients for whom efficacy data were available, there was one CR and three PR, with diarrhea being the most frequent grade 3 and 4 AE.

Another phase II study assessed the feasibility of neratinib combined with everolimus, with signs of antitumor activity: out of eight patients with heavily pretreated HER2-positive MBC, four showed a PR and one had SD for an RR of 67%.

A randomized phase II study compared neratinib with lapatinib plus capecitabine in the second line of metastatic disease. Neratinib demonstrated high antitumor activity (ORR 29%); however, it was inferior to the lapatinib/capecitabine arm in terms of mPFS, the primary endpoint of the trial (mPFS 4.5 vs. 6.8 months, HR 1.3; 95% CI, 1.0 to 1.8).

A phase III study is currently underway evaluating neratinib in patients with early-stage HER2-positive BC after completion of standard trastuzumab based adjuvant chemotherapy (NCT00878709).

Afatinib is another promising orally available irreversible pan-HER TKI under clinical development. A phase II single-arm study assessed its activity in 41 patients with trastuzumab-pretreated HER2-positive MBC, with a median of three prior chemotherapy lines (range, 0–15). Promising clinical activity was shown, with four patients (10%) having PR, 15 patients (37%) having stable disease as best response and mPFS reaching 15.1 week (95% CI, 8.1 to 16.7 weeks). The most common grade 3 AEs were diarrhea and rash. Currently, there are ongoing trials assessing afatinib in HER2-positive BC (Table 3), with a phase III trial (LUX-Breast 1, NCT01125566) randomly selecting patients with HER2-positive MBC to receive afatinib plus vinorelbine or trastuzumab plus vinorelbine as first- or second-line treatment.

**T-DM1**

T-DM1 is an ADC consisting of DM1, a maytansinoid antimitotubule agent, bound to trastuzumab through nonreducible thioether bonds. T-DM1 delivers this highly potent cytotoxic agent specifically to HER2-expressing cells. Once T-DM1 binds to HER2 on the cell surface, the T-DM1-HER2 complex is internalized and the antibody component is proteolytically degraded, releasing the DM1 into the cytoplasm. Importantly, T-DM1 retains the biologic activity of trastuzumab (i.e., HER2 signaling blockade and induction of ADCC).

These properties provided the biologic rationale for testing T-DM1 in the setting of HER2-positive BC.

A phase I study conducted in 24 patients with advanced, heavily pretreated (median, four prior chemotherapeutic agents for metastatic disease) HER2-positive BC assessed the safety and tolerability of T-DM1 in three-weekly administered ascending doses. The maximum tolerated dose (MTD) of T-DM1 was 3.6 mg/kg, with transient thrombocytopenia being a dose-limiting toxicity. The toxicity profile was favorable with common drug-related AEs being grade 2 and lower thrombocytopenia, elevated transaminases, fatigue, nausea, and anemia. Preliminary signs of antitumor activity were generated, with a CBR (defined as the objective response plus stable disease at six months) among the 15 patients treated at the MTD reaching 73%. Two consecutive phase II single-arm studies of T-DM1 in HER2-positive MBC have been reported. The first one reported an ORR of 25.9% (95% CI, 18.4% to 34.4%) by independent assessment among 112 heavily pretreated patients. There were not sufficient events to reach median duration of response, and mPFS was 4.6 months (95% CI, 3.9 to 8.6 months). Another phase II study assessed T-DM1 monotherapy in 110 patients with HER2-positive MBC, who were previously treated with trastuzumab, lapatinib, an anthracycline, a taxane, and capecitabine (median, seven prior agents for metastatic disease).

The ORR was 34.5% (95% CI, 26.1% to 43.9%), the CBR was 48.2% (95% CI, 38.8% to 57.9%), mPFS was 6.9 months (95% CI, 4.2 to 8.4 months), and median duration of response was 7.2 months (95% CI, 4.6 months to not estimable). Both studies showed a favorable toxicity profile, with most AEs being grade 1 to 2; the most frequent grade 3 and lower AEs were thrombocytopenia and fatigue.

The results of a randomized phase II trial have been also reported; that study randomly selected 137 previously untreated patients to receive either T-DM1 or trastuzumab with docetaxel. T-DM1 resulted in a significant prolongation of mPFS as compared to the comparator arm (14.2 vs. 9.2 months, p = 0.035), whereas no significant differences in ORR (64% for T-DM1 vs. 58% in the control arm) or in CBR (75% for T-DM1 vs. 81% in the control arm) were found. The toxicity profile of T-DM1 compared favorably with standard treatment, with the rates of grade 3 or 4 neutropenia being 5.8% and 61% and the rates of alopecia being 4.3% and 67%, respectively.

Positive results confirming the antitumor activity of T-DM1 in the setting of HER2-positive MBC were recently
reported from the phase III randomized trial EMILIA (NCT00829166). This study randomly assigned 991 patients, previously treated with trastuzumab and a taxane, to receive T-DM1 or lapatinib plus capecitabine. The study met its primary endpoint, with T-DM1 resulting in a significant prolongation of mPFS as compared with the lapatinib plus capecitabine arm (9.6 vs. 6.4 months, HR 0.65; 95% CI, 0.55 to 0.77, p < 0.001), assessed by independent review. The ORR also favored the T-DM1 arm (43.6% vs. 30.8%, p < 0.001), as did the median duration of response (12.6 months vs. 6.5 months). The median OS (mOS) at the second interim analysis crossed the stopping boundary for efficacy (30.9 vs. 25.1 month, HR 0.68; 95% CI, 0.55 to 0.85, p < 0.001), favoring the T-DM1 arm. In terms of toxicity, rates of grade 3 or 4 AEs were lower for T-DM1 overall (41% vs. 57%). T-DM1 was associated with higher incidences of thrombocytopenia and increased liver enzyme levels and lower incidences of diarrhea, nausea, vomiting, and palmar-plantar erythrodysschia compared with that for lapatinib plus capecitabine.

Although the results of the EMILIA study provide solid evidence for T-DM1 as the treatment of choice in the second-line setting of HER2-positive MBC, another phase III randomized trial termed MARIANNE (NCT01120184) is seeking evidence to support the use of T-DM1 in the first-line setting. With 1,092 enrolled patients, MARIANNE will compare the efficacy of T-DM1 plus pertuzumab, T-DM1 plus placebo, and the combination of trastuzumab plus a taxane. A third phase III study, TH3RESA (NCT01419197), is evaluating T-DM1 in the third or later line of therapy. In this study, patients with HER2-positive MBC who previously have received trastuzumab, lapatinib, and chemotherapy are randomly selected to receive T-DM1 or physician’s choice of therapy. Other ongoing phase II trials are currently exploring the feasibility and/or antitumor potency of T-DM1 in the adjuvant (NCT01196052) and neoadjuvant setting (NCT01745965) of HER2-positive BC.

**PI3K/Akt/mTOR BLOCKING AGENTS**

PI3K/Akt/mTOR represents an intracellular signal transduction pathway commonly deregulated in the setting of BC and potentially mediating resistance to anti-HER2-blocking agents. To date, rapalogs represent the most clinically advanced agents targeting this signaling pathway. A phase I study assessed the triple combination of everolimus with paclitaxel and trastuzumab administered weekly in 33 patients with heavily pretreated (median of two lines of chemotherapy in metastatic setting, range, 0 to 17 lines) HER2-positive MBC. Encouraging antitumor activity was reported, with an overall disease control rate 6 months or fewer of 74% and mPFS reaching 34 weeks (95% CI, 29.1 to 40.7 weeks). Grade 3 to 4 neutropenia was the most common toxicity observed (17 patients; 52%). Another phase I study evaluated a regimen of everolimus combined with vinorelbine and trastuzumab in a similar population of 47 patients, with the ORR reaching 19.1%, disease control rate 6 months or fewer of 83% and mPFS reaching 30.7 weeks (95% CI, 28 to 44.9 weeks). Neutropenia (92%) and stomatitis (70%) of any grade were the most common AEs. Results of the dual combination of everolimus with trastuzumab in the metastatic setting have been reported from a pooled analysis (47 patients) of two phase I/II studies conducted concurrently: a CBR of 34% and a mPFS of 4.1 month were reported with fatigue, infection, and mucositis being the most frequent AEs. Currently, there are two ongoing phase III trials of everolimus in the setting of HER2-positive MBC: BOLERO-1 (NCT00876395) assesses the triplet of everolimus, trastuzumab, and paclitaxel as first-line regimen, and BOLERO-3 (NCT01007942) assesses the addition of vinorelbine to the two targeted agents in patients previously treated with patients. Importantly, the clinical activity of rapalogs is dampened by their mode of molecular action, which is restricted to mTORC1 inhibition, whereas mTORC2 remains unaffected. To overcome this innate limitation, there are currently many targeted agents blocking different molecular components of the PI3K/Akt/mTOR signaling pathway under development in HER2-positive BC, namely pan-PI3K, PI3K isomorf-selective, mTORC1/2 selective, dual PI3K/mTOR, and Akt inhibitors.

**OTHER EXPLORATORY ANTI-HER2-BLOCKING STRATEGIES**

Understanding the molecular biology of HER2-positive BC coupled with the elucidation of the molecular mechanisms mediating resistance to HER2 blockade can lead to the development of effective targeted agents. Ongoing trials combining HER2-targeted agents with compounds blocking other signaling pathways hold the promise to further improve the clinical outcome of patients with HER2-positive BC. A potentially promising approach appears to be the combination of HER2 blockade with insulin growth factor receptor 1 (IGF-1R)–blocking agents. A bidirectional crosstalk of HER2 and IGF-1R is well documented, with IGF-1R inhibition restoring sensitivity to trastuzumab in preclinical models. Clinical trials evaluating this hypothesis are currently ongoing. Another rational combination is the blockade of HER2 and SRC. SRC is a nonreceptor tyrosine kinase that was recently shown to function as a common node downstream of multiple trastuzumab-resistance mechanisms, both acquired and de novo. Blocking SRC in combination with trastuzumab showed a heightened synergistic antitumor efficacy in trastuzumab-resistant tumors in vivo, underlining the potential clinical usefulness of this approach. HER3, as already highlighted preclinically, is another rational molecular target in the setting of HER2-positive BC. HER3 is a potent activator of PI3K/Akt signaling pathway that has been demonstrated to be upregulated on HER2 blockade. Importantly, a recent report suggested that dual HER2-blockade in HER2-positive BC cells does not completely abrogate HER3 function, so that the addition of a HER3-targeting monoclonal antibody, with the dual combinations of either trastuzumab/pertuzumab or trastuzumab/lapatinib, led to increased antitumor activity.
CONCLUSION

The aforementioned data underline the antitumor potency of newly developed HER2-blocking agents against HER2-positive BC. Dual HER2 blockade has resulted in clinical successes in both the neoadjuvant (Fig. 1) and metastatic setting. The development of anti-HER2 ADCs promises further treatment options for these patients, achieving selected delivery of potent chemotherapy coupled with HER2 inhibition. Further treatment options could rise from the dissection of the molecular mechanisms mediating resistance to HER2 blockade. Nevertheless, many questions need to be answered: how can we better select the optimal HER2 blockade strategy for the individual patient? Clearly, to answer this question, robust predictive biomarkers are urgently needed. To the present day, only HER2 remains a suitable biomarker for therapeutic decisions about HER2 blockade, as was exemplified by the failure of the recent biomarker analysis of the CLEOPATRA study to identify additional predictive biomarkers.44 How can we identify those patients who can be effectively treated with HER2 blockade without the addition of cytotoxic chemotherapy? Recent findings indicate that FDG-PET/CT can serve as a valuable tool to this end. The Neo-ALTTO PET study assessed whether metabolic response with anti-HER2 therapies could predict pCR at the time of surgery; pCR rates were twice as high in patients who were FDG-PET/CT responders (using European Organisation for Research and Treatment of Cancer criteria45) compared with nonresponders (41% vs. 21% with FDG-PET/CT after 2 weeks of chemotherapy-free HER2 blockade).46 Building on these promising results, the Breast International Group (BIG) is planning to launch the Integrating Imaging to De-escalate Therapy in HER2-positive breast cancer Management (DREAM) trial, aiming to define the most effective HER2-targeting strategy and identify early on the group of patients who could potentially be spared systemic chemotherapy. What is the optimal sequencing of treatment among the variety of treatment options that are being constantly added in our therapeutic armamentarium (Table 5)? Is it possible to further improve the antitumor efficacy of dual HER2 blockade regimens by either HER2 blockade triplets or through the addition of other compounds targeting anti-HER2-resistance mechanisms? Lastly, will we identify more effective anti-HER2 adjuvant regimens with acceptable toxicity? Moreover, in a financially constrained environment, issues of increasing costs for the national health care systems are of concern. These questions can be answered through properly conducted collaborative clinical trials. The near future is promising for major leaps forward toward more effective treatment of HER2-positive BC.

TABLE 5. Developmental Status of Targeted Agents in HER2-Positive Breast Cancer, Across the Different Disease Settings

<table>
<thead>
<tr>
<th>Agent</th>
<th>Adjuvant</th>
<th>Neoadjuvant</th>
<th>Metastatic, First Line</th>
<th>Metastatic ≈ Second Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab + lapatinib</td>
<td>Phase III (ongoing)</td>
<td>Phase III (completed)</td>
<td>Phase I (ongoing)</td>
<td>Application for approval withdrawn</td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>Phase III (ongoing)</td>
<td>Phase III (completed)</td>
<td>Approved</td>
<td>Phase II (ongoing)</td>
</tr>
<tr>
<td>Trastuzumab-DM1</td>
<td>Phase III (ongoing)</td>
<td>Phase II (ongoing)</td>
<td>Phase III (ongoing)</td>
<td>Approval under evaluation</td>
</tr>
<tr>
<td>Afinatinib</td>
<td>-</td>
<td>Phase II (ongoing)</td>
<td>-</td>
<td>Phase III (ongoing)</td>
</tr>
<tr>
<td>Neratinib</td>
<td>-</td>
<td>Phase II (ongoing)</td>
<td>Phase II (ongoing)</td>
<td>Phase II (ongoing)</td>
</tr>
<tr>
<td>Everolimus</td>
<td>-</td>
<td>-</td>
<td>Phase III (ongoing)</td>
<td>Phase III (ongoing)</td>
</tr>
</tbody>
</table>

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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BREAST CANCER

Finding the Balance in the Management of Low-Risk Breast Cancer

CHAIR
Julia R. White, MD
The Ohio State University
Columbus, OH

SPEAKERS
Javier Cortes, MD, PhD
Vall d’Hebron University Hospital
Barcelona, Spain

Paul Goss, MD, PhD
Massachusetts General Hospital Cancer Center
Boston, MA
The Effect of Biology in the Treatment of Small Breast Tumors
Jose Perez-Garcia, MD, Eva Muñoz-Couselo, MD, PhD, and Javier Cortes, MD, PhD

OVERVIEW

Although the outcome of small (T1a/b) node-negative breast tumors is generally excellent, in the absence of prospective clinical trials, we are limited to data derived from retrospective analyses. Overall, the 10-year overall mortality rate is approximately 20%, while the 10-year breast cancer-specific mortality is in the range of 4% to 8% among this population in the absence of systemic therapy. This clearly reflects that many patients die of causes not related to breast cancer. Due in large part to breast cancer screening programs, the incidence of small tumors is increasing. There is consequently a growing interest in identifying factors that negatively affect the prognosis of these patients. Several studies have shown that patients with triple-negative and HER2+ tumors have a worse prognosis compared with hormone-receptor-positive, HER2- small breast cancers. However, the recent explosion of knowledge of the molecular characteristics of tumors is opening a new way to address cancer. Different genomic assays are currently available to help better predict the outcome of breast cancer patients. However, none of these techniques have been specifically evaluated in patients with small (T1a/b) node-negative tumors, and only a small number of patients with these tumors were included in those studies. In addition, very limited data are available about the role of these assays in patients with triple-negative or HER2-positive cancers. Although a chemotherapy-based strategy might be useful for triple-negative or HER2-positive T1b tumors, more information is urgently needed in order to optimize the treatment of our patients.

The incidence of small (T1a/b) node-negative breast tumors is steadily increasing due in part to the implementation of breast cancer screening programs. Unfortunately, the role of adjuvant systemic therapy in this population remains unclear.

Classically, tumor size and axillary lymph node status have been considered the most important prognostic factors in patients with breast cancer. For many years, small (T1a/b) node-negative breast tumors were believed to have such a good prognosis that systemic adjuvant therapy was not viewed to be necessary. However, more recent research on this issue has shown that certain subgroups of these patients have a significant risk of systemic recurrence. The challenge is therefore to identify patients most likely to benefit from systemic adjuvant therapy based on validated prognostic and predictive factors. Although clinical and pathologic features are currently the mainstay of clinical decision making for this population, a better knowledge of the molecular biology of breast cancer and the introduction of new prognostic tools into daily clinical practice, such as MammaPrint and Oncotype Dx (and most likely the PAM50 test in the upcoming months), are establishing a new and intriguing scenario in the adjuvant treatment of these patients.

The best systemic strategies for small tumors vary across different guidelines and are a matter of current debate. For example, according to the National Comprehensive Cancer Network (NCCN) guidelines, the selection of the adjuvant systemic treatment for patients with breast cancer is based on hormone receptor and HER2 status, tumor size, and axillary lymph node status. In brief, these guidelines do not recommend administration of adjuvant chemotherapy in breast tumors that are 0.5 centimeters (cm) or smaller without axillary lymph node involvement, irrespective of hormone receptor, and HER2 status. In addition, prognostic tools such as Oncotype Dx are recommended for HER2- and hormone-receptor-positive disease that is 0.5 cm or larger with negative axillary lymph nodes, whereas adjuvant systemic therapy is recommended for triple-negative and HER2+ breast cancers between 0.6 and 1.0 cm without axillary lymph node involvement. Therefore, considering these guidelines, a patient age 40 with a stage T1aN0 triple-negative breast cancer (0.5 cm of diameter, high tumor grade, and Ki-67 index of 80%) should not receive adjuvant chemotherapy. But, are we heading in the right direction under this or other guidelines?

Thus, we discuss the outcome of small (T1a/b) node-negative breast tumors; the factors that adversely affect the prognosis among this population; and how new prognostic tools and the molecular classification of breast cancer can help us to identify those patients that might benefit from adjuvant systemic therapy.
OUTCOME OF SMALL (T1a/b) NODE-NEGATIVE BREAST CANCERS

Several authors have retrospectively analyzed the outcome of small (T1a/b) node-negative breast tumors. Overall, the 10-year breast cancer-specific mortality is in the range of 4% to 8%, while the 10-year overall mortality rate is approximately 20% among this population in the absence of systemic therapy. Most of these studies had a short follow-up and/or included a small number of patients. In addition, it is important to take into account that some of them recruited patients treated with adjuvant systemic therapy. This procedure can lead to an overestimation of the outcome that would be found in an entirely untreated population. For this reason, the studies summarized below have been selected either because they included a significant number of patients and/or because they had a longer follow-up. Moreover, only data from surgery-treated patients are reported.

Fisher et al. retrospectively evaluated the outcome and treatment of patients with breast tumors smaller than 1 cm without axillary lymph node involvement from five National Surgical Adjuvant Breast and Bowel Project (NSABP) randomized clinical trials. Two hundred and thirty-five patients with estrogen receptor (ER)-negative tumors and 1,024 patients with ER+ tumors were identified in these trials. Median follow-up was 8 years. Patients with ER+ tumors received surgery alone (26%); surgery and tamoxifen (53%); or surgery, tamoxifen, and chemotherapy (21%). Patients with ER- tumors received surgery alone (26%) or surgery and chemotherapy (74%). The 8-year recurrence-free survival (RFS) rates for women with ER- and ER+ tumors who received surgery alone were 81% and 86%, respectively. The 8-year overall survival (OS) rates for surgery-treated patients with ER- and ER+ tumors were 93% and 90%, respectively, and the 8-year OS rate for all patients was 92%, regardless of treatment and ER status. However, only half of these patients died from breast cancer.

Rosen et al. retrospectively assessed the prognosis of 767 patients with stage T1/2 node-negative breast cancer treated with surgery alone. A total of 171 patients with small (T1a/b) node-negative breast tumors were included in the analysis. Median follow-up was 18 years. This subgroup had RFS rates of 91% and 88% at 10 and 20 years, respectively. Additionally, there were 178 (23.2%) deaths caused by breast cancer among all patients included in the study. In this regard, it is important to note that the probability of death from breast cancer did not exceed the probability of death caused by other causes among women with small (T1a/b) node-negative breast tumors.

Finally, Chia et al. retrospectively evaluated the 10-year outcomes in a population-based cohort of node-negative, lymphatic, and vascular invasion-negative early breast cancers without adjuvant systemic therapy. The 430 tumors included in this analysis were smaller than 1 cm. Median follow-up of the entire series was 10.4 years. The 10-year OS rate was 79%, and the 10-year breast cancer-specific survival was 92%.

It is necessary to emphasize that one of the major weaknesses of these studies, apart from their retrospective nature, is the fact that the effect of HER2 status in the outcome of these patients was not analyzed.

In summary, the outcome of small (T1a/b) node-negative breast cancers is generally excellent. However, the breast cancer-specific mortality, probably the most appropriate end point for this particular subgroup, varies between 4% and 8% among these patients, warranting an optimization of the adjuvant systemic therapy for this population.

KEY POINTS

- Due to the increase in breast cancer screening programs, the incidence of small, node-negative tumors is increasing, which leads to a growing interest in identifying factors that negatively impact the prognosis of patients with breast cancer.
- The recent explosion of knowledge about the molecular characteristics of tumors is opening a new way to tackle cancer. In breast cancer, different genomic assays are available to better predict the outcome of patients with breast cancer. However, none of them has been specifically evaluated in patients with T1a/b node-negative tumors and the number of patients with these tumors included in these studies was very small.
- Although clinical and pathologic features are currently the mainstay of clinical decision making for this population, a better knowledge of the molecular biology of breast cancer and the introduction of new prognostic tools into daily clinical practice, such as MammaPrint and Oncotype Dx (and most likely the PAM50 test in the upcoming months), are establishing a new and intriguing scenario in the adjuvant treatment of these patients.
- The breast cancer intrinsic subtypes may represent a relevant prognostic factor regardless of tumor size. Overall, triple-negative and HER2+ breast cancers by immunohistochemistry are associated with a higher risk of recurrence among small (T1a/b) node-negative tumors.
- Although the outcome of small (T1a/b) node-negative breast tumors is generally excellent, in the absence of prospective clinical trials, we are limited to clinical data derived from retrospective analyses.

COMMONLY USED FACTORS THAT ADVERSELY AFFECT THE PROGNOSIS OF SMALL (T1a/b) NODE-NEGATIVE BREAST CANCERS

Although the risk of relapse of small (T1a/b) node-negative breast tumors is low, there is a growing interest in identifying factors that negatively affect the prognosis of these patients, to recognize which patients might benefit from adjuvant systemic therapy as well as to avoid unnecessary side effects.

In the study of Fisher et al. the risk of recurrence was greater in women who had tumors of 1 cm in size than for those women who had tumors of less than 1 cm; in women age 49 or younger compared with women age 50 or older; and in women with ductal or lobular carcinoma compared
with women who had other histologic tumor types, irrespective of ER status.\textsuperscript{10} In the analysis of Rosen et al., patients with infiltrating ductal carcinoma had a worse RFS rate than patients with special tumor types (medullary, mucinous, tubular, adenocystic, and papillary) and infiltrating lobular carcinoma.\textsuperscript{11}

In the study of Chia et al. tumor grade appeared to be an important predictor of risk of breast cancer death, regardless of tumor size.\textsuperscript{12} Finally, Hanrahan et al. also retrospectively analyzed the OS and cause-specific mortality of patients with stage T1a/bN0 breast cancer registered in the Surveillance, Epidemiology, and End Results (SEER) Program from 1988 to 2001.\textsuperscript{13} The main limitation of this analysis was that the proportion of patients who received adjuvant chemotherapy or hormonal therapy was unknown. A total of 51,246 patients were identified. Median follow-up was 64 months. In this study, a trend toward higher probability of death was found in patients younger than age 50 at diagnosis; in patients with adverse pathologic features such as high tumor grade, ER-status, and progesterone-receptor–negative status; and in patients with an inadequate axillary lymph node assessment (fewer than six nodes removed at axillary dissection).\textsuperscript{13}

Although none of these studies evaluated the influence of HER2 status in the prognosis of these small (T1a/b) node-negative tumors, other series have appropriately assessed this issue.

Gonzalez-Angulo et al. reviewed the risk of recurrence in women diagnosed with stage T1a/b, node-negative breast cancer taking HER2 status into account.\textsuperscript{14} A total of 965 patients were identified at the MD Anderson Cancer Center between 1990 and 2002. Patients who received adjuvant chemotherapy or trastuzumab were excluded. Median follow-up was 74 months. Ten percent of patients had HER2+ tumors, and the 5-year RFS rates were 77.1% and 93.7% in patients with HER2+ and HER2- tumors, respectively ($p = 0.001$). The 5-year distant RFS rates were 86.4% and 97.2% in patients with HER2+ and HER2- tumors, respectively ($p = 0.001$). Among HER2+ tumors, the risk of recurrence was similar to independent of hormone-receptor status, although the fact that up to 55% of hormone-receptor–positive patients received adjuvant hormone therapy should be taken into consideration to avoid misinterpretations. Unfortunately, the authors did not analyze the outcome of HER2+ patients according to tumor stage (T1a vs. T1b).

Curigliano et al. also identified 150 consecutive patients with HER2+ tumors among a population of 2,130 patients with stage T1a/b, node-negative breast cancers at the European Institute of Oncology between 1999 and 2006.\textsuperscript{15} A matched cohort was selected by using variables of hormone-receptor status, age, and year of surgery. No patient received adjuvant trastuzumab, but up to 50% of patients received adjuvant chemotherapy in the hormone-receptor–negative group, and more than 90% of patients received adjuvant hormone therapy, alone or in combination with chemotherapy, in the hormone-receptor–positive group. Median follow-up was 4.6 years. In the hormone-receptor–positive group, the 5-year disease-free survival (DFS) rates were 99% (95% CI, 96% to 100%) for HER2- disease and 92% (95% CI, 86% to 99%) for HER2+ disease. In the hormone-receptor–negative group, the 5-year DFS rates were 92% (95% CI, 84% to 100%) for HER2- disease and 91% (95% CI, 84% to 99%) for HER2+ disease. It is important to emphasize that in the hormone-receptor–negative group, the authors compared HER2+ with patients with triple-negative breast cancer. Moreover, the DFS rates according to tumor stage (T1a vs. T1b) and hormone receptor status were adequately assessed. Among HER2+ and hormone-receptor–positive tumors, the 5-year DFS rates were 88% (95% CI, 67% to 96%) in patients with stage T1a and 95% (95% CI, 82% to 99%) in patients with stage T1b. Among HER2+ and hormone-receptor–negative tumors, the 5-year DFS rates were 93% (95% CI, 72% to 98%) in patients with stage T1a and 85% (95% CI, 60% to 95%) in patients with stage T1b. However, considering the limited number of patients and the treatment heterogeneity, definitive conclusions cannot be drawn from this subgroup analysis.

In sum, a younger age at diagnosis, ER- status, HER2+ status, and high tumor grade adversely affect the prognosis of node-negative breast cancers smaller than 1 cm. These factors should be taken into account to define the best adjuvant systemic therapy for these patients.

How can new prognostic tools help to better identify which patients with small (T1A/B) node-negative tumors might benefit from adjuvant systemic therapy?

MammaPrint and Oncotype Dx are two prognostic tests based on retrospective analyses that are currently used in clinical practice. These tools can help physicians to determine whether or not a patient with breast cancer will benefit from adjuvant chemotherapy.

MammaPrint was initially evaluated in a series of 295 consecutive women diagnosed with stage T1/2, ER- or ER+ breast cancer, with or without axillary lymph node involvement.\textsuperscript{8} Up to 40% of patients had received adjuvant chemotherapy. At 10 years, the probability of remaining free of distant metastases was lower in the group with a poor-prognosis signature than in the group with a good-prognosis signature (HR for distant metastases = 5.1; $p < 0.001$). The prognosis profile was significantly associated with tumor grade, ER status, tumor diameter, and age at diagnosis, but not with the number of axillary positive nodes. The authors did not specifically analyze these findings among small (T1a/b) node-negative breast cancers. Subsequently, MammaPrint was validated with the same results in a series of 307 patients with stage T1/2, node-negative, ER- or ER+ breast cancer.\textsuperscript{8} No patient received adjuvant systemic therapy. Only 11 patients with small (T1a/b) tumors were included in the analysis. Of these tumors, six had a poor-prognosis signature. The authors did not evaluate in detail the characteristics of these patients, although ER- status and high tumor grade were associated with a poor-prognosis signature.
signature again. Finally, Mammaprint was assessed in 965 patients with stage T1 breast cancer irrespective of node status, age, and ER, PR, and HER2 status. Only 10% of patients had received adjuvant chemotherapy. The results were consistent with those reported in previous studies. A total of 140 small (T1a/b) tumors were included in the analysis. However, given the heterogeneity of these patients, solid conclusions should not be drawn.

Mammaprint is not only an independent prognostic factor for patients with early stage breast cancer, but it may also be predictive for the benefit of chemotherapy. Knauer et al. analyzed a pooled database from six prior studies. Five hundred and forty-one women diagnosed with stage T1–3, ER- or ER+ breast cancer, with or without axillary lymph node involvement were included. Only patients with a poor-prognosis signature had a significant benefit from chemotherapy (distant disease-free survival, 76% vs. 88%; p = 0.01), whereas patients with a good-prognosis signature did not benefit from chemotherapy treatment (distant disease-free survival, 93% vs. 99%; p = 0.2). The chemotherapy benefit among small (T1a/b) node-negative breast cancers was not assessed in this study.

In contrast, Oncotype DX was retrospectively evaluated in the NSABP trial B14, a clinical trial that analyzed the treatment with tamoxifen in node-negative, ER+ patients. The levels of expression of 21 genes were used to calculate a recurrence score (RS). The rates of distant recurrence at 10 years in the low-risk (<18), intermediate-risk, and high-risk groups (≥31) were 6.8%, 14.3%, and 30.5%, respectively. The rate in the low-risk group was significantly lower than that in the high-risk group (p < 0.001). A total of 109 patients with small (T1a/b) tumors were included in the analysis. Of these patients, 59.5% had a low RS, 25% had an intermediate RS, and 15.5% had a high RS. A high RS was also associated with a higher rate of distant recurrence at 10 years among these patients, although the differences with respect to the patients with intermediate and low RS tumors were not as significant as observed in larger tumors. Later, Oncotype DX was assessed in the NSABP trial B20 that tested the effect of adding cyclophosphamide, methotrexate, and fluorouracil or methotrexate and fluorouracil chemotherapy to 5 years of tamoxifen in the treatment of patients with node-negative, ER+ breast cancer. Patients with high RS tumors had a large benefit from chemotherapy (relative risk for distant recurrence, 0.26; 95% CI, 0.13 to 0.53), although patients with low RS tumors did not benefit from chemotherapy treatment (relative risk for distant recurrence, 1.31; 95% CI, 0.46 to 3.78). The chemotherapy benefit in patients with intermediate RS tumors remains controversial. A total of 110 patients with small (T1a/b) tumors were included in the analysis. Of these patients, 64% had a low RS, 20% had an intermediate RS, and 16% had a high RS. Unfortunately, the chemotherapy benefit according to tumor stage (T1a vs. T1b vs. others) was not evaluated in this study.

Finally, neither Mammaprint nor Oncotype Dx have been assessed among small (T1a/b) node-negative HER2+ and triple-negative breast cancers, and their use in clinical practice is not recommended in these patients.

In summary, some small (T1a/b) node-negative breast cancers are considered high-risk tumors by Mammaprint or Oncotype Dx, in particular high-grade ER+ and ER- tumors, and might benefit from adjuvant systemic therapy. However, the effect of tumor stage (T1a vs. T1b vs. others) in this benefit, its influence in the selection of the adjuvant treatment, and the optimal chemotherapy regimen merit further investigation.

**IS THE MOLECULAR CLASSIFICATION OF BREAST CANCER RELEVANT FOR DEFINING THE BEST ADJUVANT SYSTEMIC TREATMENT FOR PATIENTS WITH SMALL (T1A/B) NODE-NEGATIVE BREAST CANCERS?**

Breast cancer is an extremely heterogeneous disease with multiple clinical presentations and tumor characteristics. Gene-expression profiling studies have classified breast tumors into a number of distinct biological and intrinsic subtypes with prognostic and therapeutic implications, thus providing a new molecular classification of breast cancer. According to this classification, at least four different molecular subtypes have been identified: luminal A, luminal B, HER2-enriched, and basal-like.

Molecular profiling is not currently ready for use in clinical decision making. Therefore, a combination of immunohistochemical surrogate markers (using ER and PR status, HER2 status, tumor grade, and Ki-67 index) have been validated for molecular subtyping. However, as previously mentioned, neither HER2 status nor Ki-67 index were analyzed in some of the studies discussed above. Surrogate definitions of intrinsic subtypes of breast cancer are summarized in Table 1.

The PAM50 gene-expression assay is one of the tools in current development to classify breast cancers into intrinsic subtypes. Recently, Bastien et al. evaluated the concordance between PAM50 breast cancer subtyping and immunohistochemistry, concluding that a standard immunohistochemical panel for breast cancer does not adequately identify the PAM50 gene-expression subtypes.

Theriault et al. analyzed the outcome of patients with stage T1a/b, node-negative breast tumors according to breast can-

**TABLE 1. Surrogate Definitions of Intrinsic Subtypes of Breast Cancer**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>ER and/or PR</th>
<th>HER2</th>
<th>Ki67 Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>Positive (PR &gt; 20%)</td>
<td>Negative</td>
<td>&lt; 14%</td>
</tr>
<tr>
<td>Luminal B</td>
<td>Positive</td>
<td>Negative</td>
<td>≥ 14%</td>
</tr>
<tr>
<td>Luminal HER2 OVER EXP</td>
<td>Positive</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>HER2-overexpression</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Basal-like</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.
er subtype determined by immunohistochemistry.\textsuperscript{22} One thousand and twelve patients diagnosed between 1990 and 2002 at the MD Anderson Cancer Center who did not receive chemotherapy or trastuzumab were included. Median follow-up was around 60 months. There were 771 hormone-receptor–positive, 98 HER2+ , and 143 triple-negative breast cancers. Compared with patients with hormone-receptor–positive disease, patients with HER2+ breast cancer had 4.98-times (95% CI, 2.91–8.53) the risk of worse RFS, and patients with triple-negative breast cancer had 2.71-times (95% CI, 1.59–4.59) the risk of worse RFS. Amar et al. also evaluated the outcome of small (T1a/b) node-negative breast cancers according to hormone receptor and HER2 status.\textsuperscript{23} Of the 421 tumors identified, 364 (86.5%) were HER2-, 28 (6.7%) were HER2+, and 29 (6.9%) were triple-negative breast cancers. The median follow-up time was only 1,015 days. Unlike the previous study, 3%, 25%, and 27.6% of HER2-, HER2+, and triple-negative patients with breast cancer received adjuvant chemotherapy, respectively, and 17.8% of HER2+ patients received trastuzumab-based adjuvant therapy. During the follow-up, the tumor recurred in nine patients: four were HER2- tumors (1.1%), two were HER2+ tumors (7.1%), and three were triple-negative tumors (10.7%). Unfortunately, no correlation between immunohistochemistry and PAM50 test was performed in this both studies.

Among ER+ tumors, there is evident tumor heterogeneity by gene-expression assays. In this way, luminal B tumors are associated with poor breast cancer-specific survival than luminal A tumors.\textsuperscript{24} Both are usually ER+ tumors. However, luminal B tumors are associated with increased expression of proliferative genes resulting in higher tumor grade and Ki-67 index. In addition, up to 20% of luminal B tumors are HER2+.\textsuperscript{25} As stated above, ER- status, HER2+ status, and high tumor grade adversely affect the prognosis of small (T1a/b) node-negative breast cancers. These characteristics are very infrequent in luminal A tumors. Therefore, it could indirectly be concluded that luminal A small (T1a/b) node-negative tumors are associated with the best outcome. Nevertheless, it would have been interesting to analyze in the previously mentioned studies the outcome of ER+ patients taking into account Ki-67 index and tumor grade. In this manner, we would have been able to identify a well-defined subgroup of patients with ER+, small (T1a/b) node-negative breast cancers with an excellent prognosis, essentially composed of luminal A tumors, who might not benefit from adjuvant chemotherapy without the need to use MammaPrint, Oncotype Dx, or even PAM50 recurrence test. Additionally, the risk of relapse of luminal B small (T1a/b) node-negative tumors would have also been reported.

Unfortunately, there is no data about the prognostic effect of PAM50 intrinsic subtyping among small (T1a/b) node-negative breast cancers. For this reason, it would be interesting to evaluate the outcome of these patients according to molecular subtyping. Despite this, the breast cancer intrinsic subtypes have shown prognostic significance, remaining more significant in multivariate analyses than other standard parameters (ER status, histologic grade, tumor size, and node status), and have also been used to generate a risk of recurrence score.\textsuperscript{4} Therefore, the breast cancer intrinsic subtypes may represent an important prognostic factor despite the tumor size. Considering that only the luminal A subtype contains low-risk patients, whereas the luminal B, HER2-enriched, and basal-like subtypes include intermediate- and high-risk tumors, the PAM50-based, low-risk luminal A tumors among small (T1a/b) node-negative breast cancers could constitute a subgroup with a favorable prognosis that would not benefit from adjuvant chemotherapy. In regards to the management of PAM50-based high-risk small (T1a/b) node-negative tumors, including all the intrinsic subtypes, would tumor stage (T1a vs. T1b vs. others) have an effect in the benefit derived from adjuvant systemic therapy? Should cancer staging guide the choice of the adjuvant systemic therapy, or should we rely only on the biologic behavior? What is the breast cancer-specific mortality among the different molecular subtypes without adjuvant systemic therapy? These questions remain open and most of them will probably never be fully answered.

In sum, the breast cancer intrinsic subtypes may represent a relevant prognostic factor regardless of tumor size. Overall, triple-negative and HER2+ breast cancers by immunohistochemistry are associated with a higher risk of recurrence among small (T1a/b) node-negative tumors.

**CONCLUSION**

Although the outcome of small (T1a/b) node-negative breast tumors is generally excellent, in the absence of prospective clinical trials, we are limited to clinical data derived from retrospective analyses. Different studies have shown that a younger age at diagnosis, ER- status, HER2+ status, and high tumor grade adversely affect the prognosis of these patients and should be taken into consideration in order to define the best adjuvant systemic therapy for this population.

Additionally, several genomic assays are currently available to help better predict the outcome of breast cancer patients. In this way, some small (T1a/b) node-negative breast cancers are classified as high-risk tumors by MammaPrint or OncotypeDx, in particular high-grade ER+ and ER- tumors, and might benefit from adjuvant systemic therapy. However, the effect of tumor stage (T1a vs. T1b vs. others) in this benefit, its influence in the selection of the adjuvant treatment, and the optimal chemotherapy regimen merit further evaluation.

Finally, triple-negative and HER2+ breast cancers by immunohistochemistry have been associated with a higher risk of recurrence among small (T1a/b) node-negative tumors. Unfortunately, there is no data about the prognostic impact of PAM50 intrinsic subtyping. For this reason, it would be interesting to evaluate the outcome of these patients according to molecular subtyping in the absence of systemic therapy in order to optimize the management of these tumors.
APPENDIX 1: TREATMENT ALGORITHM FOR SMALL (T1A/B) NODE-NEGATIVE BREAST TUMORS

**ER-Positive/HER2-Negative**
1. T1a → adjuvant endocrine therapy
2. T1b
   - Adjuvant endocrine therapy in postmenopausal patients with low-grade/intermediate-grade tumors (PR positive > 20% and Ki67 < 14% are typically associated with luminal A subtype and can help to better identify those patients that are unlikely to benefit from adjuvant chemotherapy).
   - Consider MammaPrint or Oncotype Dx mainly in premenopausal women, and/or in patients with high-grade tumors:
     - High risk/high recurrence score → adjuvant chemotherapy + adjuvant endocrine therapy
     - Low risk/low recurrence score → adjuvant endocrine therapy
     - Intermediate recurrence score → adjuvant endocrine therapy

**Triple-Negative Breast Cancer**
1. T1a → consider adjuvant chemotherapy in very young women (age ≤ 40), and/or in patients with high-grade tumors
2. T1b → consider adjuvant chemotherapy

**ER-Positive/HER2-Positive**
1. T1a → adjuvant endocrine therapy. Consider adjuvant chemotherapy plus trastuzumab in very young patients (age ≤ 40), and/or in patients with high-grade tumors.
2. T1b → adjuvant endocrine therapy. Consider adjuvant chemotherapy plus trastuzumab.

**ER-Negative/HER2-Positive**
1. T1a → consider adjuvant chemotherapy plus trastuzumab in very young patients (age ≤ 40), and/or in patients with high-grade tumors.
2. T1b → consider adjuvant chemotherapy plus trastuzumab

Following these proposed guidelines, our recommendation for a 40-year-old patient with a stage T1aN0 triple-negative breast cancer (0.5 cm of diameter, high tumor grade, and Ki67 index of 80%) would be chemotherapy with taxane-based therapy.

This recommendation is based on the following:
1. ER-negative status, younger age at diagnosis, and high tumor grade are independent adverse prognostic factors among small (T1a/b) node-negative breast cancers.10,12,13
2. Triple-negative breast cancers by immunohistochemistry are associated with a higher risk of recurrence among small (T1a/b) node-negative tumors.22,23
3. Most basal-like tumors are triple-negative by immunohistochemistry.25
4. Basal-like tumors show a high chemosensitivity.26
5. Basal-like tumors have a significantly worse clinical outcome.26

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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Competing Risks in Low-Risk Breast Cancer

Kathrin Strasser-Weippl, MD, MBA, and Paul E. Goss, MD, PhD

OVERVIEW

In recent years a growing amount of data on prognostic features of breast cancer has allowed for identification of tumors with a very low risk of recurrence. Markers used to predict the risk of distant spread include classic clinicopathologic features as well as newer tumor gene signatures, which have been validated and are being used in cohorts of patients with breast cancer patients who have low-risk disease. However, the definition of “low-risk” breast cancer requires consideration of patient-related factors such as comorbidities and age in addition to tumor characteristics, as high competing risks for mortality might be more important than cancer recurrence from a patient’s point of view. In addition, identification of low-risk breast cancer cohorts is only valuable if treatment decisions are based on this information. Therefore, the magnitude of any treatment benefit in low-risk disease needs to be quantified in a comprehensible way. However, treatment benefit in low-risk situations is hard to quantify, may vary over time, and needs to be compared to individual risks for side effects. Decision models considering tumor and patient characteristics as well as predictive markers for treatment efficacy and toxicity will be needed. Tools such as Adjuvant! Online ultimately need to include information from gene signatures in order to reliably recommend specific treatment options for patients with breast cancer patients who have low-risk disease.

Historically, the decision of whether, and how, to treat patients with limited-stage breast cancer has been based on clinicopathologic parameters such as tumor size or extent of nodal involvement. In recent years however, “a trend to using tumor biology rather than numerical disease indicators such as tumor size or extent of nodal involvement” has become increasingly common. This concept was first implemented for deciding about the use of tamoxifen for estrogen receptor (ER)-positive breast cancer and developed further for decision making around the use of trastuzumab for HER2+ disease. In 2005, the St. Gallen International Breast Cancer Conference divided endocrine responsiveness of breast cancers into three categories “highly endocrine responsive,” “endocrine response uncertain,” and “endocrine unresponsive.” Increasingly, relying on predictive biomarkers for treatment decisions has become the basis of deciding on cancer therapies and is leading to approval of targeted therapies for many cancers.

Despite the value of both predictive and prognostic biomarkers in dividing high-risk patients from lower-risk patients, inevitably it remains debatable whether those with very low risk of recurrence should still be treated relatively aggressively if biomarkers point to even a small therapeutic benefit. To make these decisions numerous factors need consideration including tumor and patient characteristics, treatment side effects, economic deliberations, and patient preference. In this article we discuss the nuances of some of these factors.

DEFINING “LOW-RISK” BREAST CANCER

Before considering treatment options for low-risk patients with breast cancer, the term “low-risk disease” needs to be defined. Commonly, the term “low risk” has been used for subtypes of breast cancer with very favorable prognostic markers, predicting “objectively and independently” favorable clinical outcome “independent of systemic treatment given.” However, as today it is usual practice to treat most patients with breast cancer with endocrine therapy or chemotherapy, it is increasingly difficult to estimate the prognostic value of a new marker independent of treatment effects. In addition, a marker can be both prognostic and predictive, and the possibility of certain disease subtypes becoming “low risk” only in the presence of a specific treatment needs to be taken into account. A typical example would be if certain HER2+ ER-positive cancers become “low risk” if trastuzumab and endocrine therapy are administered.

In addition, the label “low risk” should not be used merely for breast cancers with a lower risk of recurrence or death than other breast cancers, because this ignores the patient’s perspective. Rather, “low risk” from a patient’s point of view is a disease that has little or no risk of death and/or morbidity without (systemic) treatment. A patient’s competing risks for...
survival and quality of life, accounting for her expectation of remaining quality-adjusted life years (QALY) without breast cancer need to be considered in addition to disease characteristics. This is a difficult task, as expected QALYs can only be roughly estimated and may vary considerably between patients on the basis of comorbidities, lifestyle, genetic, and other unknown factors.

In summary, the identification of patients with low-risk breast cancer needs a two-tiered approach: (1) identification of favorable tumor subtypes by prognostic and predictive biomarkers; and (2) identification of patients by markers of quality of life and life expectancy who, with a specific tumor subtype, will not be able to benefit sufficiently from (more aggressive) treatment during their remaining QALYs.

**DOES “INDOLENT” BREAST CANCER EXIST AT ALL?**

When reviewing the prognostic spectrum of breast cancers, an important question is whether breast cancers can ever be considered “indolent,” that is whether there are patients whose natural life expectancy will not be altered by the presence of the disease. This question has recently been answered in an indirect but very convincing manner. By examining the incidence of early- and late-stage breast cancers in the Surveillance, Epidemiology, and End Results Program database from 1976 through 2008, Bleyer et al. showed that in 2008 invasive breast cancer was over-diagnosed in more than 30,000 women or 20% of all invasive breast cancers diagnosed that year in the United States. These and other data demonstrate that there are a number of patients with breast cancer who are treated for breast cancers that would not have caused clinical illness had they remained undetected. If there are patients who will live to their normal life expectancy with no treatment at all, one may conclude that—across the prognostic spectrum of breast cancers—there are certainly a number of patients with breast cancer who will remain free of recurrence after surgical (and possibly radiation) treatment alone or for whom endocrine therapy is sufficient. A major goal of our efforts to tailor treatment to individual patients is to identify these groups.

**TUMOR CHARACTERISTICS**

The first step toward identifying “low-risk” patients with breast cancer is to define favorable tumor characteristics that may be purely prognostic markers, or predictive markers for non-toxic therapies that allow for the omission of more aggressive treatment modalities.

**TUMOR SIZE AND NODAL INVOLVEMENT**

It has long been acknowledged that the prognosis of patients with breast cancer varies with the size of the primary tumor and the extent of nodal involvement, because both indicate the capacity of the tumor to cause distant spread. It was also shown in the 1970s—when tumor size and nodal involvement were the only markers available—that adjuvant chemotherapy improves the survival of patients with breast cancer with node-positive disease.

However, although all these studies consistently reported a higher risk of recurrence and death with higher values of these numerical markers, they also showed that not all patients with breast cancer with large tumors and/or extensive nodal involvement ultimately suffer disease recurrence.

**ESTROGEN RECEPTOR STATUS**

The ER is predominantly used for treatment decision making, because ER expression is highly correlated with response to endocrine treatment. However, expression of the ER is also a prognostic marker as the survival curves and the corresponding hazard rates vary significantly by ER expression of the primary tumor: the hazard rate for recurrence of ER-positive breast cancer is much lower than for ER-negative breast cancer in the first years after diagnosis, but it remains almost unchanged over many years so that starting from about year 8 to 10, the yearly rate of recurrence of ER-positive breast cancer is higher than for ER-negative disease.

**GRADING AND PROLIFERATIVE INDEX**

The biomarkers tumor grade and proliferative index (Ki-67) correlate with each other and do so inversely with expression of ER. The prognostic value of Ki-67 staining for disease-free and overall survival independent of node status has also been established in numerous studies and several meta-analyses. In addition, pretreatment Ki-67 scores are predictive for response to chemotherapy.

**HER2 POSITIVITY**

Overexpression of the HER2 oncogene (HER2 positivity) has been known to be of prognostic value in breast cancer for many years. When the monoclonal antibody trastuzumab became available, HER2 moved from being a merely prognostic to being a mainly predictive biomarker for response to anti-HER2 agents. In addition, HER2 also seems to be pre-
dictive for response to certain chemotherapies, including anthracyclines and taxanes.26,27

Like ER expression, HER2 positivity moved from being a prognostic to a predictive marker, which in turn yields a favorable prognostic value in the presence of anti-HER2 treatment.

“LOW-RISK” BY STANDARD CLINICOPATHOLOGIC MARKERS

When clinical and pathologic biomarkers are combined, breast cancers can be classified into subgroups with distinct predictive and prognostic features. The “low-risk” category resulting from combinations of these markers (which is an approximation of the breast cancer subtypes identified by gene-expression profiling, see below) comprises tumors that are ER-positive, HER2-, with a low proliferative index, and low or no involvement of the axillary lymph nodes.16,28-33 In the presence of systemic treatment, the risk of distant recurrence for patients belonging to this category is below 5% at 5 to 10 years.34 On the basis of clinical information and estimates of ER, progesterone receptor, HER2, and Ki-67 (IHC4), several prognostic scores have been developed. In the neoadjuvant setting, the preoperative endocrine prognostic index score (PEPI) was able to identify three prognostically distinct groups among a cohort of 158 patients treated with preoperative letrozole or tamoxifen for stage II or III breast cancer.35 The IHC4 prognostic index proved to be a strong predictor of survival in cohorts of women with ER-positive breast cancer treated with adjuvant endocrine therapy.36

Considering that responsiveness to chemotherapy of ER-positive, HER2- tumors with a low proliferative index is low,37 it suggests that most of these low-risk patients would derive little or no benefit from adjuvant chemotherapy. For young patients without comorbidities and, thus, a high remaining-life expectancy, however, even small absolute benefits in distant recurrence rates might be worth pursuing. On the other hand, in the presence of a high competing risk of death from other causes, even tumors with a greater risk of recurrence might mean a comparably low(er) risk of death from breast cancer.

GENE-EXPRESSION SIGNATURES

In the past two decades the development of new technologies has allowed for the broad analysis of tumor tissue on a molecular level. By using gene-expression–based assays, it has been possible to identify breast cancers with highly favorable prognostic features. Several groups have used the results of multigene expression analyses in cohorts with a known outcome (i.e., study populations) to retrospectively develop multigene tests (called “signatures”) with a prognostic value that goes beyond that gained from standard clinicopathologic markers. Although development of these signatures was initially “survival driven,” they also allowed for a more precise pathologic definition of “intrinsic” breast cancer subtypes into luminal A, luminal B, HER2-enriched, and basal-like tumors.42,43 These subtypes are mainly distinguished on the basis of proliferation and ER-regulated genes, and they therefore largely overlap, but are not identical with, the risk groups identified by standard clinicopathologic markers (Table 1).

In addition to their prognostic value, gene-expression signatures can be used to obtain predictive information regarding response to treatment. It has been shown that tumors with favorable prognostic features (luminal A) may at the same time have unfavorable predictive characteristics. For example in the neoadjuvant setting pathologic complete response rates of only 7% for luminal A compared with 36% for HER2-enriched and 43% for basal-like tumors to chemotherapy can be achieved.44

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“LOW-RISK” BY GENE-EXPRESSION PROFILING

Low-risk groups defined by gene-expression profiling consist mostly of a subset of luminal A tumors45 so that the value of the signatures is mainly in the ER-positive, HER2- cohort.46 In this group, the independent prognostic information of gene signatures beyond standard clinicopathologic markers has been established in numerous studies.39,47-52 Patients with node-negative disease whose tumors fall into a low-risk molecular category have an excellent prognosis, so that in two ongoing prospective studies (MINDACT and TAILORx) the
effect of omitting chemotherapy on survival is being evaluated (Table 2).

Survival can also be predicted by gene-expression signatures within cohorts with HER2+ or triple-negative tumors, but even the prognostically favorable subgroups within this category have a relatively high risk of relapse,\(^\text{53,54}\) confining the clinical utility of gene signatures to patients with increased competing risks of death in these cohorts.

Importantly, most gene expression signatures were developed and validated in cohorts with node-negative breast cancer, making their benefit in node-positive breast cancer questionable.\(^\text{42}\) In different cohorts of patients with ER-positive, node-positive tumors, the recurrence rates in the identified "low risk" groups were too high to consider omitting chemotherapy solely on the basis of prognosis and in the absence of competing risks for survival.\(^\text{41,49,55}\) This, however, does not preclude the possibility that intermediate-risk tumors constitute a much lower risk for mortality compared with other competing risks in certain patients.

**PERSPECTIVES RELATED TO PATIENTS’ AGE AND COMORBIDITIES**

As mentioned above, apart from tumor characteristics, many patient-specific parameters need to be considered when treating patients with breast cancer. This is particularly true in the presence of “low-risk” disease, in which the absolute benefit from any treatment is bound to be small and competing causes of death may considerably reduce the possibility of long-term benefit from adjuvant therapies.

Among the patient-related factors, the patient’s age and comorbidities act as the major competing causes of mortality. In a study of 900 women with early breast cancer, those with more than two comorbid conditions were 20-times more likely to die from non-breast cancer causes.\(^\text{60}\) Any benefit from systemic adjuvant treatment therefore needs to be weighed against these competing causes of death.\(^\text{61}\)

A diagnosis of breast cancer with intermediate-risk features might turn into a “low-risk” situation in the presence of a higher likelihood of death from other causes. On the other hand, up to 40% of patients with breast cancer older than age 80 will die from their cancer,\(^\text{62}\) and age itself is known as a risk factor for under treatment, particularly when life expectancy is underestimated.\(^\text{63}\) Even in a very low-risk situation, such as extended adjuvant endocrine therapy (in women who have already survived 5 years without recurrence) it has been shown that breast cancer accounts for 30% of the observed mortality in patients age 70 or older.\(^\text{64}\) The dilemma in the treatment of elderly women with breast cancer is aggravated by the fact that frail and older individuals are often not included or not represented adequately in clinical trials because of inclusion criteria precluding their study participation or a selection bias.

On the other hand, in young and fit patients with high life expectancy, treatment decisions need to be based on studies with extended follow-up. As mentioned, the recurrence rate of ER-positive tumors remains high during years 5 to 10 and beyond, exceeding the hazard rate of the assumed higher-risk ER-negative tumors. However, most molecular biomarkers used today have been developed and validated using outcome in the first years after breast cancer diagnosis only.\(^\text{41,48}\) When the Breast Cancer Index (BCI) signature was compared with Oncotype DX and the IHC4 in patients treated with endocrine therapy in the ATAC trial, only the BCI yielded significantly more prognostic information than the “clinical tumor score” (involved nodes, tumor grade and size, and treatment) for years 5 to 0 and beyond (p = 0.0005).\(^\text{65}\)

As yet, there are few clinical tools that consider tumor and patient characteristics for breast cancer at the same time. Adjuvant! Online (AO) is an online tool attempting to estimate the natural mortality of a patient with breast cancer as well as the baseline prognosis of the disease and treatment effects considering both patient-related factors such as age and comorbidities as well clinicopathologic tumor characteristics. On the basis of these data, the absolute benefit of systemic adjuvant therapies is estimated up to 10 years after diagnosis. Despite its many limitations AO is an important tool taking into account the patient’s remaining life expectancy as well as tumor characteristics.

In the future, the information derived from gene-expression signatures of breast tumors needs to be combined with (not merely compared to) patient-related factors to be able to correctly estimate the benefit from adjuvant treatment options. For example, in a retrospective study in 265 tamoxifen-treated patients with breast cancer, the addition of AO to BCI improved predictive accuracy of the gene signature.\(^\text{66}\)
SPEAKING OF “TREATMENT BENEFIT”

As discussed, patients with “low-risk” breast cancer can be identified when both tumor and patient characteristics are combined. However, the identification of these patients is only valuable if treatment decisions are based on both of these. Therefore other parameters, for example, expected treatment benefit and toxicity, need to be taken into consideration. One might argue that if the risk of recurrence is small enough, any possible absolute treatment benefit is so small that (certain) systemic therapies can be safely omitted. However, even in healthy patients with a long life expectancy, even a small absolute treatment benefit might be worth pursuing if toxicity is low.

The “low-risk” breast cancer subgroup identified by gene-expression analysis is characterized by high ER expression and low markers of proliferation (Ki-67), thus suggesting a low expected benefit from adjuvant chemotherapy (see above) and a high sensitivity to endocrine treatment options. Considering, in addition, the relatively constant risk of recurrence (hazard rate) in ER-positive breast cancer over a long period of time after diagnosis, this argues for using endocrine treatment over a long period of time in these patients. When looking at treatment benefit, however, one needs to keep in mind that there are numerous ways to describe such benefit, including the hazard ratio of two survival curves, the absolute benefit of treatments at certain time points, measures of time such as median survival, time-to-event end points (like overall or progression-free survival), and more “economic” outcome measures (like the number-needed-to-treat or “cost per QALY”). In current oncologic literature, the hazard ratio in combination with a measure of time, commonly median survival, is generally used; however, it is not clear whether this is the best way to discuss treatment benefit for patients with low-risk breast cancer. It was shown by the EBCTCG that the hazard ratios achieved by various chemotherapies are more or less the same across most risk subgroups, but the absolute benefit and the number-needed-to-treat (being the inverse of the absolute benefit), are not. The magnitude of absolute benefit depends on the time when it is measured (assuming constant hazard rates) and tends to increase with longer follow-up, so that it may be underestimated in studies with short follow-up. It is also known from psychological studies that outcomes that are almost certain (e.g., a 96% chance of remaining recurrence-free) are underweighted and that people are willing to pay disproportionately high prices to achieve (near-)certainties (e.g., a 100% chance of remaining recurrence-free). An absolute increase in the chance of survival from 93% to 99% might therefore mean much more to a patient considering systemic treatment than an absolute increase from 60% to 66% and if absolute benefits are measured at different time points that adds to this complexity. Ultimately, the choice of adjuvant systemic treatment might be very subjective and strongly related to the risk/benefit ratio of a particular treatment.

BENEFIT COMPARED WITH SIDE EFFECTS

In treatment situations with low expected benefit and a long follow-up, the risk/benefit ratio of any therapy becomes an essential parameter. Side effects are usually diligently recorded in clinical trials, and the willingness to accept a certain level of toxicity is higher in oncology than in other areas of medicine. In low-risk cohorts with near-normal life expectancies, however, toxicities might be the deciding factor. This has been extensively shown in the breast cancer prevention situation, in which numerous clinical trials have reported a measurable benefit for selective ER modulators and an aromatase inhibitor at little toxicity, but still only a minority of women uses these agents for prevention. It has been argued that the difficulty of weighing multiple risks compared with multiple benefits, the general risk aversion of humans, and the fact that treatment side effects are often experienced early whereas benefits come later, are responsible for this phenomenon. In addition, the risk for certain side effects may vary considerably between individuals and over time, so that general recommendations are hard to make in situations in which the threshold for unacceptable toxicity is very low.

CONCLUSION

Over the past decade, understanding of breast cancer has evolved from being considered a single tumor type to now a multitude of diseases with different prognostic and predictive features. Understanding the disease on a molecular level has pointed to the existence of tumors that harbor an exceptionally low risk of recurrence, which is undoubtedly the first step toward identifying “low-risk” breast cancer. However, there are many other patient- and treatment-related factors that need to be considered to allow reliable classification of patients into a “low-risk” group. If, by “low risk,” we mean breast cancer that will not alter a patient’s expected QALYs, then her competing risks for morbidity and mortality must be taken into consideration. In addition, when talking about treatment options in this setting, the complex issue of treatment benefits that can be measured— and framed— in different ways, needs to be discussed. Those small absolute treatment benefits must then be contrasted with expected side effects, which are a fundamental basis of treatment decisions in low-risk situations with long treatment durations. Tools that take into consideration molecular information from the tumor as well as markers of patients’ life expectancy and quality of life will ultimately be needed to provide reliable treatment recommendations for patients with breast cancer who have low-risk disease.

In ongoing research, somatic mutations in breast cancer are being analyzed further, including identification of “driver” and “passenger” mutations and development of models that address the time dependence of breast cancer recurrence.
Disclosures of Potential Conflicts of Interest


References


Breast radiotherapy after lumpectomy is considered standard for nearly all patients with invasive breast cancer and is recommended for many patients after lumpectomy for ductal carcinoma in situ (DCIS). However, there is recognition that lumpectomy alone can achieve optimal cancer control for some patients with invasive breast cancer and DCIS. Patients with breast cancers with lower risk of recurrence are less likely to derive benefit from breast radiotherapy. This review will focus on defining populations of patients with invasive breast cancer and DCIS with a low risk of recurrence post-lumpectomy and the evidence supporting omission of breast radiotherapy post-lumpectomy.

**INVASIVE BREAST CANCER**

For patients with invasive breast cancer, the primary goal of breast radiotherapy after lumpectomy is to maximize in-breast cancer control so it is equivalent to that achieved by mastectomy and minimizes risk for breast cancer mortality. Secondary but important goals are to preserve a sensate, cosmetically appealing breast and to avoid mastectomy. The benefit of breast radiotherapy after lumpectomy has been studied in numerous clinical trials that have randomized women post-lumpectomy to breast radiotherapy compared with observation. Each of these trials demonstrated a highly significant reduction in cancer recurrence (typically 70% to 75%) in the treated breast with addition of breast radiotherapy. Despite fairly large reductions in local recurrence, none of these trials individually documented a significant improvement in breast cancer survival from radiotherapy after lumpectomy. However, on meta-analyses of these trials, reduction in local regional recurrence (LRR) after lumpectomy by radiotherapy resulted in a reduction in breast cancer mortality. This was demonstrated in the Early Breast Cancer Trialists Group (EBCTCG) meta-analysis that studied the relationship of local regional recurrence at 5 years and breast cancer mortality at 15 years in 7,311 patient level data from 10 randomized clinical trials. The 5-year local recurrence was 7% among those allocated radiotherapy and 26% among those observed, corresponding to an absolute reduction of 19%. The 15-year risk of death as a result of breast cancer was 30.5% among those allocated post-lumpectomy radiotherapy and 35.9% among those observed (corresponding to an absolute breast cancer mortality reduction of 5.4%, SE 0.75–0.91, p = 0.0002). Subgroup analyses demonstrated that the absolute benefit produced by radiotherapy was determined principally by the magnitude of the local recurrence risk in unirradiated women. In particular, analyses that divided absolute local recurrence risk reduction
after lumpectomy or mastectomy by 5 years into three categories of less than 10%, 10% to 20%, or greater than 20% demonstrated that for those with less than 10% absolute reduction in local recurrence by 5 years no improvement in breast cancer mortality by 15 years is seen. A more recent meta-analysis from the EBCTCG of 10,801 women in 17 trials further explored the effect of post-lumpectomy breast radiotherapy on 10-year overall breast cancer recurrence rate (locoregional or distant) and 15-year breast cancer mortality effects. The 10-year risk of any (locoregional or distant) first recurrence was 19.3% in women allocated to radiotherapy and 35.0% in women allocated to breast-conserving surgery only, corresponding to an absolute risk reduction of 15.7% (95% CI 13.7–17.7, 2p = 0.00001). Radiotherapy also reduced breast cancer death at 15 years by a moderate amount: 25.2% without and 21.4% with radiotherapy, respectively, for 15-year absolute risk reduction of 3.8% (95% CI 1.6 – 6.0, 2p = 0.00005). Analysis of 7,287 pathologically node-negative patients by patient and tumor characteristics revealed that the absolute recurrence reduction varied strongly by patient age, tumor grade, tumor size, and ER status. Older women and those with low-grade tumors had a smaller risk of recurrence without radiotherapy than younger women and those with high-grade tumors. Each woman with pN0 disease was assigned an individually predicted absolute reduction in 10-year recurrence risk from radiotherapy that was categorized into three risk groups: lower (< 10%), intermediate (10% to 19%), and large (> 20%). The 10-year recurrence risks without radiotherapy was 50.3%, 24.8%, 18.9%, compared with 26%, 12.4%, and 12% (absolute reductions of 24.3%, 12.4%, and 6.9%) with radiotherapy for patients in the large, intermediate, and lower risk groups, respectively. The corresponding absolute reductions in 15-year risk of breast cancer death in the three groups were 7.8% (95% CI 3.1–12.5), 1.1% (−2.0 to 4.2), and 0.1% (−7.5 to 7.7), respectively. These analyses suggest that patients with breast cancer post-lumpectomy with an anticipated ≤ 10% absolute reduction in locoregional risk by 5 years and overall recurrence (locoregional and distant) by 10 years can omit breast radiotherapy without risk of excess breast cancer mortality. Furthermore, older patients with node-negative, less than 2 cm, ER positive, low grade breast cancer who have taken tamoxifen characterize this lower risk group.

Table 1 lists several randomized controlled trials designed to evaluate the benefit of breast radiotherapy in patients considered to be at low risk of recurrence post-lumpectomy. The breast cancer populations in these trials were all node-negative, typically older than age 50, had received antiecdocrine therapy for cancers less than 2 cm in size that were ER/PR +, and grade 1–2. In the NSABP B21 and PMH trials, high grade lesions were associated with higher rates of in-breast recurrence. In contrast, small, grade 1 breast cancers, such as those enrolled on the BASO II study that has had limited reporting so far, have been associated with low recurrence rates after lumpectomy alone. Two trials focused specifically on populations of older patients with breast cancer and provide additional information about the secondary goals of breast conservation: namely, cosmetic appearance and avoiding mastectomy. CALGB 9343 randomized post-lumpectomy 626 women older than age 70 with hormone receptor positive breast cancer that intended to take 5 years of tamoxifen, to breast radiation compared with observation. The most recent reporting of this trial at 10.5 years follow-up demonstrated a low rate of in-breast recurrence of 8% without compared with 2% with radiotherapy. Notably, 43% of women participating in this study had died, but only 7% as a result of breast cancer, demonstrating that in this older population comorbidity was more frequently the primary health risk than the breast cancer. There was statistically similar freedom from mastectomy after lumpectomy alone; 96% compared with 98% with the addition of breast radiotherapy. A SEER-Medicare database that identified 8,724 women older than age 70 who were treated with lumpectomy for small, lymph node-negative, hormone receptor positive breast cancer demonstrated good agreement with CALGB 9242. In this study, by 8 years of follow-up, women who received breast radiotherapy post-lumpectomy had a 5.7% absolute benefit; 8% ipsilateral breast cancer event without compared with 2.3% with treatment. Healthy women ages 70 to 79 were more likely to experience the benefit associated with radiation therapy. However, a separate study of the SEER-Medicare observational cohort showed that omission of radiotherapy resulted in higher rates of mastectomy in 7,074 women aged 70 to 79 with receptor positive, node-negative breast cancer, less than 2 cm in size. Overall, the 10-year risk of mastectomy without radiotherapy was 6.3% and 3.2% with radiotherapy (p < 0.001). In particular, women ages 70 to 74 and those with

**KEY POINTS**

- Breast radiotherapy after lumpectomy is considered standard for nearly all patients with invasive breast cancer and is recommended for many patients after lumpectomy for DCIS.

- The goals of breast radiotherapy post-lumpectomy are different for patients with invasive breast cancer than for patients with DCIS.

- For patients with invasive disease, the risk of in-breast recurrence is lowest post-lumpectomy in node-negative, small, grade 1–2, hormone sensitive breast cancer in older postmenopausal women who will take antiendocrine therapy.

- For patients with DCIS, the risk of in-breast recurrence is lower postlumpectomy in mammographically detected, small, low-grade hormone sensitive DCIS with negative margins in postmenopausal women that will take tamoxifen.

- These patients with breast cancer can still have variable risks of in-breast recurrence post-lumpectomy that underlies the need for better tests to predict an individual’s risk so optimal treatment decisions can be made.
high grade breast cancer had 10-year absolute reduction in mastectomy rates of 3.8% and 6.7%, respectively. The PRIME trial of 255 women older than age 65 with negative nodes and less than 3 cm size breast cancer treated with lumpectomy and antiendocrine therapy to evaluate quality of life in those randomized to observation compared with radiotherapy. More breast symptoms were noted at 15 months follow-up in the irradiated group but no longer significant by 5 years follow-up.

In summary, for most patients with invasive breast cancer that have undergone lumpectomy, radiotherapy is needed to maximize local control and minimize breast cancer mortality. Older postmenopausal women who have node-negative, hormone receptor positive breast cancer, less than 2 cm in size and grade 1–2 have a lower risk of subsequent breast cancer recurrence and will likely not have worse breast cancer mortality with omission of radiotherapy. For woman age 70 or older, competing health risks may influence outcomes more than intrinsic risk features of the breast cancer. Additional studies are warranted in younger postmenopausal woman to further identify patients for whom the radiation therapy might be safely omitted.

### DCIS

For DCIS, the primary goal of breast radiotherapy after lumpectomy is to maximize in-breast cancer control to prevent or minimize occurrence of the first invasive breast cancer and to avoid mastectomy. In the treatment of DCIS, breast cancer mortality has not been associated with local treatment. Five randomized control trials have demonstrated that breast radiotherapy significantly reduces cancer recurrence following complete excision for DCIS. An EBCTCG meta-analysis provides a concise overview of radiotherapy effect following lumpectomy for DCIS. A total of 3,729 women were eligible for analysis from the NSABP B17, EORTC 10953, SweDCIS, and United Kingdom ANZ trials with a median follow-up of 8.9 years. Radiotherapy more than halved the 10-year rate of ipsilateral breast events (rate ratio 0.46, standard error [SE] 0.05, 2 p < 0.00001) from 28.1% with lumpectomy alone to 12.9% with the addition of breast radiotherapy, p < 0.00001. At 5 years after randomization the absolute reduction in risk was 10.5% (SE 1.2%, 7.6% vs 18.1%), and at 10 years it was 15.2% (SE 1.6%, 12.9% vs 28.1%). Radiotherapy resulted in a larger proportional reduction in the rate of ipsilateral breast recurrence for women aged more than 50 than for younger women. The proportional reduction did not differ significantly according to any other factor. There was no significant difference in the meta-analysis for breast cancer or overall mortality between treatment arms. There were 50 out of 1,878 (2.7%) deaths as a result of breast cancer for the radiotherapy groups and 44 out of 1,851 (2.3%) for observation post-lumpectomy.

Within the EBCTCG meta-analysis a "low-risk" group was sought in which the absolute risk of ipsilateral breast events was so low that the addition of radiotherapy would provide little absolute gain. There were 291 such cases of DCIS with low-grade, less than 20 mm in size and with negative surgical margins identified. Among them, the 10-year risk of an ipsilateral event in those allocated to lumpectomy alone was substantial at 30.1%, and the addition of radiotherapy resulted in a 10-year absolute gain of 18.0% (SE 5.5%).

Recently, the Radiation Therapy Oncology Group (RTOG) reported the results from its RTOG 9804 clinical trial for “Good Risk” DCIS post-lumpectomy, randomizing patients to observation compared with breast radiotherapy; seeking to determine radiotherapy benefit after lumpectomy for DCIS patients who are expected to have a low risk of ipsilateral breast recurrence. In comparison to the other randomized trials, RTOG 9804 enrolled all mammography screen detected lesions, 72.4% were less than 10 mm in size, all were low or intermediate nuclear grade, and adjuvant tamoxifen was taken by 62%. After a median follow-up of 7.2 years there have been 19 in-breast recurrences (42% invasive: 58% DCIS) in the observation arm (7-year rate 6.7%) and two (50% invasive:

### TABLE 1. “Low Risk” Invasive Breast Cancer Post-Lumpectomy: Randomized Controlled Trials Evaluating Radiotherapy Benefit

<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>N</th>
<th>F/U yrs</th>
<th>Age &gt; 50 yrs (%)</th>
<th>ER/PR+ (%)</th>
<th>Tam/Al (%)</th>
<th>Grade 1-2 (%)</th>
<th>In-Breast Recurrence (%)</th>
<th>RT</th>
<th>No RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSABP B21</td>
<td>1009</td>
<td>8</td>
<td>80</td>
<td>56.5</td>
<td>67</td>
<td>67</td>
<td>9.3  2.8</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>PMH</td>
<td>769</td>
<td>5.6</td>
<td>100</td>
<td>80.5</td>
<td>100</td>
<td>68.3</td>
<td>0.6  7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABCSG Study 8a</td>
<td>869</td>
<td>4.48</td>
<td>99</td>
<td>100</td>
<td>95</td>
<td>-</td>
<td>0.4  5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CALGB 9343</td>
<td>626</td>
<td>10.5</td>
<td>100</td>
<td>97</td>
<td>100</td>
<td>-</td>
<td>2  8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBSG-V</td>
<td>347</td>
<td>9.9</td>
<td>91.4</td>
<td>88</td>
<td>50</td>
<td>97.2</td>
<td>6  20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BASO II</td>
<td>1172</td>
<td>4.5</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>100</td>
<td>1.3  3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRIME</td>
<td>255</td>
<td>5</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>94.5</td>
<td>0  6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NSABP, National Surgical Breast and Bowel Program; PMH, Princess Margaret Hospital; ABCSG, Austrian Breast and Colorectal Cancer Study Group; CALGB, Cancer and Leukemia Group B; GBSG, German Breast Cancer Study Group; BASO, British Association of Surgical Oncology; PRIME, Postoperative Radiotherapy in a Minimum Risk Population.

1 RT = placebo, 2 RT = tamoxifen. All were >70. All were > age 65. All were grade 1.
50% DCIS) in the radiotherapy group (7-year rate 0.9%) for a hazard ratio of 0.11 (95 CI 0.03–0.47, p = 0.0003). There have been numerous retrospective studies from single institutions reporting in-breast recurrence rates of 15% to 18% at 5–6 year median follow-up from excision alone for DCIS. Two prospective single arm trials sought to establish a combination of size of lesion, grade, and surgical margin width that might define a subset of patients at low risk for local failure without irradiation, Table 2. In the Harvard study of 158 women with predominant grade 1–2 DCIS and margin of 10 mm, 13 patients developed local recurrence as the first site of treatment failure 7 to 63 months after study entry, corresponding to a 5-year rate of 12%. In the ECOG study, ipsilateral breast recurrence of 6.1% at 5 year and 10.5% at 7 years was seen in the low-intermediate grade stratum, which the authors conclude may be acceptable to many patients and physicians.

There are numerous factors associated with risk of local recurrence for a woman with DCIS who has undergone lumpectomy. In many cases it is difficult to weight the contribution of multiple risk factors to arrive at an individual estimate for in-breast recurrence. As a result, clinical tools have been developed by using these factors to estimate risk of local recurrence for an individual and to guide treatment decision making. The Van Nuys Prognostic Index was first described in 1995 using combinations of nuclear grade and necrosis. It now incorporates four statistically significant independent prognostic factors for local tumor recurrence (tumor sizes, margin width, pathology class that includes grade and necrosis, and patient age) to which a grade of 1–3 is assigned for a score of 4–12. DCIS cases with a score of 4–6 are recommended to receive lumpectomy alone without radiotherapy based on their institutional experience in 320 patients for which a 6% in-breast recurrence risk was seen. Investigators from Memorial Sloan-Kettering have developed a nomogram based on 1,868 consecutive patients treated for DCIS with lumpectomy, approximately 50% of whom received breast radiotherapy. A combination of 10 clinical, pathologic, and treatment factors associated with in-breast recurrence were studied with multivariate analysis to devise the monogram that has been validated and is available on the institution website. Gene expression has been correlated with DCIS prognosis post-lumpectomy and represents an important area of future investigation. ECOG investigators presented a “DCIS Score” subset of the 21 Gene Ontotype DX Recurrence Score for hormone sensitive invasive cancer. The DCIS Score was able to stratify cases by risk of ipsilateral invasive recurrence on a subset of 327 cases enrolled on the ECOG 5194. Similarly, loss of retinoblastoma (RB) and phosphate and tensin homolog (PTEN) suppressor genes was strongly associated with ipsilateral invasive breast cancer recurrence in 236 patients with DCIS treated with lumpectomy alone.

In summary, breast radiotherapy following lumpectomy for DCIS reduces the risk of in-breast recurrence by more than half. There has not been a subset of DCIS reproducibly identified that does not receive some benefit in reduction of local recurrences with radiotherapy. A lower risk of in-breast recurrence is seen after lumpectomy alone for mammographically detected, small less than 10 mm, grade 1–2 without necrosis, hormone receptor positive DCIS, with at least 3 mm surgical margins. Patients should be included in the treatment decision-making to learn what magnitude of risk reduction is meaningful to them.

**CONCLUSION**

It is becoming increasingly evident that not all invasive breast cancer or DCIS needs breast radiotherapy post-lumpectomy. The challenge remains to accurately predict an individual patient’s risk of recurrence post-lumpectomy to make treatment recommendations. Future development of genomic and molecular discriminators will be an important step to accurately characterize an individual woman’s personalized prognosis for local cancer recurrence. Identification of patients unlikely to benefit from radiation therapy post-lumpectomy may also serve to reduce use of mastectomy. The indications for mastectomy should be no longer be relevant if local recurrence risk is sufficiently low to omit radiotherapy post-lumpectomy.

**TABLE 2. Prospective Studies of Lumpectomy Alone for DCIS**

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>F/U (yrs.)</th>
<th>Median Age</th>
<th>Median Size (cm)</th>
<th>Grade</th>
<th>Margin Width</th>
<th>In-Breast Recurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard</td>
<td>158</td>
<td>3.6</td>
<td>51</td>
<td>0.9</td>
<td>Low-intermediate grade</td>
<td>10 mm</td>
<td>12 -</td>
</tr>
<tr>
<td>ECOG 5194</td>
<td>565</td>
<td>6.2</td>
<td>60</td>
<td>0.6</td>
<td>Low-intermediate grade</td>
<td>3 mm</td>
<td>6.1 10.5</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>6.7</td>
<td>59</td>
<td>0.5</td>
<td>High grade</td>
<td></td>
<td>15.3 18</td>
</tr>
</tbody>
</table>

Abbreviation: ECOG, Eastern Cooperative Oncology Group.


BREAST CANCER

Obesity and Inflammation: The Dangerous Duo in Breast Cancer

CHAIR
Jennifer Ligibel, MD
Dana-Farber Cancer Institute
Boston, MA

SPEAKERS
Howard Strickler, MD, MPH
Albert Einstein College of Medicine
Bronx, NY

Andrew Dannenberg, MD
Weill Cornell Medical College
New York, NY
Obesity and Inflammation: New Insights into Breast Cancer Development and Progression

Neil M. Iyengar, MD, Clifford A. Hudis, MD, and Andrew J. Dannenberg, MD

OVERVIEW

The importance of inflammation in promoting carcinogenesis and tumor progression is well recognized. Chronic inflammation caused by a variety of infectious agents can lead to the development of several common malignancies. Similarly, inflammatory bowel disease is a well-known risk factor for colorectal cancer. Much less is known about the link between inflammation and the development of breast cancer. Recent data suggest that obesity causes both in-breast and systemic inflammation that contribute to the development and progression of breast cancer. This observation has potentially important implications in terms of prevention and treatment of breast cancer, especially given the rising worldwide overweight and obesity rates. Inflamed white adipose tissue (WAT) within the breast is associated with elevated levels of proinflammatory mediators, enhanced expression of aromatase (the rate-limiting enzyme for estrogen biosynthesis), and increased estrogen receptor-α (ER-α)-dependent gene expression. Systemic consequences of obesity including altered adipokine levels, elevated circulating estrogen levels, and insulin resistance are also believed to play a role in the pathogenesis of breast cancer. Collectively, these findings suggest a significant role for inflammation in the pathogenesis of breast cancer in obese and overweight patients.

Among its wide range of clinical consequences, obesity is a well-known risk factor for the development of several common epithelial malignancies. In postmenopausal women, the risk of developing breast cancers that express the estrogen and progesterone receptors (ER and PR), is significantly elevated for those who are obese or overweight. Furthermore, obese and overweight patients, once diagnosed, suffer from worse disease-related outcomes than their leaner counterparts, regardless of breast cancer subtype. After menopause, estrogen is mostly derived peripherally from the noncyclical conversion of androgen precursors within adipose tissue. The rate-limiting step in this conversion is catalyzed by the cytochrome P450 enzyme, aromatase, which is encoded by the CYP19 gene. Circulating estrogens, such as estradiol, are known to stimulate the proliferation of breast epithelial cells and potentially exert a mutagenic effect. Higher levels of circulating estradiol as a result of increased adiposity and aromatase expression are thought to contribute, in part, to the greater risk of ER/PR-positive breast cancer in obese postmenopausal women. However, compared to the premenopausal state, circulating estradiol levels are significantly lower as the ovaries no longer produce substantial amounts of estrogen. Nevertheless, the incidence of ER-positive disease rises with age, approaching nearly 85% of breast cancers diagnosed in women during their ninth decade of life. This seemingly paradoxical association between the increasing incidence of hormone-sensitive tumors in aging women whose circulating estradiol levels have declined after menopause highlights an important scientific question: Why are hormone-sensitive breast cancers more prevalent after circulating levels of tumor-stimulating estrogens have significantly and naturally declined? One possibility is that the microenvironment in which the neoplasm arises and grows plays an important role and that it compensates for the decrease in circulating estrogens. Indeed, locally produced estrogens and, possibly, ligand-independent activation of ER-α have been associated with carcinogenesis. Local effects may also be important for understanding the link between obesity and increased risk of hormone receptor-positive breast cancer in obese postmenopausal women.

Additional evidence suggests that there are likely to be several estrogen-independent mechanisms involved in the link between obesity and breast carcinogenesis (Fig. 1). In addition to the elevated risk of hormonally sensitive breast tumors, obesity has also been associated with an increased risk of ER-negative breast cancers in some studies. This observation, in particular, points to the involvement of estrogen-independent pathways (although these may also be operative in hormone receptor-positive cancers). Obesity is associated with chronic, systemic inflammation characterized by elevated levels of circulating proinflammatory mediators known to promote tumorigenesis and growth.
systemic and local consequences of chronic adipose inflammation thus provide key potential mechanistic links between obesity and breast cancer. In addition to inflammation, other obesity-related effects promote cell proliferation and survival. These include hyperinsulinemia and increased insulin-like growth factor-1 (IGF-1) signaling, as well as altered levels of the adipokines, adiponectin, and leptin. This review addresses the complex biologic interactions between obesity and breast cancer, with a particular focus on inflammation as a key mediator.

CONNECTING OBESITY AND BREAST CANCER VIA INFLAMMATION

Tumor Microenvironment

The links between inflammation and cancer have long been described. In the mid-nineteenth century, Virchow first hypothesized that tissue injury and the ensuing inflammation promote enhanced cellular proliferation, thereby predisposing cells to neoplastic transformation.9 Subsequent observations that many cancers arise at sites of chronic inflammation, coupled with epidemiologic data demonstrating increased cancer rates in patients with underlying inflammatory conditions, have led to significant efforts to better understand the complex role of inflammation. Adding urgency to this ongoing investigation are reports linking inflammation and the inflammatory response to 15% to 20% of all cancer-related deaths worldwide.9 A critical observation is that the tumor microenvironment closely resembles that of a healing wound, including the influx of immune cells with resultant production of proinflammatory mediators as well as tissue remodeling and angiogenesis.10 Furthermore, malignant cells may co-opt the inflammatory mechanism, leading to increased growth, invasion, and metastasis.11 Together, the epidemiologic and histologic observations strongly suggest that inflammation plays a key role in tumor biology, and is not simply a result of the development of cancer.

Although many cancers including colon, lung, bladder, esophagus, liver, and others, are known to have infectious and/or inflammation-related etiologies, this link has been far less clear for breast cancer. Recently, inflammation, characterized by the hallmarks of wound healing, has been demonstrated to occur in breast tumors.12 Emerging evidence points to a causal relationship between obesity, chronic in-breast inflammation, and tumorigenesis. Increased body mass index has been associated with adipocyte hypertrophy in the breast.13 Moreover, a positive correlation has been observed between increased adipocyte size and cell death in the human breast. Adipocytes can release a variety of proinflammatory cytokines and adipokines, which are likely to contribute to the recruitment and activation of immune cells including macrophages.14 Dying adipocytes also release free fatty acids (FFA), which can stimulate the production of proinflammatory cytokines via toll-like receptor-4 (TLR-4) and NFκB signaling.

Adipocyte Interactions

A growing understanding of the complex interactions between adipocytes and immune cells within the WAT stromal vascular fraction is shedding new light on the role of adipose inflammation in tumor development and progression. The activated macrophage is a key mediator of adipose inflammation, and the adipocyte-macrophage interaction is emerging as a central theme. Adipocyte death leads to myeloid cell recruitment in a characteristic pattern whereby macrophages form a crown surrounding the dead adipocyte. This formation is histologically apparent as crown-like structures (CLS), which have been observed in subcutaneous and visceral fat in association with the metabolic syndrome.15 More recently, these inflammatory lesions were discovered to occur in the mammary gland of obese mice and the WAT of the human breast, termed CLS-B.13,16 Notably, CLS-B are detected more efficiently using immunohistochemical staining for CD68, a macrophage marker, rather than with standard hematoxylin and eosin (H&E) staining (Fig. 2).13 For this reason as well as the usual focus on tumor, rather than benign tissue, CLS-B and their associated biology may not have been previously appreciated. As in the preclinical mouse models, the presence of CLS-B in women was associated with activation of NFκB and increased levels of TNFα, IL-1β, IL-6, and COX-2-derived PGE2. Relating to the epidemiologic association of obesity and postmenopausal hormone receptor-positive breast cancer, a critical consequence of CLS-B and the associated elevation in tissue levels of proinflammatory cytokines is increased transcription of the CYP19 gene encoding aromatase.19 Indeed, several of the proinflammatory mediators associated with CLS-B, including TNFα, IL-1β, IL-6, and PGE2, are known to upregulate aromatase expression via specific promoters that give rise to unique mRNA species found in breast tissue.19-21 Increased levels and activity of aromatase lead to enhanced estrogen biosynthesis and upregulation of PR, an ER target gene. Additionally, increased circulating

**KEY POINTS**

- In obese women, low-grade or “smoldering” inflammation occurs in multiple white adipose tissue depots including the breast.
- Overweight and obesity are characterized by adipocyte hypertrophy and adipocyte death in association with recruitment of immune cells into white adipose tissue.
- Infiltrating macrophages surround dead adipocytes in breast white adipose tissue to form inflammatory crown-like structures (CLS-B) that are associated with NFκB activation and increased levels of several proinflammatory mediators that are known inducers of aromatase and thereby estrogen biosynthesis.
- Breast inflammation, quantified by the CLS-B index, correlates with increased aromatase activity, thus establishing an obesity — inflammation — aromatase signaling axis.
- Excess adiposity is associated with a state of metabolic dysregulation and systemic inflammation, which have been linked to breast carcinogenesis.
levels of proinflammatory mediators in obese patients with breast cancer correlate with poor prognosis. For example, elevated levels of IL-6 in serum have been associated with worse survival in patients with metastatic hormone-refractory breast cancer.

Given the significant biologic consequences of these inflammatory pathways and the need to further explore the histologic structures associated with their activation, a CLS-B index was developed to quantify the severity of breast WAT inflammation. This index is defined as the number of slides with histologic evidence of CLS-B compared to the number of slides examined, reported on a scale ranging from 0 to 1.0. Consistent with the hypothesis that the obesity→inflammation→aromatase axis is operative, the severity of breast inflammation as defined by the CLS-B index correlated with aromatase activity. Furthermore, the presence of CLS-B was not merely a surrogate for increasing body mass index. In support of this point, CLS-B was found in approximately 75% of obese women. Inflammatory foci can also be found in a minority of lean women. These findings are consistent with the observation that not all obese individuals suffer from the metabolic syndrome and that other factors may also induce localized inflammation in WAT. Overall, the discovery of CLS-B implicates inflammation specifically, rather than obesity alone, as a key driver of aromatase activity in the breast. Taken together, these findings demonstrate an obesity→inflammation→aromatase signaling axis, characterized by a complex system of paracrine interactions between macrophages and other cells, for example, preadipocytes, in human breast WAT (Fig. 3). This axis places inflammation at the center of ER-positive breast cancer pathogenesis for many patients and provides an important mechanistic and targetable link between obesity and breast cancer.

**FIG 1. Pathways linking obesity and breast cancer.** Reprinted with permission from Sinicrope and Dannenberg. © 2010 American Society of Clinical Oncology. All rights reserved.

Metabolic Dysfunction and Inflammation

In addition to activation of estrogen signaling pathways via inflammation-mediated upregulation of aromatase, obesity promotes breast cancer through the systemic effects of dysregulated metabolism. A primary function of adipose tissue is to store energy in the form of triglycerides within the WAT adipocytes. Hyperadiposity is characterized by a state of energy imbalance, which plays an important role in immune activation. In addition to cell wall stretch and subsequent release of proinflammatory cytokines, adipocyte hypertrophy is associated with increased lipolysis, resulting in the release of FFA. FFA are believed to stimulate inflammatory pathways in adipose tissue, but can also be deposited in various organs contributing to atherosclerosis, hypertension, insulin resistance, and glucose intolerance. Indeed, excess adiposity is associated with higher circulating triglyceride levels, reflecting the state of energy imbalance that occurs in many obese/overweight patients. As noted, FFA stimulate multiple inflammatory signaling pathways, ultimately leading to activation of the transcription factor, NFκB, which plays a key role in the immune response and adipose inflammation.22 Activation of NFκB can be mediated by TLR-4, a member of the TLR family of pattern recognition molecules that are centrally involved in the immune response.23 Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, is a prototypic TLR-4 agonist and stimulates the innate immune response. In a similar manner, TLR-4 is thought to orchestrate inflammation in response to excess endogenous lipids.23,24 FFA signaling through TLR-4 can lead to macrophage activation, resulting in elevated levels of TNFα, IL-6, and other proinflammatory mediators as detailed above.24 These cytokines stimulate further lipolysis and release of FFA, thus establishing a reinforced feedback cycle of sustained inflammation.

CONNECTING OBESITY AND BREAST CANCER VIA ENDOCRINE DYSFUNCTION

Endocrine-mediated consequences of obesity also contribute to breast carcinogenesis and tumor progression. These include elevated levels of insulin and bioavailable insulin-like growth factor-1 (IGF-1), partly related to alterations in levels of the adipokines, adiponectin, and leptin (Fig. 1). Both of these adipokines have angiogenic properties. Elevated leptin levels, as occur in obesity, stimulate breast tumor cell proliferation through several signal transduction pathways and by altering cell-cycle checkpoints via upregulation of cyclin D1 and cdk2.25,26 In contrast to leptin, adiponectin levels are diminished in obesity. Although the underlying mechanisms are not completely understood, decreased adiponectin levels are associated with hyperinsulinemia and increased breast cancer risk. These observations may be partly explained by the effects of adiponectin on the PTEN/Pi3K/mTOR, MAPK, and AMPK pathways, leading to enhanced cell proliferation and inhibition of apoptosis.1,25

Insulin resistance, characterized by elevated plasma insulin levels, occurs in association with obesity-related inflammation and is likely to play an important role in breast cancer progression.27 Both insulin and IGF-1 promote cell proliferation and inhibition of apoptosis by stimulating the PI3K/Akt and Ras/Raf/MAPK systems.28 Additionally, IGF-1 and insulin, to a lesser degree, interact with estrogen signaling pathways to promote hormone-sensitive breast cancers. IGF-1 has been shown to stimulate aromatase activity.29 Insulin, on the other hand, acts systemically by inhibiting hepatic synthesis of sex-hormone-binding globulin (SHBG), which binds and transports the hormones testosterone, dihydrotestosterone, and estradiol, in a biologically inactive state.1 Increased free estradiol, as a result of diminished SHBG levels, is then available to stimulate growth of ER-expressing breast tumors. Together, these findings suggest that obesity-associated insulin resistance has multiple local variants.
and systemic consequences that help to explain the link between obesity and breast cancer.

CONCLUSION
Rates of obesity and overweight are predicted to climb in the coming years and as such, we are likely to see a proportional increase in neoplastic disease and obesity-related mortality. Understanding the biologic underpinnings of this relationship is critical to the development of preventative and adjunctive therapies that could disrupt the obesity-cancer link even if obesity itself remains. To this end, it is becoming increasingly apparent that activation of multiple inflammatory signaling pathways provides a critical link between obesity and cancer. Recent observations reporting the interactions between adipocytes and immune cells leading to a proinflammatory local milieu are providing new insights into the wound-like tumor microenvironment described by Virchow more than a century ago. Specifically, the discovery of an obesity→inflammation→aromatase axis highlights inflammation as a central mediator of breast carcinogenesis for many patients. Identifying patients in whom this axis is active could uncover a specific population that may benefit from anti-inflammatory interventions. The potential role of behavioral interventions, nutritional changes, and pharmacologic strategies to attenuate obesity-related inflammation need to be explored. Because WAT inflammation is found in most but not all overweight and obese women, the development of noninvasive methods to detect chronic in-breast inflammation to identify the at-risk population is a key research objective.

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Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member (I), and/or their institutions (Inst). For information on the pilot program, or to provide feedback, please visit coipilot.asco.org.


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A positive association between obesity and the risk of incident postmenopausal breast cancer has been consistently observed in epidemiologic studies. Although most studies of premenopausal women have not found a similar relationship between breast cancer and obesity, the prognosis for both pre- and postmenopausal breast cancer is substantially worse among obese than normal-weight individuals. Increasing evidence suggests that these associations may be mechanistically related to sex hormones, insulin, and certain adipokines. Insulin, for example, has important mitogenic/antiapoptotic activity in addition to its metabolic effects, and many breast tumors express high levels of the insulin receptor (IR)-A isoform. Further, the use of metformin, a diabetes medication that reduces insulin levels, has been epidemiologically associated with reduced breast cancer risk among patients with diabetes, and a recent observational study found a higher rate of pathologic complete responses among patients with diabetes and breast cancer who were using metformin. Formal clinical trials of metformin as adjuvant breast cancer therapy have been initiated and are ongoing. Similarly, the effect of lifestyle changes on breast cancer outcomes is actively being investigated. Several lifestyle intervention studies have demonstrated that weight loss, increased physical activity, and dietary changes are feasible in breast cancer populations, and that individuals who make lifestyle changes after breast cancer diagnosis experience several physical and psychologic benefits. In this article, the authors review the evidence linking obesity with breast cancer risk and outcomes and provide an overview of lifestyle intervention studies in patients with breast cancer.

The prevalence of obesity among U.S. women has more than doubled since the late 1970s, with 34% of women above 20 years of age currently estimated to be obese, and an additional 27% considered overweight (Fig. 1). Although the prevalence of obese and overweight women has, in recent years, stabilized at this high level, certain risk groups have especially high prevalence of excess adiposity, including women of black or Hispanic race/ethnicity, and those of lower social economic status. Obesity is a well-established risk factor for several common conditions including diabetes, cardiovascular disease, stroke, renal disease, nonalcoholic fatty liver disease/hepatic fibrosis, and disability. It is also significantly associated with overall mortality. More recently, obesity has been associated with cancer risk and mortality. Although data suggesting this association goes back to the 1990s and earlier, it is only in the past few years that the evidence has reached a point that the obesity-cancer relationship can be said to be well-established and broadly accepted. This change in perspective reflects not only the growing number of large, well-conducted prospective investigations regarding obesity and cancer, but also the growing number of laboratory and molecular epidemiologic studies that have helped demonstrate biologic plausibility. Table 1 shows a list of the cancers reported to have a significant positive association with obesity in women. In addition to breast cancer, these tumors include endometrial, gallbladder, esophageal adenocarcinoma, renal, leukemia, thyroid, pancreas, multiple myeloma, colon, non-Hodgkin lymphoma, rectal, and possibly ovarian cancers.

The review that follows provides an overview of the evidence relating obesity with breast cancer by subtype, including the associations of obesity with cancer incidence, recurrence, and survival. The laboratory and clinical/epidemiologic data that implicate specific signaling pathways in the obesity–breast cancer relationship are also discussed, and the authors review both published and ongoing interventional studies being conducted to target these pathways. Although there are currently no randomized trials testing the effect of purposeful weight loss after diagnosis on breast cancer prognosis, dozens of smaller-scale lifestyle intervention studies have been conducted in patients with breast cancer. This review provides an overview of the growing literature on lifestyle intervention studies in breast cancer.
imported example, a prospective study in the Women’s Health Initiative found that excluding the 53% of women who were using hormone therapy (HT) increased the association of obesity and postmenopausal breast cancer from a hazard ratio [HR] of 1.13 (95% confidence interval [CI]: 0.83–1.55) to a HR of 1.91 (95% CI: 1.11–3.27). Similar results were reported in the Nurses’ Health Study 7 and in other cohorts. The effect of HT use likely reflects the strong first-pass effect of oral estrogens on the liver, which alters hepatic protein production and processing, including proteins related to glucose metabolism such as insulin and other hormones; a frequent consideration in interpreting the obesity and breast cancer literature.

In contrast, studies of obesity and premenopausal breast cancer have had conflicting findings. Most studies have reported either an inverse association 4 or no association between obesity and cancer risk among menstruating women, although a few studies found a positive association. Hypotheses to explain the possible protective effect of obesity on premenopausal breast cancer risk have largely focused on the relationship of obesity with irregular or long menstrual cycles and anovulatory infertility, as having fewer ovulations is thought to correlate with reduced estrogen exposure.

**Estrogen Receptor (ER) and Progesterone Receptor (PR) Expression**

Multiple studies of postmenopausal women have shown strong associations between obesity and the risk of ER+/PR+ breast cancer but not other breast cancer subtypes. For example, a meta-analysis of nine cohorts and 22 case-control studies found that each five unit change in BMI was associated with a 33% increased risk of ER+/PR+ postmenopausal breast cancer and a 10% decreased risk in ER+/PR+ premenopausal breast cancer, whereas no associations were observed for ER-/PR- or ER+/PR-. Although this study could not address use of HT in postmenopausal women, a large case-control study found a similar positive association of obesity with all ER+/PR+ postmenopausal breast cancer (OR = 1.3; 95% CI: 1.0–1.7) that was

**KEY POINTS**

- Obesity is associated with the risk of incident breast cancer in postmenopausal but not menstruating women and with risk of cancer recurrence and mortality in patients with early-stage breast cancer, regardless of menopausal status.
- Sex hormones, insulin, and certain adipokines may have a mechanistic role in the development of ER+ postmenopausal breast cancer.
- Metformin, a diabetes medicine that reduces insulin levels, is being studied as an adjuvant breast cancer therapy in clinical trials and has been shown in observational studies to be associated with reduced breast cancer risk and better prognosis among patients with diabetes.
- There are no data from randomized trials testing the effect of purposeful weight loss on prognosis in overweight or obese women with early breast cancer.
- Dozens of interventional studies have shown that weight loss, increased physical activity, and improvements in dietary quality are feasible in breast cancer survivors, and that individuals who make these changes feel better, improve cardiorespiratory fitness, and experience favorable changes in biomarkers linked to breast cancer risk and outcomes.

**TABLE 1. Cancers Associated with Obesity According to Meta-analysis Studies**

<table>
<thead>
<tr>
<th>Cancer Type</th>
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<tbody>
<tr>
<td>Breast</td>
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<tr>
<td>Colon</td>
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<tr>
<td>Endometrial</td>
</tr>
<tr>
<td>Esophageal adenocarcinoma</td>
</tr>
<tr>
<td>Gallbladder</td>
</tr>
<tr>
<td>Leukemia</td>
</tr>
<tr>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
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<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Renal</td>
</tr>
<tr>
<td>Thyroid</td>
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</tbody>
</table>

*Adapted from Renehan et al, 2008*
nearly two-fold higher for obesity when HT users were excluded (OR = 2.3; 95% CI:1.3–3.8). A meta-analysis that specifically addressed “triple negative tumors” (i.e., ER-/PR-/HER2-) found positive associations with obesity but only among premenopausal women, although that study could not account for HT use in postmenopausal women.

POSSIBLE MECHANISMS UNDERLYING THE OBESITY–BREAST CANCER ASSOCIATION

Most hypotheses regarding the biologic pathways that may underlie the relation of obesity with breast cancer focus on three areas: sex hormones, including both estrogens and androgens; the insulin/insulin-like growth factor (IGF)-axis; and adipokines, including both metabolic and inflammatory factors.

Sex Hormones

High endogenous serum estrogen levels have been consistently associated with breast cancer risk in postmenopausal women and are, on average, higher in overweight and obese women than in normal-weight women, likely due the activity of aromatase in adipose tissue. Estrogens are reported to be carcinogenic through at least two pathways, namely, the mitogenic/anti-apoptotic activity of estrogens in the breast as well as other tissues, and the possibly mutagenic effects of estrogen metabolites. Postmenopausal breast cancer risk has additionally been associated with endogenous androgens, including testosterone, DHEAS, and androstenedion, which may reflect the conversion of androgens to estrogens by aromatase. Serum androgen levels are, like estrogen levels, correlated with adiposity. Recent prospective data suggest that the associations with estrogens and androgens may be specific, or at least strongest, for ER+/PR+ tumors.

The Insulin/IGF-Axis

Obese women have high rates of impaired fasting glucose and diabetes, often accompanied by high circulating levels of insulin. In addition to insulin’s well-known metabolic activity, it is also an important growth factor for a wide range of tissues and plays a substantial role in normal organogenesis. Indeed, insulin shares 40% amino acid sequence homology with insulin-like growth factor (IGF)-1, the primary mediator of the effects of growth hormone, and also shares mitogenic/antiapoptotic activity and downstream signaling pathways with IGF-I.

As compared with insulin levels, however, levels of IGF-I in individuals with impaired fasting glucose and early diabetes are variable. Thus, it is insulin, and not IGF-I, that is increasingly thought to play a major role in the obesity-diabetes-cancer relationship. In animal models, high insulin levels have an independent mitogenic effect, and the insulin receptor (IR) is highly expressed in cancers of the breast. More specifically, tumors over-express the IR-A isoform, which is important in fetal development but does not have a primary role in metabolism; this observation may help explain increased cancer risk related to obesity, despite the presence of metabolic insulin resistance.

Diabetes has been prospectively associated with postmenopausal breast cancer risk in many but not all studies. A meta-analysis found an overall relative risk (RR) of 1.20 (95% CI: 1.11–1.30) for the association of diabetes and breast cancer. This modest association could reflect the complicated relationship between diabetes and levels of insulin, as patients may use antidiabetic medications, and insulin levels may fall in advanced diabetes. Although few large prospective studies of insulin using fasting blood specimens have been reported, two of the three studies that addressed HT use found a strong relation of insulin levels with breast cancer among non-HT users. The largest of these studies measured fasting insulin, estradiol and IGF-I levels, and found that the highest compared with lowest insulin quartile was associated with a HR of 2.40 (95% CI: 1.30–4.41) in nondiabetic/non-HT users. In other recent studies, it was shown that patients with diabetes who used metformin, a medication that inhibits hepatic glucose production and reduces circulating insulin levels, had lower risk of postmenopausal breast cancer risk than patients with diabetes who did not use metformin.

Adipokines

Leptin and adiponectin are the adipokines most extensively studied in relation to cancer. Obese individuals tend to have high levels of leptin, which has mitogenic/antiapoptotic activity, and low levels of adiponectin, which has antimitogenic/pro-apoptotic activity. However there are only limited, conflicting epidemiologic data regarding the relation of adiponectin levels and breast cancer risk. For example, a cross-sectional study by Gaudet and colleagues (2010) failed to find associations between circulating adiponectin or other adipokines with postmenopausal breast cancer, whereas another cross-sectional study reported that the leptin/adiponectin ratio was increased in postmenopausal breast cancer cases relative controls. Overall, there is a paucity of relevant prospective cohort data regarding adipokines and breast cancer, and future studies will need to additionally control for both insulin and sex hormone levels.

RECURRANCE AND SURVIVAL

Obesity has been repeatedly shown to be a risk factor for breast cancer recurrence and poor survival. A meta-analysis of 43 studies, for example, found a HR of 1.33 (95% CI: 1.19 – 1.50) for breast cancer-related mortality and a similar HR for all-cause mortality, when contrasting obese and non-obese patients with breast cancer. These association did not differ by menopausal status, nor have results been found to differ by the ER status of tumors. Further, a recent study that accounted for use of screening, access to
treatment, the type of treatment, use of adjuvant therapy, and tumor characteristics found a more than two-fold increased risk of recurrence (HR = 2.43; 95% CI: 1.34 – 4.41) and breast cancer-related death (HR = 2.41; 95% CI: 1.00 – 5.81) among obese patients compared with normal weight patients with breast cancer.24

Similarly, several studies found positive associations between high pretreatment insulin levels and poor prognosis.25,26 For example, in a recent study, those with high insulin levels had a two-fold worse distant disease-free survival (HR = 2.05; 95% CI: 1.16 – 3.62) and overall survival (HR = 2.57; 95% CI: 1.48 – 5.50) than other patients with breast cancer.25 Other studies have suggested that high leptin levels25 and low adiponectin levels were associated with poor prognosis.26 However, studies that concurrently address sex hormone, insulin, leptin, and adiponectin levels, before and after treatment, will be needed to comprehensively study these relationships with disease recurrence and survival.

**METFORMIN AND OTHER PHARMACOLOGIC INTERVENTIONS**

Metformin is an oral medication for treatment of type II diabetes that acts by suppressing AMPK-mediated gluconeogenesis in the liver, and results in lower serum insulin levels. It is well tolerated and does not induce hypoglycemia in nondiabetics. Metformin may reduce the risk of breast cancer and its recurrence through several mechanisms. Most importantly, in addition to its effects on insulin levels, metformin may inhibit AMP-activated protein kinase activity in the mTOR cancer-related pathway.27

Meta-analyses have shown that metformin use in patients with diabetes is associated with reduced risk of postmenopausal breast cancer OR = 0.82; 95% CI: 0.71 – 0.97),19 and reduced breast cancer mortality (summary relative risk = 0.63; 95% CI: 0.40 – 0.99).28 This included improved overall survival in patients with diabetes with HER2+ tumors, and possibly distant metastasis free survival in patients with diabetes with triple-negative breast cancer.28 There are currently several clinical trials of metformin use as adjuvant therapy in the treatment of patients with breast cancer.27,29 In particular, there is a large phase III randomized clinical trial of 3,582 women with stage I-III breast cancer (NCIC MA.32)27, which is the only study with adequate sample size to assess the effect of metformin on breast cancer recurrence and mortality. Other pharmacologic regulators of AMPK are also being developed, as are methods to increase adiponectin levels and agents that directly target the IR-A and IR/IGF-IR hybrid receptors.

However, considerable effort is additionally being put toward the development of nonpharmacologic interventions. Given that obesity is a modifiable condition, behavioral interventions designed to change dietary and exercise practices in patients represent a major opportunity to reduce breast cancer risk and improve disease outcomes.

**BEHAVIORAL INTERVENTIONS TARGETING OBESITY**

**Weight Loss Interventions in Breast Cancer Survivors**

Until recently, there were relatively few studies evaluating the efficacy and potential benefits of weight loss interventions in patients with breast cancer. Many of the early studies of lifestyle change in breast cancer survivors focused on elements of weight maintenance, such as physical activity or dietary quality (described below), and did not include weight loss as an objective. However, as the evidence linking obesity to breast cancer recurrence and mortality has grown, along with the proportion of U.S. adults who are overweight and obese, the need to develop and test weight loss interventions in breast cancer populations has become more pressing.

Several small studies have evaluated different weight loss strategies in breast cancer survivors.30-34 Studies generally demonstrate that weight loss can be successfully implemented in this population through a variety of interventions. For example, Shaw and colleagues randomized 64 obese women with early stage breast cancer to a low-fat diet group, a low-calorie diet group, or a control group, and demonstrated that participants randomized to low-fat and low-calorie diets experienced significant weight loss and reductions in body fat as compared with controls (p = 0.006 and 0.008, respectively).33 Another small study randomized 48 obese breast cancer survivors to one of three dietary intervention arms (Weight Watchers, individualized dietary counseling, or a combination of the two) or to a control group31 and found that the individual counseling and combination groups lost significantly more weight than controls (p < 0.05 at 12 months for comparison of each group to control), whereas the Weight Watchers alone group did not experience significant weight loss.

Only one large-scale weight loss study has been reported to date in breast cancer survivors. The Lifestyle Intervention Study for Adjuvant Treatment of Early Breast Cancer (LISA)35 randomized 338 postmenopausal women with hormone receptor-positive breast cancer to an educational control group or to a 2-year, telephone-based weight loss intervention, modeled on the Diabetes Prevention Program. The weight loss intervention focused on calorie reduction to attain a 500-1000 kcal per day deficit; reduction in fat intake to approximately 20% of calories; increased intake of fruits, vegetables, and grains; and increased physical activity to at least 150 minutes of moderate-intensity recreational activity per week. Intervention participants lost significantly more weight than control participants at 6, 12, 18, and 24 months (4.3 vs 0.6 kg, p < 0.001 at 6 months and 3.6 vs 0.3 kg, p < 0.001 at 24) and reported a significant improvement in physical functioning scores and higher levels of physical activity as compared with controls.35

Several other weight loss intervention studies are currently ongoing in breast cancer survivors (Table 2), although it is not clear that any of these will be adequately powered to test the effect of purposeful weight loss on the risk of breast cancer recurrence or mortality. These studies will compare the effects of different dietary interventions on weight change.
and biomarkers linked to breast cancer prognosis and will also evaluate the feasibility of in-person and distance-based weight loss interventions, thus providing information that will be essential in the design of future studies evaluating the impact of purposeful weight loss on disease outcomes in overweight and obese women with early breast cancer.

**Other Lifestyle Interventions in Breast Cancer Survivors**

Many studies have looked at the feasibility and potential benefits of modifying two key components of weight control (dietary quality and physical activity patterns) after breast cancer diagnosis. The two largest lifestyle intervention studies in breast cancer survivors to date have been the Women's Interventions Nutrition Study (WINS) and the Women's Healthy Eating and Living (WHEL) study, which both looked at the impact of dietary interventions on disease recurrence in women with early-stage breast cancer. The WHEL study randomized 3,088 breast cancer survivors to a dietary intervention focused on increasing fruits, vegetables, and fiber and lowering fat intake, or to a usual care control group. Intervention participants substantially increased intake of fruits and vegetables and decreased percentage of dietary calories from fat. The diet was designed to be isocaloric with the baseline diet of study participants, and no weight loss was observed. There was no difference in recurrence rates in the diet and control groups (16.7 vs. 16.9%, p = 0.63). In contrast, the WINS randomized 2,437 women with early-stage breast cancer to a low-fat dietary intervention or to a usual care control group. Intervention participants substantially decreased dietary fat intake, lost approximately 6 pounds, and experienced significantly better disease free survival compared with controls (HR 0.76, 95% CI 0.60–0.98, p = 0.034). With further follow-up, these findings lost statistical significance, but an exploratory subgroup analysis demonstrated a significant survival benefit of the intervention in

**TABLE 2. Select Ongoing Weight Loss Intervention Studies in Patients with Breast Cancer**

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Population</th>
<th>Study Design/Intervention(s)</th>
<th>Aims</th>
</tr>
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</table>
| CHOICE50   | 370        | - Postmenopausal breast cancer survivors | Participants are allowed to choose 1 of 3 arms:  
- BMI 25-34.9kg/m²  
- Control  
- Low-fat diet + exercise  
- Low carb diet + exercise  
- Secondary: Changes in other metabolic and inflammatory biomarkers linked to recurrence |
| DIANA-551  | 1208-2000  | - Stage I-III breast cancer within the past 5 yr | 1:1 randomization:  
- Age 35-70  
- Usual care control group  
- One of the following:  
  - “Mediterranean-Macrobiotic” lifestyle  
  - Biomarkers  
  - ER+ tumor  
  - Weight loss or maintenance  
  - Anthropometric measures  
- Metabolic syndrome  
- Physical activity: 210 min/wk  
- High serum insulin or testosterone  
- Modest calorie restriction  
- Reduction in intake of foods with high glycemic index |
| ENERGY     | 500        | - Stage I-III breast cancer  
- BMI 25-45kg/m² | 1:1 randomization:  
- Usual care control group  
- Supervised weight loss intervention  
- Quality of life  
- Calorie restriction  
- Serum biomarkers  
- Increased physical activity  
- Behavioral strategies & social support  
- Primary: Breast cancer recurrence/new primary |
| SUCCESS-C52| 1200*      | - Newly diagnosed breast cancer:  
- Node + OR | Lifestyle randomization:  
- Usual care control group  
- High risk node + ER, age ≤35, grade 3 tumor, T2-3  
- Telephone-based lifestyle intervention  
- BMI 24-40 kg/m²  
- Calorie restriction  
- Low fat diet  
- Increased physical activity to 150-200 min/wk  
- Primary: Comparison of disease free survival between groups |

*Weight loss portion of the SUCCESS-C trial will include the subset of participants with BMI 24-40 kg/m² in the parent adjuvant chemotherapy trial.
Physical activity interventions. A few recent studies have looked at the impact of increased physical activity and dietary change on quality of life and other outcomes in breast cancer survivors. The Reach Out to Enhance Wellness (RENEW) study randomized 641 survivors of breast, prostate, and colon cancers to a 1-year telephone- and print materials-based lifestyle intervention, designed to increase physical activity and improve dietary quality, or to a wait-list control group. The study demonstrated that participants randomized to the intervention group experienced significantly less decline in functional status, improvements in dietary quality, increased physical activity, and modest weight loss, as compared with control participants (all p < 0.05). The FRESH Start trial randomized 543 survivors of breast or prostate cancer to a 10-month tailored, mail-based intervention, designed to increase intake of fruit and vegetables, decrease fat intake, and increase exercise or to a control group who received nontailored materials about a healthy diet and exercise. Both groups significantly improved lifestyle behaviors (p < 0.05), but participants in the tailored intervention were more likely to improve two or more behaviors (34% vs. 18%, p < 0.001), and also experienced modest weight loss and improvements in exercise patterns and dietary quality as compared with control participants.

Lifestyle Intervention Trials with Biologic Endpoints

Lifestyle intervention studies also provide the opportunity to learn more about the complex biologic mechanisms through which obesity and other lifestyle factors could impact breast cancer risk and outcomes. Evaluating the efficacy of lifestyle interventions in affecting changes in biomarkers linked to breast cancer risk and recurrence could help determine which interventions are most promising for further testing, as well as identifying the patient populations most likely to benefit from lifestyle change after breast cancer diagnosis. For example, the Nutrition and Exercise for Women (NEW) Trial evaluated the effect of different lifestyle interventions on biomarkers linked to breast cancer in 439 postmenopausal, sedentary, overweight women. Participants were randomized to one of four groups: dietary weight loss, exercise alone, dietary weight loss + exercise, or control. Women randomized to the weight loss groups, with or without exercise, experienced the most significant reductions in insulin, other metabolic hormones, sex steroids, and inflammatory mediators. Women randomized to exercise alone experienced smaller but significant changes in estrogen, testosterone and leptin levels (p < 0.001, p = 0.04, and p < 0.01, respectively), but no significant changes in other hormones.

Several studies have looked at the impact of exercise alone on biomarkers linked to cancer risk and outcomes in breast cancer survivors. For example, Ligibel and colleagues demonstrated a 28% decrease in insulin levels in a group of sedentary, overweight breast cancer survivors participating in a 16-week mixed strength-training and aerobic exercise intervention (p = 0.07); similar effects were reported by Irwin and colleagues. Individual studies have also demonstrated a favorable impact of exercise on IGF-I and other metabolic and inflammatory hormones, but data are too limited to draw firm conclusions. More work is needed to better understand the effect of lifestyle change on biologic mechanisms linked to breast cancer.

CONCLUSION

Obesity is associated with breast cancer incidence and prognosis, and increasing evidence implicates sex hormones, insulin, adipokines, and their inter-related biologic pathways as major factors underlying these relationships. More importantly, these hormones and biologic pathways represent important potential targets for primary and secondary breast cancer prevention. The MA-32 study, for example, is the first formal randomized clinical trial of metformin use and its effect on prognosis in early-stage breast cancer. However, there are additional potential biologic targets related to the implicated signaling pathways, and appropriate pharmacologic studies to assess these opportunities for cancer prevention and treatment are needed. Furthermore, the ability of purposeful weight loss to improve prognosis has not been tested in the setting of randomized trials. Several lifestyle intervention trials have been conducted in breast cancer survivors, demonstrating the feasibility of implementing weight loss and other interventions after cancer diagnosis. Data from two large-scale dietary intervention studies provide further evidence that dietary changes that produce weight loss may improve outcomes in breast cancer survivors, and other studies suggest that weight loss induces significant favorable changes in biomarkers linked to breast cancer risk and outcomes. Adequately powered adjuvant studies are needed to
define the role of weight loss in the management of overweight and obese breast cancer survivors. Overall, the association of obesity with breast cancer incidence and prognosis represents a very significant, but still under-exploited opportunity to improve prognosis for patients with early-stage breast cancer.

**Disclosures of Potential Conflicts of Interest**

The author(s) indicated no potential conflicts of interest.

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BREAST CANCER

Optimizing Locoregional Treatment in Early-Stage Breast Cancer

CHAIR
Stephen R. Grobmyer, MD
Cleveland Clinic
Cleveland, OH

SPEAKERS
Hiram S. Cody III, MD
Memorial Sloan-Kettering Cancer Center
New York, NY

Timothy Whelan, BM, BCh, MSc
Juravinski Cancer Centre at Hamilton Health Sciences
Hamilton, ON, Canada
Which Patients with Sentinel Node–Positive Breast Cancer Can Avoid Axillary Dissection?

Alice Y. Ho, MD, MBA, and Hiram S. Cody III, MD

OVERVIEW

Sentinel lymph node (SLN) biopsy is standard care for patients with cN0 breast cancer. An extensive literature, including seven randomized trials, has established that patients with negative SLN do not require axillary dissection (ALND), that axillary local recurrence after a negative SLN biopsy is rare, that disease-free and overall survival are unaffected by the addition of ALND to SLN biopsy, and that the morbidity of SLN biopsy is substantially less than that of ALND. It is now clear that many patients with positive SLN do not require ALND. In ACOSOG Z0011, 6-year locoregional control and survival were equivalent with versus without the performance of ALND in cT1–2N0 patients with ≤2 positive SLN treated by breast conservation with whole breast radiation therapy. A small but growing body of data now suggests that ALND may not be required for selected patients outside the Z0011 eligibility criteria, specifically those treated by mastectomy (without post-mastectomy radiation therapy), by partial breast irradiation, and by neoadjuvant chemotherapy. Looking ahead, the principal goals of axillary staging, prognostication, and local control will be accomplished by SLN biopsy for a substantial majority of patients, and the role of ALND will continue to diminish.

The widespread adoption of sentinel lymph node (SLN) biopsy as standard care for axillary staging in cN0 breast cancer is supported by the results of at least 69 observational studies,1 seven randomized trials,2 3 meta-analyses,2-4 an ASCO Guideline,5 and an extensive literature covering all aspects of the procedure. These studies establish that patients with negative SLN do not require axillary dissection (ALND), that axillary local recurrence (LR) after a negative SLN biopsy is rare (0.3%),6 that disease-free (DFS) and overall survival (OS) are unaffected by the addition of ALND to SLN biopsy, and that the morbidity of SLN biopsy is less than that of ALND. The logical next question in the evolution of axillary staging is to ask whether there are SLN-positive patients who can avoid ALND, and it is clear that there are: 30% to 50% of SLN-positive patients have disease limited to the SLN.1

In a retrospective study of practice patterns in the United States, drawing on the National Cancer Data Base, Bilimoria et al7 reported on 97,314 SLN-positive patients (1998–2006); 23% with SLN macrometastases (≥2 mm, pN1) and 55% with SLN micrometastases (0.2–2 mm, pN1mi) did not have ALND, yet for both pN1 and pN1mi SLN disease, axillary local recurrence and 5 year relative survival were the same with or without ALND (Table 1). A similar study by Yi et al8 from the SEER Database of 26,986 SLN-positive patients (1998–2004) found that 11% of those with SLN micrometastases and 33% of those with SLN micrometastases did not have ALND, and that OS was unaffected at a median follow-up of 50 months. Both studies report a strong trend over time away from ALND for patients with SLN micrometastases. Nine smaller retrospective studies9 comprising 1,035 patients with positive SLN and no ALND report low rates of axillary LR, most in the range of 0% to 2%, at 28–82 months follow-up.

The most definitive data are from ACOSOG Z0011,10,11 a prospective randomized trial in which 813 SLN-positive patients with clinical stage T1–2N0 breast cancer were randomized to ALND compared with no further surgery. All were SLN-positive by routine hematoxylin and eosin staining and all had breast conservation including whole-breast radiation therapy (RT). Patients with three or more positive SLN (or with matted nodes) were excluded, and axillary-specific RT was not allowed. Additional positive nodes were found in 27% of the patients who had ALND, but at 6 years’ follow-up there were no differences between the ALND and no-ALND arms in local (3.6% vs. 1.9%), regional (0.5% vs. 0.9%), or overall locoregional recurrence (4.1% vs. 2.8%),10 nor were there any differences in DFS or OS11 (Table 2).

Critics of Z0011 have focused on issues of case selection (arguing that young women and those with estrogen receptor [ER]-negative tumors were under-represented), follow-up (arguing that 6.3 years is inadequate), and statistical power (arguing that Z0011 did not meet its planned accrual or statistical endpoints). In defense of Z0011, Morrow and Giuliano12 respond, rightly in my opinion, as follows: 1) younger
age is associated with higher rates of recurrence in the ipsilateral breast, but not in regional nodes, 2) ER-negative tumors are associated with early relapse but not with higher rates of axillary node involvement, 3) most women within the Z0011 selection criteria are postmenopausal and ER-positive, 4) axillary recurrence is an early event (virtually all occur within the first 5 years), and 5) Z0011 closed early (based on slow accrual and a lower-than-expected rate of events) but achieved its predefined goal, showing with a high level of significance that SLN biopsy alone was not inferior to ALND.

The principal implications of Z0011 are surgical, and over the last 2 years many institutions and surgeons in the United States (and to a lesser extent in Europe and worldwide) have found the results to be persuasive and practice-changing, incorporating into their treatment guidelines a policy of “no ALND” for SLN-positive patients who meet the Z0011 selection criteria. At our institution, we have done so since 2010; for “Z0011-like” patients we have largely abandoned preoperative axillary ultrasound, axillary fine needle aspiration core biopsy, and intraoperative frozen section of SLN, with a substantial decline in the rate of completion ALND for SLN-positive patients. Our observations match those of the University of Texas MD Anderson Cancer Center, which compared their “pre-Z0011” versus “post-Z0011” practices, observing declines in the rates of intraoperative pathologic assessment from 69% to 26%, and of ALND for SLN-positive patients from 85% to 24%. In a separate report, they estimated that 75% of their SLN-positive patients from the “pre-Z0011” era could have avoided ALND. Among respondents to a 2012 survey of the American Society of Breast Surgeons, 97% expressed familiarity with Z0011 and 57% said they would not perform ALND in “Z0011-like” patients. The response to Z0011 worldwide, and especially in Europe, has been mixed; the 2011 St. Gallen Consensus acknowledged the results of Z0011 without making a straightforward recommendation, and cautioned that a policy of no-ALND for SLN-positive patients should adhere strictly to the Z0011 selection criteria.

What are the implications of Z0011 for the medical oncologist? Montemurro et al have suggested that the omission of ALND could alter the choice of systemic therapy; using post hoc case review, they found that the information gained from completion ALND could have changed the indication for systemic chemotherapy in 16% of their patients. Reassuringly, two large trials which randomized SLN-positive patients to ALND versus no-ALND (ACOSOG Z0011) and ALND versus axillary RT (EORTC AMAROS) found no differences in the usage of chemotherapy, hormone therapy, or RT based on the performance of ALND.

What are the implications of Z0011 for the radiation oncologist? Positive axillary nodes were left behind in a presumed 27% of the Z0011 patients in the no-ALND arm, but only 0.9% developed axillary LR. Although Z0011 did not allow the use of supraclavicular or axillary fields, Haffty et al


<table>
<thead>
<tr>
<th>SLN micrometastases (≤2 mm, pN0i-/N1mi)</th>
<th>Relative Survival (5 yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN only (n = 802)</td>
<td>0.4% 99%</td>
</tr>
<tr>
<td>SLN/ALND (n = 2,357)</td>
<td>0.2% 98%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>SLN macrometastases (&gt;2 mm, pN1)</th>
<th>Relative Survival (5 yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN only (n = 5,596)</td>
<td>1.0% 90%</td>
</tr>
<tr>
<td>SLN/ALND (n = 22,591)</td>
<td>1.1% 89%</td>
</tr>
</tbody>
</table>

Abbreviations: SNL, sentinel lymph node; ALND, axillary lymph node dissection; yr, year.
*Adapted from Bilimoria et al

<table>
<thead>
<tr>
<th>TABLE 2. Results of ACOSOG Z0011, a Randomized Comparison of SLN Biopsy with and without ALND, in SLN-Positive Patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN Biopsy/ALND n = 388</td>
</tr>
<tr>
<td>Loco-regional recurrence (6.3 yr)</td>
</tr>
<tr>
<td>Local</td>
</tr>
<tr>
<td>3.6% 1.9% (P = NS)</td>
</tr>
<tr>
<td>Local + Regional</td>
</tr>
<tr>
<td>Survival (6.3 yr)</td>
</tr>
<tr>
<td>Disease-free survival</td>
</tr>
<tr>
<td>82% 84% (P = NS)</td>
</tr>
</tbody>
</table>

Abbreviations: SNL, sentinel lymph node; ALND, axillary lymph node dissection; yr, year; H&E, hematoxylin and eosin stain; RT, radiation therapy; NS, not significant.
*Adapted from Giuliano et al; all patients were clinical stage T1–2N0, with ≤2 H&E-positive SLN, treated by breast conservation with whole-breast and without axillary-specific RT
have suggested that irradiation of the lower axillary nodes with “high tangents” to the breast may have contributed to the low rate of axillary LR. Modern computed tomography (CT)-guided treatment planning allows identification of the axillary nodes and treatment of at least part of the axilla by adjusting the superior and deep tangent borders. Resnik et al20 have used CT to estimate the proportion of the prescription dose to the breast given to the axilla by standard breast tangents (66% to level I, 44% to level II, and 31% to level III) versus “high” breast tangents (86%, 71%, and 73%, respectively), and recommend high tangents for axillary prophylaxis. Schlembach et al21 have described a technique for high tangents that allows treatment of the SLN region and most of levels I and II. An audit of the Z0011 treatment fields is ongoing to clarify the degree to which participating radiation oncologists adjusted their tangent fields based on tumor characteristics and the extent of axillary surgery, but these nuances of intent may be difficult to extract in a retrospective study.

Some confusion comes from the recent results of the NCIC Clinical Trials MA.20,22 a trial in which patients with high-risk node-negative and 1–3 node-positive breast cancer were randomly assigned to whole breast RT with or without regional node irradiation (RNI). These patients, 85% of whom had 1–3 positive nodes, overlap somewhat with the Z0011 population, none of whom received RNI. At 5 years, MA.20 observed notable improvements for the RNI arm in isolated locoregional DFS (by 2.3%), distant DFS (by 5.4%), DFS (by 5.7%) and OS (by 1.6%, p = 0.07). These results require further study (the full paper has not yet been published) and are perplexing; inexplicably, the absolute gain in DFS exceeded the gain in local control, a result which stands in sharp contrast to the Oxford meta-analysis,23 which famously observed that the prevention of four local recurrences was required to save one life.

Looking ahead, can we extend the success of Z0011 to Z0011-ineligible patients, specifically those treated a) by mastectomy without RT, b) by partial breast RT (PBI), and c) by neoadjuvant chemotherapy (NAC)?

Regarding mastectomy, we have recently reported24 on 535 SLN-positive patients from the pre-Z0011 era who had either mastectomy or breast conservation without other axillary-specific treatment: among 234 with N1mi or N1 disease, there were no differences at 4 years in regional node recurrence between mastectomy (97 patients, 2.5%) and breast conservation (134 patients, 1.5%). On the plus side, this low event rate is encouraging but requires wider confirmation in prospective studies specific to mastectomy; on the minus side, a low event rate argues that a “Z0011-for-mastectomy” randomized trial would not be feasible. In lieu of a randomized trial, well-characterized case series with adequate follow-up of SLN-positive mastectomy patients treated without RT should be sufficient to address this issue.

Regarding PBI, the MammoSite Registry Trial (in which PBI is delivered through an intracavitary balloon) has reported 5-year axillary LR of 0.8% in SLN-negative patients,25 a result quite similar to that of negative SLN biopsy in general (0.3%). A more definitive answer comes from the TARGIT Trial,26 an international multicenter randomization of PBI given as a single intraoperative dose to the tumor site (in 1,113 patients) versus conventional whole-breast RT (in 1,119 patients); 4-year local recurrence rates were 1.20% and 0.95%, respectively (p = 0.41), and a more recent analysis has found no differences between the treatment arms in the rate of axillary LR (J. S. Vaidya, oral communication February, 2013). Both studies suggest that the effect of whole-breast RT on axillary LR in SLN-negative patients is modest at best. Another trial, NSABP B-39,27 compares PBI (delivered by intracavitary balloon, brachytherapy catheters, or postoperative external beam) with whole-breast RT and has not yet reported outcomes. There are no data reporting outcome in SLN-positive patients treated with PBI and without ALND, but it is reasonable to assume that the results would be at least as good as for mastectomy and, to the extent that the PBI patients usually have earlier-stage disease, probably better. For the same reasons noted above, a “Z0011-for-PBI” trial is unlikely. Interestingly, in the American Society of Breast Surgeons survey,15 36% of respondents would not perform ALND in patients receiving PBI, and 27% would consider omitting ALND in patients not planned to receive RT at all. These results fit the nationwide trends noted by Bilimoria et al28 in their survey of the National Cancer Data Base.

Regarding neoadjuvant chemotherapy (NAC), the false-negative rate of SLN biopsy after NAC (in 27 studies comprising 2,148 patients) is roughly comparable to that of SLN biopsy in general, 10.5%.28 Hunt et al29 have reported that for patients who were cN0 at the time of diagnosis, the false-negative rates of SLN biopsy done before versus after chemotherapy are virtually identical (4.2% vs. 5.9%). The performance of SLN biopsy following NAC in patients with biopsy-proven nodal metastases is the subject of a prospective validation study, ACOSOG 1071, recently reported by Boughey et al30 at the San Antonio Breast Cancer Symposium; 40% of patients had a pathologic complete response in their lymph nodes and among 607 patients with cN1 disease who received NAC followed by a successful SLN biopsy and a planned backup ALND, the false-negative rate was 12.8%. A new report by Mamounas et al31 on the 10-year patterns of locoregional recurrence after NAC in National Surgical Adjuvant Breast and Bowel Project (NSABP) trials B-18 and B-27 demonstrates the highest rates of regional node recurrence in clinically node-positive patients whose nodes remained positive after NAC. Taken together, these data suggest that ALND may not be required for cN0 or cN1 patients whose SLN are negative after NAC, but should be strongly considered whenever the SLN remain positive after NAC. Many questions remain unanswered, and the decision for ALND (and/or nodal RT) following NAC can be complex; patient age, pretreatment T/N stage, biologic subtype of tumor, the clinical response to NAC, the operation (mamectomy vs. breast conservation), the pathologic response in breast and nodes, and the anticipated rate of local recurrence. This complexity argues against a “Z0011-for-neoadjuvant” trial.
Looking further ahead, we must ask whether axillary staging is necessary at all; the 21-gene recurrence score appears to give better prognostication and prediction of response to therapy than conventional histopathology for node-negative and possibly for node-positive patients and is the subject of large randomized trials (TAILORx and RxPONDER [swog.org/rxponder]). It is quite possible that the "SLN biopsy of the future" may be no SLN biopsy at all and that the "ALND of the future" will be limited to the salvage of locally persistent or recurrent disease.

Disclosures of Potential Conflicts of Interest

The author(s) indicated no potential conflicts of interest.

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Boughey JC, Suman VJ, Mittendorf EA, et al. The role of sentinel lymph node surgery in patients presenting with node positive breast cancer (T0-4, N1-2) who receive neoadjuvant chemotherapy: Results from the ACOSOG Z1071 trial. Paper presented at: 2012 San Antonio Breast Cancer Symposium; Abstract S2-1; December, 2012; San Antonio, TX.


Optimizing Clinical Management of Surgical Margins in Breast-Conserving Therapy for Breast Cancer

Stephen R. Grobmyer, MD, Michael S. Cowher, MD, and Joseph P. Crowe, MD

OVERVIEW

There has been, and continues to be, significant controversy over the definition of an “optimal” surgical margin in breast-conserving therapy (BCT). The historic basis of this controversy stems from the original trials documenting the safety of BCT and many conflicting retrospective studies that have sought to define the association between surgical margin width and outcomes over the last 20 years. It is important to understand that margin assessment is an inexact science, and current laboratory approaches to surgical-margin assessment represent only a sampling of the surgical margin. Currently available evidence suggests that decisions regarding surgical margins in BCT should be made in the context of what is known about the biology of breast cancer, as well the interactions of tumor biology, adjuvant treatment for breast cancer, and outcomes. Achieving consensus on management of surgical margins in BCT should be a clinical priority as it offers the opportunity to reduce the burden of breast cancer treatment on patients without compromising cancer-related outcomes.

Breast conserving therapy (BCT) consisting of lumpectomy followed by adjuvant breast irradiation and systemic therapy has become a widely accepted approach to the treatment of early-stage breast cancer. Numerous studies have demonstrated very high rates of local recurrence (~20% at 5 years) of breast cancer in patients in whom microscopically clear margins (“negative”) are not achieved with lumpectomy even in the setting of adjuvant radiation therapy (reviewed by Klimberg et al.). Complete surgical resection of breast cancer to negative microscopic margins is therefore an essential component of BCT. There has been, and continues to be, controversy regarding the value of obtaining “wide” microscopic margins in patients undergoing BCT in an effort to improve outcomes and reduce recurrences.

Wider surgical margins are a goal of some surgeons and treatment teams who seek to remove any residual disease in the hopes of improving outcomes. However, the goal of BCT and lumpectomy is to preserve the volume and shape of the breast, and it is desirable to minimize the amount of normal tissue removed around a tumor that is associated with the minimal risk of recurrence. The “optimal” surgical margin for a lumpectomy for the purposes of this article is defined as the minimum margin of normal tissue around a breast cancer that is associated with the lowest achievable rates of recurrence and highest rates of breast cancer related survival in the setting of modern adjuvant therapies. Based on this definition, removal of more than optimal tissue has no additional value in relation to patient outcomes and can be detrimental in terms of cosmesis, increased rates of re-excision, costs, patient anxiety, and delay in adjuvant therapy.

Reporting the distance from the edge of the tumor to the edge of the surgical specimen (“margin width”) at the time of lumpectomy has become a frequently reported quality metric in breast cancer care. However, there has been no general consensus on how to use this measurement to optimally guide further surgical therapy. There is a need to develop consensus guidelines in defining the optimal surgical margin in BCT. Defining the optimal surgical margin as this has the potential to reduce overtreatment without negatively impacting the survival of patients with early-stage breast cancer. There are no prospective randomized trials that directly address the influence of measured margin status on outcomes in BCT for invasive breast cancer or ductal carcinoma in situ (DCIS).

In this article, we review the evidence concerning the relationship between margin status and outcomes in BCT. This data will be reviewed with a specific focus on the limitations of current approaches to margin assessment, other clinical and biologic factors that govern clinical outcomes, as well factors associated with a residual-disease burden following lumpectomy. This report will not address optimal surgical margins in patients with breast cancer undergoing lumpectomies who do not receive adjuvant radiotherapy.

From the Section of Surgical Oncology, Cleveland Clinic, Cleveland, OH.

Authors’ disclosures of potential conflicts of interest are found at the end of this article.

Corresponding author: Stephen R. Grobmyer, MD, Section of Surgical Oncology, Cleveland Clinic, 9500 Euclid Ave./A81, Cleveland, OH 44195; email: grobmys@ccf.org.

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THE HISTORIC BASIS OF CONCERNS OVER “CLOSE” MARGINS IN BCT

Invasive Breast Cancer

Table 1 summarizes the surgical margin criteria used by investigators in the large randomized trials that formed the basis of modern BCT. These trials varied greatly in their minimum requirements for surgical margins. All trials required at least complete gross removal of the tumor at the time of lumpectomy (Table 1). These trials, performed in the 1970s and 1980s, and now with long-term follow-up, demonstrated that patients undergoing BCT had overall survival comparable with patients having mastectomy for breast cancer. In only one of these trials, the National Surgical Adjuvant Breast and Bowel Project B-06 reported by Fisher et al., did investigators record the margin width of the lumpectomy specimens, but simply having a negative margin was satisfactory for trial-entry requirements. When viewed together, these trials suggest that variation in surgical margins and in particular wide surgical margins are not a determinant of survival in patients undergoing BCT for invasive breast cancer.

Subsequent to the publication of the prospective trials, there have been many retrospective studies that have investigated the concept of “close” margins and the effect of “close” margins on outcomes (reviewed in Singletary). In these studies, the definition of “close” margin varied and was defined by the investigators in each individual study (e.g., < 1 mm or < 2 mm). Some studies have suggested that “close” margins are associated with recurrence rates similar to those for patients with positive margins. Other studies have shown intermediate outcomes for those with close margins compared with “positive” and “negative.” Others have shown that “close” margins have no effect on relapse-free survival compared with widely negative margins. This uncertainty has prompted many to advocate greater initial tissue resection or re-excision for “close” margins in an effort to reduce local recurrence (or improve relapse-free survival) for patients with invasive breast cancer. Reported ranges of local recurrence rates at 5 years for clear margins, close margins, and positive margins are 2%-3%, 2%-8%, and 10%-25%, respectively.

Houssami et al. recently reported the results of a meta-analysis of 21 studies analyzing the relationship between margin status and outcome for patients having BCT for invasive breast cancer. The authors demonstrated that although increasing margin width has a weak correlation with increased risk for local recurrence, the effect is mitigated when adjustments are made for administration of adjuvant endocrine and radiation therapy. The authors conclude, “adoption of wider margins, relative to narrower widths, for declaring negative margins is unlikely to have a substantial additional benefit for long-term local control in BCT.”

Ductal Carcinoma In Situ

Two large randomized trials involving radiation following lumpectomy for DCIS (NSAPB 17 and NSAPB 24) defined margins as either pathologically positive or negative (i.e., no tumor touching ink). In these trials, low rates of ipsilateral recurrence have been demonstrated among patients receiving radiation therapy, and the lowest rates of recurrence were seen in those with negative margins. In another large randomized trial of radiation therapy following lumpectomy for DCIS, EORTC 10853, positive margins were defined as tumor 1 mm or smaller from the margin. On multivariate analysis in this trial, tumor-margin status and use of radiation were associated with reduced risk of local recurrence. Patients with a positive margin had a 10-year local recurrence rate of 39% without radiation and 24% with radiation.

Rudloff et al. reported a retrospective series of 294 patients treated for DCIS with lumpectomy with or without radiotherapy with a median 11-year follow-up. This series demonstrated higher recurrence rates in patients with narrow margins. Ten-year recurrence rates were 42%, 27%, and 21% for measured margins smaller than 1 mm, 1 mm to 9 mm, and 10 mm or more, respectively, in patients not receiving radiation therapy. The authors also demonstrated an

KEY POINTS

- There are currently wide variations in the practice of surgical-margin management and re-excision rates following breast-conserving surgery for breast cancer.
- Achieving pathologically negative margins should be the primary goal of breast-conserving surgery for invasive breast cancer.
- Low-volume residual disease following lumpectomy is common and has no proven effect on clinical outcomes in the setting of modern adjuvant systemic and radiation therapies.
- Adjuvant radiation therapy and chemotherapy have been associated with reduced rates of ipsilateral breast tumor recurrence.
- Decisions for surgical re-excision to obtain wider margins in BCT should be based not solely on measured margin width but in the context of tumor biology and other treatment-related factors.

<table>
<thead>
<tr>
<th>Study</th>
<th>Lumpectomy Margin Requirement</th>
<th>Follow-up Time (Years)</th>
<th>Overall Survival Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacobson, 1995</td>
<td>Gross tumor removal</td>
<td>10</td>
<td>NS*</td>
</tr>
<tr>
<td>Arriogada, 1996</td>
<td>2 cm excision margin</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Fisher, 2002</td>
<td>Histologically negative</td>
<td>20</td>
<td>NS</td>
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<tr>
<td>Van Dongen, 2000</td>
<td>Gross tumor removal*</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>Veronesi, 2002</td>
<td>2-3 cm excision, skin, fascia</td>
<td>20</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* In general, re-excision took place only when macroscopic (i.e., palpable) disease was left behind.

Table 1. Randomized Trials of Mastectomy versus Lumpectomy plus Radiation

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associated reduction in ipsilateral recurrence in all patients receiving adjuvant radiation therapy.

A meta-analysis performed by Dunne et al.\textsuperscript{13} analyzed 4,660 patients undergoing BCT (lumpectomy and adjuvant radiation therapy) for DCIS that demonstrated significantly higher recurrence rates in patients with positive margins compared with close or negative margins (p < 0.01). Further, when considering specific margin thresholds in DCIS, the authors found 2 mm was a threshold. Beyond that there was little additional value in reducing ipsilateral recurrence.

**THERE ARE CURRENTLY WIDE VARIATIONS IN DEFINING “OPTIMAL” MARGINS AND RE-EXCISION RATES IN BCT**

Defining optimal margin in BCT has been an area of controversy and debate among breast cancer specialists for many years.\textsuperscript{3,7} There is a current lack of consensus in defining optimal margins as demonstrated in several recently reported surveys of breast cancer physicians. There are also differences noted among radiation oncologists and breast cancer surgeons, and geographical differences noted between physicians in North America and Europe. Taghian et al.\textsuperscript{4} recently reported the results of a survey of 1,137 radiation oncologists regarding their approach to surgical margins in BCT. There were significant variations in how the respondents defined “negative” and “close” margins. There were significant differences in the responses of physicians in North America and Europe. Greater than 50% of respondents considered “negative” margins in BCT as being further than 1 mm from the inked specimen margin. There was also significant variation noted relative to the recommendation for surgical re-excision of margins.

In several studies, significant variations among breast cancer surgeons regarding their recommendations for re-excision of margins as part of BCT have been documented. Blair et al.\textsuperscript{14} analyzed the results of a survey of 351 breast surgeons regarding a minimal acceptable margin width with lumpectomy.\textsuperscript{14} The authors found significant variations in the recommendations of the minimal acceptable margin width for both invasive cancer and DCIS. Sixty-five percent of surgeons found 2 mm or wider margin acceptable, although 35% viewed a margin width of less than 2 mm acceptable. Similarly, Azu et al.\textsuperscript{15} found wide variations in the recommendations for safe margins, and further that subspecialization in breast cancer surgery were associated with a recommendation for a smaller acceptable margin. In aggregate, these series suggest significant variations in the current practice of surgical margin management in BCT for both invasive cancer and DCIS.

Several more recent studies have documented wide variations in the re-excision rates following lumpectomy for breast cancer among hospitals and regions. McCahill et al.\textsuperscript{6} reported that in 2,286 patients undergoing lumpectomy, 23% required additional surgery. In this study, variation in the rates of re-excision noted for patients with a negative pathologic margin ranged from 1.7% to 20.9%. Although 47.9% of patients with a clear but smaller than 1 mm margin underwent re-excision, only 20.2% of patients with margins between 1.0 and 1.9 mm underwent re-excision. Similarly, Jeevan et al.\textsuperscript{16} reported wide variations in the use of re-excision following lumpectomy for breast cancer in England.

**MICROSCOPIC RESIDUAL DISEASE IN THE BREAST IS COMMON AFTER BCT**

In now classic studies, Rosen et al.\textsuperscript{17} performed simulated lumpectomies on mastectomy specimens from patients who had mastectomy for presumed unifocal breast cancer. Interestingly, they found residual carcinoma (in situ and invasive) in other quadrants of the breast in a significant number of patients. The likelihood of finding residual disease in other quadrants of the breast increased with increasing primary tumor size. Patients with tumors smaller than 2 cm were found to have residual disease in other quadrants in 26% of cases, and patients with tumors larger than 2 cm were found to have residual disease in other quadrants in 38% of cases. These studies suggest that small-volume residual disease is common after lumpectomy. Again, these findings suggest that overly aggressive attempts to remove small volume residual disease are not warranted and not clinically meaningful particularly when radiation and systemic therapy are planned.

Multiple studies have been performed that have analyzed predictors of residual disease that may be useful in clinical decision making regarding the need for re-excision. Young age, extensive intraductal component, and triple-negative phenotype have all been associated with increased risk of residual disease following lumpectomy for invasive breast cancer.\textsuperscript{18} Other studies have demonstrated that tumor size, high grade, number of involved margins, and linear extent of disease at the margin were all associated with increased risk of residual disease at the time of re-excision.\textsuperscript{19} Factors associated with residual disease following lumpectomy for DCIS have included the presence of comedo necrosis, multifocality, margin width, and lesion size.\textsuperscript{20} An understanding of these predictors may be useful in clinical decision making regarding re-excision to achieve wider surgical margins in BCT.

**LIMITATIONS OF PATHOLOGIC ASSESSMENT OF MARGINS**

There are practical limitations of pathologic assessment of margin assessment that must be appreciated when discussing “optimal” surgical margins following lumpectomy and related clinical decision making. There is currently no standardized approach to pathologic assessment of surgical margins.\textsuperscript{21} It is critical to realize that all techniques of margin assessment are based on sampling the margin,\textsuperscript{6} and it is not practical for a pathologist to sample the entire specimen margin for microscopic analysis.\textsuperscript{21} Specimen handling, specimen processing, and the technique of margin assessment have also been shown to influence margin assessment.\textsuperscript{22} There-
fore, adherence to strict clinical decision algorithms based entirely on measured margin widths do not seem warranted nor clinically justified. It is also imperative to focus on developing standards toward uniform pathologic specimen handling and analysis, which will ultimately lead to better defining the relationship between margins and outcomes.

**ADJUVANT THERAPIES HAVE A ROLE IN REDUCING IPSILATERAL BREAST TUMOR RECURRENT**

Several reports have documented a reduction in local recurrence rates following BCT over time that are likely attributable to advances in adjuvant therapy for breast cancer.26 Cabioglu et al.23 demonstrated significant reductions in local recurrence over time that on multivariate analysis were associated not only with improving negative surgical-margin rates but also adjuvant hormonal and chemotherapy (p = 0.001). Other studies have also demonstrated that an association between endocrine therapy and chemotherapy administration on reductions in local recurrence in patients undergoing BCT.24,25 Among HER2+ patients, adjuvant trastuzumab has been associated with a reduction in locoregional recurrences.26

The addition of boost radiation therapy also has a role in optimizing local control rates in BCT. The EORTC 22881–10882 trial demonstrated a significantly lower rate of local recurrence in patients who received boost irradiation following adjuvant whole breast irradiation (6.2%) compared with those receiving whole breast irradiation alone (10.2%).

In aggregate, these observations suggest that various adjuvant treatment factors have an influence on ipsilateral breast tumor recurrence that should be considered in the context of clinical decision making regarding surgical margin management. Advances in adjuvant therapy and selection of patients for adjuvant therapy are associated with observed improvements in local recurrence over time.

**EFFECT OF PATIENT CHARACTERISTICS AND TUMOR BIOLOGY ON LOCAL RECURRENT**

Although the presence of tumor at the inked margin has clearly been associated with high rates of local recurrence, both patient and tumor characteristics have also been associated with increased rates of local recurrence in patients undergoing BCT. These factors are inherent to the patient and underlying tumor biology and their influence on outcomes are not mitigated by wider surgical margins.3

Young patient age, an extensive intraductal component, basal phenotype, HER2+ cancers, high-grade tumors, and lymphovascular invasion have all been associated with higher risk of recurrence in patients undergoing BCT.26 Recent studies have also identified gene-expression profiles that associated an increased risk of locoregional recurrence of breast cancer in patients undergoing BCT for estrogen-receptor–positive invasive breast cancer.29 Biologic features including tumor grade, size, and the presence of comedo necrosis have also been shown to be important in predicting recurrence in DCIS.30

Recent advances in genomic sequencing of human breast tumors have categorized four main breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities31 Future studies elucidating the attributes of these subtypes regarding in-breast growth and recurrence patterns have the potential to influence the acceptable surgical margin criteria for each of these subtypes.

These findings suggest that factors beyond measured surgical margin are important determinants of recurrence in breast cancer and suggest a need to better understand the biology and the relationship of tumor to recurrence and systemic treatments in optimizing decision clinical making in BCT.

**MANAGING SURGICAL MARGINS FOLLOWING BCT**

The available evidence outlined in this report suggests that having either invasive cancer or noninvasive cancer at a surgical margin is associated with a high rate of local recurrence and should be avoided in patients undergoing BCT.7 For invasive breast cancer, we are in agreement with other authors that routine re-excision of margins beyond tumor touching ink when adjuvant radiation therapy and appropriate systemic therapy are planned as part of BCT is not necessary.3 For patients with DCIS, the data suggest that routine re-excision of margins greater than 2 mm when radiation therapy follows lumpectomy is not necessary.3 Consensus on these issues would likely reduce unnecessary re-excisions and undoubtedly improve cosmesis for patients undergoing BCT.

These recommendations must be taken within the context of individual patients, and surgeons need to appreciate that measured margin width should be but one factor considered in the decision to perform a re-excision as a component of BCT.3 Decisions for re-excision beyond the above outlined parameters may be clinically indicated based on an understanding of the potential for finding a significant volume residual disease within the remaining breast, tumor biology, patient characteristics, and plans for the omission of adjuvant therapy.

**CHALLENGES FOR THE FUTURE IN MANAGING SURGICAL MARGINS WITH BCT**

Regarding surgical margins, challenges for the future are to better understand the biologic basis for both tumor growth patterns and recurrence. Also, it is desirable to better understand the interplay between tumor biology, treatments, and outcomes. Doing so may allow development of more precise and more personalized algorithms to guide treatment of patients with surgical and adjuvant therapy. It is also imperative to focus on developing standards toward uniform pathologic-specimen handling and analysis that will be ulti-
mately lead to better defining the relationship between margins and outcomes. This will streamline clinical decision making and eliminate artifacts that may lead to a misinterpretation of the true surgical margin. There is also a need to clarify “optimal” margins in patients being treated with partial breast irradiation and in those receiving neoadjuvant chemotherapy followed by BCT.

The ultimate goal of these endeavors should be to reduce the rate of re-excisions being performed as part of BCT and the associated downstream negative sequelae associated with re-excisions. Reducing the amount of nondiseased tissue removed at the time of BCT to the optimal level will minimize patient morbidity while also minimizing tumor recurrence and maximizing patient outcomes.

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BREAST CANCER

Pushing the Limits of Upfront Care and Drug Development: Neoadjuvant Opportunities in Breast Cancer

CHAIR
Angela DeMichele, MD, MSCE
Abramson Cancer Center of the University of Pennsylvania
Philadelphia, PA

SPEAKERS
Tatiana Prowell, MD
U.S. Food and Drug Administration
Silver Spring, MD

Charles E. Geyer Jr., MD
Statewide Clinical Trials Network of Texas
Addison, TX
Drug Development: Neoadjuvant Opportunities in Breast Cancer

Priya Rastogi, MD, Charles E. Geyer Jr., MD, Eleftherios P. Mamounas, MD, and Angela DeMichele, MD, MSCE

OVERVIEW

Preoperative therapy allows for a higher rate of breast conserving surgery and has been shown equivalent to adjuvant therapy. Preoperative therapy provides an opportunity to obtain insights into breast cancer biology and to accelerate the evaluation of new therapies. Clinical trials have shown that women who achieve a pathologic complete response (pCR) have substantially improved outcomes compared with those who do not achieve a pCR. The U.S. Food and Drug Administration (FDA) meta-analysis demonstrated that the association of pCR and long-term outcomes is greater in women with aggressive breast cancer subtypes. In patients with HER2+ breast cancer, the addition of trastuzumab to chemotherapy in the neoadjuvant setting has doubled pCR and correlated with improved outcomes. Clinical trials will evaluate tailoring the use of radiation therapy in patients who have received neoadjuvant therapy. Trials have established neoadjuvant endocrine therapy as a valid treatment and research option for ER-rich breast cancer. The neoadjuvant setting allows for evaluation of endocrine therapies in combination with newer targeted therapies in the appropriate patient populations. The neoadjuvant setting provides opportunity to accelerate the evaluation of new agents, improve pCR rates, and identify predictive biomarkers for response. This setting provides the opportunity for screening new agents in combination with chemotherapy while obtaining serial biopsies to understand biology of response and resistance. Although current standard therapies provide substantial benefits for patients with a pCR, patients with residual disease are at substantial risk for disease recurrence. New agents are being evaluated in patients with high-risk residual disease following standard treatment regimens.

Preoperative or neoadjuvant therapy, initially used for locally advanced breast cancers, has become more commonly used for patients with operable disease, particularly those with larger primary tumors. By reducing the size of the primary tumor, preoperative therapy allows a higher rate of breast conserving surgery.1-10 The neoadjuvant setting also provides opportunities to obtain insights into breast cancer biology and accelerate evaluation of new therapies.

CLINICAL RATIONALE FOR PREOPERATIVE (NEOADJUVANT) THERAPY

Hortobagyi and colleagues demonstrated in the 1980s that women with locally advanced breast cancer who achieved a pathologic complete response (pCR) after neoadjuvant chemotherapy (NACT) had substantially improved outcomes compared with historic controls.1-11 Use of NACT in operable breast cancer was evaluated in the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18 clinical trial in which 1,523 women with operable breast cancer were randomized to four cycles of AC (doxorubicin 60 mg/m², cyclophosphamide 600 mg/m²) every 21 days either before or after definitive surgery.5-7,10 A pCR in the breast was documented in 13% of patients, who had substantially improved outcomes relative to the larger group without pCR (disease-free survival (DFS) hazard ratio [HR] = 0.47, p < 0.0001; overall survival (OS) HR = 0.32, p < 0.0001). Pathologically negative nodes were identified in 58% of women receiving the neoadjuvant therapy compared with 42% among women undergoing initial surgery (p < 0.0001). A higher rate of breast conserving surgery was also observed in women receiving preoperative therapy (68% vs. 60%, respectively; p = 0.001). There were no statistically significant differences in DFS (HR = 0.93, p = 0.27) and OS (HR = 0.99, p = 0.90) between the two groups. This trial demonstrated that NACT was safe in operable breast cancer and that pCR status was associated with a favorable outcome.

PATHOLOGIC RESPONSE AS A SURROGATE FOR CLINICAL OUTCOME

The NSABP B-27 trial evaluated the incorporation of docetaxel into the neoadjuvant setting.8-10 Women with operable breast cancer were randomized to receive (1) preoperative AC followed by surgery, (2) preoperative AC followed by four cycles of preoperative docetaxel followed by surgery, or (3) preoperative AC followed by surgery and then four cycles of postoperative docetaxel. Preoperative docetaxel fol-
lowing AC increased the pCR rate (13% to 26%, p < 0.001), and the proportion of women with negative nodes (51% to 58%, p < 0.001) relative to AC alone. Although this larger group of women had a more favorable outcome (DFS HR = 0.49, p < 0.0001 and OS HR = 0.36, p < 0.0001), there were no statistically significant differences in DFS and OS demonstrated among the three overall treatment groups (p across all arms = 0.76).

Recently an international working group, in collaboration with the FDA, conducted a large meta-analysis of 12,993 patients treated on 12 early, randomized NACT trials with available long-term follow-up for DFS and OS. Improved outcomes in patients with pCR compared with those without were again demonstrated (event-free survival HR = 0.48, p < 0.001 and OS HR = 0.36, p < 0.001). Moreover, the magnitude of the association between pCR and event-free survival was greater in patients with aggressive breast cancer subtypes (hormone receptor (HR) +/HER2- HR = 0.49, p < 0.001 and HR+/HER2+ HR = 0.58, p = 0.001vs. HR-/HER2+ HR = 0.25, p < 0.001 and HR-/HER2- HR = 0.24, p < 0.001). However, correlation of improvement in long-term outcomes with increased pCR rates could not be established. The authors suggested this may be due to relatively low pCR rates in these early trials, the heterogeneity of the populations accrued, and the lack of targeted therapy in the majority of patients.12

The German Breast Group (GBG) and Arbeitsgemeinschaft Gynäkologische Onkologie-Breast Group (AGO-B) conducted a pooled analysis of seven randomized NACTs conducted by the groups, involving 4,193 patients, to determine whether pCR predicts improved outcomes across the intrinsic subtypes. Estrogen receptor (ER), progesterone receptor (PR), HER2 and histologic grade determined by local pathologists were used to classify patients by intrinsic subtypes according to clinicopathologic criteria recently recommended by the St. Gallen panelists, with the exception that grade was substituted for Ki-67.13 In the luminal B/HER2-, ER-/HER2+ and triple negative groups, pCR was associated with improved outcomes. However in luminal A and luminal B/HER2+ patient groups, no correlations were identified, suggesting that inclusion of these “low-risk” patients may have had a substantial adverse dilutional effect on the ability to demonstrate improved outcomes among the women with highly proliferative tumors with pCR. Analysis of the I-SPY TRIAL 1 (Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging and Molecular Analysis 1) correlated pCR, residual cancer burden (RCB), intrinsic subtypes, and three-year RFS; the trial demonstrated similar findings in low-risk subsets defined by a variety of predictive profiles. Low-risk subsets had low pCR rates but favorable outcomes regardless of the degree of response.14

The hypothesis that the lack of correlation between pCR rates and long-term outcome could be on the basis of inclusion of low-risk patients is further supported by results of the NOAH (NeoAdjuvant Herceptin) trial.15 In this study, 235 women with HER2+ locally advanced or inflammatory breast cancer were treated with NACT with or without trastuzumab. For women receiving chemotherapy and trastuzumab, the pCR rate was 38%, compared with 19% for women receiving chemotherapy alone. The doubling of pCR in this more homogeneous population with high proliferation rates correlated with improved three-year event-free survival (71% vs. 56%; HR = 0.59, p = 0.013). In aggregate, these findings support the contention that pCR is a meaningful short-term surrogate of outcome in more aggressive tumors (particularly HER2+ and triple negative tumors) and provide a strong rationale for testing new agents targeting highly proliferative tumors in the neoadjuvant setting using pCR as a primary end point.

**TAILORING LOCOREGIONAL THERAPY WITH NEOADJUVANT CHEMOTHERAPY**

Response to NACT allows for less extensive surgery to the breast and may provide similar benefits in tailoring the extent of surgical resection in the axilla (with the use of sentinel node biopsy), as well as decreasing the need and extent of postoperative radiotherapy in patients who achieve pCR. The utility of downstaging of axillary nodes with NACT has been demonstrated in several studies that have evaluated the role of node status in prognosis after neoadjuvant therapy. Historically, definitions of pCR vary across studies, ranging from the most stringent, ypT0 ypN0 (absence of invasive cancer in the breast and axillary nodes and absence of DCIS), to ypT0/Tis ypN0 (absence of invasive cancer in the breast and axillary nodes; DCIS allowed), to the most liberal, ypT0/Tis (absence of invasive cancer in the breast and DCIS allowed; regardless of nodal involvement). The pooled analysis of seven randomized neoadjuvant trials conducted by GBG and AGO found that patients with no invasive or noninvasive disease in the breast and negative axillary nodes had significantly better DFS than the group with either residual noninvasive or microinvasive breast disease or residual positive nodes.13 Hazard ratios for DFS comparing patients with or without pCR were lowest when defined as no invasive and no

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**KEY POINTS**

- Preoperative therapy provides an opportunity to obtain insights into breast cancer biology and accelerate the evaluation of new therapies.
- The neoadjuvant setting provides an opportunity to evaluate new agents with chemotherapy, improve pCR rates, and identify predictive biomarkers for response.
- Clinical trials will evaluate tailoring the use of radiation therapy in patients who have received neoadjuvant therapy.
- The neoadjuvant setting allows for evaluation of endocrine therapies in combination with newer targeted therapies in the appropriate patient populations.
- New agents are being evaluated in patients with high-risk residual disease following standard treatment regimens.
in situ residuals (0.446) and increased monotonously when in situ residuals (0.523), no invasive breast residuals but involved nodes (0.623), and focal-invasive disease (0.727) were included in the definition. However the FDA-led meta-analysis, which included the German studies, demonstrated no difference in the hazard of recurrence or death regardless of presence or absence of residual DCIS in the breast. These data suggest that clearing the nodes has prognostic significance, and therefore accurate assessment of the residual nodal burden is necessary.

An additional advantage of rendering the axillary nodes free of disease with NACT is the potential reduction in surgical morbidity and late lymphedema from the use of sentinel node evaluation. However, this advantage is predicated on the accuracy of sentinel node biopsy following NACT. Because NACT can have an adverse effect on the integrity of axillary nodal tissue (with or without eradication of disease), the accuracy of sentinel lymph node evaluation in the patient who has undergone NACT must be established independently of its validation in patients who are chemotherapy-naïve. Several retrospective and prospective, single-institution and multicenter trials, as well as two meta-analyses, have shown that sentinel node biopsy after NACT has lower identification rates but similar false-negative rates as sentinel node biopsy before NACT (about 10%).

It is important to emphasize that the available data on the performance of sentinel node biopsy after NACT are only applicable to patients who present with operable breast cancers (T1–3N0–1) and should not be extrapolated to those who present with locally advanced disease (T4, N3). Similarly, caution is required for patients who present with documented tumor involvement of the axilla (by fine needle aspiration or core biopsy) and undergo NACT. Two prospective trials presented at the 2012 San Antonio Breast Cancer Symposium demonstrated false-negative rates of 12.6% and 14.2%, respectively, slightly higher than in the upfront setting. Moreover, the number of resected sentinel nodes inversely correlated with the false-negative rate, and the data suggested that the removal of at least two sentinel nodes in this setting is important to keep the accuracy of sentinel node biopsy similar to that in the upfront setting.

A pCR to NACT may identify subsets of patients with lower risk for locoregional recurrences (LRR) that may not justify regional chest wall and/or regional nodal radiation. This approach is predicated on the demonstration that downstaging substantially lowers the rates of locoregional recurrence. The NSABP B-18 and B-27 trials provide an important data set to address these questions. In both trials, regional nodal radiation after lumpectomy or chest wall and regional nodal radiation after mastectomy were not permitted per protocol, thus avoiding the confounding effects of selective use of radiation in patients with positive nodes or other high-risk features. Data from a combined analysis of these two trials were recently published. The 10-year cumulative incidence of LRR was 12.3% for patients treated with mastectomy (8.9% local; 3.4% regional) and 10.3% for those treated with lumpectomy plus breast radiation (8.1% local; 2.2% regional). Independent predictors of LRR in patients treated with lumpectomy were age, clinical node status (before NACT), and pathologic node status/pathologic breast tumor response. In patients treated with mastectomy, independent predictors of LRR were clinical tumor size (before NACT), clinical node status (before NACT), and pathologic node status/pathologic breast tumor response. By using these independent predictors, risk of LRR can be tailored, and the need for postoperative radiotherapy could be individualized.

On the basis of these findings, a prospective, randomized phase III clinical trial led by NSABP and RTOG (NSABP B-51/RTOG 1304) has been initiated to validate the approach of tailoring the use of radiation for patients who present with documented involvement of axillary nodes before NACT but are found to have histologically negative nodes afterward. Alternatively for patients who present with documented involvement of axillary nodes before NACT and are found to be pathologically node-positive based on sentinel node biopsy following NACT, the Alliance for Clinical Trials in Oncology is conducting a phase III trial (A011202) randomizing patients to completion axillary dissection plus regional RT compared with regional RT alone.

**DEVELOPING NEW AGENTS IN THE NEOADJUVANT SETTING**

Sequential taxane/anthracycline-based three-drug regimens have become standard for neoadjuvant therapy of patients who present with stage II or III breast cancer and are candidates for chemotherapy. Trastuzumab is incorporated into these regimens in HER2+ disease. These regimens result in pCR rates of 20% to 50% in patient populations with subtypes of breast cancer associated with high proliferative rates. Although current standard therapies provide substantial benefits for patients with a pCR at surgery, patients with residual disease are at substantial risk for disease recurrence, with the majority of patients with HER2+ or triple negative breast cancers who do not achieve pCR developing distant metastatic disease within the first three years after therapy. Attempts to improve pCR rates with additional standard chemotherapeutic agents have not been successful.

Therefore efforts to improve pCR rates and develop alternative therapies for women with residual disease following standard therapy are clearly needed. Insights into the heterogeneity and biology of breast cancer obtained through gene-sequencing and other technologies have identified a large number of potential targets in these tumors for newer, more selective investigational agents (so-called targeted therapies). Toxicity of many of these agents appears to be substantially less problematic than with more nonselective agents, and combination with chemotherapy appears to be feasible. The neoadjuvant setting provides an opportunity to accelerate evaluation of these agents and identify predictive biomarkers for response, as well as to gain insights into mechanisms of resistance. Other interventions, including immunotherapy and complementary approaches...
in the neoadjuvant setting, are also being used or are under consideration.

SCREENING TRIALS
Because palpable or locally advanced breast cancer is readily accessible for serial biopsies and imaging assessment, the neoadjuvant setting provides opportunity for screening new agents in combination with chemotherapy, while also obtaining serial biopsies to understand biology of response and resistance as well as to develop early predictors of response. The prototype for this important step in drug development is the I-SPY TRIAL 2.23 This ambitious, multicenter, randomized phase II effort among academic cancer centers across the United States employs a standard regimen of weekly paclitaxel for 12 weeks followed by AC for four cycles. Trastuzumab is administered with paclitaxel for HER2+/breast cancer in the control arm and in several of the treatment arms. Potential candidates undergo biopsy for screening, which includes the 70-gene Mammaprint (MP) along with ER, PR, and HER2 assessment. Women with low-risk MP score and ER+/HER2 negative disease are excluded from entry and randomization.

Several agents (up to eight) are under evaluation at any time point, with assignment to control therapy or investigational therapy on the basis of results of the molecular profiling and an adaptive randomization design. The primary objective is to determine whether adding experimental agents to standard NACT (with or without trastuzumab) increases the probability of pCR over standard NACT for each biomarker signature established at trial entry, and to determine for each experimental agent used the predictive probability of success in a subsequent phase III trial for each possible biomarker signature. A data safety monitoring board (DSMB) meets monthly to assess toxicity and review performance of the adaptive randomization, given the potential curability of patients receiving investigational therapy. A biopsy for pharmacodynamic markers is obtained after three weeks of therapy, and serial imaging is performed throughout the trial using automated dynamic contrast enhanced MRI. As information accumulates on each agent, it is either “graduated” or “dropped for futility,” and new agents enter the trial. The basic design of the study remains unchanged so that additional agents and potential biomarkers can be more rapidly and efficiently evaluated. Although the I-SPY TRIAL 2 will not provide definitive information on a new investigational agent, it is hoped that promising agents will be quickly identified and moved into phase III trials with a much higher probability for success than has been seen in the past.

NEOADJUVANT TRIALS POWERED FOR DISEASE-FREE AND OVERALL SURVIVAL
In the potentially curative situation, it is likely that regulatory agencies will continue to require trials to conclusively demonstrate improvement in DFS and perhaps OS for approval of new agents in this setting. The FDA released a draft guidance in May 201224,25 stating that the agency would consider granting accelerated approval on the basis of a surrogate end point that is likely to predict clinical benefit, and it proposed pCR as such an end point in the neoadjuvant setting for high-risk early stage breast cancer. Confirmation of clinical benefit with an improvement in DFS or OS would still be required or the indication could be withdrawn if confirmatory trials do not show a benefit.

A potential approach would be to use a two-step design in which only neoadjuvant patients were entered in the first stage to determine whether a prespecified improvement in pCR could be demonstrated. If so, the second stage would allow entry of patients in both the neoadjuvant and adjuvant setting to achieve the sample size needed for determining DFS and OS. Interim analyses of pCR rates could be used to determine whether an accrual hiatus between the two stages was necessary. Accrual to this type of trial could be readily accomplished in cooperative group and community settings.

POSTNEOADJUVANT RESIDUAL DISEASE TRIALS
Because patients with pCR following standard adjuvant therapies have a low risk for recurrence, it may not be appropriate to evaluate new agents with moderate toxicities or which do not combine well with standard therapies in these patients. In this situation an alternative development approach would be to study new agents in patients with high-risk residual disease following standard treatment regimens.

The NSABP, GBG, and other investigators are collaborating on a global trial (KATHERINE NCT 01772474) evaluating T-DM1 as an alternative to continuation of trastuzumab in women with residual disease following neoadjuvant chemotherapy combined with HER2 targeted therapy that includes trastuzumab. Patients with residual disease following NACT and trastuzumab have a three-year RFS of only 70%,26 so they are appropriate candidates for evaluating promising new therapeutic agents such as T-DM1. If successful this trial would establish a new approach for drug development.

Two innovative trials are in progress to address residual disease in patients with triple-negative breast cancer. The Hoosier Oncology Group is currently evaluating the benefit of cisplatin with or without a PARP inhibitor, rucaparib (Clovis) in women with triple negative or ER+/BRCA-mutant breast cancer who have residual disease in the breast after neoadjuvant chemotherapy with an anthracycline and/or taxane-based regimen (NCT01074970).27 The primary end point of this trial is two-year DFS. The ABCDE trial (Adjuvant Bevacizumab, Metronomic Chemotherapy Diet and Exercise)28 being conducted within the Translational Breast Cancer Research Consortium (TBCRC) enrolls patients with triple negative or high-stage ER+ disease who have received anthracycline and/or taxane-containing neoadjuvant chemotherapy and have residual disease at surgery. In this 2 x 2 design, patients are initially randomly assigned to either “observation” (standard of care, including endocrine therapy), or “treatment” (six months of bevacizumab with
metronomic chemotherapy, followed by an additional 18 months of intermittent bevacizumab alone). Patients are additionally randomized to one of two telephone-based lifestyle interventions: diet alone or diet plus exercise.

NEOADJUVANT ENDOCRINE THERAPY TRIALS

Development of multigene expression profiles to predict the degree of benefit from specific therapeutic agents has been challenging; to date, a profile for defining benefit from specific agents has not been established. However the Oncotype DX Recurrence Score assay derived from an algorithm based on expression level of 16 breast cancer-related genes and five reference genes has been shown to consistently identify large subsets of patients with early stage, ER+ breast cancer who have a low-risk for recurrence with endocrine therapy alone.29 The assay also has apparent utility for defining potential benefit from the addition of chemotherapy in node-negative ER+ breast cancers.30 The utility of the assay in this population will be better defined by results of the TAILORx study, which has completed accrual and is in follow-up.31 SWOG is leading an effort (RxSPONDER) to evaluate the utility of the Recurrence Score for defining chemotherapy benefit in women with node-positive, ER+ breast cancers as well.32 Studies evaluating the Recurrence Score as well as other predictive assays have shown many women with early stage, ER+ breast cancer derive little or no benefit from the addition of chemotherapy to endocrine therapy. In view of these findings, it is not surprising that pCR rates in response to chemotherapy are low in these populations and that outcome is not associated with pCR.

Given these observations, the neoadjuvant setting is ideal for evaluating endocrine therapies in combination with newer targeted therapies in appropriate patient populations. Ellis and colleagues conducted a randomized phase II study of four months of neoadjuvant endocrine therapy with tamoxifen vs. letrozole and evaluated post-therapy pathology findings to derive a response-based preoperative endocrine prognostic index (PEPI) to define a subset of patients who do well with endocrine therapy alone.33 The index was validated in an independent cohort of patients from another neoadjuvant endocrine study, the IMPACT trial.34 The authors concluded that “patients with breast cancer with pathologic stage I or 0 disease after neoadjuvant endocrine therapy and a low-risk biomarker profile in the surgical specimen (PEPI score 0) have an extremely low risk of relapse and are therefore unlikely to benefit from adjuvant chemotherapy.”

ACOSOG conducted a follow-up randomized phase II neoadjuvant trial (Z1031) comparing the three approved aromatase inhibitors in postmenopausal women with ER-rich stage II to III breast cancers. They demonstrated an overall clinical response rate of 63% and that breast-conserving surgery could be performed in 50% of patients who presented with disease that would have required mastectomy at presentation. They also demonstrated that a favorable PEPI score was more common in luminal A than luminal B tumors (27.1% ± 10.7%; P = 0.004).35 The ALTERNATE trial (ACOSOG Z11103) will be a phase III study comparing anastrozole vs. fulvestrant vs. the combination as neoadjuvant endocrine therapy in postmenopausal women with ER-rich stage II and III breast cancer. The study will also prospectively evaluate the PEPI score as a predictive factor for favorable outcome with endocrine therapy alone. This innovative series of trials has established neoadjuvant endocrine therapy as a valid treatment and research option for ER-rich early breast cancer and will likely be further exploited in the future for combination endocrine/targeted therapy trials.

CONCLUSION

Neoadjuvant therapy for breast cancer has an established role in the management of women with inoperable disease and for those in whom breast conservation is a goal of therapy. Early randomized trials established pCR as a surrogate for survival outcomes, and more recent trials have refined our understanding of this relationship for different molecular subtypes of disease. Clinical management to minimize LRR continues to evolve, with recent studies evaluating the applicability of sentinel node evaluation after NACT, the relative contribution of nodal response to predicting outcome, and ongoing trials designed to optimize radiotherapy.

From a research standpoint, the neoadjuvant setting provides several important opportunities for translational drug development. Phase II randomized “screening studies” to improve pCR rates in high-risk patients with the addition of targeted therapies to standard chemotherapy are ongoing and will contribute important information on both drug activity and biomarkers identifying those patients most likely to respond. Such studies will contribute to the pipeline of agents for testing in phase III trials, with the ability to potentially accelerate approval of successful therapies by using the new FDA guidance that provides a roadmap for the use of pCR as a surrogate end point and smaller, more focused trial designs in specific biologically-defined subgroups to assess survival. Molecular and genetic profiling of residual disease after neoadjuvant therapy is an active avenue of investigation into potential targets of resistant disease, and trials of agents against these targets have the potential to improve outcome for patients with poor prognosis after standard therapy. The recognition that ER+ low-proliferation tumors are often intrinsically unresponsive to chemotherapy (and thus show low pCR rates) has led to a parallel pathway of investigation into neoadjuvant endocrine therapy, in which Ki-67 is being validated as a surrogate for response and outcome. Taken together, the advances brought about by neoadjuvant therapy in both standard and investigational settings have ushered in a more personalized approach to breast cancer therapy that could result in a reduced burden of toxicities and recurrent disease and in improved survival.
Disclosures of Potential Conflicts of Interest

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BREAST CANCER

Surveillance and Monitoring in Breast Cancer Survivors: Maximizing Benefit and Minimizing Harm

CHAIR
Maxine S. Jochelson, MD
Memorial Sloan-Kettering Cancer Center
New York, NY

SPEAKERS
Daniel F. Hayes, MD
University of Michigan Medical Center
Ann Arbor, MI

Patricia A. Ganz, MD
University of California, Los Angeles Schools of Medicine and Public Health
Los Angeles, CA
Surveillance and Monitoring in Breast Cancer Survivors: Maximizing Benefit and Minimizing Harm

Maxine Jochelson, MD, Daniel F. Hayes, MD, and Patricia A. Ganz, MD

OVERVIEW

Although the incidence of breast cancer has increased, breast cancer mortality has decreased, likely as a result of both breast cancer screening and improved treatment. There are well over two million breast cancer survivors in the United States for whom appropriate surveillance continues to be a subject of controversy. The guidelines from the American Society of Clinical Oncology (ASCO) and the American College of Physicians are clear: only performance of yearly screening mammography is supported by evidence. Although advanced imaging technologies and sophisticated circulating tumor biomarker studies are exquisitely sensitive for the detection of recurrent breast cancer, there is no proof that earlier detection of metastases will improve outcome. A lack of specificity may lead to more tests and patient anxiety. Many breast cancer survivors are not followed by oncologists, and their doctors may not be familiar with these recommendations. Oncologists also disregard the data. A plethora of both blood tests and nonmammographic imaging tests are frequently performed in asymptomatic women. The blood tests, marker studies, and advanced imaging techniques are expensive and, with limited health care funds, may prevent funding for more appropriate aspects of patient care. Abnormal marker studies lead to additional imaging procedures. Repeated CT scans and radionuclide imaging may induce a second cancer because of the radiation dose, and invasive procedures performed as a result of these examinations also add risk to patients without clear benefits. Improved adherence to the current guidelines can cut costs, reduce risks, and improve patient quality of life without adversely affecting outcome.

Society has been trained by the lay press—often stimulated by the medical field—to be aware of signs and symptoms of cancer. Although this concept is usually applicable to unaffected people, the perception that earlier detection is better has been ingrained in most patients who have been diagnosed with breast and other cancers. This concern raises issues about whether early detection of occult metastases in patients with asymptomatic cancer has clinical utility, and if so, what are the optimal tools to use for surveillance.

REASONS TO PERFORM SURVEILLANCE OF PATIENTS WITH ASYMPTOMATIC BREAST CANCER

One might monitor asymptomatic patients with breast cancer for one of several reasons. Several prospective trials of adjuvant therapy have demonstrated that early systemic therapy improves overall survival (OS) when compared with waiting until a patient has symptomatic disease, detectable by standard radiographic techniques.1-5 This observation stimulated the hypothesis that monitoring patients for early recurrence after standard adjuvant systemic therapy might permit early detection of impending metastases leading to earlier treatment, which would result in improved outcomes. The main anticipated benefit of such a strategy is to administer systemic therapy before the cancer has developed inherent resistance and therefore to improve OS. A second purported reason to monitor asymptomatic patients might be “up-front palliation.” In this scenario, early detection of impending relapse might permit initiation of a new therapeutic regimen that might delay the time to progression and cancer-related symptoms. Therefore, one might argue that even if this strategy does not improve OS, the delay in onset of cancer-related symptoms would outweigh the toxicities associated with the earlier treatment. A secondary hypothesis related to this concept would be that identification of an impending catastrophic complication of a metastasis, such as fracture, brain metastasis, or advanced visceral involvement, would be beneficial to the patient. Finally, it has been argued that no evidence of recurrence on a surveillance test is reassuring to patients who are otherwise doing well.

EVIDENCE REGARDING SURVEILLANCE OF PATIENTS WHO ARE ASYMPTOMATIC

To address this hypothesis, two prospective trials were initiated in Italy in the 1980s in which women with a prior history of breast cancer and who were asymptomatic and free of disease were randomly assigned to either routine follow-up...
without any special testing or periodic evaluation for occult metastases using standard techniques available at the time.6,7 Neither of these two trials demonstrated any of the above benefits. Indeed, in one of these trials serial quality-of-life analysis suggested that the anxiety and false-positive findings associated with surveillance were worse in the screened patients. Neither of these trials encompassed what would be considered modern diagnostic techniques (such as circulating tumor markers or new imaging techniques, including CT, PET, or MRI). In this regard, in a Finnish trial conducted in the 1990s, patients were randomly assigned to blood counts, sedimentation rate, liver enzymes, and CA15-3 every 3 or 6 months after primary treatment or to routine use of diagnostic examinations or use based on clinical grounds only if concerning clinical findings were identified.8 This trial also failed to detect any difference in outcome among any of the arms.8 A 2005 Cochrane Collaboration-sponsored meta-analysis of these trials concurred that there was no apparent benefit to intensive surveillance of patients who were asymptomatic following primary and adjuvant systemic therapy for breast cancer.9

CURRENT GUIDELINES REGARDING SURVEILLANCE OF ASYMMPTOMATIC BREAST CANCER SURVIVORS
Based on the results of the studies from two separate ASCO guidelines committees, as well as the Breast Committee of the National Cancer Center Network (NCCN), these organizations have strongly recommended against any sort of routine surveillance for metastatic (disease other than routine screening for locally recurrent tumor within the breast or new primary cancers with mammography and age-appropriate screenings have strongly recommended against any sort of routine surveillance for metastatic breast cancer.10-12 In summary, the only routine surveillance recommended by ASCO and NCCN is standard breast imaging for detection of a new primary or in-breast recurrence. Mammography is considered the standard of care in this regard. Breast MRI is only recommended in genetically high-risk women or women who had received mediastinal radiation for Hodgkin lymphoma who do not elect to have contralateral prophylactic mastectomy.13 Indeed, to address a challenge to medical specialties to identify diagnostic tests or treatments that are commonly performed but of dubious meaningful benefit to patients, ASCO’s Cost of Care Task Force included lack of adherence to breast cancer surveillance guidelines as one of the “Top Five” list for oncology.14

WHY NOT PERFORM ROUTINE SURVEILLANCE TESTING?
Overuse of imaging is associated with certain risks. These include expense, radiation risk, anxiety, and the risk of additional procedures.15

Expense
The cost of health care is a major problem in this country and has become a major political issue as well. The United States spends more on health care per capita than most other countries without better results. The cause is multifactorial but includes cultural issues and fear of lawsuits. Sometimes it is just easier to say yes to a breast cancer survivor with no additional risk factors and fatty breasts who insists on having a yearly breast MRI for her remote breast cancer than to show her why it is not indicated. The current charge for a bilateral breast MRI can be more than $4,000.00. In a cohort of more than 25,000 women diagnosed between 1998 and 2003 with stage I to II breast cancer from the SEER-Medicare linked database who survived more than 48 months, Panageas et al reported the cost of the MRIs alone would be almost $1.5 million, and even in the best hands there are many false-positive findings leading to short interval follow-up MRI, core biopsies, and occasionally surgery, making it even more expensive.16

As noted above, although occasionally only a chest X-ray is performed for surveillance, advanced imaging surveillance for metastatic disease is often performed with either CT of the chest, abdomen, and pelvis and bone scan or more recently PET/CT. Charges for a CT and bone scan can run up to $6,000 and PET/CT is even more. Women may have multiple examinations each year. There will be a number of positive findings, which will generate additional imaging, biopsies, or even surgery. It has been shown that intensive surveillance testing adds an additional $260 million to $630 million to the annual cost of caring for breast cancer survivors without demonstrated benefit compared with a strategy that follows the guidelines.17

Radiation Risk
There has been a great deal of consternation regarding the possibility of cancers developing as a result of imaging, particularly from the increasing use of CT scans. Much of the data regarding the radiation risk from imaging studies comes from studies of survivors of atomic bombs dropped in Hiroshima and Nagasaki as well as from 400,000 radiation workers in the nuclear industry.18 There are various ways to define
radiation exposure, but it is most frequently defined as the effective dose of the radiation delivered (measured in sieverts [Sv] or milli sieverts [mSv]), particularly as it refers to specific organ dose. Long-term survivors of atomic bomb exposures had an increased risk for cancer, having received 10–100 mSv. The Hiroshima and nuclear worker data are based on whole-body exposure. Although CT exposure measured in the current literature is to limited portions of the body, a single CT scan can deliver an equivalent radiation exposure. Brenner and Hall suggested that 1.5% to 2% of cancers in the United States might be attributable to CT scans.\(^{19}\) Berrington de Gonzalez et al used risk models to estimate future cancer risks from CT scan use in the United States according to age, gender, and scan type. They determined that approximately 29,000 future cancers could be the result of the 72 million CT scans performed in the United States in 2007, predominantly from CTs of the abdomen and pelvis. As demonstrated in multiple other studies, the projected number of cancers decreased with increasing age at exposure. Although pediatric patients are at highest risk, in terms of absolute numbers, the highest potential effect is in women age 35 to 54.\(^{20}\) A portion of the breast cancer survivor population falls into this category.

In trying to understand patients’ actual radiation exposure patients, Smith-Bindman et al conducted a retrospective study evaluating the doses of the 11 most common types of CT in 1,119 adult patients.\(^{21}\) Not surprisingly, doses varied among various studies. However, within each study type, they detected a 13-fold variation between the highest and lowest doses, making it very difficult to assess individual risks. More recently, a great deal of effort has been made to reduce the dose of radiation delivered by CT scans, and there are now requirements for documentation of delivered dose for each scan performed. However, the most effective dose reduction would be to eliminate unnecessary scans.

**FALSE-POSITIVE FINDINGS GENERATE ADDITIONAL EXPENSIVE AND RISKY PROCEDURES**

As mentioned previously, additional imaging exams generate additional procedures. The lower the prevalence of disease is in a population, the higher the false-positive rate will be. In a population of women who have early-stage breast cancer, recurrence rates are low. For example, Drotman et al retrospectively reviewed 2,426 pelvic CT scans in patients with breast cancer and demonstrated that not only was there a low yield of cancer detection of disease in the pelvis as the only site of metastasis, but also performance of pelvic CT led to 204 additional tests. These included 186 pelvic ultrasounds and 50 surgical procedures. These additional procedures were normal, benign, or indeterminate in 84.6%.\(^{22}\)

**DO PHYSICIANS STILL MONITOR PATIENTS WHO ARE ASYMPTOMATIC TO DETECT OCCULT METASTASES?**

Despite these data and guideline recommendations, many physicians continue to order either circulating tumor marker studies—which, if abnormal, lead to imaging studies—or imaging studies including chest radiographs; MRI; bone scans; CT scans of the chest, abdomen, and pelvis; and PET/CT scans, whereas mammography surveillance is performed less frequently than expected.\(^ {17-23}\) In a cohort of more than 11,000 Canadian women followed for 5 years, one-quarter had less than the recommended mammographic surveillance, whereas one-half had more than recommended imaging for metastatic disease.\(^ {23}\) Han et al surveyed 1,098 primary care physicians (PCPs) and medical oncologists, and 84% of PCPs and 72% of oncologists reported beliefs consistent with blood test overuse, and 50% of PCPs and 27% of oncologists had beliefs consistent with imaging test overuse.\(^ {24}\)

From a recent national survey of PCPs and oncologists—the Survey of Physician Attitudes Regarding the Care of Cancer Survivors (SPARCCS)—conducted in 2009, we learned that both physician groups recommended unnecessary tumor marker and imaging studies in response to a vignette-based case of a 4-year asymptomatic breast cancer survivor.\(^ {25}\) Specifically, 51% of PCPs and 31% of oncologists recommended tumor markers; 42% of PCPs and 22% of oncologists recommended chest X-rays; and 23% of PCPs and 3% of oncologists recommended bone scans.\(^ {25}\) Although 85% of oncologists reported being “very confident” about their knowledge related to follow-up care, only 40% of PCPs reported high confidence in perceived follow-up skills (p < 0.001). Thus, there appears to be a need to improve knowledge among PCPs regarding the follow-up care of breast cancer survivors.

The exact number of “higher technology” studies performed in the earlier investigations of breast cancer surveillance cannot be determined from the available data. These include CT, MRI, PET, and bone scans. In the cohort of women reported by Panageas, 40% had at least one high-tech examination, and 30% had CT scans. Over time, use of these tests increased from 34% of women diagnosed in 1998 to 43% in women diagnosed in 2003. The increasing use of high-tech imaging continued through 2005.\(^ {16}\)

**IS THERE A NEED TO MONITOR PATIENTS WHO ARE ASYMPTOMATIC?**

A major concern regarding the recommendations to not screen asymptomatic patients is that they are based on outdated technology, lacking sensitivity and specificity. In addition, outcome data could possibly be improved in the future with the use of new antineoplastic agents causing less toxicity and better antitumor activity (possibly resulting in improved OS compared with older therapies). For example, although trastuzumab is now used routinely as adjuvant therapy for patients with HER2-positive breast cancer, it was shown in a prospective randomized trial to provide an OS benefit for women with metastatic breast cancer.\(^ {26}\) The results of this study suggest that earlier treatment of women with HER2-positive metastatic disease is superior to waiting for later treatment, since the OS benefit was observed even though many patients were permitted to cross over from the
chemotherapy-only arm to receive trastuzumab. Therefore, one might extrapolate that data to suggest that treatment of a patient who is asymptomatic with impending relapse might be more effective than waiting for development of clinically apparent metastases.

Although these are cogent arguments, they have not been demonstrated with any level of evidence and are only theoretical at present. Investigators in the North American cooperative groups are currently considering a new prospective trial that would incorporate modern circulating tumor biomarkers and imaging techniques.

### Circulating Tumor Biomarkers

Several studies have demonstrated that circulating routine liver and bone enzymes have very poor sensitivity and specificity for detection of metastases.\(^1\)\(^1\) Circulating soluble tumor biomarkers—in particular MUC-1 protein and carcinoembryonic antigen (CEA)—have been shown to have more accurate performance characteristics in this setting. Two assays for circulating MUC-1 protein (CA15-3 and CA27.29) provide nearly identical results. MUC1 is elevated in approximately 75% of women with symptomatic metastases and approximately 50% of women with asymptomatic metastases.\(^2\)\(^7\) CEA is elevated in approximately 50% of women with symptomatic metastases and 25% of women with asymptomatic metastases. The two are complementary, raising the sensitivity in both settings by approximately 10%. However, both result in false-positive findings, which are usually associated with benign inflammatory conditions of the gut, lung, pleura and peritoneum, and liver.\(^2\)\(^7\) Depending on the cutoffs used to distinguish a positive from a negative finding, the positive predictive value (PPV) of an elevated circulating tumor biomarker ranges from 50% to 95%. As expected, the PPV is particularly high if one applies especially stringent cutoffs for “positivity” to patients with high-risk disease, such as T3 primary lesions or positive lymph nodes.

Regardless, the median lead time from rising tumor biomarker levels (again, depending on the criteria used for “positive”) ranges from 3 to 12 months.\(^2\)\(^7\)

The negative predictive value (NPV) of these markers for recurrence within the next 6 to 12 months in an asymptomatic patient is quite high, but that is based more on the relatively low risk of recurrence for any such patient, especially if she had a relatively favorable prognosis and/or is several years away from her original diagnosis.\(^2\)\(^7\)\(^2\)\(^8\) In other words, the NPV for recurrence by history and physical examination is quite high and is probably not improved by negative results of circulating biomarkers.

Recently, circulating tumor cells (CTC) have been shown to be associated with a worse prognosis in patients with either early-stage or metastatic breast cancer.\(^2\)\(^9\)\(^3\)\(^0\) However, there are no data regarding use of CTC to monitor patients who are asymptomatic.

### Educating Oncologists and Primary Care Physicians About the Lack of Evidence for Surveillance Testing

Ongoing surveillance despite the recommendations raises the question as to why physicians continue to pursue this strategy. In a subsequent analysis from the SPARCCS study, Han et al examined the beliefs about breast cancer surveillance testing among PCPs and oncologists in response to the vignette case of a 4-year asymptomatic and disease-free breast cancer survivor.\(^2\)\(^4\) Both groups of physicians reported beliefs consistent with overuse of blood and imaging tests that were not indicated for surveillance in this setting. In multivariable models, they examined physician and practice setting factors that might be associated with the beliefs in overuse of these tests and procedures. Factors significantly associated with PCP beliefs related to overuse of blood tests included working in a smaller practice setting and being a full or part owner of the practice. For imaging tests, having a lower volume of patients with breast cancer and being in a smaller metropolitan area (< 1 million population) were significantly associated with greater overuse beliefs. In the case of oncologists, factors significantly associated with greater overuse beliefs for both blood and imaging tests included older age, being internationally trained, and having lower self-efficacy (confidence in knowledge about surveillance testing) and higher perceived ambiguity about testing recommendations. Blood test overuse beliefs were significantly greater among Asian oncologists than white oncologists and were lower among employed oncologists than practice owners. Patient with breast cancer volume was significantly associated with overuse beliefs for imaging tests, with a nonlinear pattern that was difficult to interpret.\(^2\)\(^4\)

The differences in factors associated with overuse beliefs between PCPs and oncologists are interesting and point to different strategies that should be considered to improve knowledge and reduce overuse of nonindicated testing in each group of physicians. Although the SPARCCS study did not find a significant relationship between the receipt of a survivorship care plan and overuse beliefs among PCPs, it is possible that this strategy has not yet had an effect on surveillance care because of the lack of self-efficacy and ambiguity regarding these tests noted among oncologists who would be the individuals expected to prepare a survivorship care plan. For oncologists, it appears that reducing ambiguity about surveillance guidelines and improvement in self-efficacy may have value in addressing their use of unnecessary tests. Although the surveillance guidelines for patients with breast cancer have been available from ASCO since 2006, with recent reissue, adherence to these guidelines has not been regularly monitored in most clinical practice settings.\(^3\)\(^1\) Perhaps the selection of this guideline as one of ASCO’s recommendations for the American Board of Internal Medicine Foundation’s Choosing Wisely Campaign will provide an impetus for health care organizations and oncology practices to monitor the overuse of blood tests (including tumor markers) and advanced imaging tests in breast cancer survivors.\(^3\)\(^1\)
Providing feedback to oncologists regarding their use of these tests in breast cancer survivors could help them appreciate what their own practice patterns are and demonstrate their lack of awareness of the guidelines. Oncologists who work together in a practice setting should be encouraged to discuss their ambivalence about the guidelines among their peers to establish standards for their practice settings.

COMMUNICATING TO OUR PATIENTS THE RECOMMENDATIONS TO NOT SCREEN FOR ASYMPTOMATIC METASTASES

Given the common misconceptions about the benefits of early detection of recurrent breast cancer among physicians described earlier, it is not surprising that in practice, relatively few oncologists fully adhere to the ASCO and NCCN guidelines regarding lack of value of tumor markers and imaging studies in the follow-up of patients with breast cancer and survivors. When patients present for regular follow-up visits after adjuvant chemotherapy and radiation therapy, they deserve a good history and physical examination, as well as a comprehensive symptom assessment that will elicit lingering side effects from past treatment as well as ongoing endocrine therapy. These efforts also serve to identify new onset problems that might herald a recurrence of the disease. Unfortunately, cognitive services such as this have been devalued in the procedure-oriented world of fee-for-service medicine. Many providers and patients do not believe this is sufficient, leading the patient to request and the provider to order blood tests and scans. Instead, this time with the patient should be spent educating them about the natural history of breast cancer, the individual woman’s prognosis (which might be excellent), the harms of surveillance testing (e.g., false-positives, false-negatives, radiation exposure, and costs), and the lack of benefit from testing.

Fear of recurrence is extremely common, and patients do not understand that these tests provide false reassurance that everything is okay. Instead, the oncology provider, in the cognitive exchange, must reassure the patient that they should report any symptoms or concerns when they occur, and a prompt diagnostic work-up will be undertaken. Psychosocial services should be provided to help the patient cope with ongoing uncertainties and the challenges of adjusting to the “new normal” of survivorship. The use of treatment summaries and care plans can facilitate this conversation with use of the patient information sheet that ASCO has provided along with its guidelines on surveillance testing after early-stage breast cancer treatment. The treatment summary and care plan should be shared with the patient’s PCP to ensure unnecessary testing does not occur in that setting. A focus on health promotion and well-being—including physical activity, energy balance, and social engagement—should be emphasized rather than using testing as the default activity in the follow-up of the breast cancer survivor.

CONCLUSION

In summary, contrary to the perceptions held by many patients and their physicians, available data do not support routine surveillance for occult metastases asymptomatic patients with a prior history of breast cancer. It is important to continue breast imaging for a patient with intact breasts. Prospective randomized trials have failed to demonstrate improvement in any clinically meaningful endpoints for surveillance with routine blood tests, circulating tumor markers, or other imaging. Surveillance screening is associated with false-positive findings, induction of anxiety, risk of exposure to radiation, and higher medical expenses. Conveyance of this information with a thoughtful recommendation that a patient not undergo screening is an important part of clinical management of breast cancer survivors. The availability of more sophisticated screening techniques and more effective, less toxic therapies raises the possibility that surveillance might be effective using these newer modalities, but this hypothesis must be demonstrated in prospective trials before it is introduced into standard care.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated. Authors marked with an asterisk (*) are participants in ASCO’s Disclosure Management System Pilot; their disclosure is not limited to subject matter under consideration in this article and includes payments to themselves, an immediate family member (I), and/or their institutions (Inst). For information on the pilot program, or to provide feedback, please visit coipilot.asco.org.


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BREAST CANCER

Treatment Algorithms for Hormone Receptor-Positive Advanced Breast Cancer

CHAIR
Stephen R. D. Johnston, MA, MD, PhD
The Royal Marsden Hospital NHS Foundation Trust
London, United Kingdom

SPEAKERS
Todd W. Miller, PhD
Dartmouth College
Hanover, NH

Stephen K. L. Chia, MD
British Columbia Cancer Agency
Vancouver, BC, Canada
Treatment Algorithms for Hormone Receptor-Positive Advanced Breast Cancer: Applying the Results from Recent Clinical Trials into Daily Practice—Insights, Limitations, and Moving Forward

Sheridan Wilson, MBChB, and Stephen K. Chia, MD

OVERVIEW

Hormone receptor-positive (HR+) breast cancer is the most prevalent subtype of breast cancer in both early- and advanced-stage disease. Thus, the treatment of HR+ breast cancer has had the greatest global influence in improving clinical outcomes overall. Although the first-line metastatic breast cancer (MBC) trials comparing a third-generation aromatase inhibitor (AI) to tamoxifen have favored the AI, one of the challenges in translating these findings into clinical practice stems from the influence of prior adjuvant endocrine therapy, particularly the increasing use of adjuvant AIs today, on the choice of endocrine agent in the advanced setting because of the development of acquired resistance. Because the majority of patients enrolled into these studies were either endocrine-treatment naïve or exposed to tamoxifen only, the “real-life” applicability of the evidence is unclear. Because a superior dose of the selective estrogen receptor (ER) downregulator fulvestrant has now been established, its role as first-line therapy is being re-established. We are now starting to see the promise realized with blocking cross-talking growth factor pathways in addition to the ER pathway. The greatest efficacy is seen with the mammalian target of rapamycin (mTOR) inhibitor everolimus in combination with exemestane and, perhaps to a lesser extent, anti-HER2-directed therapy in combination with an AI. Future gains will likely involve a greater understanding of the redundancy and compensation induced by blocking these pathways, trials involving blocking multiple pathways in addition to hormonal agents, and the molecular interrogation of the individual’s tumor in search of predictive biomarkers and “actionable” genomic aberrations.
significantly longer for anastrozole (11.1 vs. 5.6 months) in the smaller North American trial, although no difference was observed in the larger European TARGET study (8.3 months vs. 8.2 months). It should be noted that no OS difference was demonstrated in either of the trials. A higher percentage of known HR+ patients and patients who had received adjuvant tamoxifen in the North American study may have selected for an outcome favoring the AI. The two trials were also prospectively designed to facilitate a combined analysis in which no difference in PFS was demonstrated.

In the PO25 study, letrozole was associated with a significantly improved median time to progression (TTP) (9.4 months vs. 6 months for tamoxifen, hazard ratio = 0.72 p < 0.0001). The study design built in cross-over at the time of progression. There was no significant difference in median OS (hazard ratio = 0.96; 95% CI 0.84 – 1.09). The steroidal AI, exemestane, has also been shown to improve median PFS over tamoxifen (9.9 vs. 5.8 months), without an OS benefit (hazard ratio = 1.13; 95% CI 0.85 – 1.5).

Fulvestrant, an estrogen-receptor downregulator, was initially approved for use in the second-line setting at a dose of 250 mg monthly. Over the last 5 years there has been an evolution of the recommended dose and of its position within the treatment algorithm for HR+ MBC. An updated analysis of the FIRST trial (a randomized phase II study), comparing a higher dose (HD) of fulvestrant (500 mg days 1 and 14, followed by 500 mg monthly thereafter) with anastrozole, confirmed superior TTP for fulvestrant (23.4 months vs. 13.1 month) compared with anastrozole. It is noteworthy that a high proportion of patients in both study arms were endocrine-treatment naïve (71.6% fulvestrant arm and 77.7% anastrozole arm), which may make interpretation of these results confounded in those patients relapsing on an adjuvant hormonal regimen. Both treatments were well tolerated with no important differences in prespecified events. Notwithstanding the limitations of this as a phase II open-label study, the role of fulvestrant as first-line treatment for HR+ MBC is promising and the basis for a phase III randomized control trial (FALCON).

Historically, combination regimens have failed to demonstrate a benefit over single endocrine agents. Furthermore, in the adjuvant setting the combination arm of the ATAC trial failed to show efficacy over tamoxifen alone and was discontinued. A pharmacokinetic interaction whereby anastrozole concentrations were lower in the combination with tamoxifen has been proposed as an explanation for this finding. In preclinical studies fulvestrant is active in a low estrogen environment and has demonstrated efficacy in combination with an AI. Fulvestrant competes with estrogen for binding of the ER, and reducing estrogen levels might enhance efficacy by allowing increased fulvestrant-ER binding.

Clinical studies evaluating the efficacy of fulvestrant by using a loading dose (500 mg day 1, 250 mg days 14 and 28, and every 28 days thereafter) in combination with anastrozole compared with anastrozole alone have reported mixed results. In the SWOG S0226 study a PFS and OS benefit in favor of the combination arm were seen (PFS 15 vs. 13.5 months, hazard ratio = 0.80; 95% CI 0.68 – 0.94; p = 0.007, OS 47.7 vs. 41.3 months, hazard ratio = 0.81; 95% CI 0.65 – 1.0; p = 0.049). In contrast, the similarly designed FACT trial found no statistically significant difference in either TTP or OS (TTP hazard ratio = 0.99; 95% CI 0.81 – 1.2; OS hazard ratio, 1.0; 95% CI, 0.76 – 1.32). The discordance between these studies may be partly explained by a higher proportion of patients with previous exposure to tamoxifen in the FACT study (67% compared with 40% in the SWOG trial) and the large proportion of patients with de novo metastatic disease (38.9%) in the SWOG study.

In the case of premenopausal women combining a gonadotropin-releasing hormone analog with tamoxifen was shown to be more effective than either agent alone in a three-arm study of 161 patients randomly assigned to either buserelin, tamoxifen (40 mg daily) or both. Less than 3% of the patients enrolled had received adjuvant tamoxifen. The combination arm demonstrated superior median PFS compared with the buserelin and tamoxifen arms (9.7, 6.3, and 5.6 months, respectively; p = 0.03). Median OS was also greater for the combination (3.7, 2.5, and 2.9 years; p = 0.01). Evidence for combining a gonadotropin-releasing hormone analog with an AI is less substantial (single-arm phase II trials only) and therefore this approach is best regarded as a second-line option.

The use of fulvestrant in premenopausal women is yet to be established. An observational study of fulvestrant in combination with ovarian suppression for MBC described a clinical benefit rate of 58%, suggesting that further investigation of

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**KEY POINTS**

- The treatment of HR+ breast cancer has the greatest global influence on breast cancer burden.
- It is important to confirm hormone-receptor presence in the relapsed tumor and take into consideration prior exposure and duration of exposure of prior adjuvant hormonal agent(s) when selecting hormone-based treatment options in the advanced setting.
- At present it does not appear the sequence of hormonal agents in advanced HR+ breast cancer affects overall survival (OS), rather using all of the agents available in a hormonally sensitive population is the important factor.
- We are now starting to see the promise realized with blocking cross-talking growth factor pathways in addition to the ER pathway, with the greatest efficacy seen with the mTOR inhibitor everolimus in combination with exemestane and perhaps to a lesser extent anti-HER2-directed therapy in combination with an AI.
- Future gains will likely involve a greater understanding of the redundancy and compensation induced by blocking these pathways, trials involving blocking multiple pathways in addition to hormonal agents, and the molecular interrogation of the patient’s tumor in search of predictive biomarkers and “actionable” genomic aberrations to establish that individual’s treatment algorithm.
this combination is indicated. Modern trials including ovarian suppression with a gonadotropin-releasing hormone have commonly employed a 28-day schedule of goserelin or buserelin at 6 mg 6-weekly for two treatments and 8 mg every 8 weeks thereafter. There is no clinical data assessing the relative efficacy of various gonadotrophin-releasing-hormone analogs or different dosing schedules. Despite this, it seems reasonable to substitute a long-acting agent once response has been established.

In the absence of impeding visceral crisis or knowledge of primary endocrine resistance, endocrine strategies remain the preferred first approach for the management of HR+ MBC. Recent studies have sought to establish which agent should be considered for this pole-position. One of the challenges in translating these findings into clinical practice stems from the influence of prior adjuvant endocrine therapy, particularly the increasing use of adjuvant AIs, on the choice of endocrine agent in the advanced setting. Because the majority of patients enrolled in the studies discussed above were either endocrine-treatment naïve or exposed to tamoxifen only, the “real-life” applicability of the evidence is unclear. Nonetheless the literature supports an advantage for one AI agent over another. Preclinical data indicate that letrozole is a more potent inhibitor of aromatization. In a head-to-head trial comparing letrozole with anastrozole over megestrol emerged. Although these agents have individually established efficacy in both first- and second-line trials, there is less data to support superiority of one AI agent over another. Preclinical data indicate that letrozole is a more potent inhibitor of aromatization. In a head-to-head trial comparing letrozole with anastrozole following progression on antiestrogen therapy there was no difference in TTP or OS, and as such clinically meaningful superiority for letrozole over the other AIs has not been definitively established.

Early second-line trials of fulvestrant in patients who had progressed on prior antiestrogen therapy demonstrated similar response rates and survival compared with AIs. A LD regimen (500 mg day 1, 250 mg day 14, and 28 days thereafter) was developed to hasten attainment of steady-state concentrations and was tested against exemestane arms from EFECT.

### TABLE 1. First-Line Hormone Therapy Trials in HR+ MBC

| Study       | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tam | Ana | Tap | An | }
the preferential use of the 500 mg regimen that demonstrated superior PFS (6.5 months vs. 5.5 months, hazard ratio = 0.80; 95% CI, 0.68 – 0.94; p = 0.006) without a significant increase in adverse events. A recent update from the study suggested an OS benefit for the higher dose of fulvestrant. How and where fulvestrant fits remains an area of active research. Although earlier trials demonstrated equivalence, more recent data has demonstrated that the 500 mg dose is the more efficacious dose therefore calling into question the validity of earlier studies done with a lesser-dose regimen.

Without specific sequencing trials it is not possible to establish the optimal order of exposure to hormonal agents in the advanced setting. Large PFS differences are seldom seen in either first- or second-line trials, and in the absence of significant OS benefits, the specific sequence may be less important that ensuring exposure to each class of agent over time as long the tumor(s) remain hormonally sensitive.

**THIRD-LINE AND BEYOND HORMONE THERAPY IN HR+ MBC**

There is little data on which to establish evidence-based recommendations for third-line hormonal treatment and beyond. On the basis of the EFECT study and the results of a review of cross-resistance between steroidal and nonsteroidal AIs, both fulvestrant and exemestane are reasonable options following progression on tamoxifen and a nonsteroidal AI as monohormone therapy. The preferred option, in medically fit individuals, would be the combination of exemestane and everolimus (an mTOR inhibitor)—see below. Endocrine therapy with either progestins or high-dose estrogen can be considered in selected cases. Suitability for further endocrine therapy following progression on two or more prior lines must be assessed with consideration of earlier individual drug exposures, disease burden, and previous duration of response (clinical benefit).

**HORMONE THERAPY IN COMBINATION WITH ADDITIONAL TARGETED AGENTS**

It is likely we have realized the majority of the benefit from our current hormonal agents given as monotherapy for the treatment of MBC. The major focus over the past decade has been the investigation of blocking signaling pathways that potentially cross-talk to the ER pathway and are thought to be involved in either intrinsic or acquired resistance. The pathways that have led to randomized clinical trials include the HER2 pathway, epidermal growth factor receptor (EGFR) pathway, insulin growth factor receptor pathway, and the phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of mTOR pathway.

Despite a quantitative inverse relationship between HER2 and ER (and progesterone receptor), approximately half of all HER2+ breast cancers are also HR+. HER2 overexpression confers a worse prognosis in breast cancer, regardless of the accompanying hormone-receptor status. Two phase III randomized controlled trials have been performed with the addition of targeted anti-HER2–therapy in combination with hormone therapy in HER2+ HR+ MBC. The TAnDEM study (207 patients) combined trastuzumab with anastrozole compared with anastrozole alone as first- or second-line hormone therapy in advanced stage disease. Prior tamoxifen as adjuvant or hormone therapy for MBC was allowed, though approximately 35% to 40% of patients were hormone-therapy naïve on enrollment. Although the addition of trastuzumab to an AI did have a statistically significant effect in improving the hazard ratio for PFS (hazard ratio = 0.63; 95% CI, 0.47 – 0.84), the absolute improvement was modest at best (median PFS 4.8 months vs. 2.4 months; log-rank p = 0.0016). Moreover, there was no difference in OS between the arms, with the authors stating a likely reason being that 70% of patients on the anastrozole-alone arm crossed over to receive a trastuzumab-containing regimen at some point in time postprogression. The second randomized controlled trial to mention is the phase III trial that compared the combination of letrozole plus lapatinib with letrozole with a placebo as first-line treatment of HR+ MBC, which included a population of known HER2+ tumors. In the HER2+ HR+ cohort (219 patients), the addition of lapatinib to letrozole improved PFS (hazard ratio = 0.71; 95% CI, 0.53 – 0.96), but again with no difference as of yet seen in OS (though less than 50% of deaths have occurred at time of analysis). Although

**TABLE 2. Second-Line Hormone Therapy Trials in HR+ MBC**

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**Abbreviations:** Ana, anastrozole; Exe, exemestane; Ful, fulvestrant; HR+, hormone-receptor positive; Let, letrozole; Meg, megestrol; MBC, metastatic breast cancer.
numerically the hazard ratio was less on this trial compared with the TAnDEM trial, the absolute improvement in PFS was greater (median PFS 8.2 months vs. 3.0 months). Taken together, it would appear reasonable to offer a postmenopausal woman with HR+ HER2+ advanced breast cancer not medically fit to receive chemotherapy with trastuzumab, a combination of either lapatinib or trastuzumab with an AI, with the goal of prolonging PFS but uncertain of improving OS. The other reasonable scenario would be in patients on maintenance trastuzumab (and possibly pertuzumab as well) postcombination chemotherapy to add a hormonal agent to the antibody(s) with hopes again of prolonging PFS and delaying the time to the next chemotherapy regimen.

Preclinical data has suggested that cross-talk between growth-factor receptors and the ER pathway are involved in the development of endocrine resistance. Increased expression of EGFR, HER2 and IGF-1 receptors is associated tamoxifen resistance via activation of downstream signaling pathways.28 Two randomized phase II studies have been performed of hormone therapy in combination with either an oral EGFR tyrosine kinase inhibitor (TKI) (gefitinib) or a placebo in HR+ MBC.29-30 In the smaller of the two studies (93 patients), the PFS was longer in the anastrozole and gefitinib arm compared with the anastrozole and placebo arm (hazard ratio = 0.55; 95% CI, 0.32–0.94) with a median PFS of 14.7 months versus 8.4 months, respectively.29 Numerically the clinical benefit rate was also higher in the gefitinib arm, but no results were provided in regards to the effect on OS. In the other study in which gefitinib or the placebo was combined with tamoxifen (n = 290), there was no improvement in PFS (hazard ratio = 0.84; 95% CI, 0.59–1.18).30 What was an interesting observation was that in the cohort of patients that were endocrine-therapy naïve, the hazard ratio was 0.78 (95% CI, 0.52–1.15) in favor of the gefitinib arm; however, in patients treated with prior tamoxifen or prior AIs, the hazard ratio in fact favored the placebo arms (hazard ratio = 1.45, 95% CI 0.63–3.45; hazard ratio = 1.16, 95% CI 0.69–1.93, respectively). Because these studies having conflicting results (to some degree), the phase II statistical design, relatively small sample sizes, and no demonstration of an effect on OS—it would not be advisable to prescribe gefitinib or any other EGFR TKI in combination with hormone therapy in HR+ MBC.

In the previously mentioned study by Johnston et al.,27 there was a preplanned hypothesis to explore the role of lapatinib to overcome endocrine resistance in the cohort of HR+ and HER2- patients experiencing a relapse on or less than 6 months since discontinuation of adjuvant tamoxifen. The premise being preclinical models demonstrating growth-factor activity is enhanced in association with endocrine resistance. Because lapatinib is a dual TKI against EGFR and HER2, the combination of lapatinib and letrozole would be ideal to test this hypothesis. In fact, there was a nonsignificant trend toward a prolonged PFS for lapatinib-letrozole in this cohort (200 patients), with a hazard ratio of 0.78 (95% CI, 0.57–1.07; p = 0.117) and median PFS of 8.3 months versus 3.1 months, respectively. Although it may be tempting to consider this combination in tamoxifen resistant scenarios, this was only an exploratory subgroup analysis, there are greater grade 3 and 4 toxicities (primarily diarrhea and rash) for the combination, and there is no evidence of a survival benefit to recommend this therapeutic strategy.

Perhaps the greatest promise realized so far in attempting to combine targeted agents to hormone therapy has been in blocking the PI3K-Akt-mTOR pathway. Preclinical data suggests a close interaction between this pathway and ER signaling, with a substrate of the mTOR complex 1 (mTORC1) having the ability to directly activate the ER in a ligand independent manner. In the landmark BOLERO-2 study, 724 postmenopausal women with HR+ MBC and prior exposure to a nonsteroidal AI were randomly assigned to either everolimus (a mTORC1 inhibitor) and exemestane or a placebo and exemestane.31 Despite the patient population being relatively heavily pretreated (100% prior nonsteroidal AI, approximately 50% prior tamoxifen, and 68% had received prior chemotherapy) there was an improvement in PFS with a median PFS of 6.9 months with everolimus plus exemestane compared with 2.8 months for the placebo and exemestane.

### TABLE 3. Targeting Additional Pathways in Addition to Hormone Therapy in HR+ MBC

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**Abbreviations:** Ana, anastrozole; CBR, clinical benefit rate; EGFR, epidermal growth factor receptor; Exe, exemestane; Ful, fulvestrant; HR+, hormone-receptor positive; Let, letrozole; Meg, megestrol; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; NR, not reported; PFS, progression-free survival; Tras, trastuzumab.
WHERE WILL PROGRESS BE POTENTIALLY MADE IN THE NEAR FUTURE?
Technology today is allowing us to perform genomic and transcriptomic analyses to optimize resolution in breast cancers in a timely and less-costly manner than ever before. We have now recognized from a molecular landscape not all HR+ breast cancers are identical, to now using subtype designations of luminal A and luminal B breast cancers.32 In a recent integrated clustering analysis of copy number and gene expression of close to 2,000 breast tumors, 10 novel subtypes (or clusters) were identified with distinct clinical outcomes.33 This included the discovery of a high-risk ER+ subgroup composed of 11q13/14 cis-acting luminal tumors. Several known and putative driver genes reside in this region, such as CCND1, EMSY, PAK1 and RSFI. A recent randomized phase II trial of an oral inhibitor of cyclin-dependent kinase 4/6 (PD 033299) in combination with letrozole demonstrated a significant improvement in PFS (p < 0.001) compared with letrozole alone, with preclinical studies suggesting a greater sensitivity in tumors with CCND1 amplification.34 The hope is that as the capacity increases to molecularly interrogate breast tumors on a real-time basis we can understand the mutational evolution that occurs with disease progression, identify potentially “actionable” genomic aberrations, and ultimately use the information to better select therapeutic agents more likely to be efficacious.

ER biology is inextricably linked to multiple cell-signaling pathways with known cross-talk and regulatory feedback loops between pathways. As a further example of linked pathway cross-talk, both preclinical and a clinical studies have demonstrated that blockade of mTOR can induce AKT activation and activation of IGF-1R (via induction of IRS-1) as a result of loss of a potent intrinsic-negative feedback loop.35 Thus further preclinical studies with human tumor samples and tissue-based pharmacodynamic studies will need to be performed to better understand the complexity of blocking a pathway and the resultant alterations in other linked pathways to move this field further forward. Only with this information will we have the knowledge of whether to block single or multiple pathways together with hormonal agents to delay or prevent the development of resistance to hormone therapy.

CONCLUSION
Hormonal-based treatment strategies are and will almost certainly remain the mainstay of therapy in the majority of breast cancers today and into the foreseeable future. At present it does not appear the sequence of hormonal agents effect OS rather using all of the agents available in a hormonally sensitive population is the important factor. It is likely prior exposure (duration of exposure and time from exposure) to adjuvant hormonal agent(s) and a greater understanding of the pathways involved in primary and acquired hormonal resistance will dictate the choice of the actual hormonal agent used. We are now starting to see the promise realized with blocking cross-talking growth factor pathways in addition to the ER pathway, with the greatest efficacy seen with the mTOR inhibitor everolimus in combination with exemestane and perhaps to a lesser extent anti-HER2–directed therapy in combination with an AI. Future gains will likely involve a greater understanding of the redundancy and compensation induced by blocking these pathways, trials involving blocking multiple pathways in addition to hormonal agents, and the molecular interrogation of the patient’s tumor in search of predictive biomarkers and “actionable” genomic aberrations to establish that individual’s treatment algorithm.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

Employment or Leadership Position: None. Consultant or Advisory Role: Stephen K. Chia, AstraZeneca; Genomic Health; Novartis; Roche. Stock Ownership: None. Honoraria: Stephen K. Chia, Roche. Research Funding: Stephen K. Chia, AstraZeneca; Genomic Health; Novartis; Roche. Expert Testimony: None. Other Remuneration: None.
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OVERVIEW

Overcoming de novo or acquired endocrine resistance remains critical to further enhancing the benefit of existing endocrine therapies. Recent progress has been made in understanding the molecular biology associated with acquired endocrine resistance, including adaptive “cross-talk” between ER and various growth factor receptor and cell-signaling pathways. Strategies that combine endocrine therapy with targeted inhibitors of growth factor receptors or cell-survival pathways to further enhance first-line response have largely been disappointing, suggesting that any attempts to prevent endocrine resistance by blocking specific pathways from the outset will be futile. In contrast, success has been seen by selecting patients with acquired endocrine resistance and enhancing response to further endocrine therapy by the addition of mTOR antagonists. Numerous other therapeutics are being evaluated in combination with endocrine therapies based on varying levels of preclinical science to support their use, including inhibitors of PI3K, HDAC, Src, IGFR-1, and CDK4/6. Enriching trial recruitment by molecular profiling of different ER subtypes will become increasingly important to maximize any additional benefit that these new agents may bring to current endocrine therapies for breast cancer.

Endocrine therapies are widely used in the treatment of recurrent/metastatic estrogen receptor–positive (ER+) advanced breast cancer, given their proven efficacy and generally favorable side-effect profile. Unfortunately, not all patients respond to first-line endocrine treatment due to primary de novo resistance, while others may initially respond but eventually progress with secondary acquired resistance. Recent years have witnessed major efforts to understand the various biologic mechanisms responsible for the development of endocrine resistance, with the aim of identifying new clinical therapeutic strategies to enhance the efficacy of current treatment strategies for hormone receptor–positive breast cancer. Not only has progress been made in understanding how endocrine resistance develops in laboratory models, but also new clinical strategies have emerged including the addition of mTOR antagonists to endocrine therapy for patients refractory to prior therapy. However, not all strategies that initially looked promising in experimental models have proved successful in the clinic. This article provides an update on some of the current therapeutic strategies that are being investigated as a means to further enhance the benefit of existing endocrine therapies and examines some of the clinical trial design issues that need to be considered for this approach to be successful.

REVERSING OR PREVENTING ENDOCRINE RESISTANCE: WHAT IS THE SCIENCE?

To enhance the benefit of existing endocrine therapies and improve outcomes in ER+ advanced breast cancer, it has become clear that understanding key molecular pathways in ER+ breast cancer is central to developing effective clinical strategies that might reverse or prevent endocrine resistance occurring. Acquired endocrine resistance develops as a consequence of a series of complex adaptive changes occurring in breast cancer cells, during the selective pressure of long-term endocrine treatment. Activation of various intracellular signaling pathways leads to endocrine resistance in preclinical models, and increasing evidence suggest that targeting a number of these pathways could be a valid strategy to reverse resistance to endocrine therapy. There is evidence for increased “cross-talk” between various growth factor receptor signaling pathways and ER at the time of relapse on endocrine therapy, with ER often becoming activated and supersensitized by a number of different intracellular kinases, including mitogen-activated protein kinases (MAPKs) that are activated either by peptide growth factors such as human epidermal growth factor receptors (i.e., EGFR, HER2, HER3), the insulin-like growth factor (IGF) signaling pathway, or the fibroblast growth factor receptor (FGFR). As
such, ER-mediated gene transcription and endocrine-resistant cell growth in these cells can then be interrupted by using a number of different approaches to abrogate upstream signaling, including the EGFR tyrosine kinase inhibitor (TKI) gefitinib, anti-HER2 therapies, and MEK inhibitors.\(^9,10\)

This has provided the clinical rationale for combining various growth factor therapies with endocrine therapy, and several different trials have now been completed with mixed results as discussed below.

Likewise, ER can become involved with the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway in breast cancer cells, with both genomic and nongenomic “cross talk” between this signaling pathway and ER. Because of its role in cell survival, there is evidence that the pathway becomes activated in acquired hormone-resistant breast cancer and accounts for survival of cells despite the presence of continued endocrine blockade.\(^5\)

Preclinical models of both ER+ hormone-sensitive and resistant breast cancer have been used to examine the effects of combining mTOR antagonists with endocrine therapy, with clear evidence for additive/synergistic effects.\(^11,12\) These laboratory data provided a strong rationale for combining endocrine therapy with mTOR inhibition as a means of treating endocrine resistance, which to date has proved the most successful strategy to be translated into the clinical setting – however, as discussed below it is unclear whether this approach can only be used to treat established endocrine resistance, or whether it could be used as first-line therapy to prevent or delay resistance developing in hormone-sensitive breast cancer.

**KEY POINTS**

- Endocrine resistance (either primary de novo or secondary acquired) limits the ultimate effectiveness of current first-line endocrine treatment for ER+ advanced breast cancer.
- Significant progress in both laboratory and translational studies has been made in identifying key peptide growth factor and cell-survival signaling pathways that account for endocrine resistance.
- The addition of either EGFR or HER2 targeted therapies to endocrine therapy in hormone-sensitive breast cancer does not significantly improve efficacy, although co-targeting of known ER+ HER2+ disease is an effective strategy.
- The addition of mTOR antagonists to further endocrine therapy in endocrine-resistant breast cancer significantly improves clinical outcomes and is an important advance in the second-line setting, although to date there is no benefit for the combination in the first-line setting.
- Based on scientific evidence for each signaling pathway and the availability of appropriate therapeutics, numerous targeted therapies are now being evaluated in various endocrine combinations studies in ER+ advanced breast cancer.
- Appropriate clinical trial design, patient selection, and molecular profiling of ER+ breast cancer (including biopsies of metastatic disease) will be increasingly important to ensure success for future trials of combined endocrine and targeted therapy.

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**TREATING ENDOCRINE RESISTANCE WITH TARGETED THERAPIES IN THE CLINIC: HAVE WE MADE PROGRESS RECENTLY?**

Given the involvement of both peptide growth factor signaling and cell-survival pathways in models of endocrine resistance, a number of recent trials have been conducted either with targeted therapies such as EGFR/HER2 inhibitors or mTOR antagonists given in combination with endocrine therapy.\(^13\) Some of these studies were conducted in patients with established hormonal resistance where activated growth factor or cell-survival pathways may already be operative, and it was hoped that combined endocrine and targeted therapy could be more effective than using another endocrine therapy given alone. Other randomized trials were conducted in the first-line advanced breast cancer setting with the expectation that combined endocrine and targeted therapy might delay the time to disease progression on endocrine therapy alone by blocking from the outset a key resistance mechanism (i.e., peptide growth factor signaling) that operates in ER+ breast cancer cells to cause the development of acquired resistance. Although some of these approaches have yielded success, improving the response to existing endocrine therapy is not necessarily that simple.

Two randomized trials have assessed whether co-targeting EGFR and ER could enhance the benefit of endocrine therapy in the first-line advanced breast cancer setting (Table 1). These studies set out to prove the hypothesis that combination therapy could delay the onset of acquired resistance to endocrine therapy, as demonstrated in xenograft models in vivo.\(^14\) A double-blind placebo-controlled phase II trial of tamoxifen with/without gefitinib included patients with endocrine naïve disease or who had developed greater than a year after completion of adjuvant tamoxifen (Stratum 1, n = 206), or had developed during or after AI therapy (Stratum 2 n = 84).\(^15\) In the endocrine naïve patients, there was a numerical increase in progression-free survival (PFS) from 8.8 to 10.9 months, while patients who had been pre-exposed to AIs did not gain any benefit from the combination. A second randomized trial of gefitinib and anastrozole compared with anastrozole alone in a similar first-line patient population of women with ER+ve–advanced breast cancer reported a significant prolongation of progression-free survival from a median of 8.2 months with anastrozole to 14.6 months with the combination (HR 0.55, 95% CI 0.32–0.94).\(^16\) Although the number of patients in this second study was only 93, a subsequent combined analysis of both clinical trials suggested that the benefit for the combination was seen exclusively in those patients who were endocrine-therapy naïve, including no prior endocrine therapy in the adjuvant setting. On the basis of these results, a prospective multicenter study (MINT, NCT 01151215) was conducted with a novel tyrosine kinase
inhibitor AZD8931, a potent inhibitor of EGFR, HER2, and HER3, to test the hypothesis that combined therapy of growth factor blockade together with anastrozole could delay time to progression compared with anastrozole alone in endocrine therapy naïve metastatic breast cancer. As such, the MINT study will be the definitive test of this concept that blocking this pathway could be a strategy to delay/prevent endocrine resistance from the outset.

Co-targeting HER2 in hormone receptor positive breast cancer has also been explored as a means of improving endocrine responsiveness, given the evidence that HER2 expression results in breast cancer models is associated with primary resistance to endocrine therapy. Three randomized trials,171819 have confirmed that this approach can treat this form of endocrine resistance in known ER+ HER2+ advanced disease (Table 1). In the large EGF30008, there were an additional 952 patients with ER+ HER2- negative tumors where the hypothesis that development of acquired resistance to letrozole due to adaptive EGFR or HER2 upregulation could be prevented/delayed by dual targeting. However, there was no improvement in PFS for the combination in these patients, which implies that in ER+ endocrine-sensitive breast cancer specific co-targeting of HER2 (as opposed to EGFR) together with ER from the outset does not delay resistance. Indeed, this result is consistent with experimental models that showed the failure of trastuzumab and letrozole combined together from the outset to delay endocrine resistance in hormone receptor-positive xenografts, in contrast to combined therapy that was very effective once resistance to letrozole had developed.8 As discussed below, this important lesson needs to be borne in mind when designing other combined therapy trials in the first-line setting, as improving on the efficacy of existing endocrine therapy in this setting may not be that straightforward if targeted blockade simply allows other resistance mechanisms to evolve over a similar time frame to first-line aromatase inhibition.

In contrast to the relative disappointment of first-line studies of endocrine therapy combined with EGFR/HER2-targeted therapies, a more successful approach has been the addition of the mTOR antagonist everolimus to endocrine therapy for postmenopausal women with ER+ve MBC who have already received prior endocrine therapy. In these studies, patients had often developed endocrine resistance, and as such might be expected to have acquired either cell-survival or adaptive-signaling pathways that could be susceptible to an appropriate therapeutic agent. Tamoxifen plus everolimus was investigated in patients with AI-resistant metastatic breast cancer (MBC) in the phase II TAMRAD (tamoxifen plus everolimus) study and showed that the combination therapy gave an improvement in TTP (8.6 vs. 4.5 months), 6-month CBR (61% vs. 42%) and median overall survival (OS) compared with tamoxifen alone.20 Importantly, the trial design included stratification according to type of resistance to previous treatment with AIs and showed that the greatest clinical benefit for the combination arm occurred in patients with acquired secondary resistance.

These clinical data would support the hypothesis that tumors that initially respond to AIs and then develop resistance may utilize the PI3K/Akt/mTOR cell-survival pathway and that a combined approach should be most effective in those patients with ER+ve advanced disease that progresses during or after nonsteroidal-aromatase-inhibitor therapy.21 This was confirmed in the Breast Cancer Trials of Everolimus-2 (BOLERO-2) study, a large randomized phase III trial that assigned 724 postmenopausal patients with ER-positive metastatic breast cancer in a 2:1 ratio to either exemestane alone or the combination of exemestane and everolimus.22 All patients had progressed on a nonsteroidal AI, and importantly 84% of them had demonstrated prior hormone-sensitive disease defined as “at least 24 months of endocrine therapy before recurrence in the adjuvant setting, or a response or stabilization for at least 24 weeks of endocrine therapy for

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<td>Cristofanilli et al.16</td>
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<tr>
<td>in ER+ MBC</td>
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<tr>
<td>Osborne et al.15</td>
</tr>
<tr>
<td>in ER+ MBC</td>
</tr>
<tr>
<td>TANDEM17</td>
</tr>
<tr>
<td>in ER+ HER2+ MBC</td>
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<tr>
<td>EGF3000818</td>
</tr>
<tr>
<td>in ER+ HER2+ MBC</td>
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<tr>
<td>eLEcTRA19</td>
</tr>
<tr>
<td>in ER+ HER2+ MBC</td>
</tr>
</tbody>
</table>

Abbreviations: MO, months; NR, not reported; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RCT, randomized controlled trials; TTP, time-to-progression; ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; MBC, metastatic breast cancer.

* Statistically significant difference.
advanced disease.” In BOLERO-2, there was a statistically significant and clinically relevant improvement in PFS for the combination (median 7.8 vs. 3.2 months, HR = 0.45, p < 0.0001). The clinical benefit was primarily due to better control of the disease, although there was a significant improvement in tumor response rates from only 0.4% in the exemestane-alone group to 9.5% in the everolimus/exemestane group (p = 0.001).

A key question remains as to whether the combination of an mTOR inhibitor with endocrine therapy will only be effective for endocrine-resistant breast cancer, or whether this is a new option for endocrine-sensitive MBC in the first-line setting that could delay or prevent endocrine resistance developing. A large first-line phase III study (HORIZON) recently reported the efficacy for the oral mTOR antagonist temsirolimus (30 mg orally for 5 days every 2 weeks) in combination with letrozole vs. letrozole/placebo in 1,112 patients with AI-naïve ER+ advanced breast cancer. In contrast to BOLERO-2, the population in this larger study was mainly totally endocrine therapy naïve (approximately 60%), and had received no prior AI therapy for locally advanced/metastatic disease. In HORIZON, there was no improvement in PFS overall (median 9 months, HR = 0.90, p = 0.25), or in the 40% patient subset who had received prior adjuvant endocrine therapy. These data suggest that as first-line therapy the combination may not be any better than an AI alone.

FUTURE STRATEGIES TO CO-TARGET ENDOCRINE AND OTHER SIGNALING PATHWAYS

The emergence of endocrine resistance during prolonged therapy is complex, and it is unlikely that any single mechanism is operative. Although the EGFR/HER2 and mTOR pathways have been studied extensively, numerous other signaling pathways may also be implicated. Both preclinical and early-phase clinical research is now trying to identify various other strategies to overcome endocrine resistance, based on the availability of targeted therapeutics that can be combined with endocrine therapy. Some of the key areas are discussed below, together with a list of current randomized trials that are investigating various signal-transduction inhibitors in combination with endocrine therapy in ER+ advanced breast cancer (Table 2).

Agents Targeting PI3K/AKT/MTOR Pathway

Despite the encouraging results reported for the combination of everolimus plus exemestane in the BOLERO-2 trial, at a molecular level, two key regulatory loops may limit the effectiveness of current mTOR inhibitors. A negative-feedback loop exists downstream in the PI3K/Akt/mTOR pathway whereby the mTOR activated kinase S6K1 phosphorylates de-stabilizes the IRS1 and IRS2 proteins in insulin-like growth factor (IGF) responsive cells. In these cells, mTOR inhibition leads to a reduction in S6K1 activity, which in turn allows IRS1/2 expression to be increased with associated enhanced activation of IGFR-1 dependent Akt activity. Clinically phosphorylated Akt is upregulated in both tumor and skin biopsies of patients treated with everolimus, and as such this loss of negative feedback may overcome the antitumor effectiveness of mTOR blockade. In addition, a positive regulatory loop exists involving the mTORC2 complex that can be activated more directly by growth factors and activated phosphorylated Akt. Therefore the inability of some rapamycin derivatives to block mTORC2 could result in increased Akt signaling that result in ER phosphorylation on Serine 167 negating the effect of combined aromatase inhibition.

Although these two mechanisms may limit the benefit of the current generation of mTOR inhibitors in combination with endocrine therapy, several other drugs that target the PI3K/AKT pathway upstream of mTOR are currently being tested in phase I/II trials in patients with advanced ER+ breast cancer in the hope that may prove more specific and effective than current mTOR inhibitors. These include pan or isoform-specific PI3K inhibitors, dual PI3K/mTOR inhibitors, and AKT inhibitors (Table 3). For example, BKM120 is a potent oral pan-PI3K inhibitor that when given either continuously or intermittently in combination with letrozole in a phase I study has been demonstrated to be safe, with evidence of antitumor efficacy as assessed by FDG-PET scans. The combination of BKM120 with fulvestrant has also been investigated, and a randomized phase III study of BKM120 with fulvestrant in patients with HR+/HER2-negative locally advanced/metastatic breast cancer who have progressed after prior AI therapy (BELLE-2) is recruiting a second-line patient population very similar to that in the BOLERO-2 trial (NCT 00863655). Given the likely increased use of everolimus in combination with exemestane in the second-line setting, a further trial will assess the role of BKM-120 with fulvestrant who have progressed on or after mTOR inhibitors (BELLE-3) (NCT01633060).

Another approach is to develop drugs that target PI3K and mTOR together, and two pharmaceutical companies have set up studies comparing these dual inhibitors with pan-inhibitors of PI3K, both in combination with endocrine therapy compared with endocrine therapy alone. For example either XL147 (inhibitor of PI3K) or XL765 (dual inhibitor of PI3K and mTOR) are being combined with letrozole in a phase I/II trial (NCT01082068) in ER+ advanced breast cancer. Likewise, FERGI is a multicenter, international, randomized, double-blinded, placebo-controlled phase II trial recruiting patients with advanced or MBC who have previously received treatment with an AI, randomized to receive either GDC-0941 (pan PI3K inhibitor) + fulvestrant or GDC-0980 (dual inhibitor of PI3K and mTOR) + fulvestrant, or placebo + fulvestrant (NCT01437566). Whether these dual-targeted drugs are more effective than panisoform PI3K–inhibitors remains to be seen, together with early assessments of toxicities that sometimes can be greater for drugs with broader target specificities.
Histone Deacetylase Inhibitors (HDACi)

Another possible approach to reverse hormone resistance is the use of histone deacetylase inhibitors (HDACi) to re-sensitize breast cancer cells to hormone manipulation. It has been shown that in some breast cancers, expression of ER can be repressed/lost by epigenetic modifications such as methylation and histone deacetylation, and this could be a mechanism for endocrine resistance. Entinostat is an HDACi that has been shown to increase expression of both ER and the enzyme aromatase in a dose-dependent manner both in vitro and in vivo, which then sensitized breast cancer cells to estrogen and subsequent inhibition by the aromatase-inhibitor letrozole.32 Furthermore, in xenograft experiments, the combination of letrozole plus entinostat was significantly more effective at inhibiting xenograft growth than either therapy alone. In a randomized phase II trial (ENCORE 301, NCT00676663), entinostat in combination with exemestane was compared with exemestane/placebo in patients who had received prior hormone therapy.33 This trial showed prolongation of median PFS (4.3 vs. 2.3 months) and extension of OS benefit (26.9 vs. 19.8 months), and a randomized phase III trial is being planned. Similarly, a phase II study testing vorinostat and tamoxifen in 43 patients with ER-positive–MBC progressing on endocrine therapy showed an overall response rate (ORR) of 19% and a median response duration of 10.3 months. Correlative studies suggest that HDAC2 expression could be a predictive biomarker, and that histone hyper-acetylation may be a valid pharmaco-dynamic marker for the efficacy of this combination.

Antiangiogenic Agents

Preclinical data35 and retrospective clinical data36 suggest that high vascular endothelial growth factor (VEGF) levels in breast tumors are associated with a decreased response to endocrine therapy. As several phase II studies had suggested the feasibility and activity of the combination of bevacizumab with endocrine agents,37,38 a randomized phase III study (LEA) was conducted to test the hypothesis that anti-VEGF treatment with bevacizumab could prevent resistance to hormone therapy (either letrozole 2.5 mg/day or fulvestrant 250 mg/4 weeks) given as first-line therapy in endocrine-responsive–advanced breast cancer.39 The PFS was better with the

### TABLE 2. Randomized Clinical Trials Investigating Signal Transduction Inhibitors (STIs) Plus Endocrine Agents in MBC

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Stage and Study Number</th>
<th>Estimated Enrollment (n pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K/AKT/mTOR</td>
<td>XL147 (inhibitor of PI3K) or XL765 (dual inhibitor of PI3K and mTOR) plus letrozole</td>
<td>Phase I/I (NCT01082068)</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>GDC-0941 + fulvestrant or GDC-0980 + fulvestrant or placebo + fulvestrant</td>
<td>Phase II (NCT01437566)</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>BKM120 (pan-PI3K inhibitor) plus fulvestrant vs. placebo plus fulvestrant</td>
<td>Phase III (NCT01633060)</td>
<td>615</td>
</tr>
<tr>
<td></td>
<td>MK-2206 (Akt inhibitor) plus anastrozole, or letrozole, or exemestane, or fulvestrant</td>
<td>Phase I (NCT01344031)</td>
<td>54</td>
</tr>
<tr>
<td>Histone deacetylase (HDAC)</td>
<td>entinostat (SNDX-275) plus exemestane versus placebo plus exemestane</td>
<td>Phase II (NCT00676663)</td>
<td>125</td>
</tr>
<tr>
<td>Vascular endothelial growth factor/angiogenesis</td>
<td>Bevacizumab plus tamoxifen or letrozole vs. tamoxifen or letrozole alone</td>
<td>Phase III (NCT00509000)</td>
<td>502</td>
</tr>
<tr>
<td></td>
<td>Bevacizumab plus letrozole or fulvestrant vs. letrozole or fulvestrant alone</td>
<td>Phase III (NCT00545077)</td>
<td>378</td>
</tr>
<tr>
<td></td>
<td>BMS-690514 (inhibitor of EGFR, HER2, and VEGF receptor kinases) plus letrozole versus lapatinib plus letrozole</td>
<td>Phase II (NCT01068704)</td>
<td>140</td>
</tr>
<tr>
<td>Proteasome (NF-kB pathway)</td>
<td>Bortezomib plus fulvestrant vs. fulvestrant alone</td>
<td>Phase II (NCT0142401)</td>
<td>118</td>
</tr>
<tr>
<td>Src kinase</td>
<td>Dasatinib plus fulvestrant vs. fulvestrant alone</td>
<td>Phase II (NCT00754325)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Dasatinib plus exemestane vs. exemestane alone</td>
<td>Phase II (NCT00767520)</td>
<td>157</td>
</tr>
<tr>
<td>Fibroblast growth factor receptor (FGFR)</td>
<td>AZD4547 plus fulvestrant vs. fulvestrant alone</td>
<td>Phase I/I (NCT01202591)</td>
<td>120</td>
</tr>
<tr>
<td>Insulin-like growth factor type 1 (IGF-I)</td>
<td>MEDI-573 (Dual IGF-I/II-neutralizing antibody) plus AI or AI alone</td>
<td>Phase Ib/I (NCT00446559)</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>BMS-754807 plus letrozole vs. BMS-754807 alone</td>
<td>Phase II (NCT01225172)</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>MM-121 plus exemestane vs. exemestane alone</td>
<td>Phase II (NCT0105046)</td>
<td>130</td>
</tr>
<tr>
<td>Cyclin dependent kinase (CDK) 4/6</td>
<td>PD-0332991 plus letrozole vs. letrozole alone</td>
<td>Phase I/I (NCT00724099)</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>PD-0332991 plus letrozole vs. placebo plus letrozole</td>
<td>Phase III (NCT01740427)</td>
<td>450</td>
</tr>
</tbody>
</table>

Abbreviations: AI, aromatase inhibitor; mTOR, mammalian target of rapamycin; N, number; PI3K, phosphatidylinositol-3-kinase; PTS, patients.
TABLE 3. Novel Agents under Investigation Targeting the PI3K/ AKT/mTOR Pathway

<table>
<thead>
<tr>
<th>Target(s)</th>
<th>Drug</th>
<th>Pharmaceutical Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3Kα</td>
<td>BYL719</td>
<td>Novartis</td>
</tr>
<tr>
<td>PI3Kβ</td>
<td>GDC-0032</td>
<td>Genentech</td>
</tr>
<tr>
<td>PI3Kγ</td>
<td>MLN-1117</td>
<td>Millennium</td>
</tr>
<tr>
<td>PI3Kδ</td>
<td>CAL-101</td>
<td>Calistoga</td>
</tr>
<tr>
<td>Pan-PI3K</td>
<td>XL-147</td>
<td>Exelixis/Sanofi</td>
</tr>
<tr>
<td>Pan-PI3K</td>
<td>BKM120</td>
<td>Novartis</td>
</tr>
<tr>
<td>Pan-PI3K</td>
<td>GDC-0941</td>
<td>Genentech</td>
</tr>
<tr>
<td>Pan-PI3K</td>
<td>PKI-587</td>
<td>Pfizer</td>
</tr>
<tr>
<td>PI3K/mTOR</td>
<td>XL-765</td>
<td>Exelixis/Sanofi</td>
</tr>
<tr>
<td>PI3K/mTOR</td>
<td>BEZ235</td>
<td>Pfizer</td>
</tr>
<tr>
<td>PI3K/mTOR</td>
<td>GDC-0980</td>
<td>Genentech</td>
</tr>
<tr>
<td>PI3K/mTOR</td>
<td>PF-4691502</td>
<td>Pfizer</td>
</tr>
<tr>
<td>TORC1/2</td>
<td>MLN-128</td>
<td>Millennium</td>
</tr>
<tr>
<td>TORC1/2</td>
<td>OSI-027</td>
<td>OSI Pharma</td>
</tr>
<tr>
<td>AKT (catalytic)</td>
<td>AZD2041</td>
<td>AstraZeneca</td>
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<tr>
<td>AKT (allosteric)</td>
<td>MK-2206</td>
<td>Merck</td>
</tr>
<tr>
<td>AKT (catalytic)</td>
<td>GDC-0068</td>
<td>Genentech</td>
</tr>
</tbody>
</table>

combination of bevacizumab plus endocrine therapy than with endocrine monotherapy (18.4 vs. 13.8 months), but this was not statistically significant. The combination had a significantly higher incidence of hematologic and nonhematologic toxicities and does not appear to be a promising approach to enhance first-line therapy.

Agents Targeting Src Kinase

Results from preclinical studies showed that ER-Src kinase axis play an important role in promoting hormonal resistance by proto-oncogenes such as HER2, PELP1, and that blocking this axis prevents the development of hormonal independence in vivo.40 Dasatinib is a potent, broad spectrum ATP–competitive inhibitor of Src tyrosine kinase. However, the addition of dasatinib to fulvestrant in a randomized phase II study in ER-positive postmenopausal MBC patients who had progressed after a NSAI did not improve PFS, CBR, or OS.41 Similarly, 157 patients were randomized in a double-blind phase II trial (CA180 –261) to receive dasatinib (100 mg daily) or matched placebo in combination with exemestane (25 mg daily). Although the PFS difference was not significant in overall study population, a higher clinical benefit rate (CBR) in the dasatinib arm and higher PFS in patients with symptomatic bone metastasis (HR = 0.68) suggested that dasatinib may have efficacy in a subset of patients.42

Agents Targeting FGFR Pathway

Several studies have shown that the fibroblast growth factor receptor-1 gene (FGFR1) is amplified in approximately 10% of all breast cancers, correlating with increased FGFR1 mRNA or protein expression.43 Amplification of FGFR1 is enriched in up to 20% of ER-positive breast cancers. Amplification and over-expression of FGFR1 may be a major contributor to poor prognosis in luminal-type B breast cancers, driving anchorage-independent proliferation and endocrine-therapy resistance.43 AZD4547 is a potent selective inhibitor of FGFR-1, 2, and 3 receptor tyrosine kinases (enzyme and cellular phosphorylation endpoints) and has a significantly lower potency for inhibition of IGF1R and KDR.44 The co-administration of an FGFR inhibitor and exemestane has the potential to improve outcome for patients with aggressive disease or resistance to endocrine therapy. Therefore, GLOW is a randomized double-blind phase IIa study (with phase I combination safety run-in) designed to assess the safety and efficacy of AZD4547 in combination with exemestane vs. exemestane alone in patients with ER-positive and FGFR1 amplified (FISH ≥ 4) breast cancer who have failed treatment with one prior endocrine therapy (adjuvant or first-line metastatic) (NCT01202591).

Agents Targeting Insulin-Like Growth Factor Type 1 (IGF-1)

The role of the insulin-like growth factor (IGF) system in endocrine-resistant breast cancer has been studied, and inhibitors of this pathway are currently in clinical trials in ER+ve patients who have progressed on prior endocrine therapy. MEDI-573 is a dual-targeting human antibody that neutralizes IGF-1/-II ligands and inhibits insulin-like growth factor receptor 1 (IGF-1R) and insulin receptor-A (IR-A) signaling pathways, which play a role in breast and other epithelial cancers. By sparing insulin receptor-B (IR-B) and its hybrid receptors, MEDI-573 is expected to achieve antitumor activity without perturbing glucose homeostasis and has showed acceptable safety and favorable PK profiles without significant changes in glucose levels.45 A biomarker-rich phase Ib/II study of MEDI-573 with an aromatase inhibitor in patients with advanced ER+ve breast cancer is ongoing (NCT01446159).

Inhibitor of Cyclin-Dependent Kinase (CDK) 4/6

Modulating the cell cycle has always been an attractive therapeutic target in cancer, and previously published data have suggested that CDK 4/6 inhibition may play a key role in the treatment of subsets of breast cancers.46,47 PD 0332991 is a novel oral selective inhibitor of cyclin-dependent kinase (CDK) 4/6, which prevents cellular DNA synthesis by blocking cell-cycle progression from G1 to S phase. Recently, it was reported that the combination of PD 0332991 and letrozole significantly improved median PFS in a randomized phase II study in patients with advanced ER-positive breast cancer, including those with identified cyclin D1 amplification and/or p16 loss in whom CDK 4/6 inhibition is expected to be most effective.48 A PFS of 26.1 month was observed for patients in the combination arm compared with 7.5 months for patients treated with letrozole alone. In patients with measurable disease, an improved response rate was seen (45% compared with 31%), and the toxicity profile for the combination was favorable with the most common adverse
events being (uncomplicated) neutropenia, leukopenia, anemia, and fatigue. On the basis of this extremely promising result, a randomized, multicenter, double-blind first-line study of PD-0332991 plus letrozole compared with letrozole/placebo in postmenopausal women with ER+ HER2- MBC who have not received any prior systemic anticancer treatment for advanced disease will open to recruitment soon. (NCT01740427)

As such, CDK 4/6 inhibition seems one of the more promising approaches to enhance endocrine response in ER+ endocrine-sensitive—breast cancer and could potentially produce that quantum leap in response to first-line endocrine therapy that to date has eluded this area of clinical research in ER+ advanced breast cancer. However, appropriate clinical trial design, patient selection, and biomarker research remains crucial to enhancing the chance of success.

ISSUES FOR FUTURE CLINICAL TRIAL DESIGN IN ER+ BREAST CANCER

It is becoming clear that improving endocrine therapy by the addition of targeted therapy is not that simple.49 Undoubtedly, the significant efficacy for the combination of the mTOR antagonist everolimus and exemestane in those patients refractory to prior AI therapy is a major advance in providing greater clinical benefit compared with the use of just further endocrine therapy alone for these patients, which may spare the use of palliative chemotherapy for a period of time. As for identifying untreated ER+ MBC patients that would benefit from a given combined targeted and endocrine therapy in the first-line setting, this appears much trickier—so far it would appear that “blind” co-treatment of hormone sensitive ER+ MBC with a given drug combination in the hope of delaying resistance and improving the benefit already obtained with first-line AI therapy does not work. It is possible that some ER+ tumors are inherently primed to respond to a given combination, as shown by expression of PIK3CA mutations in a neoadjuvant study of letrozole and everolimus,50 or co-expression of HER2 and ER in the studies cited above (Table 1). However, the challenge in daily clinical practice is to identify the relevant pathways that are operative in individual patients with ER+ve MBC, which may or may not benefit from a given targeted-therapy combination.

Although overexpression of any given oncogene or molecular target could identify the best group of ER+ patients to treat with any given novel-targeted therapeutic in a combination strategy to enhance endocrine responsiveness, it remains likely that only a proportion will gain benefit because of other coexisting mutations and molecular alterations within the complex web of inter-related signaling networks that will determine response/resistance to inhibition of any key target (e.g., either loss of the PTEN tumor suppressor gene or activating mutation of PI3-kinase may modulate response to trastuzumab in HER2+ve breast cancer). As such, translational studies in real-time metastatic samples from patients will remain crucial for optimizing clinical benefit from these new therapies, whether it is the original primary tumor or the relapsed sample where the molecular profile may have changed.

In future, all clinical studies should make a greater effort to enrich their trial population with the most appropriate patients, with a tumor molecular profile likely to benefit from targeting the given pathway. In the absence of the ability to identify the molecular target in samples of metastatic disease (i.e., only the original primary tumor is available, as often is the case), understanding the inherent biology in the primary tumor that accounts for relapse/resistance may become crucial is determining which population to select for these studies. Genomic profiling in ER+ve breast cancer may help identify those more likely to develop resistance to endocrine therapy, or indeed the pathways that these tumors are most likely to utilize as escape mechanisms, which in turn may guide appropriate selection of target therapies to add in at the time of relapse. Recent studies have started to show common oncogenic pathways that intrinsic subtypes of breast cancer will utilize, thus allowing strategies to be developed for combinations of various signaling agents to be used in an attempt to enhance responsiveness to current therapies.51 In particular, in ER+ve breast cancer, gene expression profiling has identified that in the luminal B subtype activation of growth factor signaling pathways occurs, often independent of HER2 overexpression, thus contributing to their poorer prognosis.52 Selection of this subgroup for future combination strategies may yield answers faster than treating a more heterogeneous unselected group of patients with ER+ breast cancer.

CONCLUSION

There are now a multitude of targeted therapeutics in various stages of clinical development for breast cancer (Table 2) that are based on a rationale that the target in question is valid in the pathogenesis of ER+ breast cancer. Although the integration of targeted therapies with conventional therapeutics in breast cancer has been pioneered by the combination of trastuzumab with chemotherapy, substantial research is ongoing into combining targeted therapeutics with endocrine therapy to enhance responsiveness and delay resistance.13 The emphasis is now shifting toward targeting networks and pathways with combinations of signaling drugs, either in parallel (so-called combined horizontal blockade) or in series (combined vertical blockade). Selection of which approach is valid depends on key preclinical studies that need to be undertaken in various relevant models, in order to guide which combinations need testing in early phase clinical trials. This will ensure rapid and efficient transition from proof of concept studies into pivotal efficacy studies, thus maximizing the likelihood of success.
Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.


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Endocrine Resistance: What Do We Know?

Todd W. Miller, PhD

OVERVIEW

Adjuvant therapy with antiestrogens targeting estrogen receptor α (ER) signaling prevents disease recurrence in many patients with early-stage ER+ breast cancer. However, a significant number of cases exhibit de novo or acquired endocrine resistance. While other clinical subtypes of breast cancer (HER2+, triple-negative) have disproportionately higher rates of mortality, ER+ breast cancer is responsible for at least as many deaths because it is the most common subtype. Therefore, identifying mechanisms that drive endocrine resistance is a high clinical priority. A large body of experimental evidence indicates that oncogenic signaling pathways underlie endocrine resistance, including growth factor receptor tyrosine kinases (HER2, epidermal growth factor receptor [EGFR], fibroblast growth factor receptor 1/2 [FGFR], insulin-like growth factor-1 receptor [IGF-1R]/insulin receptor [InsR]), PI3K/AKT/ mTOR, MAPK/ERK, Src, CDK4/CDK6, and ER itself. Combined targeting of ER and such pathways may be the most effective means to combat antiestrogen resistance, and clinical trials testing such strategies show promising results. Herein, we discuss pathways associated with endocrine resistance, biomarkers that may be useful to predict response to targeted agents, and avenues for further exploration to identify strategies for the treatment of patients with endocrine-resistant disease.

More than 75% of breast cancers express estrogen receptor α (ER) and/or progesterone receptor (PR, an ER-regulated gene product). These cancers comprise the so-called “ER+” subtype, and the number of ER+ breast cancers is projected to increase. Such patients are typically treated with antiestrogen therapies that inhibit ER function. There are 3 main types of anti-estrogens: (1) selective ER modulators (SERMs) such as tamoxifen (which has mixed agonist/antagonist activity); (2) selective ER downregulators (SERDs) such as fulvestrant; (3) agents that reduce circulating estrogen levels such as aromatase inhibitors (AIs; letrozole, anastrozole, exemestane). Such endocrine therapies are some of the most effective targeted anticancer agents in histology. Combined targeting of ER and such pathways may be the most effective means to combat antiestrogen resistance, and clinical trials testing such strategies show promising results.

ER+ breast cancers can exhibit de novo or acquired endocrine resistance. Common clinical scenarios of endocrine resistance include: (1) de novo resistance where a patient presents with metastatic disease resistant to all hormonal therapies, or recurs soon after starting adjuvant hormonal therapy and does not respond to further endocrine manipulation; (2) de novo resistance to some but not other hormonal therapies; (3) acquired resistance after initial response to endocrine therapy, followed by temporary response to additional hormonal therapies until the cancer becomes refractory to all endocrine agents; (4) progression after initial response to a hormonal agent, and then transient response to the same agent introduced years later. Preclinical evidence suggests that the cellular and molecular mechanisms governing de novo versus acquired resistance may be the same, and that mechanisms of resistance to different classes of antiestrogens are similar. However, such findings may be biased by our ER+ models. Instances of cancers that respond to the AI exemestane following progression on a nonsteroidal AI (letrozole or anastrozole), or that respond to fulvestrant following progression on an AI, support the existence of agent-specific and class-specific types of endocrine resistance.

GROWTH FACTOR RECEPTOR SIGNALING PATHWAYS PROMOTE ENDOCRINE RESISTANCE

The only clinically available marker of antiestrogen resistance is HER2 overexpression. The findings that HER2 overexpression promotes the agonistic effects of tamoxifen, and that AI therapy is more effective than tamoxifen in patients with ER+/HER2+ disease prompted a switch to non-SERM therapies for these patients. However, less than 10% of ER+ breast cancers overexpress HER2, and HER2 is viewed...
as clinically more important than ER because HER2+ breast cancer is generally more aggressive. Thus, such patients are often treated with HER2-directed (trastuzumab) and endocrine therapies, either sequentially or in combination. Retrospective clinical data suggest that endocrine therapy is beneficial in combination with anti-HER2 therapy, but these observations await confirmation in a prospective study.

Preclinical studies have implicated additional growth factor receptor signaling pathways in endocrine resistance. EGFR, IGF-1R, InsR, Ron, and FGFR1 activation promote anti-estrogen resistance in model systems. Such receptors converge on the PI3K/AKT/mTOR and MEK/ERK pathways, which have also been implicated in antiestrogen resistance. Components of these pathways are often genomically altered in human cancers. Whether mechanisms of resistance to tamoxifen, fulvestrant, and AIs are common remains to be determined, but activation of the PI3K or MEK pathways confers resistance to all forms of endocrine therapy. Many of these findings are supported by correlative, retrospective clinical evidence. For example, patients with FGFR1-overexpressing ER+ tumors had shorter distant recurrence-free survival following adjuvant treatment with tamoxifen compared with patients with FGFR1-normal tumors. Patients with ER+ tumors exhibiting a (phospho)protein signature of PI3K hyperactivation exhibited shorter recurrence-free survival following adjuvant endocrine therapy compared with patients with PI3K-low tumors. Similarly, patients with ER+ tumors exhibiting a gene expression signature of IGF-1R activation had a worse prognosis compared with patients with IGF-1R-inactive tumors. Such findings have led to ongoing trials testing novel agents targeting these signaling pathways in combination with antiestrogens.

**KEY POINTS**

- Over 75% of breast cancers express estrogen receptor α (ER). Patients with such tumors are treated with antiestrogens to block ER signaling. Many patients exhibit antiestrogen-resistant disease, making ER+ breast cancer a significant contributor to breast cancer mortality.
- Growth factor receptor signaling pathways that activate PI3K/AKT/mTOR promote antiestrogen resistance.
- Cell-cycle deregulation that circumvents the requirement for estrogen receptor α (ER) confers antiestrogen resistance. This may occur in the form of CDK4/CDK6 hyperactivation or Rb loss.
- ER itself can drive endocrine-resistant cell proliferation, and ER downregulators may be superior to estrogen-deprivation therapy with aromatase inhibitors (AIs).
- Drugs targeting the PI3K/AKT/mTOR and CDK4/CDK6 pathways are being tested in ongoing trials in patients with ER+ breast cancer.

**ER PROMOTES ENDOCRINE RESISTANCE: CROSS-TALK BETWEEN ER AND GROWTH FACTOR RECEPTOR PATHWAYS**

The best-characterized mechanism of ER signaling involves estrogen-induced dimerization and phosphorylation of ER to promote transcriptional activity. However, growth factor receptor signaling pathways can modulate ER activation. The PI3K effector AKT, the TORC1 effector p70S6K, and ERK can phosphorylate ER to promote estrogen-induced, tamoxifen-induced, and ligand-independent ER transcriptional activity. PI3K, Ras, and ERK can also promote activation of ER cofactors (Fig. 1). In turn, ER drives expression of genes encoding growth factor receptor pathway components. Neoadjuvant estrogen deprivation therapy with an AI reduced AKT and mTOR activation in ER+ tumors in patients, and such reductions correlated with improved clinical response and outcome, suggesting that estrogen-induced signaling activates these pathways.

While clinical findings imply that antiestrogens should suppress growth factor receptor signaling, preclinical evidence shows that such crosstalk is more complex. Forced PI3K pathway activation (by knockdown of PTEN, or overexpression HER2, IGF-1R, or activated AKT1) confers resistance to tamoxifen, fulvestrant, and estrogen deprivation in ER+ breast cancer cell lines. Such resistance is typically reversible by inhibition of PI3K. ER+ breast cancer xenografts with acquired resistance to tamoxifen exhibit increased expression of IGF-1R, HER2, and EGFR. AI-resistant MCF-7/aromatase cells and xenografts exhibit increased levels of HER2 and the EGFR ligand amphiregulin, and lower levels of ER. Long-term estrogen-deprived, ER+ breast cancer cell lines (which model AI resistance) exhibit hyperactivation of IGF-1R/InsR/PI3K/AKT/mTOR signaling. MCF-7 cells and xenografts with acquired fulvestrant resistance shown similar changes. At first glance, such findings seem to conflict with clinical observations. However, cells may escape estrogen/ER dependence via upregulation of growth factor receptor pathways by an alternative mechanism(s) (i.e., independent of ER). The majority of well-characterized breast cancer cell lines are ER-, so generation of more ER+ models may help capture the heterogeneity of ER+ breast cancers. Patient-derived ER+ breast cancer xenografts may also present clinically-relevant models to study endocrine resistance. While patient-derived ER+ tumors do not graft well in immunodeficient mice, a recently derived immunodeficient knock-in mouse expressing human prolactin (hPRL) has an ER+ tumor graft success rate of 43.1% and is expected to increase the capacity to generate such models.

Preclinical and clinical evidence implicate ER itself in endocrine resistance. Most breast cancers that progress on antiestrogen therapy retain ER. Following progression on an AI, patients are often switched to fulvestrant, which effectively inhibits and partially downregulates ER. Approximately 30% of patients who progress on an AI respond to second-line fulvestrant. High-dose fulvestrant may
provide a longer time-to-progression than estrogen deprivation with the AI anastrozole as first-line treatment for advanced breast cancer. In ER-/H11001 cell lines with acquired resistance to estrogen deprivation, ER remains transcriptionally active, and treatment with fulvestrant or knock-down of ER expression inhibits cell growth. These data suggest that ER may remain active under estrogen-depleted (AI-treated) conditions, and that further inhibition/downregulation of ER (with fulvestrant) may be superior to AI therapy.

**COMBINED TARGETING OF ER AND GROWTH FACTOR RECEPTOR PATHWAYS ABROGATES ENDOCRINE RESISTANCE**

PI3K/AKT/mTOR signaling is required for growth of endocrine-resistant breast cancer cell lines. The estrogen-independent growth of long-term estrogen-deprived cells is inhibited by the PI3K/mTOR inhibitor BEZ235, or the TORC1 inhibitor everolimus (Afinitor, now approved for treatment of advanced ER+ breast cancer in combination with the AI exemestane). Similarly, the pan-PI3K inhibitor buparlisib (BKM120) inhibits estrogen-independent growth of MCF-7 xenografts in mice, and the PI3K/mTOR inhibitor wortmannin inhibits growth of letrozole-resistant MCF-7/aromatase xenografts. Estrogen stimulation blocks the apoptotic effects of BEZ235 in ER+ cells, suggesting that combined blockade of ER and PI3K may be most effective. Indeed, combined treatment with fulvestrant and buparlisib induced regression of MCF-7 xenografts, and fulvestrant plus the IGF-1R/InsR inhibitor OSI-906 completely inhibited tumor growth, while single-agent treatments only slowed growth.

**CELL CYCLE DEFECTS PROMOTE ANTIESTROGEN RESISTANCE**

Dysregulation of cell cycle checkpoints is common in cancer. Since ER regulates the expression of many genes involved in cell cycle progression, and antiestrogens block such ER functions, antiestrogen resistance can arise by genetic alterations that circumvent the requirement for ER. A commonly deregulated checkpoint involves the Cyclin-D/CDK4/CDK6/Rb pathway. Cyclin-D1/CDK4 and Cyclin-D3/CDK6 complexes phosphorylate Rb family proteins, thereby promoting activation of E2F transcription factors to drive expression of genes encoding proteins required for cell cycle progression. Genes encoding Cyclin-D1, Cyclin-D3, CDK4, and CDK6 are amplified, and the Rb tumor suppressor is lost or mutagenically inactivated in many cancers. Rb loss confers tamoxifen resistance in ER+ models. Patients with ER+ breast cancer exhibiting a gene expression signature of Rb loss had shorter recurrence-free survival following adjuvant tamoxifen. A tumor gene expression signature of E2F activation was associated with higher residual tumor cell proliferation following presurgical AI therapy. Therefore, activation of the CDK4/CDK6/E2F axis promotes endocrine resistance, and treatment with a CDK4/6 inhibitor or knockdown of CDK4 expression abrogates endocrine-resistant cell proliferation. For reasons that remain unclear, ER+ breast cancer cell lines are much more sensitive to CDK4/6 inhibition.
than ER- cell lines. In a recent phase II trial, the CDK4/6 inhibitor PD-0332991 in combination with the AI letrozole extended progression-free survival compared with letrozole alone as first-line treatment for metastatic ER+/HER2-breast cancer. This drug combination is being tested in a phase III study.

BIOMARKERS OF RESPONSE TO TARGETED AGENTS (?)

Following the identification of growth factor receptor pathways as causes of endocrine resistance, much effort was expended to identify biomarkers predictive of sensitivity to drugs targeting the various nodes of these pathways in order to identify patient subpopulations that may reap greater benefit from treatment. Such biomarkers were typically identified by screening large panels of cell lines, and searching for genetic or proteomic markers enriched in sensitive or resistant lines. The most mature targeted agent to combat endocrine resistance mediated by growth factor receptor signaling is everolimus, but biomarkers associated with everolimus sensitivity in breast cancer remain unclear.

Mutations in PIK3CA, the gene encoding the p110α catalytic subunit of PI3K, occur in 28-47% of ER+ breast cancers, and are associated with sensitivity to PI3K and AKT inhibitors in cancer cell lines. Thus, PIK3CA was a logical biomarker to select patients for inclusion in early-phase clinical trials with PI3K/AKT inhibitors. However, this genetic biomarker has not been evaluated in the context of PI3K inhibition with endocrine therapy. Early clinical data showed that among 8 patients with advanced ER+ breast cancer who exhibited a response (by [18F]FDG-PET) to buparlisib plus an AI, only two patients had PIK3CA-mutant tumors. In another study considering patients enrolled in phase I trials testing PI3K/AKT/mTOR inhibitors, there was a higher response rate in tumors containing a PIK3CA mutation compared with those with wild-type PIK3CA (30% vs. 10%). These data imply that (1) a significant fraction of PIK3CA-wild-type tumors can respond to PI3K inhibition, and (2) a better biomarker of response is needed.

Genetic biomarkers are easier to measure and more reliable in archived tissue than (phospho)protein biomarkers. While it has been considered that the degree of PI3K pathway activation may predict sensitivity to pathway inhibition, the levels of phospho-AKT were not associated with sensitivity to AKT inhibition in cancer cell lines. Furthermore, PIK3CA mutations are not associated with PI3K pathway activation in cell lines as assessed by AKT phosphorylation. The most appropriate biomarker of sensitivity to PI3K pathway inhibitors may be pharmacodynamic. If a tumor is sensitive to a drug, the tumor should exhibit a rapid metabolic response. This concept is being pursued in ongoing clinical trials testing novel agents. Early clinical data suggest that a metabolic response to buparlisib plus an AI as assessed by [18F]FDG-PET after two weeks of therapy is associated with longer time-on-study, and thus improved response, in patients with metastatic disease. With such a pharmacodynamic biomarker, each patient serves as their own control, and drug response can be assessed within weeks of initiation of therapy. A similar strategy is being tested in the presurgical/neoadjuvant setting, where changes in molecular markers (e.g., phospho-S6 as a marker of TORC1 activation) are detected by comparing pre- and post-treatment tumor tissues.

In contrast to PI3K pathway biomarkers, cancer cell sensitivity to CDK4/6 inhibitors may be more predictable using genetic biomarkers. Genomic loss of Rb renders a cell insensitive to CDK4/6 inhibition, so patients with Rb-deficient tumors should not be treated with a CDK4/6 inhibitor. Amplification of the gene encoding Cyclin-D1, or loss of the gene encoding p16INK4A, may be indicative of cells that have hyperactivated CDK4/6 signaling. Whether these genetic lesions are associated with greater sensitivity to CDK4/6 inhibition is being tested in the trials with letrozole + PD-0332991.

CONCLUSION

Over two decades of research has led to the implication of two major signaling axes in endocrine resistance: (1) growth factor receptor/PI3K/AKT/mTOR, and (2) CDK4/CDK6/Rb/2E2F. Drugs targeting these pathways are being tested clinically in combination with antiestrogens, and have shown promising results thus far. A looming concern is that inherent biases in the model systems used to identify mechanisms of endocrine resistance guided pathway identification. For example, there is an over-representation of PIK3CA mutations among ER+ breast cancer cell lines compared with ER+ tumors. If PIK3CA mutations confer sensitivity to PI3K inhibitors, most ER+ breast cancer cell lines will likely respond. However, many ER+ breast tumors are PIK3CA-wild-type, and available ER+ models to study this genotype are few. With the establishment of more ER+ breast cancer cell lines and patient-derived tumor xenografts, it is hoped that we will establish a broader genotypic repertoire of ER+ breast cancer models, and begin to identify novel drug targets for endocrine-resistant cancers.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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