Modern Risk Assessment for Individualizing Treatment Concepts in Early-stage Breast Cancer

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Validated prognostic and predictive factors currently play an important role in treatment planning for patients with early-stage breast cancer. The role of personalized medicine has led to the search for markers that can be applied to individual patients to optimize treatment regimens. In addition to traditional clinicopathologic measures, scores and gene tests have been developed to independently predict risk of patients in the neoadjuvant and adjuvant settings. The discovery of these markers provides the opportunity to identify patients at such low risk of recurrence that toxic therapy side effects are not justified. Selection and management of patients with early-stage, hormone receptor-positive breast cancer who are appropriately treated with endocrine therapy alone after receiving locoregional therapy but do not necessarily require adjuvant chemotherapy is currently problematic. This article reviews the current state-of-the-art biomarker assessment methods and discusses the potential role for the prediction of chemotherapy benefit focusing on endocrine sensitive disease.

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Breast cancer is the most common form of malignancy and still the second leading cause of cancer mortality in women.[1] Despite an upward trend in incidence rates, a sustained decline in mortality rates can be reported over the past several years, resulting in incremental mature adjuvant regimens.[2,3] A shift toward the detection of early-stage (< 2 cm) node-negative breast cancer is being reported, demonstrating the result of improved screening programs and public education.

However, this satisfying development poses a challenge for clinicians with regard to the optimal adjuvant treatment choice, as patients generally have improved outcomes. High percentages of these patients with improved outcomes are treated by adjuvant chemotherapy in everyday practice, depending on the extent of tumor growth and stage. Nevertheless, the benefit of preventive adjuvant chemotherapy is not clearly demonstrated in early-stage disease. Novel treatment decisions are made by trying to individually estimate the absolute benefit of systemic chemotherapy, taking into consideration which potential toxic side effects might occur. An international survey rated the development of molecular signatures and biomarkers to identify patients who could be spared chemotherapy as the highest translational research priority for breast cancer.[4] Serious treatment-related adverse events are common in chemotherapy, and one might hypothesize that the risk of death from causes other than tumor-associated ones becomes increasingly likely with the toxicity of chemotherapeutic agents. In times of individualized care, clinicians should aspire to avoid overtreatment and prevent toxic therapy side effects. During the past decade, many different tests have been developed to assist the process of improving the accuracy of prediction. However, the current situation, with a broad choice of parameters and biomarkers, often constitutes a challenge in everyday practice. With regard to breast cancer, it can clearly be stated that mortality is associated with distant recurrence of cancer at an advanced stage of disease. Therefore, prediction of breast cancer recurrence at the time of diagnosis could optimize individual treatment decisions and could avoid overtreatment by unnecessary chemotherapy. Consequently, there is an upcoming trend of individualizing therapy regimens according to calculated recurrence risk estimates. Increasing effort is being paid to discovery and development of biomarkers and gene tests in order to improve risk stratification and personalized treatment of breast cancer. Furthermore, predictive markers that are the potential target of a specific therapy itself are of continued interest. Tools for prediction and risk estimation are in great demand by physicians trying to determine whether patients with intermediate recurrence risk could be spared the toxicity and side effects of chemotherapy. The substantial number of tests claiming to stratify the risk of recurrence and provide clinicians with more information on the treatment outcomes of using chemotherapy, endocrine therapy, or combination therapies complicates treatment decisions for oncologists.

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Established Biomarkers

The Estrogen Receptor

The estrogen receptor (ER) is one of the most established prognostic and predictive markers for adjuvant treatment decisions. Approximately 80% of all breast cancer patients have ER-positive disease, meaning that their tumors grow in response to estrogen.[5] The tumor is considered to be ER positive if 10% or more of the cells stain positive by an immunohistochemical (IHC) assay,[2] thereby providing the index for sensitivity to endocrine treatment. ER-positive tumors do respond to endocrine agents in approximately 50% to 60% of cases, showing a greater benefit from endocrine therapy if they are strongly positive. ER is the direct target of endocrine therapy; ER-positive breast cancer can be classified into two intrinsic molecular subtypes with different prognosis and response to treatment, based on the biology of the underlying disease pathways.[6,7] The so-called luminal A and luminal B subtypes are characterized by low and high proliferation levels, respectively.[8-10] In 1998, the Early Breast Cancer Trialists' Collaborative Group[11] reported higher levels of ER being associated with lower recurrence risk in patients receiving adjuvant tamoxifen. Several subsequent trials, such as the National Surgical Adjuvant Breast and Bowel Project (NSABP)-14 trial,[12] and more recent trials comparing aromatase inhibitors and tamoxifen,[13,14] demonstrated an association of improved outcome of both endocrine treatment options with higher ER levels. As the situation in ER-positive disease is clear, the American Society of Clinical Oncology and the College of American Pathologists recommended the standard IHC assessment of both the estrogen and progesterone receptors. According to the expert panel, they should be determined in all invasive breast cancers and recurrences in a standardized algorithm.[15]

The HER2/neu *Oncogene*

Predictive markers may also be the target of a specific therapy itself. The advent of specific tumor characteristics that are treatable by such targeted therapies has significantly improved the outcome of these patients. The human epidermal growth factor receptor (*HER2*) is the most prominent representative of this group. A positive *HER2* status

can be detected in approximately 15% of all primary breast cancers. In cases of overexpression of the oncogene *HER2*, patients are more likely to relapse and tend to have shorter overall survival.[16] *HER2* status predicts good response to anti-*HER2* therapy, being the specific target of anti-*HER2* drugs such as the monoclonal antibody trastuzumab and the tyrosine-kinase inhibitor lapatinib.[17] Clearly, *HER2* plays a predictive and prognostic role in breast cancer. *HER2* amplification status should obligatory be assessed by immunohistochemistry and/or fluorescence in situ hybridization in every breast cancer patient. This enables targeted treatment concepts for patients with over-expression of *HER2*. The gene amplification is being assessed by calculating the ratio of two locus-specific probes using ISH techniques.[18] The test result is considered *HER2*-positive if the ratio of the centromere of chromosome 17 and *HER2* is above 2.2. Aside from many studies that have described the benefit of targeted therapy in cases of *HER2* positivity, recent studies have also reported an association of *HER2* amplification with benefit from adjuvant anthracycline therapy and/or from paclitaxel. With regard to hormone receptor-positive disease, it is clear that aromatase inhibitors and tamoxifen are equally effective in patients with *HER2* amplification. Although anti-*HER2* treatment is generally well tolerated, clinicians should bear in mind that some patients may develop symptomatic or asymptomatic cardiotoxicity, forcing its discontinuation.[19] The risk of trastuzumab-related cardiotoxicity can be minimized by a priori identifying patients who may be at high risk in whom additional anthracycline-based chemotherapy should be omitted, as well as by specific cardiac monitoring. Currently, trials to define, refine, and optimize the use of *HER2*-targeted agents in patients with *HER2*-positive early stage breast cancer are ongoing. Promising new agents, also with minimal cardiotoxicity, are currently under investigation.

New Designs and Biomarkers

The Neoadjuvant Setting

Randomized clinical trials have evaluated neoadjuvant treatment in comparison with adjuvant systemic regimens.[20,21] However, superior outcomes after preoperative treatment could not be reported.[22] Chemotherapy administration before surgery has been used for treatment of breast cancer since the late 1970s, when De Lena and collegues[23] reported tumor response and local control by chemotherapy and subsequent surgery plus radiotherapy. Following preoperative chemotherapy, endocrine treatment was then evaluated in the preoperative setting. Since then, improvements in surgical outcomes have been reported with use of presurgical endocrine therapy agents; studies have confirmed elevated breast conservation rates in postmenopausal women with ER-positive disease. The role of presurgical therapies has since evolved, showing advantages, such as tumor down-staging, and assessment of tumor sensitivity to the chosen regimen, while improving the chance of breast conserving surgery. However, one should be aware that these advantages are only achievable by selecting a defined cohort of patients for preoperative therapy. The preoperative setting allows the opportunity to assess the effects of systemic treatment and certain regimens in prospective trials. It offers the possibility to identify prognostic and predictive significance by using biomarkers as primary endpoints of studies. The burning question of which combination of markers and tumor characteristics might be the best for risk assessment and which should be neglected could thereby be answered.

One of the most important breast cancer trials that has evaluated outcomes after presurgical endocrine agent administration is the Immediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen (IMPACT) trial.[24,25] This randomized double-blinded study assessed the outcome of 330 postmenopausal women with ER-positive disease, receiving either the aromatase inhibitor anastrozole or tamoxifen alone or in combination. As uncontrolled proliferation is one of the hallmarks of cancer, the proliferation marker Ki67 (discussed below) was used as primary endpoint in the IMPACT trial. The neoadjuvant design allowed comparing the levels of Ki67 suppression following 2 and 12 weeks of administration of the particular endocrine agent. The greater decrease of Ki67 at 2 and 12 weeks with anastrozole than that seen with tamoxifen or their combination demonstrated its predominant position in endocrine treatment and predicted results of the much larger adjuvant Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial.[25,26] Other studies subsequently compared the effects of endocrine treatment agents in the neoadjuvant setting using Ki67 as marker for volume and clinical

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response.[27,28] This novel approach with proliferation response to a short-term induction therapy is distinct from designs that simply match baseline levels and mutations with relapse-free survival. These considerations highlight the importance of designing trials in which emerging biomarkers can be evaluated for their prognostic and/or predictive value and therapy regimens for their effectiveness. Such trials provide a great opportunity for detailed study of the determinants of response and resistance to endocrine agents.

Recent data confirm the fact that post-neoadjuvant measurement of Ki67 is a strong predictor of long-term disease outcome, such as overall and recurrence-free survival.

Ki67 and Treatment Benefit

An alternative to conventional primary endpoints of neoadjuvant endocrine therapy trials is the proliferation marker Ki67, already being tested in numerous studies. Ki67 was named after the city of Kiel, Germany, where it was first described in the 1980s, when researchers identified a monoclonal antibody against the nuclear antigen from a Hodgkin lymphoma cell line.[29] After administration of an endocrine agent, tumor levels of this proliferation marker are more prognostic than after baseline analysis. The change in the expression of this antigen after short-term exposure to particular agents is frequently used as a dynamic marker for treatment efficacy. Ki67 is absent in quiescent cells, and is therefore a reliable parameter for measurement of proliferation. The expression levels are determined by the percentage of tumor cell nuclei stained positively. Unfortunately, a standard cutoff value has not been defined thus far.[30] Various studies have investigated the prognostic role of the nuclear proliferation marker, showing an association with shorter overall survival for Ki67 positivity. Interestingly, baseline Ki67 has been found to predict response to chemotherapy, and serial measurements are still under investigation in neoadjuvant settings. Recent data confirm the fact that post-neoadjuvant measurement of Ki67 is a strong predictor of long-term disease outcome, such as overall and recurrence-free survival. Studies detecting decreasing levels of Ki67 after therapy were attracting enormous interest. Just as with the IMPACT trial, the Perioperative Endocrine Treatment for Individualizing Care (POETIC) trial[31] evaluates levels of Ki67 and treatment outcomes. Ki67 levels were measured in postmenopausal patients with hormone receptor-positive disease 2 weeks before and 2 weeks after surgery. The authors reported that higher Ki67 levels after 2 weeks of endocrine treatment were statistically significantly associated with lower recurrence-free survival, whereas higher Ki67 levels at baseline were not. Moreover, their data showed poorer recurrence-free survival rates in patients with larger tumor sizes and lower ER levels at baseline. In this study, short-term changes in proliferation in the neoadjuvant setting were able to predict outcome during adjuvant use of the agents. Again, this study uses the new trial design as a chance to investigate the optimal agent and time for administration. However, paradoxical Ki67 increases after neoadjuvant endocrine treatment have also been reported.[32,33]

Further advances in this field have been made to combine Ki67 with other IHC measurements, such as hormone receptors and *HER2*, which should all routinely be assessed in every breast cancer work-up.[34] A recent study by Cuzick and colleagues[35] evaluating 1125 ER-positive patients from the ATAC trial, showed that accurate quantitative IHC measurements of this standard parameter provides additional prognostic information, and are at least as informative as other genetic tools such as the recurrence score (RS), which is known to provide a very accurate prediction of the likelihood of recurrence.

Another combination of Ki67 with other parameters is considered in the preoperative endocrine prognostic index (PEPI) score. This score has integrated Ki67 measurements in a model with pathologic stage and ER levels. Patients with a PEPI score of 0 (suppressed Ki67 level and persistent ER expression after endocrine treatment) showed a statistically significantly lower risk of relapse, so that they could potentially be spared adjuvant chemotherapy.[36] One interesting study evaluating the PEPI score as secondary endpoint is the recent study by Ellis and colleagues.[28] Their major interest was the response rates to the three different aromatase inhibitors letrozole, anastrozole, and exemestane. The trial took place in the neoadjuvant setting and was initially designed to select agents for further phase III investigation. A total of 377 postmenopausal women with ER-positive disease were enrolled and clinical response was selected as the primary endpoint. Breast-conserving surgery, Ki67, and PEPI score changes, as well as the bioclassifier panel *PAM50*-based intrinsic subtype analysis, were selected as secondary endpoints. The study selected letrozole and anastrozole for further investigation on the basis of clinical response rates but could not report differences

in clinical response rates between the different hormonal agents. Interestingly, luminal A breast cancer subtypes seemed to be more suitable for neoadjuvant endocrine therapy without chemotherapy, as 25% of the patients had a PEPI score of 0. Further trials in this area are urgently needed. Currently, in Germany, the West German Study Group Adjuvant Dynamic Marker-Adjusted Personalized Therapy (WSG ADAPT)[37] trial uses the approach of Ki67 response to short-term therapy in all subtypes of early breast cancer.

Modern Risk Assessment

The Adjuvant! Online Software

For a long time, prognostic estimates were just based on stage of disease, pathologic tumor characteristics and treatment efficacy.[11,38] Classical clinicopathologic characteristics such as the TNM-score have been used in clinical practice for many years for assessing patient prognosis. However, they are insufficient for today's complex treatment decisions.[39,40] The Web-based Adjuvant! Online software (www.adjuvantonline.com [Adjuvant!, Saint Cloud, MN]) is one of the most widely used prognostic tools to help inform clinicians and patients in decision making about therapeutic regimen.[41] It was first described in 1995, utilizing life table analytic techniques and tumor characteristics to project outcomes and thus aid clinicians. It requires the entry of patient age, menopausal status, comorbidities, tumor size, number of positive lymph nodes, and ER status.[42] This Web-based tool calculates individualized 10-year survival probabilities and predicts benefit of adjuvant systemic therapy. Estimates of relapse risk in Adjuvant! Online are based on the 10-year observed overall survival for women with early-stage breast cancer, validated by various registries and databases.[4,43] In a study involving more than 4000 women with early-stage localized breast cancer, Adjuvant! Online was shown to overestimate overall survival, breast cancer-specific survival, and event-free survival in patients younger than 35 years who had additional adverse prognostic factors such as lymphatic or vascular invasion.[43] The study reported a reliable predictive quality of Adjuvant! Online for the majority of patients, but it showed overly optimistic results in subgroups that were not accounted for in the model. The authors recommended a manual adjustment using the prognostic factor impact calculator, which uses a Bayesian method to make adjustments based on relative risks and prevalence of positive test results.[39] Consequently, in a time when IHC and genetic tests were way below today's standards, the call for further adjustment of risk estimation, especially for the subgroups with adverse prognostic factors, came up. In response to this, a rising number of novel biomarkers, scores, and gene tests have been developed for prognostic and predictive purposes. However, only a few markers have reached highest levels of evidence and consequentially made their way into clinical routine.

Multigene Signatures

In recent years, numerous multigene signatures have been identified that aim to outperform traditional prognostic markers. Novel high-throughput technologies for gene expression profiling have been introduced to identify women with early-stage breast cancer who are most likely to benefit from adjuvant treatment. Several genomic tests have been developed to assist in this process, claiming to revolutionize the predictive and prognostic assessment in the clinic.[44-46] Subsequent studies have demonstrated an additional contribution of gene expression profiles to current clinicopathologic factors that are routinely evaluated.[7,12,47-50] Currently, their prognostic role is under investigation in various clinical trials. Three prognostic biomarkers that are already used in clinical practice are the 70-gene MammaPrint signature (Agendia Inc., Irvine, CA), the 21-gene Oncotype DX panel (Genomic Health, Redwood City, CA), and the EndoPredict test (Sividon Diagnostics, Köln, Germany). These tests analyze genes that are involved in the cell cycle, invasion, angiogenesis, and metastasis of the cancer, and could influence clinical care based on the individual molecular profiles of each patient.[44]

Three prognostic biomarkers that are already used in clinical practice are the 70-gene MammaPrint signature, the 21-gene Oncotype DX panel, and the EndoPredict test.

The Oncotype DX assay is a reverse transcriptase polymerase chain reaction assay, analyzing a panel of 21 genes, involving 16 cancer-related genes that are strongly correlated with distant recurrence-free survival, as well as 5 control genes.[12] It is still the most frequently used genomic test in clinical practice in the United States and in many countries in Europe.[51] It has been reported that this test is prognostic for hormone receptor-positive, postmenopausal, tamoxifen-treated patients with negative and positive nodes.[52,53] Oncotype DX determines an RS represented by a number between 0 and 100. The number corresponds with the

specific likelihood of breast cancer recurrence within 10 years of the initial diagnosis and is classified by low, intermediate, or high risk. The RS categorizes hormone receptor-positive lymph node-negative disease into low (RS < 18), intermediate (RS 18-31) or high (RS > 31) risk groups. In 2004, Paik and colleagues[12] reported distant recurrence rates of 6.8%, 14.3%, and 30.5% in the different risk groups, respectively. The RS assay may also predict the magnitude of chemotherapy benefit.[54,55] Unlike the MammaPrint assay, Oncotype DX does not require freshly prepared tissues. In collaboration with several independent investigators, the test has been evaluated in numerous studies involving over 3300 patients. The Southwest Oncology Group 8814 analysis, for instance, demonstrated both prognostic and predictive significance of Oncotype DX in women with ER-positive early breast cancer and positive lymph nodes.[56] The NSABP B-14 and B-20 studies clinically validated a major role of Oncotype DX in recurrence risk estimation and also demonstrated a possible prediction of the magnitude of chemotherapy benefit.[57] Generally, patients with a high RS showed greater benefit from additional chemotherapy than patients with a low RS.[47] Chemotherapy seems to provide little, if any, benefit for patients with low RS, despite the presence of a low number of positive nodes.[54] Another large clinical study conducted by Kaiser Permanente confirmed in a community setting that Oncotype DX helps to predict the likelihood of breast cancer survival at 10 years among ER-positive tamoxifen-treated and systemically untreated patients.[58] According to the National Comprehensive Cancer Network Guidelines, Oncotype DX should be considered as an option for patients with node-negative, hormone receptor-positive, *HER2*-negative tumors 0.6 to 1 cm in size that are moderately to poorly differentiated, or those with angiolymphatic invasion, high nuclear or histologic grade, and tumors > 1 cm in size.[59] To assess the important question regarding how to manage the large population of patients at intermediate risk (RS between 11-25), the Trial Assigning Individualized Options for Treatment (Rx) (TAILORx)[60] was launched in May, 2006. In this prospective trial enrolling over 10,000 patients, Oncotype DX is currently being evaluated in node-negative, ER-positive breast cancer. It remains to be seen whether trial results will be presented in 2014, as expected. Results of the TAILORx trial will provide important information on how to manage the large population of intermediate risk patients. The translational arm of the ATAC study (TransATAC)[61] assessed biomarkers for their prediction of overall and distant recurrence in the translational arm of the ATAC study. The authors found out that each of the factors, ER, progesterone receptor, *HER2*, and Ki67, was associated with the risk of recurrence. Interestingly, the 21-gene RS Oncotype DX provided equal prognostic value as the combination of these four major characteristics did. However, the combination of molecular profiles with classical clinicopathologic variables provided the most accurate prediction of outcome and was even superior to the 21-gene RS. If the multivariate model of ER, progesterone receptor, *HER2,* and Ki67 (so-called IHC4), is being combined with classical clinicopathologic parameters, it could provide similar predictive value to genetic scores as the 21-gene RS. The reclassification in the intermediate RS risk group will surely be a topic for upcoming trials.[34] With regard to the correlation between the RS and Adjuvant! Online, studies showed poor results but commented on a possible benefit of the combination of both prognostic tools.[61]

The MammaPrint is a diagnostic test to determine patient risk and identify which patients will benefit from chemotherapy treatment, based on the Amsterdam 70-gene breast cancer gene signature. The assay tests 70 genes that are focused primarily on proliferation with additional genes associated with invasion, metastasis, stromal integrity, and angiogenesis. The assessment of these 70 risk profile messenger RNAs (mRNAs) and 536 quality and reference mRNAs requires fresh tissue for the microarray analysis.[48] MammaPrint can be used for lymph node-negative tumors < 5 cm in patients of all ages and every ER status. Initially, it was developed to predict the risk of developing distant metastases in 5 years for node-negative patients < 55 years.[48] Validation studies then demonstrated its prognostic value and independent clinical risk classification.[62-64]

MammaPrint was the first fully commercialized microarray-based multigene assay designed to individualize treatment for breast cancer patients, which was approved by the US Food and Drug Administration.[65,66] In 2007, the Microarray in Node Negative and 1-3 Positive Lymph Node Disease May Avoid Chemotherapy (MINDACT) trial,[67,68] sponsored by the European Organization for Research and Treatment of Cancer, began to clinically validate this genomic microarray assay. This multi-institutional, prospective, phase 3 randomized trial compared MammaPrint with Adjuvant! Online, enrolling over 6600 patients with negative or one to three positive nodes for adjuvant chemotherapy. In MINDACT,

patients with both clinical and genomic high risks were offered adjuvant chemotherapy. Those with both clinical and genomic low risks only received endocrine treatment. Patients with discordant risk by MammaPrint and clincopathologic factors were randomized to receive either chemotherapy followed by endocrine therapy or endocrine therapy alone. Preliminary data confirmed the trial hypothesis, but final data have not yet been published.[69] Various trials already confirmed the clinical value of MammaPrint in accurately selected patient cohorts. In a prospective multicenter study enrolling 427 patients, 37% of treatment recommendations were altered due to the use of MammaPrint, sparing 20% of patients from chemotherapy.[70] However, this 70-gene prognosis signature is rather expensive; its cost of approximately $4200 is not routinely reimbursed by social insurance services. Health economic studies evaluating the cost effectiveness of MammaPrint reported that it is a more cost-effective choice compared with Oncotype DX at a threshold willingness-to-pay of $50,000 per quality-adjusted life year.[51] Regardless of its high cost, MammaPrint seems to be a cost-effective strategy to guide adjuvant treatment, especially in younger patients.[71] Recently, Oncotype DX has also been shown to be cost effective in Germany.[72]

The third gene assay currently used is the EndoPredict test. This risk score provides additional prognostic information regarding the risk of distant recurrence in patients with hormone receptor-positive, nodal-negative breast cancer. The comprehensive EP-clin score is generated as a combination between clinicopathologic parameters and the EndoPredict score. It was the first RNA-based prognostic test for breast cancer to outperform all conventional clinicopathologic risk factors alone or in combination.[73,74]

Future Perspective and Summary

Many modern tools aid clinical decision making but still have limitations that must be considered in their clinical application. Classical tools are critically dependent on accurate measurements of clinicopathologic parameters, whereas modern gene assays are appealing because of their presumed objectivity, which is strongly dependent on thorough quality assurance. In the near future, identification of predictive biomarkers for specific chemotherapy sensitivity may potentially allow targeted use of the available agents. A variety of evolving factors already exist that have the potential to become relevant, and new trial designs have been established to assess their potential and identify patients who benefit from adjuvant chemotherapy. Drug targets and targetable signaling pathways may ultimately revolutionize adjuvant treatment regimens in breast cancer. Just as with *HER2*, additional specific targets remain to be identified.

Moreover, there is still a range of protein-based biomarkers that might play a potential role. Urokinase-type plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1) for instance, are tumor tissue-associated cancer biomarkers that have also been validated for their prognostic and predictive value by a meta-analysis in more than 8000 patients, as well as in a prospective clinical trial with 10-year follow-up.[75] Their role in cell migration and invasion is well known. Patients with high tumor tissue antigen content of uPA and/or PAI-1 tend to have more aggressive tumors and poor clinical outcomes. They also benefit significantly from adjuvant chemotherapy.[76] Although the German Working Group for Gynecological Oncology strongly recommends their use as risk group classification markers for routine clinical decision making, they are still not used by the majority of clinicians, likely due to the need for fresh-frozen tumor tissue.[77]

The integration of pre- and post-treatment biomarker assessment could also improve prognostic algorithms. However, not all currently available biomarkers and risk estimates provide accurate prediction of the likelihood of recurrence; some need to be combined with clinical information. It is still difficult to decide which constellation of biomarker assessment results helps select the correct treatment regimen. The decision to abstain from a potentially life-saving treatment can cause uncertainty and anxiety for clinicians as well as patients. Further improvements should focus on the optimal choice and combination of markers and tests, to safely avoid the use of chemotherapy and its accompanying side effects. ■

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References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*. 2011;61:212-236.
2. Friedenreich CM, Woolcott CG, Mctiernan A, et al. Alberta physical activity and breast cancer prevention trial: sex hormone changes in a year-long exercise intervention among postmenopausal women. *J Clin Oncol*. 2010;28:1458-1466.
3. Robert Koch Institut Web site. German Centre for Cancer Registry Data. www.krebsdaten.de. Accessed November 12, 2013.
4. Dowsett M, Goldhirsch A, Hayes DF, et al. International Web-based consultation on priorities for translational breast cancer research. *Breast Cancer Res*. 2007;9:R81.
5. Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest*. 2006;116:561-570.
6. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical

implications. *Proc Natl Acad Sci U S A*. 2001;98:10869-10874.
7. Loi S, Haibe-Kains B, Desmedt C, et al. Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol*. 2007;25:1239-1246.
8. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406:747-752.
9. Sørlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A*. 2003;100:8418-8423.
10. Sotiriou C, Neo SY, Mcshane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci U S A*. 2003;100:10393-10398.
11. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet*. 1998;351:1451-1467.
12. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351;2817-2826.
13. Mouridsen H, Giobbie-Hurder A, Goldhirsch A, et al; BIG 1-98 Collaborative Group. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *N Engl J Med*. 2009;361:766-776.
14. Forbes JF, Cuzick J, Buzdar A, et al; Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol*. 2008;9:45-53.
15. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010;28:2784-2795.
16. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177-182.
17. Pegram MD, Konecny G, Slamon DJ. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. *Cancer Treat Res*. 2000;103:57-75.
18. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2007;25:118-145.
19. Slamon D, Eiermann W, Robert N, et al; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med*. 2011;365:1273-1283.
20. Bonadonna G, Valagussa P, Brambilla C, et al. Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. *J Clin Oncol*. 1998;16:93-100.
21. Fisher B, Saffer E, Rudock C, et al. Effect of local or systemic treatment prior to primary tumor removal on the production and response to a serum growth-stimulating factor in mice. *Cancer Res*. 1989;49:2002-2004.
22. Wolmark N, Wang J, Mamounas E, et al. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr*. 2001;30:96-102.
23. De Lena M, Varini M, Zucali R, et al. Multimodal treatment for locally advanced breast cancer. Result of chemotherapy-radiotherapy versus chemotherapy-surgery. *Cancer Clin Trials*. 1981;4:229-236.
24. Smith IE, Dowsett M, Ebbs SR, et al; IMPACT Trialists Group. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J Clin Oncol*. 2005;23:5108-5116.
25. Dowsett M, Smith IE, Ebbs SR, et al; IMPACT Trialists Group. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst*. 2007;99:167-170.
26. Cuzick J, Sestak I, Baum M, et al; ATAC/LATTE Investigators. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol*. 2010;11:1135-1141.
27. Harper-Wynne CL, Sacks NP, Shenton K, et al. Comparison of the systemic and intratumoral effects of tamoxifen and the aromatase inhibitor vorozole in postmenopausal patients with primary breast cancer. *J Clin Oncol*. 2002;20:1026-1035.
28. Ellis MJ, Suman VJ, Hoog J, et al. Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype--ACOSOG Z1031. *J Clin Oncol*. 2011;29:2342-2349.
29. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer*. 1983;31:13-20.
30. Fasching PA, Heusinger K, Haeberle L, et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. *BMC Cancer*. 2011;11:486.
31. Dowsett M, Smith I, Robertson J, et al. Endocrine therapy, new biologicals, and new study designs for presurgical studies in breast cancer. *J Natl Cancer Inst Monogr*. 2011;2011:120-123.
32. Dowsett M, Smith IE, Ebbs SR, et al. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. *Clin Cancer Res*. 2005;11(2 Pt 2):951s-958s.
33. Ellis MJ, Coop A, Singh B, et al. Letrozole inhibits tumor proliferation more effectively than tamoxifen independent of HER1/2 expression status. *Cancer Res*. 2003;63:6523-6531.
34. Barton S, Zabaglo L, A'Hern R, et al. Assessment of the contribution of the IHC4+C score to decision making in clinical practice in early breast cancer. *Br J Cancer*. 2012;106:1760-1765.
35. Cuzick J, Dowsett M, Pineda S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. *J Clin Oncol*. 2011;29:4273-4278.
36. Ellis MJ, Tao Y, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst*. 2008;100:1380-1388.
37. Harbeck N, Rody A. Lost in translation? Estrogen receptor status and endocrine responsiveness in breast cancer. *J Clin Oncol*. 2012;30:686-689.
38. Polychemotherapy for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet*. 1998;352:930-942.
39. Ravdin PM, Siminoff LA, Davis GJ, et al. Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J Clin Oncol*. 2002;19:980-991.
40. Harris L, Fritsche H, Mennel R, et al; American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol*. 2007;25:5287-5312.
41. Mook S, Schmidt MK, Rutgers EJ, et al. Calibration and discriminatory accuracy of prognosis calculation for breast cancer with the online Adjuvant! program: a hospital-based retrospective cohort study. *Lancet Oncol*. 2009;10:1070-1076.
42. Ravdin PM. A computer based program to assist in adjuvant therapy decisions for individual breast cancer patients. *Bull Cancer*. 1995;82(suppl 5):561s-564s.

MAIN POINTS

- Breast cancer is the most common form of malignancy and still the second leading cause of cancer mortality in women. The role of personalized medicine has led to the search for markers that can be applied to individual patients to optimize treatment regimens.
- With regard to breast cancer, mortality is clearly associated with distant recurrence of cancer at an advanced stage of disease; therefore, prediction of recurrence at the time of diagnosis could optimize individual treatment decisions and avoid overtreatment with unnecessary chemotherapy.
- Three prognostic biomarkers currently used in clinical practice are the 70-gene MammaPrint signature, the 21-gene Oncotype DX panel, and the EndoPredict test. These tests analyze genes that are involved in the cell cycle, invasion, angiogenesis, and metastasis of the cancer, and could influence clinical care based on the individual molecular profiles of each patient.

43. Olivotto IA, Bajdik CD, Ravdin PM, et al. Population-based validation of the prognostic model ADJUVANT! for early breast cancer. *J Clin Oncol*. 2005;23:2716-2725.
44. Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. *N Engl J Med*. 2009;360:790-800.
45. Ross JS, Hatzis C, Symmans WF, et al. Commercialized multigene predictors of clinical outcome for breast cancer. *Oncologist*. 2008;13:477-493.
46. Marchionni L, Wilson RF, Wolff AC, et al. Systematic review: gene expression profiling assays in early-stage breast cancer. *Ann Intern Med*. 2008;148:358-369.
47. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006;24:3726-3734.
48. van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*. 2002;415:530-536.
49. Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst*. 2006;98:262-272.
50. Hu Z, Fan C, Oh DS, et al. The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics*. 2006;7:96.
51. Yang M, Rajan S, Issa AM. Cost effectiveness of gene expression profiling for early stage breast cancer: a decision-analytic model. *Cancer*. 2012;118:5163-5170.
52. Esteban J, Baker J, Cronin M, et al. Tumor gene expression and prognosis in breast cancer: multi-gene RT-PCR assay of paraffin-embedded tissue. *Proc Am Soc Clin Oncol*. 2003;22:A3416.
53. Cobleigh MA, Bitterman P, Baker J, et al. Tumor gene expression predicts distant disease-free survival (DDFS) in breast cancer patients with 10 or more positive nodes: high throughput RT-PCR assay of paraffin-embedded tumor tissues. *Proc Am Soc Clin Oncol*. 2003;22:850.
54. Albain KS, Barlow WE, Shak S, et al; Breast Cancer Intergroup of North America. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. 2010;11:55-65.
55. Andre F, Delaloge S. First-generation genomic tests for breast cancer treatment. *Lancet Oncol*. 2010;11:6-7.
56. Albain KS, Barlow WE, Ravdin PM, et al; Breast Cancer Intergroup of North America. Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. *Lancet*. 2009;374:2055-2063
57. Tang G, Shak S, Paik S, et al. Comparison of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. *Breast Cancer Res Treat*. 2011;127:133-142.
58. Habel LA, Shak S, Jacobs MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res*. 2006;8:R25.
59. National Comprehensive Cancer Network Clinical Practice Guidelines. 2009.
60. Trial Assigning Individualized Options for Treatment (Rx). Hormone Therapy With or Without Combination Chemotherapy in Treating Women Who Have Undergone Surgery for Node-Negative Breast Cancer (The TAILORx Trial). ClinicalTrials.gov Web site. http://clinicaltrials.gov/show/NCT00310180. Accessed November 12, 2013.
61. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol*. 2010;28:1829-1834.
62. van de Vijver MJ, He YD, Van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med*. 2002;347:1999-2009.
63. Bueno-de-Mesquita JM, Linn SC, Keijzer R, et al. Validation of 70-gene prognosis signature in node-negative breast cancer. *Breast Cancer Res Treat*. 2009;117:483-495.
64. Buyse M, Loi S, van't Veer L, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst*. 2006;98:1183-1192.
65. Mook S, Schmidt MK, Weigelt B, et al. The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age. *Ann Oncol*. 2010;21:717-722.
66. Wittner BS, Sgroi DC, Ryan PD, et al. Analysis of the MammaPrint breast cancer assay in a predominantly postmenopausal cohort. *Clin Cancer Res*. 2008;14:2988-2993.
67. Cardoso F, Piccart-Gebhart M, van't Veer L, Rutgers E; TRANSBIG Consortium. The MINDACT trial: the first prospective clinical validation of a genomic tool. *Mol Oncol*. 2007;1:246-251.
68. Cardoso F, Van't Veer L, Rutgers E, et al. Clinical application of the 70-gene profile: the MINDACT trial. *J Clin Oncol*. 2008;26:729-735.
69. Rutgers E, Piccart-Gebhart MJ, Bogaerts J, et al. The EORTC 10041/BIG 03-04 MINDACT trial is feasible: results of the pilot phase. *Eur J Cancer*. 2011;47:2742-2749.
70. Bueno-de-Mesquita JM, van Harten WH, Retel VP, et al. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). *Lancet Oncol*. 2007;8:1079-1087.
71. Chen E, Tong KB, Malin JL. Cost-effectiveness of 70-gene MammaPrint signature in node-negative breast cancer. *Am J Manag Care*. 2010;16:e333-e342.
72. Blohmer JU, Rezai M, Kummel S, et al. Using the 21-gene assay to guide adjuvant chemotherapy decision-making in early-stage breast cancer: a cost-effectiveness evaluation in the German setting. *J Med Econ*. 2013;16:30-40.
73. Filipits M, Rudas M, Jakesz R, et al; EP Investigators. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res*. 2011;17:6012-6020.
74. Denkert C, Kronenwett R, Schlake W, et al. Decentral gene expression analysis for ER+/Her2- breast cancer: results of a proficiency testing program for the Endo-Predict assay. *Virchows Arch*. 2012;460:251-259.
75. Harbeck N, Schmitt M, Meisner C, et al; Chemo-N 0 Study Group. Ten-year analysis of the prospective multicentre Chemo-N0 trial validates American Society of Clinical Oncology (ASCO)-recommended biomarkers uPA and PAI-1 for therapy decision making in node-negative breast cancer patients. *Eur J Cancer*. 2013;49:1825-1835.
76. Harbeck N, Kates RE, Schmitt M, et al. Urokinase-type plasminogen activator and its inhibitor type 1 predict disease outcome and therapy response in primary breast cancer. *Clin Breast Cancer*. 2004;5:348-352.
77. Annecke K, Schmitt M, Euler U, et al. uPA and PAI-1 in breast cancer: review of their clinical utility and current validation in the prospective NNBC-3 trial. *Adv Clin Chem*. 2008;45:31-45.