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Editorial

The oestrogen receptor and its selective modulators in gynaecological and breast cancer

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Following it's first international meeting on 'Tamoxifen and the Endometrium' [1], the Flemish Gynaecological Oncology Group organised a second international meeting on: 'the Oestrogen Receptor and it's Selective Modulators in Gynaecological and Breast Cancer' on 3–4 December 1999. Over 300 participants from more than 20 countries attended this meeting in Brussels on 3–4 December 1999; 50 papers were presented together with 30 manuscripts selected for a poster exhibition. This editorial summarises the huge amount of information presented at this meeting and illustrates the most recent advances in research on the oestrogen receptor and it's selective modulators.

1. The oestrogen receptor and molecular mechanisms of oestrogenic action

Today, it is generally accepted that oestrogens increase the mitotic activity in breast and endometrial tissue and that errors in the mechanics of cell division lead to malignancy. Although tamoxifen has few toxic side-effects [1] and is very efficacious in all stages of breast cancer, new anti-oestrogens were developed, the majority of which are classified within a group of compounds called selective oestrogen receptor modulators (SERMs). The ideal compound is the one with an anti-oestrogenic activity in the breast and uterus but an oestrogenic effect on bone and the cardiovascular system [2].

The genomic pathway of oestrogen activation involves interaction of oestrogen with a nuclear receptor protein that retains oestrogen in target cell nuclei. The oestrogen receptor (ER)α protein consists of 595 amino acids, and is, like the recently discovered 485-amino acid ERβ, a ligand regulated transcription factor. Both receptors, expressed in different amounts in different tissues, can be activated with different affinities by steroid and non-steroidal compounds. Both ER isoforms have been cloned in humans and rodents and possess six (A–F) similar functional domains, each responsible for different functions like hormone-binding, hormone response elements, transcription activation functions (AFs), initiation of gene transcription [3]. The amino-terminal A/B-domain (AF-1) has the least amino acid sequence identity, while the C-domain (DNA-binding) has the highest amino acid sequence identity. There is a second activation function, AF-2 which is located in the oestrogen-binding domain (E/F). The ER interacts with the compounds of the core transcriptional complex via these AF's. Oestrogens diffuse into oestrogen-dependent cells and are retained by the ER. When activated by hormone binding, the ER undergoes transformation which enables it to bind to specific DNA sequences, termed oestrogen responsive elements (EREs) found in the vicinity of target genes. Subsequently, recruitment of co-activators and general transcription factors results in the formation of an active transcriptional complex and enhancement of target gene transcription. One or more of these AF's may be required for SERM gene transactivation, depending on the cell and gene promotor context. The ability to affect differentially the 3-dimensional structure of the AF-2 region after ligand binding has been proposed as an important mechanism contributing to the selective actions of different ER ligands. For example, following oestrogen binding in the uterus and breast, there is increased cellular proliferation. ERs have also been located in, for example, the hypothalamus, cortex, pituitary, limbic system and osteoclasts. The nature of the ER ligand is clearly not the only factor that accounts for SERM tissue specificity. There is the distribution and localisation of different ER's, the cell- and/or gene-specific presence of activating or inhibiting protein cofactors (i.e. co-activators and co-repressors), the activation of different hormone-binding domains and, hence, the interaction of transcription

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factors and co-activators, determines an agonist, antagonist or mixed response of each SERM in different tissues.

During recent years we have seen a paradigm shift in our understanding of oestrogen action. This is mainly due to the discovery of a second ER, ERβ, which is encoded by a separate gene from that of ERα [4]. The presence of ERs has been demonstrated in numerous normal and pathological tissues. Many organs express both ER-subtypes, but different cell-types within each organ may express different ERs indicating that ERα and ERβ have distinct functions in some instances. Distribution of human ER subtypes is mainly based on measurements of ER mRNA levels. This pattern awaits confirmation at the protein level when subtype-specific monoclonal antibodies (MAbs) become available. ERβ is expressed in partially different tissues from ERα, notably prostate, ovary, lung, kidney, gastrointestinal tract and especially colon, as well as in tissues where ERα is also found, e.g. breast, central nervous system (CNS), testis [5]. ERα predominates in the uterus and liver.

The conformational effects of a variety of ligands on the structures of both ER isoforms have been investigated by Hubbard and colleagues [6]. Each class of ligand (agonists, partial agonists, and SERMs) induces a unique conformation in the receptor's ligand-dependent transcriptional activation function. The ability of ER to bind a wide repertoire of compounds stems from both the size and shape of the hormone binding cavity which permits a variety of ligand binding modes. A key feature of the structure is the ability of ERα — its carboxy terminus at DNA helix 12 — in the ligand-binding site to fold into the complex after oestradiol is bound. Whether ER activation following binding of different ligands leads to an agonist or antagonist activity depends on the position adopted by the bound ligands towards this carboxy terminal helix of ERα (H12). Raloxifene's large side chain displaces helix 12 via direct interaction with aspartate 351 of ERα disrupting ER's interaction with co-activators in some way [7]. The antagonistic effects of SERMs appear to arise from a ligand-induced, sub-optimal conformation of ER that is unable to communicate with the cellular transcriptional machinery.

It has clearly been shown that the benefit from endocrine therapy is directly proportional to the amount of ER present in the tumour. The ER has recently been determined immunohistochemically (IHC) or with cytosol measurements. The advantages of the cytosol measurements via a ligand binding assay, include the generation of numerical results across the whole of the likely concentration range, good reproducibility, full technical and clinical validation, inclusion of measures of receptor functionality and existing quality assurance schemes. Disadvantages include a relative large amount of tissue required, the necessary care over handling, storage, assay and data processing, the labour intensive nature of the assay, and the lack of information about the nature of the tissue being homogenised [8]. As improved anti-ER antibodies became available, IHC assay began to replace the ligand binding assay (LBA). However, there were again advantages and disadvantages. Advantages of IHC included the fact that routine, fixed material could be used; archival material could be assayed retrospectively; only small quantities of tissue were needed; receptor content could be related to morphology and there was a measure of cellularity; internal positive control was often provided by the normal epithelial tissue in the section. The disadvantages included the subjectivity; the lack of quantitation; the absence of any indication of functionality of the receptor; the lack of standardisation of staining; the absence of an appropriate quality assurance scheme and the lack of clinical validation. An 8-point scoring system from 0–5 points according to the proportion of nuclei staining and 0–3 for the intensity of staining, with corresponding clinical recommendations according to the final score has been proposed by Harvey and colleagues [9]. It was concluded by these authors that IHC was probably the more clinical useful score.

In another study by Gordts and colleagues [10] with 299 breast cancer cases IHC was used in a semi-quantative method and scored between 0 and 300 (the H-score). In this study concordant results between LBA and IHC were observed in 230 out of 299 cases (77%) while 69 patients had discordant results (kappa = 0.537).

Hawkins [11] concluded that both for the LBA and IHC assay, the use of a single cut-off should be avoided and activity quantified, or stratified into categories.

2. The oestrogen receptor in breast, uterus and other gynaecological tissues

Khan and colleagues [12] examined the relationship between breast cancer diagnosis and the presence and level of ER expression in benign breast epithelium [13]. The level of ER expression was higher in breast cancer patients than in control subjects (i.e. those undergoing breast biopsy for benign conditions), and it was related to breast cancer risk in postmenopausal patients. Furthermore, they observed that the median labelling indices for ER in breast epithelium from postmenopausal women with two new antibodies 6F11 and TE111, were significantly higher choosing these antibodies compared with previous studies.

The ER and progesterone receptor (PR) are co-expressed in approximately 10–20% of normal human breast epithelial cells, but expression of Ki67 proliferation associated antigen segregates to a separate population [14]. Loss of this separation between cell proliferation and steroid receptor expression may be an early event in breast cancer genesis as it occurs in

atypical ductal hyperplasia and ductal carcinoma *in situ*, as well as in invasive carcinoma. The fate of proliferating breast epithelial cells over time and in relation to the expression of the $p27^{KIP1}$ inhibitor of cyclin-dependent kinase activity which has been shown to be a differentiation marker in other tissues such as the ovaries and testes, were examined by Clarke and colleagues [15]. These authors observed the process of terminal differentiation in normal human breast epithelium, whereby some proliferating cells undergo a final round of division before becoming quiescent and switching on $p27^{KIP1}$ and steroid receptor expression. These data support the hypothesis developed to explain the dissociation between steroid receptor expression and proliferation seen in the normal breast.

Furthermore, it has been shown that glandular epithelium and stroma of the endometrium showed typical behavioural patterns in the expression of ER due to both endogenous and exogenous hormonal influence. Under the influence of progesterone, the ER disappears during the luteal phase and is not even detectable after day 21 [16]. During menopause, the atrophic endometrium typically shows very little, if any, ER expression. In cases of oestrogen induced hyperplasias ER can again be demonstrated.

While ER has been identified in the epithelium of the vulva, vagina and ovary by biochemical, IHC and molecular techniques, none of the epithelial malignancies arising from these sites are routinely hormonally sensitive [17].

Fujimoto and colleagues [18] observed that the relative overexpression of ER exon 5 splicing variant (ER E5SV) ERβ and PR might be related to metastatic potential and partially cause deviation from steroidal dependency in endometrial cancers.

ER downregulators, of which ICI 182,780 (faslodex) is a prototype, are a novel class of highly effective steroidal antitumour agents that reduce cellular levels of ER. Using transient and where appropriate stable gene transfection experiments, Madden and colleagues [19] found that constitutive overexpression of ER antisense RNA and a hormone-binding domain compromised dominant negative ER mutant (DNER-1), were most effective at downregulating ER expression and/or activity *in vitro*. Constitutively expressed ER antisense RNA's and ER dominant negative mutants appear feasible alternatives to current ER ligand derivatives as a means of ER downregulation.

3. Tamoxifen and the endometrium

Uterine side-effects of tamoxifen have been extensively detailed in recent years [1]. Prospective and retrospective studies have shown a higher incidence of endometrial carcinoma (relative risk, RR, 2.0–2.5) in long-term users. The consensus we agreed upon 2 years ago [1] has not changed and has even become more important at a time when many healthy women start using tamoxifen for breast cancer prevention. Those who advocate screening should start with a pretreatment uterine assessment using transvaginal ultrasonography or outpatient hysteroscopy. Symptom-free women with a normal pretreatment uterine cavity can be screened yearly with transvaginal sonography from 2–3 years after the start of tamoxifen. Saline infusion sonography or hysteroscopy will be required if there is endometrial thickening (double endometrial thickness >5 mm) because the only value of transvaginal ultrasonography is a normal finding being a thin rectilinear endometrium.

In a study of 575 postmenopausal breast cancer patients with an intact uterus, who were to receive tamoxifen as adjuvant therapy Berlière and colleagues [20] concluded that the group of women exhibiting increased sensitivity to the oncogenic potential of tamoxifen can be identified by pretreatment uterine evaluation. Indeed, atypical lesions were observed in 10 out of 567 person-years in patients with initial endometrial lesions compared with only three atypical endometrial lesions out of 2574 person-years in a group of patients without initial endometrial lesions. Furthermore, these authors observed that endometrial resection did not protect against the subsequent development of atypical lesions or endometrial cancer.

In contrast, Vosse and colleagues [21] observed that with a conservative strategy with transvaginal ultrasound as first-line screening test and yearly follow-up starting only 3 years after the start of tamoxifen, resulted in good results in terms of compliance, cancer detection rate, sensitivity and positive predictive value.

Finally, another Belgian study supported the hypothesis that tamoxifen-associated endometrial polyps may not be induced simply by the drugs oestrogen agonistic activity but that they may be formed from 'pre-existing' focal endometrial lesions [22]. These findings confirm earlier data on chromosomal changes detected in tamoxifen-associated polyps [23].

Carmichael and colleagues [24] speculated that the epigenetic mechanisms of carcinogenicity of tamoxifen on the endometrium, involving growth factor modulation may be responsible. To examine this further transforming growth factor β (TGFβ) immunoreactivity was determined in endometria from non-drug-therapy and tamoxifen-treated patients [25]. They displayed greater levels of endometrial dysplasia and glandular hyperplasia, in addition to a statistically significant elevation of endometrial gland-associated TGFβ1.

Furthermore, Oesterreich and colleagues [26] showed that the inhibition of ER activity by the co-repressor HET/SAF-B is relieved by blockade of histone deacetylase activity.

Karck and Kommoss [27] studied the expression of ER and PR in postmenopausal women, receiving tamoxifen for breast cancer. In this study, they demonstrated consistent expression of ER and PR in the endometria from patients receiving tamoxifen, with a trend towards a higher proportion of receptor positive specimens during tamoxifen. In breast cancer tissue the ER content seemed to be reduced under tamoxifen. After short time exposure to tamoxifen the PR appeared to be increased, longer treatment caused the PR to go down to pretreatment levels or below that.

4. SERMs in the prevention of breast cancer

The oestrogen responsiveness of breast tumours suggested the use of a therapeutic antagonist to the action of oestrogen, a SERM, in the prevention of breast cancer. Accumulating evidence from breast cancer treatment trials pointed to the ability of the first SERM, tamoxifen, to prevent second primary breast cancers in the contra-lateral breast in women being treated in the adjuvant setting for established invasive disease [28–32]. Encouraged by this data, in 1992 the National Surgical Adjuvant Breast and Bowel Project (NSABP) started the Breast Cancer Prevention Trial (P-1: BCPT) to test the ability of tamoxifen to prevent breast cancer in women at increased risk for the disease [33]. The resulting data showed that through 69 months of follow-up tamoxifen reduced the risk of invasive breast cancer, primarily ER-positive tumours, by 49% with a cumulative incidence of 175 versus 89 cases of invasive breast cancer in the placebo and the tamoxifen groups, respectively. As a secondary endpoint, tamoxifen reduced the incidence of fractures by 19%, approaching but not reaching statistical significance. Another secondary endpoint, the rate of ischaemic heart disease, was unaffected by tamoxifen. The major toxicity was an increase in the rate of endometrial cancer (RR 2.53; 95% confidence interval (CI): 1.35–4.97) [34]. Based on these data, in the US, tamoxifen is now indicated for use in healthy women — ''to reduce the incidence of breast cancer in women at high risk for breast cancer''.

In the Italian Tamoxifen Prevention Study, 5408 healthy women, aged 35–70 years, who had a total hysterectomy for reasons other than neoplasms were included [35]. Women were randomised to receive tamoxifen 20 mg/day or placebo for 5 years. The preliminary results of the study after a median of 46 months showed no difference in the incidence of breast cancer between the two arms. Among the women on tamoxifen for more than 1 year, there was a trend towards a beneficial effect of tamoxifen (11 in the tamoxifen arm versus 19 in the placebo arm, not significant). Interestingly, a borderline significant reduction of breast cancer was observed among women who were on hormone replacement therapy (HRT) and received tamoxifen. Compared with the 8 cases of breast cancer occurring among the 390 HRT users who were on placebo, there was one case of breast cancer among the 362 HRT users who were receiving tamoxifen. Veronesi [36] pointed out that although the Italian study was regarded as being affected by a higher drop-out rate a subsequent analysis comparing the three primary prevention trials of tamoxifen indicated that the number of discontinuations for reasons other than major events, was 20.7, 28.8 and 35.5% in the Italian, NSABP and Marsden trial (see below), respectively. Decensi [37] suggested that using the combination of tamoxifen and HRT, in order to reduce the risks while retaining the benefits of both agents, or the use of a lower daily dose of tamoxifen should be further investigated.

In the Marsden trial, 2471 women, followed for a median of 70 months, a relative risk of 1.06 was observed for the tamoxifen versus the placebo group [38]. A variety of explanations has been offered for the discrepancy between these three trials. Strong family history, suggestive of genetically determined disease in the Marsden trial; and the use of HRT in both the British and Italian trials, 42 and 14%, respectively [34].

A fourth trial, the International Breast Cancer Intervention Trial was initiated in 1992. This trial is still ongoing and now has 5596 patients and is expected to close at the end of the year 2000 [39].

Clinical trials carried out in Europe and the US have demonstrated that raloxifene decreases bone turnover and increases bone mineral density in postmenopausal women [30]. This trial had 40 months follow-up and showed that raloxifene significantly reduced the risk of breast cancer among postmenopausal women with osteoporosis compared with placebo. 13 cases of invasive breast cancer were observed in 5129 women assigned to take raloxifene and 27 in 2576 women assigned to take placebo. Raloxifene reduced the risk of invasive ER-positive breast cancer by 90% but did not influence the risk of ER-negative cancer. Raloxifene has similar desirable effects to tamoxifen on biochemical markers of cardiovascular risk [40] but is less stimulatory to the uterus [41,42]. Therefore, a new study of tamoxifen and raloxifene (STAR) began in July 1999 at almost 500 centres in North America. The plan is to randomise 22 000 eligible women to tamoxifen 20 mg or raloxifene 60 mg/day for 5 years. Eligible women must be postmenopausal, 35 years or older and have an increased risk reflected in a $\geqslant 1.66\%$ projected 5-year probability of developing invasive breast cancer according to the breast cancer risk assessment profile (RAP) [42,43].

Goldstein [44] presented a uterine safety study on 415 patients who demonstrated a lack of uterine pathology by transvaginal ultrasound, saline-infusion sonohysterography and negative endometrial biopsy, randomised

to receive either placebo, conjugated equine oestrogen (0.625 mg), raloxifene 60 mg or raloxifene 120 mg over 1 year. This study showed that raloxifene is not stimulatory to the endometrium in postmenopausal women and is not tamoxifen-like resulting in no cancers, no hyperplasia and no polyps.

5. SERMs and other anti-oestrogens for use in gynaecological and breast cancers

Kaufman conducted a randomised trial including 380 patients with endometrial cancer who received for 2 years either medroxyprogesterone acetate (MPA) ($n=133$) or tamoxifen ($n=121$) orally or were observed only ($n=134$) after surgical therapy. In this study, slightly less recurrences and deaths were observed in the tamoxifen group compared with the control or the MPA group. This study is clearly contradictory to the increased incidence of endometrial carcinoma observed with tamoxifen in the prevention or adjuvant trials in breast cancer. In addition, in ovarian carcinoma, tamoxifen seems to have a beneficial effect in patients with relapsing disease. In an overview by Tropé and colleagues [45] of 633 patients, 11% of the patients responded objectively.

Another drug which has been used in the treatment of breast cancer is toremifene. Currently available for the treatment of advanced disease, toremifene has been found to be as effective and at least as well tolerated as tamoxifen [46]. Based on a small number of studies, the same appears to apply for the adjuvant setting [47]. After a total cumulative clinical exposure to toremifene of 140 000 patient/years only 9 cases of endometrial carcinoma have up to now been reported. At this moment there are no clinical data implying that toremifene *per se* causes endometrial carcinoma [46]. However, compared with tamoxifen, where patient exposure is now estimated to be over 10 million patient years, it is premature to conclude that toremifene will not be associated with the numbers of endometrial carcinomas observed in tamoxifen-treated patients.

Idoxifene is the third SERM. This drug acts as an oestrogen agonist in osteoblastic cells via an ER/ERE-mediated mechanism [48]. In breast cancer cells, both raloxifene and idoxifene have been shown to be potent antagonists of ERE-mediated 17β-oestradiol action suggesting an ERE-dependent mechanism of action for both ligands in these cells.

Other synthetic non-steroidal SERM's which have as such been tested or are still in development include a wide variety of structural families, among others triphenylethylenes like tamoxifen (toremifene, raloxifene, idoxifene, GW 5638), benzothiophenes (raloxifene, SERM III — LY 353381), naphtalenes (nafoxidine, trioxifene, lasofloxifene-CP 336,156), benzopyran (centchromans — levormeloxifene, SCH 57050) and indoles (zindoxifene). Some of these compounds have similar effects on the endometrium as tamoxifen, have no breast cancer advantage over tamoxifen or have other unacceptable side-effects and are, therefore, no longer in clinical study (nafoxidene, trioxifene, zindoxifene, levormeloxifene) [49]. Nafoxidene and trioxifene have been tested in phase I and II breast cancer trials with similar objective results as tamoxifen but an unacceptable side-effect profile. Zindoxifene, a member of the indole derivatives, demonstrated only a marginal therapeutic activity in advanced breast cancer with a strong oestrogenic activity leading to an increase in sex hormone binding globulin (SHBG)-levels. In connection with long-term use of levormeloxifene (and others), an increased endometrial thickness, urinary incontinence and utero-vaginal prolapse have been reported. This drug, however, is no longer in development. Droloxifene, with its 20–60-fold higher affinity for the ER, its advantage over tamoxifen in preclinical breast and uterine studies has been compared with tamoxifen in women with advanced breast cancer. These studies have been prematurely stopped because no advantage was observed of droloxifene over tamoxifen. Of the other new compounds in development are GW 5638 and lasofoxifene or CP-336,156 already referred to as SERM IV [50,51]. SCH 57050, a benzopyran, has potent anti-oestrogenic characteristics in MCF-7 human breast cancer cells and it also prevents the development of dimethylbenz(a)anthracene (DMBA)-induced mammary tumours. This drug is the most potent known anti-oestrogen and most importantly, devoid of the oestrogenic activity observed with SERMs such as tamoxifen, toremifene, droloxifene and raloxifene [52]. LY-353381 is more potent than raloxifene as an ER-antagonist in the endometrium and breast and is more potent than raloxifene as an oestrogen-agonist on bone and serum cholesterol levels [53].

Another interesting drug is the non-agonist (pure) anti-oestrogen ICI 182,780 (faslodex). Faslodex leads to downregulation and loss of the ER [54]. This results in the complete abrogation of the ER function. Furthermore, faslodex inhibits the growth of tamoxifen resistant cell lines, inhibits the uterotrophic effects of tamoxifen and doubles the duration of response seen in the MCF-7 human breast tumour model [55]. In addition, in a small phase II study of 19 postmenopausal patients with advanced breast cancer, failing on tamoxifen therapy 37% of the patients achieved a partial response and a further 32% achieved stabilisation [56]. Faslodex is currently in phase III advanced breast cancer trials in postmenopausal women versus anastrozole and tamoxifen, with data versus anastrozole due to be available in the near future. Another steroidal anti-oestrogen believed to be devoid of any oestrogen-agonist activity is the Roussel compound RU 58668. At the

present time, there are no clinical data available for this anti-oestrogen.

6. How to manage the menopause following therapy for a hormone-dependent cancer?

The use of classical hormone therapy following breast cancer or endometrial cancer remains a very controversial area. Opinions range from those who maintain that HRT should never be prescribed, to those who give HRT on the basis that the potential risk of an increase in chance of recurrence is unproven. The results of ongoing randomised studies investigating the role of HRT in breast and endometrial cancer patients will have to be available before conclusions can be made on this issue.

Other type of agents that have been proposed as alternatives to hormone replacement therapy are soybean phyto-oestrogens (isaflavones). Early interest in this field was fueled by the lower rates of coronary heart disease (CHD) and breast and prostate cancer in Asia. Focus on soy is due largely to the high concentrations of isoflavones in soybeans. The primary isoflavones in soybeans are genestein and daidzein. Isoflavones are weakly oestrogenic. Depending upon the assay employed isoflavones possess between 1×10^{-4} and 1×10^{-2} the activity of 17β oestradiol on a molar basis. However, genistein binds with 5 to 20 times more affinity to ERβ than ERα [57]. Thus far, no controlled studies have examined whether soy or isolated isoflavones reduce cardiac events [58]. Although findings are somewhat inconsistent overall, they suggest that isoflavone-rich soy products favourably affect bone turnover and reduce bone loss at the lumbar spine. Findings from studies on the effects of soyfood on menopausal symptoms are mixed but they suggest that if soy does have an effect, it is a relatively modest one [59]. Case–control and prospective studies do not show that soy consumption is associated with a reduced risk of postmenopausal breast cancer [58]. Interestingly, human data indicate that soy does not increase endometrial cell proliferation and in animals soy consumption has been shown to inhibit the stimulatory effect of oestrogen on breast and endometrial cell proliferation. Messina [58] concluded that there are not sufficient data to recommend that soy and/or isoflavones be used in place of HRT.

Another natural product is tangeretin, a molecule present in citrus fruits and in certain 'natural' menopausal medications. Tangeretin is an effective tumour growth and invasion inhibitor *in vitro* of human MCF 7/6 breast cancer cells [60]. However, when added to the drinking water of MCF 7/6 tumour bearing mice it neutralises the beneficial tumour suppressing effect of tamoxifen [61].

Another drug, proposed as an alternative to HRT in patients with breast- or endometrial carcinoma is raloxifene. Raloxifene decreases bone turnover and increases bone mineral density. However, raloxifene does not alleviate early menopausal symptoms such as hot flushes and urogenital atrophy and may even exacerbate some of them [62]. Sismondi concluded that raloxifene may be an alternative for the prevention of long-term effects of oestrogen deficiency (osteoporosis and heart disease) in women with previous breast cancer not having hot flushes. For symptomatic patients different drugs which have demonstrated efficacy in the control of vasomotor symptoms are now under evaluation.

Tibolone is a well-known progestagen with partly oestrogenic and androgenic effects, and is now proposed as an alternative to classical HRT. Tibolone is known to reduce breast density on mammography when used in postmenopausal patients. Gompel and colleagues [63] showed that in breast cells tibolone slowed down the proliferation rate and increased differentiation and apoptosis. Tibolone is known to increase bone density in a similar way as oestrogens. However, clear data on the incidence of breast cancer in patients on tibolone are not available.

7. New aromatase-inhibitors in breast cancer

The first generation aromatase inhibitor, aminoglutethimide, has been replaced by more potent and less toxic inhibitors belonging to the triazole class (anastrozole and letrozole) and, more recently the steroidal aromatase inactivator exemestane [64]. An interesting observation is that patients may respond to oestrogen deprivation independent of their pre-treatment plasma oestrogen level. Thus, pre- as well as postmenopausal patients may respond to castration or use of an aromatase inhibitor, respectively, and there is evidence that patients previously exposed to hypophysectomy or adrenalectomy as well as patients on therapy with a low potent aromatase inhibitor may respond to further treatment with the new more potent aromatase inhibitors [65]. Lack of cross-resistance between the different endocrine treatment regimens suggests endocrine therapy may be improved in the advanced as well as in the adjuvant setting and sequential therapy with tamoxifen, followed by an aromatase inhibitor as well as the use of both drugs together is currently compared with tamoxifen alone for adjuvant therapy.

Based on the findings of two large identical phase III studies comparing anastrozole (1 and 10 mg) with megestrol acetate (160 mg qid) as second-line therapy in 764 postmenopausal patients with advanced disease, anastrozole has been shown to be associated with

significant improvements in both median survival and 2 years survival rates compared with the progestin [66]. Also letrozole (2.5 mg daily), which is also now commercially available, was reported as having a significant advantage over megestrol acetate in terms of objective response, duration of response and time to treatment failure [67].

While anastrazole and letrozole have shown clear evidence of benefit over existing agents as second-line treatments, the data regarding fadrozole, which is available only in Japan, and vorozole, which has not progressed beyond phase III clinical development, are more equivocal [68]. In a randomised double-blind multicentre trial performed in the US and Canada, designed to demonstrate equivalent efficacy of anastrozole (1 mg daily) relative to tamoxifen (20 mg daily) in ER-positive and/or PR-positive or unknown receptor status, patients eligible for endocrine therapy, included a total of 353 patients who were followed for a median of 18 months. Anastrozole was as effective as tamoxifen in terms of OR, with clinical benefit (CR + PR + SD > 24 weeks) observed in 59% of patients on anastrozole and 46% on tamoxifen. Anastrozole had a significant advantage over tamoxifen in terms of TTP ($P = 0.005$). Both treatments were well tolerated, however, thrombo-embolic and vaginal bleeding were reported in fewer patients on anastrozole. In a similar randomised European trial, 668 patients were randomised on a 1:1 basis between anastrozole and tamoxifen as first-line treatment in advanced breast cancer. In this study anastrozole was shown to be at least equally effective as tamoxifen as first-line hormonal therapy based on OR, clinical benefit and TTP. Furthermore, fewer cases of thrombo-embolic events and vaginal bleeding were seen in patients taking anastrozole. Based on the combined data from both these first-line studies, anastrozole showed superiority to tamoxifen in TTP ($P = 0.022$) in those patients known to be ER+ and/or PR+ [69]. Based on the findings of these trials versus tamoxifen, anastrozole has recently been approved for the first-line treatment of advanced breast cancer in postmenopausal women.

The steroidal aromatase inhibitor exemestane was evaluated in 769 postmenopausal patients with advanced breast cancer failing tamoxifen. The patients were randomised between exemestane and megestrol acetate in this double-blind trial [70]. Objective response rate was similar between treatments. Median TTP, time to treatment failure and overall survival was significantly longer with exemestane than with megoestrol acetate. Hence, on the basis of available data exemestane like letrozole and anastrozole should be considered in the treatment of postmenopausal patients with advanced breast cancer who have failed on tamoxifen.

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