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Oestrogen receptor mutations and their influence on breast cancer growth

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CHAPTER TWO

LITERATURE REVIEW

As a prelude to this chapter, it should be mentioned that the discovery of ER± prompted intensive research with regar Subsequently, when a second ER, ER², was discovered, t interest in the two ERs. However in recent years, this foc certain extent and this is reflected in the paucity of curre with the regulatory effects of epigenetics and microRNA Nevertheless, oestrogen receptors and their splice variants re area of study and this is reflected in the current literature that encompasses the field at large.

2.1 Hormones and Breast Cancer

2.1.1 Historical Perspective

A relationship between ovarian secretions and the growth o cancers was suggested as early as 1836 when Cooper noted fluctuati in tumour growth during different phases of the menstrual The use of oophorectomy for the treatment of inoperable breas

described by Beatson in 1896 (Beatson, 1896). This introd
ovarian secretions were somehow necessary for the mainte
breast tissue. In the 1940 s introduced was the treatmen
prostatic cancer (Huggins and Hodges, 1941) thereby revivi
Beatson s observations. Ablative endocrine therapy, re
oestrogenic hormones, either by oophorectomy or by abla
sites of oestrogenic hormone production, became an accept
with advanced breast cancer. Additive hormonal treatment became avai
1950 s with the commercial production of steroid hormon
described to cause regression of female breast cancer (Nathan
oestrogen, which appeared to be necessary for the mainte
breast cancer in younger women, was found to be be
postmenopausal women (Haddow, 1944; Carter, 1977; Henderson, 1987
Other agents used in additive hormone therapy include oestrogen antagonists
such as tamoxifen (Cole, 1971; Ward, 1973)

2.1.2

The Role of Hormones in Breast Cancer

The mammary gland, which is not completely formed at bir
early puberty when the primitive ductal structures enlar
branching of the ductal system becomes more complex an
form at the ends of terminal ducts to produce terminal lobu
menstrual cycles have been shown (Anderson, 2002). The development of norm
mammary tissue is the result of complex interactions be

hormones and growth factors including steroids, insulin
factors. Genomic mediated steroids, which include induc
mitogenesis, occur following significant perturbations in th
number of genes (Parker, 1983). Oestrogen and progesterone are
alveolar formation, ductal branching (Meites et al 1975) and for
full lobulo-alveolar development (Warner, 1978).

The ductal system of the human mammary gland is lined by
luminal epithelial cells surrounded by a layer of myoepith
direct contact with the basement membrane. The TDLUs are surrounded
delimiting fibroblasts and are embedded in mammary adipose
carcinomas appear to arise from epithelial rather than my
glandular epithelium, progress either intraductal or intralobu
Epidemiological evidence indicates that exposure of oest
mammary gland is a potent factor in breast cancer risk (Henderson et al 1988).
Increased exposure to endogenous oestrogens may occur as a result of e
menarche, late menopause (Pike et al 1983) and late age-at-first full-
pregnancy (Henderson et al 1988). Obesity has also been implicated
breast cancer risk possibly due to increased peripheral conversion of
androgens to oestrogens (Siiteri et al 1981, Helmrich et al 1989).

Oestrogen stimulated cell growth appears to require the pr
affinity, low capacity binding sites (Horwitz et al 1976). In human mammary
epithelial cells the "classical" oestrogen receptor (ER) is
receptor (King and Greene, 1984; Nelson et al 1990). ER is thought to be the

factor mediating the cellular response to ER. The presence of ER has been detected in cells of developing breast tissue (Boyd et al 1996; Keeling et al 2000) but in normal mature human breast tissue strong clearly ER negative (Boyd et al 1996). A small proportion (6 7%) of epithelial cells ER is positive (Peters et al 1987). Levels of ER fluctuate with the menstrual cycle (Baetkar et al 1987). The myoepithelial cells appear ER negative (Jacquemier et al 1990). ER has been observed in both epithelial and stromal tissue (Carlsson et al 1998; Palmieri et al 2002).

The presence of ER in breast tumours is an indication that these arise from within ER positive epithelial cell populations of normal tissue (Clarke et al 1990). Approximately two-thirds of all breast tumours exhibit detectable levels (McGuire et al 1975) and the majority of ER positive tumours are also associated with adjacent tissue which is also positive (Nettoo et al 1990).

There is already a marked heterogeneity of ER expression by the time a breast tumour mass is detected (Van Nieuwenhuyzen et al 1988) and this may be due to dedifferentiation of ER positive populations resulting in the formation of negative populations. ER negative breast tumour cells have been observed less well differentiated than positive (Naeb et al 1988) and poorly differentiated tumours (histological grade all) are negative new compared to well differentiated tumours (grades all 1988; Singh et al 1988). Some predominantly ER negative tumours may also

arise from within the normal populations (Bruner et al 1990). The stages at which cell dependence on oestrogen and t expressed ER and thus acquire resistance to endocrine manipula the progression of the disease.

There has been a statistically significant association bet progesterone receptor (PR) status (Kahan et al 1994). PR synthesis is a kn consequence of oestrogen-ERDNA binding (Sherman et al 1972; Fuber et al 1978) and therefore the determination of PR may be used as a index of oestrogenic action, hormiveness (Horwitz et al 1975; De Sombre et al 1979; McGuire, 1980) and prognosis in breast ca 1977; Mayer et al 1978; Coot et al 1979; Pietron et al 1980; Clark et al 1983; Sotez et al 1958.

2.1.2.1

Mechanism of action of ER

Recent evidence suggests that oestrogen ER is located in or near plasma membrane and rapidly activate other oestrogen signalling p action in the nucleus (genomic action) has now been termed nuclear steroid signal (NISS) and oestrogen action at the non-plasma m genomic action (termed membrane-initiated steroid signalling (MISS) et al 2003).

2.1.2.1.1

Nuclear-initiated steroid signalling (NISS)

The hormone-regulated nuclear transcription factor, ERα, can induce expressio
of a number of genes such as PRG (Horwitz and McGuire, 1978) and bind
activation, ERα binds to oestrogen response elements (EREs) in
recruits a coregulator complex (CoReg), and regulates transcription spe
(Figure 1, pathway A) (Beato, 1989; Horwitz et al., 1996). ERα may also act
without DNA binding by modulating gene expression at regu
as AP-1 (Kushner et al., 2000), Sp1 (Safe, 2001), nuclear factor κB
(USF) sites (Gao and Archer, 1996) (Figure 1, pathway B).

Coactivators and corepressors are coregulatory proteins that
and modulate chromatin structure to facilitate or repres
(Dobrzycka et al., 2003; Smith and O'Malley, 2004). The recruitment of
coregulatory proteins to the receptor complex is influen
accessibility of the various coactivators and corepressors
Selective ER modulators (SERMs) such as tamoxifen, that ha
agonist/antagonist properties, may therefore have tissue-specific effects
to the absolute and relative levels of ER coregulator pro
associated with both de novo and acquired resistance to endocrine therap
2003).

The expression of ERα is regulated through DNA methylatio
condensation of promoter regions via hypermethylation of C

promoter which is associated with a marked expression in E
among ER± expressing cancer cell lines. The inhibition of DNA
methyltransferases reactivates ER± expression in these cel
1994).

ER± may also interact with other nuclear proteins involved w
Interaction with BRCA1 results in a reduction of ER (Fan et al
1999), may be due to increased CpG methylation of the ER± p
(Archey et al, 2002). Similarly, Da1 may be regulated by ER± (Zwijsen et al, 1997; Liu et al, 2002).

2.1.2.1.2

Membrane-initiated steroid signalling (MISS)

There is growing evidence that oestrogen can have rapid cell
long before its effects on gene transcription. This suggest
action (Levin, 1999). ER± can be detected in membrane, the
plasma membrane, where it can interact with and modulate several signalling
type of signalling is known as membrane-initiated steroid signalling or
(Figure 1, pathway c).

Figure 1. Oestrogen Receptor Action

(adapted from Ali 2005)

NISS, nuclear-initiated steroid signalling; MISS, membrane-initiated steroid signalling; ER, oestrogen receptor; CoR, Coregulator; P, ERE, oestrogen response element; E2, estradiol; SERM, selective oestrogen receptor modulator.

This membrane ER has been demonstrated by numero
immunohistological and genetic methods (Li et al., 2002; P
Razandi et al., 2003; Pedram et al., 2006) and can potentially interact wi
activate phosphorylation several membrane tyrosine kinases
cells. Direct interaction of ER± with HER2 in the membrane has
protect HER2-expressing breast cancer cells from tamo
apoptosis (Chung et al., 2002). Interaction of the growth factor recept
(IGFR) and membrane ER± has been described to activatio
IGFR downstream signalling that is stimulated by tamoxifen
either fulvestrant, oestrogen (Huynh and Pollack, 1993), or
mitogen activated protein kinase (MAPK) (Kahlert et al., 2002).

Epidermal growth factor receptor (EGFR) can also be p
activated by oestrogen activated membrane ER± in a process involv
of G-protein, Src, and matrix metalloproteinases (MMP3) (Razan
Again this is stimulated by oestrogen and SERMs like tam
fulvestrant.

2.1.3 Prognostic Indicators in Clinical Breast Cancer

The steroid receptor content of human breast cancer has
valuable both in providing prognostic information in pati
cancer and in identifying patients who are likely to respond
to endocrine treatments (Davies et al., 2003). There are also

other valuable prognostic indicators such as epidermal g
Her-2/neu oncogene, ploidy and S phase fraction.

2.1.3.1

Oestrogen Receptor Status

ER status is useful as a prognostic indicator of the course
with ER positive breast tumours have a longer disease-free interval and bette
overall survival than those with ER negative tumours, regardless of lym
status (Reitch, 1978). ER positivity has also been linked to histol
differentiated tumours. The more poorly differentiated ER negative tumours
appear to recur earlier than poorly differentiated ER positive tumours and have
very unfavourable prognosis (Mayr, 1978). Smaller tumours sho
higher rate of ER positivity (Clark, 1984). ER status is significantl
associated with age and menstrual status (Tasto, 1983). Thus cancer
arising in young women is low in ER-NA (Anderson, 2008)
Post-menopausal women show the highest prospectivity of ER
increases with each decade of age after the menopause.
studies have found to be ER independent prognostic factor i
positive and node negative early breast cancer (Mayr, 1978; Thomas
1987).

2.1.3.2

Progesterone Receptors

Progesterone receptors (PR) are ligand-activated transcription factors
steroid hormone family of nuclear receptors which include the ERs. There are two
isoforms of PR in human cells, PR-A and PR-B. The molecular weights of t
forms are 95000 and 120000 respectively and they appear t
alternative translation start sites on the same RNA (Kastner, 1990). transcr
Human PR-B is 933 amino acids in length and contain a unique activation
function AF3 (Sartorius, 1994). PR-A lack the 164-N terminal residues that
contain AF3 is 769 amino acids in length. The breasts of normal wom
express equimolar ratios of PR-A to PR-B (Mote, 2002).

The inclusion of total PR measurements has been observed to in
predictive values of steroid receptor analyses in certain cancers. In particu
the ER-positive/PR-negative tumour type is less likely to respond
tumours that are positive for both receptors. Primary tumo
and PR contents are also associated with favourable survive
al, 1984; Sahnek Godolphin, 1989; Koenders, 1992; McCormack, et al
2007). Elevated PR levels significantly and independently cor
probability of response, longer time to treatment failure, an
overall survival in patients with metastatic disease (Reynolds et al, 1992; Stearns et al
2006). Response is positively and directly related to PR le
with ER± (Elledge et al, 2000). During tamoxifen therapy, levels of bo
ER decrease but PR levels decrease more dramatically tha

been observed that up to half of the tumours lose PR exp
they develop tamoxifen resistance (Ravdin et al 1984). PR excess has also been
reported to be associated with resistance to tamoxifen (Hop
clinical outcome (Mohr et al 2002). PR-B, on the other hand, are str
transcriptional activation increased production of PR-B, resulting from a
functional promoter polymorphism, is associated with an in
cancer (De Vivo et al 2003). PR isoform ratios therefore also be impor
predictive factors for endocrine therapies.

2.1.3.3

Epidermal Growth Factor Receptor (EGFR)

The EGFR belongs to the erbB family of four closely rel
receptors, namely, EGFR (HER1-B), erbB-2 (HER2), erbB-3 (HER3),
and erbB-4 (HER4), and is believed to play an important canc
development (Cohen et al 1998; Gullick and Srinivasan et al 1998; Kl
2000; Holbro et al 2003). These receptors are transmembrane gl
consisting of an extracellular domain binding ligand to transmembrane
and a cytoplasmic tail that involves a domain tyrosine kinase for signal
transduction. EGFR activation occurs when a ligand, such
factor (EGF), transforming growth factor (TGF) or amphireulin, binds to
extracellular domain, the receptor to dimerize. Tyrosine kin
tyrosine autophosphorylation then occur, leading to mitoge
cellular activities (Alroy and Yarden, 1997).

Activation of EGFR has been shown to enhance processes r
growth and progression, including the promotion of prolif
and invasion/metastasis, and inhibition of apoptosis (Wo
2000). Overexpression of EGFR has been associated with en
potential of some breast cancer cell lines (Bitzer et al. 1984; Patrick 1986)
and has been correlated with disease progression, poor
response to therapy in clinical studies (Salmon et al. 1987; Tosi et al. 1991
Anders et al. 2008).

21.3.4

HER2/neu oncogene

The HER2/neu oncogene codes for a transmembrane tyrosine k
been suggested as an etiologic factor in several kinds of ca
growth factor receptor-2 positivity (HER2) has been found in up to 25%
with early breast cancer and has been associated with a
higher likelihood of recurrence after initial treatment, and
et al. 2006). A number of studies have also shown that increased expressi
HER2/neu has significant prognostic value for decreased s
negative breast cancer (Rolkl et al. 1994; Reed et al. 1995; Rosen et al. 1995;
Press et al. 1997; Reed et al. 2000; Anders et al. 2008). HER2/neu is routinely
measured in patients to predict the likelihood of their response to Trastuzumab
(Herceptin®, F. Hoffman-LaRoche Ltd., Basel, Switzerland), a r
antibody directed against the extracellular domain of HER2/neu. Should they relapse
after standard adjuvant-therapy, HER2/neu positivity may also predict respons

other adjuvant therapy regimes. HER-2 impositive tumours treated w doxorubicine (Pai et al 1998) or paclitaxel (Blasberg et al 1997) have shown a significantly improved response.

2.1.3.5

Ploidy and Phase Fraction

Analysis of tumour ploidy (DNA content) and SPF (phase fraction) has been demonstrated to be important as a marker for neoplastic progression and differential descriptive versus malignant tumour with high tumour proliferation indicates an adverse prognostic factor in surgicall (Barlogie et al 1980; Kute et al 1981). Previous methods of cell kinetic using tritiated thymidine labelling were expensive and time DNA flow cytometry is the powerful technique used to evaluate content and cell proliferation of breast cancer. Cell material (Spiratos obtained by fine needle aspirate is particularly suitable f analysis as it contains a lower proportion of non-tumour cells than tissue sam (Gazet et al 2008). Knowledge of the cell cycle is necessary in o ploidy and SPF

Cells which are not involved in cell division are referred $_0$ phase. Once cell division is triggered in the $_1$-phase of the cell cycle during which the amount of RNA increases and certain p replication of DNA are made. As the cells start to synthesi the synthetic phase, or S phase of the cell. During this phase the D

content of the cells increases until it has doubled. It is thi
the proliferative capacity of the cells. DNA synthesis enters the
G_2-phase. Finally, the cells dividing by mitosis and, if cell
division is to be sustained, return to the G_1-phase. The cells return to the G
if they are to divide (Figure 2).

Cells in the G_0-phase have diploid (2n) DNA content and ar
designated the G_0/G_1-phase. Cells in the G_2-phase have tetraploid (4
DNA content and are referred to as G_2-phase cells. Cells in the S-phase have a
DNA content varying between these two extremes. The mean DNA content o
diploid G_0/G_1-phase cells is a measure of the cell population
Aneuploidy is the term used for an abnormal number of ch
reflected by a change in content of DNA. Tumours with a
less than 2n are considered hypodiploid whereas greater
hyperdiploid.

Figure 3 shows a wide variety of DNA histogram types in
tumours analyzed by flow cytometry (Coulson et al. 1984). Normal human
mammary tissue (Figure 3A) and diploid breast cancer cell
aneuploid peaks. The first peak represents the G_0/G_1 nuclei containing the 2
(2c) complement of DNA. The smaller peak represents the G_2-nuclei containing
4n (4c) amounts of DNA, with the population between these
nuclei. Peaks which are greater than G_0/G_1 and G_2-peaks contain
aneuploid nuclei.

Diploidy, hyperdiploidy, and tetraploidy have been associa
survival (Toikkanen, 1989; Fisher et al, 1991). A significantly poorer s
has been observed in breast cancer patients with hypodiploid, hypotetra
hypertetraploid tumours (Bleeman et al, 1990; Fernöl et al, 1992; Balslev et al,
1994). Hypodiploidy and hypotetraploidy are associated w
high histological grade, and low expression of ER and PR (Fernöl et al, 1992,
Balslev et al, 1994). Hypertetraploidy has been associated with
and advanced tumour stage (Bleeman et al, 1990) and the worst clinical o
(Pinto et al, 1999). High SPF has been found to indicate an
recurrence and mortality in patients with breast cancer (Me
et al, 1993; Campleje et al, 1995; Gazic et al, 2008) and is associated with nuc
grade, tumour size, ER, PR, lymph node metastasis, and a
Fernöl et al, 1992; Stål et al, 1993).

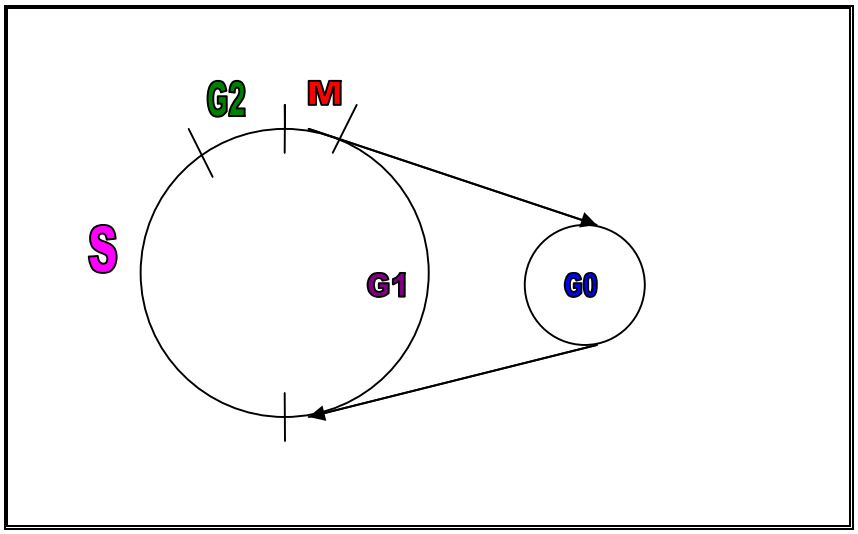

Figure 2: Diagram Illustrating the Cell Cycle
(adapted from Ormerod, 1990)

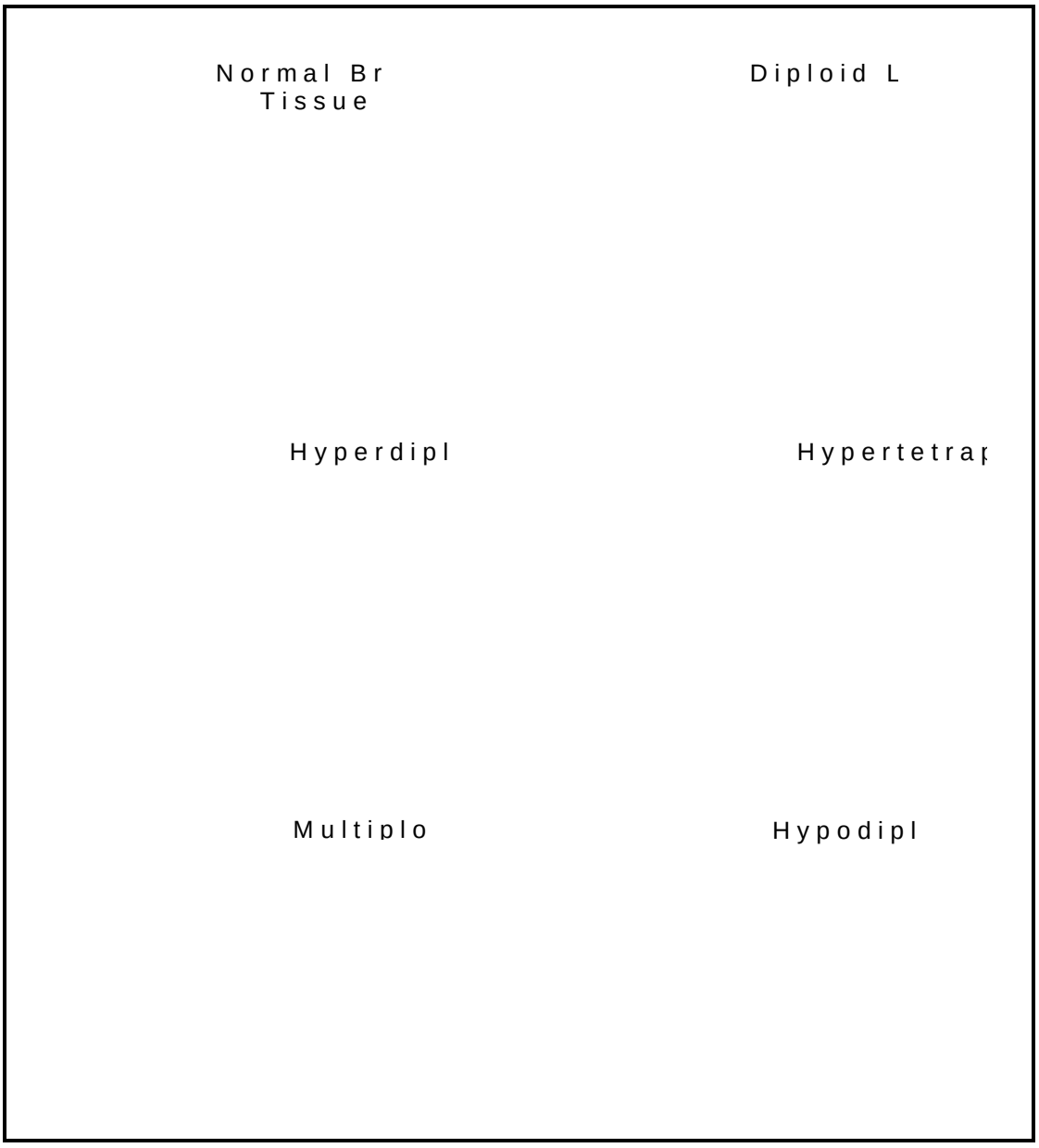

Figure 3. DNA flow cytometer histograms of mammary tissue

(Coulson et al, 1984)

Each section represents the number of nuclei measured (Y
relative intensity of fluorescence (DNA-axis) per nucleus
2c = 2n and 4c = 4n (in the text).

2.1.4

Choice of Treatment

2.1.4.1

Oestrogen Receptor Antagonists

The presence of ER has been shown to assist in the identif
to respond to endocrine (Osborne et al., 1980). ER positive tumours
respond to endocrine therapy 50 to 70% of the time whi
associated with a response rate of less than 10% (McGui
incidence of response to hormone therapy increases with
(Wittliff, 1984). The oestrogen antagonist, tamoxifen,
ethylene derivative (Figure 4) and is frequently used first line endocrine ther
for breast cancer as it interrupts the stimulatory effects of
and blocking the ER (Encarnación et al., 1993; Figure 5). The response to this
drug has been shown to depend on the presence of ER (Rose et al., 1985;
Glauber and King, 1992) and therefore reduces the incidence of
tumours but has no effect on ER negative tumours. Tamoxifen also decrease
circulating cholesterol which is thought to be beneficial in osteoporosis
However, tamoxifen has shown oestrogen agonist properties in
increased risk of endometrial cancer in postmenopausal women (Fisher
1998)

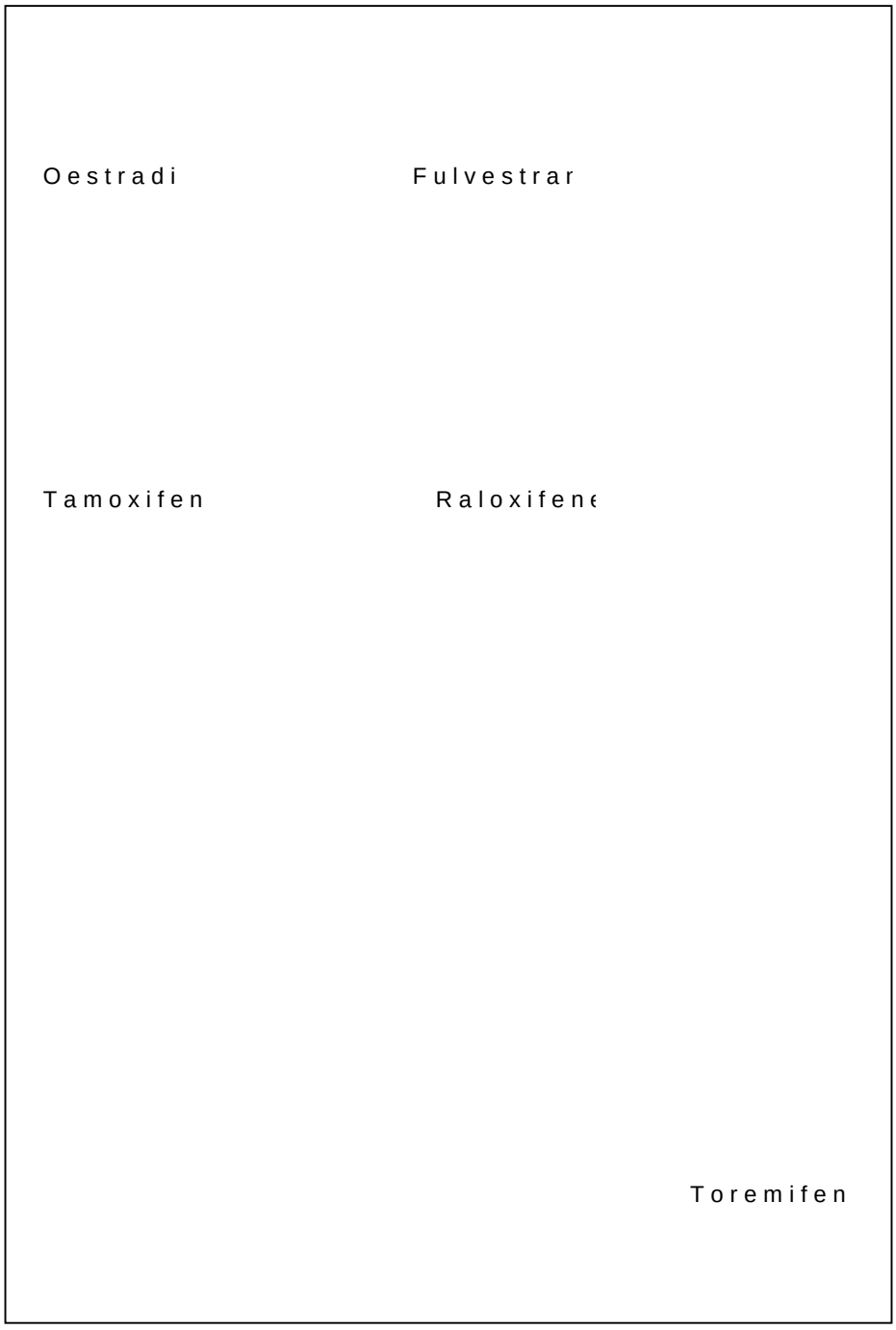

Figure 4 Chemical structures of oestradiol, tamoxifen, fulvestrant, raloxifene and toremifene

Clinical resistance to antioestrogens like tamoxifen is a
treatment of hormone-dependent breast cancers. It has been hypo
mutations in the ER could mechanism by which breast tumo
from a hormone-dependent to a hormone-independent phenotype (K
1994). It has also been suggested that the resistance to tam
an increase in cell surface signalling through the Insulin l
growth factors that promote phosphorylation of the ER
(Jordan, 2004; Gupta and Jordan, 2008) which in turn activates breast
cell growth.

First-generation SERMs, such as tamoxifen (Fareston®), toremifene
(Figure 4) droloxifene and idoxifene, were based on the struct
showed no benefits over tamoxifen and are all partial agoni
second generation SERM (Evista®), a fixed benzothiophene
derivative (Figure 4) exerts oestrogenic activity in the breast and
agonistic activity in bone and the cardiovascular system (
2001). Initially raloxifene (originally named keoxifene) was
cancer treatment (Celtek, 1983) but its use was abandoned in
1980s as clinical trials indicated no superiority to tamoxifen
et al., 1988). According to the more recent Study of Tamoxifen
(STAR) trial both SERMs are equivalent in the prevention of inva
cancer, but tamoxifen is slightly better in preventing non-invasive breast cancer
Raloxifene, however, has fewer side effects than tamoxifen

The discovery of an ER subtypeb (Kuiper, 1996; Mosselman et al, 1996), which is highly homologous with the classical ERa and has been shown to bind oestrogens with an affin ERa, has added a new dimension to the problem of tamoxifen resistance an progression of the disease (Sedeas 2.3).

A more recent oestrogen receptor antagonist used in the treatment of adva ER+ positive breast cancers fulvestrant (®), a 7-alkylsulphinyl analogue of 17-oestradiol that is structurally distinct from the non oestrogen antagonists (Fig 4). The C7 side chain of fulvestrant dif from oestradiol and its position, length and flexibility antagonistic activity (Bowler, 1989). Fulvestrant, which competitively bin the oestrogen receptor with a much greater affinity than ta 1991), prevents oestrogen receptor dimerization, inhibits oes binding and leads to a down-regulation of oestrogen receptor (Wakeling et al, 2000; Figure 6). Fulvestrant may help to circumvent hormone pathways of receptor cross-reducing cellular ER levels (Hutc 2003). After fulvestrant treatment tumour sensitivity to sub therapies appears to be maintained (Vergote et al 2003; Robertson 2005).

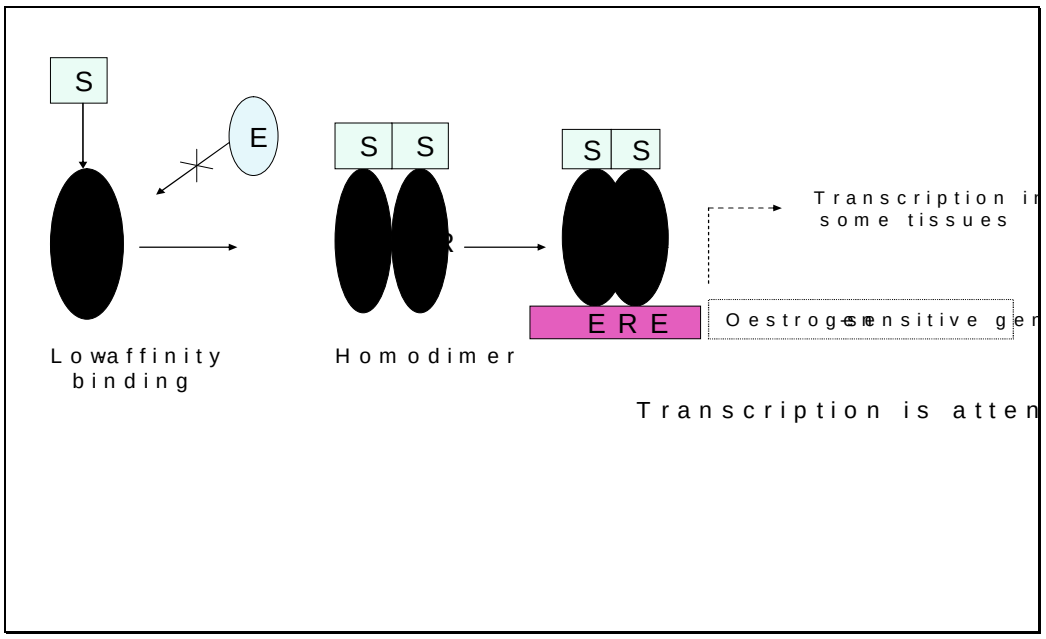

Figure 5. SERMs action on ER in breast cancer

(adapted from Morris and Wakeling, 2002)

SERMs (S) compete with oestrogen (E) for binding to the transcription of oestrogen sensitive genes. ERE, oestrogen response elemen

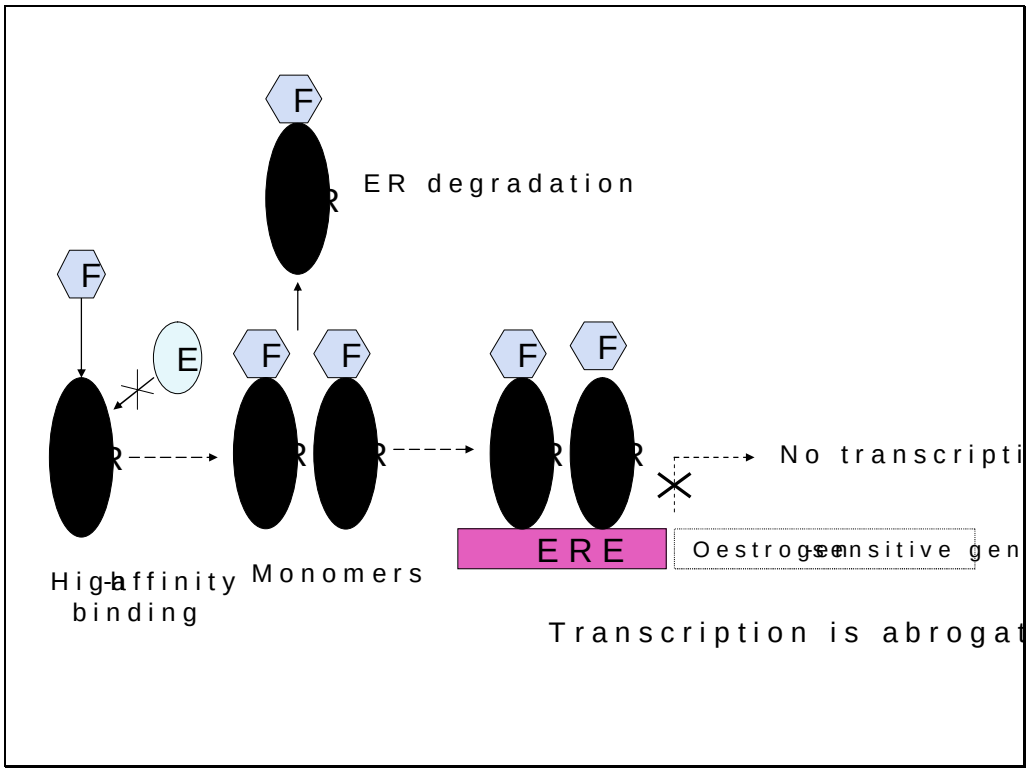

Figure 6 Fulvestrant action on ER in breast cancer (adapted from Morris and Wakeling, 2002)

Fulvestrant (F) competitively inhibits the binding of oestrog prevents dimerization, potentiates degradation and prevents transcription of oestrogen-sensitive genes. ERE, oestrogen response element.

2.1.4.2

Aromatase Inhibitors

The aromatase inhibitors (AIs) act by blocking aromatas
catalyzes the final and rate-limiting step in the synthesis of oestrogen
is an enzyme complex consisting of a cytochrome P450 protein and
NADPH cytochrome P450 reductase (Howell and Buzdar, 2001). Aromatization of
the androgen, androstenedione, proceeds via three successive oxidatio
resulting in the formation of the oestrogen product, oestrone (Figure 7),
oestradiol.

The expression of aromatase is tissue specific and is c
different promoters. The most significant site of aromatase activity in premenopa
women is in the ovaries where it is regulated by the gon
hormone (LH) and follicle stimulating hormone (FSH). Aromatase
expressed at lower levels in nonovarian tissues such as mu
tissue in both premenopausal and postmenopausal woma
postmenopausal women that these nonovarian tissues becor
sources of oestrogen, where the expression of aromatase is not regu
FSH (Hemsell et al., 1974). Aromatase has also been measured in the
component of normal breast and breast tumours. The expre
highest in or near breast tumour tissue (Miki et al., 2005; Suzuki et al., 2003).

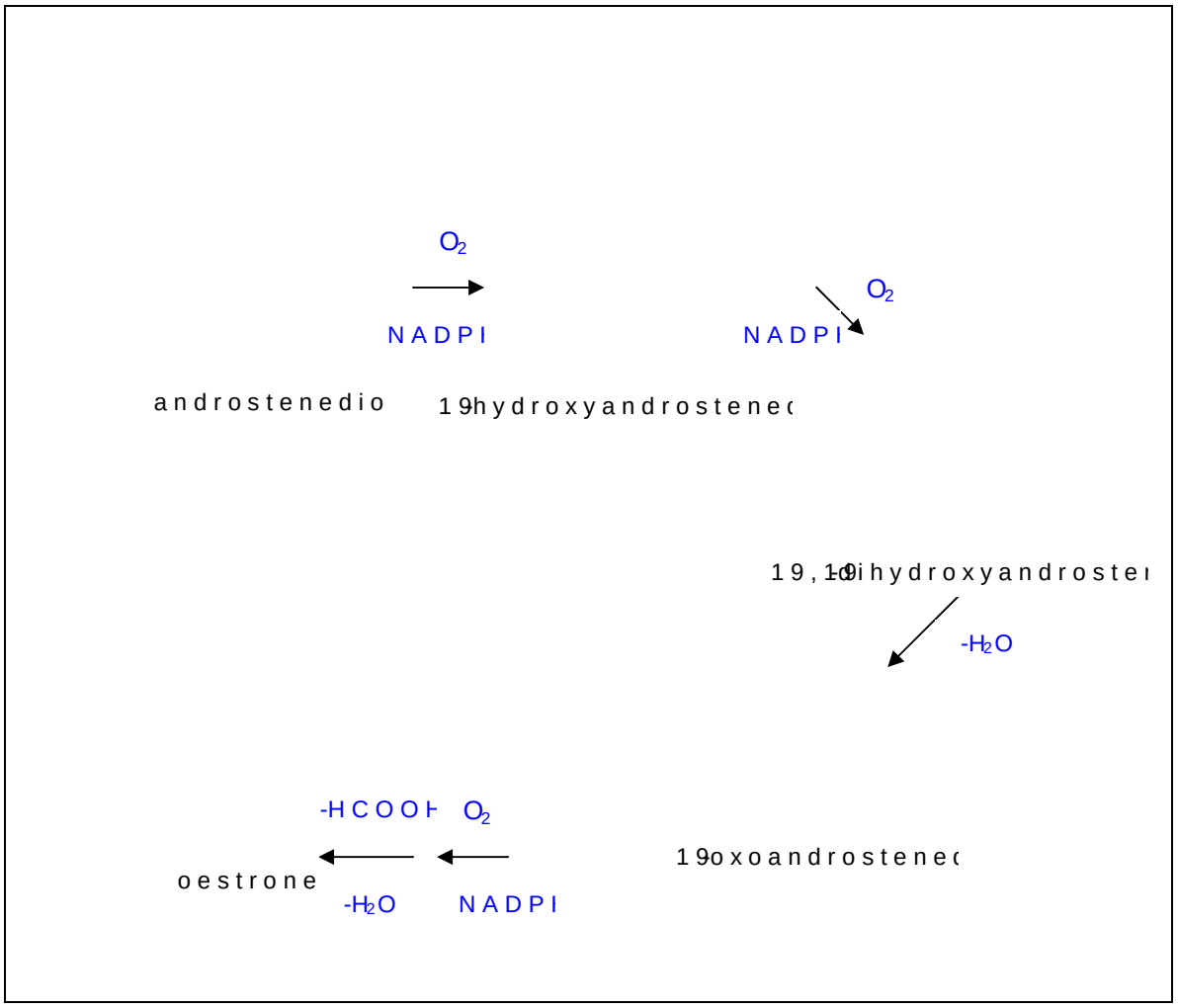

Figure 7. Aromatase enzyme reaction (from Brueggemeier 2005)

Aromatase inhibitors block the conversion of androstenedione and testosterone to oestradiol (Figure 8) and lower the leve tumour. This is particularly relevant in postmenopausal women as oestrogen source is derived from the conversion of androst the adrenals) to oestrone and oestradiol in the peripheral premenopausal women AIs induce increased regional drop in ovarian stimulation and potential increase in ovarian size ovarian cysts. AIs are therefore not indicated in premenopa 2008).

Other steps in the synthesis pathway of catalyzed hormone enzymes related to aromatase. Early aromatase inhi aminoglutethimide, had limited selectivity over these oth treatment of patients with these inhibitors required corticosteroids with toxic rtesulcities. A-generation 4AI, hydroxyandrosterone (formerstane and fadrozole) had increased selectiv and fewer-side effects than aminoglutethimide but not shown any benefit o tamoxifen (Wiseman and McTavish, 1993).

Aromatase inhibitors are categorized by generation and by mech There are two types of third-generation aromatase inhibitors, irrevers activators and reversible non-steroidal inhibitors (Figure 9). Both types interfere with the step of oestrogen biosynthesis but they mechanism (Figure 8). Steroidal agents, such as exemestane®, have an an androgen structure and compete with the aroma

androstenedione. They bind irreversibly to the catalyse caus
loss of enzyme activity. Before oestrogen biosynthesis
aromatase enzyme must be produced. This is reflected by the decre
aromatase activity observed in vitro systems (Miller, 2000).
steroidal agents are often referred to as suicide inhibitor
inactivators. Due to their steroidal structure, exemes
hydroexemestane metabolite have potentially androgenic ef

Nonsteroidal imidazole agents, which include anastrozole (Arimidex®) and
letrozole (Femara®), bind reversibly to the haem portion of th
molecule (Buzdar and Howell, 2001) and are associated
aromatase activity when tested in vitro systems. This may occur as
biosynthesis of oestrogens in the presence of reversible ste
require synthesis of new aromatase (Miller, 2000). This i
activity may be important to the development of tumour res
long-term treat. Both anastrozole and letrozole have sh
advantages over tamoxifen in postmenopausal patients a
(Bonneterre, 2000; Nabholtz, 2000; Mouridsen, 2001).

Aromatase inhibitors are supplanting tamoxifen as the most widely used hormo
agent in the treatment of breast cancer particularly in pos
they improve disease-free survival and decrease the risk of endo
(Coombes, 2004; Boccardo, 2005).

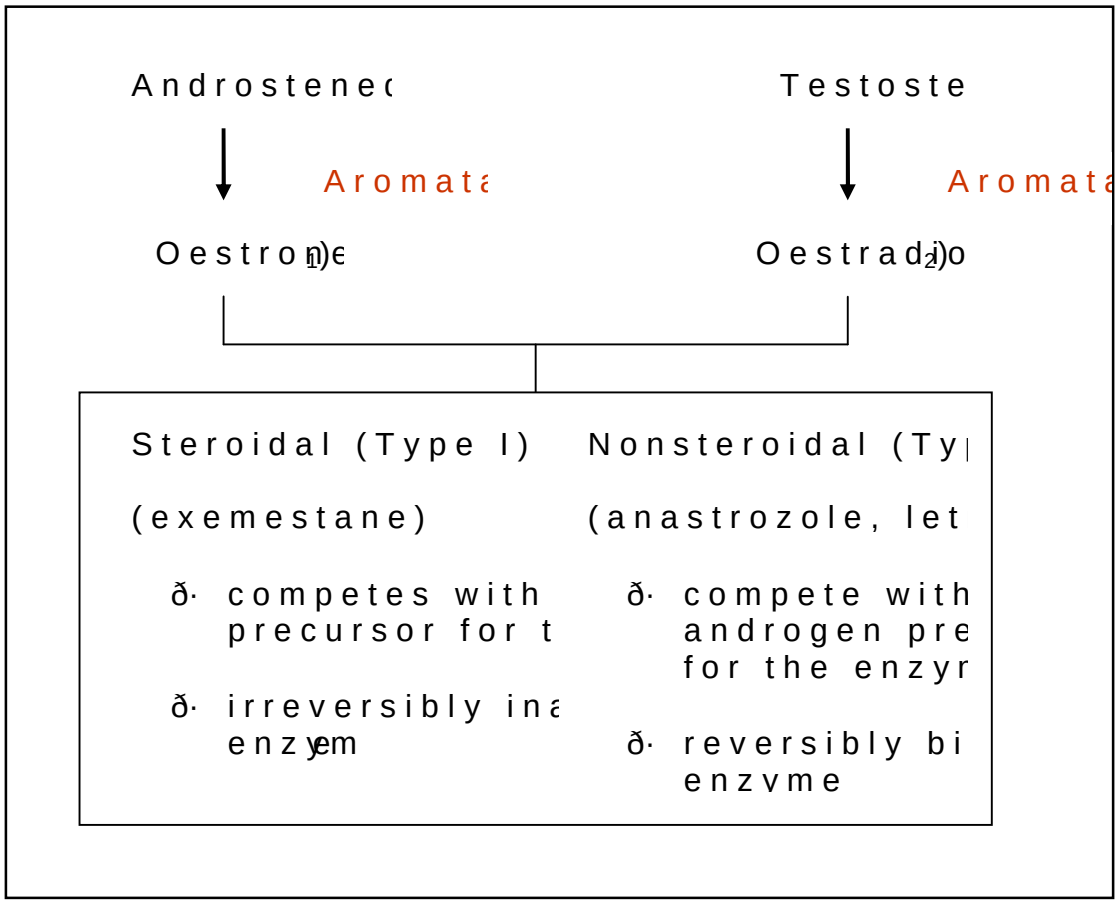

Figure 8. The third generation aromatase inhibitors: mechanism of action (adapted from Lake and Hudis, 2002)

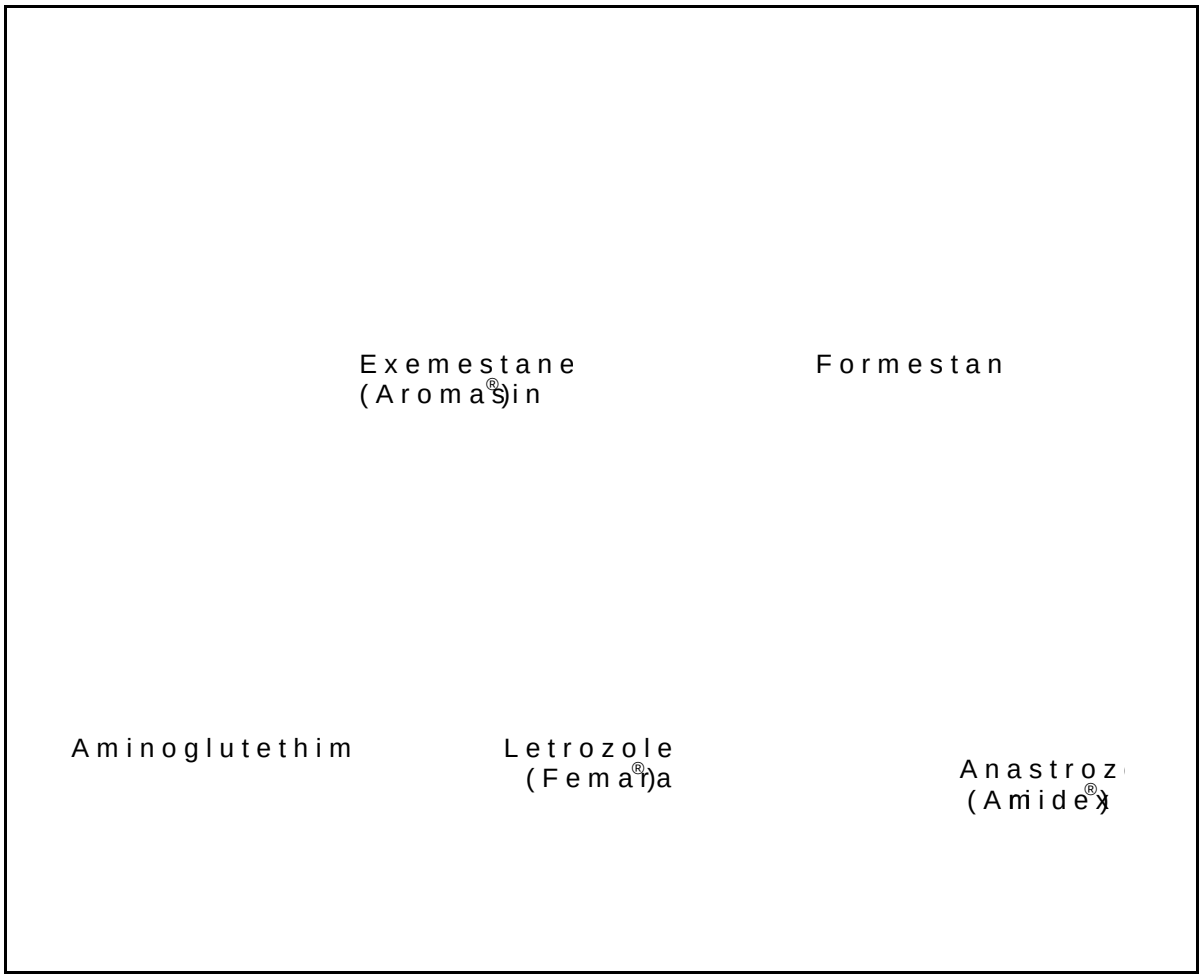

Figure 9. Chemical structure of different aromatase inhibito

(adapted from Lønning 2004, Brueggemeier et al. 2005)

Current data suggest that there is a lack of cross-resistance between steroidal nonsteroidal agents as clinical benefits may occur when prescribed following a steroidal agent, and vice versa, anastrozole given after exemestane (Lonning, 2000) shows benefit as does exemestane anastrozole (Bertelli, 2001). This allows for another hormonal therapy before switching to chemotherapy in women with advanced b

Recently, new synthetic AIs obtained by modifications of in the androstenedione, 3-ethyl-androstene-17-one and 3-ethyl-4-5-androstene-17-one, have shown to be strong inhibitors of aromatase (Cepa et al, 2005) and cancer cell proliferation (Cepa et al, 2008).

2.1.4.3

Progestagens

Although anti-oestrogens have been the first choice of hormon therapy for human breast cancer, progestagens of the pre-menopausal breast cancer has also been used (progesterone and synthetic progesterone-like compounds) mainly through the progesterone which has been described in 3.2. Five different progestagens have been used historically in various concentrations, route schedules and lengths of treatment. These include 17-progesterone (Figure 1.7), hydroxyprogesterone, norethisterone acetate (NA), medroxy (MPA), and megestrol acetate (MA) (Setdale and Horwitz, 1984). The synthetic progestagen, medroxyprogesterone acetate (Figure 1.0) is a 17-

hydroxyprogesterone derivatives frequently until the early 1
second-line hormonal therapy for metastatic breast cancer (Pacaprenist 1993).
Following the advent of tamoxifen and aromatase inhibit
decline but there has been renewed interest in alternative h
including MPA, for use both in early disease and in the a
conventional therapies (Fiocentini 2004, Lauchet al 2004). Their apps
limited, however, by the occurrence of weight gain and oedema
cardiovascular and thromboembolic side effects. Recent studies indicate that
and other progestagens may have an impact on the progression of
breast cancer by enhancing the ability of cancer cells to interact with
extracellular environment and to invade the surrounding virgin tissue (Fuettsal
2008). These results suggest that PR activation may play
positive breast cancer progression.

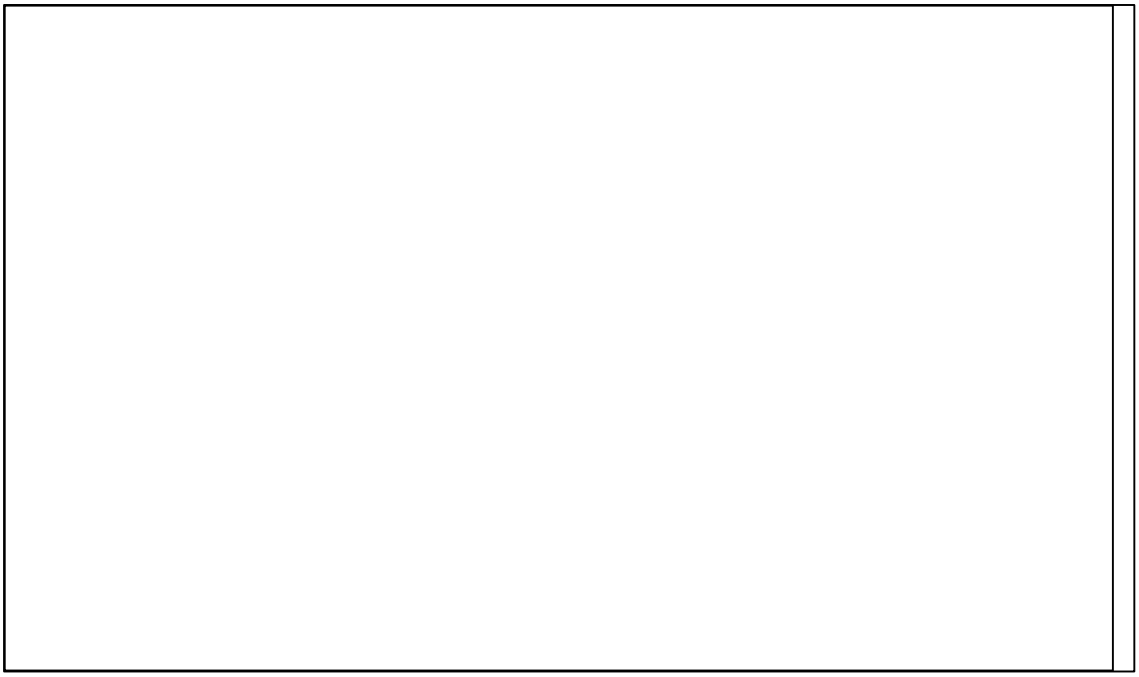

Figure 10. Chemical structures of MPA and DHT,

(adapted from Ghattas et al 2005)

DHT, dihydrotestosterone; MPA, medroxyprogesterone aceta

The pharmaceutical name and manufacturer's text are shown in ita

2.2

Oestrogen Receptor

The classical ER, now, was cloned by Walter and co-workers in 1985. The cDNA sequence was determined in 1986 by G genomic organization was described by Pongkolaisan ligand-activated transcription factor which binds to specific responsive DNA elements which behave as enhancers (Yar ER gene has been localized to human-chromosome 1985 2.4 (Wadsworth, 1985).

2.2.1

Molecular Structure of the ER gene

ER is composed of several domains important for hormone b and activation of transcription. The 66 kDa protein coded b 6.6 kb mRNA, comprised of six functional domains de (Figure 1).

Region A (encoded by part of exon 1) and region B (encod and 2) constitute the terminal part of the receptor in which transactivation function elements (AF1) is located (Kumar et al. 1987). In the A/B region contains a cyclic domain, which binds ER coactivators and corepressors-mediated transcriptional acti

Region C is a 55 amino acid region which has the potential to fo
zinc-finger motifs which are responsible for binding the ER to
site called the oestrogen responsive-element (ERE) (Klein
This region is encoded by exon 3 and parts of exons 2
Chambon, 1987). It also contains a nuclear localization signal so that
targeted to the nucleus after translation.

Exons 2 and 3 are separated by an intron of greater than
between the two zinc fingers (Ponglikitmongkol et al., 1988). Point mutation
replacing two cysteines by two histidines in the first zinc finger of the DNA b
shown to prevent the activation of gene transcription.

The replacement of the 66 amino acid region of the human ER
glucocorticoid receptor (hGR) in the rat has resulted in the expression of a glucoco
inducible gene, not an oestrogen inducible gene, in the p
(Green and Chambon, 1987) thus indicating that not only is
activation of transcription but it also contributes to the specificity of ta
genes.

Region E, encoded by exons 5 to 7 and parts of exons 4 a
binding domain and is involved in several other functions
and hormone-dependent transcriptional activity (AF-2) (Tora et al., 1989). The
domain folds to form a hydrophobic pocket, which binds th
(Green et al., 1986). The presence of an unoccupied domain in the
results in the masking of the DNA-binding domain. Oestrogen binding h

shown to cause phosphorylation and induction of conformatio
(Beekman et al, 1993; Kraus et al, 1995). This change releases the heats
proteins and allows ER homodimerization, DNA binding, a
proteins called coactivator and the histone acetylase, p300/CBP,
activation of transcription. Antioestrogens do not cause thi
and are therefore unable to fully activate the receptor.

The hinge (D) domain contributes flexibility to the DNA-binding
domain and has been shown to influence the DNA-binding properties of indivi
receptors. It may also serve as an anchor to protein complexes (Bea
et al, 1995). Part of the ligand-dependent, transactivation domain-2 is located in AF
in this region and lies between amino acids (Noel et al, 1997). 282A
portion of the ER nuclear localization signal is also located

Region F contains a time 42 acid carboxyl-terminal region of ER
together with region E, involved in receptor dimerization, the
coregulatory proteins, and the binding of chaperone protei
proteins (hsp) 70 and 90 (Chambraud et al, 1990). Region F differs from region E
in that it is not well conserved in different vertebrates. Montano
and workers (1995) suggest that the F domain specifies a
modulatory function that affects the agonist/antagonist
oestrogens and the transcriptional activity of the ligan

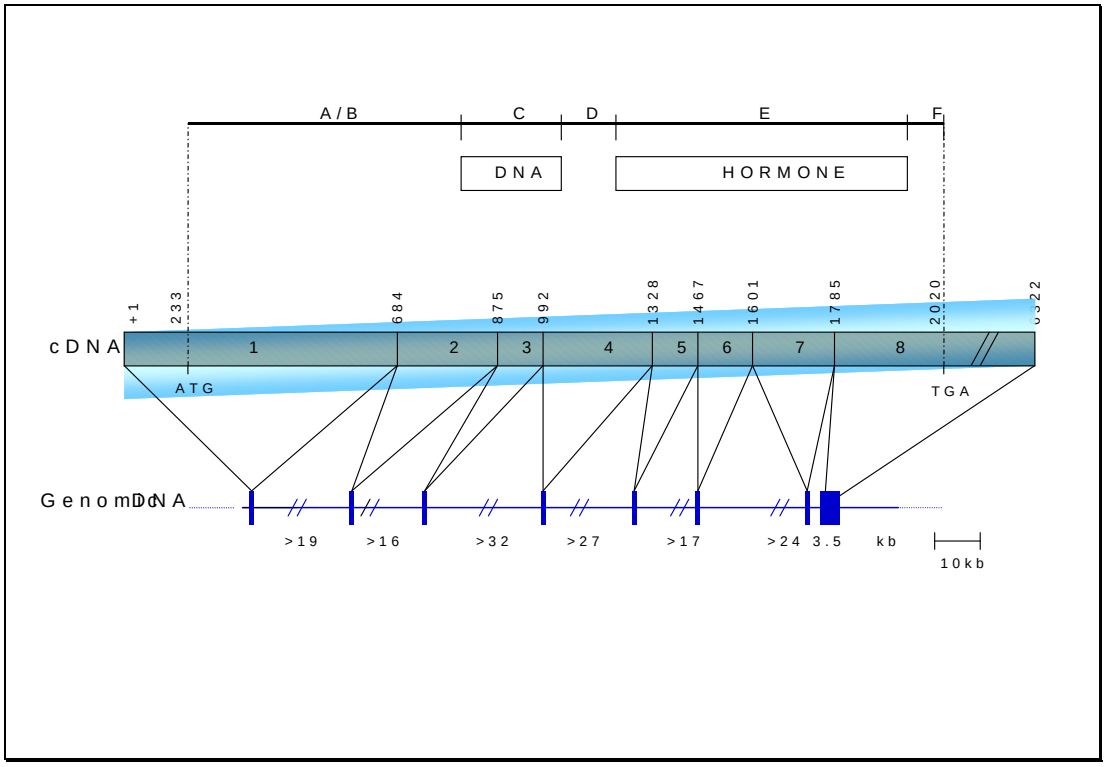

Figure 1. The Oestrogen Receptor

(adapted from Ponglikitmongkol, 1988)

The solid boxes (genomic DNA) represent the eight exons gene with the minimum of each indicated between each box. The cDNA is shown above with the corresponding positions of the eight e The numbers at the borders refer to the nucleotides (Gre position of the translation (ATG) and termination (TGA) are indicated. The division of the human ER (AF) together with the location of the DNA (region) and hormone binding domains are shown above the cDNA.

Their data indicates that the conformation of the ligand-receptor complex is different with oestrogen as compared to an antiwhile wild-type compared to region F, deleted that the potential for interac protein cofactors or transcriptional factors is markedly different.

2.2.2

Oestrogen Receptor Variants

Several ER variants have been identified in breast cancer cel tumours. These variants are generally found to exist with wild-type ER and may interfere with normal function (Zhang, 1996). Two main structural patterns of ER variant mRNAs have been consistently identifie ER mRNAs (Dotzlaw, 1992) and the deleted ER (Fuqua, 1993; Poola, 2000) (Figure 1.2). Cancer tumours appear to exhibit a hi total number of splice variants and have more multiple exo with normal breast (Taylor, 2010).

2.2.2.1

ER± exon deletion (ER±"2)

The deletion variant, ER±"2, is prematurely terminated afte missing the DNA binding domain. The resulting 17 kDa transcriptional variant (Miksicek, 1991) only the AB and domains of ER± where AF-1 is located is encoded. It also lacks the

domain and therefore does not affect wild-type signalling (Wang and Miks
1991). Experiments using tamoxifen-resistant and tamoxifen-sensitive MCF-7 cell
lines have found no differences in expression of this variant
ER±”2 does not play a role in tamoxifen resistance (Mad
et al 1997). ER±”2 se
has been identified in both normal and diseased tissue (Ley
et al 1996).

2.2.2.2

ER± exon 3 deletion (ER±”3)

The ER±”3 splice variant is missing part of the DNA bindin
an inability to bind DNA and therefore results in a 61 kDa
negative activity which is unable to induce transcriptional
activity (Wang and Miksicek, 1991, Han 2004). This dominant nega
activity may serve to reduce normal oestrogenic signalling
tumour progression and growth. This variant has been dete
ER-positive, PR-negative tumours (Zhang et al 1996, Taylor et al 2010).

2.2.2.3

ER± exon 4 deletion (ER±”4)

ER±”4 is one of the most abundant ER± deleted variants (
Pfeffer, 1996) and results in an in-frame deletion encoding a protein l
nuclear localization signal, the AF-2 activation domain and part of t
binding domain (Figure 12). This variant is unable to bind D

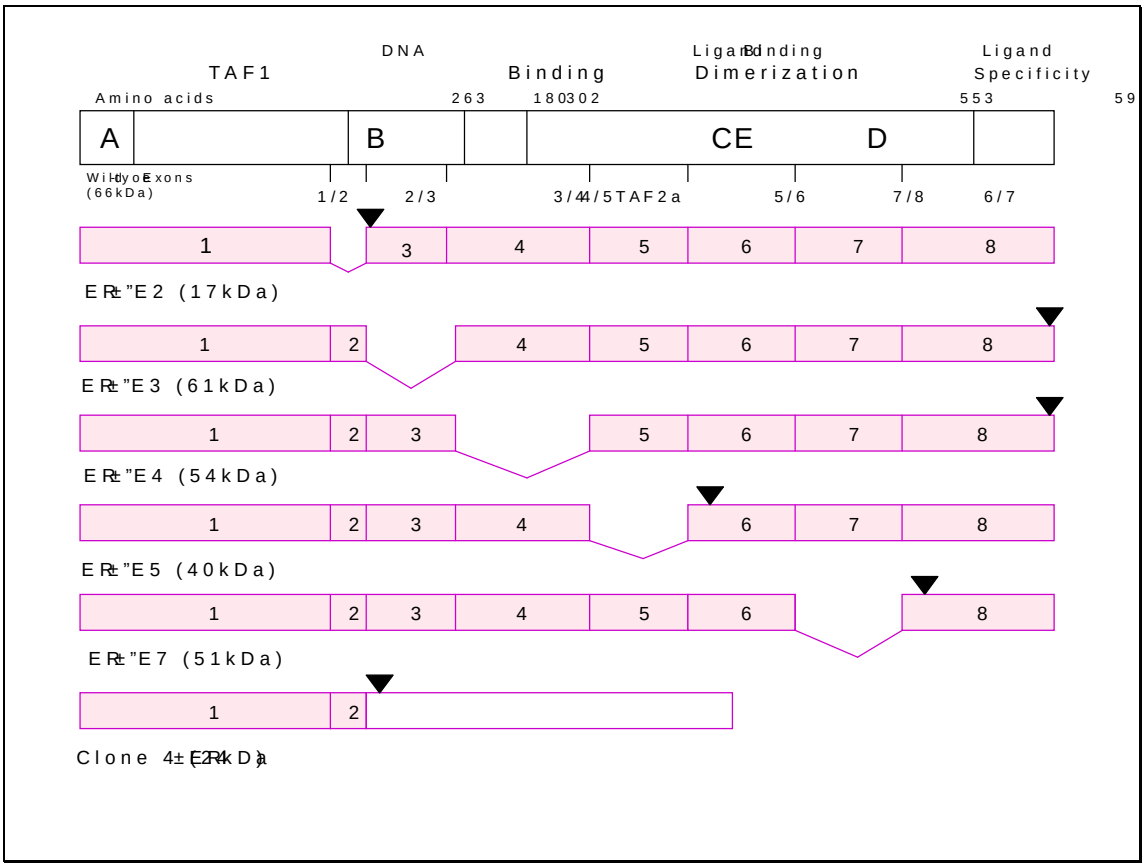

Figure. 1.2 Schematic diagram of ER± exon deletions (adapted from Ferguson and Davidson, 1997; Murphy et al, 1999)

A-F represent the structural and functional domains of the ER±. 1-8 represent the 8 different exons within the ER± cDNA. The a termination, and the estimated molecular mass (kDa = kilodaltons) of ea open reading frame is shown in bracke

therefore has no basal-induced transcriptional activity and appear to interfere with wild-type ER± activity (Koelbost et al 1994). The RNA expression of this variant has, however, been associated cancer and high PR levels which are biomarkers of a more outcome (Leygue et al 1996; Zhang et al 1996). ER±"4 is also more common PR-positive breast cancer (Leygue et al 1996; Zhang et al 1996).

2.2.2.4

ER± exon 5 deletion (ER±"5)

The ER±"5 is a form of the first ER splice variant described and l 8, which includes the hormone. The ground contains a protein predicted molecular weight of 4.01 kDa (Figure 1.2-4). Ligand activity and DNA binding is retained and therefore the encoded p constitutively (Castles et al 1993). ER±"5 can form heterodimers wit type ER± when coexpressed in the same cell. A number of studies have atten to correlate expression of ER±"5 with tamoxifen resistance in clinical Madsen et al (1997) found similar levels of ER±"5 mRNA in b resistant and tamoxifen-MCF-7 cells. Daft et al (1995) and Zhang et al (1996) supported these in vivo contrast. Fuqua et al (1993; 1995) have shown that overexpression of ER±"5 confers relativ tamoxifen resistance. ER±"5 has also been found in norma expressed at increased levels in breast cancer (Leygue et al 1996; Zhang et al 1996; Detsai et al 1997). No association has been shown be

ER±”5 levels and clinical prognostic indicators such as ER
size, or SPF (Zhang et al 1996).

2.2.2.5

ER± exon 6 deletion (ER±”6)

There have been few reports of an ER± exon 6 deletion (Poola et al 2000) although duplications are more common in this region of th
al, 1996). This region encodes part of the hormone binding domain and may play an important signalling role in ER±

2.2.2.6

ER± exon 7 deletion (ER±”7)

ER±”7 is the most frequently observed variant in breast ca
ER status of the tumour (Mc Guire et al 1992; Zhang et al 1996; Poola et al
2000). This variant encodes a 51kDa truncated protein (Wang and Miksicek, 199
Fuqua et al 1992) (Figure 1.2) lacking the AF-2 domain and part of the lig
binding domain. ER±”7 has not been found to be constitu
been demonstrated to act as a negative dominant form of ER² (Fuqua et al 1992; Garcia Pedrero et al 2003) and may contribute towar
disease progression. This variant also does not appear to play a si
tamoxifen resistance (Zhang et al 1996; Maetsch et al 1997).

2.2.2.7 Multiple ERα exon deletions

A number of multiple exon deletion variants have also been identified by Pfeffer et al. (1995). Double, triple and quadruple exon deletion mRNAs, ERα Δ2, ERα Δ2-3, ERα Δ2-4, ERα Δ2-5, ERα Δ2-7, ERα Δ3-4, ERα Δ3-5, ERα Δ3-7, ERα Δ4-5, ERα Δ4-7, ERα Δ5-7, ERα Δ3-4-5, ERα Δ3-4-7, ERα Δ3-5-7 and ERα Δ4-5-7 were identified in breast tumours. The double exon deletion variants, ERα Δ2-3, ERα Δ3-4, ERα Δ4-5 and ERα Δ5-7, and the triple exon deletion variant ERα Δ3-4-5 has been observed in breast tumour tissue (Pfeffer and Speirs, 2001).

2.2.2.8 ERα insertion and exon duplications

Studies have shown a number of ERα variants with exon duplications (Murphy et al. 1996). Such duplications result in a truncation immediately after the duplicated exon and produces a 50 kDa protein that lacks the ligand binding and dimerization domains (Murphy et al. 1996). A duplication of exons 6 and 7 has been found to result in an 80 kDa protein that lacks the ability to bind oestrogen or tamoxifen (Pink et al. 1996; Pink and Jordan, 1996). A 69 nucleotide insertion, resulting from a point mutation between exons 5 and 6 has also been identified in three breast tumours analyzed (Murphy et al. 1996).

2.2.2.9

Truncated ER± transcripts

These altered ER± transcripts are significantly smaller than t
mRNA. Dotzlaw (1992) identified truncated transcripts that ar
the 3 end compared to ER± mRNA. These 3 truncated trans
contain entire exon sequences of at least two of the 5 ER
then diverge into ER± unrelated sequences. Several diff
mRNAs have been cloned, some of which were found to be
breast tumour. Clone 24 truncated ER± mRNA (Dotzlaw
clone, clone 4 truncated ER± mRNA, has been found to be
in human breast tumours. Clone 4 encodes a protein of 22
molecular mass of 23.9 kDa (Dotzlaw (1992) Figure. 12). The functional
significance of these variant ERs is not known.

2.3

Oestrogen Receptor

Since the cloning of the ER (Greene et al 1986), it was
believed that only a single ER was responsible for mediating the
oestrogens on target tissues. Recently, the cDNA of a seco
oestrogen receptor (ER), was cloned and sequenced from the rat
1996), the human (Mosselman et al 1996) and mouse (Tremblay et al 1997).

2.3.1

Molecular Structure of the ER

Like ER and other members of the steroid hormone receptor ER gene has been shown to be encoded by eight exons (E (Figure 13). The longest reading frame of human ER proteins of 530 amino acid residues with a molecular weight of 59.2 1998a). This contrasts with the 6 Greene, 1986). The ER² gene has been mapped to human chromosome 14q22 (Enmark, 1997). The ER DNAs in humans, rats and mice have all shown sig homology. ER also highly homologous to DNA (96%) and liga binding (58%) domains, the A/B domain, hinge region, and not well conserved (Mosselman, 1996; Figure 14)

ER has been shown to bind oestrogen specifically and with as to stimulate transcription of an ER target gene in an manner (Kuiper, 1996; Kuiper, 1997). The classical ER response element (ERE) is composed of two inverted hexanucleotide repeats. T ligand-bound ER binds to the ERE as a homodimer

ER and ER both activate transcription of target genes through sin (Pace, 1997) and may form heterodimers with each other (Ogawa, 1998a). This heterodimer complex has been shown in vivo irrespective of ligand. Differential activation of ER by the

antioestrogen hydroxytamoxifen has been shown with reporter genes (Watanabe, 1997) and because heterodimerization of E has been demonstrated, putative cross signalling pathwa been suggested (Cowley, 1997). There may thus be three possible of oestrogen signalling. In those tissues that exclusively ERb, signalling would be via the specific receptor, where expressing both subtypes, signalling would be mediated b heterodimers (Spelrs, 1999). ERa and ERb proteins may interact leadin differential responses to oestrogens or anti

The ER also mediates gene transcription from an AP1 en requires ligand and the AP1 transcription factors Fos and activation (Gaub, 1990; Umayahara, 1994). In transactivati experiments, the antioestrogen, tamoxifen inhibits the transcriptio are related by a class of ER, but like the natural oestroge oestradiol, tamoxifen activates the transcription of genes t of an AP1 element (Webb, 1995). ERa and ERb respond differently to cer ligands at an AP1 element. At an AP1 site oestrogens act as trans activators and antioestrogens inhibit the transcription of gen elements show the reverse ie. antioestrogens act as trans oestrogen as inhibitors (Paech, 1997). This suggests different regul functions for the two ER subtypes.

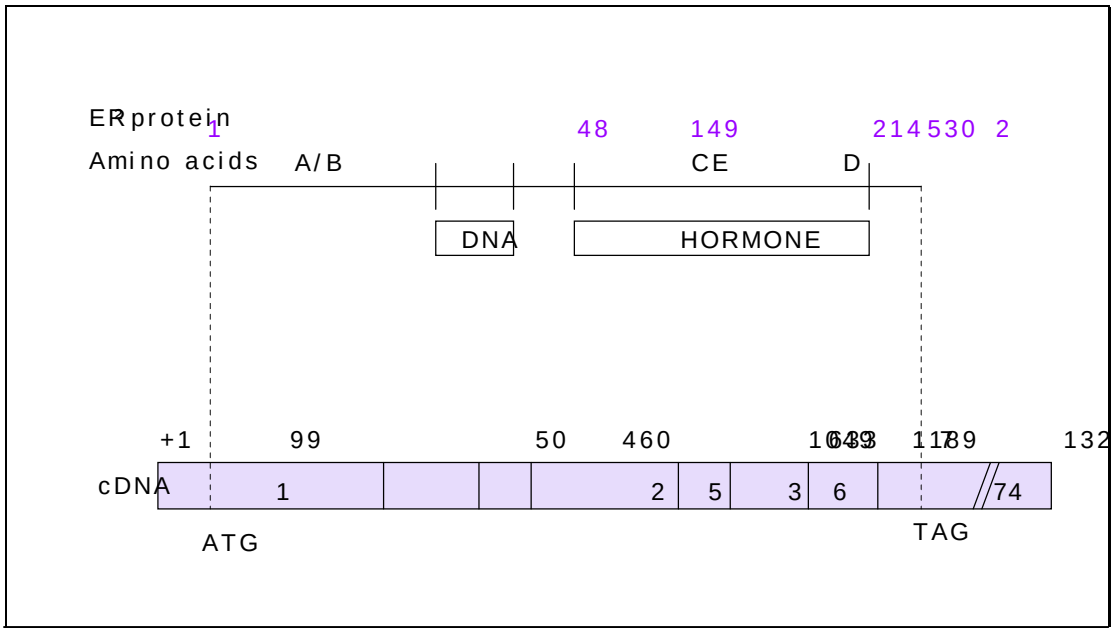

Figure 13. The Oestrogen Receptor

(adapted from Mosselman et al 1996; Enmark et al 1997; Ogawa et al 1998

The ATG start codon and TAG stop codon are indic
(blocked region). Exons are shown with the nucleotide num
The protein domains are shown with the relative positions
Ligand-Binding domains indicated below the solid line. Amino
indicated by coloured numbers above the solid line

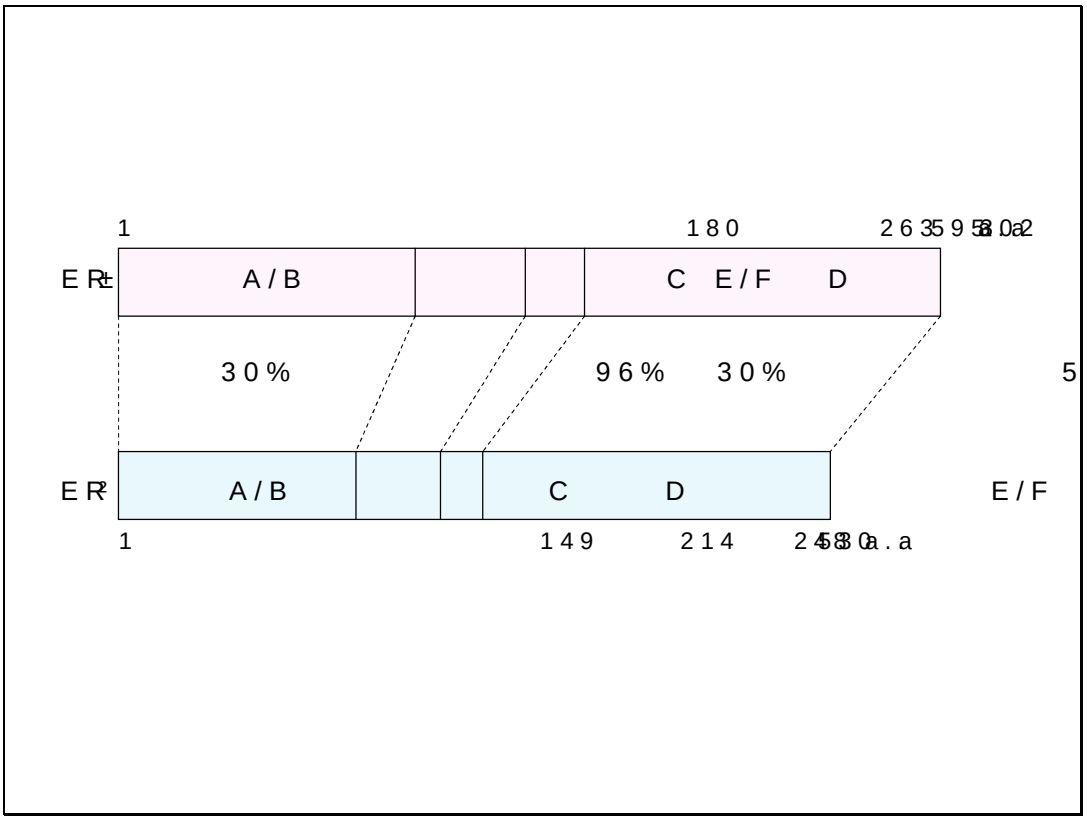

Figure 14. Comparison of the structures between ERα and ERβ (from Ogawa et al., 1998a)

The functional A to E/F domains are schematically represented. Numbers of amino acid residues indicated. Percentage identity of amino acid sequences depicted.

The demonstration of ER expression in both human breast tumours
al, 1997; Enmark, 1997; Vladusic, al 1998) and normal breast tiss
(Enmark, al 1997; et al 1998) suggests that the well documented role of
oestrogen in breast tumourigenesis (Henderson, al 1982) may also involve b
receptors. Using RT-PCR the ratio of ER to ER has been shown to be signific
higher in breast tumours than it is in adjacent normal tissu
alter during carcinogenesis, suggesting that ER and ER pathways may
have definitive roles in this process (Wenger, al 1998). When ER are
coexpressed, it has been observed that ER² exhibits an inh
mediated gene expression (Lindberg, al 2001; Lindberg, al 2003).

2.3.2

ER in clinical breast cancer

It has been estimated that only 7 to 10% of the epithelial c
breast express ER±, and it has been shown that this expres
menstrual cycle (Pt, al 1987; Markopoulos, al 1988; Ricketts, al
1991). In contrast, ER² expression is relatively high in the n
of the cells expressing ER² (Palmieri, al 2002). ER² expression also do
appear to change during the cycle (Shaw, al 2002; Cutchey, al 2003).
The potential role of ER² in breast cancer pro
controversial with some studies suggesting that ER² expre
prognostic indicator due to the inverse correlation (Rogers, al 2000; Roger, al 2001) on an RNA level, or its correlation
biomarkers such as low histological grade, ER± and PR

disease-free survival, and response to tamoxifen in metal protein
2000; Mann, al 2001; Omoto, al 2001; Omoto, al 2002; Fuqua, al 2003).
Other studies suggest that ER² expression is associated w
poor clinical outcome such as EGF receptor expression an
and an inverse correlation between ER² expression and PR statu
1999; Speirs, al 1999; Iwaa, al 2000). According to Speirs and cowor
(1999), most breast tumours, as expressed in ER combination with
Those tumours that coexpressed ER were node positive and tende
be of higher grade. Dotzlaw, al (1999) found no significant correlatio
ER mRNA levels and ER status (as determined by ligand binding assays). They
did, however, observe a significant difference in ER mRNA levels in those
tumours that were ER positive and progesterone receptor
expression) and those tumours that were ER negative and
expression) which could be interpreted to mean that both E
could influence ER mRNA expression. It has also been suggested that the
individual levels of ER± or ER² are not as clinically rel
ER±:ER² that may change during tumorigenesis (Lazennec, al 2001; Stoica, al 2008).

2.3.3

ERöb Variants

ERöb splice variants have been described in a number of breast
significantly fewer than those described for ERöb. Tesnc rixed for ERöb variants
schematically represented in Figure 15.

2.3.3.1

ER²2 or ER²cx

Ogawa et al (1998b) identified and characterized a novel isoform, an ER
ERbetacx, which is truncated at the C-terminal region but has an extra 2
acids due to alternative splicing. ER²cx mRNA is missing the wild-type exon 8
sequence and contains extra sequence, which is located
downstream of exon 8 on chromosome 14 (Pengo et al, 2001). Has no ligand
binding ability and exhibits reduced DNA. This study also indicat
that this isoform potentially dimerizes ER²cx. Omoto et al
(2002), in a study analyzing ER²cx in normal and breast
revealed that 54% of breast cancers express ER²cx variant
normal breast express ER²cx. ER²cx expression in ER+ PR negati
tumours has been found to exhibit a poor response to tamoxifen (S
2002a), whereas tumours lacking ER²cx expression respon
(Saji et al, 2002b)

2.3.3.2

ER² exon 2 deletion (ER²”2)

The most common ER² splice variant has been found to be
(Poola et al, 2002). It is frequently associated with deletion of
(Figure 15). The deletion of exon 2 causes a frame shift mutation resulting i
premature termination codon leading to a prot ein truncated a protein (Poola
2002). No significant differences in expression have been
breast cancer or matched normal breast from a distant sit
suggesting ER²”2 may not play a significant role in breast tumour genesis

2.3.3.3

ER² exon 3 deletion (ER²”3)

ER²”3 does not result in a disruption of the ER² mRNA ope
produces a protein with internal deletions that remain included
half of the DNA binding domain, including the second zin
(Poola et al, 2002a). This variant was initially identified in normal
2002a) and although it has not been identified in breast ca
in differential interactions with other transcription factors

2.3.3.4

ER² exon 4 deleted (ER²"4n)

The loss of only ER² exon 4 does not result in a disruptio
frame of ER² mRNA but the translated protein lacks the nuc
and remains in the cytoplasm (Pedrola et al 2000). ER²"4 has been identified in
breast cancer but no significant differences have been obs
breast and breast cancer (Pozol et al 2000).

2.3.3.5

ER² exon 5 deleted (ER²"5n)

Vladusic and coworkers (1998) detected a corresponding deletion variant
precisely to the deletion of the entire exon 5 of ER (Figure 15). This variant was
coexpressed with the ER-negative, oestrogen independent
cancer cell line MB-231 and in malignant breast tumour sp
Sequence analysis revealed the absence of 139 bp within the ho
domain (exon 8). After amino acid 468 ER²"5 is predicted to enco
amino acids with resulting protein a predicted molecular mass
kDa (Pentgal et al 2003). The ER²"5 is localized to the nucleus and
not have any differential effect on ER² (Inoue et al 2000).
ER²"5 acts as a dominant negative regulator of transactivation
of both ER± and ER² in a dose dependent manner (Inoue et al 2000; Pentgal et al 2003).

Expression of ER²”5 could impair the formation of both EURs and thu contribute to altered oestrogen binding and, consequently, th oestrogenic response.

2.3.3.6

ER² exon 6 deletion (ER²”6)

Vladusic (1998) also detected precise deletion of Exons 5+6 (Figure. 15). These variants would all be expected to encode pr unlikely to bind as they result in a truncated translation prod 2002a). Unlike ER± mRNA, ER²”6 appears to be one of the deleted exons of ER² mRNA, although it is found in slightly lowe breast cancer compared to normal breast, the differences h be statistically significant (Pool 2002). ER²”6 may not play a sig role in breast tumourigenesis.

2.3.3.7

ER² exon 7 deletion (ER²”7)

Deletion of exon 7 is rare in ER² whereas deletion of exon et al, 2002a). This suggests that both exon 6 in ER± and exo important roles in ER function (Herynk and Fuqua, 2004).

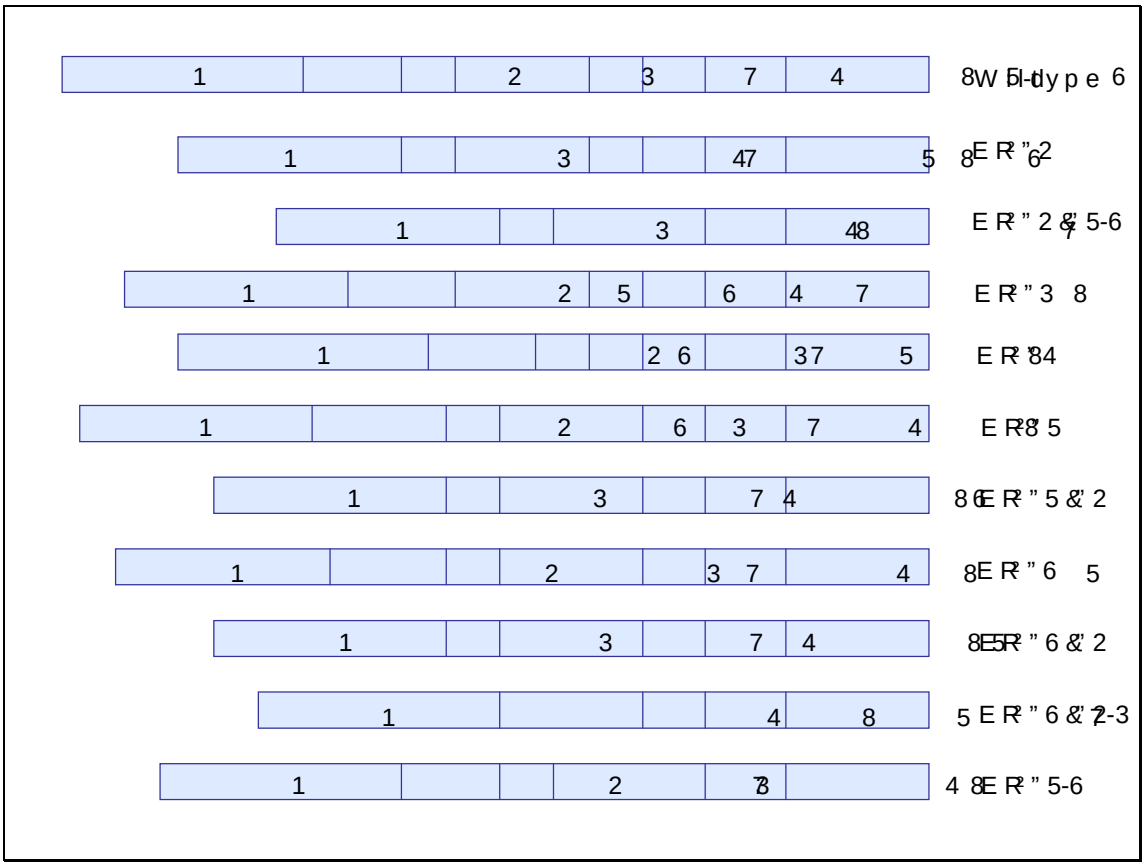

Figure 15. Schematic diagram of EGFR exon deletion variants (adapted from Pao et al 2002a)

ER² mRNA exons are labelled 1-8

2.4

Tissue Culture as a model of Tumour Progression a

Oestrogen

2.4.1

Mechanisms of Tumour Progression

The alterations in cellular behaviour and morphology tha
progression have been well described (Foulds, 1975; Nicol
Biochemical, cytogenetic, molecular biological and immun
that most neoplasms arise from a single altered cell, with
expanding as a neoplastic clone (Nowell, 1976; Klein, 19
It has also been hypothesized that clonal evolution may
genetic instability within the tumour cell population. Thi
probability of further genetic alterations and their subseq
1975; Nowell, 1976; Klein, 1979; Sager, 1985). Most vari
tumour cell population do not survive but those that have
growth advantage become predominant subpopulations with
demonstrate characteristics of tumour progression. There
the clonal evolution concept from chromosome studies where adv
show more extensive chromosomal aberrations than do earl
(Rowley, 1980; Nowell, 1982). It has also been shown that most
are more genetically unstable than normal cells in vitro
vitro neoplastic cells may be more susceptible to chromoson

disjunction and ploidy changes, sister chromatid exchang
alterations such as translocations, deletions and inversion (Petrishad, 1979; German, 1983; Lin, 1985; Sager, 1985). This genetic instability
continuous process that lends to expansion and contract
populations and a relentless remodelling of the tumour (Gra et al, 1990).

2.4.2. Tissue Culture as a Technique: a Brief History

Tissue culture was first devised at the beginning of the twe
1907; Carrel, 1912) as a method for studying the behaviou
controlled environment. Initially the technique involved the maintenan
undisaggregated fragments of tissue and growth was restric
cells from the tissue fragment, with occasional mitoses
Embryonated hen's egg was used as a source of cell divers
techniques for the long term propagation of continuously g
were established (Earle et al, 1943). The demonstration that human tu
give rise to continuous cell lines (Gey et al, 1952; Lacour, 1955) reaged interes
human tissue. Since then many continuously growing cell
have been described.

2.4.3

Human Breast Tumour Cell Lines

Cell lines established from human tumours provide an unl
source of malignant cells free of contaminating stromal cells. As earl
attempts to culture breast cells were reported (Cameron a
but it was not until 1958 that Lasfargues and Ozzello repo
long-term culture of a breast tumour (BT-20). Since the establishment of the
cell line in 1958, 15 additional putative breast cancer c
tumours have been established and a greater number of
derived from solid tumours and pleural ortiasis with effusi
metastatic disease (Engel and Young, 1978). Breast canc
MCF-7, ZR-75-1, MDA-MB231 and T-47D, are ideal model systems for s
the mechanisms of steroid action in tumours

2.4.3.1

MCF-7 Human Breast Cancer Cell Line

The MCF-7 cell line was derived from a malignant pleural eff
69-year old female patient with metastatic breast cancer (Soule
et al (1973) established this cell line-responsive to human cance
cell line and cytogenetic studies indicated a distinct stem line
The oestrogen receptor was first described by Benda (1973)
by both Scatchard and sucrose density gradient analysis.
(1975) demonstrated that the oestrogen tamoxifen inhibited the g

MCF-7 cells, but that this could be reversed by the pres
Receptors for glucocorticoids, progesterone and oestrogens were also iden
in MCF-7 cells (Horwitz, 1975). Some groups, however, were unable
show stimulatory effects of oestrogen (Pongal, 1983; Soto and
Sonnenschein, 1985). One factor causing this may have been the
growth factors within the foetal calf serum (Pagetsar, 1983). Pol red,
the indicator used routinely in commercial cell culture med
reminiscent of non-steroidal oestrogens first synthesized by Dod
Lawson, 1936) and the removal of this compound from the med
result in increased oestrogen sensitivity in MCF-7 cells (Bentham, 1986).
Oestrogen receptor studies now utilize FCS (Aakvaag, 1990) in phenol-red free culture media.

Extensive research-7 cells MCF-7 cells led to the development of monoclonal
antibodies to the human ER (Greene, 1980) which are now used routinely
detection of ER in clinical cancer samples to predict the likely out
Tamoxifen (Jordan, 1986; Mudduwa and Liyanage, 2009). This
development also facilitated the cloning and sequencing of
1985; Greene, 1986; Greene, 1986; Lippman, 1987) utilized the
MCF-7 cell line as a model for growth regulation of human bre
through growth factor use.

MCF-7 cells have the ability to exhibit resistance to tamoxifen has
been observed to stimulate growth in this cell line (Wolf, 1993; Bolrine, 1994; Wandl, 1994). Over the years the use of the MCF-7 cell line

led to the evolution of distinct MCF-7 lines (Ketwal, 1981; Westley, 1984; Bronzert, 1985). Documented differences in cellular sensitivities to oestrogen-stroma and differential expression of oestrogen and differences in tumorigenicity and proliferation (Oliver, 1987; Perry, 1995). These differences may be explained by the fact polyclonal cell line consisting of heterogeneous canc phenotypically and cytogenetically (Resnick, 1987; Nugoli, 2003). Differential expression of ERs in MCF-7 was observed by Lotz and the ability to undergo DNA fragmentation (Gaynor, 1999).

MCF-7 human breast cancer cells continued to be used in ga oestrogen-responsive breast (Lacroix and Leclercq, 2004). Although investigators should carefully document the source of cells and id used in published experiments as it has been demonstrat (1987) and Jones and workers (2000). MCF-7 lines from different laboratories may have unique properties despite having simila karyotype. MCF-7 cells from ATCC, in particular, exhibit en properties to other MCF-7 sources.

2.4.3.

T-47D Human Breast Cancer Cell Line

The T-47D line was originally isolated from a pleural effusion year old female patient with an infiltrating duct carcinoma al, 1979). It was found to have a modal chromosome containe

both ER and PR. The sensitivity of the T-47D cells to the antiestrogen tamoxifen is similar to that of the other ER positive breast cancer cell lines (Reddel et al 1985). However, T-47D cells stand as a model of genetic instability among hormonally responsive cell lines as sublines have positive and oestrogen responsive (Chalbos et al 1982), as ER positive and oestrogen resistant (Horwitz et al 1982), and as ER negative (Chalbos et al 1982). In addition to changes in steroid receptor levels and the T-47D cell line has been shown to be genetically unstable, changing DNA ploidy (Reddel et al 1986, Graham et al 1989, Fernandez et al 1998) where spontaneous DNA duplication was observed to lead to from the hyperdiploid state to a hypertetraploid state. Based it may be postulated that T-47D cells are in transition from the oestrogen responsive, ER positive state to the oestrogen resistant, ER negative state and may thus serve as a model for this process. T-47D breast cancer cells express high levels of PR and have therefore also become the major actions of progesterone and synthetic progestins in human arteries (Lin et al 1994).

The regulation of mRNA in T-47D cells has been reported to be different that observed in MCF-7 cells. Oestrogen exposure in T-47D cells was observed to give rise to an increase in mRNA. Whereas the effect of oestrogen on mRNA levels in MCF-7 cells was dependent on the prior growth history (Reddel et al 1989, Fernandez et al 1998). Oestrogen withdrawal, on the other hand, causes a decrease in ER expression in T47D cells (Pink et al 1996). In contrast to MCF-7 cells which show an increase in ER expression following

oestrogen deprivation. The effects of oestrogen-like compounds on cell proliferation seem to be dependent on ER±:ER² expression (Soto et al. 2008). T-47D cells may therefore be suitable for the study of oestrogens and oestrogen antagonists and their effects on ERR mRNA.