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Review

What clinicians need to know about antioestrogen resistance in breast cancer therapy

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ABSTRACT

Tamoxifen is the drug most used for early breast cancer treatment in oestrogen receptor (ER) positive patients. Unfortunately, despite high ER tumour levels in a tumour, resistance to endocrine therapy, either *de novo* or acquired after prolonged treatment, can occur. In this review, we will try to summarise the postulated mechanisms of hormonal-resistance, namely, the role of co-regulators and the crosstalk between the HER-2, IGF-IR, Cox-2 and ER pathways. Other predictive markers of tamoxifen-resistance/response, such as cyclin E and UPA/PAI-1, are also discussed.

1. Introduction

Tamoxifen has been the drug most widely used for breast cancer treatment. Administered after loco-regional and adjuvant chemotherapy treatment of early breast cancer, it significantly reduces the risk of relapse and death in women with hormone-receptor positive disease. Specifically, 5 years of tamoxifen reduces the annual risk of recurrence and death by 47% and 26%, respectively.[1] In addition, tamoxifen has been shown to reduce the risk of contralateral breast cancer by almost 50%.[2] Tamoxifen is beneficial irrespective of age, nodal and menopausal status. The magnitude of the effect of adjuvant tamoxifen is directly correlated to duration of treatment and to oestrogen receptor (ER) status in the primary tumour, with no effect on ER-negative cancers.[1] Unfortunately, many patients experience resistance to endocrine therapy either *de novo* (at the beginning of the treatment) or acquired (after prolonged use), despite detectable levels of ER in their tumours. Several mechanisms could contribute to the development of this resistant phenotype. These include the following: loss of ER in the tumour; selection of ER mutations; alteration in the intracellular pharmacology and/or

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binding of antioestrogens to breast cancer cells; perturbation of the interactions between ER-coregulatory proteins;[3,4] and crosstalk between the ER and the growth factor receptor pathways [c-erbB2/neu (HER-2) and EGFR and/or their downstream effectors].[5–8] or other pathways, such as IGF-IR[9] and Cox-2.[10] In addition to these already identified mechanisms, the development of tamoxifen resistance is the subject of intense ongoing research, which includes the interaction with other (ER-independent) signalling pathways, such as those driven by protein kinase C (PKC) and oxidative stress.[11,12] The role of the non-genomic effects of tamoxifen, mediated by membrane ER, is also being evaluated. Very recently another pool of ER has been identified in the mitochondria of several cell types including MCF-7 breast cancer cells, and it is thought that oestradiol can act on this ER pool, preventing the activation of the intrinsic mitochondrial death pathway, and thus providing an additional mechanism for cancer cell survival and possibly treatment resistance.[13]

In the last St. Gallen consensus panel, the experts agreed that, rather than focusing on patient's risk of relapse, treatment decisions should first take into account the tumour's 'endocrine responsiveness'. Three categories were defined (endocrine responsive, endocrine response uncertain and endocrine unresponsive) in which any detectable steroid hormone receptor indicates some degree of endocrine responsiveness.[14] This 2005 St. Gallen breast cancer conference also emphasised the importance of the rapid progress made in understanding the biology of the ER function, including the characterisation of a large number of proteins that participate in oestrogen signalling. It is hoped that this knowledge will lead to improved tailoring of effective endocrine therapy, according to ER status and other biological predictive markers. Notwithstanding this progress, nowadays the only predictive markers for endocrine therapy that yield sufficient level of evidence to be recommended for routine clinical practice are the presence and the level of ER and PgR, and to a lesser extent HER-2 status.

A number of alternative endocrine treatments have been developed. These include several selective oestrogen receptor modulators (SERMS) and selective oestrogen receptor down regulators,[15] which compete with oestrogens for binding to ER. Fulvestrant (ICI 182,780) is a specific antioestrogen that binds, blocks and accelerates the degradation of ER protein, leading to complete inhibition of oestrogen signalling through ER. Fulvestrant has no agonist effects,[16,17] contrary to tamoxifen, which has a mixed oestrogen antagonist/agonist effect (Fig. 1). Preclinical studies have demonstrated that a fraction of ER positive, tamoxifen resistant breast tumours are still sensitive to fulvestrant.[18,19] This has been confirmed also in clinical studies.[20,21] Recently, a possible mechanism for this difference has been suggested: resistance to tamoxifen in these breast tumours was mediated by a modification of ER by protein kinase A (PKA), which converted the antagonist tamoxifen into an agonist; consequently tamoxifen's effect on tumour cell growth was reversed, whereas the tumour's sensitivity to fulvestrant remained unaltered.[22] Moreover, recent studies identified differences in the effects of different classes of antioestrogens on cell-cycle arrest. In fact, tamoxifen arrests

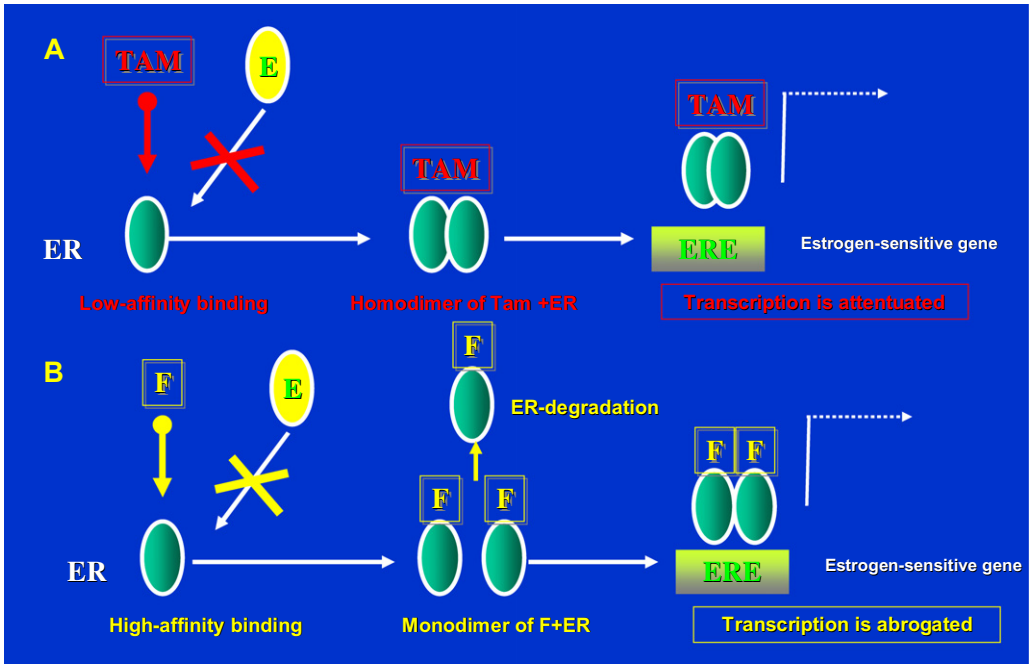

Fig. 1 – This figure shows the difference between tamoxifen and fulvestrant. (A) tamoxifen competes with oestrogen for binding to ER and inhibits the transcription of oestrogen-sensitive genes to a greater or lesser degree depending on the target tissue. Tamoxifen exhibits both oestrogen agonist and antagonist effects; in the breast, it acts primarily as an oestrogen-antagonist, whereas in bone, liver, and in the uterus, it acts predominantly as an oestrogen-agonist. (B) fulvestrant competitively inhibits the binding of oestrogen to ER, prevents dimerisation, promotes ER degradation and prevents transcription of oestrogen-sensitive genes. Fulvestrant is a pure antioestrogen. *ER = oestrogen receptor; E = oestrogen; TAM = tamoxifen; F = fulvestrant; ERE = oestrogen response elements.*

cells in the early G1-phase, while fulvestrant appear to arrest cells in a quiescent G0 state through upregulation of p27Kip1. In this way, fulvestrant induces insensitivity to mitogenic growth factors in treated cells, which may contribute to its efficacy in tamoxifen-resistant disease.[23]

The mechanisms behind the development of fulvestrant-resistance remain unknown. Preclinical studies have demonstrated that cells resistant to fulvestrant exhibit cross-resistance to tamoxifen[24] revealing an increased dependence on EGFR-mediated signalling.[25] In fact, the addition of the EGFR-tyrosine kinase inhibitors, such as gefitinib ('Iressa' ZD 1839), increased the antitumour effect of fulvestrant.[26] Taken together, these data suggest that the full mechanisms of the development of endocrine-resistance to tamoxifen and other antioestrogen agents are still unclear.

In this review, we summarise the postulated mechanisms of endocrine resistance, particularly coregulators, and cross-talk between the HER-2, IGF-IR, Cox-2 and ER pathways.

2. Coregulators of oestrogen receptor action and antioestrogen resistance

It has been known for some time that coregulator proteins can significantly influence ER-mediated transcription.[27] Depending on the ligand, ER interacts with corepressors[28] or coactivators[29,30] that inhibit or enhance its transcriptional activity on target genes (Fig. 2). These intracellular factors have the capacity to modulate the relative agonist/antagonist activity of mixed antioestrogens, such as 4-hydroxytamoxifen (4HT)'.[28,31]

A clinically relevant example of these coactivators is AIB1 (also named nuclear coactivator 3, RAC3, ACTR, SRC-3, or p/CIP in mice),[4,29] which is amplified in certain breast cancers[32,33] and thought to contribute to an antioestrogen-resistance phenotype[34] through crosstalk between the growth factors and ER signalling pathways. In fact, Osborne and colleagues showed that AIB1, when overexpressed in cultured cells, reduces the antagonist activity of tamoxifen, especially in tumour cells that also overexpress the HER-2 receptor. AIB1 is phosphorylated and thereby functionally activated by MAPKs, which are downstream effectors of HER-2.[35] The predictive value of AIB1 was further evaluated in a retrospective study: patients receiving adjuvant tamoxifen therapy who had high HER-2 and high AIB1 expression (25 patients) had a lower 5-year disease free survival (DFS) (42%, 95% CI = 22% to 63%) than the three other groups of patients combined (35 patients = high HER-2/low AIB1; 21 patients = low HER-2/high AIB1; 106 patients = low HER-2/low AIB1), for which the 5-year DFS was 70% (95% CI = 62% to 77%, $p = 0.002$). Interestingly, the group of patients with high HER-2 expression but low AIB1 expression had favourable DFS, despite showing HER-2 overexpression (5-year DFS = 77%, 95% CI = 63% to 92%). For this reason, the authors assumed that high HER-2 is an indicator of poor outcome under tamoxifen treatment only if high levels of AIB1 are available to mediate these adverse effects.[35]

Recently, another study using microarray technology showed a number of profound changes in the expression of genes associated with AIB1 overexpression, such as the up-

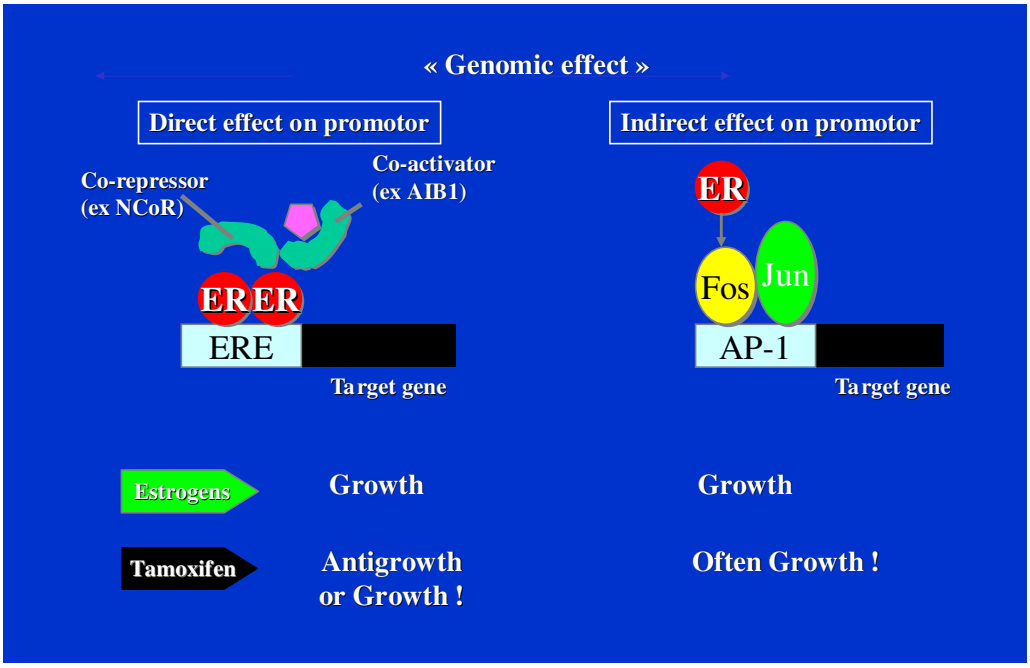

Fig. 2 – In the genomic pathway, ligand binding activates the receptor that binds a variety of co-regulatory molecules; subsequent binding to an ER response element in the promoter region of target genes (ERE) will alter gene transcription. The co-regulatory molecules may act as co-activators (AIB1) to amplify ER-mediated gene transcription or co-repressors (NCOR) to inhibit this function by preventing chromatin unwinding. Data also suggest that ER through protein:protein interaction with other transcription factors can itself function as coactivator for these alternative pathways. ER has been reported to bind to fos/jun complexes bound to their specific response elements in the promoter of AP-1 responsive genes, and, thereby, it can increase the transcription of these genes. *ERE = oestrogen response element; ER = oestrogen receptor; AIB1 = co-activator of oestrogen receptor; NCOR = co-repressor of oestrogen receptor.*

regulation of cyclin D3 and downregulation of NFkB.[36] Endocrine resistance mechanisms are even more complex, also involving corepressors molecules, such as NCOR1. *In vitro* studies have established that NCOR1 protein binds ER and inhibits the partial agonist activity of tamoxifen.[37] Additionally, a recent study demonstrated that low NCOR1 expression was associated with significantly shorter relapse-free survival ($p = 0.0076$) in tamoxifen-treated patients, while patients with high NCOR1 and normal HER-2 fared best on tamoxifen therapy.[38] Taken together, these observations suggest that NCOR1 is as a promising predictor of tamoxifen resistance.

Finally, data also suggest that ER, through protein:protein interaction with other transcription factors, can itself function as a coactivator for these alternative pathways. ER has been reported to bind to fos/jun complexes bound to their specific response elements in the promoter of AP-1 responsive genes, and thereby to increase the transcription of these genes that are normally thought to be oestrogen targets (Fig. 2).

3. HER-2 signalling and antioestrogen resistance

HER-2, a member of the EGFR family, is amplified and/or overexpressed in 20 to 30% of breast cancers and appears to be associated with a more aggressive phenotype.[39] High HER-2 expression has been shown to correlate with tamoxifen resistance,[40,41] but other studies have failed to confirm this association.[42,43] The biology of ductal carcinoma *in situ* (DCIS)[44] supports a direct role of HER-2 in promoting progression towards invasive carcinoma, as well as downregulation of ER and endocrine- resistance.

The retrospective analysis of some clinical trials has suggested a negative interaction between HER-2 overexpression and tamoxifen effect. Frequently cited is the GUN-1 trial, in which 433 patients were randomly assigned to 2 years of tamoxifen ($n = 206$) or observation ($n = 227$) and followed for a median of 15 years. In this study, overexpression of HER-2 was found to predict for poor outcome on tamoxifen (HR = 1.09 95% CI: 0.63–1.87), especially in the subgroup of patients with steroid receptor-positive tumours (HR = 1.33, 95% CI: 0.70–2.51).[41] Many other retrospective analyses supported this negative interaction between HER-2 expression and tamoxifen effect,[45–49] while a few did not.[50,51] These studies are summarised in Table 1 and should only be viewed as 'hypothesis-generating'. (See Table 2)

A growing body of evidence suggests that ER can regulate cellular function through non-classical mechanisms of action. Recent studies indicate that ER not only resides in the nucleus, but also resides in the cytoplasm or in or near the plasma membrane[52] (Fig. 3). This non-nuclear ER is important

Table 1 – Retrospective studies: HER-2/neu and adjuvant tamoxifen

Author	Study arms	N. of pts	% with HER-2	Results
De Placido et al.[41]	Tamoxifen No Tamoxifen	433	57 %	Her-2 is a strong predictor of resistance to Tam, independently of ER
Stal et al.[45]	Tamoxifen 2 years Tamoxifen 5 years	871	66 %	HER-2 overexpression decreases the benefit of adjuvant Tam.
Climent et al.[46]	Tamoxifen not randomised after mastectomy or BCS	283	88 %	The treatment with adjuvant Tam had significantly longer DFS and OS when HER-2 was negative
Sjogren et al.[48]	Tamoxifen/RT/CHT	298	31 %	HER-2 overexpression has a predictive value associated to low survival in tumours N+ treated with Tam
Pinto et al.[47]	Tamoxifen/RT/CHT	295	14.6 %	HER-2 seems to predict response to tamoxifen therapy, by identifying breast cancer ER+ pts with poor prognosis
Ferrero-Pous et al.[49]	Tamoxifen/RT/CHT	488	13.3 %	HER-2 overexpression may be a better predictor of the response to Tam than is ER status alone

Tam = tamoxifen; ER = oestrogen receptor; DFS = disease free survival; OS = overall survival; pts = patients; RT = radiotherapy; CHT = chemotherapy; N+ = axillary lymph-nodes.

Table 2 – Neoadjuvant trials with aromatase inhibitors

Author	N. of pts (n) Characteristics	Study arms	Results subgroup HER2 and ER positive	% Pts with HER2 overexpression
Ellis et al.[55]	$n = 250$ Phase III randomised postmenopausal ER+ and /or PgR+ ErbB-2 + or neg	Tamoxifen 20 mg daily versus letrozole 2.5 mg daily for 4 months	RR 21% versus 88% ($p = 0.0004$)	14%
IMPACT trial[56]	$n = 330$ Phase III randomised postmenopausal ER+	Tamoxifen 20 mg daily versus anastrozole 2.5 mg daily versus combination of T and A	CR 22% versus 58% versus 31% (ns)	14%

ER = oestrogen receptor; PgR = progesterone receptor; RR = response rate; CR = clinical response.

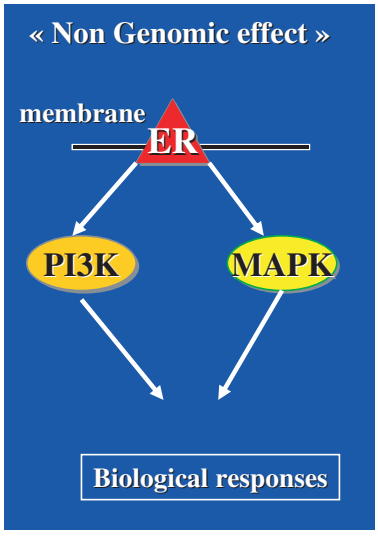

Fig. 3 – In the non-genomic pathway, occupancy of a putative membrane receptor (which in some instances might be a membrane-associated nuclear receptor) by the steroid hormone can lead to the initiation of rapid responses that are coupled through appropriate second-messenger systems, either directly to the generation of the end biological response(s) or indirectly through modulation of genomic responses. *ER = oestrogen receptor; PI3K = phosphatidylinositol 3-kinase; MAPK = mitogen-activated protein kinase pathway.*

in breast cancer because it may directly and indirectly activate several growth-factor-signalling pathways. The binding of oestrogen, and even tamoxifen, to the membrane ER can activate the PI3K/AKT cell survival pathway and it can also activate the epidermal growth factor (EGF) family receptors. Therefore, there is a possibility that in cells with abundant EGFR and/or HER-2 in addition to ER, the administration of tamoxifen, like oestrogen, might stimulate cell proliferation and cell survival through these alternative pathways. The growth factor signalling can, in turn, functionally activate ER and its coregulatory proteins. This is the crosstalk between the ER pathways and pathways mediating a variety of other important cellular functions that can contribute to resistance to specific endocrine therapies (Fig. 4).

In short, this triumvirate, composed of ER, HER-2, and ER-coactivators such as AIB1, appears to regulate the intrinsic or rapidly acquired resistance observed with tamoxifen therapy.[53]

Of great interest are the recently published results of an *in vivo* study using a xenograft model of ER-positive human breast cancer cells engineered to overexpress HER-2 (MCF-7/HER-2). Here the use of gefitinib, an EGFR receptor tyrosine kinase inhibitor, completely restored tamoxifen-growth inhibition, blocking the agonist properties of tamoxifen induced by HER-2 overexpression. Additionally, the combination of gefitinib plus trastuzumab, a humanised monoclonal antibody, resulted in a marked delay in the development of hormone-resistance, making this combination of agents, in association to ER-targeted therapy, an attractive strategy to explore in clinical trials.[54]

Published studies in the adjuvant setting have shown the superiority of aromatase inhibitors (AI) over tamoxifen. However, in view of the potential benefit associated to extended adjuvant hormone therapy beyond 5 years and considering the largely unknown consequences of long-term oestrogen-deprivation, it is crucial to determine which patients need an AI upfront and which are better served with an initial period of tamoxifen. In fact, the data available from some trials suggest that specific subgroups of patients, depending on the characteristics of their tumours, may derive greater benefits from an AI than from tamoxifen. In this regard, two small neoadjuvant trials support the hypothesis that HER-2 positive tumours may be better treated with an AI. The first study demonstrated that, compared with letrozole, tamoxifen produces an inferior clinical response rate in patients with ER positive tumours that overexpress HER-2/neu and/or EGFR. Probably EGFR and/or HER-2 signalling promotes the agonist effects of tamoxifen,[55] through molecular communication from their intracellular kinases (PI3K/Akt), downstream to the ER pathway, altering its function. In the second study (IMPACT trial), 330 patients ER+ were randomised to neoadjuvant treatment with anastrozole (113 patients), tamoxifen (108 patients) or the combination (109 patients). Preliminary data indicated similar efficacy in the overall population (OR was achieved in 37.2%, 36.1%, and 39.4% of patients on anastrozole, tamoxifen or combination, respectively), but a trend for higher antitumour activity of anastrozole in the ER/HER-2 positive subset.[56]

It has also been shown that in ER positive tumours, PgR levels are markedly suppressed by aromatase inhibitors but not tamoxifen.[56] In the ATAC (arimidex and tamoxifen alone or in combination) trial,[57] a randomised double-blind placebo controlled study, 9,366 postmenopausal patients with operable BC were randomised to receive anastrozole (1 mg daily), tamoxifen (20 mg daily), or the combination for 5 years. In this very large trial, 84% of patients had ER positive and/or PgR positive tumours. The last update of results at a median follow-up of 68 months[58] favours anastrozole in patients with ER-positive disease, with statistically significant hazard ratios (HR) for DFS (primary endpoint of the study), time to recurrence, contralateral BC, and time to distant recurrence. However, there is as yet no statistically significant difference between the anastrozole and tamoxifen arms in terms of overall survival (OS). A retrospective subgroup analysis showed a 57% reduction in the HR for recurrence with anastrozole compared to tamoxifen in women with ER positive and PgR negative tumours.[59] However, this observation is not supported by the published results of BIG 1-98. This trial compared (A) tamoxifen (5 years) to (B) letrozole (5 years), to (C) tamoxifen (2 years) followed by letrozole (3 years), to (D) letrozole (2 years) followed by tamoxifen (3 years). The first results of 8028 patients, based on the comparison of initial treatment assignment to letrozole in arms B and D versus initial assignment to tamoxifen in arms A and C were presented after a median follow-up of 25.8 months.[60] A statistically significant difference in DFS, time to recurrence, and time to distant metastases favours the use of letrozole, but the subgroup analysis did not demonstrate a benefit for ER positive and PgR negative tumours.

Interestingly, in a breast cancer xenograft model, TAM-resistant tumours show a switch in molecular phenotype

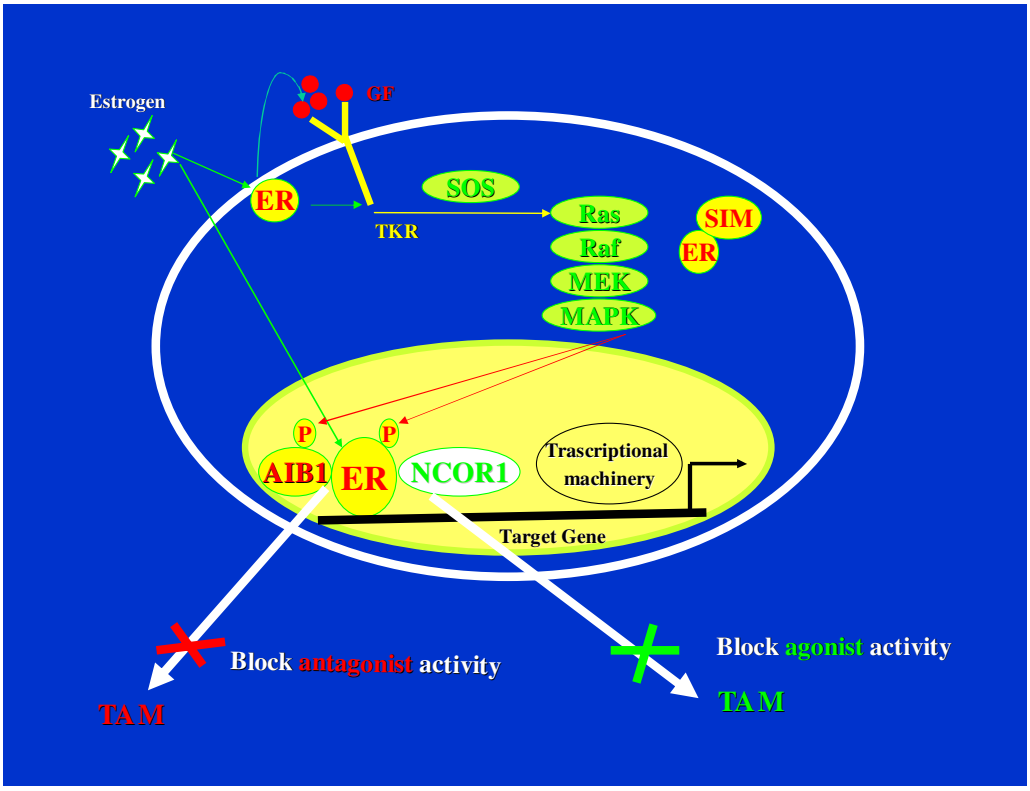

Fig. 4 – This figure shows that the ligand oestrogen can turn on nongenomic membrane and/or citoplasmic ER, which, in turn, through multiple interactions with sign intermediate molecules (SIM), can activate tyrosine kinase receptors (TKR) and cellular kinase cascades. Subsequent phosphorylation (P) of the ER and its coativators (eg, AIB1) and corepressors (eg, NCOR1) by these kinases then potentiates the genomic-nuclear ER activity. The activation of the tamoxifen-ER complex, either directly (through phosphorylation cascades) or indirectly (through phosphorylation of the coactivator) explains endocrine-resistance through blockade of the antagonist activity of tamoxifen.Conversely, NCOR1 protein binds ER and inhibits the partial agonist activity of tamoxifen and its physiological metabolite (4-hydroxy-tamoxifen). In a mouse model of breast cancer, decreased NCOR1 protein expression correlated with acquired tamoxifen resistance. *ER = oestrogen receptor; E = oestrogen; TAM = tamoxifen; AIB1 = co-activator of oestrogen receptor; NCoR1 = co-repressor of oestrogen receptor.*

from ER/PgR positive to ER+/PgR negative concomitantly to the upregulation of EGFR/HER-2. This suggests that loss of PgR in ER positive breast cancer may be a distinctive molecular event associated with the activation of the EGFR/HER-2 pathway.[61] This hypothesis was corroborated by the results of a study in which the clinical and biological features of 29,047 patients with ER/PgR positive tumours were compared with those of 12,358 patients with ER+/PgR negative tumours to determine if these subtypes of ER positive breast cancer represent distinct biological and clinical entities. Overall, ER+/PgR negative tumours were found more frequently in older patients. These tumours were modestly larger in size, had a higher number of positive axillary nodes, expressed higher levels of EGFR than ER/PgR positive tumours (25% versus 8%, $p < 0.001$) and HER-2 expression was significantly higher (24% versus 14%, $p < 0.001$). Importantly, HER-2 expression was significantly associated with a worse OS in the ER+/PgR negative group (HR, 2.2; 95% CI, 1.0, 4.8; $p = 0.04$). Clinical and biological features of 31,415 patients with ER+/PgR+ breast tumours were compared with those of 13,404 patients with ER+/PgR- tumours. A subset of 11,399 patients receiving adjuvant tamoxifen therapy had the analysis of the association between DFS and HER-1 and HER-2 status. Among tamoxifen-treated women with ER+/PgR- tumours, both EGFR expression (HR = 2.4, 95% CI = 1.0 to 5.4; $P = 0.36$) and HER-2 overexpression (HR = 2.6, 95% CI = 1.1 to 6.0; $P = .022$) were associated with a higher likelihood of recurrence.[62] It is possible that the loss of PgR in ER positive tumours is a surrogate marker for aberrant growth factor signalling and thus could serve as a marker of tamoxifen resistance.[63]

More recently, tumour tissue samples of 65 metastatic patients treated with letrozole were evaluated to see whether single nucleotide polymorphisms (SNPs) of the CYP19 aromatase gene had a predictive value. The presence of wild type (WT) or SNPs was detected by PCR. Time to treatment progression was longer in patients with SNPs of CYP19 than those with WT CYP19 (525 versus 196 days, $p = 0.02$). Therefore, it appears that the presence of SNPs is associated with improved treatment efficacy and may help in selecting patients for letrozole therapy.[64]

In conclusion, a number of preclinical studies and retrospective analyses of clinical trials provide support for the view that HER-2 has predictive value with regard to tamoxifen or AI response. Further translational studies utilising the ongoing/closed adjuvant endocrine therapy trials and prospective studies powered to test this biological hypothesis

are crucial in order to fully understand the role of the HER-family pathway in clinical endocrine resistance. At the same time, factors relate to the host, such as SNP's in the aromatase genes should receive increasing attention in the next generation of clinical trials.

4. Insulin-like growth factors pathway and antioestrogen-resistance

The IGF-IR/IGF-I pathway is involved in tumour growth,[65] transformation,[66] development and apoptosis.[67] Therefore, its association with an increased risk of cancer, and breast cancer in particular, is not surprising.[68]

In breast cancer specimens, expression of the IGF-I receptor (IGF-IR) is positively correlated with that of ER[69] while in the laboratory, cross-talk has been shown to take place between the IGF-IR and ER signalling pathways to stimulate proliferation in normal and malignant human mammary epithelial cells.[70,71] Moreover, there is reciprocal crosstalk between the ER and IGF-IR pathways to produce antioestrogen resistance. Indeed, the PI3K/Akt cell survival pathway is not only activated by HER-2 but also by IGF-IR mediated signalling.[72] The downstream effectors PI3K and Akt are involved in the ability of HER-2 and/or IGF-IR to abrogate tamoxifen antagonist action through the phosphorylation of specific amino acids of ER (Ser-167)[72] (Fig. 5).

Many studies[73] but not all[74] have shown that oestrogen induces IGF-IR expression,[75,76] whereas tamoxifen inhibits IGF-I's ability to phosphorylate the IRS-1. IGF-IR, upon activation, regulates the expression genes that are otherwise regulated by oestrogen,[74] and the growth of human breast cancer cells is inhibited by an antibody that blocks ligand binding to the IGF-IR.[9] While a small number of human breast cancer cell lines express IGF-I/II mRNA, significant IGF-I/II mRNA expression is observed in the stromal components of a number of breast tumours, implying a potential paracrine role of IGFs.[77] Other studies have shown an alteration of insulin-like growth factors-binding proteins (IGF-BP) that generally inhibit IGF function; it seems that the regulation of these proteins could play a role in the development of endocrine-resistance. Supporting this hypothesis are the fact that tamoxifen-resistant cells secrete lower levels of IGF-BP-2 and IGF-BP4,[78] while fulvestrant is very effective in abolishing the tamoxifen-resistant proliferation through up-regulation of IGF-BP-5[76] or IGF-BP-3.[79]

In summary, antioestrogen resistance could be related to changes in IGF-IR signalling, changes in systemic IGFI/IGF-BP secretion, and/or by autocrine / paracrine interactions mediated by IGFs.

5. COX-2 pathway and antioestrogen-resistance

Cycloxygenases or prostaglandin endoperoxide synthases (COXs) are key enzymes in the conversion of arachidonic acid (AA) to prostaglandins (PG) and other eicosainoids. There are two isoforms of this enzyme, COX-1 and COX-2. Whereas COX-1 is expressed constitutively in many tissues, COX-2 is induced by many growth factors, cytokines and tumour promoters, with increased expression observed in many tumours, including breast cancers.[80,81] A well-known function of COX-2 is its ability to induce angiogenesis and metastasis, through the activation of matrix-metalloproteinases.[81,82] COX-2 overexpression is significantly associated with less differentiated and more aggressive breast carcinomas, high p53 expression and HER-2 amplification.[80,83,84]

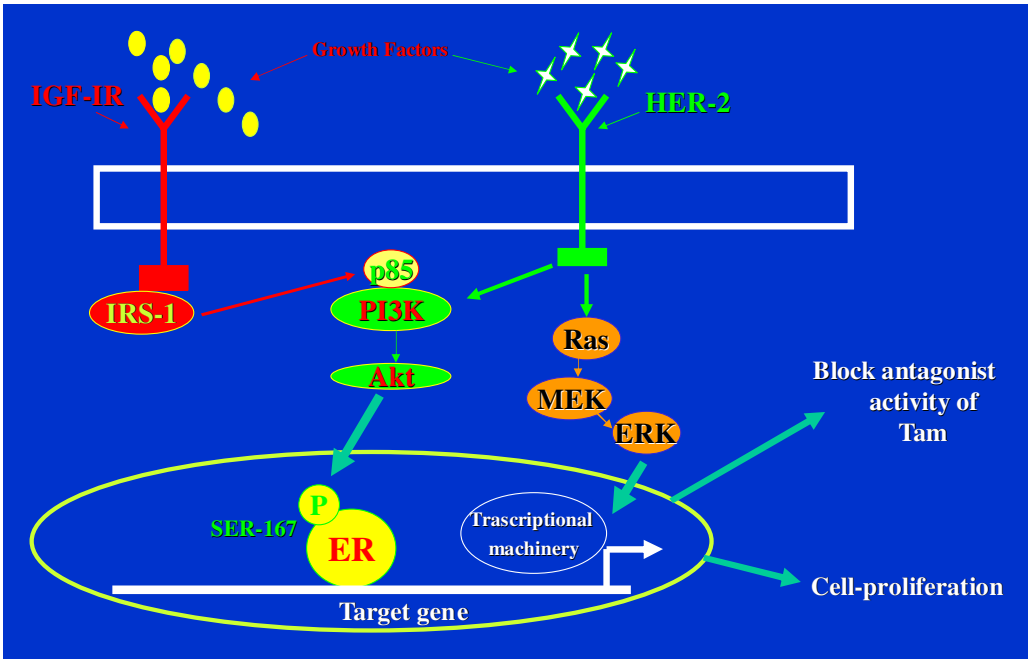

Fig. 5 – The crosstalk between IGF-IR/HER-2 and ER is schematically represented. PI3K/Akt represented the main cell-survival pathway, which is activated by IGF-IR and HER-2 pathways. Activated PI3K/Akt phosphorylate ER in a specific site (SER-167); and induce apoptosis inhibition, cell proliferation and blockade of the antagonist activity of tamoxifen. *IGF-IR = insulin-like growth factor receptor; ER = oestrogen receptor; TAM = tamoxifen.*

The inactivation of tumour suppressor genes, like p53, and the activation of oncogenes, such as HER-2, have been implicated in the induction of COX-2 expression.[85] Moreover, elevated COX-2 expression is associated with poor survival, especially in ER-positive tumours. A possible explanation for this observation is that prostaglandins, particularly PGE2, enhance stromal cell aromatase expression.[86,87] This paracrine effect of PGE2 potentiates local biosynthesis of oestrogen, providing a critical link between the PG-cascade and deregulation of oestrogen biosynthesis in mammary carcinogenesis. All the above observations support a role for COX-2 in resistance to hormonal therapy. This potential predictive value of COX-2 overexpression is worth studying in the context of clinical trials assessing the efficacy of aromatase inhibitors versus tamoxifen.[86,87] For example the combination of celecoxib, a specific COX-2 inhibitor, with aromatase inhibitors appears to be synergistic in the laboratory and so could potentially improve the efficacy of anti-aromatase therapy in the clinic. In an ongoing phase II trial, 27 patients with ER positive advanced breast cancer have been enrolled to receive exemestane plus celecoxib. All patients had received prior tamoxifen, 12 as adjuvant therapy, 12 for metastatic disease and three for both indications. A first analysis showed that clinical benefit (complete + partial response + stable disease) for the whole group was 19/27 (70%).[88] Due to this promising early activity, this trial will continue until 53 patients are recruited. In the meantime, the National Cancer Institute of Canada Clinical Trials Group (NCI-CTG) has initiated a randomised adjuvant trial that will compare two aromatase inhibitors given together with celecoxib or a placebo.

6. Predictive markers of response to tamoxifen

Thus far, only hormonal receptors have achieved level 1 evidence to support their routine use as predictive markers of response to endocrine therapy. However, even in hormonal receptor positive patients only about 2/3 of responses are obtained, a clear indication that additional markers are urgently needed.

6.1. *Progesterone receptor (PGR)*

Results from the NSABP B09 trial,[6] that randomised node positive patients to receive CT (L-phenilalanine mustard and 5FU) with or without tamoxifen, shed light on the value of PgR as predictive factor of tamoxifen benefit. In the subgroup analysis, patients who were PgR+, whether the ER status interaction was controlled for or whether they were considered simultaneously, always derived higher benefit from tamoxifen therapy. As discussed above, an unplanned subset analysis of the ATAC[57] trial showed that the ER+/PgR- tumours derived less benefit from tamoxifen than anastrozole, although these findings were not confirmed in the BIG 1-98 trial.[60] Additional data regarding the role of PgR was given by the two already described neoadjuvant AI trials,[55,56] both showing that tumours that are ER+/PgR- respond better to an AI than to tamoxifen. Also as discussed above it is most likely that the loss of PgR in ER+ tumours is a surrogate marker for aberrant growth factor signalling and could serve as a marker of tamoxifen resistance.

6.2. *Cyclin E*

E-type cyclin controls the G1 to S-phase transition during normal cell cycle progression and is a critical component of steroid-induced mitogenesis in breast epithelial cells. Cyclin E1 is abnormally expressed in approximately 40% of breast cancers, in which the protein is overexpressed as a series of five low-molecular-weight isoforms (ranging in size from 34 to 49 kD). These isoforms, which lack the amino terminus, are hyperactive when compared with the full-length protein with respect to phosphorylation substrates and inducing progression from the G to the S phase.[89–91]

High cyclin E mRNA levels were associated with a poor-relapse-free survival only in patients treated with adjuvant endocrine therapy.[92] Additionally, the predictive value for endocrine therapy of cyclin E1/E2 was evaluated by quantitative RT-PCR in early breast cancer patients. Cyclin E appeared to be predictive for endocrine responsiveness in 112 ER-positive patients (HR = 2.79, for cyclin E1 $p = .005$ and HR = 1.97, for cyclin E2 $p = .05$).[93] Cyclin-E levels were also associated with poor RFS in 108 patients treated with adjuvant endocrine therapy ($P = 0.01$, HR = 3.04, 95% CI: 1.30–7.09), independent of ER status.[92] Overall, these results support that cyclin E could be a predictor of failure of endocrine therapy.

6.3. *uPA/uPAI-1: Predictive markers of tamoxifen resistance?*

The urokinase-type plasminogen activator (uPA), its receptor (uPAR, CD87) and its main inhibitor (PAI-1), through the activation of several matrix metalloproteinases play a central role in the processes leading ultimately to the development of metastases.[94,95] Many studies have shown the strong and independent prognostic value of uPA and PAI-1 in node negative breast cancer.[96] Furthermore, high tumour levels of uPA and PAI-1 predict a poor outcome in patients treated with tamoxifen for advanced disease.[97] The predictive value of these proteolytic factors was analysed by evaluating the association of their tumour expression level and the efficacy of first-line tamoxifen therapy in patients with recurrent breast cancer. High tumour levels of uPA ($P < 0.001$), uPAR ($P < 0.01$), and PAI-1 ($P = 0.01$) were associated with lower efficacy of tamoxifen therapy. In multivariable analysis, uPA ($P < 0.001$) provided additional information independent of the traditional prognostic and predictive factors of tamoxifen benefit, namely age and menopausal status, nodal status, tumour size, grade, ER/PgR status, dominant site of relapse, and disease-free interval.[98]

In the adjuvant setting, patients with high uPA/PAI-1 levels seem to derive a larger benefit from adjuvant chemotherapy than those with low levels.[99,100] In one study, 3424 primary breast cancer patients from two different data sets (Department of Obstetrics and Gynaecology, Technical University of Munich, Germany and Department of Medical Oncology, Rotterdam Cancer Institute) were analysed. The tumour levels of UPA/PAI-1 were used retrospectively to stratify patients' risk of relapse and to indicate whether adjuvant therapy might

be beneficial.[99,100] In the low-uPa/PAI-1 group (n = 1418; 5-year relapse rate 20%; 9% receiving hormonal therapy 17% receiving chemotherapy), it turned out that neither of the adjuvant therapy forms yielded significant benefit: at the 95% CI the HR was 0.60–1.22, and 0.59–1.44, respectively for chemo and hormonal therapy. In contrast, in the high- uPa/PAI-1 group (n = 1174; 5-year relapse rate 38%; 10% receiving hormonal therapy; 19% receiving chemotherapy), both adjuvant therapy forms were significantly and strongly beneficial, with HR of 0.51 (0.33–0.78).

6.4. *Bcl-2*

Bcl-2, the protein product of the bcl-2 gene, is a member of the Bcl-2 family of proteins that play a crucial role in the complex mechanism of apoptosis. The prognostic value of bcl-2 has been studied and, interestingly, its expression has been associated with favourable prognostic factors such as small size, ER-positivity and low nuclear grade.[101] Bcl-2 expression was also associated with better response to hormonal therapy and longer DFS and OS.[102,103] Recently, the expression patterns of Bcl-2, ER, and PgR and their association with other clinicopathological parameters were evaluated in 71 primary invasive breast carcinomas. At a median follow up of 57 months, higher expression of Bcl-2 was associated with longer OS (p = 0.02) and RFS (p = 0.03), and these results were independent of lymph node status and tumour size in Cox multivariate analysis.[104] The potential predictive value of Bcl-2 with respect to tamoxifen response was evaluated in a retrospective analysis of 287 patients, selected from a multicentric phase III trial that compared a moderate dose of EC (epirubicin-cyclophosphamide) with a full dose of EC and CMF (cyclophosphamide-methotrexate-5-fluorouracil), as adjuvant therapy for node-positive breast cancer, followed by five-years of tamoxifen given to postmenopausal ER/PgR-positive or unknown patients. Despite the relatively small number of patients in each subgroup, there was an observed trend towards a greater benefit of tamoxifen in ER/Bcl-2-positive patients as opposed to ER+/Bcl-2-negative ones.[105] These findings are surprising in view of the known anti-apoptotic role of Bcl-2. However, other reports have demonstrated that increased expression of Bcl-2 might not always predict a favourable clinical outcome,[106,107] so the exact value of this marker is still unknown.

6.5. *ER-β*

ER-α and ER-β are both ligand-induced transcription factors that can modulate the expression of specific target genes. ER-β binds oestrogen with similar affinity as ER-α, but unlike ER-α, antioestrogen-occupied ER-β can activate transcription via nonclassical ER-signalling pathways. This has led some investigators to speculate that ER-β could play a role in tamoxifen resistance through the agonist activity of tamoxifen. ER-β protein levels were measured by immunoblot analysis in a retrospective bank of 305 axillary node-positive patients. A total of 119 received no adjuvant therapy, and 186 were treated with tamoxifen only. At a follow-up of 65 months, patients with lower ER-β values were 2.04 times more likely to relapse than patients with higher ER-β values, suggesting that ER-β is an independent predictive factor for disease-free survival in treated patients.[108]

7. New predictive 'molecular signatures' identified through the use of high throughput technologies

In the last few years, the use of microarrays for genome-wide expression profiling has provided a refined molecular classification of human breast cancer.[109] The major subdivision is between basal-like (positivity for keratin 5/6 and 17, B4 and laminin, and mainly ER negative) and luminal-like (positivity for GATA-binding protein 3, and mainly ER positive) breast cancer. These two groups have completely distinct outcomes and their characteristics correspond predominantly to ER negative and positive breast cancer.[110–112] These types of breast cancer can be further subdivided into subgroups. The luminal-like group, roughly corresponding to ER positive breast cancer, may be composed of luminal A, B and C subtypes, each associated with different outcomes.[113,114] Gene expression studies have consistently confirmed the heterogeneity of ER positive breast cancer, and they may provide new insights into the mechanisms of resistance to endocrine therapy.

Current research efforts are directed at the discovery of molecular signatures that might identify those patients most responsive to tamoxifen. In one study, Paik and colleagues described a recurrence-score (Oncotype™) obtained through the RT-PCR evaluation of 21 genes in paraffin-embedded tumour material from node-negative ER-positive breast cancer patients.[115] This tool seems to accurately identify a group of patients with excellent prognosis when treated with adjuvant tamoxifen. Notably, the predictive power of this recurrence score was independent of age and tumour size ($P < 0.001$), and also provided significant information beyond tumour grade.

Another study, conducted in 60 ER-positive breast cancer patients treated with adjuvant tamoxifen alone, suggested the utility of a simple two-gene expression ratio of HOXB13 to IL17BR in identifying a subset of patients with ER-positive breast cancer who are at risk for tumour recurrence when receiving adjuvant tamoxifen, and who may therefore benefit from alternative therapeutic options (H0XB13 was overexpressed in recurrent cases while IL17BR was overexpressed in non recurrent cases).[116] However these results have not been confirmed by Reid and colleagues.[117] This group has attempted to validate the performance of the two-gene predictor on an independent cohort of 58 patients with resectable oestrogen receptor–positive breast cancer.

Another retrospective study developed a risk score to predict distant relapses on tamoxifen in early-stage breast cancer. The risk score was based on 62 probe sets, after testing it on a training set of 99 patients, and was applied to an independent validation set consisting of a total of 156 patients from two different institutions. The results found that 66% of patients were classified in the low-risk group with a 3-year distant metastasis-free survival of 91%. It was suggested that a group of genes could identify breast cancer patients at risk of early distant relapse when treated with tamoxifen.[118] In addition, a microarray approach was also used to identify DNA methylation markers in a population of 278 ER-positive and node-negative patients who were treated with tamoxifen

alone. By combining three classical markers (grade, tumour size and ER expression level) with DNA methylation, an independent patient cohort with excellent outcome when treated with tamoxifen alone (DFS 95% at 10 years versus 62% in the poor prognosis group) was isolated.[119] These low risk node negative ER-positive breast cancer patients identified by DNA-methylation markers have an excellent outcome when treated with tamoxifen alone and may therefore not require additional chemotherapy, or more 'potent' endocrine agents such as aromatase inhibitors.

Taken together, these discoveries have a clear potential for an improved selection of patients in need of adjuvant therapy, as well as for tailored treatment approaches. Interestingly, the HOXB13/IL17BR, DNA methylated markers and the Oncotype™ recurrence score are able to identify a subset of patients with a <10 % distant relapse failure rate at 10 years when treated with tamoxifen alone, as well as a subset of patients that have a poor outcome when treated with this agent. A 'biological signature' of tamoxifen resistance could help determine the patient population for which alternative endocrine strategies and/or cytotoxic therapies are clearly needed. The Oncotype™ multi-gene predictor was able to identify a group of patients with a rate of distant recurrence at 10 years of 30.5% — a risk similar to that observed among node positive breast cancer patients — despite treatment with tamoxifen, which indicates a clear need for more effective therapies in these group of patients.[120] While a new door has been opened in the field of predictive and prognostic markers with these newer high throughput technologies, proper validation of the putative predictive signatures is required including prospective clinical trials in which the prognostic or predictive question can be directly addressed as a function or objective of the trial design.[121]

8. Novel approaches to delay the onset of tamoxifen resistance in the clinical setting

Prospective trials are ongoing with the aim being to discover ways to delay or overcome the development of resistance to tamoxifen. These trials are exploring the potential usefulness of endocrine therapy with simultaneous blockage of different signal transduction pathways driven by EGF-like growth factors and their receptors. An example of these agents is gefinitb (Iressa®, ZD-1839), a tyrosine kinase inhibitor, with which responses have been seen in breast cancer cell lines resistant to endocrine therapy.[122] In addition, three different studies have recently demonstrated that a synergistic growth inhibition occurs when HER-2-overexpressing human breast cancer carcinoma cells are treated with a combination of trastuzumab and gefinitb.[123,124] However, and to much disappointment, in a recently presented phase I/II clinical trial that tested this combination in advanced breast cancer, the DFS did not meet the predetermined statistical endpoints required for the study to continue beyond the first planned interim analysis, and consequently the study was stopped.[125]

Much interest surrounds agents that block several HER-family receptors, such as GW-572016 (lapatinib),[126] a dual inhibitor of both EGFR and HER-2, and CI-1033,[127] a potent and irreversible pan-HER tyrosine kinase inhibitor that modulates EGFR/HER-2 phosphorylation and inhibits tumour cell proliferation.

Combinations of endocrine agents with these new HER-family inhibitors are being explored. Examples of such studies are the ongoing trials that combine trastuzumab with tamoxifen or with an aromatase inhibitor (anastrozole or letrozole), gefitinib with tamoxifen or with anastrozole, and lapatinib with letrozole.

9. Final comments

Treatment tailoring is one of the most important goals of modern oncology. Convergent observations suggest the existence of at least four distinct ER-positive phenotypes: (a) the oestrogen-dependent phenotype 1, responsive to both antioestrogens and aromatase inhibitors. This phenotype requires adequate oestrogenic stimulus for proliferation; (b) the oestrogen-dependent phenotype 2 resistant to tamoxifen, but possibly responding to an aromatase inhibitor; (c) the oestrogen-independent phenotype, antioestrogen responsive and possibly responsive to aromatase inhibitors. This phenotype does not require but may be stimulated by available intracellular oestrogens; (d) the oestrogen-independent but unresponsive phenotype, cross-resistant to all hormonal therapies. This phenotype does not require, and will not respond to, available intracellular oestrogenic stimuli even if oestrogen is present.[7]

Recent years have witnessed tremendous progress in our understanding of the antioestrogen resistant mechanisms developed by breast tumours. However, this progress has yet to translate into improved patient management. Once we are able to ascertain *de novo* resistance upfront and the features of each individual tumour, we will be better able to tailor therapy. For example, we will know when to select tamoxifen or an aromatase inhibitor, or when to prescribe endocrine therapy combined with biological agents capable of delaying or avoiding the onset of resistance. Only well designed randomised clinical trials with a strong translational research component will allow for this rapid transfer of knowledge from bench to bedside.

Conflict of interest statement

None declared.

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