Biomarker Research in Breast Cancer

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Breast cancer is a heterogeneous disease with vast differences between patients regarding treatment response and prognosis. Therefore, strategies for individualizing care are needed. The rapid developments in biomarker research in breast cancer are making personalized breast cancer therapy a reality. A biomarker is defined as an objectively measured characteristic that can be evaluated as an indicator of normal biologic processes, pathogenic processes, or therapeutic responses. Biomarkers can have prognostic or predictive value. A small group of individual biomarkers has been used in the management of breast cancer, including estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. Advances in molecular biology and an increased understanding of tumor cell biology have led to the discovery of a vast array of promising new biomarkers, including cancer stem cells, circulating tumor cells, gene-expression profiles, individual response markers, disease subtypes, predictors of metastasis, and mutation markers. To be adopted into routine practice, these candidate biomarkers will require extensive clinical validation. The improved application of traditional biomarkers and the discovery of additional markers will undoubtedly change the face of breast cancer care.

At a Glance

- Information provided by prognostic biomarkers (overall outcomes) and predictive biomarkers (the effect of a particular therapy) allows for better individualization of treatment, possibly resulting in improved clinical outcomes.
- Although familiar biomarkers continue to undergo additional characterization, tests for newer biomarkers and multigene signatures are in development.
- Information on breast cancer biomarkers is evolving, so nurses should be prepared to answer patients’ questions on this topic and have a predetermined communication plan that encompasses the entire healthcare team when relevant.

After a discussion of traditional and emerging biomarkers for breast cancer and their application to clinical practice, a case study of L.C., a 42-year-old woman whose gene expression profile results helped determine the most appropriate treatment for her disease, will be provided.
**The Evolution of Biomarkers in Breast Cancer Treatment**

The evolution of biomarkers has occurred concomitantly with the development of new treatments for breast cancer. Historically, radical mastectomies have been performed since the 19th century for the treatment of breast cancer, with modified radical mastectomies introduced in 1940. Despite these surgical techniques, prognosis remained poor (Donegan, Sugarbaker, Handley, & Watson, 1970). The approval of tamoxifen in 1977 by the U.S. Food and Drug Administration (FDA) marked a significant advancement in breast cancer treatment because approval was based on one of the earliest known biomarkers for breast cancer therapy—the estrogen receptor (ER) (FDA, 2010). During the early- to mid-1970s, more treatment options emerged with the introduction of breast-conserving surgery (i.e., lumpectomy) and combination chemotherapy (Deemarsky & Chernomordikov, 1970). With the addition of highly active agents such as anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin) in the 1980s and taxanes (e.g., paclitaxel, docetaxel) in the 1990s, the chemotherapeutic armamentarium continued to grow. In the early 1990s, researchers found a link between familial breast cancer and mutations in the BRCA genes—another major biomarker (Hall et al., 1992). In addition, in 1998, the FDA approved the use of trastuzumab, the first agent designed to specifically block the activity of a breast oncoprotein (human epidermal growth factor receptor 2 [HER2]) (FDA, 2010), marking the beginning of targeted breast cancer therapy with monoclonal antibodies. Subsequently, the medical community has continued to research the contribution of genetics to the clinical heterogeneity of breast cancer: Individuals with the same disease stage and similar pathologic diagnoses could experience very different clinical courses, presumably because of the genetic variability of patients and tumors. The completion of the human genome project in 2000 allowed researchers to investigate the relevance of tens of thousands of genes (Aldhous, 2000). At approximately the same time, gene-profiling studies began to gate the relevance of tens of thousands of genes (Aldhous, 2000). In 1992, researchers introduced in 1940. Despite these surgical techniques, prognosis remained poor (Donegan, Sugarbaker, Handley, & Watson, 1970). The approval of tamoxifen in 1977 by the U.S. Food and Drug Administration (FDA) marked a significant advancement in breast cancer treatment because approval was based on one of the earliest known biomarkers for breast cancer therapy—the estrogen receptor (ER) (FDA, 2010). During the early- to mid-1970s, more treatment options emerged with the introduction of breast-conserving surgery (i.e., lumpectomy) and combination chemotherapy (Deemarsky & Chernomordikov, 1970). With the addition of highly active agents such as anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin) in the 1980s and taxanes (e.g., paclitaxel, docetaxel) in the 1990s, the chemotherapeutic armamentarium continued to grow. In the early 1990s, researchers found a link between familial breast cancer and mutations in the BRCA genes—another major biomarker (Hall et al., 1992). In addition, in 1998, the FDA approved the use of trastuzumab, the first agent designed to specifically block the activity of a breast oncoprotein (human epidermal growth factor receptor 2 [HER2]) (FDA, 2010), marking the beginning of targeted breast cancer therapy with monoclonal antibodies. Subsequently, the medical community has continued to research the contribution of genetics to the clinical heterogeneity of breast cancer: Individuals with the same disease stage and similar pathologic diagnoses could experience very different clinical courses, presumably because of the genetic variability of patients and tumors. The completion of the human genome project in 2000 allowed researchers to investigate the relevance of tens of thousands of genes (Aldhous, 2000). At approximately the same time, gene-profiling studies began to reveal distinct subgroups of breast cancer based on their expression of a panel of genes, rather than single biomarkers (Perou et al., 2000). These disease subgroups have similar patterns in tumor aggressiveness and treatment outcome.

**Prognostic Versus Predictive Biomarkers**

Biomarkers differ depending on their function. For instance, a **prognostic biomarker** provides information about the patient’s overall cancer outcome, independent of therapy (Oldenhuis et al., 2008). The presence or absence of such a marker can be useful when selecting a specific treatment, but it does not predict the response to this treatment. Two main categories of prognostic biomarkers exist: those that give information on the risk of recurrence in patients receiving treatment and those that give information on the probable duration of progression-free survival in patients with metastatic disease. Conversely, a **predictive biomarker** provides information on the effect of a therapeutic intervention in a patient and also may be the target of therapy (Oldenhuis et al., 2008). In addition, some biomarkers have mixed prognostic and predictive associations, contingent on the treatment given (Dowsett & Dunbier, 2008). For example, some

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**Traditional Biomarkers**

A small group of individual biomarkers has been used to manage breast cancer, including ER, progesterone receptor (PR), and HER2 (Dowsett & Dunbier, 2008) (see Table 1). Both the American Society of Clinical Oncology (ASCO) and NCCN recommend routine measurement of ER, PR, and HER2 and provide guidance for their uses (Harris et al., 2007; NCCN, 2010a). Given the importance of the traditional biomarkers for breast cancer management, assays must be robust and quality controlled and interpretation should be standardized.

**Estrogen Receptor**

ER status is the most powerful individual predictive factor examined in breast cancer (Dowsett & Dunbier, 2008). In fact, the biologic characteristics of breast cancer segregate differentially between patients with ER-positive and ER-negative tumors. The relevance of ER as a biologic marker stems from receptor-binding proteins, particularly estradiol, that are essential for growth and survival of ER-positive breast cancer: About two-thirds of women younger than age 50 with breast cancer and approximately 80% of women older than age 50 have ER-positive tumors (Anderson, Chatterjee, Ershler, & Brawley, 2002). Therefore, ER inhibition (either through modulation of estrogen synthesis or antagonist binding to the ER) is the mainstay of adjuvant and metastatic breast cancer treatment (Dowsett & Dunbier, 2008). The hormone status of breast cancer in a patient can vary between tumor sites and stages of disease (Oldenhuis et al., 2008). In fact, the ER status of metastatic disease differs from that of the primary tumor in approximately 20% of cases (Franco, Col, & Chlebowski, 2004).

ER expression is measured routinely in clinical practice (Dowsett & Dunbier, 2008) because it strongly predicts the efficacy of antiestrogen treatments such as tamoxifen and aromatase inhibitors. Patients with ER-negative tumors do not significantly benefit from five years of tamoxifen treatment (Early Breast Cancer Trialists' Collaborative Group, 2005). Even among patients with ER-positive tumors, higher ER expression predicts better response to tamoxifen in metastatic and primary disease (Byar, Sears, & McGuire, 1979; Paik et al., 2005). Both the ASCO and NCCN guidelines recommend measuring ER on every primary invasive breast cancer and on metastatic lesions if the results would influence treatment planning (Harris et al., 2007; NCCN, 2010a).

ER expression can be measured using reverse transcription polymerase chain reaction (RT-PCR) techniques or DNA microarrays, two assays that use a labeled RNA probe molecule to
predictor of response to hormonal therapy. In the adjuvant setting, patients with ER-positive disease may derive less benefit from anthracycline- and paclitaxel-based chemotherapies than patients with ER-negative cancers; overall mortality rate reductions associated with chemotherapy were 23% and 55%, respectively, in a retrospective analysis of three trials (Berry et al., 2006). Greater chemosensitivity of ER-negative tumors may be partly related to their higher proliferation rate (Dowsett & Dunbier, 2008).

**Progesterone Receptor**

Expression of PR is closely related to estrogen (i.e., regulated by ER) and is, therefore, rarely seen in ER-negative tumors (Dowsett & Dunbier, 2008). Like ER expression, PR expression is an independent prognostic factor in breast cancer (Oldenhuis et al., 2008). Patients with ER- and PR-positive tumors had greater five-year overall survival (all stages) than patients with double-negative tumors (83% versus 69%) (Grann et al., 2005).

Patients with ER- and PR-positive tumors may be more likely to respond to tamoxifen in both the adjuvant (Bardou, Arpino, Elledge, Osborne, & Clarke, 2003) and metastatic settings (Ravdin et al., 1992) than PR-negative and ER-positive tumors. The predictive value of PR positivity in the absence of ER expression remains controversial (Elledge et al., 2000). PR expression is strongly prognostic but poorly predictive of response to adjuvant tamoxifen therapy (Dowsett et al., 2006), meaning that patients with PR-negative tumors have poorer outcomes than patients with PR-positive tumors when treated with tamoxifen, but the relative benefit from tamoxifen is similar in both groups (Dowsett et al., 2006; Early Breast Cancer Trialists’ Collaborative Group, 2005).

PR expression may change with disease course; upon metastasis, 40% of previously PR-positive tumors are found to be PR negative (Oldenhuis et al., 2008). Both the ASCO and NCCN guidelines recommend that PR be evaluated in every primary invasive breast cancer and in metastatic lesions (if the results influence treatment planning) (Harris et al., 2007; NCCN, 2010a).

Another important biomarker in patients with breast cancer is HER2, also known as ErbB2 (Dowsett & Dunbier, 2008; Ross, 2009a). HER2 protein overexpression or amplification of the HER2 gene has been identified in 15%–30% of newly diagnosed breast cancers and is associated with increased proliferation, invasiveness, potential for metastases, angiogenesis, and resistance to programmed cell death or apoptosis (Ross, 2009a). Most studies that have evaluated HER2 overexpression in tumor tissue have suggested that HER2 positivity is associated with poorer prognosis in untreated patients (Paik et al., 1990; Slamon et al., 1987; van

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<th>Table 1. Summary of Breast Cancer Biomarkers</th>
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<td>Breast cancer gene expression ratio</td>
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<td>Cellular markers (e.g., cancer stem cells, circulating tumor cells)</td>
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<td>Emerging predictive markers</td>
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<td>Topoisomerase II</td>
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HER2—human epidermal growth factor receptor 2; TMEM—tumor microenvironment of metastasis

de Vijver, Mooi, Wisman, Peterse, & Nusse, 1988). Likewise, the majority of studies of serum HER2 extracellular domain have found a similar association with higher tumor stage and increased tumor burden (Colomer et al., 2000; Kandl, Seymour, & Bezwoda, 1994; Willsher et al., 1996). Therefore, based on the data, a patient with untreated HER2-positive breast cancer would have a worse prognosis than a patient with untreated HER2-negative disease. Similar to ER and PR expression, HER2 expression can vary between lesions within a patient and can increase or decrease over the disease course (Gancberg et al., 2002; Gong, Booser, & Sneige, 2005; Meng et al., 2004; Oldenhuis et al., 2008; Zidan et al., 2005).

Although first identified as a potential prognostic marker, the predictive value of HER2 became apparent with the development of trastuzumab (Dowsett & Dunbier, 2008; Ross, 2009a). Patients with HER2-overexpressing tumors benefit from trastuzumab in the adjuvant and metastatic settings (Oldenhuis et al., 2008); therefore, analysis of HER2 has become a standard of care (Mass et al., 2005).

In a prospective, randomized study, recurrence-free survival was similar among patients with HER2-negative tumors after chemotherapy alone and in those with HER2-positive tumors treated with chemotherapy plus trastuzumab, suggesting that the prognostic value of HER2 overexpression may be neutralized by trastuzumab therapy (Joensuu et al., 2006). The NCCN (but not the ASCO) guidelines state that HER2 status may be used for prognostic purposes if the patient is node-negative. However, the pure prognostic value of HER2 expression in the absence of adjuvant therapy is not completely understood (Ross, 2009a). In contrast, both ASCO and NCCN guidelines recommend that HER2 expression at the time of diagnosis or recurrence can predict whether patients may benefit from trastuzumab (Harris et al., 2007; NCCN, 2010a).

HER2 levels are typically measured from tissue samples; however, whether circulating levels of the HER2 in the extracellular domain—which can be measured from a serum sample—yield results that are as reliable as traditional HER2 testing from tissue is unclear (Harris et al., 2007). To date, trastuzumab is indicated in tumors displaying clear HER2 positivity by IHC (3+ staining) or FISH (amplification of greater than two), so accurate methodology and interpretation of testing for HER2 in tissue are critical for the correct clinical application of trastuzumab (Dowsett & Dunbier, 2008; Harris et al., 2007). An expert panel from ASCO and the College of American Pathologists has developed guidelines to optimize HER2 testing, strongly recommending that all laboratories performing HER2 assays be accredited annually to ensure that standards of quality control are being upheld (Wolff et al., 2007). The NCCN guidelines recommend use of a laboratory that can document 95% agreement between the results of IHC or FISH analyses. Given these assumptions of quality control, NCCN gives no preference as to whether initial HER2 testing should be IHC- or FISH-based, stating that absence of clear HER2 positivity or negativity (i.e., a borderline result) warrants retesting by the same method or additional analysis by the other method (NCCN, 2010a).

HER2 is likely predictive for therapies that target the HER2 protein, such as tyrosine kinase inhibitors (e.g., lapatinib) and monoclonal antibodies (e.g., investigational anti-HER2 pertuzumab) (Widakowich, Dinh, de Azambuja, Awada, & Piccart-Gebhart, 2008). Patients with tumors exhibiting HER2 amplification and overexpression also derived greater benefit from standard doses of doxorubicin-based adjuvant chemotherapy (Dressler et al., 2005; Muss et al., 1994). A retrospective analysis of an adjuvant chemotherapy trial (CALGB 9344) suggested that adding paclitaxel after doxorubicin plus cyclophosphamide resulted in improved progression-free survival and overall survival among patients with HER2-positive tumors as compared to patients with HER2-negative tumors (Hayes et al., 2006, 2007). In fact, analysis of tissue specimens from women who received adjuvant doxorubicin plus cyclophosphamide, with or without subsequent paclitaxel, suggested that sequential paclitaxel was not beneficial in HER2-negative, ER-positive, node-positive tumors (Hayes et al., 2007). As such, although the predictive value of HER2 in adjuvant anthracycline-based therapy is supported by convincing data, the benefit of using HER2 to determine the use of taxane-based therapy remains controversial and definitive conclusions have yet to be reached (Harris et al., 2007; NCCN, 2010a).

**Emerging Biomarkers**

Advances in molecular biology and an increased understanding of tumor cell biology have led to the discovery of many promising new biomarkers. Candidate biomarkers include cancer stem cells, circulating tumor cells, gene expression profiles, individual markers, disease subtypes, predictors of metastasis, and mutation markers. Although these various biomarkers are being intensively investigated, the current data are not yet sufficient to support their use in routine clinical practice of breast cancer.

**Cancer Stem Cells**

Because of their association with cancer progression, cancer stem cells, or tumor-initiating cells, are being assessed as possible tumor biomarkers. Cancer stem cells are a distinct subset of cells within a tumor that have stem cell-like properties (Zardawi, O’Toole, Sutherland, & Musgrove, 2009) and are able to proliferate extensively, forming new tumors (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003). Cancer stem cells undergo processes that resemble the self-renewal and differentiation of normal stem cells, but they are epithelial in origin (Al-Hajj et al., 2003). Representing a minor population (10%–20%) within primary tumors (Abraham et al., 2005), cancer stem cells are phenotypically distinct from the larger population of tumor cells (Al-Hajj et al., 2003). Cancer stem cells have been detected in primary breast tumors as well as distant metastases (Balic et al., 2006) and may be characterized as having high expression of the cell surface marker CD44 (CD44-positive) and no or low expression of the CD24 surface marker (CD24-negative/low), without specific lineage markers (Lin-negative) (Al-Hajj et al., 2003). In preclinical trials, as few as 100 cells with this pattern of surface markers was sufficient to regenerate tumors, whereas tens of thousands of cells lacking this phenotype were unable to give rise to tumors (Al-Hajj et al., 2003). The role of cancer stem cells in breast cancer metastasis was further exemplified by the detection of the CD44-positive CD24-negative phenotype in the majority (71%) of disseminated tumor cells within the bone marrow of patients with stage I or IIA breast cancer (Balic et al., 2006). In addition, CD44-positive CD24-negative/low breast...
Circulating Tumor Cells

Circulating tumor cells with epithelial characteristics are rare in the blood of healthy individuals and those with benign breast disease, but can be detected in the blood of patients with metastatic and primary carcinomas (Cristofanilli et al., 2004). Consequently, circulating tumor cells are being investigated for their prognostic and predictive value (Cristofanilli et al., 2005). In a prospective study of patients with metastatic breast cancer, those in whom more than five circulating tumor cells were detected in 7.5 ml of blood at diagnosis and before initiation of first-line therapy had significantly shorter progression-free survival (2.7 months versus 7 months, p < 0.001) and overall survival (10.1 months versus more than 18 months, p < 0.001) than patients with fewer than five circulating tumor cells per 7.5 ml of blood (Cristofanilli et al., 2005). In addition, a decrease in the number of circulating tumor cells to fewer than five from baseline to first follow-up (3–4 weeks after initiation of new therapy) and at the time of restaging (9–12 weeks) was predictive of treatment efficacy, progression-free survival, and overall survival. These results suggested that, among patients with metastatic breast cancer, the number of circulating tumor cells could predict disease progression and survival earlier than traditional imaging techniques. Preliminary data also indicate that circulating tumor cell levels may identify residual disease following adjuvant therapy (Ignatiadis, Georgoulias, & Mavroudis, 2008).

The development of newer assays, such as the CellSearch® system (Veridex, LLC), has allowed accurate enumeration of circulating tumor cells at extremely low frequencies. To date, very little margin of error exists regarding sample numbers used to measure circulating tumor cell levels. Sample collection guidelines must be followed to maintain the validity of this test (consult the individual laboratory for specific recommendations). Currently, the ASCO guidelines recommend that the measurement of circulating tumor cells not be used to make the diagnosis of breast cancer or to influence any treatment decisions in patients with breast cancer (Harris et al., 2007). Likewise, the use of the CellSearch assay in patients with metastatic breast cancer is not recommended until additional validation confirms the clinical value of this test (Harris et al., 2007).

Possible Treatment Response Associations With Gene Expression Profiles

Preliminary evidence suggests that the gene expression profiles of tumors that are highly sensitive to chemotherapy are different from those that are resistant to treatment. For example, a gene expression analysis of two randomized trials in ER-negative patients with breast cancer identified sets of molecular markers that predicted a response to ixabepilone but not to paclitaxel (Wu et al., 2007). Ixabepilone, an epothilone, and paclitaxel, a taxane, both are microtubule-stabilizing agents that bind to β-tubulin and promote tubulin polymerization, causing cell cycle arrest and tumor cell apoptosis (Vahdat, 2008). However, ixabepilone binds to β-tubulin in a qualitatively different manner than taxanes. Ixabepilone also has demonstrated clinical activity in large randomized trials of patients with taxane-refractory breast cancer, which resulted in FDA approval (Bristol-Myers Squibb, 2010). The analysis revealed four candidate models that might differentiate response to ixabepilone treatment and taxane-containing therapy (paclitaxel and fluorouracil-doxorubicin-cyclophosphamide): Two of the models were based on expression levels for single microtubule-related genes, whereas the remaining two models were based on expression levels for 26 and 20 genes (Wu et al., 2007). A clinical trial is being conducted to further evaluate their ability to differentiate response to ixabepilone- and taxane-containing regimens. In a phase II study of preoperative (neoadjuvant) ixabepilone for invasive breast cancer, a 10-gene model (developed from 200 genes predictive of ixabepilone sensitivity) had a higher positive predictive value (45%) and comparable negative predictive value (89%) than did ER gene expression (Baselga et al., 2009).
Microtubule-Associated Protein Tau

Expression of markers, such as the microtubule-associated protein tau, might be predictive of treatment response among patients with breast cancer. Microtubule-associated protein tau binds to the same pocket as paclitaxel on the inner surface of microtubules, interfering with the drug’s actions (Kar, Fan, Smith, Goedert, & Amos, 2003; Rouzier et al., 2005). Breast cancer cells exhibiting high tau expression were found to be less susceptible to paclitaxel (Kar et al., 2003; Pusztai, 2007; Rouzier et al., 2005). In addition, tau expression might be predictive of response with ixabepilone (Baselga et al., 2009).

Beta III-Tubulin

The use of βIII-tubulin as a single marker that predicts treatment response is being investigated. βIII-tubulin is an alternative isotype of β-tubulin that destabilizes microtubules, counteracting the effects of taxanes (Pusztai, 2007). Overexpression of βIII-tubulin may be predictive of taxane resistance, as illustrated in a study where 38% of patients with advanced breast cancer expressing high levels of βIII-tubulin experienced progression versus 2% of patients with low expression (Paradiso et al., 2005).

Notably, ixabepilone shows activity against tumors expressing βIII-tubulin (Vahdat, 2008) and also is one of the few agents to receive FDA approval to treat metastatic breast cancer after taxanes have failed (Bristol-Myers Squibb, 2010). In contrast to the correlation between high βIII-tubulin expression and poor response to taxane-based therapy, high βIII-tubulin expression may predict a good response to ixabepilone therapy, at least in the neoadjuvant setting, although more data are needed to confirm this relationship (Horak, Lee, Xu, Galbraith, & Baselga, 2009).

Topoisomerase II

Topoisomerase II, or topo-II, is a DNA-modifying enzyme that binds to the double helix and promotes DNA replication by creating double-strand breaks. Agents that interfere with topo-II (i.e., anthracyclines, etoposide, teniposide, and amsacrine) covalently bind to topo-II, prevent DNA religation, and induce lethal cellular damage. Therefore, as a marker, topo-II could identify patients who are most likely to respond to these agents (Dowsett & Dunbier, 2008; Pritchard et al., 2008). Several retrospective analyses have shown that increases in topo-II protein levels are associated with a positive response with anthracyclines (Coon et al., 2002; MacGrogan et al., 2003; O’Malley et al., 2006; Tanner et al., 2006). However, a topo-II deletion was found to have the same effects as its amplification (Knoop et al., 2005; O’Malley et al., 2006), complicating its use as a biomarker. Because topo-II often is co-expressed with HER2, research efforts are focusing on the clinical significance of this co-expression. In the Breast Cancer International Research Group 006 trial, patients with early breast cancer co-expressing topo-II and HER2 demonstrated equivalent responses to anthracycline-containing therapy (doxorubicin and cyclophosphamide followed by paclitaxel) and trastuzumab-containing therapy (docetaxel, carboplatin, and trastuzumab) in the adjuvant setting, suggesting that topo-II amplification was a good surrogate marker (Press et al., 2005; Slamon et al., 2006). However, available data remain insufficient for topo-II to be used to assign patients to prognostic groups (Harris et al., 2007).

Thymidine Phosphorylase

Another potential marker, thymidine phosphorylase (TP), is an enzyme highly expressed in some cancers. TP-expressing tumors are believed to be more aggressive because of this enzyme’s antiapoptotic, proangiogenic, and prometastatic activities (Andreetta et al., 2009). TP is upregulated by stress induced by hypoxia, radiation, and chemotherapies such as anthracyclines and taxanes. TP also is involved in the enzymatic conversion of the oral prodrug capecitabine to 5-fluorouracil, suggesting that TP expression might be a marker for sensitivity of tumor cells to capecitabine. In a retrospective analysis of cases of TP-expressing metastatic breast cancer, patients treated with capecitabine after anthracyclines and taxanes had a significantly longer median time to progression than those treated with capecitabine without these prior therapies (7.5 versus 3.3 months; p = 0.01) (Andreetta et al., 2009). Similarly, median time to progression was significantly longer in patients with TP-expressing tumors who received capecitabine after taxane therapy than in patients who received capecitabine without prior taxane therapy (7.3 versus 5.4 months; p = 0.053) (see Figure 1). The preliminary data provide evidence that TP expression in breast cancer could represent a biomarker of sensitivity to capecitabine treatment.

Disease Subgroups

In addition to identifying multiple gene profiles and single marker expression, molecular techniques are classifying breast cancer tumors into prognostically valuable subgroups. Large-scale gene expression analyses using complementary DNA microarrays have identified four subgroups of breast cancer: luminal A, luminal B, HER2 positive, and basal-like (Pusztai, 2008). These tumor subtypes represent biologically distinct disease entities in terms of invasiveness, metastatic potential, and prognosis (Sørlie et al., 2003). Luminal-A tumors are ER positive and patients have the most favorable long-term survival if they receive endocrine therapy (Pusztai, 2008). Luminal-B tumors also are ER positive but possess a gene expression pattern that differs from that of luminal-A tumors. Luminal-B tumors are associated with poorer prognosis than luminal-A tumors (Sørlie et al., 2005). HER2-positive tumors are typically ER negative and, compared with luminal tumors, have a shorter time to metastasis and overall survival and may be more sensitive to chemotherapy (Sørlie et al., 2003). Basal-like tumors represent about 15% of all breast cancers and are composed almost entirely of the “triple-negative” cancers, which are characterized by the lack of ER and PR immunoreactivity or HER2 overexpression (i.e., ER negative, PR negative, and HER2 negative). In comparison with luminal tumors, basal-like tumors exhibit a gene signature for a high proliferation rate, have a poorer prognosis, are more sensitive to chemotherapy, and are more strongly associated with BRCA1 mutations (Pusztai, 2008; Sørlie et al., 2003). In addition, more than half of basal-like tumors overexpress epithelial growth factor receptor (EGFR) (Nielsen et al., 2004), and EGFR overexpression in this tumor type correlates with worse...
Anthracyclines and Taxanes

Figure 1. Effect of Prior Chemotherapy on Response to Capecitabine Treatment in Patients With Metastatic Breast Cancer Positive for Thymidine Phosphorylase Expression

Note. Based on information from Andreetta et al., 2009.

Predictors of Metastasis

At presentation, some gene expression features of primary tumors can predict the course of the disease (Chung, Bernard, & Perou, 2002). In the early 2000s, microarray analysis in patients with early breast cancer at diagnosis (in which the distance across the primary tumor was 2-5 cm, with no involvement of nearby lymph nodes [T1/T2/N0]) identified a group of 70 genes that, when analyzed together, predicted lymph node metastasis within five years with sensitivity and specificity of approximately 80% (van’t Veer et al., 2002). The full clinical significance of this information is still under investigation, but it could be used to develop a rationale for adjunctive treatment.

Rodent models of mammary tumors suggest that a tumor microenvironment of metastasis, which was defined as a tripartite arrangement of an invasive carcinoma cell, a macrophage, and an endothelial cell, is essential to metastasis (Wyckoff et al., 2007). Clinical findings confirmed that tumor microenvironment of metastasis density was a strong predictor of developing systemic, hematogenous metastases (p = 0.00006), independent of lymph node status and other standard prognostic indicators (Robinson et al., 2009). With additional study, tumor microenvironment of metastasis density may someday be a useful prognostic marker for metastatic disease.

Mutation Markers

Inherited germ-line gene mutations can predispose individuals to tumor development in specific tissues; these tumors may have unique molecular portraits and frequently resemble basal-like cancers (Chung et al., 2002). Among women with mutations in the BRCA1 or BRCA2 genes, an estimated 50%-80% will develop breast cancer (Bordeleau, Panchal, & Goodwin, 2010). Because only 5%-10% of breast cancers are hereditary, testing for BRCA1 or BRCA2 mutations is reserved for cases of a strong family history of breast cancer (Eitan, Michaelson-Cohen, Levavi, & Beller, 2009). NCCN (2010a, 2010b) discourages any genetic testing without formal genetic counseling and also outlined specific guidelines for determining whether the patient’s family history means she may be at an increased risk for breast cancer. Discovery of a BRCA1 or BRCA2 mutation may warrant increased surveillance, prophylactic surgery, risk avoidance through lifestyle changes, or chemoprevention (Eitan et al., 2009). Many women who already have had BRCA-related breast cancer opt for prophylactic contralateral mastectomy, which may reduce the incidence of contralateral breast cancer by almost 97%, but not increase survival (Bordeleau et al., 2010). A multiplex laboratory panel has been developed that identifies deleterious mutations more precisely and less expensively than complete sequencing of the BRCA genes in high-risk populations (Voelker, 2009).

Multigene Signatures

High-throughput genomic technology has enabled the development of multigene signatures for tumors and, as a result, dramatically increased the rate of discovery of new potential biomarkers (Dowsett & Dunbier, 2008). A multigene signature is a gene expression profile composed of two to several thousand genes that provides prognostic or predictive information about a tumor. Assays for gene signatures may be based on familiar methods such as IHC or FISH, or, alternatively, they may be based on newer techniques such as RT-PCR and microarrays (Ross, Hatzis, Symmans, Pusztai, & Hortobagyi, 2008). However, use of genomic prognostic assays remains low, both in routine practice and in clinical trials, presumably because of the lack of validation in different regimens (Pusztai, 2008). Common multigene signature assays include Oncotype DX® (Genomic Health), MammaPrint® (Agendia), and Rotterdam (76-gene) signature.

Oncotype DX is a 21-gene assay that uses a mathematical algorithm to calculate a recurrence score based on RT-PCR data (Dowsett & Dunbier, 2008). The algorithm was created by analyzing the results of three independent preliminary studies
of 447 patients and 250 candidate genes (Cobleigh et al., 2003; Esteban et al., 2003; Paik et al., 2003). Recurrence score is a continuous risk measure used to identify three risk groups: low, intermediate, and high with distant recurrence rates of less than 10%, 10%-30%, and greater than 30%, respectively. Recurrence score predicts distant recurrence independently of age and tumor size (p < 0.001) and also predicts overall survival (p < 0.001) (Sparano & Paik, 2008) (see Figure 2). Oncotype DX was validated in a prospective study of patients with ER-positive, node-negative breast cancer treated with tamoxifen in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 trial (Paik et al., 2004). The extent to which Oncotype DX is predictive of the magnitude of response to adjuvant therapy was determined in a prospective analysis of archival tissue from patients in the NSABP B-20 trial, also ER positive and node negative (Paik et al., 2006). Collectively, Oncotype DX has been clinically validated in women with node-negative, ER-positive invasive disease, as well as postmenopausal women with node-positive and ER- and PR-positive invasive disease (Oncotype DX, 2010). The only multigene assay endorsed by ASCO (Harris et al., 2007), Oncotype DX is commercially available in the United States (Ross et al., 2008) and covered by Medicare and most private insurers (Oncotype DX, 2010). Oncotype DX is being evaluated in the Trial Assigning Individualized Options for Treatment clinical trial, which is part of the National Cancer Institute’s Program for the Assessment of Clinical Tests.

The MammaPrint 70-gene signature, the first fully commercial breast cancer microarray system, has been approved by the FDA and is commercially available in the United States (Ross 2009b). The genes used in the test were identified in a case-control study of young women with node-negative primary breast cancer with 10 years or more of follow-up (van’t Veer et al., 2002). In this study, tumors from patients with early metastatic relapse had gene expression profiles distinct from those of patients who remained metastasis-free. As a result, the 70-gene signature predicted outcomes more accurately than classical clinical criteria. The ongoing Microarray in Node Negative Disease May Avoid Chemotherapy clinical trial is currently evaluating the usefulness of the MammaPrint gene set in determining systemic adjuvant therapy for patients with node-negative breast cancer (Cardoso, Piccart-Gebhart, van’t Veer, Rutgers, & the TRANSBIG Consortium, 2007).

The Rotterdam (76-gene) signature also is microarray-based, but it shares no common genes with Oncotype DX or MammaPrint (Ross et al., 2008). This assay is predictive of distant metastatic recurrence in lymph node-negative breast tumors, outperforming standard clinical variables (Pusztai, 2008). It has not yet received commercial approval (Ross et al., 2008).

Several other multigene assays have been validated externally. The two-gene signature test measures the expression ratio of two estrogen-regulated genes, HOXB13 and IL17BR (Ma et al., 2008). The ratio was a significant and independent predictor of disease-free survival in patients with lymph node-negative, ER-positive breast cancer receiving tamoxifen therapy. The more complex 97-gene genomic grade index classifies histologic grade 2 tumors into low- and high-risk groups alongside grade 1 and grade 3 tumors, respectively (Sotiriou et al., 2006). Among untreated and tamoxifen-treated patients with purely ER-positive tumors, this signature identifies two groups with distinct outcomes (Loi et al., 2007).

Clinical Application

Clearly, breast cancer research has made great strides in biomarker identification and validation, but the full value of many emerging biomarkers, and even some longstanding biomarkers, such as HER2, remains under investigation. More and more, breast biomarkers are being used in routine clinical practice.

With the advent of the Internet, women with breast cancer (and their caregivers) have access to a range of information regarding breast biomarkers. Some of this information may be outdated, erroneous, biased, or too technical to be useful to patients, raising the distinct possibility that this vast pool of Internet resources may be doing more harm than good. Regardless of the validity of Internet sources on biomarkers, most patients with breast cancer most likely do not have the background and medical knowledge to properly evaluate and process the voluminous amount of information available to them. If taken out of context, information about breast biomarkers may instill patients with an unfounded fear regarding their expression of a poor prognostic marker or a false sense of hope regarding their risk for recurrence or the efficacy of certain treatment options. Nurses and other healthcare personnel must mitigate the outcomes of this “information overload” in their patients with breast cancer. Nurses who are prepared to answer patients’ questions concerning the relevance of breast biomarkers and how they affect prognosis or treatment would provide a great service to their patients.

The topic of biomarkers in breast cancer is confounded by the large volume of ongoing research. Legitimate scientific information is constantly emerging or changing, challenging nurses and clinicians to keep abreast of the most current data. The healthcare team should have a solid, reactive communication plan in place should patients have questions about breast biomarkers or panel results. A clear chain of communication

Figure 2. Risk of Distant Recurrence According to Oncotype DX® Recurrence Score Group in the National Surgical Adjuvant Breast and Bowel Project B-14 Validation Study

Note. Based on information from Sparano & Paik, 2008.
Case Study: Confirming a Breast Cancer Subtype With a Multigene Assay

L.C. is a 42-year-old woman with an unremarkable medical history (i.e., menarche occurred at age 14, she is premenopausal, not taking hormone-replacement therapy, and has no history of ovarian cancer) and whose prior mammograms were negative. Notably, L.C. has a history of smoking and a paternal aunt who was diagnosed with breast cancer in her 70s. A routine mammogram indicated a positive result for the left breast only and, following a core biopsy, L.C. was diagnosed with infiltrating ductal cell carcinoma, grade 3 out of 3. A pathological examination confirmed the tumor to be high-grade and necrotic, with no signs of carcinomatosis at the margins of the specimen. A positron-emission tomography scan did not show distal spread or axillary lymph node involvement, corroborated by a positive result for only one of three sentinel nodes. The dimensions of her tumor indicated a relatively large mass (2.5 cm x 2.5 cm x 1.3 cm). Before beginning treatment, L.C. elected to have a bilateral mastectomy. Scores from estrogen receptor (ER) and progesterone receptor (PR) status tests were 1+ (weakly positive) and 0, respectively. Cytologic examination by immunohistochemistry for human epidermal growth factor receptor 2 (HER2) status resulted in a score of 2+, later confirmed as negative by fluorescence in situ hybridization. Subsequent lymph node dissection showed 14 more negative nodes, with a distance across the tumor of 2–5 cm (T2), spread to 1–3 axillary lymph nodes, with small amounts of metastasis to the internal mammary lymph nodes on sentinel node biopsy (N1), and no metastasis to distant sites (M0mi). Because of her nodal status and an inconclusive ER status, L.C. was a candidate for the Oncotype DX® assay. Her recurrence score was 67, with a 34% rate of distant recurrence at 10 years. L.C. was considered to be at high risk for recurrence. In addition, her ER, PR, and HER2 status were 4.6, less than 3.2, and 8.3, respectively, confirmed her breast cancer to be the aggressive triple-negative breast cancer subtype.

After L.C.’s diagnosis of breast cancer, routine clinical tests for the presence or absence of traditional biomarkers (ER, PR, and HER2) showed her tumor to be weakly positive for ER and negative for PR and HER2. L.C.’s case study is a testament to the quantitative power of assays that measure RNA expression, such as Oncotype DX, which, in fact, confirmed L.C.’s tumor to be ER negative. Because L.C.’s tumor type was the more aggressive and difficult to treat triple-negative, her healthcare provider tailored her treatment regimen accordingly to achieve an optimal clinical benefit.

should be agreed on by all clinical staff, and nurses should have a firm understanding of what information they should communicate to patients and what information they should communicate to the physician, midlevel provider, or, in some cases, a genetic counselor. Preemptive steps should ensure that all clinical staff members agree on who communicates and explains panel results with the patient and when such communication should occur. Technologic advances in clinical practice, such as electronic charts, should aid in achieving such alignment among all team members. Through electronic medical records, a patient’s history and test results may be readily available to any relevant team member.

Conclusions

Since the early 2000s, an exponential increase has been seen in information concerning prognostic and predictive biomarkers in breast cancer. The use of traditional markers (i.e., ER, PR, and HER2) is well established, and many others are approaching widespread clinical use. Validation will be required if newer markers are to be adopted into routine practice; this will require scrupulous attention to assay design and validation and the collection of large numbers of high-quality tissue specimens from suitably designed trials. Looking ahead, numerous multigene profiles are in development and are correlative (to varying degrees) with prognosis and response to treatment. Microarray platforms may be capable of identifying novel genes associated with disease development and clusters of genes predicting clinical outcome of an individual tumor. Among the different high-throughput technologies used in molecular tumor analysis, DNA microarrays are the closest to becoming routine diagnostics. An important potential of microarray-based tests is that multiple predictions, including prognosis and sensitivity to various treatment modalities, may be generated from a single experiment. Proper comparisons between new and existing biomarkers are required to properly contextualize the clinical role of novel discoveries. Genomic tumor profiling has the potential to change the prognostication and treatment options for patients with breast cancer, and offers a clear rationale for individualized therapeutic strategies. In conclusion, the advent of personalized therapy through application of biomarkers is changing the face of breast cancer treatment and management. The fields of oncology and oncology nursing are in the midst of exciting change. Through appropriate communication channels and proactive patient management, healthcare personnel can help to ensure that their patients realize the full extent of breast cancer biomarker research.

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References


Gancberg, D., Di Leo, A., Cardoso, F., Rouas, G., Pedrocchi, M.,


Slamon, D.J., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., & Pawlicki, M. (2006, December). BCIRG 006: 2nd interim analysis phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC-T) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (AC-T) with docetaxel, carboplatin and trastuzumab (TCH) in Her2neu positive early breast cancer patients [Abstract LBA 52]. Presented at the San Antonio Breast Cancer Symposium, San Antonio, TX.


tissue sections of human breast tumors with amplified neu DNA. *Oncogene*, 2, 175–178.


