The selective estrogen receptor (ER)\(^2\) modulator tamoxifen has been used for more than 30 years to treat and, more recently, to prevent breast cancer. Unfortunately, even among patients with ER-positive tumors, the response achieved with tamoxifen treatment is variable. Recent years have seen a sharp increase in the number of studies suggesting that the efficacy and safety of anticancer therapies such as tamoxifen depend not only on tumor characteristics but also on characteristics of the host. It is probable that future prescribing of truly “personalized” treatment approaches for our patients will be determined after analysis of both sets of traits. This Perspective summarizes recent basic and translational studies of putative mechanisms of tamoxifen resistance.

Tumor Characteristics

Resistance to tamoxifen therapy may be intrinsic or acquired. Breast cancers that produce either ER or progesterone receptor (PR) have the potential to respond to tamoxifen; however, a proportion of ER-positive tumors are intrinsically resistant to the drug. Historically, metastatic breast cancer that is both ER and PR positive (suggesting that the ER is functional) has an approximately 80% rate of response to antihormonal therapy, whereas tumors that are ER positive but PR negative have lower response rates, approximately 40%. Tumors that do not produce ER or PR are not expected to respond to tamoxifen.

Investigators have proposed several mechanisms of intrinsic resistance, including cross talk with growth factor–signaling pathways and a balance of ER coregulators. In addition, alterations in epigenetic regulation may cause a lack of ER production or lead to the acquisition of resistance to hormone therapy.

Recently, breast tumor phenotypes have been further characterized with DNA microarrays, and such studies have identified 5 clinically distinct subtypes: luminal A, luminal B, ERBB2-amplified, basal-like, and normal breast-like. So-called luminal B breast cancers that are classically associated with amplification of ERBB2\(^3\) [\(\text{v-erb-b2 erythroblastic leukemia viral oncogene homolog 2}\), neuro/glioblastoma derived oncogene homolog (avian)], with resulting overproduction of HER2/neu growth factor receptor (HER2) in addition to hormone receptor production, are now recognized as being relatively resistant to hormonal manipulations.

Molecular tumor signatures that can help stratify tumors before treatments are recommended are increasingly being introduced to the clinic. The 21-gene assay recurrence score (Oncotype DX) has been shown to predict distant recurrence more accurately than classic clinicopathologic features in patients with ER-positive breast cancer and ER-negative axillary nodes treated with adjuvant tamoxifen. Validation studies with this population led to the approval of Oncotype DX as a diagnostic test, and it is now commonly used to assess the benefit of adding chemotherapy to adjuvant hormonal therapy (1).

Other investigations have clearly demonstrated that the ratios of coactivators and cosuppressors in target tissues contribute to the tamoxifen-associated benefit. Tamoxifen has been noted to act as an agonist in experimentally engineered breast cancer cells with high concentrations of HER2 and the coactivator SRC3 (also known as AIB-1). A study that evaluated the possible connection between HER2, ER, PR, and tamoxifen resistance in a tissue database linked to clinical outcomes found that intrinsic tamoxifen resistance was associated with HER2, ER-positive, and PR-negative tumors that have increased AIB-1 concentrations (such tumors represent about 10%–15% of breast cancers) (2).

Epigenetic changes such as gene hypermethylation have been implicated in carcinogenesis and associated with resistance to common therapies. In breast cancer,
multiple genes are methylated, and thus silenced, compared with noncancerous tissue. The gene encoding ER has a CpG island (a dinucleotide of cytosine preceding a guanosine in the DNA sequence that is methylated by DNA methyltransferase) in its A and B promoters and the first exon. CpG islands in gene promoter regions are usually unmethylated, allowing active gene transcription; thus, the gene encoding ER is unmethylated at its CpG island in healthy tissues and in several ER-positive human breast cancer cell lines. Investigators have shown that only 36% of human breast cancers that produce the ER and PR proteins are methylated at the ER promoter, compared with 72% of tumors that are ER positive but PR negative, and 100% of tumors that are both ER and PR negative (3).

**PAX2 Appears to Mediate ER Repression of HER2 by Tamoxifen**

Cross talk between the ER and the HER2 pathways has long been implicated in the response to a breast cancer drug, but a direct connection at the transcriptional level was not understood. Hurtado and colleagues proposed that the antiproliferative effects of tamoxifen treatment require repression of ERBB2 and that breast cancers can potentially acquire tamoxifen resistance by amplifying the HER2 locus or by deregulating the control mechanisms that normally repress ERBB2 transcription (4). Their experiments with human cell lines demonstrated that estrogen–ER and tamoxifen–ER complexes directly repress ERBB2 transcription by means of a cis regulatory element within the ERBB2 gene. The investigators identified an ER-binding site within the intron of the ERBB2 genomic region that is bound by the product of the PAX2 gene (paired box 2). PAX2 was found to be recruited to this ER-binding site after treatment with either estrogen or tamoxifen. When PAX2 was silenced with short interfering RNA and transfected into ER-positive MCF-7 cells, ERBB2 transcription and HER2 protein concentrations were increased. Relative to the control, tamoxifen treatment of cells transfected with PAX2 short interfering RNA produced an increase in cell number. Expression of the NCOA3 gene (nuclear receptor coactivator 3) produced the protein AIB-1, which competed with PAX2 for binding to the ERBB2 cis regulatory element, leading to an increase in ERBB2 transcription and an increase in cell proliferation in the presence of tamoxifen. Thus, increased AIB-1 concentrations block PAX2 binding and ERBB2 gene repression, thereby reversing the antiproliferative effects of tamoxifen (4).

These investigators further confirmed these findings in primary breast cancer. PAX2 immunohistochemistry analysis was performed with 109 ER-positive samples from women with tamoxifen-treated breast cancer. Women with PAX2-positive tumors had a significantly improved recurrence-free survival time compared with women with PAX2-negative tumors \((P < 0.0001)\). Cox regression analysis confirmed an inverse relationship between PAX2 and AIB-1 concentrations in determining relapse \((P < 0.03)\). Tumors that were PAX2 positive and AIB-1 negative had the lowest percentage of HER2-positive staining, supporting the hypothesis that a balance between PAX2 and AIB-1 ultimately determines HER2 production and tamoxifen efficacy (4).

**Host Characteristics**

The role of inherited polymorphisms with respect to drug targets or drug-metabolizing enzymes has recently been recognized in resistance to common therapies. Tamoxifen is biotransformed to a potent antiestrogen, endoxifen, almost exclusively via the cytochrome P450 2D6 (CYP2D6) isoform. Perhaps the most established cause of intrinsic resistance to tamoxifen is a genetic polymorphism in the CYP2D6 gene (cytochrome P450, family 2, subfamily D, polypeptide 6). The balance of the currently available evidence (from at least 11 studies correlating CYP2D6 variants and clinical outcome) suggests that single-nucleotide polymorphisms in the CYP2D6 gene, and 2 null alleles in particular, predict altered tamoxifen metabolism and possibly a poorer outcome than that expected in patients with a wild-type genotype. Translation of a predictive biomarker into a diagnostic test, however, requires well-designed clinical trials for proof of benefit. To date, most of the studies that have evaluated the impact on long-term outcome of genetic polymorphisms producing a CYP2D6 enzyme with reduced or no activity have been retrospective and conducted on archival formalin-fixed, paraffin-embedded tissue blocks or on tissue samples obtained during previous prospective studies of tamoxifen. As the demand for molecular diagnostic tests increases, investigators must reconsider methods of handling clinical tissue samples. Well-powered prospective randomized studies are warranted to provide definitive data on this issue (5).

**Acquired Tamoxifen Resistance**

A high percentage of ER-positive breast cancers that respond to initial tamoxifen treatment subsequently develop resistance. ER production itself is maintained in the majority of tamoxifen-resistant tumors, suggesting that the unresponsive phenotype is caused by a complex, multifactorial change in the expression of a network of genes. A greater understanding of these factors may assist in the development of methods to overcome this resistance. Continuous tamoxifen treat-
...implantation of athymic mice implanted with ER-positive and PR-positive MCF-7 tumors eventually produces tamoxifen-stimulated tumors that grow in response to either tamoxifen or estradiol. Epidermal growth factor, insulin-like growth factor I, and heregulin-β1 have been shown to modulate the production and activity of ER-α via the phosphatidylinositol 3-kinase/Akt pathway (2). Constitutive activation of this pathway (e.g., by phosphorylation of ER-α at serine 167) can lead to a reduced sensitivity to tamoxifen (2).

**Implications and Treatment Recommendations**

As insight into tumor biology and host factors expands in concert with technologies that make such analyses readily available, it is likely that we will soon use personalized treatment approaches when prescribing hormonal therapies to breast cancer patients. Transitioning the data obtained from preclinical studies or retrospective analyses to their application in the clinic remains challenging and must be evaluated with caution and ideally within well-designed prospective clinical trials. The 21-gene assay recurrence score is already identifying patients likely to obtain sufficient benefit from adjuvant hormonal therapy and for whom chemotherapy may be avoided. Other commercial tests (e.g., for determination of CYP2D6 status) are available, but their clinical utility has not been established for general use in patients.

Trials are ongoing to determine whether the addition of agents that target epidermal growth factor pathways may reverse hormone resistance. Likewise, novel approaches with agents such as histone deacetylase inhibitors, either alone or in combination with existing therapies, are being tested to reverse epigenetic alterations and to restore a functional ER. Preclinical data suggest that histone deacetylase inhibitors and demethylating agents can induce increased production of ER mRNA and increased sensitivity to tamoxifen, even in ER-negative breast cancer cell lines (3).

**Conclusions**

Recent exciting advances are allowing a better understanding of the resistance to tamoxifen. Translation of laboratory discoveries to useful biomarker testing in the clinic, however, requires well-designed clinical trials for proof of benefit. We hope that treatment recommendations for breast cancer patients will soon be tailored through the use of tumor genomic profiling, as well as analysis of host characteristics. In this way, patients likely to benefit maximally from tamoxifen therapy can be selected, and other patients for whom alternative hormonal manipulations should be considered can be identified.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors’ Disclosures of Potential Conflicts of Interest:** Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

**Employment or Leadership:** None declared.

**Consultant or Advisory Role:** V. Stearns, Otsuka America Pharmaceutical.

**Stock Ownership:** None declared.

**Honoraria:** None declared.

**Research Funding:** V. Stearns, Merck & Co.

**Expert Testimony:** None declared.

**Role of Sponsor:** The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

**References**