

# NCCN Task Force Report: Estrogen Receptor and Progesterone Receptor Testing in Breast Cancer by Immunohistochemistry

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## Key Words

Breast cancer, immunohistochemistry, estrogen receptor, progesterone receptor, endocrine therapy, NCCN Clinical Practice Guidelines in Oncology, technical validation, clinical validation, false-negative rate, quality control, quality assurance

## Abstract

The NCCN Task Force on Estrogen Receptor and Progesterone Receptor Testing in Breast Cancer by Immunohistochemistry was convened to critically evaluate the extent to which the presence of the estrogen receptor (ER) and progesterone receptor (PgR) biomarkers in breast cancer serve as prognostic and predictive factors in the adjuvant and metastatic settings, and the ability of immunohistochemical (IHC) detection of ER and PgR to provide an accurate assessment of the expression of these biomarkers in breast cancer tumor tissue. The task force is a multidisciplinary panel of 13 experts in breast cancer who are affiliated with NCCN member institutions and represent the disciplines of pathology, medical oncology, radiation oncology, surgical oncology, and biostatistics. The main overall conclusions of the task force are ER is a strong predictor of response to endocrine therapy; ER status of all samples of invasive breast cancer or ductal carcinoma in situ (DCIS) should be evaluated by IHC; IHC measurements of PgR, although not as important clinically as ER, can provide useful information and should also be performed on all samples of invasive breast cancer or DCIS; IHC is the main testing strategy for evaluating ER and PgR in breast cancer and priority should be given to improve the quality of IHC testing methodologies; all laboratories performing IHC assays of ER and PgR should undertake formal validation studies to show both technical and clinical validation of the assay in use; and all laboratories performing IHC assays of hormone receptors in breast cancer should follow additional quality control and assurance measures as outlined in the upcoming guidelines from the American Society of Clinical Oncology and College of American Pathologists. (*JNCCN* 2009;7[Suppl 6]:S1–S21)

## Task Force Rationale

Estrogen receptor (ER) status is a powerful predictor of breast cancer response to endocrine therapy. Results from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) overview show that tamoxifen substantially reduces risk for breast cancer recurrence and death across all age groups in patients with ER-positive early-stage breast cancer, whereas patients with ER-negative disease do not show benefit from tamoxifen.<sup>1,2</sup> ER status also predicts response to endocrine therapy in the metastatic setting.<sup>3,4</sup> In addition, retrospective evidence suggests that the benefits of chemotherapy are significantly higher in patients with ER-negative compared with ER-positive early-stage, node-positive breast cancer, indicating that information on ER tumor status can also impact treatment decision-making regarding adjuvant chemotherapy.<sup>5,6</sup>

Virtually all determinations of the ER and progesterone receptor (PgR) status of breast cancers are performed today using immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tissue. Nevertheless, several problems have been associated with IHC tests of these hormone receptors, the most important being a high false-negative rate. For example, the false-negative rate of samples evaluated for ER by IHC in one consult practice was estimated at approximately 20% to 30% (with possibly higher rates for PgR; Craig Allred, MD, personal communication). This problem has recently received increased attention after reports of false-negative rates of 30% to more than 60% in several different settings.<sup>7–11</sup>

In contrast, results from ECOG E2197 study showed a 90% concordance when IHC test results of

ER in breast cancer tissue samples evaluated in local laboratories were compared to results obtained in a central laboratory.<sup>12</sup> Members of the task force acknowledged that although more typical false-negative rates of IHC determinations of ER status in breast cancer may not be represented by the more extreme cases reported, this is nonetheless a relatively common problem (i.e., estimated at 20% in the United States).

Ramifications of a false-negative test result for ER/PgR tumor status are potentially devastating for women with breast cancer, resulting in the withholding of a highly effective therapy with a good safety profile (Figure 1). Furthermore, the potential impact of decreasing the number of false-negative results is great because approximately 70% to 80% and more than 50% of women with breast cancer in higher-income countries have ER-positive and PgR-positive disease, respectively,<sup>13–15</sup> although these values may not be as high in some lower-income countries.

The task force was formed to facilitate interaction between pathologists and clinicians to enhance their cooperation in addressing the existing problems related to hormone receptor testing in breast cancer. The purpose of the following task force report is several-fold:

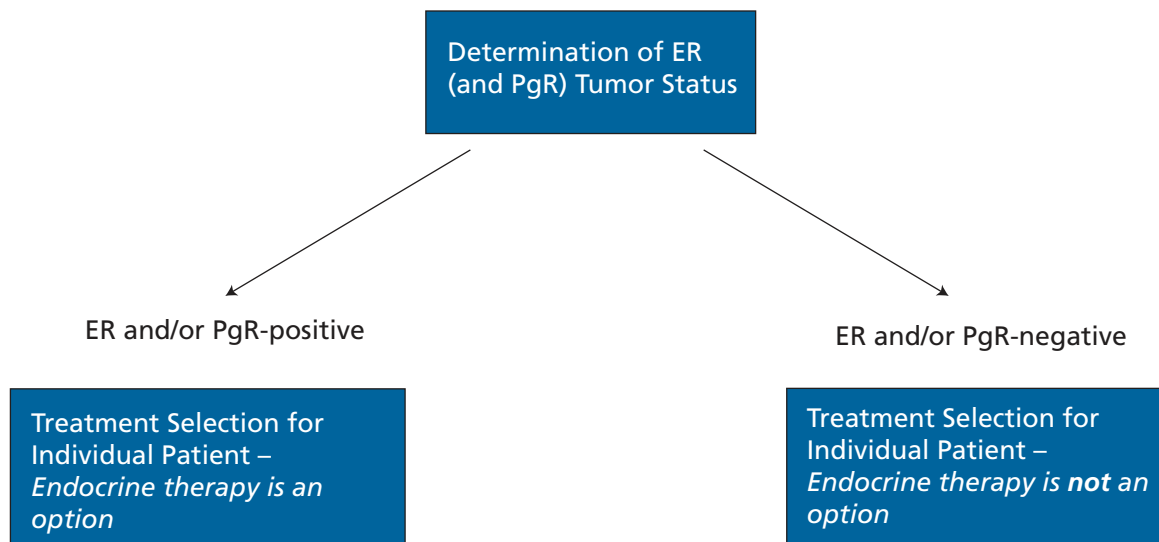
- To provide clinicians with insight into strengths and weakness of tests used to determine ER and PgR status in breast cancer, with emphasis on

IHC tests of these hormone receptors.

- To provide pathologists with insight into the impact of results of ER and PgR testing on clinical decision-making for patients with breast cancer.
- To provide pathologists with recommendations for improving the quality of IHC testing of ER and PgR in breast cancer.
- To provide clinicians with information to assess quality and interpret results of IHC tests of ER and PgR in breast cancer.
- To incorporate information and recommendations into NCCN Clinical Practice Guidelines in Oncology: Breast Cancer (to view the most recent version of these guidelines, visit the NCCN Web site at [www.nccn.org](http://www.nccn.org)).

### Task Force Process

All 13 members of the task force are affiliated with NCCN member institutions, and some of the participants are also members of the NCCN Breast Cancer Guidelines Panel. Medical specialties of the participants included pathology, medical oncology, surgical oncology, radiation oncology, and biostatistics. A formal meeting agenda was developed by the task force chairs before the meeting, and individual participants presented focused didactic presentations based on identification of key references, followed by group discussion. An evidence-based



**Figure 1** Detection of ER (estrogen receptor; and PgR [progesterone receptor]) in breast cancer tissue determines whether a patient is a candidate for endocrine therapy.

consensus approach<sup>16</sup> was used to formulate recommendations relating to the pathologic and clinical application of the evidence to the evaluation and care of patients with breast cancer. In areas of controversy, this process extended beyond the meeting to achieve consensus. Draft versions of this report were circulated among all task force members for review and comment.

## Biology of Steroid Hormone Receptors

Estrogen and progesterone are steroid hormones that play a central role in regulating the growth and differentiation of normal breast epithelium and in promoting the development of breast cancer (especially in a setting of prolonged and/or high levels of exposure). The functions and effects of these hormones are mediated through the binding and activating of specific receptors. An ER, first identified in 1962,<sup>17–19</sup> was subsequently named *ER-alpha* after the more recent discovery of another ER, termed *ER-beta*.<sup>20,21</sup> Two different genes encode for the 2 types of ER.<sup>22</sup> Two PgRs, the isoforms PgR-A and PgR-B (the former being a truncated form of the latter), have also been identified and characterized.<sup>23,24</sup>

ERs and PgRs are members of the class I nuclear receptor superfamily. Radiographic crystallographic studies have shown that ER-alpha, ER-beta, and isoforms of PgR have similar 3-dimensional structures, particularly with respect to protein domains involved in receptor dimerization, and the binding of ligand, DNA, and transcriptional cofactors.<sup>25,26</sup> This section focuses on the biology of ER-alpha, the most well studied of these receptors. Other hormone receptors (e.g., ER-beta and PgR, and its isoforms) participate in regulating some of the same functions as ER-alpha, but they also possess distinct properties. When not otherwise specified, “ER” refers to ER-alpha and PgR refers to both PgR-A and PgR-B.

ER-alpha is a soluble protein, with a molecular weight of 66,000 Da,<sup>27</sup> although many smaller so-called splice variants, formed through posttranscriptional modification, are translated into lower molecular weight forms of the protein.<sup>28,29</sup>

The classic mechanism of action for ER-alpha is based on its ability to act as a ligand-dependent transcription factor.<sup>25</sup> This process is initiated by diffusion of the hydrophobic estrogen molecule across the plasma membrane of the cell. Estrogen then binds

to ER-alpha located in either the cytoplasm or the nucleus of the cell. Ligand binding induces a protein conformational change that facilitates receptor dimerization, with subsequent translocation of the cytosolic receptor dimer across the nuclear membrane. The receptor dimer within the nucleus is able to bind to specific transcriptional cofactors and interact either directly or indirectly with DNA through association with other DNA-binding proteins to activate or repress the transcription of estrogen-responsive genes (see Figure 2). Manifestation of the transcriptional impact of this type of regulation has been estimated to require a timeframe of minutes to days.

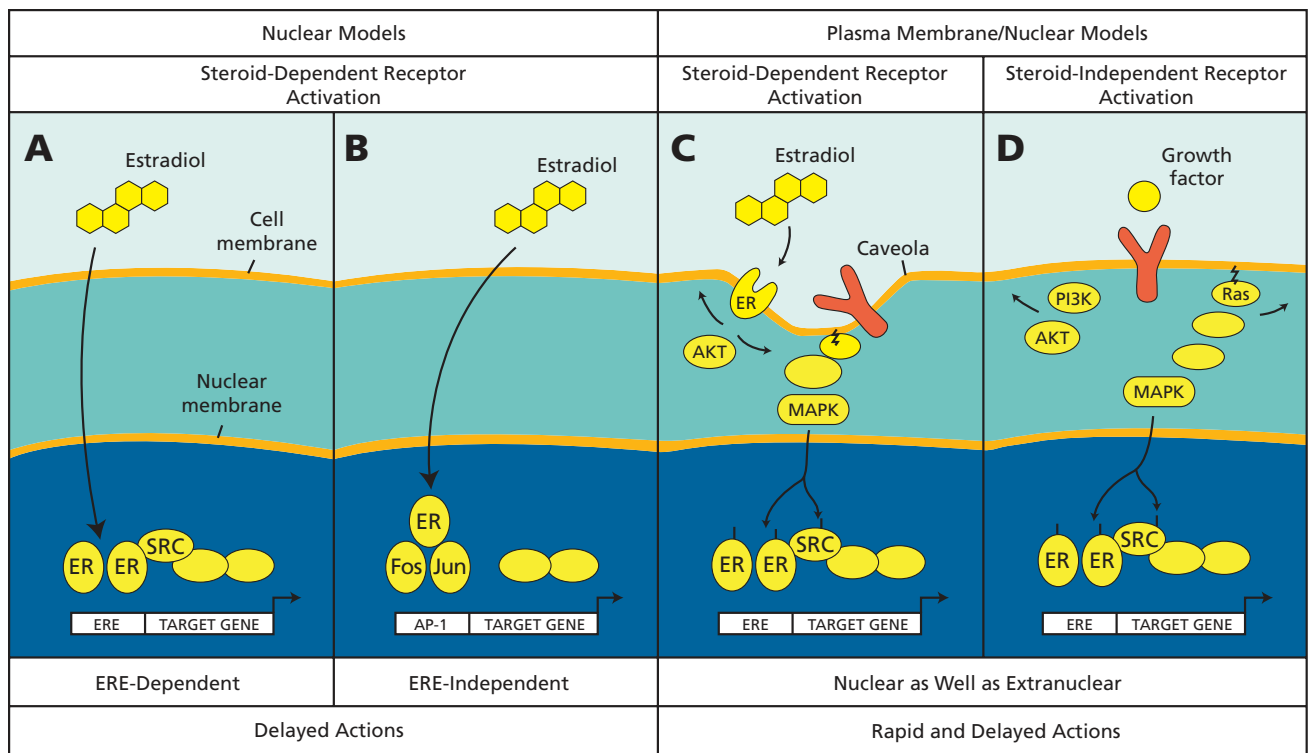
Some estrogen-induced cellular processes also occur over much shorter periods (i.e., seconds to minutes). Although first reported more than 30 years ago, experts have recently established that ER can also associate with the plasma membrane of the cell.<sup>30</sup> Evidence shows that protein palmitoylation of the receptor facilitates its association with caveolin-1, a “lipid raft” protein that is believed to play a role in membrane trafficking.<sup>31</sup> This, in turn, promotes localization of the receptor to the plasma membrane.<sup>32</sup> The membrane-bound form of ER is known to be capable of initiating rapid cellular signaling on estrogen binding through activation of particular kinases.<sup>33,34</sup> These signaling processes ultimately influence nongenomic cellular processes and genomic cellular responses through “crosstalk” with other signaling cascades or production of second messengers (see Figure 2), resulting in stimulation of cellular proliferation and suppression of apoptosis.<sup>25,34</sup>

Phosphorylation of the receptor at specific amino acid residues can modulate its activity in nuclear and plasma membrane-bound environments.<sup>25,35</sup> A phosphorylated form of membrane-bound ER is present in invasive breast cancer but not in ductal carcinoma in situ (DCIS) or normal breast tissue.<sup>36</sup> Phosphorylated forms of ER attached to the plasma membrane or residing in the nucleus may be capable of initiating cell signaling processes independent of bound ligand.<sup>35,37</sup>

## Endocrine Therapy: Mechanisms of Action

Endocrine therapies for the treatment of breast cancer include a wide variety of endocrine agents, including the antiestrogens, tamoxifen and fulves-

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**Figure 2** Model of estrogen action in target cells. In many prevailing models (A, B), estrogen freely enters all cells and is retained only in those cells in which it binds to its receptor. (A) In the nucleus, estrogen binding to ER (estrogen receptor) is believed to favor enhanced gene transcription with coactivator proteins and certain estrogen-responsive elements (EREs) on specific genes, leading to selective gene transcription. (B) This nuclear model accounts for ERE-independent genomic activation; ER interacts with activating protein-1 (AP-1) to facilitate gene transcription. (C) In this model, estrogen binds to a plasma-membrane associated ER to promote hormonal responses through another pathway that “crosstalks,” or interacts directly with, a genomic mechanism. (D) In this steroid-independent model of estrogen action, activation of ER occurs through mediation by growth factor receptor signaling pathways. Abbreviations: AKT, protein kinase B; Fos and Jun, transcription factors; SRC, steroid receptor coactivator; MAPK, mitogen-activated protein kinases; PI3K, phosphatidylinositol-3-kinase.

From Pietras RJ. Biologic basis of sequential and combination therapies for hormone-responsive breast cancer. *Oncologist* 2006;11:704–717; with permission.

trant, and reversible (e.g., letrozole, anastrozole) and irreversible (e.g., exemestane) aromatase inhibitors. Tamoxifen, the most extensively studied of these therapies, is converted by the enzyme CYP2D6 to endoxifen, its active metabolite, which then competes with estrogen for the ligand binding site of ER. Tamoxifen acts as either an estrogen antagonist or agonist, depending on the species, target tissue, and end point studied. For example, tamoxifen displays antiestrogen activity in breast tissue but acts as an estrogen agonist with respect to bone and endometrial tissue.<sup>38,39</sup> Fulvestrant also binds to ER at the estrogen binding site but is considered to be a pure antiestrogen without agonist activity in any organ. It is known to interfere with several cellular processes involving ER, such as the binding of certain cofactors, receptor dimerization, and translocation of the

receptor to the nucleus.<sup>40,41</sup> Reversible nonsteroidal and irreversible steroidal aromatase inhibitors do not interact directly with ER. Instead, they interfere with the enzymatic conversion of androgens to estrogen, thereby lowering the estrogen concentration available for binding to ER in tissues.<sup>42</sup>

Importantly, not all ER-positive breast cancers respond to endocrine therapy. In the setting of metastatic breast cancer, almost all tumors exhibiting a response to endocrine therapy will eventually experience disease progression despite continued treatment. The molecular basis for endocrine refractory/resistant disease is unclear and likely to be diverse.<sup>43,44</sup> Proposed mechanisms include post-translational modification of ER and its transcriptional co-activators and repressors, and crosstalk with other signaling pathways. The 2 processes may be linked.

For example, breast cancer tumors that overexpress human epidermal growth factor receptor 2 (HER2) are more likely to be resistant to endocrine therapy compared with HER2-negative tumors.<sup>45</sup> HER2 overexpression has been reported to trigger signaling for increased ER phosphorylation.<sup>46</sup> Phosphorylation at particular sites on ER may increase the binding affinity of ER for estrogen, interfere with endoxifen-ER association, or provide for ligand-independent activation of ER.<sup>34,35</sup> Other possible causes of resistance to endocrine therapy may involve an increase in aromatase levels.<sup>34</sup>

### Methods of Detecting ER and PgR in Breast Cancer

Most ER and PgR testing is performed using IHC assays, although other methods of detecting ER and PgR in breast cancer tissue are available. Several fundamental principles of tumor marker testing should be considered when addressing ER and PgR testing of breast cancer.<sup>47-51</sup>

First, the most important purpose of evaluating the status of a tumor marker is to predict whether a patient is likely to experience a clinically important benefit from a particular therapy.<sup>52</sup> In the case of endocrine therapy, this would ideally involve an assessment of the functionality of ER and PgR, including an evaluation of the activated downstream proteins of these receptors. None of the currently available tests provide this assessment, although expression of PgR is known to be regulated by estrogen-activated ER; hence, a PgR-positive status is one indication of functional ER.<sup>53,54</sup> Nevertheless, measurement of ER and PgR status in breast cancer tissue meets the criterion of usefulness with respect to clinical decision making (see Clinical Issues, page S-9).

Second, the test used to measure the tumor marker should be technically validated. A technically validated assay is sensitive, specific, and reproducible and allows for a uniform interpretation of results.<sup>52</sup> The process of technical validation involves measuring the degree of concordance between tumor marker results obtained using the test assay and another assay previously shown to be technically valid. Furthermore, technical validation of an IHC test against another technically valid IHC method for assessing ER status involves using the same method to quantify and interpret signals in both assays.

Finally, measurement of a tumor marker using a technically validated assay should be clinically validated in that it should be calibrated to identify groups of patients with significantly different risks of relapse, survival, or treatment response, preferably as shown in multiple randomized clinical trials.<sup>52</sup> In other words, it involves correlation between clinical outcome and the status of the tumor marker as measured by a particular assay; this information is necessary to establish a clinical cutoff value separating a positive from a negative result. In fact, the term *clinical validation* can be applied to both a particular assay and the biomarker that is measured in the context of that assay. Some specific examples of the processes used to technically and clinically validate particular IHC assays of ER and PgR are described later.

ER has been measured in breast cancer tissue for more than 35 years. In 1973, McGuire<sup>55</sup> used a dextran-coated charcoal ligand assay to show the broad concentration range of ER in breast cancer tissue, and LB assays were routinely used for this purpose for many years. An advantage to using the LB assay for measuring ER is that it is a quantitative method (showing a continuum of ER concentrations in breast cancer tissue) that has been correlated with clinical outcome.<sup>52,56,57</sup> Nevertheless, the LB assay is difficult and expensive to perform. Other disadvantages include the need for large frozen specimens and radioisotopes, and the fact that receptors occupied by endogenous substances are not detectable. Perhaps most importantly, the LB assay is a “grind-up” method that measures ER in all cells of the sample tissue (i.e., tumor and normal breast tissue).

In 1985, McCarty et al.<sup>58,59</sup> showed a correlation between measurements of ER in breast cancer tissue obtained using the LB assay and those using an IHC assay with a highly specific monoclonal antibody for the receptor. The methodology of IHC testing is outlined in Table 1 for ER, although the same general schema applies to IHC testing of PgR. The IHC test is typically performed on thin slices of formalin-fixed, paraffin-embedded tissue. An example of a stained specimen of breast cancer tumor tissue processed according to the IHC assay for ER is shown in Figure 3.

Advantages of IHC over the LB assay include the ability to be performed inexpensively on small specimens, frozen or fixed, without radioactivity. In addition, it allows pathologists to restrict their evaluation of ER and PgR status to tumor cells only.<sup>58-60</sup>

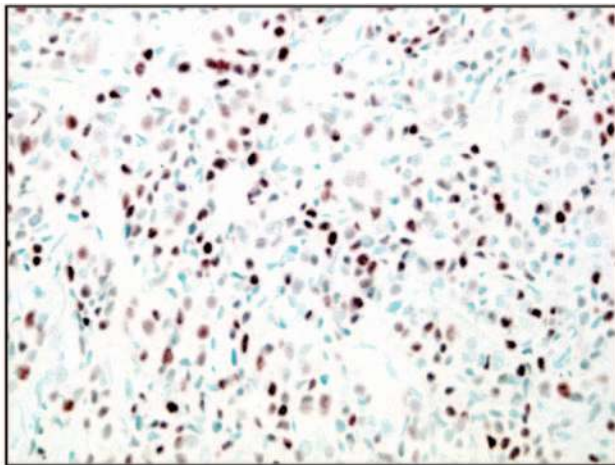


**Table 1 Shared Elements of IHC Assays of ER and PgR**

- Sample is formalin-fixed paraffin-embedded tissue
- Antigen retrieval process (many methods)
- ER or PgR specific antibody (several/well validated)
- Generation of a color signal
- Quantification of signal
- Interpretation of signal

Abbreviations: ER, estrogen receptor; IHC, immunohistochemistry; PgR, progesterone receptor.

IHC tests of hormone receptors provide either dichotomous or more quantitative results. Several IHC systems have been devised to score results from IHC tests of ER in breast tissue, including the Allred score. The Allred score provides an overall score of either 0 or 2–8 through summation of a proportion score and an intensity score; first the proportion of cells with positive staining is estimated with a score of 0 to 5, then an average staining intensity is determined with a score of 0 to 3.<sup>52</sup> Another method, called the “H-score,” provides an overall score (0–300) based on the sum of weighted percentages of cells stained as weak, moderate, or strong.<sup>45,61,62</sup> A dichotomous interpretation of results (i.e., positive



**Figure 3** Representative invasive breast cancer sample with immunostaining. ER-positive cells are stained brown or black. Note cells characterized by the absence or presence of stain and variations in the stain intensity of stained cells. Using the Allred score as an example, this sample would have a proportion score of 4 (1/3–2/3 “positive” cells) and an average intensity score of 2 (intermediate) to give a final Allred score of 6/8. Courtesy of D. Craig Allred, MD.

vs. negative) is also used.<sup>5,13,63,64</sup> Automated systems are also available for the quantitative analysis of ER expression in breast cancer tissue.<sup>65</sup>

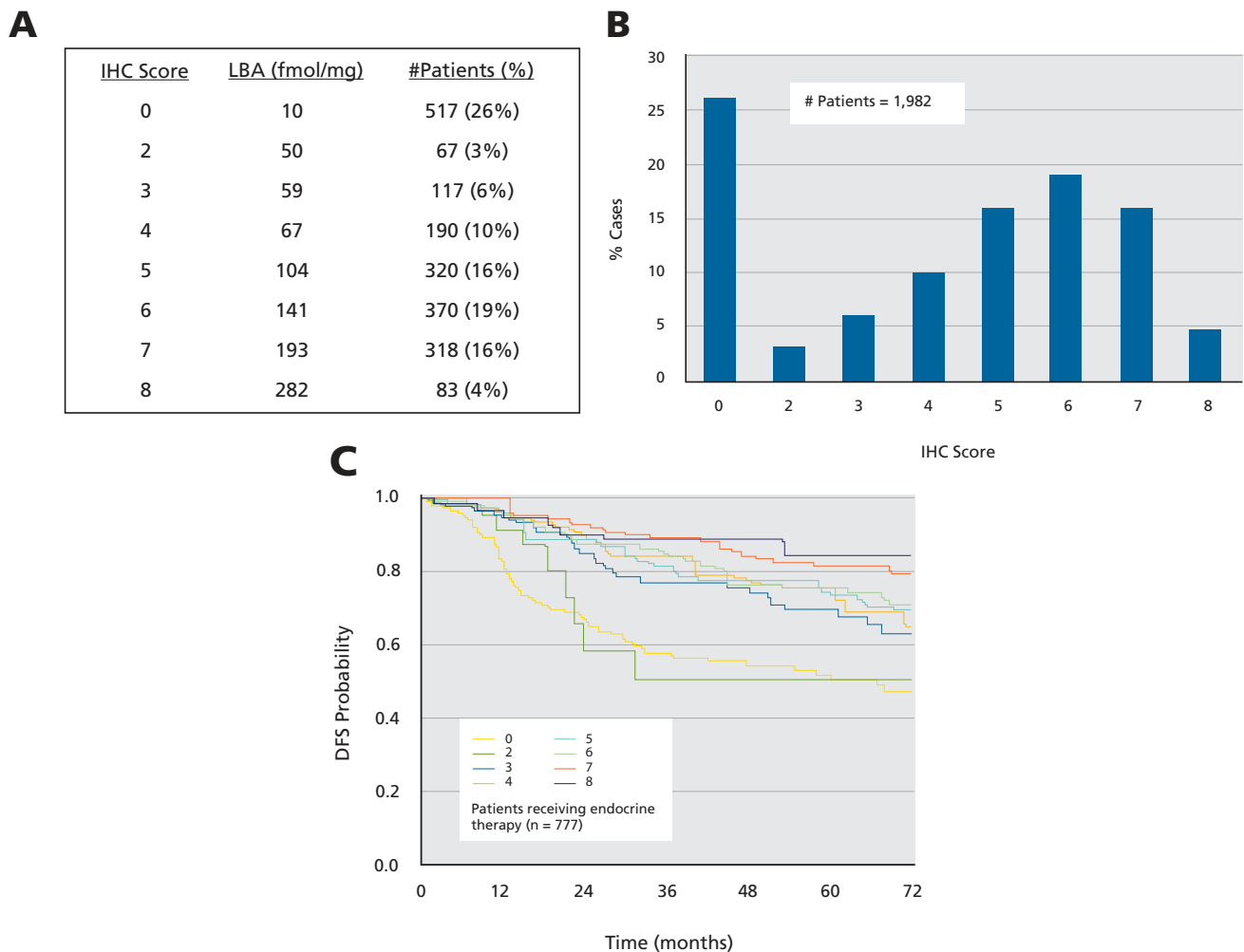
Studies evaluating the correlation of measurements of ER and PgR in breast cancer tissue performed using either IHC testing or the LB assay (as a gold standard) have typically shown discordance rates ranging from 10% to 30%.<sup>60,66–71</sup> In some studies, IHC was found to be superior to the LB assay in predicting clinical response to endocrine therapy in certain patient populations,<sup>66,69–71</sup> although other studies showed the LB assay to be more accurate.<sup>60,72</sup>

In a study published in 1999, Harvey et al.<sup>66</sup> described the development of a standardized procedure for detecting ER in breast tissue using IHC, and provided detailed information on the processes followed for technical and clinical validation of the assay in a large population of women with breast cancer (Figure 4). The original study used samples prepared in an unconventional manner (particulate frozen tissue left over from the LB assay, which was concentrated using centrifugation, fixed in 10% neutral buffered formalin for 8–12 hours, and processed to formalin-fixed, paraffin-embedded tissue samples). However, this procedure has been validated in other studies based on conventionally prepared samples.<sup>14,73–76</sup>

The 6F11 monoclonal antibody to ER was shown to be sensitive and specific for detecting ER in tumor cells using IHC through a comparison of assay results from IHC and LB assays performed on the same tumor samples; assay concordance was 86%. Using the Allred method, a range of IHC scores were observed, representing a nearly linear correlation between ER concentrations detected using IHC and the LB assay. A cutoff distinguishing a positive from a negative IHC result was determined through calibrating assay results with clinical outcome (i.e., disease-free survival in patients treated with adjuvant tamoxifen). In addition, a distribution of IHC scores was shown to correlate with a distribution of responses to tamoxifen. Interestingly, 3 was the lowest score predicting for a positive response to hormonal therapy, corresponding to as few as 1% to 10% of weakly positive tumor cells.

Another IHC assay uses a cocktail of ER.2.123 and 1D5 as primary antibodies. The test kit of this assay is the only FDA-approved IHC assay of ER in breast cancer;<sup>77</sup> FDA approval was made after this assay was carefully validated against that of Harvey et al.<sup>66,78</sup>

## Estrogen Receptor and Progesterone Receptor Testing in Breast Cancer



**Figure 4** Example of study showing relationship of ligand binding (LB) assay measurements, distribution of IHC scores, and clinical response. (A) Comparison of measurements of ER as determined by immunohistochemistry (IHC) and LB assay. (B) Distribution of IHC scores using the Allred scoring method. (C) Comparison of disease-free survival curves for all possible Allred IHC scores allows for determination of cutoff score distinguishing a positive from a negative result. A positive score is Allred score  $\geq 3$  (indicating  $> 1\%$  of cells with weak staining is a positive result).

Panels A and C from Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999;17:1474–1481. Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved.

Other examples of standardized, comprehensively validated IHC assays for ER were reported by Cheang et al.<sup>72</sup> In this study, tissue microarrays were constructed from formalin-fixed tumor specimens from a large population of women in British Columbia with newly diagnosed invasive breast cancer. Most of the specimens had previously been evaluated for ER using the LB assay. Two different IHC assays were performed on these samples using the SP1 and 1D5 monoclonal antibodies to ER. In most cases, results were reported as either negative ( $< 1\%$  of cells stained) or positive ( $\geq 1\%$  of cells stained). This cut-

off value was subsequently shown to correlate with overall survival in a retrospective study of patients from the NSABP B-09 trial in which the ER status of tumor samples was retested using an IHC method and compared with results obtained with other testing methods (e.g., LB assay).<sup>64</sup>

A comparison with results from the LB assay showed the IHC assays using SP1 and 1D5 antibodies to be sensitive and specific for detecting ER in breast tumor tissue. Concordance of ER tumor status with respect to the LB assay was 86.8% and 80.5% for the IHC assay using SP1 and 1D5 antibodies, re-

spectively. In addition, both IHC assays and the LB assay were shown to be good predictors of breast cancer-specific and relapse-free survival.

Because not all patients with ER-positive disease benefit from endocrine therapy, one of the initial driving forces behind the evaluation of tumor PgR status in breast cancer was to identify additional markers of endocrine therapy response. Several comprehensively validated IHC assays for PgR have been developed.<sup>11,45,70</sup> Table 2 provides a list of standard IHC methods for evaluating ER and PgR in breast cancer tumor samples, which have also been comprehensively validated. These assays include those that have been clinically validated in patients treated with tamoxifen and an aromatase inhibitor.

Tests for measuring ER and PgR in breast cancer are evolving. The task force recognizes that new methods are being developed that may replace IHC at some point in the future. Nevertheless, IHC is likely to be the main testing strategy for the evaluation of these receptors in breast cancer for several years, and therefore it is the central focus of this report.

Promising new strategies being developed for the measurement of ER and PgR in breast cancer tissue involve a fluorescence-detected IHC assay and several gene-based assays. For example, fluorescent probes used in an IHC assay using an automated image immunofluorescence detection system (AQUA, Automated Quantitative Analysis)<sup>79</sup> have been reported to have an advantage over traditionally used chromogenic (i.e., brown) stains in that they can provide a broader dynamic range for

detecting the wide spectrum of ER concentrations in breast tissue.<sup>79–82</sup>

Other alternative methods for measuring ER and/or PgR in breast cancer tissue include gene-based assays evaluating mRNA expression. High concordance rates were reported when quantitative ER and PgR results obtained using reverse-transcription polymerase chain reaction (RT-PCR) methods according to the Oncotype DX assay (and using predetermined positive/negative cutoff points<sup>83</sup>) were compared with results of IHC assays performed locally and centrally.<sup>12</sup> The Oncotype DX assay has been validated<sup>84,85</sup> and results of a recent study comparing measurements of ER using quantitative RT-PCR and IHC suggest that the former methodology may be superior in predicting breast cancer recurrence.<sup>12,86</sup> In addition, a study comparing determination of ER and PgR breast cancer tumor status in fresh-frozen tissue using microarray expression analysis with custom-made arrays showed good concordance for ER compared with IHC (concordance rate, 94%), although assay agreement with IHC was lower for PgR (concordance rate, 80%).<sup>87</sup>

Another approach under investigation involves measuring the levels of gene expression regulated by estrogen in estrogen receptor-positive breast cancer tissue.<sup>88</sup> A disadvantage of using gene expression assays to measure ER and PgR in breast cancer tissue is that they are “grind-up” methods that lack the ability of IHC to define the cytologic/histologic localization of the receptors. The cost and availability of these assays and the possibility of true biologic dis-

**Table 2** Comprehensively Validated IHC Assays for Evaluating ER and PgR in Breast Cancer

Assay Reference	Cutoff Value Used to Define “Positive” Result	
	IHC Assays for ER	Primary Antibody
Harvey et al. <sup>66</sup>	Allred Score $\geq 3$ (1%–10% weakly positive cells)	6F11
Regan et al., <sup>69</sup> and Viale et al. <sup>5,11</sup>	1%–9% (low) and $\geq 10\%$ (high)	1D5
Cheang et al. <sup>72</sup>	$\geq 1\%$	SP1
Phillips et al. <sup>78</sup>	Allred Score $\geq 3$ (1%–10% weakly positive cells)	ER.2.123 + 1D5 (cocktail)
Dowsett et al. <sup>45</sup>	H-score $> 1$ ( $\geq 1\%$ )	6F11
<b>IHC Assays for PgR</b>		
Mohsin et al. <sup>70</sup>	Allred Score $\geq 3$ (1%–10% weakly positive cells)	1294
Regan et al., <sup>69</sup> and Viale et al. <sup>5,11</sup>	1%–9% (low) and $\geq 10\%$ (high)	1A6
Phillips et al. <sup>78</sup>	Allred Score $\geq 3$ (1%–10% weakly positive cells)	1294
Dowsett et al. <sup>45</sup>	$\geq 10\%$	312



cordances between protein and mRNA<sup>12</sup> are also potential impediments to more widespread use of these assays in evaluating hormone receptors in breast cancer tissue.

## ER/PgR Testing

### Clinical Issues: Correlation of ER/PgR with Clinical Outcome in the Absence and Presence of Therapy

Both individually and across the spectrum of breast cancer, breast cancers show a high degree of molecular heterogeneity.<sup>89</sup> Therefore, attempts to correlate a single tumor marker with clinical outcome are likely to have limitations. Furthermore, the clinical studies cited later used many different assay methods to determine the ER and PgR status in samples of breast cancer tumors and, in some cases, the level of validation of these tests is unclear.

**ER/PgR as Prognostic Factors in Breast Cancer:** A prognostic factor provides information on the intrinsic biology and natural history of disease. In breast cancer, it has been defined as a “measurement available at the time of diagnosis or surgery that is associated with clinical outcome in the absence of systemic adjuvant therapy.”<sup>70</sup> The prognostic significance of ER and PgR in breast cancer is still being debated. Many studies addressing the prognostic significance of hormone receptors in patients with early-stage breast cancer are confounded by the administration of adjuvant therapy. In addition, small sample sizes, short follow-up, differences in clinical characteristics of patient populations, and exclusion of certain pathologic or clinical features from statistical analyses can contribute to different findings on the prognostic significance of ER and PgR in breast cancer.<sup>12</sup>

Some evidence shows that ER and/or PgR are favorable prognostic factors in patients not undergoing adjuvant therapy.<sup>90–92</sup> For example, Bardou et al.<sup>92</sup> showed ER and PgR to have a modest independent prognostic benefit over a 3-year period when evaluated in women with breast cancer who did not undergo adjuvant therapy. In a population-based study, positive ER tumor status correlated with improvements in breast cancer–specific survival and recurrence-free survival over a long-term period in women with breast cancer not treated with adjuvant therapy.<sup>72</sup> However, the survival advantage for patients with ER-positive disease decreased over time

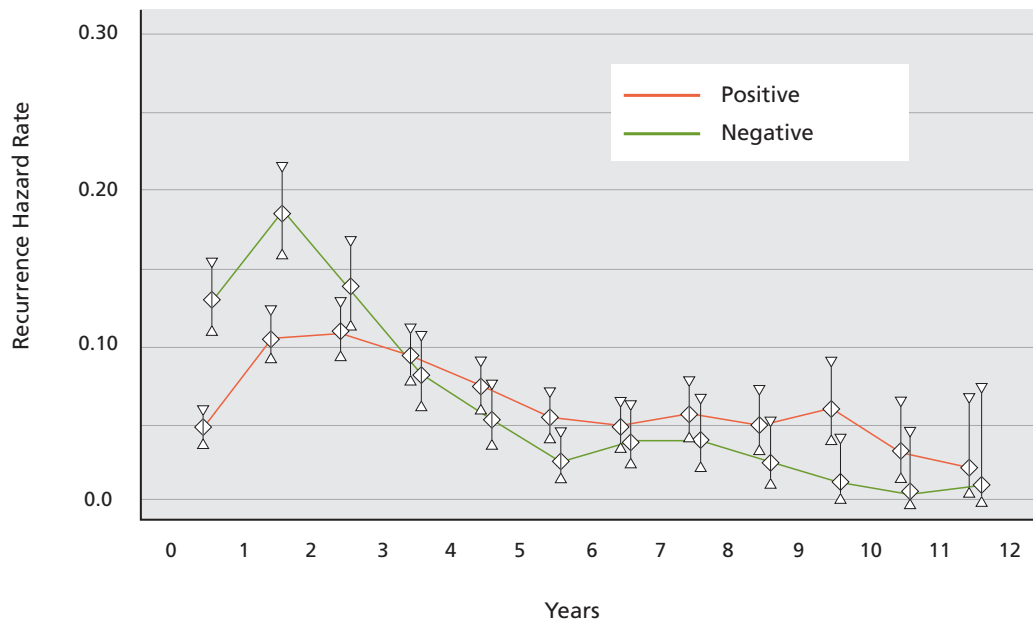
and crossed with the curve representing ER-negative patients at 18 years.

Furthermore, several additional studies with long follow-up confirm that the initial prognostic advantages of tumor ER-positivity diminish over time, suggesting that this tumor marker may provide information on the rate at which disease recurs but not on the long-term outcome of patients with ER-positive disease.<sup>93–96</sup> Additional support for this conclusion comes from a retrospective analysis of the recurrence rate of 3585 patients enrolled in 7 ECOG trials of patients treated for nonmetastatic breast cancer, although this data set includes patients undergoing adjuvant therapy. In that study, the annual hazard rate of recurrence was initially lower for patients with ER-positive disease after surgery compared with those with ER-negative disease, but this trend was reversed at approximately year 4 (see Figure 5).<sup>97</sup>

One explanation for the long-term observations represented in Figure 5 is that the population of patients with ER-negative disease is heterogeneous so that only some patients with ER-negative disease have a higher rate of recurrence and death in the first few years after diagnosis. Thus, a “good prognosis” group with ER-negative disease remains recurrence-free over a long-term period. Furthermore, this study suggests that a diagnosis of ER-positive disease is not necessarily a marker of favorable long-term prognosis.

Another study found no differences in outcome with different amounts of ER in early-stage, node-negative, ER-positive patients with breast cancer not undergoing adjuvant therapy. This suggests that ER may be a weak prognostic factor.<sup>91</sup> It has also been proposed that ER may not be an independent predictor of outcome but, instead, may be associated with other clinicopathologic features, such as tumor grade or proliferation rate<sup>91</sup> with ER-positive tumors more likely than ER-negative tumors to be lower-grade<sup>13,60,95,98</sup> (see Table 3).

Gene expression profiling involving cDNA microarray analyses of several hundred genes showed that breast cancers are biologically and clinically diverse.<sup>99,100</sup> This type of analysis has been used to identify 5 intrinsic molecular subtypes of breast tumors called luminal A, luminal B, basal, HER2, and normal breast–like. These tumor subtypes are characterized by differences in clinical behavior based on the expression of a large number of genes, with ER-associated genes as key markers in distinguishing



**Figure 5** Recurrence rate by estrogen receptor (ER) status. Annual hazard of recurrence of 3562 patients separated by ER status. The mean follow-up times for ER-positive and ER-negative patients were 8.1 and 8.0 years, respectively. (ER status was missing for 23 patients.) From Saphner T, Tormey DC, Gray R. Annual hazard rates of recurrence for breast cancer after primary therapy. *J Clin Oncol* 1996;14:2738–2746. Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved.

the subtypes.<sup>101–103</sup> For example, the luminal subtypes make up the ER-expressing breast cancers, and tumor proliferation has been identified as a significant discriminator in determining whether an ER-positive tumor is classified as luminal A or B.<sup>104,105</sup> The luminal A subtype typically has a higher expression of ER-related genes and a lower expression of proliferative genes than the luminal B subtype, and has been associated with a better prognosis.<sup>104,106–108</sup> ER-negative tumors can be subdivided into HER2, basal, and normal breast tissue subtypes. Although these types of molecular signatures have been shown to be capable of providing powerful prognostic and predictive information, their integration into routine clinical practice is limited by several technical factors and the vast molecular diversity of breast cancer.<sup>109,110</sup>

Recent studies have investigated the ability of small panels of biomarkers evaluated using IHC to act as practical surrogates in the identification of intrinsic tumor subtype in invasive breast cancer<sup>104,107,111</sup> and DCIS.<sup>112–114</sup> For example, Livasy et al.,<sup>111</sup> using IHC to measure several tumor markers in invasive breast cancer, identified an IHC-based phenotype of basal-like carcinoma. Nevertheless, the prognostic significance (i.e., information on the aggressiveness of tumors cells regardless of treatment)

of these molecular subtypes, based on either gene sets or immunopanel of tumor markers, is still unclear. The benefit of this type of tumor classification may be primarily to provide predictive information on response to particular therapies.<sup>107,115</sup>

**ER/PgR as Predictive Factors in Breast Cancer:** A predictive factor is defined as a “measurement associated with a response or lack of response to a particular therapy.”<sup>70</sup> ER expression as determined by the LB assay, IHC, and RT-PCR has been shown to be a powerful predictor of breast cancer response to endocrine therapy; it is predictive of benefit from tamoxifen in patients with DCIS,<sup>116</sup> and from tamoxifen and aromatase inhibitors in early-stage and advanced breast cancers.<sup>1,3–5,11,12,45,66,70,72,75,117–119</sup> Furthermore, endocrine therapy in patients with DCIS or invasive breast cancer has been shown to have no significant benefit if the tumor is ER- and/or PgR-negative.<sup>1–5,11,14,45,66,70,72,73,116,119,120</sup>

Numerous studies have shown that the proportional benefits of tamoxifen vary with the relative quantitative expression of ER. Higher amounts of ER have also been positively correlated with increased clinical response rates and/or improvements in clinical outcome in patients with invasive breast cancer undergoing tamoxifen therapy in the adju-

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**Table 3 Selected Characteristics Among Breast Cancer Cases by Hormone Receptor Status: SEER Program, 1990–2001**

	ER+/PgR+ (n = 98,463)	ER+/PgR- (n = 19,886)	ER-/PgR+ (n = 4896)	ER-/PgR- (n = 31,930)
<b>Tumor Histology</b>	(%)			
Ductal	62.7	12.3	3.2	21.8
Lobular	73.6	17.7	2.3	6.4
Ductal/lobular	76.7	13.6	2.4	7.3
Inflammatory	37.8	13.3	6.3	42.6
Mucinous	80.9	13.5	1.1	4.5
Tubular	79	15.2	2.1	3.7
Comedo	43.6	12.0	6.7	37.7
Medullary	14.7	8.2	5.4	71.7
Papillary	79.0	7.9	1.3	11.8
Other	49.4	12.2	3.9	34.5
<b>Tumor Grade</b>	(%)			
1	81.1	13.0	1.8	4.1
2	74.2	13.0	2.4	10.4
3	44.4	12.1	4.4	39.1
4	45.0	10.5	4.4	40.1

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; SEER, Surveillance, Epidemiology, and End Results.

Adapted from Dunnwald LK, Rossing MA, Li CI. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Res* 2007;9:R6; with permission.

vant setting,<sup>1,45,72,121</sup> and in the setting of advanced disease.<sup>3,4,14,122</sup> In addition, increased quantities of ER and/or PgR have also been shown to correlate with a favorable benefit from aromatase inhibitor therapy in women with early-stage hormone receptor–positive breast cancer.<sup>45</sup>

Finally, a retrospective analysis of ER expression in patients with DCIS enrolled in NSABP B-24 suggest that increased levels of expression predict for tamoxifen benefit in terms of risk reduction for the development of both ipsilateral and contralateral breast cancer after breast-conserving therapy.<sup>116</sup>

Whether quantification of ER and tumors markers should be routinely performed in the evaluation of patients with breast cancer is controversial. The National Institutes of Health consensus statement on adjuvant therapy in breast cancer concluded that, “While the likelihood of benefit correlates with the amount of hormone receptor protein in tumor cells, patients with any extent of hormone receptor in their tumor cells may still benefit from hormonal therapy.”<sup>123</sup> Likewise, recommendations in the NCCN Clinical Practice Guidelines in Oncology: Breast Cancer (to view the most recent version of these guidelines, visit

the NCCN Web site at [www.nccn.org](http://www.nccn.org)) regarding endocrine therapy for particular populations of patients with hormone receptor–positive disease are not contingent on tumor hormone receptor levels.<sup>124</sup>

Tumor hormone receptor levels also seem to impact the effectiveness of cytotoxic chemotherapy. The predicted benefit from chemotherapy in the adjuvant setting has been assessed in several studies of patients with ER-positive tumors undergoing chemotherapy plus tamoxifen compared with those undergoing chemotherapy alone for the treatment of ER-negative breast cancer.<sup>1,6</sup> These analyses suggest that the benefits of chemotherapy are significantly greater in patients with ER-negative disease, although studies incorporating other tumor markers, such as HER2 and Ki-67, also show that particular tumor subtypes characterized by HER2 and/or Ki-67 overexpression may exhibit chemosensitivity despite being ER-positive.<sup>107</sup> Furthermore, several studies have shown that the level of tumor ER may help select the subsets of patients with ER-positive disease who are likely to benefit from the addition of chemotherapy to endocrine therapy.<sup>5,6,85,125</sup>

Although the NCCN Breast Cancer Guidelines

do not include specific recommendations for the evaluation of the quantity of ER and PgR in tumor cells, the Oncotype DX assay (which provides a recurrence score that includes weighted contributions from continuous ER and PgR scores based on the levels of expression of these 2 tumor markers in addition to contributions from 14 other biomarkers) is listed as an option for some patients with ER-positive disease.<sup>124</sup> Similar to the NCCN guidelines, the 2009 St. Gallen guidelines recommend endocrine therapy for all patients with ER-positive disease regardless of the level of ER.<sup>126</sup> The 2007 version of the St. Gallen guidelines included a description of 3 categories of endocrine responsiveness: “highly endocrine responsive” (tumors express high levels of both steroid receptors in the majority of cells), “incompletely endocrine responsive” (some expression of steroid hormone receptors but at lower levels or lacking either ER or PgR), and “endocrine nonresponsive disease” (tumors have no detectable expression of steroid hormone receptors).<sup>127</sup> The most recent version of those guidelines mentions consideration of these categories of endocrine responsiveness in the context of guiding decisions regarding use of chemohormone therapy in patients with ER-positive, HER2-negative disease.<sup>126</sup>

### Biologic Issues

#### **ER/PgR Tests: Surrogates of Biologic Processes:**

The most important purpose of evaluating the ER/PgR tumor status for individual patients is to predict whether a clinically important benefit from a particular therapy is likely.<sup>50,51</sup> This would ideally involve a comprehensive assessment of the functionality of ER and PgR, including an evaluation of the activated downstream proteins of these receptors. However, IHC assays of ER and PgR are limited to determining whether these receptors are present in tumor cells and providing some information on the levels of ER and PgR in breast cancer tissue. Although some versions of the Oncotype DX and MammaPrint assays focus on determining a continuous range of values to represent expression of ER and PgR genes (Methods of Detecting ER and PgR in Breast Cancer, page S-5), these results provide information on steroid receptor mRNA levels only.<sup>12,87</sup> High-throughput gene expression microarrays have the potential to provide information on the mechanisms behind the development of endocrine resistance and causes of endocrine refractory disease in hormone receptor–

positive tumors. Nevertheless, technical issues and the enormous complexity of the molecular impact of estrogen and progesterone on breast cancer tissue limit the integration of these types of assays into routine clinical practice. For example, gene expression profiling analyses of an ER-positive breast cancer cell line have identified more than 800 genes that are regulated by estrogen.<sup>88</sup>

**ER Tumor Status: Fixed or Dynamic?:** Whether breast cancer tumor hormone receptor phenotype is stable throughout disease progression has been addressed in several studies with variable results. A prospective trial evaluating ER/PgR tumor status in 29 samples from patients with newly suspected metastatic breast cancer and previous sampling of primary tumor showed significant discordances between sample sets (i.e., 40% of patients had a change in hormone receptor status).<sup>128</sup> In another study comparing primary tumors and paired metastases from 75 patients with breast cancer, a change in either ER or PgR status was seen in 21% of cases.<sup>129</sup> An investigation of 789 patients with metastatic breast cancer showed ER and PgR discordance rates of 18.4% and 40.3%, respectively, between primary and metastatic disease sites.<sup>130</sup> Finally, a report from a study that compared tissue from the primary breast cancer tumor with paired axillary lymph node metastases showed a change in tumor status in only approximately 4% to 6% of patients.<sup>131</sup> The stability/mutability of breast cancer hormone receptor status requires further investigation. The 2009 NCCN Breast Cancer Guidelines include a recommendation for a biopsy of first recurrence of disease with determination of ER and PgR receptor status, if possible.

**Histologic Subtype/Tumor Grade and Hormone Receptor Status:** Table 3 summarizes data on the association between tumor hormone receptor status and tumor histology for 155,175 women with invasive breast cancer included in the Surveillance, Epidemiology, and End Results (SEER) registry from 1990 to 2001.<sup>98</sup> One major limitation of registry data such as these is that hormone receptor determinations were made in many different laboratories using various methods and the possibility of misclassifications cannot be excluded. Nonetheless, these data indicate that tubular and mucinous breast cancers are virtually always ER- and/or PR-positive. Table 3 also provides evidence of an association between low tumor grade (i.e., grade 1 and 2) and tumor hormone

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receptor status, showing the occurrence of a grade 1 or 2 tumor with a negative hormone receptor status to be unlikely.

### ER/PgR Testing by IHC: Methodologic Issues

This section discusses the testing techniques and reporting procedures that may impact the results and interpretation of IHC tests of ER and PgR in breast cancer tissue.

Reports have shown interlaboratory variability for ER and PgR testing with IHC.<sup>10,132–136</sup> Many laboratories performing IHC tests of ER and PgR in breast cancer tissue are using assays that have not been comprehensively validated technically or clinically (see Methods of Detecting ER and PgR in Breast Cancer, page S-5). Multiple sources of variability with IHC testing involve preanalytic, analytic, and postanalytic factors (see Table 4). Some of the variability includes use of diverse methods of preparing tissue; diverse reagents; diverse methods of staining; diverse methods of scoring; arbitrary definitions of “positive;” and inadequate quality control.<sup>78,81,137–140</sup> For example, considerable variability exists in the monoclonal antibodies used and, most importantly, in the interpretation of results in the context of clinical outcome.<sup>52</sup> Rigorous standardization, quality control, validation, and quality assurance of the methodologies used to perform these tests are needed.

**Preanalytic Factors:** Hormone receptor testing can be performed on tissue obtained from a needle core biopsy and/or a breast resection specimen. Variations in the fixation and preparation of breast resection and core biopsy tissue specimens are sources of test variability. IHC tests of ER on matched samples from core biopsies and excisional biopsies have documented good correlation.<sup>141</sup> Nevertheless, the ER positivity rate was higher in the core biopsy specimens, and the resection specimens showed variable rates of ER positivity according to distance from the tumor edge (higher ER positivity noted at the outer edges of the tissue), suggesting incomplete fixation of the resection samples. More recent studies showed nearly 100% concordance for IHC tests of ER and PgR using matched specimen samples.<sup>142–144</sup>

Reports have shown that the likelihood of obtaining an ER-negative breast cancer specimen can vary according to the day of surgery.<sup>145</sup> In this case, overfixation of samples brought to the laboratory on a Friday or Saturday leading to an increased frequen-

cy of ER-negative tests caused by antigen lability was suggested as an explanation for this observation. Goldstein et al.<sup>146</sup> reported that a minimum of 6 to 8 hours of tissue exposure to formalin was needed to obtain consistent and reproducible ER results, irrespective of specimen type. Consistency in the process of embedding the sample in paraffin and sectioning the tissue has also been identified as important to obtaining reproducible results (see Table 1).<sup>138</sup>

**Analytic Factors:** Many analytic factors can influence the determination of tumor ER and PgR status in breast cancer tissue as measured using IHC methods. Of paramount importance is the level of training and competency of the laboratory staff.<sup>52,137</sup> The sensitivity of the assay has also been repeatedly identified as being particularly significant, because inadequate sensitivity can cause weak staining and increase the likelihood of a false-negative result. The

**Table 4 Sources of Marker Testing Variation**

Preanalytic
<ul style="list-style-type: none"> <li>• Time to fixation</li> <li>• Method of tissue processing</li> <li>• Time of fixation</li> <li>• Type of fixation</li> </ul>
Analytic
<ul style="list-style-type: none"> <li>• Assay validation</li> <li>• Equipment calibration</li> <li>• Use of standardized laboratory procedures</li> <li>• Training and competency assessment of staff</li> <li>• Type of antigen retrieval</li> <li>• Test reagents</li> <li>• Use of standardized control materials</li> <li>• Use of automated laboratory methods</li> </ul>
Postanalytic
<ul style="list-style-type: none"> <li>• Interpretation criteria</li> <li>• Use of image analysis</li> <li>• Reporting elements</li> <li>• Quality assurance procedures               <ul style="list-style-type: none"> <li>◦ Laboratory accreditation</li> <li>◦ Proficiency testing</li> <li>◦ Pathologist competency assessment</li> </ul> </li> </ul>

From 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. Reprinted with permission. ©2008 American Society of Clinical Oncology. All rights reserved.



sensitivity of the IHC assay should be appropriate to capture the full dynamic range of expression. Analytic factors contributing to assay sensitivity include the quality of tissue fixation; quality and concentration of the primary antibody; antigen retrieval procedure used; and staining and detection processes.<sup>133,147</sup> *Antibody Issues:* Numerous monoclonal antibodies are available against ER and PgR, although most of these have not been validated clinically.<sup>143</sup> Furthermore, not all of these antibodies are very sensitive and specific for detecting ER and PgR. The following antibodies against ER have been well characterized as being suitable for clinical use: 6F11<sup>66</sup> 1D5,<sup>11,45,67,148</sup> SP1,<sup>72</sup> and the cocktail of ER.2.123 plus 1D5.<sup>78</sup> Likewise, IHC tests using the 1294, 1A6, and 312 antibodies against PgR, which react with both isoforms of PgR, have been shown to be equivalent or superior to the LB assay with respect to predicting clinical outcome.<sup>45,69,70</sup>

Antigen retrieval involves sample heating, which is believed to disrupt some of the protein crosslinks induced by formalin fixation and to restore immunoreactivity;<sup>149</sup> it is an important component in the optimization of assay sensitivity (see Table 1). Rhodes et al.<sup>133</sup> found that inadequate antigen retrieval contributed substantially to a lowering of the sensitivity of IHC for detecting ER and PgR, and this conclusion has been supported in several studies.<sup>63,78,137</sup>

*Quality Control:* Reference materials, serving as both negative and positive controls, and subject to the same fixation, processing, and testing conditions as used for the test sample, are necessary to standardize and calibrate results of a test.<sup>137,138</sup> Two types of controls are used: batch controls and biologic internal controls. All IHC analyses must include both a positive and negative control for each batch run. The test sample itself may not have an internal positive control but will almost always have an internal negative control (e.g., endothelial cells, lymphocytes). Traditionally, normal, nonneoplastic breast tissue embedded within the same block as the tumor tissue has been used as an internal positive control.<sup>139</sup> External controls include endomyometrial and cervical tissue (i.e., as positive controls) and pellets of cultured cells embedded within paraffin blocks (i.e., Quicgel system).<sup>78,139,150</sup> These cell lines can be either ER- and PgR-positive (i.e., positive controls) or ER- and PgR-negative (i.e., negative controls). The assay should also include a negative control reagent (i.e., substitution of a nonspecific,

or nonsense, primary antibody for the hormone receptor-specific monoclonal antibody in the assay).<sup>63</sup>

Synthetic peptides with primary sequences corresponding to the ER and PgR epitopes recognized by various monoclonal antibodies used for IHC testing are another potential source of reference material.<sup>143,151–154</sup> Specific concentrations of these peptides are attached to isocyanate-activated slides. This is a promising source of standard/control material for IHC testing of ER and PgR, although additional studies are needed to further characterize this technology. *Detection Systems/Staining Issues:* Classic IHC assays of ER and PgR of breast cancer tissue involve deposition of a chromogenic stain at the binding sites of monoclonal antibodies targeted to these hormone receptors (Table 1 and Figure 3). However, the relationship between the actual concentration of a particular hormone receptor in the tissue and the amount of the receptor protein detected through the IHC staining process is complex, and may be influenced by the tissue fixation process (see Preanalytic Factors, page S-13) and sensitivity of the assay.<sup>155</sup> For example, highly sensitive IHC assays increase the proportion and intensity of cells stained compared with less-sensitive IHC assays performed on the same tissue, and the relationship between levels of hormone receptors determined using an enzyme immunoassay is not linearly correlated with hormone receptor level in a highly sensitive IHC assay.<sup>155,156</sup>

Whether the chromogenic stain used in these assays has sufficient dynamic range for capturing and quantifying the broad spectrum of hormone receptor concentrations found in breast cancer tissue is a subject of debate.<sup>81</sup> However, use of stain intensity in addition to the extent of staining to score IHC test results may facilitate interpretation of slides with low levels of ER expression. The intensity of hormone receptor immunostaining can be assessed visually or through computerized image analysis.<sup>65</sup> Although the latter method provides a more accurate assessment of stain intensity, it is limited with respect to several factors, such as its ability to precisely distinguish between benign and malignant lesions.<sup>65</sup>

*Technical and Clinical Validation:* Each laboratory performing IHC assays of ER and PgR must show that those assays are technically valid (see Methods of Detecting ER and PgR in Breast Cancer, page S-5). This requirement exists despite whether the assay in use is FDA-approved. A laboratory can accomplish

technical validation of an assay in 2 ways: external validation using a “split sample” technique (i.e., performing a concordance study of assay results obtained on a set of samples in that laboratory, with results obtained on the same sample set in another laboratory known to have a technically valid assay [i.e., an “expert laboratory”]) or internal validation using a set of standard samples provided by an organization such as the College of American Pathologists (CAP) or National Institute of Standards and Technology (NIST), with established ER and PgR status determined through IHC testing using a technically validated assay. Clinical validation of an assay can be inferred if the technically validated reference assay is one that has been shown to be clinically valid (see Table 2).<sup>78</sup> Alternatively, clinical validation can be inferred if high concordance is seen between results obtained on the standard set of samples and the original determination of ER/PgR status of those samples was performed using a clinically valid assay (see Table 2).

**Postanalytic Factors: Interpretation of Results:** Interpretive variables in IHC testing include the scoring system used and established cutoff value distinguishing a positive from a negative result. Numerous systems have been used to score IHC test results for ER and PgR in breast tissue, including a strictly dichotomous assessment (i.e., positive vs. negative),<sup>13</sup> and more quantitative systems such as those based on proportion of cells stained only, and scoring systems that include both the proportion of cells stained and the intensity of the staining (see Methods of Detecting ER and PgR in Breast Cancer, page S-5).<sup>10,45,52,66,68,70</sup>

Ideally, the cutoff value used in clinical practice should be calibrated to clinical outcome.<sup>47,52</sup> A wide range of cutoff values have been used to interpret results of IHC assays of hormone receptors (e.g., 1%, 5%, and 10% of tumor cells), although most have not been clinically validated. Although cutoff values distinguishing a positive from a negative result cannot be assumed to be transferable from one assay method to another, studies of most well-validated IHC assays have shown that very low levels of proportion of cells stained ( $\geq 1\%$  of cells) are clinically important (see Table 2).

Controversy continues as to whether quantification of ER and PgR beyond a dichotomous “positive” or “negative” is warranted. In 2 recent studies of more than 7000 samples combined, a bimodal dis-

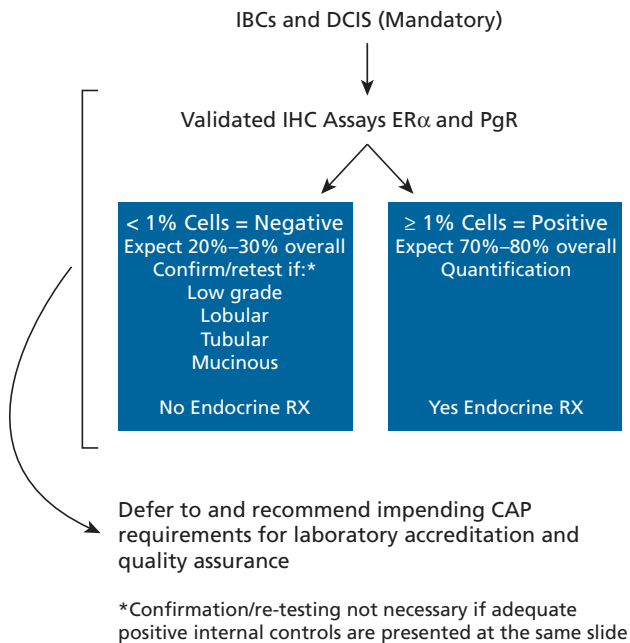
tribution of ER values (i.e., completely ER-negative or strongly ER-positive) was observed in more than 90% of the samples using contemporary IHC methodology.<sup>13,63,155</sup> Alternatively, the possibility that future drugs may benefit only patients with high levels of ER expression provides some support for quantifying IHC test results.<sup>143</sup> Whether in fact measuring stain intensity in IHC assays of hormone receptors has any clinical significance is another topic of debate.<sup>137</sup> However, some evidence indicates that accounting for both proportion and intensity parameters may provide a test score that correlates more closely with clinical outcome.<sup>70</sup>

**Reporting of Results:** Reports of IHC test results of breast cancer tumor ER and PgR status must provide sufficient information for informed clinicians to effectively use the results in clinical decision making. For example, the time of tumor fixation, type of fixative, and primary antibody used should be included in the report. Alternatively, information regarding the details of the specific assay used can be summarized in detail in laboratory standard operating procedures and referenced in the report. Other important information includes a clear statement of the ER and PgR status of the tumor (i.e., positive or negative) and the criteria used to determine the status (see Methodologic Recommendations, page S-16), and how the results were quantified. ASCO/CAP guidelines should be consulted for more detailed information regarding elements of the report. Clinicians responsible for evaluating tests of ER and PgR should be familiar with these criteria and their clinical ramifications.

**External Quality Assurance:** Laboratory quality assurance programs are based on the principles of external monitoring and enforcement. Ongoing evaluations by external agencies (e.g., CAP) of IHC testing proficiency and external reviews of laboratory documentation on the competency of the pathologists determining the ER and PgR status of breast cancer tumors are key components of these programs. Laboratory accreditation by CAP will be based on results of these ongoing evaluations (see ASCO/CAP guidelines).

## Task Force Recommendations

The recommendations of the task force are summarized in Figure 6.



**Figure 6** IHC testing algorithm.

### Clinical Recommendations

- Both ER and PgR status should be determined for all invasive breast cancers and DCIS.
- If a core biopsy is negative for ER/PgR, and without internal controls that are positive (i.e., non-neoplastic tissue), testing should be repeated on the excisional specimen.
- ER- and PgR-negative tumor status should be retested if tumor histology is lobular, tubular, or mucinous, because these types of tumors are rarely negative, and confirmation of histologic diagnosis should be performed. An exception to the need for confirmation/retesting is when adequate positive internal controls are present on the same slide.
- ER- and PgR-negative tumor status should be retested if tumor grade is 1 because these tumors are rarely negative. An exception to the need for confirmation/retesting is when adequate positive internal controls are present on the same slide.
- ER/PgR testing should be performed at primary diagnosis and first relapse.
- The option of endocrine therapy for patients with tumors characterized by ER- and/or PgR-positive status does not depend on level of hormone receptor expression.

### Methodologic Recommendations

- Priority should be given to improve the quality

of IHC testing and testing methodologies.

- IHC tests of ER and PgR should not be restricted to those that are FDA-approved.
- All assays of ER and PgR, including preanalytic processes, must be technically validated with a demonstrated 95% concordance with another technically validated assay.
- Technical validation should be performed on samples representative of those typically tested in the laboratory
- All assays of ER and PgR must be clinically validated. This can be accomplished by technically validating an assay against one that is technically and clinically validated (as listed in Table 2) or by technically validating an assay which has been shown to be clinically validated (as listed in Table 2).
- Tissue fixation in 10% neutral buffered formalin fixative for 12 to 48 hours is recommended.
- All IHC analyses must include both a positive and a negative control for each batch run.
- Antibodies recommended for IHC testing of ER include ID5, 6F11, SP1, or ER.2.123 + 1D5 (cocktail).
- Antibodies recommended for IHC testing of PgR include 1294, 1A6, or 312.
- A cutoff value of 1% (i.e., < 1% is negative; ≥ 1% is positive) is recommended to distinguish a positive from a negative result when interpreting an IHC test of ER or PgR tumor status.
- Hormone receptor quantification should be maintained when scoring IHC assays of ER and PgR. This includes evaluation of both proportion/percentage of cells stained and stain intensity using one of several acceptable, validated methods, including H-score, Allred score, computer image analysis, absolute counting, or point counting. An additional evaluation of stain intensity should also be performed if either absolute counting or point counting scoring methods are used.
- The test report should document information on tumor characteristics, tissue handling, testing processes, and laboratory procedures. It should also provide informed clinicians with the information necessary for effective clinical decision making. Guidelines from ASCO and CAP to be published in the near future will provide comprehensive information regarding essential ele-

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ments to include in the test report.

- Laboratories should meet ongoing requirements of quality assurance parameters specified in the ASCO/CAP guidelines that will be published in the near future.

## Conclusions

Measurements of ER, and to a lesser extent PgR, provide crucial information in the treatment planning of patients with breast cancer. Currently, almost all testing of these hormone receptors is performed using IHC, and this methodology is expected to continue to be the main testing strategy for evaluating hormone receptor status in breast cancer for some time. Nevertheless, a high false-negative rate has been associated with this type of testing when laboratory quality control and assurance measures are inadequate. All laboratories performing breast cancer hormone receptor testing using IHC must ensure pathologic expertise of laboratory personnel, demonstrate both technical and clinical validation of the standardized IHC assay being used, keep comprehensive records, provide complete test reports, and meet ongoing quality assurance and proficiency testing requirements.

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