Hindawi Publishing Corporation
New Journal of Science
Volume 2014, Article ID 390618, 27 pages
http://dx.doi.org/10.1155/2014/390618

Review Article

Endocrine Resistance in Breast Cancer

J. M. Dixon

Edinburgh Breakthrough Breast Cancer Research Group, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland

Correspondence should be addressed to J. M. Dixon; mike.dixon@ed.ac.uk

Received 27 April 2014; Accepted 23 July 2014; Published 17 September 2014

Academic Editor: Heidi Fiegl

Around 70% of all breast cancers are estrogen receptor alpha positive and hence their development is highly dependent on estradiol. While the invention of endocrine therapies has revolusioned the treatment of the disease, resistance to therapy eventually occurs in a large number of patients. This paper seeks to illustrate and discuss the complexity and heterogeneity of the mechanisms which underlie resistance and the approaches proposed to combat them. It will also focus on the use and development of methods for predicting which patients are likely to develop resistance.

1. Introduction

Approximately 70% of breast cancers are considered estrogen receptor alpha (ERα) and/or progesterone receptor (PR) positive, and the hormone estrogen (17β-estradiol, E_2) represents the primary stimulant in the growth and development of these tumours [1]. Thus deprivation of estrogen signalling through endocrine-targeted therapy has become the mainstay of treatment in ERα-positive disease. Despite the benefits, endocrine therapy resistance eventually occurs in a large number of patients and represents a significant issue for optimal clinical management [2]. In recent years a large body of work has focused on trying to understand the underlying mechanisms leading to resistance and approaches for their circumvention, as well as developing methods to predict which patients are likely to develop resistance and are therefore in need of additional or alternative therapies. While numerous mechanisms have been proposed in addition to those discussed in this review, a major lesson from the laboratory has been the realisation that resistance is both highly complex and heterogeneous and it is clear that more work is needed to identify and improve clinical outcome of patients with ER-positive breast cancers.

2. Estrogen Production

In both pre- and postmenopausal woman estrogen production occurs locally in the normal tissues of subcutaneous fat, the breast, muscle tissue and bone, where it is produced by the enzymatic conversion of androgens (androstenedione and testosterone) by aromatase [1, 3–8] (Figure 1). Within breast cancer tissue, expression of aromatase occurs mainly in fibroblasts [9]. Residual levels of estrogen are also commonly found circulating in the blood and are around 20-fold higher in postmenopausal women compared with premenopausal women, despite the loss of ovarian estrogen production [4, 5, 10]. In postmenopausal women there is a noticeable correlation between risk of breast cancer and levels of circulating estrogen in the blood plasma [11]. Aromatase transcription and protein levels have been shown to differ between the quadrants of the breast in women with breast cancer, being considerably increased in the quadrant containing the tumour [12, 13]. Evidence suggests that this is primarily due to tumour cell-released factors such as prostaglandins and inflammatory cell released cytokines such as IL6, IL11 and TNFα, which enhance the activity of aromatase in fibroblasts through intracellular cAMP signalling and regulation of the aromatase gene (CYP19) via an alternative non-standard promoter [1, 14, 15].

3. Estrogen Signalling

In hormone-dependent cancers, estrogen taken up from the blood plasma or from local production diffuses into the cancer cell and binds ER, thus causing the dissociation of

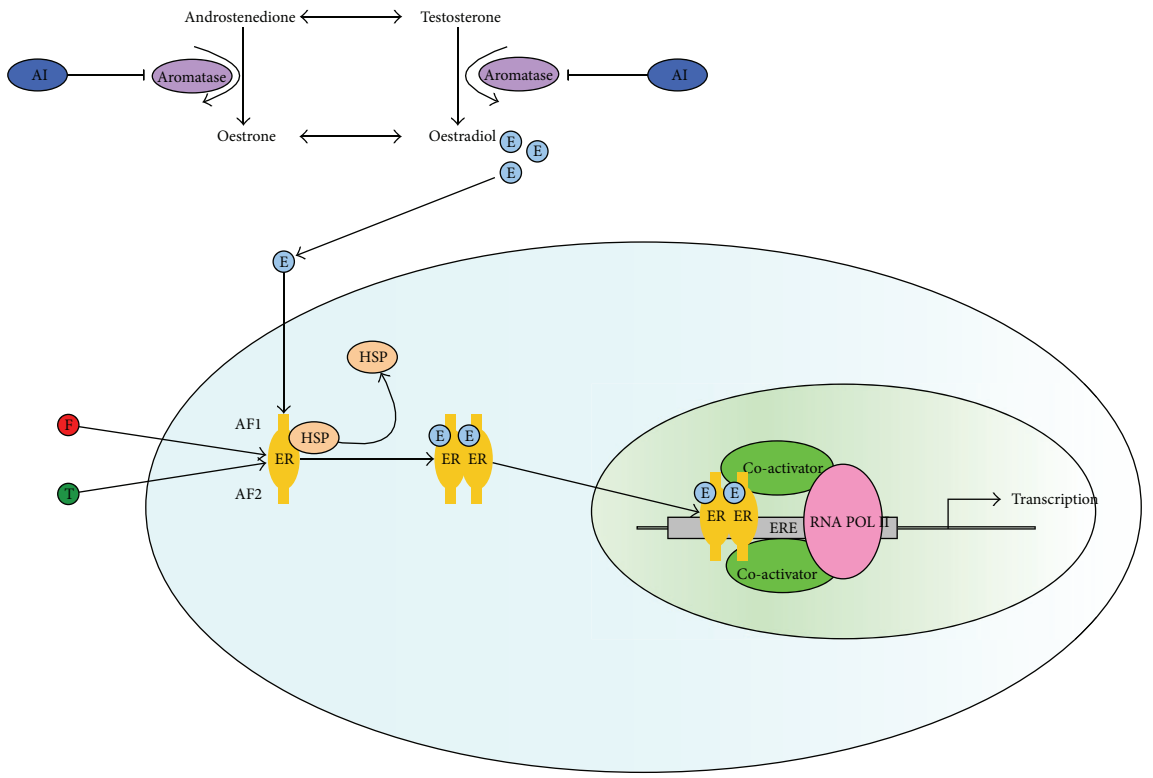

Figure 1: Diagram showing local estrogen production via the enzymatic conversion of androgens to estrogen by aromatase and estrogen receptor signalling in breast cancer. Estrogen (E), estrogen receptor alpha (ER), estrogen response element (ERE) and heat-shock proteins (HSP). The targets of commonly used endocrine therapies in the pathway are shown: aromatase inhibitors (AI), tamoxifen (T) and fulvestrant (F).

heat shock proteins from the ER molecule. The ligand-bound molecule dimerises, associates with other coactivator or corepressor proteins and subsequently binds to conserved estrogen response element (ERE) sequences within the promoter regions of genes over which it exerts transcriptional control [1, 16] (Figure 1). ER is a nuclear receptor encoded by the ESR1 gene; it comprises two distinct transactivation domains: activation function (AF)-1 in the amino-terminal region and AF2 in the carboxy-terminal region [17–19]. AF1 is regulated by growth factors acting through the MAPK pathway whereas AF2, located in the ligand binding region of ER is activated by estrogen [17, 20]. Full agonist activity requires both AF1 and AF2 to be active [21]. Studies have shown that ERE bound ER is ubiquitinated and targeted for proteosomal degradation, suggesting that each ER molecule is destined for only one cycle of estrogen signalling [22]. EREs were first discovered flanking the regions of estrogen-regulated vitellogennin genes in *Xenopus laevis* and have since been found in the promoter regions of several hundred human genes, with a minimum consensus sequence consisting of a 13 base-pair palindromic inverted repeat: 5′-GGTCANNNTGACC-3′ (N: any nucleotide) [23–25]. Some EREs have been identified which have imperfect palindromic sequences, differing from the consensus sequence by one or more nucleotides and are often less responsive to ligand-bound ER than consensus sequence EREs [26]. The association of ligand-bound ER with EREs is thought to be achieved via either of two mechanisms: (i) "direct binding" in which the molecule binds to the EREs and associates directly with coactivator/corepressor molecules and the RNA polymerase II transcription initiation complex, or (ii) "tethering" whereby the ligand-bound ER does not bind DNA but rather interacts with another DNA-bound transcription factor either stabilising that factor, or recruiting additional cofactors to the complex [27, 28]. The latter is thought to be the mechanism by which ligand-bound ER associates with transcription factor SP1 [27]. Via these transcriptional associations estrogen can induce proliferation of cancer cells which overexpress ER. It should be noted that non-ligand-bound ER complexes can also bind to EREs, without the ability to regulate gene expression it was thought [17, 29–31] however, recent chromatin immunoprecipitation sequencing data (ChIP-seq) from MCF7 breast cancer cell lines suggests that endogenous ERα can bind DNA in the absence of the ligand and that transcripts are produced at those sites [32].

Although the exact mechanisms by which estrogen drives proliferation are yet to be fully elucidated, a number of potential models have been proposed [33]. In normal human breast tissue, cells which do not express ER proliferate via paracrine signalling, whereas in tumours an autocrine action occurs in which ER-positive cells proliferate [34]. It has been reported that estrogen promotes transition through the G1/S phase of cell cycle via a number of pathways involving the induction of cyclin-D1 expression by ligand-bound ER,

mediated by one or more transcription factors including: c-Jun, c-Fos, and ATF-2 at the AP1 promoter site, or via an SP1 transcription factor dependent pathway [35–41]. Furthermore, ligand-bound ER has also been shown to bind cyclin-D1 and, as a complex, regulate the expression of that gene and other downstream genes [39, 42]. Cyclin-D1 subsequently binds and activates CDK4 and CDK6, which regulate G1/S phase transition through the phosphorylation of RB1. The latter can no longer inhibit E2F/DP1 complexes, thus allowing them to activate the transcription of S-phase entry genes such as those encoding cyclin-E1 and cyclin-A1 [35, 36, 43, 44]. Other studies suggest that ligand-bound ER may promote G1/S phase transition by induction of c-MYC which leads to activation of CDC25A and CDK4 gene transcription [45–47]. Active CDC25A dephosphorylates CDK2 leading to the inhibition of RB1 and p130 and transcriptional activation of E2F/DP1 complexes which in turn upregulates S-phase entry genes [36, 48, 49]. Alternatively, it has been proposed that ER could activate G1/S phase transition via redistribution and downregulation of p21 and P27KIP1 thus removing their inhibitory control over key cell cycle progression proteins such as CDK2 [49, 50]. It is thought this might be achieved by ubiquitin targeting for proteosomal degradation or by nuclear export via membrane-bound ER associated with ERK2 and CRM1 [51, 52].

4. Endocrine Therapy

In 1896 George Beatson, a Glasgow surgeon, showed that patients with advanced breast cancer had regression of metastatic disease following oophorectomy, providing the first contemporaneous published evidence of a link between hormones and breast cancer. By 1937 Dodds and Robertson had synthesised diethylstilboestrol and its anti-tumour activity was demonstrated although its use was limited by severe side effects (Figure 2) [53]. In 1973, the synthetic estrogen blocker tamoxifen was licensed for use in the treatment of hormone-positive breast cancer and became the mainstay of endocrine therapy for the next 30 years [54]. Today, endocrine therapy constitutes a major treatment modality in ER-positive breast cancer and can be used alone or in addition to chemotherapy, which has more associated toxicity [55–58]. Indeed, studies have shown it to provide more benefit in the adjuvant setting in postmenopausal women with ER-positive breast cancer than doxorubicin or taxane-containing chemotherapy [59, 60]. Endocrine therapies work by manipulating endocrine signalling by the exogenous administration of hormone antagonists designed to inhibit the biosynthesis and/or activity of estrogen. Endocrine therapies are considered to be cytostatic rather than cytotoxic, leading to reduced proliferation and reduction of growth rate [61]. At the simplest molecular level, they achieve this through the arrest of cell cycle in G1/S phase [62]. Today, several types of endocrine therapies exist and are used commonly in the treatment of ER-positive breast cancer in postmenopausal women.

First synthesised in the 1960s, tamoxifen is a selective estrogen receptor modulator (SERM) (Figures 1 and 2).

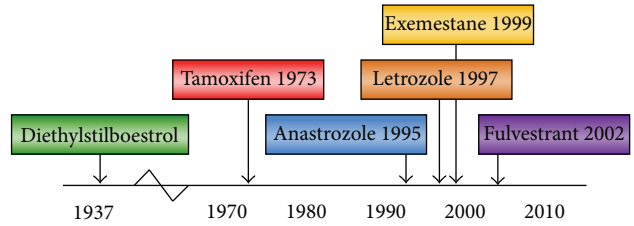

Figure 2: Timeline of approval for endocrine therapy agents.

It functions by disrupting the estrogen signalling pathway by competitive intranuclear binding to ER, causing a conformational change to the subsequently formed ER dimer involving the shift of helix 12 into an adjacent coactivator site (AF2), thus blocking the binding of the coactivator, which significantly reduces the level of estrogen-regulated gene transcription [21, 63, 64]. However, this complex has been shown to exhibit partial estrogen-agonist properties due to the remaining activity of AF1 [1, 21]. A newer yet similar class of endocrine therapies also exist which are known as selective estrogen receptor downregulators (SERDs) and these include fulvestrant, which was first approved for use in 2002. Fulvestrant functions to downregulate ER by competitive binding to ER dimers and by causing immobilisation of ER in the nuclear matrix which is accompanied by degradation via the ubiquitin-proteasome pathway [65] (Figures 1 and 2). It has been shown to be more potent than tamoxifen *in vitro* and does not exhibit the partial estrogen-agonist properties associated with tamoxifen in murine models. This is due to the fact that both AF1 and AF2 activities are suppressed, blocking cofactor recruitment at the ERE site of estrogen responsive genes [21, 66, 67].

Another major group of endocrine therapies in routine clinical use are third-generation aromatase inhibitors (AIs), which comprise two drug types. Firstly, the irreversible steroidal inhibitors (type 1), including exemestane which are androstenedione analogues and secondly, the non-steroidal inhibitors (type 2), which include letrozole and anastrozole [1, 68–73] (Figures 1 and 2). AIs seek to disrupt estrogen signalling by either: irreversible and inactivating binding (type 1), or reversible and competitive binding (type 2) to the aromatase enzyme; thus significantly reducing local estrogen biosynthesis and hence intratumoural levels of estrogen [1, 74–76]. Indeed, in the adjuvant setting, letrozole, anastrozole and exemestane have been shown to be more effective than tamoxifen with a significant reduction in the rate of relapse [73, 77–79]. Endocrine therapies can also be used in the treatment of ER-positive breast cancer in premenopausal women where their use is usually combined with drugs such as goserelin (zoladex) to suppress ovarian estrogen production [80].

In postmenopausal women, adjuvant treatment represents the major clinical setting for endocrine therapy, where long-term adjuvant systemic treatment is targeted against micro-metastatic disease [2]. Indeed, several studies have reported overwhelming evidence of a high correlation between the adjuvant use of endocrine therapy and reduction

in the risk of recurrence [81]. Endocrine therapy can also have an important role in the neoadjuvant setting where systemic treatment may be indicated for 3 to 4 months prior to surgery in postmenopausal women with large and/or technically inoperable tumours. This treatment is intended to shrink the tumour so that, in locally advanced disease surgery becomes possible and in large operable breast cancers, breast conserving surgery (BCS) can be performed [2, 56]. The Immediate Preoperative Anastrozole or Combined with Tamoxifen (IMPACT) study was a phase 3 clinical trial which showed that 46% of 124 ER-positive postmenopausal women initially recommended for mastectomy became eligible for BCS following 3 months of neoadjuvant anastrozole [82]. Similar results were found in a study of exemestane in which 85% of 40 patients deemed unfit for BCS at diagnosis became eligible following 6 months of neoadjuvant therapy [83]. Indeed by measuring proliferation levels of malignant cells using the expression of nuclear antigen Ki67, studies have shown a reduction in proliferation in approximately 90% of ER-positive primary breast tumours treated with and responsive to AIs, confirming that these tumours derive significant proliferative stimulus from estrogen and that this can be potently supressed by endocrine therapy [84, 85]. Endocrine therapy can also be used in the treatment of advanced or metastatic disease to prolong survival, either as a monotherapy or as part of a sequenced treatment regimen with chemotherapy, palliative surgery or radiotherapy [86].

5. Resistance

Despite the benefits of endocrine therapy in the treatment of ER-positive breast cancer, resistance to treatment eventually occurs in a large number of patients [2]. Clinically, resistance can manifest as relapse or cancer recurrence during or after completion of adjuvant therapy, following surgery or in rare cases after complete pathological response (elimination of all cancer tissue) following a period of drug therapy. Alternatively, in the neoadjuvant setting, resistance can be observed as clinical progression of primary disease, usually constituting an increase in primary tumour size or disease spread to regional nodes or beyond to more distant metastatic sites. Pathological changes such as increased tumour grade or increased proliferation are indicators of potential resistance to therapy. In the neoadjuvant setting, resistance occurs as either a primary lack of response (no change or an increase in tumour size and no evidence of pathological response) early in treatment, implying innate resistance, or later following a period of response, suggesting acquired resistance [87]. Studies have shown that recurrence on adjuvant endocrine therapy occurs in approximately 10–15% of patients with early stage ER-positive breast cancer within 5 years [88] and recurrence rates are has high as 30% by 15 years [77]. Others have suggested that as many as 40–50% of all ER-positive patients treated with adjuvant endocrine therapy will eventually relapse [89]. In the neoadjuvant setting, an observable clinical response occurs in 50–70% of patients [74, 83, 90]. The majority of patients with advanced or metastatic ER-positive disease acquire resistance within 2-3 years of commencing endocrine therapy [91–93].

6. Studying Endocrine Therapy Resistance

Resistance to endocrine therapy has been investigated using a number of different approaches which extend from fundamental cell line studies in culture or as xenografts in immunosuppressed animals, to clinical adjuvant and neoadjuvant treatment studies [2].

6.1. Cell Line Studies. A number of cell line based studies, both as *in vitro* cultures or as *in vivo* xenografts, have been used to investigate endocrine therapy resistance and have involved endocrine therapy-treated breast cancer cells transfected with the aromatase gene (CYP19) [78, 94, 95]. Such studies have elucidated important findings and have advantages including the ability to assess dynamic changes with numerous time-points which is not practical in studies involving patients [96]. However, several limitations exist which include an inability to accurately model the heterogeneity known to be present between and within individual primary breast tumours due to their clonality. They are also unrepresentative of the local tumour microenvironment found in patients; in particular, they lack stromal and immune components [2]. Cell line models do not represent a realistic model to evaluate endocrine resistance as seen in clinical practice, as many of the therapies such as aromatase inhibitors, which in patients target the peripheral tissue sites of aromatase activity, cannot be reliably studied. Furthermore, many of the mechanisms identified in cell lines have been found to have no or limited clinical utility.

6.2. Adjuvant Setting. Investigations involving adjuvant treatment are limited because the primary tumour has been surgically removed and is thus not available for on-treatment molecular analysis. Furthermore, measuring the response to treatment relies on monitoring survival and disease recurrence. These require long-term follow up with carefully documented study outcomes which are difficult to monitor successfully, as recurrence may be the result of inherent cancer aggressiveness rather than acquired resistance to therapy. Additionally, for studies to produce meaningful results the patient sample size must be sufficiently large to be statistically valid and data collection on relevant outcomes will take considerable time and effort to acquire [2].

6.3. Neoadjuvant Setting. The neoadjuvant setting presents a number of advantages for investigating the characteristics of response and resistance. The primary tumour remains in place during treatment and clinical response can be determined by considering changes in tumour volume as measured by 3D ultrasound or mammography. In addition, tumours can be biopsied, often at multiple and sequential times, allowing for assessment of dynamic changes in gene expression or protein levels as treatment continues. These data can be related to clinical response allowing for a dynamic

comparison of clinical and molecular response in both responsive and resistant tumours [2, 74, 97, 98].

7. Mechanisms of Resistance

Several different resistance mechanisms have been described which set the basis for continued research aimed at improving the outcome of endocrine treatment. Response to endocrine therapy essentially manifests at a molecular level as a G1/S phase arrest in cell cycle, a feature which is lacking in resistance cancer cells [62] (Figure 3). The resistant phenotype is characterised by maintained/high expression of the cell cycle machinery genes including molecules such as: cyclin-D1 and cyclin-E1, essentially driving proliferation [99] often in tandem with pro-survival signals including high expression of anti-apoptotic proteins such as BCL2 and low levels of pro-apoptotic proteins such as BAK, BIK and caspase 9 [100, 101].

High expression of cyclin-D1 is associated with activation of CDK4 and CDK6 and progression to the S-phase of cell cycle. Studies have linked high expression of cyclin-D1 to tamoxifen resistance and high expression of cyclin-E1 to letrozole resistance [102, 103]. Another study identified a correlation between high expression of cyclin-E2 and resistance to tamoxifen [104]. Several investigations have also demonstrated the aberrant expression of other key players associated with the regulation of cell cycle including: C-MYC, RB1, p21 and P27KIP1, which have all been implicated with endocrine therapy resistance [49, 105–107]. While activity of the cell cycle machinery is central to the maintenance of proliferation in endocrine therapy-resistant cells, the underlying mechanisms which regulate and control the machinery, and hence resistance to therapy, are complex, highly heterogeneous, vary with regard to estrogen-dependence and remain poorly understood. Work to-date in this field has been geared towards predicting which patients are likely to have or acquire resistance to therapy and to stratify subgroups of high-risk patients, in a move towards personalised therapy, based on their underlying molecular characteristics of resistance so as to develop tailored, novel or combinatorial treatment protocols to circumvent resistance and improve outcome in the population as a whole.

7.1. ER Expression in Tumours with Innate Resistance. Studies have suggested that innate resistance may be linked to lower levels of ER, which might suggest that the drive to proliferation of these cancers is not as dependent on estrogen as those expressing higher levels of ER. The current use of the Allred score for assessing ER levels by IHC staining categorises all tumours with greater than 1% of positively stained cells as potentially ER-positive despite the enormous variation within that group and does not cope well with intratumoural heterogeneity, with parts of the tumour less ER-positive than others [108, 109]. However, rather than a mechanism explaining resistance this suggests that endocrine therapy alone may be not be an appropriate treatment option for these patients.

7.2. Progesterone Receptor. Clinically, the decision to treat with endocrine therapy is primarily based on ER status; however, IHC levels of progesterone receptor (PR) are also determined at diagnosis. The steroid hormone progesterone is the ligand for the PR and its binding causes restructuring with dimerization and dissociation of the complex to the nucleus where it binds DNA and modulates transcription. Estrogen signalling via the ER has been shown to upregulate the expression of the PR and thus the majority of ER-positive patients are also PR-positive. However, a subset is PR-negative, and some studies have linked this genotype with innate resistance. Indeed, tumours which are ER-positive and PR-negative display a poorer response to endocrine therapy and a more aggressive phenotype than ER-positive/PR-positive tumours, and some reports have shown that the ER-positive/PR-positive population has a significantly better prognosis compared with the ER-positive/PR-negative group [110]. Studies looking at differential chromosomal loss and gain between ER-positive/PR-negative and ER-positive/PR-positive tumours have demonstrated loss of regions containing genes associated with tumour suppression and apoptosis in the PR-negative genotype. Furthermore, gains have been identified in regions of PR-negative tumours which encode genes including: MAP3K3, RPS6KB1 and ZNF217. Amplification of these genes could lead to activation of the PI3K-AKT-mTOR pathway, which has been implicated with endocrine therapy resistance [111]. However, despite the better prognosis associated with the ER-positive/PR-positive genotype, some patients still fail to respond to endocrine therapy. A recent study suggested that this may be due to the progesterone mediated assembly and activation of a transcriptional enhanceosome complex involving AP1, STAT3, PR and HER2 at the cyclin-D1 promoter which drives breast cancer growth and development [112].

7.3. Estrogen Receptor

7.3.1. ER Posttranslational Modifications. A number of post-translational modifications of ER have been reported, including phosphorylation, methylation and sumoylation which influence its interaction with other members of the ER signalling pathway. It has been suggested that aberrations in the posttranslational modification of ER could be linked to endocrine therapy resistance [113, 114]. ER can be phosphorylated at a number of different sites including serine-118, serine-167 and threonine-311 within the AF1 binding domain as well as in other domains. Phosphorylation and activation of ER at key positions can result from a number of pathways including: the MAPK/ERK pathway in response to growth factors such as epidermal growth factor (EGF), the PI3K-AKT pathway in response to insulin-like growth factors and the p38-MAPK pathway in response to stress or various cytokines [115, 116]. As tamoxifen can still bind partially activated ER, overexpression and cross-talk between these pathways regulating ER activation might explain the partial agonist capabilities of the drug.

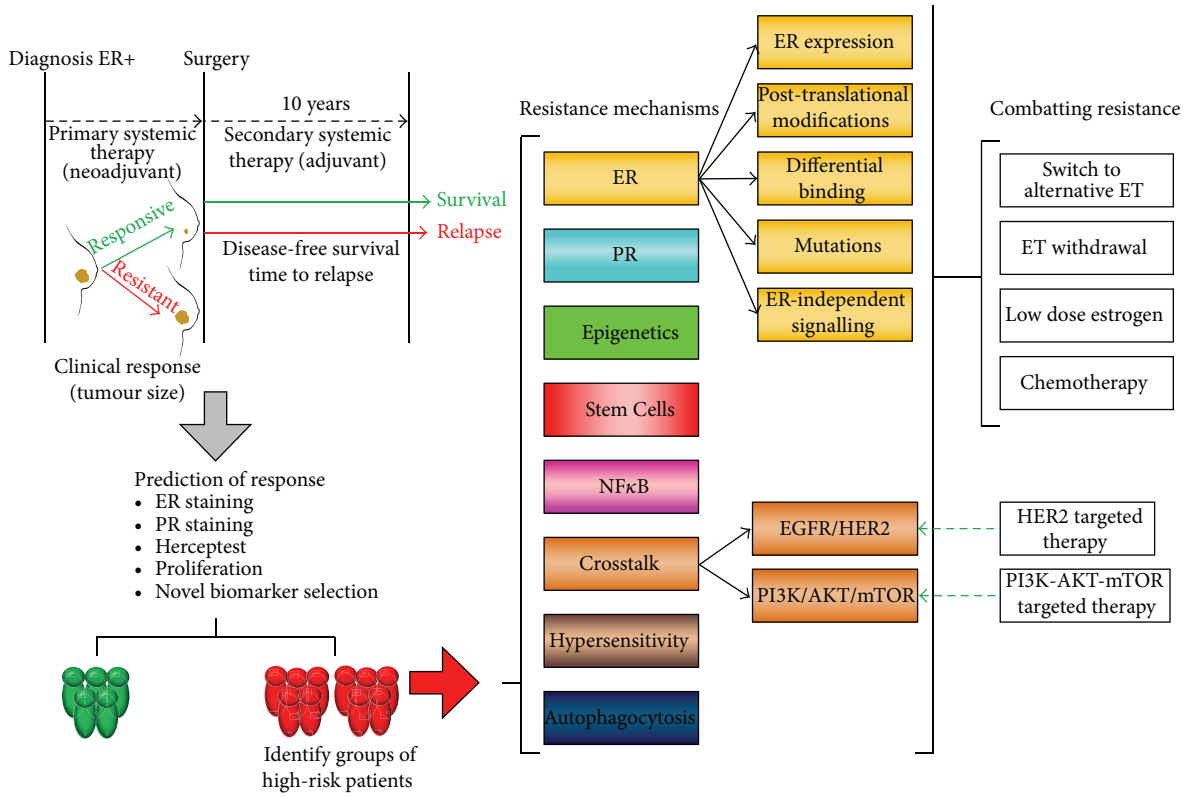

FIGURE 3: Summary of resistance in breast cancer showing the clinical manifestations of resistance in the neoadjuvant and adjuvant settings, the clinical need to accurately identify high risk patients, an overview of some of the best described resistance mechanisms and potential treatments and therapeutic strategies currently under investigation to combat resistance.

7.3.2. Differential ER Binding. A recent study looked at genome-wide ER binding events in primary breast tumours of patients sensitive and resistant to tamoxifen and revealed that in tamoxifen-resistant cancers ER is still recruited to the chromatin and binds regulatory regions in a pattern that is unique to resistant tumours [117]. The resistant phenotype may be due to selection and expansion of a resistant subpopulation of cells, or alternatively could involve the rapid reprogramming of ER binding by FOXA1, which has a known role in ER-chromatin interactions in response to growth stimuli [117–120]. Forkhead motifs and EREs were found to be enriched within the DNA regions which showed increased ER binding in tamoxifen-resistant cell lines and in primary tumour specimens of patients with a poor clinical outcome, providing further evidence for the FOXA1-mediated reprogramming model of ER binding [117]. These findings suggest that ER may have an important role to play in tamoxifen resistance by binding to a distinct set of regulatory elements giving rise to a unique gene expression profile which promotes tumour progression and confers resistance to therapy.

7.3.3. Activating Mutations in ER. A recent clinical sequencing study in patients with advanced ER-positive breast cancer identified a D538G mutation within ER in endocrine therapy resistant patients causing a change from aspartic acid to glycine at position 538 within the ligand binding domain. Importantly, the mutation was found in distant metastatic sites but not in the primary tumour. The D538G mutant ER was found to confer constitutive ligand-independent transcriptional activity which mimicked that of estrogen-bound wild-type ER with reduced tamoxifen binding affinity. Overexpression of mutant ER was found to enhance proliferation and confer resistance to tamoxifen [121]. Similar studies have also identified additional ER mutations in the ligand-binding domain which also result in constitutive activity and may represent potential mechanisms for acquired endocrine therapy resistance [122, 123].

7.3.4. ER-Independent Signalling. Other reports suggest that innate resistance may be a feature of ER-positive tumours in which proliferation is regulated by an ER-independent signalling mechanism. Some studies have shown that acquired resistance can occur in tumours with low levels of ER resulting from loss of expression or mutations inactivating its encoding gene (ESR1), suggesting an ER-independent driving mechanism for proliferation [124, 125]. However, loss of ER is only seen in approximately 15–20% of resistant breast cancers and the incidence of inactivating ER mutations is even lower, with less than 1% of resistant cases reported having this genotype [126–129]. Alternative mechanisms involve expression of truncated isoforms of ER such as ERα36 or other

estrogen-related receptors such as estrogen-related receptor gamma (ERRγ), both of which have been associated with reduced response to tamoxifen [130, 131]. Tamoxifen works by inactivating estrogen binding to wild-type ER, resistance in the case of ERα36 overexpression could be a feature of lower binding affinity to the truncated isoform of the molecule. Studies have shown that expression of ERα36 can be induced by BMP2, a member of the bone morphogenetic protein family of proteins which are known to have roles in regulation of cell fate and cancer development, suggesting a potential role for this molecule in endocrine therapy resistance [132]. Resistance related to ERRγ overexpression might suggest an important role for this molecule in an alternative estrogen signalling pathway [133, 134]. Furthermore, it should be noted that the estrogen receptor exists as two distinct isoforms: ER (ERα) and ERβ. The exact role of ERβ is not clear, however studies have shown that tamoxifen can bind ERβ and that tamoxifen-bound ERβ can activate AP1 regulated genes, possibly by altering the balance of associated coactivators and corepressors at the promoter site [135, 136]. Indeed, increased ERβ expression has been reported in tamoxifen resistant breast cancers and data from a recent study suggested that the ratio of ERα to ERβ may be important in predicting response to tamoxifen and anastrozole in the neoadjuvant setting [137, 138].

7.4. Crosstalk with Growth Factor Signalling Pathways

7.4.1. EGFR and HER2 Signalling. Resistance to endocrine therapy is common in ER-positive breast cancers that overexpress HER2 [139]. Many studies have reported cross-talk between ER and receptor tyrosine kinases (RTKs) such as HER1 and HER2, which are receptors for epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF1, somatomedin 1) [140, 141]. Overexpression of these receptors suggests that tyrosine kinase signalling is driving proliferation and evasion of apoptosis in these cancers, representing either a primary mechanism in the case of innate resistant tumours or a switch in driving mechanisms to evade the action of endocrine therapy in tumours with acquired resistance [142, 143]. Studies have also shown that EGF signalling can lead to an EGF-induced ER with regulatory control, dependent on AP1, over a set of genes commonly overexpressed in HER2-positive breast cancer, which are distinct from those regulated by estrogen-induced ER [139]. These data suggest that proliferation may be driven by a distinct EGF-dependent mechanism which is independent of estrogen signalling. One recent study suggested that long-term endocrine therapy facilitates the translocation of GPR30 to the cell surface, promoting activity of the EGFR pathway [144]. One group has suggested that EGF-induced ER might arise from EGF signalling in response to soluble stromal factors including fibronectin and matrix metalloproteases, secreted into the tumour microenvironment by fibroblasts, which associate with tumour cell membrane-bound β1 integrin, thus activating the PI3K/AKT pathway and MAPK/ERK pathway leading to ER phosphorylation and activation [145]. Alternatively, another study demonstrated that dysfunctional TP53 can lead to activation of the EGFR pathway, thus decreasing estrogen dependence for proliferation [146]. Furthermore, this indicates that patients with ER-positive/HER2-positive breast cancer could benefit from endocrine therapy combined with immune therapy targeted at EGF receptors. This mechanism fits well with studies which have suggested that ligand-bound ER is associated with repression of EGFR and HER2 [147]. Depletion of estrogen by endocrine therapy could lead to expression of these molecules via loss of activated ER repression. Studies have shown that expression of a transcription factor known as PAX2 is associated with reduced incidence of endocrine therapy resistance due to its role in the ER-mediated repression of HER2 [148]. One study reported that the response of cells to tamoxifen is regulated by competition between the ER coactivator AIB1 and PAX2 binding to the *cis*-regulatory elements in intron 4 of HER2. Indeed, they showed that a decrease in expression of PAX2 in tamoxifen resistant cells correlated with an increase in HER2 expression. Furthermore immunohistochemical staining of tamoxifen treated-ER-positive breast cancer tumours showed that PAX2 expression in the absence of AIB1 correlated with recurrence-free survival (RFS) and a low rate of HER2 expression. Conversely, patients with higher expression levels of PAX2 and AIB1 had a higher rate of RFS [148, 149]. It should also be noted that the mechanisms behind overexpression of HER2 are complex and have been shown to involve genetic and epigenetic modification, as well as alterations in upstream regulators such as FOXP3 and transcription factor GATA4 [150, 151].

7.4.2. PI3K-AKT-mTOR Pathway and Somatic Mutations. PI3K is activated by growth factor receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs). PI3K phosphorylates PIP2 to produce PIP3 which recruits several molecules such as PDK1 and AKT to the plasma membrane which, on activation, drive cell cycle progression and survival [152–154]. The pathway is negatively regulated by PTEN and INPP4B which dephosphorylate PIP3 and PIP2 respectively [155, 156]. AKT activates mTORC1 which regulates protein synthesis. The PI3K-AKT-mTOR pathway interacts with ER both directly and indirectly. AKT can phosphorylate ER, which increases estrogen-induced, tamoxifen-induced and ligand-independent ER transcriptional activity [157, 158]. In addition, PI3K promotes c-Jun phosphorylation, which complexes with c-Fos to form the AP-1 complex, known to be involved with ER transcription [159–161]. Studies have also shown that the PI3K pathway can be activated by HER2, the overexpression of which has been linked to a weaker response to endocrine therapy and poor prognosis following adjuvant therapy. In this model, activation of the PI3K pathway confers resistance to tamoxifen, fulvestrant and deprivation of estrogen [143, 157, 162]. Somatic mutations, which represent the most common in ER-positive breast cancer, have been described in key members of the PI3K-AKT-mTOR pathway including PI3KCA, PIK3CB, AKT1, AKT2, PTEN, and INPP4B, which have been implicated with aberrant activation and potential dependence on the pathway [163]. Interestingly,

a recent study showed increased expression of key PI3K-AKT-mTOR pathway members including phosphorylated mTOR, 4EBP1, and P70SK6 in metastatic sites compared with primary sites in patients who received adjuvant endocrine therapy, as opposed to no difference in expression in an untreated cohort, suggesting the compensatory activation of the PI3K-AKT-mTOR pathway as a possible mechanism leading to acquired resistance [164].

7.5. NFκB Signalling and Inflammation. NFκB plays an important role in processes such as cell survival and proliferation [165]. It can promote proliferation through regulation of key cell cycle genes including cyclins and CDKs and can mediate growth and survival signals via the PI3K-AKT-mTOR pathway [166, 167]. Additionally, NFκB has been shown to have a role in blocking apoptosis through cross-talk with ER and regulation of the BIRC3 gene [168]. NFκB has been reported as overexpressed in some endocrine therapy resistant breast tumours and many groups have alluded to its role in endocrine therapy resistance [169–175]. Some investigations have reported cross-talk between ER and NFκB signalling in which cooperative binding to transcriptional response elements can lead to specific gene expression [176]. Conversely, ER has also been shown to inhibit NFκB via a mechanism involving displacement of NFκB coregulators such as CBP at NFκB response element sites [177]. One study suggested the involvement of TGFβ-activated TAB2 in tamoxifen resistance in which a phosphorylated, active form of TAB2 exports the corepressor protein NCoR from the nucleus, thus translocating it from EREs, resulting in loss of response to tamoxifen [178]. TAB2 has also been implicated in the activation of NFκB via the IKK complex in response to IL1 stimulation [179].

Alterations in the NFκB cascade have also been identified in endocrine therapy resistant cells. In tamoxifen resistant cells, expression of the NFκB subunit p50 was increased. Nevertheless, it remained unchanged in fulvestrant resistant cells compared with the sensitive cells. Conversely, expression of the p65 subunit was found to be increased in fulvestrant resistant cells but remained unchanged in tamoxifen resistant compared with sensitive cells. The most abundant form of NFκB is the p50–p65 heterodimer and these findings suggest that resistant cells may utilise different strategies for upregulating the activity of this molecule [165]. Furthermore, the phosphorylation levels of p65 at its serine-536 site were found to be increased in endocrine therapy resistant cells. Phosphorylation at this site is necessary for optimal activity of the molecule and was shown to enhance its transactivation potential [180].

Some risk factors identified for breast cancer, including increased age and menopause, are associated with increases in indicators of systemic inflammation such as higher levels of circulating proinflammatory cytokines [181, 182]. Other risk factors such as pregnancy and obesity have been linked to the promotion and maintenance of a local inflammatory microenvironment in the breast [183]. Tumour associated macrophages (TAMs) have been found to comprise up to 50% of a breast tumour mass in some patients. Indeed, increased macrophage infiltration in breast tumours has been positively correlated with increased angiogenesis as well as reduced relapse-free and overall survival [184, 185]. One study in which TAMs were cocultured with ER-positive breast cancer cells revealed an increase in cancer cell invasiveness compared with cultures with no TAMs present. This was reported to involve a mechanism in which an inflammatory cytokine known as TNFα, produced by macrophages, led to activation of NFκB and JNK pathways [186]. Indeed, high expression of the classic macrophage marker CD68 in breast cancer is associated with poor prognosis and lack of response to endocrine therapy [187]. Furthermore, increased circulating levels of TNFα have been associated with advanced tumour stage, lymph node metastasis and local invasion [188, 189]. TNFα has been shown to stimulate proliferation in some ER-positive cell lines through increased expression of cyclin-D1 by a mechanism dependent on NFκB [190, 191]. Upon TNFα stimulation the p65 NFκB subunit is phosphorylated at serine-536 via the IKK complex. Following IL1 stimulation the PI3K-AKT pathway mediates phosphorylation of p65 at serine-536 via an unknown mechanism, although TBK, IKK, and p38 have all been implicated with IL1-induced p65 phosphorylation at serine-536 [192, 193]. TNFα-induced transcriptional activity of NFκB has been found to be significantly increased in endocrine therapy resistant cells compared to sensitive cells. This is thought to be due to the increased expression of NFκB subunits and increased levels of p65 phosphorylation. Interestingly, PR also has a known anti-inflammatory role in breast cancer cells and the loss of its expression in a subset of endocrine therapy resistant ER-positive cell lines which overexpress NFκB has been reported [194, 195].

7.6. Breast Cancer Stem Cells. There is now a large body of evidence suggesting an important role for stem cells in the development of breast tissue and that cancer stem cells (CSCs) can be found in breast cancers [196]. In breast cancer these undifferentiated, clonogenic cells are linked to increased invasive and metastatic phenotype. However, their frequency is dependent on tumour grade, disease, stage and molecular subtype [197–201]. Normal breast stem cells are thought to be basal-like and mainly ER-negative. Consequently, it is thought that CSC development is not influenced greatly by hormones such as estrogen and that these cells may be resistant to endocrine therapy as a result of low ER expression, with any partial response attributed to paracrine signalling from nearby differentiated ER-positive tumour cells. Normal breast stem cells are regulated by EGF receptor and other growth factor receptor signalling. Some groups have suggested that the observed increase in EGF receptor expression in endocrine therapy resistant tumours may reflect a greater proportion of CSCs selected by endocrine therapy [196]. Furthermore, one study showed that letrozole-treated tumours appeared to have an expression signature that was more like "claudin-low" subgroup of tumours originally described by Perou et al. (2000) and that posttreatment tumours were enriched for stem cells compared to pretreatment samples [202, 203]. A recent

study reported a mechanism for tamoxifen resistance which involved the SOX2-dependent activation of Wnt signalling in CSCs. Silencing of the SOX2 gene reduced the CSC population and restored sensitivity to tamoxifen as did inhibition of the Wnt signalling pathway [204].

7.7. Hypersensitivity to Residual Estrogen. Hypersensitivity to estrogen has also been suggested as a potential mechanism of endocrine therapy resistance [1]. Aromatase inhibitors function to dramatically reduce estrogen biosynthesis, although residual amounts of estrogen remain in tissues [205]. Cell line studies have shown that after prolonged deprivation of estrogen some cells develop hypersensitivity to residual estrogen. Reports have suggested that this phenomenon is associated with an increase in expression of HER2 and subsequent overactivity of the MAPK pathway resulting in changes in the phosphorylation of ER conferring its hypersensitivity to residual estrogen [206, 207]. Other researches have indicated that mutations in ESR1 may give rise to mutated ER which has increased interactions with the protooncogene tyrosine-protein kinase (SRC) family of coactivators and changes in promoter binding dynamics linked to hypersensitivity to estrogen [208].

7.8. Epithelial-Mesenchymal Transition. Epithelial-mesenchymal transition (EMT) is a morphological change which has been demonstrated in some epithelial tumours [209, 210]. EMT is associated with a loss of differentiation and loss of intracellular adhesion, a feature of an invasive phenotype characterising advanced metastatic disease [211–213]. Intracellular adhesion is an important feature of tissue architecture maintenance and can limit cell movement and proliferation. It is primarily mediated through the adherens junction (AJ) which are complexes of calcium-dependent transmembrane cadherin receptors, the cytoplasmic domains of which link to the actin cytoskeleton via α-catenin and β-catenin [214]. One study reported that development of tamoxifen resistance in cell lines is associated with an enhanced motile and invasive phenotype characterised by loss of intracellular adhesion and partial EMT. This phenomenon is thought to be brought about by the EGF-signalling mediated activation of β-catenin and the subsequent increased expression of β-catenin regulated genes known to be involved in tumour progression. Inhibition of EGF signalling in the same cells reduced β-catenin activity and promoted intracellular adhesion [215]. This suggests a possible role for EGF signalling (involving β-catenin) in the manifestation of an aggressive phenotype of endocrine therapy resistant tumours. In another study, overexpression of the transcription factor zinc finger protein SNAI1 (Snail) resulted in EMT. In this case ER expression was also lost as a result of Snail binding to the promoter region of ESR1 and causing deacetylation of histone H3K9 [216]. This represents a potential EMT-associated mechanism by which endocrine resistance could develop and might suggest the involvement of epigenetic mechanisms.

7.9. Epigenetics. Epigenetic mechanisms including DNA methylation have been shown to be responsible for determining and maintaining cell fate and for the stable differentiation of cells [217]. An increasing body of evidence is building to suggest an important role for DNA methylation in cancer including the silencing of tumour suppressing genes, activation of oncogenes, and promotion of metastasis [218–220]. More recently, changes in DNA methylation have been linked to endocrine therapy resistance [221, 222]. One study described a correlation between silencing of the promoter region of ER by methylation and reduction in ER expression as a potential mechanism leading to resistance [223]. Studies comparing endocrine therapy (tamoxifen or fulvestrant) resistant and sensitive cell lines have identified a pattern of methylation characterised by promoter hypomethylation in the resistant cell line compared with the sensitive [221]. This mechanism of hypomethylation was further outlined in a report showing the development of tamoxifen resistance in sensitive cell lines treated with a DNA methylation inhibitor (5-azacytidine) [222]. One study also suggested that the hypermethylation of the ERβ gene is associated with tamoxifen resistance [224]. Additionally, it has been shown that ER itself participates in epigenetic control. When ER binds EREs within the genome it recruits cofactors involved in epigenetic control including: NCOR1, NCOR2, SRC1, and AIB1 [225, 226]. Tamoxifen resistance has been linked with dysregulation of these cofactors suggesting a possible role for epigenetic mechanisms in endocrine resistance. Indeed, a recent study suggested that a novel concept that prolonged tamoxifen exposure could induce epigenetic silencing of a set of estrogen responsive genes with functions linked to the negative control of proliferation [227].

7.10. Autophagocytosis. Autophagocytosis is a cellular catabolic degradation process involving the lysosomal machinery whereby aggregated proteins, unfolded or misfolded proteins, or damaged subcellular organelles are degraded in response to stress or nutrient deprivation in an attempt to restore metabolic homeostasis. While the role of autophagocytosis in endocrine therapy resistance remains poorly understood, the process is known to be both prosurvival and prodeath with the final cell fate dependent on its extent and duration [228, 229]. Some studies have shown that inhibition of autophagocytosis can resensitise resistant cells to tamoxifen suggesting that it might have a role in resistance in some cancers [228]. One study identified a protein known as HSBP8 as having a role in regulating autophagocytosis in endocrine therapy resistant cells [230].

8. Predicting Response to Endocrine Therapy

There is an urgent need to identify early on treatment those patients who are unlikely to gain any benefit from endocrine therapy, thus sparing them from prolonged periods of ineffectual and redundant therapy and possibly exposing them to high risk side-effects. This is prudent in neoadjuvant treatment, where the aim is to down stage large or locally advanced tumours in order that they become operable or less

extensive surgery becomes possible; and in the adjuvant setting, to identify patients who would benefit from additional or alternative therapies following relapsed disease (Figure 3).

8.1. Pathological and Clinical Response. Following three to four months of neoadjuvant endocrine therapy, 60–80% of tumours demonstrate signs of pathological response. This usually includes both a decrease in overall tumour volume, and at a pathological level, a decrease in tumour cellularity with an increase in fibrosis or formation of fibrous connective tissue, and in some cases, a decrease in histological grade [231, 232]. Complete pathological response (CPR) to endocrine therapy is rare, but the incidence of CPR increases with increasing length of treatment [233]. It should be noted that quantitative measurements of partial pathological response rely on subjective assessment without robust formal criteria, and agreement between pathologists is variable; reported to be in the range of 50–86% [234, 235]. Indeed, whilst the clinical significance of tumour grade is well recognised, almost half of all tumours are histologically classified as grade 2, and interobserver reproducibility of grade is lacking, although there has been great effort to improve reproducibility [89]. A number of pathologically responding tumours decrease in size and this constitutes the clinically used definition "clinically responsive" [2]. Indeed, clinical and pathological response correlates significantly in the majority of tumours [233]. However, approximately 20% of tumours are discordant in this respect, showing either a clinical response (decrease in overall tumour volume) without evidence of pathological response or demonstrating pathological evidence of response whilst not reducing in volume [235, 236]. These clinical and pathological determinants of response require repeated clinical or radiographical measurements and biopsy histological assessment. Whilst they offer an effective end-point for categorising overall response, they often do not manifest soon enough to be suitable for predicting, early-on-treatment, which tumours are likely to respond or otherwise to endocrine therapy [2].

8.2. Proliferative Response. Significant decreases in proliferation are seen in approximately 80% of ER-positive tumours after 3 months of endocrine therapy and can be seen as early as 10–14 days of treatment in some tumours. Levels of proliferation are routinely established by measuring changes in expression of the protein Ki67. However, some tumours display variable patterns of Ki67 expression such as an initial reduction followed by recovery to pretreatment levels, a delayed change, and in some cases little difference over a treatment period [236]. Ki67, discovered in 1989, is a nuclear nonhistone protein which was reported to be universally expressed in proliferating tissues and to be absent in quiescent cells, establishing it as a marker of proliferation. More recently however we have learned that expression of Ki67 varies throughout the cell cycle which could influence the identification of proliferating cells [237]. Indeed some studies have demonstrated Ki67 expression in the G1 phase of cell cycle to be minimal [238, 239]. That being said, Ki67 expression has been shown to correlate positively with alternative markers of proliferation such as proliferating nuclear antigen and minichromosome maintenance protein 2 (MCM2) [240, 241]. Whilst the association between Ki67 and response to neoadjuvant chemotherapy has been demonstrated [242, 243], no significant correlation has been reported with neoadjuvant endocrine therapy [244–246]. Indeed, whilst an early reduction in proliferation is positively and significantly correlated with both clinical and pathological response (sensitivity) to endocrine therapy, there is discordance in a number of cases, and so this alone lacks specificity in determining which cancers are resistant to endocrine therapy [236, 247].

8.3. Molecular Response Markers. The most commonly used predictive molecular marker for endocrine therapy is ER. It has a strong negative predictive value with ER-negative tumours hardly, if ever, responding to endocrine therapy and around 50–70% of ER-positive tumours responding well. However, still around 30–50% of ER-positive patients will exhibit innate or acquired resistance to endocrine therapy [87]. Another commonly used molecular marker is PR. This molecule is regarded as a classical marker of estrogenic activity and is reduced in 70–80% of cases treated with endocrine therapy [233, 236]. However, loss of PR expression can occur independently of clinical and pathological response [236, 248]. Indeed, studies have shown increased response rates in PR-positive tumours compared with PR-negative tumours; however response to endocrine therapy can occur in both [248–250]. A recent study which sought to determine the prognostic significance of PR reported that, in multivariate models including ER and other standard clinicopathological features, PR did not contribute significant prognostic information [251]. Similar results were found with other classical markers of estrogenic activity including pS2 [248]. It has been shown that ER-positive tumours overexpressing HER2 are less likely to respond to endocrine therapy, although the number of such cases is small and they do not account for all resistant cases [252, 253]. The use of single molecular markers to predict response is far from adequate given their poor correlation with clinical and pathological response [254, 255]. Even expression of ER, clinically the most widely used molecular marker, has only around 50–70% accuracy in endocrine therapy response prediction in ER-positive cases.

8.4. Multigene Signatures

8.4.1. Gene Expression Profiling. The introduction of high-throughput gene expression profiling technologies, such as gene expression microarrays, applied to translational research has revolutionised the way breast cancer is understood, highlighting the importance of heterogeneity and the fact that distinct molecular subtypes of the disease exist which can affect the same anatomical site [256]. Indeed, much research has focused on the development of multigene signatures using high-throughput gene expression microarray technology, which have been used for molecular subtype classification, confirming the importance of key disease drivers such as ER and HER2 signalling, and for prognosis [256–258]. A number of studies have focused on identifying

subsets of patients with a favourable prognosis, in whom the absolute benefit of systemic adjuvant chemotherapy is small compared with associated toxicity, in order that they could forgo this treatment [259]. Mammaprint and Oncotype DX are two such prognostic signatures available for clinical use, both developed to estimate recurrence risk in node-negative early breast cancer [187, 260, 261].

8.4.2. Commercial Profiling: Prognostic Assays. The 70-gene Mammaprint test uses fresh tissue and microarray technology to evaluate expression of genes associated with proliferation, invasion, metastasis, tumour stroma, and angiogenesis in both ER-positive and ER-negative cancers [261]. It was given Food and Drug Administration (FDA) approval in 2007 to be used as a prognostic predictor (indicating risk of relapse within 5 years) for breast cancer patients aged under 61 years of age, with lymph node negative stage 1 or 2 tumours up to 5 cm in size. Those patients with poor prognosis and ER+ tumours are recommended to receive adjuvant chemotherapy in combination with endocrine therapy, whereas those with favourable prognosis and ER-positive tumours are recommended to receive only adjuvant endocrine therapy [256]. It should be noted that Mammaprint is of limited clinical use in ER-negative breast cancer with only 0–4% of such patients predicted to have a good prognosis [262–266]. An independent validation of the Mammaprint signature demonstrated that prognostic accuracy is highly time-dependent and may be more suitable as a predictor of early relapse [261, 265]. Furthermore, no clinical validation studies of the Mammaprint signature have been performed in randomised trial populations [267–269].

The 21-gene Oncotype DX test uses "formalin fixed paraffin embedded" (FFPE) tissue with quantitative real-time PCR (qRT-PCR) to measure expression of 16 genes associated with proliferation, estrogen regulation, HER2, and invasion in hormone receptor positive cancers, as well as 5 reference genes [187, 260, 270]. The test outcome is presented as a recurrence score (RS) ranging from 0 to 100 to predict the risk of 10 year distant recurrence. In clinical use the RS is subdivided into three groups: low (<18), intermediate (18–31), and high (>31), and several publications have shown that ER-positive breast cancer patients with low RS have a low risk of recurrence and derive little benefit from chemotherapy, whereas the reverse is true for those with high RS [270–274]. According to the National Comprehensive Cancer Network (NCCN) guidelines for breast cancer treatment, patients with a low RS should receive endocrine therapy alone, whilst those with a high RS should also receive additional chemotherapy. However, the ideal clinical management strategy for those with an intermediate RS remains at present unclear; emphasising a considerable limitation with the use of Oncotype DX [256].

More recently, a microarray diagnostic test known as Map*Quant* DX was launched to accurately measure tumour grade and risk of metastasis, predict response to chemotherapy, and indicate proliferation. It measures expression of a published 97-gene signature to determine a "genomic grade index" (GGI) [275–278]. When applied to an independent validation cohort the GGI had strong association with histological grade, although 9% of grade 1 tumours were classified as having high GGI and 14% of grade 3 tumours were classified as having low GGI. However, GGI was found to be more strongly associated with relapse free survival than histological assessment of grade. Furthermore, GGI was able to stratify histological grade 2 tumours into prognostically significant high or low grade groups. In independent validation studies GGI has also been shown to be independently prognostic of outcome (risk of recurrence) in tamoxifen treated patients. Tumours with high GGI profiles were also reported to respond well to neoadjuvant chemotherapy despite having a significantly worse outcome than low GGI tumours [275]. The Map*Quant* DX test was recently converted to an 8 gene assay (PCR-GGI) based on quantitative real-time PCR (qRT-PCR) measurements [279]. While the evidence for genomic grading is compelling, independent validation of the Map*Quant* DX and PCR-GGI systems has not yet been documented. Furthermore, their ability to discriminate between high and low grade in ER-negative tumours is limited [280, 281].

Theros is a qRT-PCR based assay designed for lymph node negative ER-positive breast cancer which has been treated with surgery alone. The test is reported to assess risk of recurrence and benefit from endocrine therapy. The test measures the ratio of expression of homeobox gene HOXB13 to interleukin IL17BR [256]. It has been shown to identify ER-positive tamoxifen-treated patients with a high risk of recurrence and predict outcome in ER-positive patients, either adjuvant systemic therapy naïve or tamoxifen treated. However, its ability to predict benefit from neoadjuvant hormone therapy or from chemotherapy remains to be seen [282, 283]. Furthermore, its use has limitations in lymph node positive disease [284]. It should also be noted that neither the Map*Quant* DX/PCR-GGI nor Theros tests have been included in the NCCN guidelines for breast cancer treatment [256].

8.4.3. Prognostic Signature Research. Several other gene signatures, which are not as yet commercially available, have also been reported in an attempt to refine tumour classification and improve prognostication.

The "sensitivity to endocrine therapy" (SET) index comprises a signature of 165 genes coexpressed with ER in 437 patients (unrelated to treatment or outcome). The association of the signature with distant relapse risk was evaluated in 5 independent validation cohorts: 2 cohorts (n = 225 and 298) of patients who received adjuvant endocrine therapy, a cohort who received adjuvant chemotherapy followed by endocrine therapy (n = 122), and two cohorts who received neither adjuvant endocrine nor chemotherapy (n = 208 and 133). The SET index was found to be significantly associated with distant relapse and risk of death in endocrine therapy and chemotherapy plus endocrine therapy treated cohorts, but not in systemic therapy naïve cohorts. Whilst this signature has been extensively validated in independent cohorts and been shown to predict survival benefit, there is as yet no evidence suggesting accurate prediction of response to therapy or inherent prognosis [285].

More recently a further PCR based assay—EndoPredict (EP), has been validated to predict the likelihood of distant recurrence in patients with ER-positive, HER2-negative breast cancer treated with adjuvant endocrine therapy. The test uses RNA levels of a panel of 8 genes (plus 3 reference genes), as determined from RT-PCR of FFPE tissue, which are used to calculate an EP score. A combination of EP score, nodal status, and HER2 status is used to calculate a comprehensive risk score: EPclin. Using this, 58%–68% of women from two large phase III trials, who were classified as having high/intermediate risk of recurrence according to clinical guidelines, were predicted to have a low recurrence risk based on their EPclin score. The rate of occurrence of distant metastases in this group was found to be only 5%. Further validation work has shown reproducible performance of the assay and negligible interlaboratory variation. The authors suggest that the EPclin score may be used to identify ER-positive postmenopausal women with a limited number of clinical risk factors who may not benefit from adjuvant chemotherapy [286–289]. EPclin does show promise in identifying subgroups of patients with a low risk of recurrence which may out-perform currently utilised clinical guidelines; however there is once again no evidence, as yet, to suggest that it can predict response to therapy or inherent prognosis. Furthermore, as with other tests such as Oncotype DX, patients' risk is defined with a score on a continuous scale and the optimal clinical management approach for those with intermediate scores remains unclear.

8.5. Lessons from Prognostic Signature Research. Numerous prognostic signatures have been proposed beyond those already discussed including signatures assessing: amplification of cyclin-D1, inactivation of p53, activation of PI3K pathway, MAPK cascade, and prediction of invasion to name a few [290–295]. Most prognostic signatures have significant agreement in their prediction of outcome and identification of similar groups of patients despite the fact that the overlap of individual gene lists is negligible [280, 281, 296–302]. Meta-analysis of different prognostic signatures, looking at genes, pathways, and networks revealed that classification of patients into favourable or poor prognostic groups relies heavily on the expression of proliferation-associated genes. Indeed, some signatures were found to perform better when only proliferation genes were used to predict prognosis [281]. Furthermore, this analysis confirmed that the prognostic power of most signatures, particularly the four commercially available assays (Mammaprint, Oncotype DX, Map*Quant* DX, and Theros) was limited to the ER-positive/HER2-negative subgroup of breast cancers, providing more evidence for proliferation as the key determinant of prognosis in this subgroup [280, 281]. The study and use of prognostic signatures has disclosed important heterogeneity features of breast cancer, including the fact that within all ER-positive disease arises a spectrum of tumours ranging from low proliferative favourable outcome tumours to highly proliferative poor outcome disease. ER-negative tumours conversely represent distinct entities, driven by distinct molecular aberrations, whose prognosis may not be determinable using proliferation gene based signatures [273, 303, 304]. Indeed, recent work has suggested the importance of immune response genes in the prediction of outcome in ER-negative breast cancer [305–307].

8.6. Predicting Response to Endocrine Therapy. Unlike prognostic signatures, relating gene expression to risk of relapse; predictive signatures determine features associated with response, or lack of it, to a particular therapy [256]. Such research is aimed at determining the ideal systemic therapy and the magnitude of benefit derived from it for individual patients, in a move towards personalised therapy [256, 296].

A number of predictive multigene signatures have been reported which directly relate gene expression to the clinical and pathological response to endocrine therapy in ER-positive patients, the majority of which have not yet been validated for clinical use. One of the first signatures developed for endocrine therapy came from a study to predict clinical response to tamoxifen in patients with recurrent disease (local/regional relapse or distant metastasis). Gene expression profiles of primary tumour samples from 112 patients who later recurred and received tamoxifen were derived. Of the 112 patients, 52 responded with an objective decrease in size of recurrent mass and 60 had progressive disease. A 44-gene signature was derived, which included genes associated with estrogenic regulation, apoptosis, extracellular matrix remodelling, and immune response. The signature was reported to predict endocrine therapy outcome and time to progression in ER-positive patients with recurrent disease. In independent validation the gene signature was shown to predict response to tamoxifen in 77% of patients, outperforming the commonly used ER marker which predicts response in 50–60% of metastatic patients [308]. However, it should be noted that this signature has not yet been shown to be of significant predictive value in patients with early stage disease.

One of the first gene expression studies focused on response to aromatase inhibitors was published in 2007 by Mackay et al. [309] and reported response to letrozole and anastrozole during a short preoperative (14 days) treatment period using sequential biopsies from 34 patients. The study revealed that short term estrogen deprivation by aromatase inhibition led to profound changes in transcriptomic profile, including genes associated with proliferation and estrogen signalling. While many of the changed genes reported had been previously identified in cell line studies, many additional estrogen responsive genes were identified in this study. Importantly, the study revealed complex changes in matrix remodelling and stromal interactions which cannot be easily studied in cell lines. The reported response gene changes were integrated into a global index of dependence on estrogen (GIDE), a measure of the number of genes with at least a 2-fold change on treatment. The GIDE was found to be significantly associated with on-treatment changes in Ki67 and with pretreatment levels of HER2 [309]. While time-course studies in cell lines have revealed much about the response to estrogen deprivation, cell lines are not representative of the *in vivo* tumour microenvironment and may

not be an ideal model for investigating endocrine therapy resistance. This pilot study was one of the first to use multiple biopsies from the same patient, allowing for an assessment of changes in gene expression rather than static expression levels at a given time point. By comparing on-treatment with pretreatment in the same patient, this valuable approach has allowed for identification of key genes which are consistently changed over a number of patients as a direct result of a given therapy. While the design of this study is novel and may have potential for determining key predictive genes associated with sensitivity or resistance to endocrine therapy, several limitations are apparent. Firstly, the study is underpowered with only 34 patients, and no independent validation was performed to confirm the findings. Furthermore, given that the study was based on only 14 days of treatment prior to surgery, clinical response could not be directly evaluated, as changes in tumour size take longer than 14 days to manifest. Instead, changes in immunohistochemical Ki67 were used as a surrogate for clinical response, which despite correlation with both clinical and pathological response, has been shown to be a suboptimal predictor of response due to discordancy in some patients. As a result, there is currently no conclusive evidence demonstrating the predictive capacity of this signature.

In 2007, Miller et al. [97] published a similar study designed to investigate changes in gene expression associated with short-term neoadjuvant letrozole therapy also using pretreatment and on-treatment (14 days) biopsies from the same patient. From gene expression profiling of 58 patients, 143 genes were identified which were consistently changed between pretreatment and 14 days biopsies. Using the most significantly and consistently changed genes, patients were stratified into 4 distinct molecular groups by hierarchical clustering, reinforcing the concept of heterogeneity of response [97]. However, the clinical significance of the molecular subgroups remains to be seen. The molecular changes observed in both Mackay et al. [309] and Miller et al. [97] studies were largely consistent, with aromatase inhibitor treatment leading to suppressed expression of genes associated with proliferation and estrogenic signalling and increased expression of genes involved with stromal remodelling, cell adhesion, and immune response [2]. Importantly, these changes were identifiable within two weeks of treatment, long before clinical changes and pathological changes in morphology could be seen, raising the potential for determining early-on-treatment which patients are likely to respond to and adapting therapeutic protocols in resistant patients.

In 2008, Harvell et al. [310, 311] published one of the first gene expression signatures reported to discriminate between tumours clinically responsive and non-responsive to endocrine therapy, which used clinical response after extended therapy (4 months) as the end-point criteria. They published a 50 gene signature which was later refined to a 25 gene signature based on pretreatment gene expression levels. However, the signature was based on expression profiles from only 6 patients, (3 responsive and 3 non-responsive), rendering the study significantly underpowered. In addition, no independent validation of their findings was carried out.

In 2009, Miller et al. [74] published the largest study of its type at the time in which they presented a gene expression signature reported to discriminate between tumours clinically responsive and nonresponsive to aromatase inhibitors. Findings were based on the same microarray gene expression dataset used in their 2007 publication [97]. Neoadjuvant clinical response to letrozole in 58 patients was evaluated by changes in 3D ultrasound measurements taken over a 3 month treatment period (immediately prior to surgery), giving rise to 37 responding tumours and 15 showing lack of response. Gene expression analysis revealed 205 covariables consistently differentially expressed between clinically responding and resistant tumours, which distinguished between the two response groups. Of the 205 genes, 69 were differentially expressed in pretreatment samples, 45 were differentially expressed in 14-day samples, and 91 were significantly different when considering expression changes between pretreatment and 14-day samples. Hierarchical clustering based on 205 genes separated clinically responding and resistant tumours into two distinct groups. The most predictive genes were found to be associated with protein biosynthesis, in particular, ribosomal proteins. Interestingly, changes in proliferation associated genes and estrogenic signalling genes were found to occur in both clinically responding and nonresponding cases [74]. The major limitation of this study is the lack of validation of predictive capacity in an independent cohort of patients which as yet has not been reported. In addition, the assessment of clinical response (at least 50% reduction in tumour volume by 3 months) was an arbitrary threshold and does not allow for a satisfactory and clear differentiation between clinically responsive and resistant tumours; for example, a tumour with a reduction of 51% would be classed as clinically responding whereas a tumour with a 50% reduction would be resistant.

8.7. Considerations When Developing Predictive Gene Signatures

8.7.1. Sample Size and Patient Heterogeneity. A number of multigene signatures have been reported to predict subgroups of ER-positive patients unlikely to respond to endocrine therapy. However, many of these studies are underpowered, with findings based on relatively small numbers of patients in the training set and as a result may not be representative of the population [312]. When considering gene signatures derived from a single time point, such as before treatment, this problem may be confounded by patient heterogeneity (variables such as age, BMI, tumour size, tumour grade, tumour histological subtype, lymph node involvement, metastasis, additional medical conditions or drug regimens, and inherent genetic differences). By considering consistent changes in gene expression between sequential biopsies (before and after/during treatment) from the same patient in a pairwise fashion, the issues of patient heterogeneity can be somewhat minimised and significantly changed genes are more likely to be directly related to treatment response. Nevertheless, for statistical validity it is desirable to recruit the largest number and most representative patients as possible.

8.7.2. Microarray Bias. The predictive capacity of many reported signatures is often not reproducible in external datasets [258]. This can be in part due to the small unrepresentative sample size in the original training set, although, it can also be related to issues of microarray bias. Several studies have alluded to microarray bias as a major contributory factor affecting the reproducibility of microarray data derived results [258, 312, 313]. Bias can occur at all stages of microarray experiments from patient selection and sample processing to choice of microarray platform. Bias of this nature can lead to results becoming dataset-specific. Some studies have reported methods which can lead to a reduction in microarray bias, which have been shown to significantly improve reproducibility [312, 313].

8.7.3. Independent Validation. A major limitation of published predictive gene signatures is a lack of independent validation. This is essential to confirm that the reported findings are real, strongly associated with clinical outcome and that they out-perform or improve upon currently clinically used predictors of response. Successful validation is an essential first step if any new predictive signatures are to be endorsed in the clinic to improve patient care [257]. Furthermore, most predictive signatures are derived from microarray data, which should be considered a surrogate for gene expression, implying a further need for validation of results in external independent datasets and by alternative methods for measuring gene expression such as qRT-PCR. Independent validation is often carried out using publically available external datasets which may or may not be similar in design to the pilot study. Important factors for consideration when selecting an appropriate validation study for endocrine therapy response might include: the patient cohort (age range, BMI range, and menopausal status), disease features of the cohort (tumour size, spread to nodes, metastasis, and ER/PR/HER2 status), treatment (drug, length of treatment window), and assessment criteria used for response. With this in mind, it should be noted that, while novel experimental designs, such as gene expression profiling of multiple biopsies taken from the same patients over a period of treatment, are promising and may yield important information, a lack of similar external experimental datasets with which to validate findings is likely.

8.7.4. Response Criteria. Predictive multigene signatures for neoadjuvant response to therapy are designed by comparing differences in gene expression, or differences in changed gene expression, between tumours defined as responsive or nonresponsive. The predictive capacity of such signatures is highly dependent on the criteria used for response assessment. Such criteria include clinical, pathological, and molecular assessment of response and, while these show reasonable concordance, they do not agree for every case. Therefore, if the aim of a neoadjuvant predictive signature is based primarily on the clinical need to reduce tumour size sufficiently to allow either surgery if the cancer was inoperable or breast conservation if it was operable only by mastectomy, then it would seem logical to use clinical response assessment (as determined by changes in periodic 3D ultrasound over the treatment period) as the primary end point [2]. However, pathological and proliferative responses do represent useful secondary end points. Nevertheless, it should be noted that using proliferation as an end-point might lead only to identification of proliferation associated genes, which have been indicated as being poor predictors of clinical response to aromatase inhibitors [74]. If clinical response (changes in tumour size or volume) is to be used as the main end-point, then attention must be given to the cut-offs applied for each response group. Rather than applying a single arbitrary cut-off where all tumours above a certain value are classed as resistant and all below this value are responsive, it may be beneficial to design a predictive signature based on 2 well characterised groups of tumours that respond well or not at all. For example, tumours which reduce by a least 70% by 3 months would be classed as responsive, while tumours which increase or decrease by no more than 50% by 3 months would be classed as nonresponsive; all tumours in-between (intermediate clinical response) could be excluded from the development of a predictive signature design. This approach may lead to the identification of a predictive signature that has greater power to differentiate between responsive and nonresponsive tumours. It may also be able to subsequently stratify the intermediate clinical response group into clinically relevant responsive or nonresponsive subgroups, ultimately identifying which individual patients are likely to benefit from alternative or combination therapy.

8.7.5. Heterogeneity in the Resistant Patients. Previous studies have alluded to heterogeneity within the clinically resistant group despite a similar clinical response to treatment [314]. However, predictive multigene signatures for endocrine therapy in ER-positive patients to-date have failed to take this heterogeneity into account, instead considering all clinically resistant cases as belonging to the same molecular group. For this reason, predictive capability is likely to be dataset-specific, with reproducibility highly dependent on the frequency and distribution of distinct molecular subtypes within validation datasets compared with the training set. The exploration and characterisation of distinct molecular subgroups within the clinically resistant patients may be an important consideration in the development of signatures with greater predictive accuracy and reproducibility. Indeed, it should be noted that the accurate elucidation of distinct molecular subtypes will doubtless require a large sample number, once again reinforcing the importance of this factor in the experimental design.

8.7.6. Clinical Application of a Predictive Signature. The application of gene expression technology in the field of breast cancer research has yielded much important information and has vastly improved understanding of the disease at a molecular level [258]. However, there are a number of important considerations to address before it can be readily applied in a clinical setting to aid diagnostic and treatment decisions [257]. Foremost, a predictive signature must demonstrate clear clinical benefit for patients. Furthermore, it must be

shown, ideally with prospective independent validation, to out-perform or improve upon currently used parameters for guiding clinical decisions. In addition, thought must be given to the specific technology chosen for the assay and to its implementation. Technologies range from expensive high-throughput commercial or custom microarray platforms to low cost lower-throughput qRT-PCR assays. While most predictive signatures are developed from microarray data, there may be potential to convert resultant signatures to lower cost technologies such as qRT-PCR as was the case with Oncotype DX and Map*Quant* DX [187, 260, 270, 273]. Another benefit of qRT-PCR is that the assay can be effectively performed on formalin-fixed paraffin embedded (FFPE) tissue with greater ease than with microarray systems. Importantly, FFPE tissue is far easier to collect, store, and work with than fresh frozen tissue. A number of factors can affect the choice of technology: firstly, costs can be prohibitive and studies must be conducted to assess the cost-to-benefit ratio [257]; secondly, the number of genes included in the assay is a point for consideration, as large numbers of genes may preclude the application of lower-throughput technologies such as qRT-PCR. There is also a known issue with reproducibility; microarray bias can significantly impact the reproducibility of results and can manifest from differing technical variables (platforms, RNA extraction, processing, and hybridisation techniques) used across different sites [312]. It may be possible to minimise bias by instigating a strict common protocol for implementation of a predictive assay at different sites. However, commercial enterprises including Oncotype DX and Mammaprint address the issue by only offering their assay at one controlled site using the same validated technology and delivered by the same technicians.

9. Combating Resistance

A number of treatment strategies have been used clinically and assessed in trials to counteract endocrine therapy resistance in breast cancer, including alternating or combining agents [315–317] (Figure 3). While combining tamoxifen and aromatase inhibitors simultaneously does not appear of benefit, patients resistant to tamoxifen have been shown to respond when treatment was switched to an aromatase inhibitor [1]. A number of studies have also assessed the combination of endocrine therapy with the selective estrogen receptor downregulator, fulvestrant. One study combining fulvestrant with anastrozole reported an improved overall survival of 6 months [318]. However, this study included a primary endocrine therapy-naïve cohort of patients treated with this combination as a first line therapy and the results suggested that the benefit was limited to this group alone. The FACT study was a randomised trial of anastrozole with or without fulvestrant for patients pretreated with endocrine therapy and they reported no benefit from the combined therapy [319]. However, it should be noted that the results of the CONFIRM trial which set out to compare 500 mg fulvestrant with 250 mg in women who progressed after previous endocrine therapy suggested that the higher dose was associated with statistically significant increases in progression-free survival (PFS) and no increase in toxicity, a finding which brings into question the conclusions of the previous trials in which not enough drug was being administered [320].

Another approach has involved augmenting standard endocrine therapy with agents designed to resensitise resistant tumours to endocrine therapy by targeting pathways and molecules recognised as drivers of resistance. One such approach has been the combination of endocrine therapies with HER2-targeted therapies such as trastuzumab and lapatinib, which have shown some promise in endocrine therapy resistant cancers which overexpress HER2 [321, 322]. A number of studies have also shown that the use of PI3K-AKT-mTOR pathway targeted therapies such as the mTOR inhibitor everolimus and EGFR inhibitor gefitinib can reverse the PI3K-AKT-mTOR mediated resistance to endocrine therapy when used in combination with endocrine therapy [321, 323–327]. Indeed, everolimus is now in wide use in combination with exemestane (a steroidal aromatase inhibitor) after being shown to improve PFS in second or third line treatment of patients with ER-positive metastatic breast cancer [163, 328, 329]. A recent study also reported benefit from everolimus plus fulvestrant in tamoxifen resistant ER-positive metastatic breast cancer [330].

Lab-based studies have also yielded some promising results. One cell line study revealed that cells resistant to tamoxifen can be resensitised to its growth-inhibitory effects by targeting and blocking the action of the NFκB pathway [165]. Another group suggested that by inhibiting O-6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein they identified as being overexpressed in some tamoxifen resistant cell lines, sensitivity to tamoxifen could be restored [331]. Another reported that a CDK2 inhibitor could reverse endocrine therapy resistance in tumours over-expressing cyclins-E1 and E2 [104].

A novel approach involved the withdrawal of endocrine therapy. Cell line studies have provided evidence for the growth-inhibitory effects of withdrawal from endocrine therapy, although there is little clinical evidence to support this. One group reported a clinical trial which suggested that resistance to endocrine therapy could be minimised by intercalating therapy with periods of withdrawal [332]. A further treatment option was suggested in which low-dose estrogen is intercalated with aromatase inhibitors [333]. Indeed, before the development of drugs such as tamoxifen, high-dose estrogen represented a major therapy for the treatment of hormone-dependent breast cancer. It was thought to work by inducing apoptosis via extrinsic Fas/Fas ligand and intrinsic mitochondrial pathways [334]. Another study reported that the apoptosis inducing action of estrogen involves endoplasmic reticulum stress response and inflammatory response genes [335]. One group demonstrated in hormone-dependent xenograft models that loss of response to letrozole was accompanied by upregulation of HER2 and MAPK pathways and downregulation of ER and aromatase activity, which was reversed by replacing aromatase inhibitors with low dose estrogen treatment for a short period of time, thus resensitising the cells to estrogen and hence aromatase inhibition [333]. The major problem with these lab based cell

line studies is that they take no account of the wide range of cancers seen in clinical practice. Their value at best is to provide leads for pathways or mechanisms of resistance that might be targeted.

10. Conclusions

A wide range of distinct mechanisms have been described that have been implicated in endocrine therapy resistance in breast cancer including lower levels and heterogeneity of ER expression, posttranslational modifications and differential binding of ER, ER-independent signalling including EGF, HER2, and PI3K-AKT-mTOR pathways, and NFκB signalling, as well as phenomena such as stem cells, EMT, epigenetics, estrogen hypersensitivity, and autophagocytosis. Together, these findings suggest that resistance to endocrine therapy is complex and heterogeneous and may differ from patient to patient, between primary and acquired resistance and even between endocrine therapy types. Improved understanding of the underlying mechanisms will significantly aid the development of new therapeutic strategies including novel drug targets with which to combat resistance. The majority of trials thus far have focused on combining or alternating endocrine therapy agents, intercalating treatment with PI3K-AKT-mTOR, EGF, or HER2 pathway targeted therapy or the addition of chemotherapy. Some trials have shown promise; however, it seems clear that given the heterogeneity of resistance mechanisms and the toxicity and side effects associated with some alternative treatments, biomarker selection to stratify patients into clinically meaningful high and low risk groups in a move toward personalised therapy will be a crucial part of successfully combatting resistance to endocrine therapy in the ER-positive population.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

References

[1] S. R. D. Johnston and M. Dowsett, "Aromatase inhibitors for breast cancer: lessons from the laboratory," *Nature Reviews Cancer*, vol. 3, no. 11, pp. 821–831, 2003.

[2] A. A. Larionov and W. R. Miller, "Challenges in defining predictive markers for response to endocrine therapy in breast cancer," *Future Oncology*, vol. 5, no. 9, pp. 1415–1428, 2009.

[3] M. Smuk and J. Schwers, "Aromatization of androstenedione by human adult liver in vitro," *Journal of Clinical Endocrinology and Metabolism*, vol. 45, no. 5, pp. 1009–1012, 1977.

[4] E. R. Simpson and M. Dowsett, "Aromatase and its inhibitors: significance for breast cancer therapy," *Recent Progress in Hormone Research*, vol. 57, pp. 317–338, 2002.

[5] A. A. Larionov, D. A. Vasyliev, J. I. Mason, A. F. Howie, L. M. Berstein, and W. R. Miller, "Aromatase in skeletal muscle," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 84, no. 4, pp. 485–492, 2003.

[6] E. Perel and D. W. Killinger, "The interconversion and aromatization of androgens by human adipose tissue," *Journal of Steroid Biochemistry*, vol. 10, no. 6, pp. 623–627, 1979.

[7] C. Longcope, J. H. Pratt, S. H. Schneider, and S. E. Fineberg, "Aromatization of androgens by muscle and adipose tissue in vivo," *Journal of Clinical Endocrinology and Metabolism*, vol. 46, no. 1, pp. 146–152, 1978.

[8] H. Sasano, M. Uzuki, T. Sawai et al., "Aromatase in human bone tissue," *Journal of Bone and Mineral Research*, vol. 12, no. 9, pp. 1416–1423, 1997.

[9] W. R. Miller and A. P. M. Forrest, "Oestradiol synthesis by a human breast carcinoma," *The Lancet*, vol. 2, no. 7885, pp. 866–868, 1974.

[10] J. R. Pasqualini, G. Chetrite, C. Blacker et al., "Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 4, pp. 1460–1464, 1996.

[11] T. J. Key, "Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies," *Journal of the National Cancer Institute*, vol. 94, no. 8, pp. 606–616, 2002.

[12] W. R. Miller and J. O'Neill, "The importance of local synthesis of estrogen within the breast," *Steroids*, vol. 50, no. 4–6, pp. 537–548, 1987.

[13] S. E. Bulun, G. Sharda, J. Rink, S. Sharma, and E. R. Simpson, "Distribution of aromatase p450 transcripts and adipose fibroblasts in the human breast," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 3, pp. 1273–1277, 1996.

[14] N. Harada, T. Utsumi, and Y. Takagi, "Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 23, pp. 11312–11316, 1993.

[15] M. P. Schrey and K. V. Patel, "Prostaglandin E2 production and metabolism in human breast cancer cells and breast fibroblasts. Regulation by inflammatory mediators," *British Journal of Cancer*, vol. 72, no. 6, pp. 1412–1419, 1995.

[16] K. B. Horwitz, T. A. Jackson, D. L. Bain, J. K. Richer, G. S. Takimoto, and L. Tung, "Nuclear receptor coactivators and corepressors," *Molecular Endocrinology*, vol. 10, no. 10, pp. 1167–1177, 1996.

[17] V. Kumar, S. Green, G. Stack, M. Berry, J. Jin, and P. Chambon, "Functional domains of the human estrogen receptor," *Cell*, vol. 51, no. 6, pp. 941–951, 1987.

[18] M. Beato, "Gene regulation by steroid hormones," *Cell*, vol. 56, no. 3, pp. 335–344, 1989.

[19] M. Tsai and B. W. O'Malley, "Molecular mechanisms of action of steroid/thyroid receptor superfamily members," *Annual Review of Biochemistry*, vol. 63, pp. 451–486, 1994.

[20] S. Kato, H. Endoh, Y. Masuhiro et al., "Activation of the estrogen receptor through phosphorylation by mitoqen-activated protein kinase," *Science*, vol. 270, no. 5241, pp. 1491–1494, 1995.

[21] A. E. Wakeling, "Similarities and distinctions in the mode of action of different classes of antioestrogens," *Endocrine-Related Cancer*, vol. 7, no. 1, pp. 17–28, 2000.

[22] G. Reid, M. R. Hübner, R. Métivier et al., "Cyclic, proteasome-mediated turnover of unliganded and liganded ERα on responsive promoters is an integral feature of estrogen signaling," *Molecular Cell*, vol. 11, no. 3, pp. 695–707, 2003.

[23] V. Bourdeau, J. Deschênes, R. Métivier et al., "Genome-wide identification of high-affinity estrogen response elements in human and mouse," *Molecular Endocrinology*, vol. 18, no. 6, pp. 1411–1427, 2004.

[24] L. Klein-Hitpass, G. U. Ryffel, E. Heitlinger, and A. C. B. Cato, "A 13 bp palindrome is a functional estrogen responsive element and interacts specifically with estrogen receptor," *Nucleic Acids Research*, vol. 16, no. 2, pp. 647–663, 1988.

[25] S. Kamalakaran, S. K. Radhakrishnan, and W. T. Beck, "Identification of estrogen-responsive genes using a genome-wide analysis of promoter elements for transcription factor binding sites," *The Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21491–21497, 2005.

[26] C. J. Gruber, D. M. Gruber, I. M. L. Gruber, F. Wieser, and J. C. Huber, "Anatomy of the estrogen response element," *Trends in Endocrinology & Metabolism*, vol. 15, no. 2, pp. 73–78, 2004.

[27] A. Scholz, M. Truss, and M. Beato, "Hormone-induced recruitment of Sp1 mediates estrogen activation of the rabbit uteroglobin gene in endometrial epithelium," *The Journal of Biological Chemistry*, vol. 273, no. 8, pp. 4360–4366, 1998.

[28] C. M. Klinge, "Estrogen receptor interaction with estrogen response elements," *Nucleic Acids Research*, vol. 29, no. 14, pp. 2905–2919, 2001.

[29] M. Brown and P. A. Sharp, "Human estrogen receptor forms multiple protein-DNA complexes," *Journal of Biological Chemistry*, vol. 265, no. 19, pp. 11238–11243, 1990.

[30] J. A. Lees, S. E. Fawell, and M. G. Parker, "Identification of two transactivation domains in the mouse oestrogen receptor," *Nucleic Acids Research*, vol. 17, no. 14, pp. 5477–5488, 1989.

[31] V. Kumar and P. Chambon, "The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer," *Cell*, vol. 55, no. 1, pp. 145–156, 1988.

[32] L. Caizzi, G. Ferrero, S. Cutrupi et al., "Genome-wide activity of unliganded estrogen receptor-alpha in breast cancer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 13, pp. 4892–4897, 2014.

[33] E. A. Musgrove and R. L. Sutherland, "Biological determinants of endocrine resistance in breast cancer," *Nature Reviews Cancer*, vol. 9, no. 9, pp. 631–643, 2009.

[34] R. B. Clarke, A. Howell, C. S. Potten, and E. Anderson, "Dissociation between steroid receptor expression and cell proliferation in the human breast," *Cancer Research*, vol. 57, no. 22, pp. 4987–4991, 1997.

[35] O. W. J. Prall, B. Sarcevic, E. A. Musgrove, C. K. W. Watts, and R. L. Sutherland, "Estrogen-induced activation of Cdk4 and Cdk2 during G 1-S phase progression is accompanied by increased cyclin D1 expression and decreased cyclin-dependent kinase inhibitor association with cyclin E-Cdk2," *Journal of Biological Chemistry*, vol. 272, no. 16, pp. 10882–10894, 1997.

[36] J. S. Foster, D. C. Henley, A. Bukovsky, P. Seth, and J. Wimalasena, "Multifaceted regulation of cell cycle progression by estrogen: regulation of Cdk inhibitors and Cdc25A independent of cyclin D1-Cdk4 function," *Molecular and Cellular Biology*, vol. 21, no. 3, pp. 794–810, 2001.

[37] M. Sabbah, D. Courilleau, J. Mester, and G. Redeuilh, "Estrogen induction of the cyclin D1 promoter: involvement of a cAMP response-like element," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 20, pp. 11217–11222, 1999.

[38] M. M. Liu, C. Albanese, C. M. Anderson et al., "Opposing action of estrogen receptors α and β on cyclin D1 gene expression," *The ournal of Biological Chemistry*, vol. 277, no. 27, pp. 24353–24360, 2002.

[39] L. Cicatiello, R. Addeo, A. Sasso et al., "Estrogens and progesterone promote persistent CCND1 gene activation during G1 by inducing transcriptional derepression via c-Jun/c-Fos/estrogen receptor (progesterone receptor) complex assembly to a distal regulatory element and recruitment of cyclin D1 to its own gene promoter," *Molecular and Cellular Biology*, vol. 24, no. 16, pp. 7260–7274, 2004.

[40] S. Safe, "Transcriptional activation of genes by 17β-estradiol through estrogen receptor-Sp1 interactions," *Vitamins and Hormones*, vol. 62, pp. 231–252, 2001.

[41] P. Webb, G. N. Lopez, R. M. Uht, and P. J. Kushner, "Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens," *Molecular Endocrinology*, vol. 9, no. 4, pp. 443–456, 1995.

[42] E. Neuman, M. H. Ladha, N. Lin et al., "Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4," *Molecular and Cellular Biology*, vol. 17, no. 9, pp. 5338–5347, 1997.

[43] M. Meyerson and E. Harlow, "Identification of G1 kinase activity for cdk6, a novel cyclin D partner," *Molecular and Cellular Biology*, vol. 14, no. 3, pp. 2077–2086, 1994.

[44] C. E. Caldon, R. J. Daly, R. L. Sutherland, and E. A. Musgrove, "Cell cycle control in breast cancer cells," *Journal of Cellular Biochemistry*, vol. 97, no. 2, pp. 261–274, 2006.

[45] D. Dubik and R. P. C. Shiu, "Mechanism of estrogen activation of c-myc oncogene expression," *Oncogene*, vol. 7, no. 8, pp. 1587–1594, 1992.

[46] K. Galaktionov, X. Chen, and D. Beach, "Cdc25 cell-cycle phosphatase as a target of c-myc," *Nature*, vol. 382, no. 6591, pp. 511–517, 1996.

[47] H. Hermeking, C. Rago, M. Schuhmacher et al., "Identification of *CDK4* as a target of c-MYC," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 5, pp. 2229–2234, 2000.

[48] S. Lacy and P. Whyte, "Identification of a p130 domain mediating interactions with cyclin A/cdk 2 and cyclin E/cdk 2 complexes," *Oncogene*, vol. 14, no. 20, pp. 2395–2406, 1997.

[49] O. W. J. Prall, E. M. Rogan, E. A. Musgrove, C. K. W. Watts, and R. L. Sutherland, "c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry," *Molecular and Cellular Biology*, vol. 18, no. 8, pp. 4499–4508, 1998.

[50] M. D. Planas-Silva and R. A. Weinberg, "Estrogen-dependent cyclin E-cdk2 activation through p21 redistribution," *Molecular and Cellular Biology*, vol. 17, no. 7, pp. 4059–4069, 1997.

[51] J. S. Foster, R. I. Fernando, N. Ishida, K. I. Nakayama, and J. Wimalasena, "Estrogens down-regulate p27Kip1 in breast cancer cells through Skp2 and through nuclear export mediated by the ERK pathway," *Journal of Biological Chemistry*, vol. 278, no. 42, pp. 41355–41366, 2003.

[52] G. Castoria, A. Migliaccio, M. di Domenico et al., "Role of atypical protein kinase C in estradiol-triggered G_1/S progression of MCF-7 cells," *Molecular and Cellular Biology*, vol. 24, no. 17, pp. 7643–7653, 2004.

[53] A. Haddow, J. M. Watkinson, E. Paterson, and P. C. Koller, "Influence of synthetic oestrogens on advanced malignant disease," *British Medical Journal*, vol. 2, no. 4368, pp. 393–398, 1944.

[54] V. C. Jordan, "Tamoxifen: a most unlikely pioneering medicine," *Nature Reviews Drug Discovery*, vol. 2, no. 3, pp. 205–213, 2003.

[55] P. Schick, J. Goodstein, J. Moor, J. Butler, and K. L. Senter, "Preoperative chemotherapy followed by mastectomy for locally advanced breast cancer," *Journal of Surgical Oncology*, vol. 22, no. 4, pp. 278–282, 1983.

[56] M. Kaufmann, G. N. Hortobagyi, and A. Goldhirsch, "Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update," *Journal of Clinical Oncology*, vol. 24, no. 19, p. 3221, 2006.

[57] V. F. Semiglazov, V. V. Semiglazov, G. A. Dashyan et al., "Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer," *Cancer*, vol. 110, no. 2, pp. 244–254, 2007.

[58] M. Perloff and C. J. Lesnick, "Chemotherapy before and after mastectomy in stage III breast cancer," *Archives of Surgery*, vol. 117, no. 7, pp. 879–881, 1982.

[59] M. Colleoni, S. Gelber, A. S. Coates et al., "Influence of endocrine-related factors on response to perioperative chemotherapy for patients with node-negative breast cancer," *Journal of Clinical Oncology*, vol. 19, no. 21, pp. 4141–4149, 2001.

[60] L. Gianni, J. Baselga, W. Eiermann et al., "Feasibility and tolerability of sequential doxorubicin/paclitaxel followed by cyclophosphamide, methotrexate, and fluorouracil and its effects on tumor response as preoperative therapy," *Clinical Cancer Research*, vol. 11, no. 24, part 1, pp. 8715–8721, 2005.

[61] M. Dowsett, I. E. Smith, S. R. Ebbs et al., "Proliferation and apoptosis as markers of benefit in neoadjuvant endocrine therapy of breast cancer," *Clinical Cancer Research*, vol. 12, no. 3, part 2, pp. 1024s–1030s, 2006.

[62] S. F. Doisneau-Sixou, C. M. Sergio, J. S. Carroll, R. Hui, E. A. Musgrove, and R. L. Sutherland, "Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells," *Endocrine-Related Cancer*, vol. 10, no. 2, pp. 179–186, 2003.

[63] A. K. Shiau, D. Barstad, P. M. Loria et al., "The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen," *Cell*, vol. 95, no. 7, pp. 927–937, 1998.

[64] C. M. Klinge, S. C. Jernigan, S. L. Smith, V. V. Tyulmenkov, and P. C. Kulakosky, "Estrogen response element sequence impacts the conformation and transcriptional activity of estrogen receptor α," *Molecular and Cellular Endocrinology*, vol. 174, no. 1-2, pp. 151–166, 2001.

[65] X. Long and K. P. Nephew, "Fulvestrant (ICI 182,780)-dependent interacting proteins mediate immobilization and degradation of estrogen receptor-α," *Journal of Biological Chemistry*, vol. 281, no. 14, pp. 9607–9615, 2006.

[66] A. Howell, J. F. R. Robertson, P. Abram et al., "Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial," *Journal of Clinical Oncology*, vol. 22, no. 9, pp. 1605–1613, 2004.

[67] A. U. Buzdar, "Fulvestrant—a novel estrogen receptor antagonist for the treatment of advanced breast cancer," *Drugs of Today*, vol. 44, no. 9, pp. 679–692, 2008.

[68] P. E. Goss, J. N. Ingle, S. Martino et al., "A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer," *The New England Journal of Medicine*, vol. 349, no. 19, pp. 1793–1802, 2003.

[69] A. U. Buzdar, "Aromatase inhibitors: changing the face of endocrine therapy for breast cancer," *Breast Disease*, vol. 24, no. 1, pp. 107–117, 2005.

[70] Y. Kao, L. L. Cam, C. A. Laughton, D. Zhou, and S. Chen, "Binding characteristics of seven inhibitors of human aromatase: a site- directed mutagenesis study," *Cancer Research*, vol. 56, no. 15, pp. 3451–3460, 1996.

[71] R. Carpenter and W. R. Miller, "Role of aromatase inhibitors in breast cancer," *British Journal of Cancer*, vol. 93, supplement 1, pp. S1–S5, 2005.

[72] R. C. Coombes, E. Hall, L. J. Gibson et al., "A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer," *The New England Journal of Medicine*, vol. 350, no. 11, pp. 1081–1092, 2004.

[73] I. E. Smith and M. Dowsett, "Aromatase inhibitors in breast cancer," *The New England Journal of Medicine*, vol. 348, no. 24, pp. 2431–2442, 2003.

[74] W. R. Miller, A. Larionov, L. Renshaw et al., "Gene expression profiles differentiating between breast cancers clinically responsive or resistant to letrozole," *Journal of Clinical Oncology*, vol. 27, no. 9, pp. 1382–1387, 2009.

[75] A. Howell and M. Dowsett, "Endocrinology and hormone therapy in breast cancer: aromatase inhibitors versus antioestrogens," *Breast Cancer Research*, vol. 6, no. 6, pp. 269–274, 2004.

[76] W. R. Miller and J. Jackson, "The therapeutic potential of aromatase inhibitors," *Expert Opinion on Investigational Drugs*, vol. 12, no. 3, pp. 337–351, 2003.

[77] Early Breast Cancer Trialists' Collaborative Group, "Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials," *The Lancet*, vol. 365, no. 9472, pp. 1687–1717, 2005.

[78] A. Brodie, L. MacEdo, and G. Sabnis, "Aromatase resistance mechanisms in model systems *in vivo*," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 118, no. 4-5, pp. 283–287, 2010.

[79] M. J. Ellis, V. J. Suman, J. Hoog et al., "Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype—ACOSOG Z1031," *Journal of Clinical Oncology*, vol. 29, no. 17, pp. 2342–2349, 2011.

[80] R. Bartsch, Z. Bago-Horvath, A. Berghoff et al., "Ovarian function suppression and fulvestrant as endocrine therapy in premenopausal women with metastatic breast cancer," *European Journal of Cancer*, vol. 48, no. 13, pp. 1932–1938, 2012.

[81] J. C. Doughty, "When to start an aromatase inhibitor: now or later?" *Journal of Surgical Oncology*, vol. 103, no. 7, pp. 730–738, 2011.

[82] I. E. Smith, M. Dowsett, S. R. Ebbs et al., "Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: The Immediate Preoperative Anastrozole, Tamoxifen, or Combined With Tamoxifen (IMPACT) multicenter double-blind randomized trial," *Journal of Clinical Oncology*, vol. 23, no. 22, pp. 5108–5116, 2005.

[83] G. Mustacchi, M. Mansutti, C. Sacco et al., "Neo-adjuvant exemestane in elderly patients with breast cancer: a phase II, multicentre, open-label, Italian study," *Annals of Oncology*, vol. 20, no. 4, pp. 655–659, 2009.

[84] M. J. Ellis, Y. Tao, O. Young et al., "Estrogen-independent proliferation is present in estrogen-receptor HER2-positive

primary breast cancer after neoadjuvant letrozole," *Journal of Clinical Oncology*, vol. 24, no. 19, pp. 3019–3025, 2006.

[85] R. Burcombe, G. D. Wilson, M. Dowsett et al., "Evaluation of Ki-67 proliferation and apoptotic index before, during and after neoadjuvant chemotherapy for primary breast cancer," *Breast Cancer Research*, vol. 8, no. 3, article R31, 2006.

[86] V. Guarneri and P. F. Conte, "The curability of breast cancer and the treatment of advanced disease," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 31, supplement 1, pp. S149–S161, 2004.

[87] A. M. Gonzalez-Angulo, F. Morales-Vasquez, and G. N. Hortobagyi, "Overview of resistance to systemic therapy in patients with breast cancer," *Advances in Experimental Medicine and Biology*, vol. 608, pp. 1–22, 2007.

[88] M. Dowsett, J. Cuzick, J. Ingle et al., "Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen," *Journal of Clinical Oncology*, vol. 28, no. 3, pp. 509–518, 2010.

[89] C. X. Ma, C. G. Sanchez, and M. J. Ellis, "Predicting endocrine therapy responsiveness in breast cancer," *Oncology*, vol. 23, no. 2, pp. 133–142, 2009.

[90] M. Colleoni and E. Montagna, "Neoadjuvant therapy for ER-positive breast cancers," *Annals of Oncology*, vol. 23, supplement 10, pp. x243–x248, 2012.

[91] Y. Takatsuka, E. Yayoi, H. Inaji, and T. Aikawa, "A comparison of two doses of tamoxifen in patients with advanced breast cancer: 20 mg/day versus 40 mg/day," *Gan To Kagaku Ryoho*, vol. 16, no. 5, pp. 2093–2097, 1989 (Japanese).

[92] A. Ring and M. Dowsett, "Mechanisms of tamoxifen resistance," *Endocrine-Related Cancer*, vol. 11, no. 4, pp. 643–658, 2004.

[93] S. P. Hasson, T. Rubinek, L. Ryvo, and I. Wolf, "Endocrine resistance in breast cancer: focus on the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin signaling pathway," *Breast Care*, vol. 8, no. 4, pp. 248–255, 2013.

[94] X. Sun, D. Zhou, and S. Chen, "Autocrine and paracrine actions of breast tumor aromatase. A three-dimensional cell culture study involving aromatase transfected MCF-7 and T-47D cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 63, no. 1–3, pp. 29–36, 1997.

[95] S. Masri, S. Phung, X. Wang et al., "Genome-wide analysis of aromatase inhibitor-resistant, tamoxifen-resistant, and long-term estrogen-deprived cells reveals a role for estrogen receptor," *Cancer Research*, vol. 68, no. 12, pp. 4910–4918, 2008.

[96] K. J. Taylor, A. H. Sims, L. Liang et al., "Dynamic changes in gene expression in vivo predict prognosis of tamoxifen-treated patients with breast cancer," *Breast Cancer Research*, vol. 12, no. 3, article R39, 2010.

[97] W. R. Miller, A. A. Larionov, L. Renshaw et al., "Changes in breast cancer transcriptional profiles after treatment with the aromatase inhibitor, letrozole," *Pharmacogenetics and Genomics*, vol. 17, no. 10, pp. 813–826, 2007.

[98] W. R. Miller, A. Larionov, L. Renshaw et al., "Aromatase inhibitors—gene discovery," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 106, no. 1–5, pp. 130–142, 2007.

[99] R. L. Sutherland and E. A. Musgrove, "Cyclins and breast cancer," *Journal of Mammary Gland Biology and Neoplasia*, vol. 9, no. 1, pp. 95–104, 2004.

[100] R. B. Riggins, A. H. Bouton, M. C. Liu, and R. Clarke, "Antiestrogens, aromatase inhibitors, and apoptosis in breast cancer," *Vitamins and Hormones*, vol. 71, pp. 201–237, 2005.

[101] S. Mandlekar and A.-N. T. Kong, "Mechanisms of tamoxifen-induced apoptosis," *Apoptosis*, vol. 6, no. 6, pp. 469–477, 2001.

[102] Y. Ishii, S. Waxman, and D. Germain, "Tamoxifen stimulates the growth of cyclin D1-overexpressing breast cancer cells by promoting the activation of signal transducer and activator of transcription 3," *Cancer Research*, vol. 68, no. 3, pp. 852–860, 2008.

[103] S. Akli, T. Bui, H. Wingate et al., "Low-molecular-weight cyclin E can bypass letrozole-induced G1 arrest in human breast cancer cells and tumors," *Clinical Cancer Research*, vol. 16, no. 4, pp. 1179–1190, 2010.

[104] C. E. Caldon, C. M. Sergio, J. Kang et al., "Cyclin E2 overexpression is associated with endocrine resistance but not insensitivity to CDK2 inhibition in human breast cancer cells," *Molecular Cancer Therapeutics*, vol. 11, no. 7, pp. 1488–1499, 2012.

[105] M. Venditti, B. Iwasiow, F. W. Orr, and R. P. C. Shiu, "C-myc gene expression alone is sufficient to confer resistance to antiestrogen in human breast cancer cells," *International Journal of Cancer*, vol. 99, no. 1, pp. 35–42, 2002.

[106] S. Cariou, J. C. H. Donovan, W. M. Flanagan, A. Milic, N. Bhattacharya, and J. M. Slingerland, "Down-regulation of p21WAF1/CIP1 or p27KiP1 abrogates antiestrogen-mediated cell cycle arrest in human breast cancer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 16, pp. 9042–9046, 2000.

[107] S. Sengupta, M. C. Biarnes, and V. C. Jordan, "Cyclin dependent kinase-9 mediated transcriptional de-regulation of cMYC as a critical determinant of endocrine-therapy resistance in breast cancers," *Breast Cancer Research and Treatment*, vol. 143, no. 1, pp. 113–124, 2014.

[108] N. Harbeck and A. Rody, "Lost in translation? Estrogen receptor status and endocrine responsiveness in breast cancer," *Journal of Clinical Oncology*, vol. 30, no. 7, pp. 686–689, 2012.

[109] T. Iwamoto, D. Booser, V. Valero et al., "Estrogen Receptor (ER) mRNA and ER-related gene expression in breast cancers that are 1% to 10% ER-positive by immunohistochemistry," *Journal of Clinical Oncology*, vol. 30, no. 7, pp. 729–734, 2012.

[110] L. K. Dunnwald, M. A. Rossing, and C. I. Li, "Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients," *Breast Cancer Research*, vol. 9, no. 1, article R6, 2007.

[111] A. Carracedo, M. Salido, J. M. Corominas et al., "Are ER+PR+ and ER+PR- breast tumors genetically different? A CGH array study," *Cancer Genetics*, vol. 205, no. 4, pp. 138–146, 2012.

[112] M. C. Diaz Flaque, N. M. Galigniana, W. Béguelin et al., "Progesterone receptor assembly of a transcriptional complex along with activator protein 1, signal transducer and activator of transcription 3 and ErbB-2 governs breast cancer growth and predicts response to endocrine therapy," *Breast Cancer Research*, vol. 15, no. 6, p. R118, 2013.

[113] A. E. Gururaj, S. K. Rayala, R. K. Vadlamudi et al., "Novel mechanisms of resistance to endocrine therapy: genomic and nongenomic considerations," *Clinical Cancer Research*, vol. 12, part 2, no. 3, pp. 1001s–1007s, 2006.

[114] V. C. Jordan, "Selective estrogen receptor modulation: concept and consequences in cancer," *Cancer Cell*, vol. 5, no. 3, pp. 207–213, 2004.

[115] J. M. Knowlden, I. R. Hutcheson, H. E. Jones et al., "Elevated levels of epidermal growth factor receptor/c-erbB2 heterodimers mediate an autocrine growth regulatory pathway in tamoxifen-resistant MCF-7 cells," *Endocrinology*, vol. 144, no. 3, pp. 1032–1044, 2003.

[116] R. A. McClelland, D. Barrow, T. Madden et al., "Enhanced epidermal growth factor receptor signaling in MCF7 breast cancer cells after long-term culture in the presence of the pure antiestrogen ICI 182,780 (Faslodex)," *Endocrinology*, vol. 142, no. 7, pp. 2776–2788, 2001.

[117] C. S. Ross-Innes, R. Stark, A. E. Teschendorff et al., "Differential oestrogen receptor binding is associated with clinical outcome in breast cancer," *Nature*, vol. 481, no. 7381, pp. 389–393, 2012.

[118] J. S. Carroll, X. S. Liu, A. S. Brodsky et al., "Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1," *Cell*, vol. 122, no. 1, pp. 33–43, 2005.

[119] M. Lupien, J. Eeckhoute, C. A. Meyer et al., "FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription," *Cell*, vol. 132, no. 6, pp. 958–970, 2008.

[120] A. Hurtado, K. A. Holmes, C. S. Ross-Innes, D. Schmidt, and J. S. Carroll, "FOXA1 is a key determinant of estrogen receptor function and endocrine response," *Nature Genetics*, vol. 43, no. 1, pp. 27–33, 2011.

[121] K. Merenbakh-Lamin, N. Ben-Baruch, A. Yeheskel et al., "D538G mutation in estrogen receptor-α: a novel mechanism for acquired endocrine resistance in breast cancer," *Cancer Research*, vol. 73, no. 23, pp. 6856–6864, 2013.

[122] D. R. Robinson, Y. M. Wu, P. Vats et al., "Activating ESR1 mutations in hormone-resistant metastatic breast cancer," *Nature Genetics*, vol. 45, no. 12, pp. 1446–1451, 2013.

[123] R. Jeselsohn, R. Yelensky, G. Buchwalter et al., "Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor-positive breast cancer," *Clinical Cancer Research*, vol. 20, no. 7, pp. 1757–1767, 2014.

[124] I. Barone, L. Brusco, and S. A. W. Fuqua, "Estrogen receptor mutations and changes in downstream gene expression and signaling," *Clinical Cancer Research*, vol. 16, no. 10, pp. 2702–2708, 2010.

[125] S. R. D. Johnston, G. Saccani-Jotti, I. E. Smith et al., "Changes in estrogen receptor, progesterone receptor, and pS2 expression in tamoxifen-resistant human breast cancer," *Cancer Research*, vol. 55, no. 15, pp. 3331–3338, 1995.

[126] M. C. Gutierrez, S. Detre, S. Johnston et al., "Molecular changes in tamoxifen-resistant breast cancer: relationship between estrogen receptor, HER-2, and p38 mitogen-activated protein kinase," *Journal of Clinical Oncology*, vol. 23, no. 11, pp. 2469–2476, 2005.

[127] R. Clarke, H. W. Ressom, A. Wang et al., "The properties of high-dimensional data spaces: implications for exploring gene and protein expression data," *Nature Reviews Cancer*, vol. 8, no. 1, pp. 37–49, 2008.

[128] M. H. Herynk and S. A. W. Fuqua, "Estrogen receptor mutations in human disease," *Endocrine Reviews*, vol. 25, no. 6, pp. 869–898, 2004.

[129] R. B. Riggins, R. S. Schrecengost, M. S. Guerrero, and A. H. Bouton, "Pathways to tamoxifen resistance," *Cancer Letters*, vol. 256, no. 1, pp. 1–24, 2007.

[130] G. Li, J. Zhang, K. Jin et al., "Estrogen receptor-α36 is involved in development of acquired tamoxifen resistance via regulating the growth status switch in breast cancer cells," *Molecular Oncology*, vol. 7, no. 3, pp. 611–624, 2013.

[131] M. M. Heckler, H. Thakor, C. C. Schafer, and R. B. Riggins, "ERK/MAPK regulates ERRgamma expression, transcriptional activity and receptor-mediated tamoxifen resistance in ER+ breast cancer," *FEBS Journal*, vol. 281, no. 10, pp. 2431–2442, 2014.

[132] D. Wang, P. Huang, B. Zhu, L. Sun, Q. Huang, and J. Wang, "Induction of estrogen receptor α-36 expression by bone morphogenetic protein 2 in breast cancer cell lines," *Molecular Medicine Reports*, vol. 6, no. 3, pp. 591–596, 2012.

[133] L. Shi, B. Dong, Z. Li et al., "Expression of ER-α 36, a novel variant of estrogen receptor α, and resistance to tamoxifen treatment in breast cancer," *Journal of Clinical Oncology*, vol. 27, no. 21, pp. 3423–3429, 2009.

[134] R. B. Riggins, J. P.-J. Lan, U. Klimach et al., "ERRγ mediates tamoxifen resistance in novel models of invasive lobular breast cancer," *Cancer Research*, vol. 68, no. 21, pp. 8908–8917, 2008.

[135] K. Paech, P. Webb, G. G. J. M. Kuiper et al., "Differential ligand activation of estrogen receptors ERα and ERrβ at AP1 sites," *Science*, vol. 277, no. 5331, pp. 1508–1510, 1997.

[136] P. J. Kushner, D. A. Agard, G. L. Greene et al., "Estrogen receptor pathways to AP-1," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 74, no. 5, pp. 311–317, 2000.

[137] V. Speirs, C. Malone, D. S. Walton, M. J. Kerin, and S. L. Atkin, "Increased expression of estrogen receptor β mRNA in tamoxifen-resistant breast cancer patients," *Cancer Research*, vol. 59, no. 21, pp. 5421–5424, 1999.

[138] M. Madeira, A. Mattar, A. F. Logullo, F. A. Soares, and L. H. Gebrim, "Estrogen receptor alpha/beta ratio and estrogen receptor beta as predictors of endocrine therapy responsiveness—a randomized neoadjuvant trial comparison between anastrozole and tamoxifen for the treatment of postmenopausal breast cancer," *BMC Cancer*, vol. 13, article 425, 2013.

[139] M. Lupien, C. A. Meyer, S. T. Bailey et al., "Growth factor stimulation induces a distinct ERα cistrome underlying breast cancer endocrine resistance," *Genes and Development*, vol. 24, no. 19, pp. 2219–2227, 2010.

[140] J. R. C. Sainsbury, J. R. Farndon, G. V. Sherbet, and A. L. Harris, "Epidermal-growth-factor receptors and oestrogen receptors in human breast cancer," *The Lancet*, vol. 1, no. 8425, pp. 364–366, 1985.

[141] M. Koga, E. A. Musgrove, and R. L. Sutherland, "Modulation of the growth-inhibitory effects of progestins and the antiestrogen hydroxyclomiphene on human breast cancer cells by epidermal growth factor and insulin," *Cancer Research*, vol. 49, no. 1, pp. 112–116, 1989.

[142] R. X.-D. Song, Y. Chen, Z. Zhang et al., "Estrogen utilization of IGF-1-R and EGF-R to signal in breast cancer cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 118, no. 4-5, pp. 219–230, 2010.

[143] J. Shou, S. Massarweh, C. K. Osborne et al., "Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer," *Journal of the National Cancer Institute*, vol. 96, no. 12, pp. 926–935, 2004.

[144] Z. Mo, M. Liu, F. Yang et al., "GPR30 as an initiator of tamoxifen resistance in hormone-dependent breast cancer," *Breast Cancer Research*, vol. 15, no. 6, article R114, 2013.

[145] O. Pontiggia, R. Sampayo, D. Raffo et al., "The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through β1 integrin," *Breast Cancer Research and Treatment*, vol. 133, no. 2, pp. 459–471, 2012.

[146] M. Rieber and M. Strasberg-Rieber, "p53 inactivation decreases dependence on estrogen/ERK signalling for proliferation but promotes EMT and susceptility to 3-bromopyruvate in ERα + breast cancer MCF-7 cells," *Biochemical Pharmacology*, vol. 88, no. 2, pp. 169–177, 2014.

[147] K. Lindberg, L. A. Helguero, Y. Omoto, J. Gustafsson, and L. Haldosén, "Estrogen receptor β represses Akt signaling in breast cancer cells via downregulation of HER2/HER3 and upregulation of PTEN: implications for tamoxifen sensitivity," *Breast Cancer Research*, vol. 13, no. 2, article R43, 2011.

[148] A. Hurtado, K. A. Holmes, T. R. Geistlinger et al., "Regulation of *ERBB2* by oestrogen receptor-PAX2 determines response to tamoxifen," *Nature*, vol. 456, no. 7222, pp. 663–666, 2008.

[149] D. Beauchemin, C. Lacombe, and C. van Themsche, "PAX2 is activated by estradiol in breast cancer cells of the luminal subgroup selectively, to confer a low invasive phenotype," *Molecular Cancer*, vol. 10, article 148, 2011.

[150] T. Zuo, L. Wang, C. Morrison et al., "FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene," *Cell*, vol. 129, no. 7, pp. 1275–1286, 2007.

[151] G. Hua, B. Zhu, F. Rosa et al., "A negative feedback regulatory loop associates the tyrosine kinase receptor ERBB2 and the transcription factor GATA4 in breast cancer cells," *Molecular Cancer Research*, vol. 7, no. 3, pp. 402–414, 2009.

[152] M. Whitman, C. P. Downes, M. Keeler, T. Keller, and L. Cantley, "Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate," *Nature*, vol. 332, no. 6165, pp. 644–646, 1988.

[153] K. E. Anderson, J. Coadwell, L. R. Stephens, and P. T. Hawkins, "Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B," *Current Biology*, vol. 8, no. 12, pp. 684–691, 1998.

[154] T. F. Franke, S. I. Yang, T. O. Chan et al., "The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase," *Cell*, vol. 81, no. 5, pp. 727–736, 1995.

[155] T. Maehama and J. E. Dixon, "The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate," *The Journal of Biological Chemistry*, vol. 273, no. 22, pp. 13375–13378, 1998.

[156] C. Gewinner, Z. C. Wang, A. Richardson et al., "Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling," *Cancer Cell*, vol. 16, no. 2, pp. 115–125, 2009.

[157] R. A. Campbell, P. Bhat-Nakshatri, N. M. Patel, D. Constantinidou, S. Ali, and H. Nakshatri, "Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor α: a new model for anti-estrogen resistance," *The Journal of Biological Chemistry*, vol. 276, no. 13, pp. 9817–9824, 2001.

[158] R. L. Yamnik, A. Digilova, D. C. Davis, Z. N. Brodt, C. J. Murphy, and M. K. Holz, "S6 kinase 1 regulates estrogen receptor α in control of breast cancer cell proliferation," *The Journal of Biological Chemistry*, vol. 284, no. 10, pp. 6361–6369, 2009.

[159] O. A. Coso, M. Chiarielio, J.-C. Yu et al., "The small GTP-binding proteins rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway," *Cell*, vol. 81, no. 7, pp. 1137–1146, 1995.

[160] A. Minden, A. Lin, F. Claret, A. Abo, and M. Karin, "Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPses Rac and Cdc42Hs," *Cell*, vol. 81, no. 7, pp. 1147–1157, 1995.

[161] L. N. Petz, Y. S. Ziegler, M. A. Loven, and A. M. Nardulli, "Estrogen receptor α and activating protein-1 mediate estrogen responsiveness of the progesterone receptor gene in MCF-7 breast cancer cells," *Endocrinology*, vol. 143, no. 12, pp. 4583–4591, 2002.

[162] T. W. Miller, M. Pérez-Torres, A. Narasanna et al., "Loss of phosphatase and tensin homologue deleted on chromosome 10 engages ErbB3 and insulin-like growth factor-I receptor signaling to promote antiestrogen resistance in breast cancer," *Cancer Research*, vol. 69, no. 10, pp. 4192–4201, 2009.

[163] T. W. Miller, J. M. Balko, and C. L. Arteaga, "Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer," *Journal of Clinical Oncology*, vol. 29, no. 33, pp. 4452–4461, 2011.

[164] R. R. Saleh, N. Bouganim, J. Hilton, A. Arnaout, and M. Clemons, "Neoadjuvant endocrine treatment for breast cancer: from bedside to bench and back again?" *Current Oncology*, vol. 21, no. 1, pp. e122–e128, 2014.

[165] C. W. Yde, K. B. Emdal, B. Guerra, and A. E. Lykkesfeldt, "NFκB signaling is important for growth of antiestrogen resistant breast cancer cells," *Breast Cancer Research and Treatment*, vol. 135, no. 1, pp. 67–78, 2012.

[166] H. Shen and V. Tergaonkar, "NFκB signaling in carcinogenesis and as a potential molecular target for cancer therapy," *Apoptosis*, vol. 14, no. 4, pp. 348–363, 2009.

[167] L. V. Madrid, M. W. Mayo, J. Y. Reuther, and A. S. Baldwin Jr., "Akt stimulates the transactivation potential of the RelA/p65 Subunit of NF-κ B through utilization of the Iκ B kinase and activation of the mitogen-activated protein kinase p38," *The Journal of Biological Chemistry*, vol. 276, no. 22, pp. 18934–18940, 2001.

[168] J. Frasor, A. Weaver, M. Pradhan et al., "Positive cross-talk between estrogen receptor and NF-κB in breast cancer," *Cancer Research*, vol. 69, no. 23, pp. 8918–8925, 2009.

[169] R. Nehra, R. B. Riggins, A. N. Shajahan, A. Zwart, A. C. Crawford, and R. Clarke, "BCL2 and CASP8 regulation by NF-κB differentially affect mitochondrial function and cell fate in antiestrogen-sensitive and -resistant breast cancer cells," *FASEB Journal*, vol. 24, no. 6, pp. 2040–2055, 2010.

[170] Y. Zhu, B. Singh, S. Hewitt et al., "Expression patterns among interferon regulatory factor-1, human X-box binding protein-1, nuclear factor kappa B, nucleophosmin, estrogen receptor-alpha and progesterone receptor proteins in breast cancer tissue microarrays," *International Journal of Oncology*, vol. 28, no. 1, pp. 67–76, 2006.

[171] Z. Gu, R. Y. Lee, T. C. Skaar et al., "Association of interferon regulatory factor-1, nucleophosmin, nuclear factor-κB, and cyclic AMP response element binding with acquired resistance to Faslodex (ICI 182,780)," *Cancer Research*, vol. 62, no. 12, pp. 3428–3437, 2002.

[172] Y. Zhou, C. Yau, J. W. Gray et al., "Enhanced NFκB and AP-1 transcriptional activity associated with antiestrogen resistant breast cancer," *BMC Cancer*, vol. 7, article 59, 2007.

[173] L. A. deGraffenried, B. Chandrasekar, W. E. Friedrichs et al., "NF-κB inhibition markedly enhances sensitivity of resistant breast cancer tumor cells to tamoxifen," *Annals of Oncology*, vol. 15, no. 6, pp. 885–890, 2004.

[174] Y. Zhou, S. Eppenberger-Castori, U. Eppenberger, and C. C. Benz, "The NFκB pathway and endocrine-resistant breast cancer," *Endocrine-Related Cancer*, vol. 12, supplement 1, pp. S37–S46, 2005.

[175] G. Morrison, X. Fu, M. Shea et al., "Therapeutic potential of the dual EGFR/HER2 inhibitor AZD8931 in circumventing endocrine resistance," *Breast Cancer Research and Treatment*, vol. 144, no. 2, pp. 263–272, 2014.

[176] M. Pradhan, L. A. Bembinster, S. C. Baumgarten, and J. Frasor, "Proinflammatory cytokines enhance estrogen-dependent

expression of the multidrug transporter gene ABCG2 through estrogen receptor and NFκB cooperativity at adjacent response elements," *Journal of Biological Chemistry*, vol. 285, no. 41, pp. 31100–31106, 2010.

[177] K. W. Nettles, G. Gil, J. Nowak, R. Métivier, V. B. Sharma, and G. L. Greene, "CBP is a dosage-dependent regulator of nuclear factor-κB suppression by the estrogen receptor," *Molecular Endocrinology*, vol. 22, no. 2, pp. 263–272, 2008.

[178] S. Cutrupi, S. Reineri, A. Panetto et al., "Targeting of the adaptor protein Tab2 as a novel approach to revert tamoxifen resistance in breast cancer cells," *Oncogene*, vol. 31, no. 40, pp. 4353–4361, 2012.

[179] G. Takaesu, S. Kishida, A. Hiyama et al., "TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway," *Molecular Cell*, vol. 5, no. 4, pp. 649–658, 2000.

[180] P. Viatour, M. Merville, V. Bours, and A. Chariot, "Phosphorylation of NF-κB and IκB proteins: implications in cancer and inflammation," *Trends in Biochemical Sciences*, vol. 30, no. 1, pp. 43–52, 2005.

[181] H. Bruunsgaard, M. Pedersen, and B. K. Pedersen, "Aging and proinflammatory cytokines," *Current Opinion in Hematology*, vol. 8, no. 3, pp. 131–136, 2001.

[182] J. Pfeilschifter, R. Köditz, M. Pfohl, and H. Schatz, "Changes in proinflammatory cytokine activity after menopause," *Endocrine Reviews*, vol. 23, no. 1, pp. 90–119, 2002.

[183] S. C. Baumgarten and J. Frasor, "Minireview: inflammation: an instigator of more aggressive estrogen receptor (ER) positive breast cancers," *Molecular Endocrinology*, vol. 26, no. 3, pp. 360–371, 2012.

[184] P. M. A. Kelly, R. S. Davison, E. Bliss, and J. McGee, "Macrophages in human breast disease: a quantitative immunohistochemical study," *British Journal of Cancer*, vol. 57, no. 2, pp. 174–177, 1988.

[185] R. D. Leek, C. E. Lewis, R. Whitehouse, M. Greenall, J. Clarke, and A. L. Harris, "Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma," *Cancer Research*, vol. 56, no. 20, pp. 4625–4629, 1996.

[186] T. Hagemann, J. Wilson, H. Kulbe et al., "Macrophages induce invasiveness of epithelial cancer cells via NF-κB and JNK," *The Journal of Immunology*, vol. 175, no. 2, pp. 1197–1205, 2005.

[187] S. Paik, S. Shak, G. Tang et al., "A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer," *New England Journal of Medicine*, vol. 351, no. 27, pp. 2817–2826, 2004.

[188] S. Sheen-Chen, W. Chen, H. Eng, and F. Chou, "Serum concentration of tumor necrosis factor in patients with breast cancer," *Breast Cancer Research and Treatment*, vol. 43, no. 3, pp. 211–215, 1997.

[189] D. W. Miles, L. C. Happerfield, M. S. Naylor, L. G. Bobrow, R. D. Rubens, and F. R. Balkwill, "Expression of tumour necrosis factor (TNFα) and its receptors in benign and malignant breast tissue," *International Journal of Cancer*, vol. 56, no. 6, pp. 777–782, 1994.

[190] M. A. Rivas, R. P. Carnevale, C. J. Proietti et al., "TNFα acting on TNFR1 promotes breast cancer growth via p42/P44 MAPK, JNK, Akt and NF-κB-dependent pathways," *Experimental Cell Research*, vol. 314, no. 3, pp. 509–529, 2008.

[191] M. F. Rubio, S. Werbajh, E. G. A. Cafferata et al., "TNF-α enhances estrogen-induced cell proliferation of estrogen-dependent breast tumor cells through a complex containing nuclear factor-kappa B," *Oncogene*, vol. 25, no. 9, pp. 1367–1377, 2006.

[192] H. Buss, A. Dörrie, M. L. Schmitz, E. Hoffmann, K. Resch, and M. Kracht, "Constitutive and interleukin-1-inducible phosphorylation of p65 NF-kappaB at serine 536 is mediated by multiple protein kinases including IkappaB kinase (IKK)-α, IKKβ, IKKε, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated interleukin-8 transcription," *The Journal of Biological Chemistry*, vol. 279, no. 53, pp. 55633–55643, 2004.

[193] Y. Song, K. Jen, V. Soni, E. Kieff, and E. Cahir-McFarland, "IL-1 receptor-associated kinase 1 is critical for latent membrane protein 1-induced p65/RelA serine 536 phosphorylation and NF-κB activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 8, pp. 2689–2694, 2006.

[194] D. B. Hardy, B. A. Janowski, C. Chen, and C. R. Mendelson, "Progesterone receptor inhibits aromatase and inflammatory response pathways in breast cancer cells via ligand-dependent and ligand-lndependent mechanisms," *Molecular Endocrinology*, vol. 22, no. 8, pp. 1812–1824, 2008.

[195] S. Kobayashi, J. P. Stice, D. Kazmin et al., "Mechanisms of progesterone receptor inhibition of inflammatory responses in cellular models of breast cancer," *Molecular Endocrinology*, vol. 24, no. 12, pp. 2292–2302, 2010.

[196] C. S. O'Brien, G. Farnie, S. J. Howell, and R. B. Clarke, "Breast cancer stem cells and their role in resistance to endocrine therapy," *Hormones and Cancer*, vol. 2, no. 2, pp. 91–103, 2011.

[197] G. Farnie, R. B. Clarke, K. Spence et al., "Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways," *Journal of the National Cancer Institute*, vol. 99, no. 8, pp. 616–627, 2007.

[198] S. Y. Park, H. E. Lee, H. Li, M. Shipitsin, R. Gelman, and K. Polyak, "Heterogeneity for stem cell-related markers according to tumor subtype and histologic stage in breast cancer," *Clinical Cancer Research*, vol. 16, no. 3, pp. 876–887, 2010.

[199] S. Pece, D. Tosoni, S. Confalonieri et al., "Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content," *Cell*, vol. 140, no. 1, pp. 62–73, 2010.

[200] C. Sheridan, H. Kishimoto, R. K. Fuchs et al., "$CD44^+/CD24^-$Breast cancer cells exhibit enhanced invase properties: an early step necessary for metastasis," *Breast Cancer Research*, vol. 8, no. 5, p. R59, 2006.

[201] A. Ouhtit, Z. Y. Abd Elmageed, M. E. Abdraboh, T. F. Lioe, and M. H. G. Raj, "*In vivo* evidence for the role of CD44s in promoting breast cancer metastasis to the liver," *The American Journal of Pathology*, vol. 171, no. 6, pp. 2033–2039, 2007.

[202] C. J. Creighton, X. Li, M. Landis et al., "Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 13820–13825, 2009.

[203] C. M. Perou, T. Sørile, M. B. Eisen et al., "Molecular portraits of human breast tumours," *Nature*, vol. 406, no. 6797, pp. 747–752, 2000.

[204] M. Piva, M. Rábano, B. M. Simões et al., "Sox2 promotes tamoxifen resistance in breast cancer cells," *EMBO Molecular Medicine*, vol. 6, no. 1, pp. 66–79, 2014.

[205] M. J. Sikora, V. Strumba, M. E. Lippman, M. D. Johnson, and J. M. Rae, "Mechanisms of estrogen-independent breast cancer

growth driven by low estrogen concentrations are unique versus complete estrogen deprivation," *Breast Cancer Research and Treatment*, vol. 134, no. 3, pp. 1027–1039, 2012.

[206] C. M. W. Chan, L. Martin, S. R. D. Johnston, S. Ali, and M. Dowsett, "Molecular changes associated with the acquisition of oestrogen hypersensitivity in MCF-7 breast cancer cells on long-term oestrogen deprivation," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 81, no. 4-5, pp. 333–341, 2002.

[207] L. A. Martin, I. Farmer, S. R. D. Johnston, S. Ali, C. Marshall, and M. Dowsett, "Enhanced estrogen receptor (ER) α, ERBB2, and MAPK signal transduction pathways operate during the adaptation of MCF-7 cells to long term estrogen deprivation," *The Journal of Biological Chemistry*, vol. 278, no. 33, pp. 30458–30468, 2003.

[208] M. H. Herynk, T. Hopp, Y. Cui, A. Niu, A. Corona-Rodriguez, and S. A. W. Fuqua, "A hypersensitive estrogen receptor α mutation that alters dynamic protein interactions," *Breast Cancer Research and Treatment*, vol. 122, no. 2, pp. 381–393, 2010.

[209] J. P. Thiery, H. Acloque, R. Y. J. Huang, and M. A. Nieto, "Epithelial-mesenchymal transitions in development and disease," *Cell*, vol. 139, no. 5, pp. 871–890, 2009.

[210] A. Voulgari and A. Pintzas, "Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic," *Biochimica et Biophysica Acta: Reviews on Cancer*, vol. 1796, no. 2, pp. 75–90, 2009.

[211] I. R. G. Beavon, "The E-cadherin-catenin complex in tumour metastasis: structure, function and regulation," *European Journal of Cancer*, vol. 36, no. 13, pp. 1607–1620, 2000.

[212] C. Faleiro-Rodrigues, I. Macedo-Pinto, D. Pereira, V. M. Ferreira, and C. S. Lopes, "Association of E-cadherin and β-catenin immunoexpression with clinicopathologic features in primary ovarian carcinomas," *Human Pathology*, vol. 35, no. 6, pp. 663–669, 2004.

[213] S. Hirohashi and Y. Kanai, "Cell adhesion system and human cancer morphogenesis," *Cancer Science*, vol. 94, no. 7, pp. 575–581, 2003.

[214] A. Nagafuchi, "Molecular architecture of adherens junctions," *Current Opinion in Cell Biology*, vol. 13, no. 5, pp. 600–603, 2001.

[215] S. Hiscox, W. G. Jiang, K. Obermeier et al., "Tamoxifen resistance in MCF7 cells promotes EMT-like behaviour and involves modulation of β-catenin phosphorylation," *International Journal of Cancer*, vol. 118, no. 2, pp. 290–301, 2006.

[216] A. Dhasarathy, M. Kajita, and P. A. Wade, "The transcription factor snail mediates epithelial to mesenchymal transitions by repression of estrogen receptor-α," *Molecular Endocrinology*, vol. 21, no. 12, pp. 2907–2918, 2007.

[217] M. P. Trimarchi, M. Mouangsavanh, and T. H. Huang, "Cancer epigenetics: a perspective on the role of DNA methylatiion in acquired endocrine resistence," *Chinese Journal of Cancer*, vol. 30, no. 11, pp. 749–756, 2011.

[218] M. Esteller, "Epigenetic changes in cancer," *F1000 Biology Reports*, vol. 3, no. 1, article 9, 2011.

[219] M. Hatziapostolou and D. Iliopoulos, "Epigenetic aberrations during oncogenesis," *Cellular and Molecular Life Sciences*, vol. 68, no. 10, pp. 1681–1702, 2011.

[220] M. Szyf, "Epigenetics, DNA methylation, and chromatin modifying drugs," *Annual Review of Pharmacology and Toxicology*, vol. 49, pp. 243–263, 2009.

[221] M. Fan, P. S. Yan, C. Hartman-Frey et al., "Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant," *Cancer Research*, vol. 66, no. 24, pp. 11954–11966, 2006.

[222] T. van Agthoven, T. L. A. van Agthoven, A. Dekker, J. A. Foekens, and L. C. J. Dorssers, "Induction of estrogen independence of ZR-75-1 human breast cancer cells by epigenetic alterations," *Molecular Endocrinology*, vol. 8, no. 11, pp. 1474–1483, 1994.

[223] J. Martinez-Galan, B. Torres-Torres, M. Isabel Núñez et al., "*ESR1* gene promoter region methylation in free circulating DNA and its correlation with estrogen receptor protein expression in tumor tissue in breast cancer patients," *BMC Cancer*, vol. 14, article 59, 2014.

[224] H. G. Chang, S. J. Kim, K. Chung et al., "Tamoxifen-resistant breast cancers show less frequent methylation of the estrogen receptor β but not the estrogen receptor α gene," *Journal of Molecular Medicine*, vol. 83, no. 2, pp. 132–139, 2005.

[225] T. van Agthoven, A. M. Sieuwerts, J. Veldscholte et al., "CITED2 and NCOR2 in anti-oestrogen resistance and progression of breast cancer," *British Journal of Cancer*, vol. 101, no. 11, pp. 1824–1832, 2009.

[226] I. Girault, I. Bièche, and R. Lidereau, "Role of estrogen receptor α transcriptional coregulators in tamoxifen resistance in breast cancer," *Maturitas*, vol. 54, no. 4, pp. 342–351, 2006.

[227] A. Stone, F. Valdés-Mora, J. M. W. Gee et al., "Tamoxifen-induced epigenetic silencing of oestrogen-regulated genes in anti-hormone resistant breast cancer," *PLoS ONE*, vol. 7, no. 7, Article ID e40466, 2012.

[228] K. L. Cook, A. N. Shajahan, and R. Clarke, "Autophagy and endocrine resistance in breast cancer," *Expert Review of Anticancer Therapy*, vol. 11, no. 8, pp. 1283–1294, 2011.

[229] R. Clarke, "Cannibalism, cell survival, and endocrine resistance in breast cancer," *Breast Cancer Research*, vol. 13, no. 4, article 311, 2011.

[230] L. Gonzalez-Malerva, J. Park, L. Zou et al., "High-throughput ectopic expression screen for tamoxifen resistance identifies an atypical kinase that blocks autophagy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 5, pp. 2058–2063, 2011.

[231] J. M. Dixon, C. D. B. Love, L. Renshaw et al., "Lessons from the use of aromatase inhibitors in the neoadjuvant setting," *Endocrine-Related Cancer*, vol. 6, no. 2, pp. 227–230, 1999.

[232] W. R. Miller, J. M. Dixon, L. Macfarlane, D. Cameron, and T. J. Anderson, "Pathological features of breast cancer response following neoadjuvant treatment with either letrozole or tamoxifen," *European Journal of Cancer*, vol. 39, no. 4, pp. 462–468, 2003.

[233] W. R. Miller, J. M. Dixon, D. A. Cameron, and T. J. Anderson, "Biological and clinical effects of aromatase inhibitors in neoadjuvant therapy," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 79, no. 1–5, pp. 103–107, 2001.

[234] P. Robbins, S. Pinder, N. de Klerk et al., "Histological grading of breast carcinomas: a study of interobserver agreement," *Human Pathology*, vol. 26, no. 8, pp. 873–879, 1995.

[235] W. R. Miller, "Aromatase inhibitors: prediction of response and nature of resistance," *Expert Opinion on Pharmacotherapy*, vol. 11, no. 11, pp. 1873–1887, 2010.

[236] W. R. Miller, S. White, J. M. Dixon, J. Murray, L. Renshaw, and T. J. Anderson, "Proliferation, steroid receptors and clinical/pathological response in breast cancer treated with letrozole," *British Journal of Cancer*, vol. 94, no. 7, pp. 1051–1056, 2006.

[237] J. H. van Dierendonck, R. Keijzer, C. J. H. van de Velde, and C. J. Cornelisse, "Nuclear distribution of the Ki-67 antigen during the cell cycle: comparison with growth fraction in human breast cancer cells," *Cancer Research*, vol. 49, no. 11, pp. 2999–3006, 1989.

[238] J. Gerdes, H. Lemke, and H. Baisch, "Cell cycle analysis of a cell proliferation associated human nuclear antigen defined by the monoclonal antibody Ki-67," *Journal of Immunology*, vol. 133, no. 4, pp. 1710–1715, 1984.

[239] S. du Manoir, P. Guillaud, E. Camus, D. Seigneurin, and G. Brugal, "Ki-67 labeling in postmitotic cells defines different Ki-67 pathways within the 2c compartment," *Cytometry*, vol. 12, no. 5, pp. 455–463, 1991.

[240] T. Haerslev, G. K. Jacobsen, and K. Zedeler, "Correlation of growth fraction by Ki-67 and proliferating cell nuclear antigen (PCNA) immunohistochemistry with histopathological parameters and prognosis in primary breast carcinomas," *Breast Cancer Research and Treatment*, vol. 37, no. 2, pp. 101–113, 1996.

[241] M. A. Gonzalez, S. E. Pinder, G. Callagy et al., "Minichromosome maintenance protein 2 is a strong independent prognostic marker in breast cancer," *Journal of Clinical Oncology*, vol. 21, no. 23, pp. 4306–4313, 2003.

[242] I. F. Faneyte, J. G. Schrama, J. L. Peterse, P. L. Remijnse, S. Rodenhuis, and M. J. van de Vijver, "Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome," *The British Journal of Cancer*, vol. 88, no. 3, pp. 406–412, 2003.

[243] T. Petit, M. Wilt, M. Velten et al., "Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy," *European Journal of Cancer*, vol. 40, no. 2, pp. 205–211, 2004.

[244] C. L. Harper-Wynne, N. P. M. Sacks, K. Shenton et al., "Comparison of the systemic and intratumoral effects of tamoxifen and the aromatase inhibitor vorozole in postmenopausal patients with primary breast cancer," *Journal of Clinical Oncology*, vol. 20, no. 4, pp. 1026–1035, 2002.

[245] J. Chang, T. J. Powles, D. C. Allred et al., "Prediction of clinical outcome from primary tamoxifen by expression of biologic markers in breast cancer patients," *Clinical Cancer Research*, vol. 6, no. 2, pp. 616–621, 2000.

[246] A. Makris, T. J. Powles, D. C. Allred et al., "Changes in hormone receptors and proliferation markers in tamoxifen treated breast cancer patients and the relationship with response," *Breast Cancer Research and Treatment*, vol. 48, no. 1, pp. 11–20, 1998.

[247] Y. Tao, A. Klause, A. Vickers, K. Bae, and M. Ellis, "Clinical and biomarker endpoint analysis in neoadjuvant endocrine therapy trials," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 95, no. 1–5, pp. 91–95, 2005.

[248] M. J. Ellis, A. Coop, B. Singh et al., "Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial," *Journal of Clinical Oncology*, vol. 19, no. 18, pp. 3808–3816, 2001.

[249] R. M. Elledge, S. Green, R. Pugh et al., "Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immuno-histochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study," *International Journal of Cancer*, vol. 89, no. 2, pp. 111–117, 2000.

[250] P. M. Ravdin, S. Green, T. M. Dorr et al., "Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest oncology group study," *Journal of Clinical Oncology*, vol. 10, no. 8, pp. 1284–1291, 1992.

[251] M. M. Hefti, R. Hu, N. W. Knoblauch et al., "Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype," *Breast Cancer Research*, vol. 15, article R68, 2013.

[252] C. Wright, S. Nicholson, B. Angus et al., "Relationship between c-erbB-2 protein product expression and response to endocrine therapy in advanced breast cancer," *British Journal of Cancer*, vol. 65, no. 1, pp. 118–121, 1992.

[253] P. Lal, L. K. Tan, and B. Chen, "Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast carcinomas," *American Journal of Clinical Pathology*, vol. 123, no. 4, pp. 541–546, 2005.

[254] X. J. Ma, R. Salunga, J. T. Tuggle et al., "Gene expression profiles of human breast cancer progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 10, pp. 5974–5979, 2003.

[255] M. Dowsett, J. Houghton, C. Iden et al., "Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status," *Annals of Oncology*, vol. 17, no. 5, pp. 818–826, 2006.

[256] B. Weigelt, F. L. Baehner, and J. S. Reis-Filho, "The contribution of gene expression profiling to breast cancer classification, prognostication and prediction: a retrospective of the last decade," *Journal of Pathology*, vol. 220, no. 2, pp. 263–280, 2010.

[257] A. H. Sims, K. R. Ong, R. B. Clarke, and A. Howell, "High-throughput genomic technology in research and clinical management of breast cancer. Exploiting the potential of gene expression profiling: is it ready for the clinic?" *Breast Cancer Research*, vol. 8, no. 5, p. 214, 2006.

[258] A. H. Sims, "Bioinformatics and breast cancer: what can high-throughput genomic approaches actually tell us?" *Journal of Clinical Pathology*, vol. 62, no. 10, pp. 879–885, 2009.

[259] L. J. Van't Veer, H. Dai, M. J. van de Vijver et al., "Gene expression profiling predicts clinical outcome of breast cancer," *Nature*, vol. 415, no. 6871, pp. 530–536, 2002.

[260] J. A. Sparano and S. Paik, "Development of the 21-gene assay and its application in clinical practice and clinical trials," *Journal of Clinical Oncology*, vol. 26, no. 5, pp. 721–728, 2008.

[261] M. Buyse, S. Loi, L. van't Veer et al., "Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer," *Journal of the National Cancer Institute*, vol. 98, no. 17, pp. 1183–1192, 2006.

[262] M. J. van de Vijver, Y. D. He, L. J. van 'T Veer et al., "A gene-expression signature as a predictor of survival in breast cancer," *The New England Journal of Medicine*, vol. 347, no. 25, pp. 1999–2009, 2002.

[263] J. M. Bueno-de-Mesquita, W. H. van Harten, V. P. Retel et al., "Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER)," *The Lancet Oncology*, vol. 8, no. 12, pp. 1079–1087, 2007.

[264] M. J. van de Vijver, J. M. Bueno-de-Mesquita, S. C. Linn et al., "Validation of 70-gene prognosis signature in node-negative breast cancer," *Breast Cancer Research and Treatment*, vol. 117, no. 3, pp. 483–495, 2009.

[265] F. Cardoso, L. Van't Veer, E. Rutgers, S. Loi, S. Mook, and M. J. Piccart-Gebhart, "Clinical application of the 70-gene profile: the MINDACT trial," *Journal of Clinical Oncology*, vol. 26, no. 5, pp. 729–735, 2008.

[266] M. E. Straver, A. M. Glas, J. Hannemann et al., "The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer," *Breast Cancer Research and Treatment*, vol. 119, no. 3, pp. 551–558, 2010.

[267] R. Simon, "Development and validation of therapeutically relevant multi-gene biomarker classifiers," *Journal of the National Cancer Institute*, vol. 97, no. 12, pp. 866–867, 2005.

[268] R. Simon, "Roadmap for developing and validating therapeutically relevant genomic classifiers," *Journal of Clinical Oncology*, vol. 23, no. 29, pp. 7332–7341, 2005.

[269] R. Simon, M. D. Radmacher, K. Dobbin, and L. M. McShane, "Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification," *Journal of the National Cancer Institute*, vol. 95, no. 1, pp. 14–18, 2003.

[270] S. Paik, "Development and clinical utility of a 21-gene recurrence score prognostic assay in patients with early breast cancer treated with tamoxifen," *Oncologist*, vol. 12, no. 6, pp. 631–635, 2007.

[271] L. J. Goldstein, R. Gray, S. Badve et al., "Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinicopathologic features," *Journal of Clinical Oncology*, vol. 26, no. 25, pp. 4063–4071, 2008.

[272] L. A. Habel, S. Shak, M. K. Jacobs et al., "A population-based study of tumor gene expression and risk of breast cancer death among lymp node-negative patients," *Breast Cancer Research*, vol. 8, no. 3, p. R25, 2006.

[273] S. Paik, G. Tang, S. Shak et al., "Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer," *Journal of Clinical Oncology*, vol. 24, no. 23, pp. 3726–3734, 2006.

[274] L. Mina, S. E. Soule, S. Badve et al., "Predicting response to primary chemotherapy: gene expression profiling of paraffin-embedded core biopsy tissue," *Breast Cancer Research and Treatment*, vol. 103, no. 2, pp. 197–208, 2007.

[275] C. Sotiriou, P. Wirapati, S. Loi et al., "Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis," *Journal of the National Cancer Institute*, vol. 98, no. 4, pp. 262–272, 2006.

[276] S. Loi, B. Haibe-Kains, C. Desmedt et al., "Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade," *Journal of Clinical Oncology*, vol. 25, no. 10, pp. 1239–1246, 2007.

[277] O. M. Filho, M. Ignatiadis, and C. Sotiriou, "Genomic Grade Index: an important tool for assessing breast cancer tumor grade and prognosis," *Critical Reviews in Oncology/Hematology*, vol. 77, no. 1, pp. 20–29, 2011.

[278] A. Tordai, J. Wang, F. Andre et al., "Evaluation of biological pathways involved in chemotherapy response in breast cancer," *Breast Cancer Research*, vol. 10, no. 2, article R37, 2008.

[279] J. Toussaint, A. M. Sieuwerts, B. Haibe-Kains et al., "Improvement of the clinical applicability of the genomic grade index through a qRT-PCR test performed on frozen and formalin-fixed paraffin-embedded tissues," *BMC Genomics*, vol. 10, article 424, 2009.

[280] C. Desmedt, B. Haibe-Kains, P. Wirapati et al., "Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes," *Clinical Cancer Research*, vol. 14, no. 16, pp. 5158–5165, 2008.

[281] P. Wirapati, C. Sotiriou, S. Kunkel et al., "Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures," *Breast Cancer Research*, vol. 10, no. 4, article R65, 2008.

[282] M. P. Goetz, V. J. Suman, J. N. Ingle et al., "A two-gene expression ratio of homeobox 13 and interleukin-17B receptor for prediction of recurrence and survival in women receiving adjuvant tamoxifen," *Clinical Cancer Research*, vol. 12, no. 7, pp. 2080–2087, 2006.

[283] X. Ma, S. G. Hilsenbeck, W. Wang et al., "The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer," *Journal of Clinical Oncology*, vol. 24, no. 28, pp. 4611–4619, 2006.

[284] J. F. Reid, L. Lusa, L. De Cecco et al., "Limits of predictive models using microarray data for breast cancer clinical treatment outcome," *Journal of the National Cancer Institute*, vol. 97, no. 12, pp. 927–930, 2005.

[285] W. F. Symmans, C. Hatzis, C. Sotiriou et al., "Genomic index of sensitivity to endocrine therapy for breast cancer," *Journal of Clinical Oncology*, vol. 28, no. 27, pp. 4111–4119, 2010.

[286] P. Dubsky, M. Filipits, R. Jakesz et al., "Endopredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer," *Annals of Oncology*, vol. 24, no. 3, pp. 640–647, 2013.

[287] R. Kronenwett, K. Bohmann, J. Prinzler et al., "Decentral gene expression analysis: analytical validation of the Endopredict genomic multianalyte breast cancer prognosis test," *BMC Cancer*, vol. 12, article 456, 2012.

[288] M. Filipits, M. Rudas, R. Jakesz et al., "A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors," *Clinical Cancer Research*, vol. 17, no. 18, pp. 6012–6020, 2011.

[289] C. Denkert, S. Loibl, A. Noske et al., "Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer," *Journal of Clinical Oncology*, vol. 28, no. 1, pp. 105–113, 2010.

[290] L. D. Miller, J. Smeds, J. George et al., "An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 38, pp. 13550–13555, 2005.

[291] K. Holm, J. Staaf, G. Jönsson et al., "Characterisation of amplification patterns and target genes at chromosome 11q13 in CCND1-amplified sporadic and familial breast tumours," *Breast Cancer Research and Treatment*, vol. 133, no. 2, pp. 583–594, 2012.

[292] S. Loi, B. Haibe-Kains, S. Majjaj et al., "PIK3CA mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 22, pp. 10208–10213, 2010.

[293] C. J. Creighton, A. M. Hilger, S. Murthy, J. M. Rae, A. M. Chinnaiyan, and D. El-Ashry, "Activation of mitogen-activated protein kinase in estrogen receptor α-positive breast cancer cells in vitro induces an in vivo molecular phenotype of estrogen receptor α-negative human breast tumors," *Cancer Research*, vol. 66, no. 7, pp. 3903–3911, 2006.

[294] R. Liu, X. Wang, G. Y. Chen et al., "The prognostic role of a gene signature from tumorigenic breast-cancer cells," *The New England Journal of Medicine*, vol. 356, no. 3, pp. 217–226, 2007.

[295] S. A. Amundson, K. T. Do, L. C. Vinikoor et al., "Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institute Anticancer Drug Screen," *Cancer Research*, vol. 68, no. 2, pp. 415–424, 2008.

[296] J. S. Reis-Filho, C. Westbury, and J.-Y. Pierga, "The impact of expression profiling on prognostic and predictive testing in breast cancer," *Journal of Clinical Pathology*, vol. 59, no. 3, pp. 225–231, 2006.

[297] A. A. Ahmed and J. D. Brenton, "Microarrays and breast cancer clinical studies: forgetting what we have not yet learnt," *Breast Cancer Research*, vol. 7, no. 3, pp. 96–99, 2005.

[298] J. D. Brenton, L. A. Carey, A. Ahmed, and C. Caldas, "Molecular classification and molecular forecasting of breast cancer: ready for clinical application?" *Journal of Clinical Oncology*, vol. 23, no. 29, pp. 7350–7360, 2005.

[299] M. Thomassen, Q. Tan, F. Eiriksdottir, M. Bak, S. Cold, and T. A. Kruse, "Comparison of gene sets for expression profiling: prediction of metastasis from low-malignant breast cancer," *Clinical Cancer Research*, vol. 13, no. 18, part 1, pp. 5355–5360, 2007.

[300] B. Haibe-Kains, C. Desmedt, F. Piette et al., "Comparison of prognostic gene expression signatures for breast cancer," *BMC Genomics*, vol. 9, article 394, 2008.

[301] B. Haibe-Kains, C. Desmedt, C. Sotiriou, and G. Bontempi, "A comparative study of survival models for breast cancer prognostication based on microarray data: does a single gene beat them all?" *Bioinformatics*, vol. 24, no. 19, pp. 2200–2208, 2008.

[302] C. Fan, D. S. Oh, L. Wessels et al., "Concordance among gene-expression-based predictors for breast cancer," *New England Journal of Medicine*, vol. 355, no. 6, pp. 560–569, 2006.

[303] R. Natrajan, M. B. K. Lambros, F. C. Geyer et al., "Loss of I6q in high grade breast cancer is associated with estrogen receptor status: Evidence for progression in tumors with a luminal phenotype?" *Genes Chromosomes and Cancer*, vol. 48, no. 4, pp. 351–365, 2009.

[304] R. Natrajan, M. B. Lambros, S. Maríarodríguez-Pinilla et al., "Tiling path genomic profiling of grade 3 invasive ductal breast cancers," *Clinical Cancer Research*, vol. 15, no. 8, pp. 2711–2722, 2009.

[305] F. Reyal, M. H. van Vliet, N. J. Armstrong et al., "A comprehensive analysis of prognostic signatures reveals the high predictive capacity of the Proliferation, Immune response and RNA splicing modules in breast cancer," *Breast Cancer Research*, vol. 10, no. 6, article R93, 2008.

[306] A. E. Teschendorff and C. Caldas, "A robust classifier of high predictive value to identify good prognosis patients in ER-negative breast cancer," *Breast Cancer Research*, vol. 10, no. 4, article R73, 2008.

[307] A. E. Teschendorff, A. Miremadi, S. E. Pinder, I. O. Ellis, and C. Caldas, "An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer," *Genome Biology*, vol. 8, article R157, no. 8, 2007.

[308] M. P. H. M. Jansen, J. A. Foekens, I. L. Van Staveren et al., "Molecular classification of tamoxifen-resistant breast carcinomas by gene expression profiling," *Journal of Clinical Oncology*, vol. 23, no. 4, pp. 732–740, 2005.

[309] A. Mackay, A. Urruticoechea, J. M. Michael et al., "Molecular response to aromatase inhibitor treatment in primary breast cancer," *Breast Cancer Research*, vol. 9, no. 3, p. R37, 2007.

[310] D. M. E. Harvell, J. K. Richer, M. Singh et al., "Estrogen regulated gene expression in response to neoadjuvant endocrine therapy of breast cancers: tamoxifen agonist effects dominate in the presence of an aromatase inhibitor," *Breast Cancer Research and Treatment*, vol. 112, no. 3, pp. 489–501, 2008.

[311] D. M. E. Harvell, N. S. Spoelstra, M. Singh et al., "Molecular signatures of neoadjuvant endocrine therapy for breast cancer: Characteristics of response or intrinsic resistance," *Breast Cancer Research and Treatment*, vol. 112, no. 3, pp. 475–488, 2008.

[312] A. H. Sims, G. J. Smethurst, Y. Hey et al., "The removal of multiplicative, systematic bias allows integration of breast cancer gene expression datasets—improving meta-analysis and prediction of prognosis," *BMC Medical Genomics*, vol. 1, article 42, 2008.

[313] R. R. Kitchen, V. S. Sabine, A. H. Sims et al., "Correcting for intra-experiment variation in Illumina BeadChip data is necessary to generate robust gene-expression profiles," *BMC Genomics*, vol. 11, no. 1, article 134, 2010.

[314] W. R. Miller and A. Larionov, "Changes in expression of oestrogen regulated and proliferation genes with neoadjuvant treatment highlight heterogeneity of clinical resistance to the aromatase inhibitor, letrozole," *Breast Cancer Research*, vol. 12, no. 4, article R52, 2010.

[315] B. J. Long, D. Jelovac, V. Handratta et al., "Therapeutic strategies using the aromatase inhibitor letrozole and tamoxifen in a breast cancer model," *Journal of the National Cancer Institute*, vol. 96, no. 6, pp. 456–465, 2004.

[316] M. Dowsett, C. Pfister, S. R. D. Johnston et al., "Impact of tamoxifen on the pharmacokinetics and endocrine effects of the aromatase inhibitor letrozole in postmenopausal women with breast cancer," *Clinical Cancer Research*, vol. 5, no. 9, pp. 2338–2343, 1999.

[317] D. Jelovac, L. Macedo, O. G. Goloubeva, V. Handratta, and A. M. H. Brodie, "Additive antitumor effect of aromatase inhibitor letrozole and antiestrogen fulvestrant in a postmenopausal breast cancer model," *Cancer Research*, vol. 65, no. 12, pp. 5439–5444, 2005.

[318] R. S. Mehta, W. E. Barlow, K. S. Albain et al., "Combination anastrozole and fulvestrant in metastatic breast cancer," *New England Journal of Medicine*, vol. 367, no. 5, pp. 435–444, 2012.

[319] J. Bergh, P. Jönsson, E. K. Lidbrink et al., "FACT: an open-label randomized phase III study of fulvestrant and anastrozole in combination compared with anastrozole alone as first-line therapy for patients with receptor-positive postmenopausal breast cancer," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1919–1925, 2012.

[320] A. Di Leo, G. Jerusalem, L. Petruzelka et al., "Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer," *Journal of Clinical Oncology*, vol. 28, no. 30, pp. 4594–4600, 2010.

[321] P. Fedele, N. Calvani, A. Marino et al., "Targeted agents to reverse resistance to endocrine therapy in metastatic breast cancer: where are we now and where are we going?" *Critical Reviews in Oncology/Hematology*, vol. 84, no. 2, pp. 243–251, 2012.

[322] R. Nahta and R. M. O'Regan, "Therapeutic implications of estrogen receptor signaling in HER2-positive breast cancers,"

Breast Cancer Research and Treatment, vol. 135, no. 1, pp. 39–48, 2012.

[323] A. Barnadas, L. G. Estévez, A. Lluch-Hernández, A. Rodriguez-Lescure, C. Rodriguez-Sanchez, and P. Sanchez-Rovira, "An overview of letrozole in postmenopausal women with hormone-responsive breast cancer," *Advances in Therapy*, vol. 28, no. 12, pp. 1045–1058, 2011.

[324] C. M. Barnett, "Everolimus: targeted therapy on the horizon for the treatment of breast cancer," *Pharmacotherapy*, vol. 32, no. 4, pp. 383–396, 2012.

[325] A. Cavazzoni, M. A. Bonelli, C. Fumarola et al., "Overcoming acquired resistance to letrozole by targeting the PI3K/AKT/mTOR pathway in breast cancer cell clones," *Cancer Letters*, vol. 323, no. 1, pp. 77–87, 2012.

[326] C. Villarreal-garza, J. Cortes, F. Andre, and S. Verma, "mTOR inhibitors in the management of hormone receptor-positive breast cancer: the latest evidence and future directions," *Annals of Oncology*, vol. 23, no. 10, Article ID mds075, pp. 2526–2535, 2012.

[327] S. R. D. Johnston, "BOLERO-2—will this change practice in advanced breast cancer?" *Breast Cancer Research*, vol. 14, no. 3, article 311, 2012.

[328] J. Baselga, M. Campone, M. Piccart et al., "Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer," *The New England Journal of Medicine*, vol. 366, no. 6, pp. 520–529, 2012.

[329] M. Gnant, R. Greil, M. Hubalek, and G. Steger, "Everolimus in postmenopausal, hormone receptor-positive advanced breast cancer: summary and results of an Austrian expert panel discussion," *Breast Care*, vol. 8, no. 4, pp. 293–299, 2013.

[330] S. Massarweh, E. Romond, E. P. Black et al., "A phase II study of combined fulvestrant and everolimus in patients with metastatic estrogen receptor (ER)-positive breast cancer after aromatase inhibitor (AI) failure," *Breast Cancer Research and Treatment*, vol. 143, no. 2, pp. 325–332, 2014.

[331] G. C. Bobustuc, J. S. Smith, S. Maddipatla et al., "MGMT inhibition restores ERα functional sensitivity to antiestrogen therapy," *Molecular Medicine*, vol. 18, no. 6, pp. 913–929, 2012.

[332] A. Agrawal, J. F. R. Robertson, and K. L. Cheung, "Clinical relevance of "withdrawal therapy" as a form of hormonal manipulation for breast cancer," *World Journal of Surgical Oncology*, vol. 9, article 101, 2011.

[333] G. Sabnis, O. Goloubeva, R. Gilani, L. Macedo, and A. Brodie, "Sensitivity to the aromatase inhibitor letrozole is prolonged after a "break" in treatment," *Molecular Cancer Therapeutics*, vol. 9, no. 1, pp. 46–56, 2010.

[334] J. S. Lewis-Wambi and V. C. Jordan, "Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit?" *Breast Cancer Research*, vol. 11, no. 3, article 206, 2009.

[335] E. A. Ariazi, H. E. Cunliffe, J. S. Lewis-Wambi et al., "Estrogen induces apoptosis in estrogen deprivation-resistant breast cancer through stress responses as identified by global gene expression across time," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 47, pp. 18879–18886, 2011.