

REVIEW ARTICLE

Is overcoming hormone resistance in breast cancer possible?

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Summary

The administration of hormones plays a major role in the treatment of hormone receptor-expressing breast cancers (BCs), both in the adjuvant setting as well as in advanced disease. Hormone-responsive tumors almost uniformly develop resistance, either independently or as a result of exposure to

hormones. Overcoming this phenomenon constitutes a perpetual challenge to researchers and clinical oncologists. Understanding the mechanisms leading to hormone resistance is crucial to its inhibition or, at least, its delayed onset. This manuscript provides a brief review of this topic.

Key words: hormone resistance, breast cancer

Introduction

BC follows lung cancer as the second most common neoplasm in women of the Western world. Its association with female hormones and its dependence on them has been known for decades, while studies of hormone receptors on the surface of breast cells have contributed to a greater understanding of the biology of the disease as well as to the association of estrogens and other hormones with BC initiation and progression. Although for the past two decades tamoxifen has constituted the cornerstone of the hormone therapy of BC, the last few years have witnessed a shift in interest towards depriving the tumor of the stimulatory activity of estrogens. However, as we learn more, the complexity involving the hormone-receptor interaction and the manner with which this interaction exerts an inhibitory effect on the reception, processing and, transduction of extracellular signals to the cell's interior and, in particular, to the nucleus, are becoming more evident.

The text that follows will present an overview of the relationship between estrogens and BC, the mechanisms of resistance development in an initially hormone-responsive tumor and possible means of reversing this resistance.

Estrogen biosynthesis during productive years and menopause

It is well known that in pre-menopausal women estrogens are being produced mainly by the ovaries. Following menopause, their synthesis continues in the adrenals as well as in the adipose tissue by the enzyme-mediated conversion of androgens to estrogens. Last in the cascade of successive conversion, is the enzyme aromatase, part of the cytochrome P450 system, which mediates the conversion of androstenedione to estrone and of testosterone to estradiol [1]. Estrogen production has been found in mammary [2] and in muscle tissue, while 2/3 of BCs express aromatase [3] (Figure 1). Estrogen receptors (ER) are intracellular receptors. Following hormonal binding, the complex is transported into the cell's nucleus where it activates genes that induce cell proliferation. 50% of premenopausal and 60-70% of postmenopausal BC patients are hormone-receptor positive.

Inhibition of estrogen activity

Attempts to inhibit ER binding with estrogens

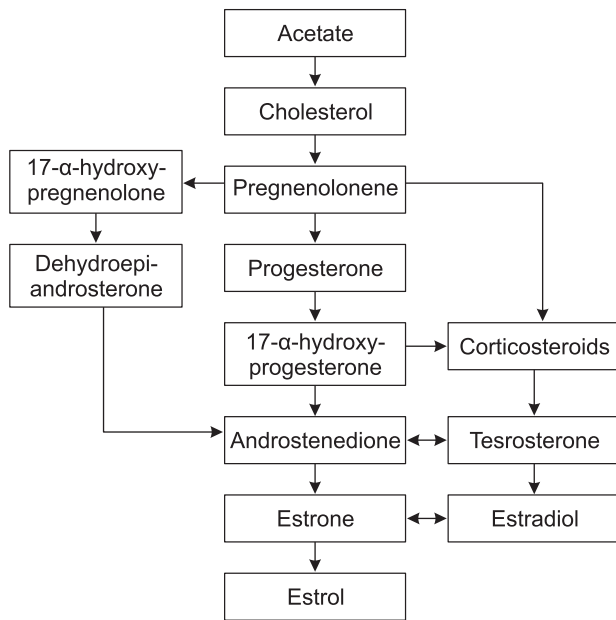


Figure 1. Estrogen biosynthesis.

have been the focus of intensive research over the last 50 years. There are two ways to achieve this effect: a) to reduce the production of estrogens and b) to block hormone-ER binding by using agents that competitively bind the receptor.

a) Reduction of estrogen production

Deprivation of the source of estrogens was historically the first approach to solving the problem. Initially, surgical removal of the organs-sources of estrogen synthesis (ovaries and adrenals in premenopausal and postmenopausal women, respectively) was practised, a procedure that, despite its unquestionable results, was quickly abandoned due to its side-effects (accelerated loss of bone mineral density), but mainly with the emergence of antiestrogens, and in particular of luteinizing hormone releasing hormone (LHRH) analogues.

Contemporary pharmacology has developed new means of reducing the production of estrogens. In premenopausal women where ovaries synthesize estrogens via the hypothalamus - pituitary - ovary axis (LHRH - LH [luteinizing hormone] - estrogen, respectively), cessation of estrogen production, termed pharmaceutical castration, is accomplished by the administration of LHRH analogues, which “purge” the pituitary LH and, therefore, deprive the ovaries of excitatory signals. By contrast, in postmenopausal women, where the basic source of estrogens results

from the conversion, mainly in the adipose tissue, of adrenal androgens into weak estrogens via aromatases, administration of aromatases inhibitors or inactivators (anastrozole, letrozole, borozone and exemestane) leads to an almost complete lack of estrogens.

b) Receptor blockage

A second mechanism involves estrogen receptor blockage by antiestrogens. Tamoxifen, the prototype antiestrogen, has been the principal medication of the last 30 years. It has been well-studied and until recently, has constituted the basis of hormonal therapy in BC. Lately, the term antiestrogen has been replaced by the term SERMs (selective estrogen receptor modulators), since these drugs exert dual receptor action, which differs from organ to organ and is not simply and exclusively antiestrogenic. Tamoxifen acts as an antagonist to estrogen binding to ER in BC cells and as partial agonist in other tissues, namely bone, endometrium etc, with a different range of pharmaceutical actions and side-effects.

Fulvestrant, a pure antagonist of estrogens, which recently entered the drug market, is of paramount interest while progestins (medroxyprogesterone acetate/MPA, megestrol acetate/MGA), androgens and high dose estrogens have been gradually withdrawn due to higher toxicity in relation to newer drugs. Antiprogestins are still being studied in clinical trials.

Hormone dependence - hormone resistance

BC is mostly hormone-dependent so that the development and application of drugs that interrupt this property is crucial to treatment. Even so, a significant percentage of women do not respond to hormone therapy, while a fraction of initial responders eventually develop resistance. An even smaller group of women does not possess ER, thus precluding hormonal therapy.

Tamoxifen has been the basis of hormonal therapy of BC. It is the most extensively studied drug with proven efficacy, which is still under active investigation. Newer drugs, such as aromatase inhibitors and fulvestrant have not succeeded entirely to replace tamoxifen. The progressive development of resistance to tamoxifen and the complexity of its action (antagonist-agonist) has led to a closer study of the mechanisms connected with the development of resistance and the determination of ways to circumvent this phenomenon in order to gain clinical benefit.

The concept that a ligand (in this instance, estro-

gen) binds to a receptor and that this association causes signal transduction to the cell's nucleus leading to gene expression, is probably oversimplified. The cell surface contains hundreds of receptors and each has its own specific ligand. These ligands, which function mostly as growth factors, can activate more than one receptor while there are also receptors that form dimers and trimers before their activation. Subsequent events are even more complex. Signal transduction from the activated receptor to the nucleus requires prior activation of other proteins via the phosphorylation cascade. Each receptor has its own transduction signal but often some steps in this pathway are common. Thus, stimulation of one receptor may activate other pathways (crosstalk). The ER (which is intracellular) appears to share common steps with other pathways (Figure 2) [4], so that the development of hormone resistance may possibly be due to a bypass from a blocked locus to another one. The association of ER with epidermal growth factor receptor (EGFR) and HER-2 has been studied to a great extent.

It is known that overexpression of EGFR and HER-2 [5,6] has been correlated with reduced sensitivity to antiestrogens and a poor prognosis. It has also been found that once tumors, initially responsive to hormone blockage, develop resistance, there is an increase in the expression of EGFR and HER-2, as well as in signal transduction via the Ras-Raf-Mek-Erk pathway [7]. In this way, the tumor cell escapes from the regulatory exerted by the antiestrogenic agent and

finds other means to survive and proliferate. As mentioned above, it should be noted that EGFR stimulation can be achieved by other ligands as revealed by reverse transcriptase-polymerase chain reaction (RT-PCR); additionally, the ER may be activated by insulin-like growth factor I (IGF-I) while its receptor (IGF-IR) can be stimulated by estradiol [8].

When EGFR and HER-2 are overexpressed, tamoxifen possibly acts as an agonist rather than an antagonist, thereby favoring tumor progression [9]. Therefore, the benefit of administering tamoxifen to ER⁺ and HER-2⁺ patients is being questioned. However, since this perception has not been confirmed, co-administration of tamoxifen and trastuzumab (herceptin) may restore cell susceptibility to tamoxifen.

EGFR is overexpressed in about 50% of BCs. In the MCF-7 cell line, EGFR was overexpressed when the cells did not respond to tamoxifen, which in turn led to an increase in cell proliferation and a reduction of apoptosis [10]. At cell level, there is an activation of signaling pathways associated with EGFR and HER-2, such as phospholipase C- γ 1, Ras-Raf mitogen activated protein, phosphatidyl -inositol kinase and its target serine threonine kinase Akt, stress activated protein kinase and others [11]. Akt kinase causes ER transcription in the absence of estrogens. Mitogen activated protein kinase (MAPK) inhibition with UO126 promotes tamoxifen's ability to inhibit the development of ER⁺ cells. The activation of the MAPK/Ras signaling pathway leads to phosphorylation of ER α [11-13] at serine 118, which

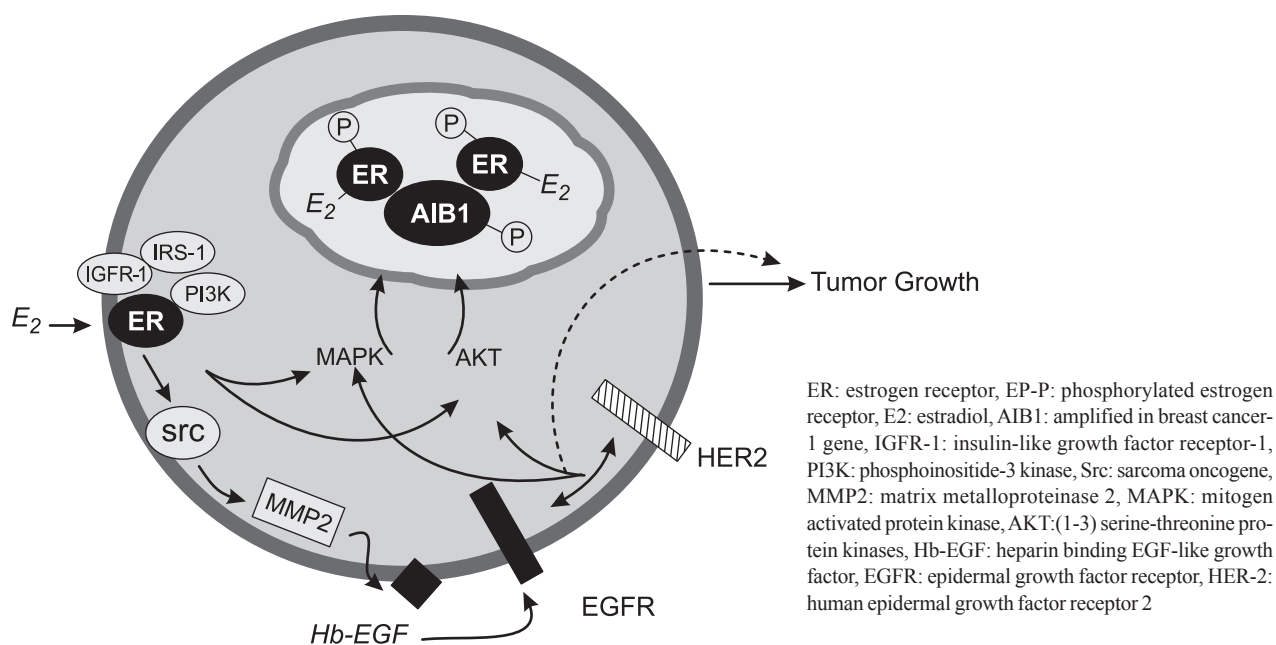


Figure 2. Crosstalk of the estrogen receptor with other intracellular pathways. (From Shiff and Osborne [4]).

results in receptor activation in the absence of estrogens [11,14] and loss of the inhibitory action of tamoxifen [11] (Figure 3). The co-administration of ZD1839 and UO126 (inhibitors of HER-2 and MAPK, respectively) decreases the level of ERser-118 and increases sensitivity to tamoxifen. The P13k/Akt system can phosphorylate ER α [15] at serine 167 and thereby activate it. Tumors overexpressing Akt are candidates for simultaneous administration of both antiestrogens and Akt inhibitors. P13k may be activated by IGF-I as well.

In ER⁺ EGFR⁺ patients who developed resistance to tamoxifen, the administration of tamoxifen can decrease the fraction of cells in S phase and arrest them in the G0/G1 phase [10] for up to 6 months [8]. Gefitinib may be effective in ER⁻ and ER⁺ tumors following development of resistance or in order to delay its appearance [16], while the combined efficacy of gefitinib with fulvestrant and anastrozole is under study in clinical trials [17].

Other receptors that can mediate effective hormonal blockage are those binding transforming growth factors (TGF- β , β_1 - β_3) [18-20]. These polypeptides regulate cell development, differentiation, morphogenesis and the production of extracellular matrix. Their expression in BC varies; they function as autocrine regulators of both cancer and normal breast cells. TGF- β appears to stimulate angiogenesis and stroma production in cancer cells, while also reducing immunological surveillance. Overexpression of TGF- β is associated with a decreased response to tamoxifen and shorter survival. Studies *in vitro* with cell lines revealed that use of TGF- β neutralizing antibodies and antisense oligodeoxynucleotides restored sensitivity to cells that had become resistant to tamoxifen [18]. However, when these experiments were repeated in mice with defective natural killer (NK) cell activity, no reversal to tamoxifen sensitivity was observed, which suggests that immunological responses are possibly involved in the suppressive activity tamoxifen exerts on cancer cells. In any case, it has been shown that tamoxifen can stimulate NK cell activity, as well as cancer cell susceptibility to NK cell function via a mechanism independent of the existence of ER which may, in turn, explain the 10% response rate of ER⁻ patients to tamoxifen [21,22].

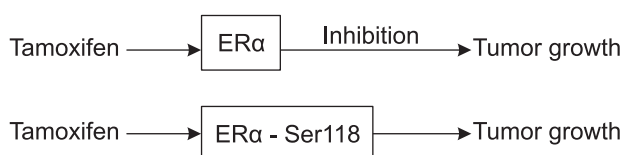


Figure 3. Phosphorylation of the ER α at serine 118 leads to tumor growth despite the presence of the antiestrogen tamoxifen.

Therefore, in ER⁺ tumors that do not respond to tamoxifen or recur rapidly after a short response, there could be an overproduction of TGF- β leading to an increase in angiogenesis and a decrease of immunological surveillance. These patients would benefit from alternative targeted therapies that suppress cytokine overproduction and restore sensitivity to tamoxifen.

A third noteworthy receptor is Fas, a widely expressed transmembrane receptor that promotes apoptosis once it binds to Fas ligand, a member of the tumor necrosis factor (TNF) family which is expressed to a lesser degree than Fas [23,24]. Fas ligand is mostly found in activated T-cells, in the monocyte-macrophage system and in some cancers [25]. Studies have shown that overexpression of Fas ligand yielding a Fas ligand/Fas ratio >1 constitutes a negative independent prognostic factor in BC that is associated with a reduced response to tamoxifen [23]. In one study involving 215 patients, the group with a Fas ligand/Fas ratio >1 had a 14-month shorter disease-free survival, a relative likelihood of relapse of 3.0, a relative likelihood of death of 3.65 and a shorter overall survival rate. The Fas-1/Fas system is believed to play a major role in the regulation of cell death in response to hormonal changes (atrophy of the thymus gland results from induction of apoptosis by estrogens via the Fas-1/Fas system [26]).

Tamoxifen appears to regulate the expression of Fas ligand by exerting its effect directly on its gene [27]. If the above observations are confirmed by other studies, then the Fas ligand/Fas ratio could constitute a new prognostic factor with values >1 leading to a choice of either an aromatase inhibitor or cytotoxic therapy instead of tamoxifen for these particular patients.

In addition to IGF-I, insulin also plays a definitive role in BC. Obesity is associated with increased morbidity and mortality in BC due to sustained aromatase activity and hyperinsulinaemia [28]. This also applies to ER⁻ tumors (which would not be the case if increased aromatase amounts only were involved). In addition, the distribution of fat (androidal and abdominal which is known to be related to higher insulin resistance) appears to increase the danger of relapse and mortality [29]. Accordingly, insulin and IGF-I are believed to function as growth factors. Patients with a history of diabetes are at similar risk.

BC cells have been found to overexpress insulin and IGF-I receptors as well as a hybrid receptor [30]. Moreover, there is crosstalk between ER and IGF-IR, EGFR/HER-2 with IGF-IR and the insulin receptor. Hyperinsulinaemia appears to interfere with ER blockage by tamoxifen, presumably due to activation of insulin and IGF-I receptors. This condition possibly leads to resistance to herceptin via the same mechanism [31].

All these said, it appears that in most cases blockage of ER is not sufficient and that other associated receptors or intracellular mechanisms need to be blocked. Therefore, the simultaneous administration of STIs (signal transduction inhibitors), TKIs (tyrosine kinase inhibitors), FTIs (farnesyl transferase inhibitors), Raf kinase inhibitors, cell cycle inhibitors (e.g. cyclin-dependent kinases or cyclins which modulate transitions between cycle phases [16]) will be crucial factors in estrogen inhibition and improvement of treatment efficacy.

Tamoxifen and mechanisms of resistance development

Besides the existence of other metabolic routes that interfere with ER blockage, tamoxifen loses its inhibitory effect on cancer cells over time by mechanisms that have not been clearly elucidated. Possible reasons for resistance development include the localized metabolism of tamoxifen to weak metabolites [32,33] loss or modification of the target receptor [31,34], and changes in tamoxifen-ER association whereby the hormone behaves more as an agonist rather than as an antagonist [5]. Tamoxifen is known to typically function as an agonist on bone and endometrium and as an antagonist on breast tissue. Its affinity to the ER is approximately 2.5% of that of estradiol [2].

Higher estrogen levels due to an increase in aromatase within tumor cells may also induce resistance. The ATAC study revealed synergy between these mechanisms, i.e. although the combined use of tamoxifen and anastrozole should have been proven superior, it appears that since estrogen eventually starts acting as an agonist it is preferable to administer anastrozole only as monotherapy [35,36].

There are two types of ERs, α and β . Whereas ER α is responsible for ductal elongation, ER β plays a role in the differentiation of the lobules [37].

The existence of ER β to which antiestrogens bind as agonists rather than as antagonists (potential transcriptional activators) may also be implicated in resistance development. Co-expression of ER α and ER β is predictive of a worse prognosis [32]. In two groups of patients (sensitive and resistant to tamoxifen) ER β expression was approximately 2-fold in the resistant population. It has been shown that in normal tissue, ER β expression is more common. Furthermore, the ER α / ER β ratio changes with disease progression and is inversely proportional to the degree of tumor's malignant potential [38]. However, perhaps ER β is not solely responsible for hormone resistance; rather the

combination with other intracellular transcriptional factors (some have already been mentioned) may induce hormone resistance. ER β presence is conversely related to that of progesterone receptors (PgR) whose expression is associated with a more favorable prognosis.

Aromatase inhibitors

Over the last years aromatase inhibitors have been successfully used as first-line therapy in postmenopausal women with advanced disease, either following tamoxifen administration or instead of tamoxifen, as adjuvant therapy in early disease. Aromatase inhibitors are divided into steroidal, such as anastrozole and letrozole, and non-steroidal, namely exemestane. Their mode of action is similar; they all compete with androstenedione and testosterone for the active site of aromatase, and although nonsteroidal aromatase inhibitors cause reversible inhibition of aromatase as opposed to steroidal inhibitors which irreversibly inhibit the enzyme [39], they seem to have the same effectiveness. Their main difference in terms of toxicity is that steroidal inhibitors have a more favorable profile in bone density and blood lipids [40]. Clinical studies have shown that 25-50% of patients who initially respond to tamoxifen treatment and subsequently undergo relapse, will benefit from aromatase inhibitors administration as second-line therapy. Their efficacy appears to result from the following:

a) tamoxifen has antagonistic (breast) and agonistic (uterus, bone, liver, pituitary) activity. Its utility as an antagonist is greater at 5 years as compared to 10 years of administration in the adjuvant setting [41]. Following 5 years of adjuvant treatment, tamoxifen appears to exert agonistic activity on breast cancer cells, resulting in disease relapse [42]. Thus, switching to aromatase inhibitors after 5 years of tamoxifen could be beneficial [3].

b) After an initial blockage by tamoxifen, cancer cells develop hypersensitivity to estradiol (as demonstrated in MCF-7 cell lines) which will subsequently lead to disease relapse. Reduced levels of circulating estrogens achieved with the addition of aromatase inhibitors at this stage will, in turn, induce tumor shrinkage.

Since the efficacy of aromatase inhibitors in BC management is becoming more evident, ongoing clinical trials are attempting to characterize the optimal combination scheme with tamoxifen as well as the most appropriate timing to initiate their administration. Table 1 summarizes the latest studies on the adjuvant use of aromatase inhibitors in early BC.

Table 1. Studies on adjuvant hormonal therapy in early breast cancer

<i>Study</i>	<i>Compared schemes</i>	<i>Results</i>
ATAC [35,36]	tamoxifen × 5 years anastrozole × 5 years tamoxifen + anastrozole × 5 years	Increased disease free survival with anastrozole
BIG 1-98 [54]	tamoxifen × 5 years tamoxifen × 2 years → letrozole × 3 years letrozole × 2 years → tamoxifen × 3 years letrozole × 5 years	Increased disease free survival with letrozole
ABCSG-8/ARNO 95 [55]	tamoxifen × 2 years → anastrozole × 3 years tamoxifen × 5 years	Reduced risk of disease recurrence/improved OS with switch to anastrozole
IES [56]	tamoxifen × 3 years → exemestane × 2 years tamoxifen × 5 years	Increased disease free survival with switch to exemestane
MA17 [57]	tamoxifen × 5 years → letrozole × 5 years tamoxifen × 5 years → placebo	Increased disease free survival with extended adjuvant with letrozole
NSABP B33 [58]	tamoxifen × 5 years → exemestane × 2 years tamoxifen × 5 years → placebo	Increased disease free survival with switch to exemestane

An attempt to group the accumulated results from aromatase inhibitor studies was presented at the American Society of Clinical Oncology (ASCO) 2005 meeting. A Markov model (a technique used to estimate clinical outcomes of a variety of treatment scenarios) [43] was used to propose adjuvant treatment with tamoxifen for 2.5 years followed by 5 years of anastrozole as the optimal administration regimen in postmenopausal women. Currently, there is no indication for aromatase inhibitor use in pre-menopausal women [44].

It remains unclear why steroidal and non-steroidal aromatase inhibitors do not exhibit cross resistance and can be given in succession [45]. The most effective treatment scheme also eludes clinicians. It appears that initial use of exemestane and, upon conditional failure, anastrozole or letrozole, is more effective in metastatic disease [46].

A possible explanation for the absence of cross resistance is the androgenic activity of exemestane (despite the conventional small dosage) and also, its variable effect on aromatase (inactivation of the enzyme by exemestane as opposed to inhibition by the other two molecules) [47].

As regards potential side effects, in contrast to tamoxifen, aromatase inhibitors do not affect the endometrium and do not increase thromboembolic episodes [34]; they do, however, limit the beneficial action of estrogens on bone (promote fractures), lipids and the cardiovascular system [35,48]. Exemestane may present an exception in terms of side effects perhaps due to its androgenic activity [49].

Fulvestrant

A new antiestrogenic factor has relatively recently been added to the armamentarium of medications used in the hormonal treatment of BC. Fulvestrant is a pure antiestrogen that deprives the endometrium of tamoxifen's agonistic activity [50], it presents greater affinity to the ER than tamoxifen and does not display any cross-resistance to tamoxifen [51]. Its affinity to the ER approximates 90% that of estradiol. It has been shown to be as effective as anastrozole in clinical studies [52], while it has absolute indication in patients whose treatment with tamoxifen and anastrozole have failed [53]. Studies to evaluate its efficacy as first-line therapy are awaited.

Conclusions

We delineated above some basic principles concerning hormonal therapy in BC, the mechanisms of resistance that develop during treatment with hormones, as well as the means to reverse this resistance, which to date remains mostly experimental and under study. In conclusion, we would like to express the view that the most recent accumulation of knowledge on this topic along with the rapid development of effective medications and targeted therapies will soon change the settings in hormonal therapy and thereby yield more promising results aiming towards optimal clinical benefit to the patient.

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