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Personalized medicine for breast cancer: it is a new day!

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Abstract
Breast cancer remains the most common cancer diagnosed in women in the United States and is second only to lung cancer as a cause of cancer mortality. Breast cancer has become the prototypical solid tumor where targets have been identified within the tumor allowing for a personalized approach of systemic therapy.
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Breast cancer continues to be the most common cause of cancer in women and the second most common cause of cancer death. Breast cancer will account for 29% of all newly diagnosed cancers and for 14% of cancer deaths in women in 2013.1 Although the incidence of breast cancer has remained relatively flat since 2005, the mortality rate has dropped about 2% per year since 1998. These improvements in breast cancer mortality can be attributed to both improved early detection from screening and to improvements and access to treatment. Breast cancer, more than any other female cancer, has been recognized as having identifiable targets for therapy. With the discovery of these various targets, new methods for identifying the targets and new agents that recognize them have been created.

Sir George Beatson, a surgeon in Glasgow, United Kingdom, published the often-quoted article in The Lancet in 1895 with the following title: “On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases.”2 He described performing an oophrectomy on 3 women with advanced breast cancer, all of whom experienced dramatic regression of their cancer. But what if these 3 women had been postmenopausal or had cancers that did not express an estrogen receptor (ER)? Although this was a targeted therapy, it was not a personalized medicine.

Estrogen Receptor

The ER was the first of many targets that has been described in most breast cancers. It is a target that is most commonly assessed by immunohistochemical staining of breast cancer tissue. About 75% of newly diagnosed breast cancers are estrogen receptor positive (ER+), and ER status is a modest predictor of disease-free and overall survival. More importantly, ER strongly predicts for benefit from hormonal manipulation. A National Comprehensive Cancer Network task force in association with a panel of the College of American Pathologists has issued recommendations on

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ER and progesterone receptor testing for breast cancer. Multiple studies have been published that demonstrate that the percentage and intensity of cells staining positive correlates with long-term clinical outcome.

The function of the ER is similar when comparing the interaction of estrogen versus tamoxifen. Estradiol binds to the ER and recruits activation factor (AF)-1. Subsequently, the bound ER dimerizes with another ER, and AF-2 is activated. The dimerized complex moves into the nucleus, binds to ER elements, and recruits multiple coactivators. This complex leads to transcription and cell division. In contrast, when tamoxifen binds to the ER, there is a reduction in both transcription and cell division. Tamoxifen binds to the ER in the same manner as estrogen, but the tamoxifen–ER complex has only activation function AF-1. The ER dimerizes, and only AF-1 is active and AF-2 remains inactive. There is nuclear localization of a partially active ER and a reduced amount of coactivator function. Thus, there is reduced transcription and cell division when compared with estrogen, which explains the partial agonist effect of tamoxifen and other selective ER modifiers. Despite the recognition that ER+ breast cancers can be treated with estrogen or ER blockade, the timeline for approval of various therapies has spanned multiple decades, with modest improvements in outcome.

The US Food and Drug Administration approved Tamoxifen in 1977 for women with advanced breast cancer. No new agents were introduced in the subsequent 18 years until the aromatase inhibitor, anastrozole, was approved in 1995. Since then, 2 other aromatase inhibitors and the ER downregulator, fulvestrant, have been approved. The aromatase inhibitors function by blocking the conversion of adrenal androgens into estrogen in postmenopausal women. Thus, rather than competing with estrogen for binding to the ER as do the selective ER modifiers, the aromatase inhibitors completely block the production of estrogen. Fulvestrant, approved in 2002, forms a complex with the ER, and neither AF-1 nor AF-2 is active. The complex does not dimerize, and localization to the ER element is markedly reduced. There is no coactivator recruitment and no estrogen-dependent cell division. In addition, the fulvestrant–ER complex rapidly degrades, resulting in “downregulation” of the ER.

The options for hormone manipulation in women with a hormonally sensitive breast cancer must take into consideration the woman’s menopausal status. Women who are premenopausal may undergo modulation of estrogen synthesis either through the administration of luteinizing hormone releasing hormone (LHRH) or through an oophorectomy. Tamoxifen is equally effective regardless of menopausal status. Postmenopausal women may block estrogen conversion via the use of aromatase inhibitors or may be placed on the ER downregulator, fulvestrant. Up to 80% of women with advanced breast cancer treated with estrogen blockade will have either a clinical response or stable disease for a minimum of 24 weeks. With each subsequent hormonal therapy, the clinical benefit will be reduced by 25% to 30%. Ultimately, malignant breast cancer cells will develop resistance, which represents an unmet need for personalized therapy.

**HER2 neu**

The second most commonly described target in breast cancer is the HER2 protein. The HER family of genes and their HER transmembrane proteins were first described in 1978, when ErbB-1 was discovered. The derivation of the term Erb-b originated with the Erb-b gene, which is responsible for the avian erythroblastosis virus. The human gene ErbB-1 is also known as HER1 or epidermal growth factor receptor. Subsequently, the neu oncogene was discovered in 1982, and HER2 was cloned in 1984. Work was subsequently initiated on the development of a monoclonal antibody against the HER2 receptor. In 1992, humanized HER2 monoclonal antibody was created, and clinical trials in humans were subsequently initiated. Trastuzumab was approved in 1998 for use in women with metastatic HER2+ breast cancer, and in 2006, the antibody was approved for use in women with HER2+ early breast cancer when given with chemotherapy.

HER2 positivity can be described in 1 of 2 ways. Normal breast ductal epithelial cells have 1 copy of the HER2 gene on each chromosome 17. Thus, each normal breast cell and each “normal” breast cancer cell should have 2 copies of each, maintaining a 1:1 ratio. The HER2 gene encodes for a 185-kDa HER2 protein, which is a transmembrane receptor with cytoplasmic tyrosine kinase activity. There are approximately 20,000 HER2 receptors in each normal breast epithelial cell. The HER2 gene may be amplified in up to 25% of cases of metastatic breast cancer. Amplification refers to an increased number of HER2 gene copies in relationship to the cell’s chromosome 17. If the ratio rises to more than 2 copies of HER2 gene to each chromosome 17, then the gene is amplified. As a consequence of this gene amplification, the HER2+ breast cancer cell may have up to 2 million receptors on the cell surface. This is referred to as HER2 overexpression. There are 2 common methods used to test for the HER2 status of a breast cancer cell. Immunohistochemistry (IHC) is used to detect the HER2 receptor and the expression is subjectively measured as 0, 1+, 2+, or 3+, depending on the percentage of cells staining. IHC 3+ usually corresponds to gene amplification, whereas 0 or 1+ rarely does. Tumors with 2+ expression are usually tested by fluorescent in situ hybridization. Alternatively, the tumor cells can be tested by the fluorescent in situ hybridization method, which directly stains both the HER2 gene and the centromeres on chromosome 17. The HER2 ratio is the expression of the HER2 gene copies/chromosome 17 centromere copies. A ratio ≥2.0 is considered amplified.

HER2 is a member of the human epidermal growth factor family of receptors. This family consists of 4 transmembrane proteins (HER 1 to 4) each of which has different properties,
but all of which are involved in regulation of angiogenesis, cell growth, and survival. HER 1, 3, and 4 each have a ligand-binding domain and bind to various specific growth factors. When ligand binding occurs, there results tyrosine kinase activity within the cytoplasmic domain and subsequently activation of a cascade of intracellular intermediates that promote the growth and survival of the cell.10 HER2 has no natural ligand and remains in a fixed open conformational state, which allows it to interact easily with the other members of the HER2 family. HER2, along with other members of the HER family, must dimerize with another member of the family to activate the signal transduction cascade. The heterodimerization of HER2/HER3 results in the most highly proliferative signaling activity of the different dimerized complexes.

Approximately 25% of metastatic breast cancers are known to be HER2	extsuperscript{+}. HER2 positivity is strongly associated with larger tumor size, higher tumor grade, higher mitotic index, number of lymph nodes involved, and reduced expression of the progesterone receptor.11 In addition, before the availability of the HER2 antibody trastuzumab, survival rates were significantly worse in women with HER2	extsuperscript{+} breast cancer in both the early and metastatic setting. There are likely multiple mechanisms of action of trastuzumab, but the most commonly cited is the immune effect through the activation of antibody-dependent cell-mediated cytotoxicity and subsequent degradation of the HER2	extsuperscript{+} cells.12 Subsequently, several additional agents were developed which also are effective in the attenuation of HER2	extsuperscript{+} breast cancer cells. Lapatinib is a small-molecule inhibitor of the intracellular tyrosine kinase domain of both HER1 and HER2. This agent, along with trastuzumab, has been shown to block the downstream signaling pathways of HER2.13 Most recently, a third agent, pertuzumab, has been released which functions by inhibiting HER2 dimerization with other HER family receptors.14 All 3 agents are approved for use in the metastatic setting, whereas only trastuzumab is approved in the adjuvant setting. Pertuzumab is only approved to be used in conjunction with trastuzumab and a taxane chemotherapy. These FDA approvals are based on the significant improvements in relapse-free and/or overall survival when applied in the setting of HER2	extsuperscript{+} breast cancer.

### Triple-Negative Breast Cancer

Tumors that express neither the ER, the progesterone receptor, and do not show amplification of the HER2 gene (HER2 negative) are labeled as triple-negative breast cancer (TNBC). With the lack of a specific target, these tumors have become thought of as being the worst form of breast cancer with a high propensity for relative chemoresistance and the rapid development of distant metastases. A great deal of research has been undertaken to discover molecular targets for these tumors toward which novel agents can be developed. To better understand which molecular targets are of significance, the technology had to be advanced past protein expression of tumors as measured by IHC. In 2003, Sorlie et al15 published a seminal article describing the repeated observation of breast tumor subtypes across independent gene expression data sets. The technology used to define these subtypes is that of DNA microarrays. By evaluating tumors through gene expression profiles, the authors were able to describe breast cancer subtypes which could have the

<table>
<thead>
<tr>
<th>Molecular subtype</th>
<th>Frequency</th>
<th>ER/PR, HER2</th>
<th>Proliferative genes</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal like</td>
<td>10%–20%</td>
<td>ER−, PR−, HER2−</td>
<td>High</td>
<td>Poor</td>
</tr>
<tr>
<td>HER2+</td>
<td>10%–15%</td>
<td>ER−, PR−, HER2+</td>
<td>High</td>
<td>Poor</td>
</tr>
<tr>
<td>Normal breast like</td>
<td>5%–10%</td>
<td>ER+/−, HER2−</td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Luminal A</td>
<td>50%–60%</td>
<td>ER+, PR+, HER2−</td>
<td>Low</td>
<td>Good</td>
</tr>
<tr>
<td>Luminal B</td>
<td>10%–20%</td>
<td>ER+/−, PR+/−, HER2+/−</td>
<td>High</td>
<td>Intermediate/poor</td>
</tr>
<tr>
<td>Claudin low</td>
<td>12%–14%</td>
<td>ER−, PR−, HER2−</td>
<td></td>
<td>Poor</td>
</tr>
</tbody>
</table>

*ER = estrogen receptor; PR = progesterone receptor.*

### Table 2 Triple-negative breast cancer classification

<table>
<thead>
<tr>
<th>Triple-negative subtypes</th>
<th>Gene expressions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal-like 1</td>
<td>Cell cycle, DNA repair, and proliferation genes</td>
</tr>
<tr>
<td>Basal-like 2</td>
<td>Growth factor signaling (EGFR, MET, Wnt, IGF1R)</td>
</tr>
<tr>
<td>Immunomodulatory</td>
<td>Immune cell processes (medullary breast cancer)</td>
</tr>
<tr>
<td>Mesenchymal like (M)</td>
<td>Cell motility and differentiation, epithelial to mesenchymal transition processes</td>
</tr>
<tr>
<td>Mesenchymal stem like</td>
<td>Similar to M, but growth factor signaling, and low levels of proliferation genes (metaplastic cancers)</td>
</tr>
<tr>
<td>Luminal androgen receptor</td>
<td>Androgen receptor and downstream genes, luminal features</td>
</tr>
</tbody>
</table>

*EGFR = epidermal growth factor receptor; IGF1R = insulin-like growth factor 1 receptor.*
potential to provide not only prognostic but predictive information. The most common subtypes that have been described are as follows: luminal A, luminal B, HER2+/ER−, basal like, and normal breast like, and claudin low. These subtypes express characteristic genes and are associated with varying prognoses (Table 1).16 Although this new classification allows us to better understand the gene expressions that may be driving the tumors, microarray technology is currently a research tool and is not yet applicable for use in routine clinical practice. However, there is growing use of this technology in prospective clinical trials.

To further characterize the TNBC subtype, Lehmann et al17 described their molecular subtyping of 587 TNBC cases to see if this could become a model for targeted therapies. Their group has identified 6 TNBC subtypes with specific gene expression profiles. These types are labeled as follows: basal-like 1, basal-like 2, immunomodulatory, mesenchymal like, mesenchymal stem like, and luminal androgen receptor. Each subtype expresses specific genes that allow segregation of these subtypes (Table 2).

These subtypes have been shown to demonstrate different responses to both chemotherapy and targeted therapies in vivo and are currently being incorporated into various clinical trials. This subtyping has been evaluated prospectively in a clinical trial performed at the Memorial Sloan Kettering Cancer Center, where 424 TNBC tumors were screened for androgen expression. Of these, 51 expressed the androgen receptor, of which 26 eligible patients were initiated on bicalutamide. Although this trial had a small number of patients, the clinical benefit rate was 21% and a larger phase II trial is ongoing.18

Pathways of Growth and Resistance

As our ability to test for gene function improves, we continue to recognize that cancer is a more complex entity than we have imagined. Sjöblom et al19 described their work in 2006 when they undertook a systematic analysis of genetic alterations in 22 human breast and colorectal cancers. Of the more than 13,000 genes analyzed, they found an average of 90 mutated genes per case. Furthermore, only a subset (about 11 per case) was thought to be relevant to the neoplastic process. We are recognizing that there are tumors with what is described as an oncogene addiction. These are tumors that are being driven by 1 very specific genetic mutation, such as epidermal growth factor receptor–mutated non–small cell lung cancer, gastrointestinal stromal tumors (GIST), or chronic myelogenous leukemia. However, most solid tumors are being driven by a myriad of genetic mutations, and it will become increasingly more important to decipher the relative importance of 1 gene mutation and 1 growth pathway from others if we are to “personalize” our therapy for breast cancer.

Despite our ability to characterize tumors as being driven through the ER or through HER2, treatments targeted to alter or bind to these receptors will ultimately meet the cell’s resistance to that therapy. This has led to the use of combination of agents that are non-cross-resistant. Although this may lead to cures in the early breast cancer setting, more advanced cancers will eventually develop resistance. Defining common resistance pathways and subsequently being able to test cancer cells for activation of these pathways are additional ways in which we might think about personalizing our therapy of breast cancer. One of the most common growth factor pathways is the PI3-Kinase-Akt-mTOR pathway, activation of which can lead to cellular growth, proliferation, and angiogenesis.20 An emerging mechanism of endocrine resistance involves aberrant signaling through this pathway. Thus, the inhibition of this pathway is a rational treatment strategy in the setting of endocrine resistance in estrogen-dependent breast cancer cells.

Everolimus is an inhibitor of mTOR, currently being investigated in multiple Breast Cancer Trials of Oral Everolimus-2 trials. The BOLERO-2 trial was created to evaluate the efficacy of the combination of everolimus and the aromatase inhibitor exemestane in patients with hormone receptor–positive advanced breast cancer refractory to nonsteroidal aromatase inhibitors.21 The phase III double-blind randomized trial met its statistical end point with a 45% relative improvement in progression-free survival when adding the mTOR inhibitor to exemestane vs exemestane plus placebo. This study represents a proof of concept that breast cancer cells may be targeted not only through their primary pathway of growth but also through their pathways of resistance. As a result of these significant findings, many more agents are in clinical trials to target not only this common growth and proliferation pathway, but multiple other pathways as well.

Conclusions

When considering women with either early or advanced breast cancer, not all will benefit equally from endocrine therapy, chemotherapy, or targeted therapy. We now have the tools to better understand the unique biologic and genomic characteristics of a person’s breast cancer. Currently, we await the broad application of those tools and the rapid development of new targeted agents specific to an individual’s tumor genomic mutations that drive breast cancer growth and metastases.

References