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The Paradox of Oestradiol-Induced Breast Cancer Cell Growth and Apoptosis

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Abstract

High dose oestrogen therapy was used as a treatment for postmenopausal patients with breast cancer from the 1950s until the introduction of the safer antioestrogen, tamoxifen in the 1970s. The anti-tumour mechanism of high dose oestrogen therapy remained unknown. There was no enthusiasm to study these signal transduction pathways as oestrogen therapy has almost completely been eliminated from the treatment paradigm. Current use of tamoxifen and the aromatase inhibitors seek to create oestrogen deprivation that prevents the growth of oestrogen stimulated oestrogen receptor (ER) positive breast cancer cells. However, acquired resistance to antihormonal therapy does occur, but it is through investigation of laboratory models that a vulnerability of the cancer cell has been discovered and is being investigated to provide new opportunities in therapy with the potential for discovering new cancer-specific apoptotic drugs. Laboratory models of resistance to raloxifene and tamoxifen, the selective oestrogen receptor modulators (SERMs) and aromatase inhibitors demonstrate an evolution of drug resistance so that after many years of oestrogen deprivation, the ER positive cancer cell reconfigures the survival signal transduction pathways so oestrogen now becomes an apoptotic trigger rather than a survival signal. Current efforts are evaluating the mechanisms of oestrogen-induced apoptosis and how this new biology of oestrogen action can be amplified and enhanced, thereby increasing the value of this therapeutic opportunity for the treatment of breast cancer. Several synergistic approaches to therapeutic enhancement are being advanced which involve drug combinations to impair survival signaling with the use of specific agents and to impair bcl-2 that protects the cancer cell from apoptosis. We highlight the historical understanding of oestrogen's role in cell survival and death and specifically illustrate the progress that has been made in the last five years to understand the mechanisms of oestrogen-induced apoptosis. There are opportunities to harness knowledge from this new signal transduction pathway to discover the precise mechanism of this oestrogen-induced apoptotic trigger. Indeed, the new biology of oestrogen action also has significance for understanding the physiology of bone remodeling. Thus, the pathway has a broad appeal in both physiology and cancer research.

Keywords

Breast cancer; oestrogen receptor; oestrogen action; apoptosis; tamoxifen; selective oestrogen receptor modulators

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HISTORICAL ORIGINS OF TARGETED HORMONAL THERAPY

In 1896 Beatson [1] published his findings of the beneficial effects of oophorectomy in premenopausal patients with advanced breast cancer. Beatson had based his approach on the role of the ovaries in mammalian lactation, and presumed that there would be a similar mechanism for breast cancer growth. The successful treatment of breast cancer by oophorectomy indicated that there was a principle in the ovary that travels around the body to control the growth of a cancer. The mechanism, however, was to remain unknown until 1923 when Allen and Doisy [2] showed that there is an ovarian hormone that they called oestrogen, which caused vaginal cornification and estrus in ovariectomized mice. Their method, employing ovariectomized mice, was subsequently used to test synthetic compounds for oestrogenic activity. Sir Charles Dodds [3] discovered the potent oestrogenic activity of the stilbenes and he was to discover and define the biological properties of diethylstilboestrol. The oestrogenic properties of triphenylethylene was also described around this time, but in contrast to diethylstilboestrol, triphenylethylene produces long term oestrogenic effects for many weeks [4].

Sir Alexander Haddow was particularly interested in the idea of chemical therapy so he initiated a program to explore the antitumour properties of polycyclic hydrocarbons in laboratory animals. Several compounds caused tumour regression but the fact that the chemicals were carcinogenic prohibited testing in humans. He reasoned that the triphenylethylene based oestrogens had a structural similarity to the polycyclic hydrocarbons and he found they also caused tumour regression in animals. He therefore chose to evaluate the application of oestrogens for the treatment of breast and prostate cancer. In 1944 Haddow [5] published the results of his clinical trial with the synthetic oestrogens triphenylchloroethylene, triphenylmethylethylene, and stilboestrol administered at high doses. He found that 10 out of 22 postmenopausal patients with advanced mammary carcinomas, who were treated with triphenylchloroethylene, had significant regression of tumour growth. Five patients out of 14 who were treated with high dose stilboestrol produced similar responses. Two patients with prostate cancer (out of 30 with diseases other than breast cancer) had a response. The finding that high doses of synthetic oestrogens induced regression of tumour growth in some, but not all patients with breast cancer was somewhat reminiscent of the apparent random responsiveness of oophorectomy in premenopausal patients with metastatic breast cancer [6]. However, Haddow [5] noted that the first successful use of a chemical therapy to treat cancer (breast and prostate) also had significant systemic side effects, such as nausea, areola pigmentation, uterine bleeding, and edema of the lower extremities.

By the 1960's, the standards for the endocrine treatment of breast cancer were established. Premenopausal women should be treated with ovarian irradiation therapy or bilateral oophorectomy. However, based on data from clinical trials, post-menopausal patients with advanced breast cancer should be treated with high dose of the most potent oestrogenic compound diethylstilboestrol [7]. Overall, one could anticipate that 36 % of patients would respond favorably to high dose oestrogen therapy [8].

The question that needed to be answered if endocrine therapy was to be further advanced was, who to treat? In other words, could one develop a test to predict responsiveness? Even as recently as 1970 Haddow [9] was not enthusiastic about the overall prospects of chemical therapy. He stated that there was unlikely to be a *chemotherapy specifica* in Ehrlich's sense because cancer cells are so similar to normal tissue. Also, unlike the antibiotics where one could pre-test responsiveness of the disease to a particular therapy, no such tests existed for cancer. Additionally, it was important that safer less toxic "oestrogens" were developed that might expand therapeutic use. There were clues that deciphering the mysteries of endocrine therapy could be of major benefit for patients. Haddow [10] noted that high dose oestrogen

therapy was more successful as a treatment for breast cancer the farther the woman was from the menopause. Oestrogen-withdrawal somehow played a role in sensitizing tumours to the antitumour actions of oestrogen.

By the 1970's, interest in endocrine therapy waned with the advent of "successful" combinations of cytotoxic chemotherapies for the treatment of metastatic breast cancer. "Coopers Cocktail" of five different chemotherapeutic agents demonstrated dramatic response rates of up to 80% [11]. Cytotoxic chemotherapy was about to become the answer to cancer. But what happened to the triphenylethylene-based oestrogens? Was there another way to improve cancer therapy and to discover the cellular mechanisms of oestrogen action that control the life and death of breast cancer cells?

NON-STEROIDAL ANTIOESTROGENS: EVOLUTION TO TARGETED THERAPY

Leonard Lerner [12] reported the pharmacological properties of the first non-steroidal antioestrogen MER25 or ethamoxytriphetol. The compound was antioestrogenic in all species tested and exhibited no oestrogenic properties. However, the finding that MER25 was a postcoital contraceptive in laboratory animals [13] ignited an intense search of the structure activity relationships by medicinal chemists in the pharmaceutical industry. The goal was to find safer, more potent agents for clinical evaluation. The method chosen for drug discovery was Ehrlich's i.e.: study the structural organic chemistry using the clues provided by the lead compound MER25. The plan of the chemists was simple: place a strategically located alkylaminoethoxy side chain on numerous nonsteroidal oestrogens and then test them as postcoital contraceptives in rats and mice [14]. Although, the oestrogen receptor (ER) had been proposed as the conduit of oestrogen action in its target tissues [15], the actual ER protein was not isolated until 1966 [16]. As a result potential antioestrogens were not screened and identified using an ER assay but drug discovery followed Ehrlich's dictum of a laboratory model *thought* to represent human physiology. When the first clinically useful compound MRL41 or clomiphene was tested in women, it was not a contraceptive, but did the opposite; it induced ovulation. The basic reproductive endocrinology of the rat is completely different than that of a woman. Clomiphene is still used today as a profertility agent in subfertile women [17]. However, clinical trials of clomiphene in the early 1960's did move forward to evaluate activity in the treatment of breast cancer, but were terminated by the company because of concerns about the drug's potential to cause cataracts [17].

The story of the early development of tamoxifen (ICI 46,474) was similar to clomiphene's but the reason that clinical trials were terminated was not because of concerns about toxicity but rather the economic issue of insignificant projected profits that would accrue from marketing a drug for the induction of ovulation and a few thousand patients with metastatic breast cancer, treated for about a year [17].

Arthur Walpole was the leader of the antifertility program at Imperial Chemicals Industry (ICI) Pharmaceuticals (now Astra Zeneca), when ICI 46,474 the pure trans-isomer of a substituted triphenylethylene was discovered and described as a postcoital contraceptive in the rat [18]. Earlier in his career [19], Walpole was interested in carcinogenesis and cancer chemotherapy. He also attempted to discover (unsuccessfully) why only some post-menopausal women with metastatic breast cancer respond to high dose oestrogen therapy [20]. It was Walpole who ensured that ICI 46,474 was tested in the clinic and placed on the market as an orphan drug while ICI invested in the scientific research by others in academia to conduct a systematic study of the anticancer actions of tamoxifen and its metabolites [21]. This investment reinvented tamoxifen as the first anticancer agent specifically targeted to the ER in the tumour and created the scientific principles to ultimately establish tamoxifen as the "gold standard" for the adjuvant

therapy of breast cancer and as the first chemopreventative agent that reduces the incidence of breast cancer in women with elevated risk [22,23].

OESTROGEN RECEPTORS

The existence of the ER was predicted after Elwood Jensen [15] described the retention of subcutaneously administered tritiated 17β -oestradiol (E2) in target tissues such as uterus and vagina of the immature rats, but not in nontarget tissues such as kidney, liver, and muscle. The actual ER protein was extracted and identified from rat uterus in 1966 by Toft and Gorski [16]. Twenty years later in 1986 the ER gene was cloned [24], but afterwards was renamed as ER α because a second ER was cloned from a rat prostate cDNA based on the sequence similarity to ER α , and called ER β [25]. Both ERs Fig. (1) are members of nuclear hormone receptor superfamily and bind oestrogens with high affinity and regulate transcription of oestrogen responsive genes [26].

The ER α gene is located on chromosome 6q25.1 [27] and encodes a 595 amino acid, 66 kDa protein composed of six domains [28] (Fig. (1)). The first domain is called the amino-terminal A/B region and contains the ligand-independent and transcriptionally minor activating function-1 (AF-1). The second domain is called the C region that contains the DNA-binding domain (DBD), whose zinc fingers are responsible for ER α 's binding to oestrogen response elements (EREs) found in the promoters of oestrogen responsive genes. The third domain is called the D region, which contains the nuclear localization signal. The fourth domain is called the E region and contains the ligand binding domain (LBD). The ligand-dependent and transcriptionally major activating function-2 (AF-2) is found in the LBD which mediates binding of the coactivators *via* nuclear receptor boxes composed of LXXLL-like motif [29]. The LBD is composed of 12 α helices, of which helix H3-H12 form a ligand-binding cavity with H12 acting as a "lid" for the cavity. The carboxy-terminal region of the receptor is called the F region. In the nucleus, unliganded monomeric ER α is bound with heat shock proteins (HSPs). Once in the nucleus, oestradiol binds to the LBD of the ER α -HSP complex, and leads to disassociation of HSPs. The LBD then undergoes a crucial conformational change in which H12 caps the ligand binding cavity, and the receptor homodimerizes with another ER α molecule and then binds with high affinity to oestrogen responsive elements (EREs) in the promoters, introns, or 3' untranslated regions of target genes [30]. The ERE is composed of a 15 base pair palindromic inverted repeat consensus sequence: 5'-AGGTCAnnnTGACCT-3' (n = any nucleotide). In addition to direct ERE binding, there is also evidence that ligand-bound ERs can interact with other transcription factor complexes like Fos/Jun (AP-1-responsive elements) [31,32] or SP-1 (GC-rich SP-1 motifs) [33] and influence transcription of genes whose promoters do not harbor EREs (i.e. "tethering mechanism). ER α and ER β also interact with NF- κ B to inhibit transcription [34]. Ligand-activated ERs mediate their genomic effects through interactions with coactivators (i.e. steroid receptor coactivator 1, 2, and 3) that recruit chromatin remodeling complexes, alter nucleosomal structure, enhance recruitment of general transcription factors, and increase recruitment of RNA polymerase II to transcribe target genes [35].

In addition to regulation by ligand binding and coregulator protein recruitment, ERs are regulated by phosphorylation in both a ligand-dependent [36] and ligand-independent manner [37]. Depending on which sites of ER α are phosphorylated, phosphorylation can either increase [38,39] or inhibit ER α -DNA binding [40] and alter nuclear localization [41], and thus alter gene transcription.

The ER β gene is located on chromosome 14q23.2 and encodes a 530 amino-acid protein. As mentioned above ER α and ER β share some sequence similarity, in particular they share the highest degree of amino acid sequence, 61 and 97% in LBD and DBD respectively, however

A/B and D domains have only 27% and 26% amino acid homology respectively. ER β is expressed in the testis, prostate, ovary, developing uterus, breast, vascular endothelium, smooth muscle, immune system, bone and some neurons. With lack of homology of A/B domain between ER α and ER β , functional studies have indicated that ER β lacks AF-1 activity [42].

However the real significance of ER β in breast cancer remains unclear [43], and ER α is considered the molecular target for treating and preventing cancer [44], with the SERMs tamoxifen and raloxifene.

SELECTIVE OESTROGEN RECEPTOR MODULATION

Tamoxifen was originally referred to as a non-steroidal antioestrogen [18]. As more has become known about its molecular pharmacology it has become the pioneering Selective Oestrogen Receptor Modulator (SERM). Tamoxifen was first described as both a partial oestrogen agonist and antagonist in the rat uterus, and a full oestrogen in the mouse uterus and vagina [18]. These were the first important facts which helped to clarify the target-site-specific actions of SERMs. The concept of SERM action was defined by four main pieces of laboratory evidence: 1) ER-positive breast cancer cells inoculated into athymic mice grew into tumours in response to oestradiol, but not to tamoxifen (antioestrogenic action), however both oestradiol and tamoxifen induced uterine weight increase in mice (oestrogen action) [45]; 2) raloxifene (another nonsteroidal antioestrogen), which is less oestrogenic in rat uterus, maintained the bone density in ovariectomized rats (oestrogen action), as did tamoxifen [46], and prevented mammary carcinogenesis (antioestrogenic action) [47]; 3) tamoxifen blocked oestradiol-induced growth of ER-positive breast cancer cells in athymic mice (antioestrogenic action), but induced rapid growth of ER-positive endometrial carcinomas (oestrogenic action) [48]; 4) raloxifene was less effective in promoting endometrial cancer growth (less oestrogenic action) [49]. These laboratory results translated well into the clinic where it was shown that tamoxifen effectively can reduce the incidence of breast cancer in high-risk pre- and postmenopausal women, however increases the incidence of blood clots and endometrial cancer, which is linked to oestrogen-like actions of tamoxifen in these tissues in postmenopausal women, who have a low-oestrogen environment [23]. Furthermore, raloxifene maintains bone density in postmenopausal women and reduces fractures [50], but simultaneously reduces the incidence of breast cancer without increasing the incidence of endometrial cancer [51]. In the study of tamoxifen and raloxifene (STAR) both SERMs were equivalent at reducing the incidence of invasive breast cancer in high risk post-menopausal women, but raloxifene appeared to have a safer toxicity profile. There was, however, a nonstatistically significant higher risk of non-invasive breast cancer (DCIS) with raloxifene compared to tamoxifen [52]. The molecular reason for this difference is not known. With the recognition [53] and effective transition of SERMs to clinical practice, it is now important to understand their mechanism of action so new and novel applications can be developed [54].

MECHANISM OF SERM ACTION

The mechanism of SERM action is dependent upon a complex decision network in target tissues to program the cells to express oestrogenic or antioestrogenic actions. There are two ER's: ER α and ER β and it is possible that a different ratio of both ER's could be important for chemoprevention with SERMs. A high ER α -ER β ratio correlates with high cellular proliferation; in contrast the low ratio correlates with low cellular proliferation. In other words ER β tends to suppress cell proliferation and may enhance apoptosis [55]. This is probably because ER α and ER β have functional differences that can be traced back to differences in the AF1 domain, in particular that they share only 27% of homology in the amino-terminus of the protein. In contrast both ER's differ only by one amino acid in the C region (DNA binding region) and both ER's are able to regulate transcriptional activity of genes regulated by

oestrogen response elements (EREs). In this regard ER β does not have an active AF1 region which is the reason for its inhibitory properties within the cell.

Extensive structure-function relationship studies were initially used to develop a molecular model of oestrogen and antioestrogen action [56–58]. The hypothetical model required the envelopment of a planar oestrogen within the LBD of the ER complex. In contrast, the three-dimensional triphenylethylene binding in the LBD cavity prevents full ER's activation by keeping the LBD open. This structural perturbation of the ER complex is achieved by a correctly positioned bulky alkylaminoethoxy side chain on the SERM. This model was enhanced following studies to solve the X-ray crystallography of the LBD ER's bound with an oestrogen or an antioestrogen. The LBD of ER α is formed by H2-H11 helices and the hairpin β -sheet, while H12, in the agonist bound conformation closes over the LBD cavity filled with E2. The steroid is sealed within the hydrophobic pocket. Oestrogen is aligned in the cavity by hydrogen bonds at both ends of the ligand, particularly the 3-OH group at the A-ring end of E2 forms a hydrogen bond network with Glu353 and Arg394, while E2's 17 β -OH group at the D-ring end of the ligand forms a hydrogen bond with ER's His524. This allows hydrophobic van der Waals contacts along the lipophilic rings of E2, in particular between Phe404 and E2's A-ring, to promote a low energy conformation [59] (Fig. (2A)). This results in sealing of the ligand-binding cavity by H12, and exposes the AF-2 surface for interaction with coactivators to promote transcriptional transactivation. In contrast, 4-hydroxytamoxifen binding to ER's LBD blocks the closure process by relocating H12 away from the binding pocket, thus preventing coactivator molecules from binding to the appropriate site on the external surface of the complex [60] (Fig. (2A)). Therefore, it is the external shape of the ERs that is being modulated by the ligand which dictates the binding of coactivator molecules. In other words, the shape of the ligand actually causes the receptor to change shape and programs the ER complex to be able to bind coregulator molecules. However, the simple model of a coregulator controlling the biology of an ER complex is not that simple (Fig. (2B)). The modulation of the oestrogen target gene is in fact, regulated by a dynamic process of assembly and destruction of transcription complexes at the promoter site of a target gene.

COREGULATORS AND OESTROGEN RECEPTOR ACTION

After ER is bound to an agonist ligand, its conformation changes allowing coregulator molecules to bind to the complex, for example, SRC-3. SRC-3 is a core coactivator that also attracts other coregulators that do not directly bind to ER, such as p300/CBP histone acetyltransferase, CARM1 methyltransferase, and ubiquitin ligases UbC and UbL. All of these coregulators perform specific subreactions within the protein complex of ER and DNA necessary for transcription of target genes, such as chromatin remodeling through methylation and acetylation modifications, and also direct their enzymatic activity towards adjacent factors, which promote dissociation of the coactivator complex and subsequent ubiquitination of select components for proteosomal degradation. As a result, this allows the next cycle of coactivator-receptor-DNA interactions to proceed and the binding and degradation of transcription complexes sustain gene transcription (Fig. (2B)).

It is well established that ER is downregulated in the presence of E2 through ubiquitin proteasome pathway. Downregulation is crucial for ER's transcriptional activity. O'Malley [61] used ER positive MCF-7 cells to demonstrate that the 26S proteasome inhibitor MG132 ablated the transcriptional activity of ER, in luciferase activity assays as well as the endogenous transcription of oestrogen responsive genes, such as pS2 or progesterone receptor (PR) gene. Indeed this is a general principle as proteasome-mediated degradation is crucial for other nuclear receptors to function, such as PR and thyroid hormone receptor. The ubiquitin proteasome pathway is responsible for degradation and turnover of a number of transcriptional factors, such as NF- κ B, and fos/jun. Through a number of enzymes (ubiquitin-activating

[UBA], with which ubiquitin protein forms a high-energy thioester bond, and ubiquitin-conjugating/ubiquitin ligase enzymes), the ubiquitin protein covalently binds to proteins marked for degradation by the 26S proteasome, which subsequently degrades the targeted protein molecules. Lonard and coworkers [61] have demonstrated that blocking proteasomal degradation with MG132 attenuates the transcriptional activity through both the AF-1 and AF-2 domains, demonstrating that proteasome function is required for efficient transcription through either activation function. At the same time disruption of coactivator binding sites abrogates the ligand-mediated downregulation of the ER.

Armed with the knowledge that oestrogen agonists induce a conformation of the ER that stabilizes coactivator binding, it was logical to ask a question whether the binding of coactivators to ER cause a reciprocal stabilization of agonist ligand binding. This question was answered in studies with use of peptides with sequences derived from coactivator (SRC-1) binding sites on the ER (Nuclear-Receptor boxes), and tetrahydrochrysene-ketone (THC-ketone), DES and E2 as oestrogen antagonists [62]. Overall, coactivator peptides can stabilize the complex between the ER and agonist ligands (E2, DES, THC-ketone), with a marked reduction in ligand dissociation rate from the ligand-receptor complexes. Nevertheless, these coactivator peptides were much less effective in stabilizing ER-antagonist complexes, which was demonstrated in reporter-gene assays, where the elevation of SRC-1 levels increased the potency of E2, it decreased the potency of antioestrogens.

With this brief background of the molecular biology of oestrogenic and antioestrogenic modulation in target tissues we will survey the practical application of this knowledge for the treatment and prevention of breast cancer.

CLINICAL APPLICATIONS OF SERMS

The clinical application of the laboratory principle of targeting the ER with long-term antihormonal therapy [21] to treat breast cancer has become the standard of care. Two different approaches to adjuvant antihormonal therapy have been developed in the past 30 years: first, is the blockade of oestrogen-stimulated growth [44] at the tumour ERs, and the second one, is the use of aromatase inhibitors to block oestrogen biosynthesis in postmenopausal patients [63]. Aromatase inhibitors have an advantage in the therapy of post-menopausal patients over tamoxifen, firstly, because there are fewer side effects, such as blood clots or endometrial cancer, and aromatase inhibitors have a small, but still significant efficacy in increasing disease free survival [64]. However, most postmenopausal patients worldwide continue to undergo treatment with tamoxifen, either for economic reasons or because they were hysterectomized and also have a low risk of developing blood clots (low body mass index and are athletically active). In premenopausal women, long term tamoxifen is the antihormonal therapy of choice for the treatment of ductal carcinoma in situ (DCIS) [65], the treatment of ER-positive breast cancer [22] and the reduction of breast cancer incidence in those premenopausal women at elevated risk [23]. It is important to stress that premenopausal women treated with tamoxifen do not have elevations in endometrial cancer and blood clots, thus risk: benefit ratio is in favor of tamoxifen treatment [66].

The development of raloxifene from a laboratory concept [67] to an effective clinical strategy to prevent both osteoporosis and breast cancer [51], [52] has opened new opportunities for clinical applications of SERMs. However, the biggest advantage of raloxifene is that it does not increase the incidence of endometrial cancer [52], which was noted in post-menopausal women taking tamoxifen [23].

The current trend is to employ long-term treatment durations to treat disease with SERMs or aromatase inhibitors. Decades of raloxifene must be used to treat and prevent osteoporosis [68]. Additionally longer treatment trials for breast cancer with either aromatase inhibitors or

tamoxifen are increasing the duration of therapy. Already aromatase inhibitors are used for a full 5 years after 5 years of tamoxifen [69] and there is an ongoing trial ATLAS testing the effectiveness of long (10 years) against short (5 years) adjuvant tamoxifen treatment of breast cancer. The introduction of extended antihormonal therapy to treat and prevent breast cancer therefore has consequences with the development of antihormonal drug resistance.

Though, the clinical application of the SERM concept has proven itself to be successful, drug resistance remains an important issue arising from long-term SERM treatment. Studies have shown that after long-term SERM treatment, the pharmacology of the SERMs changes from an inhibitory antioestrogenic state to a stimulatory oestrogen-like response [70].

ANTIHORMONE DRUG RESISTANCE

Clinical and laboratory studies have identified three possible mechanisms for the antihormone drug resistance to tamoxifen: the patient can influence the metabolism of tamoxifen, the ER-positive tumour can be intrinsically resistant or the ER positive tumour can initially respond and subsequently develop acquired tamoxifen resistance.

Activation of tamoxifen occurs when it is metabolized *via* demethylation to N-desmethyltamoxifen and subsequently gets transformed to the hydroxy metabolite endoxifen [71]. Endoxifen is formed by the CYP2D6 enzyme system [72], but there are genetic variants in the population that can influence drug metabolism. It is estimated that mutant alleles of the wild-type CYP2D6 enzyme variants are present in 10% of the population, thus meaning that these patients should be considered for an antioestrogenic therapy, other than tamoxifen i.e. aromatase inhibitors if they are postmenopausal. Side effects, that arise during treatment with tamoxifen, influence compliance and efficacy. An important side effect of tamoxifen is hot flashes and many patients become non compliant and stop therapy or use selective serotonin reuptake inhibitors (SSRIs) to reduce hot flashes. But it appears that hot flashes are good as tamoxifen must be metabolized into the potent antioestrogen endoxifen by the CYP2D6 enzyme. Unfortunately, SSRIs (fluoxetine and paroxetine) are also potent inhibitors of the CYP2D6 enzyme [73]. Therefore, symptom treatment can potentially undermine the efficacy of treatment with tamoxifen if the incorrect SSRI is employed. Venlafaxine is the recommended SSRI as there is a low affinity for the CYP2D6 enzyme system.

Forty percent of ER-positive metastatic breast cancers are intrinsically resistant to tamoxifen treatment. These tumours are identified as ER-positive and PR-negative tumours and only 40% [74] respond to antihormonal therapy. In contrast, ER/PR-positive tumours have an 80% response rate to endocrine therapy. In early studies it was noted that PR induction by oestrogen is impaired, through the epidermal growth factor receptor 1 (HER-1; EGFR) pathway [75], and that paracrine growth factor stimulation undermines the effectiveness of antioestrogen therapy [76]. These observations were expanded using breast cancer cells transfected with insulin-like growth factor receptor and by the examination of large tumour databases [77,78]. Insulin-like growth factor also reduces PR synthesis, so a general mechanism emerged that growth factor pathways impair ER signal transduction to initiate PR induction. Intrinsic tamoxifen resistance occurs in HER-2/neu-, PR negative, ER positive breast cancer cells that also have increased levels of SRC-3 coactivator [79]. Though, this patient category is only 10 to 15%, it provides a clue about who to test to avoid tamoxifen treatment. A retrospective analysis showed that patients with ER positive, PR-negative tumours would most likely respond better to aromatase inhibitor treatment than to tamoxifen [80], however, these data have subsequently not been confirmed [80,81].

Finally, and most intriguingly, long-term tamoxifen treatment can induce acquired resistance in breast cancers that are ER/PR-positive. Acquired resistance to tamoxifen is unique as the tumours are SERM stimulated for growth [82]. The first laboratory model [48,49,70] of

transplantable tamoxifen resistant tumours demonstrated that 1) tamoxifen or oestrogen can cause tumours to grow, 2) tumours require a liganded receptor to grow, 3) an aromatase inhibitor (oestrogen deprivation) or a pure antioestrogen that causes ER destruction would be useful second line agents, 4) there was cross resistance with other SERMs [83].

However, it is the study of acquired antihormone resistance that has not only allowed the development of appropriate second line treatment strategies for patients (aromatase inhibitors or fulvestrant), but also has advanced our understanding of the apoptotic biology of high dose oestrogen as an effective therapy for breast cancer in the 1940's [5].

EVOLUTION OF ANTIHORMONE DRUG RESISTANCE

An obstacle to progress in therapeutics is a clear understanding of the changes that occur in the breast cancer cell, as a consequence of exhaustive antihormonal therapies. It is presumed that the cancer cell must create a sophisticated survival network and suppress the natural process of apoptosis to subvert the continuous inhibitory signal through the ER. Currently, numerous model systems exist to study antihormone resistance. Some are engineered to enhance the likelihood of resistance [79] and others are engineered by transfection of the aromatase gene to study resistance to aromatase inhibitors and compare them with tamoxifen [84]. In contrast, others have chosen to develop models naturally through selective pressure either *in vivo* or *in vitro*. The natural selection approach is either to continuously transplant the resulting SERM resistant breast cancer into SERM-treated athymic animals [85,86] or to employ strategies *in vitro* that use continuous SERM treatment [87–89] or long term oestrogen deprivation in culture [90,91].

In order to better understand the biological consequences of extended antioestrogen treatment on the survival of breast cancer, we have elucidated distinct phases of resistance with the use of unique models of tamoxifen-resistant breast cancer developed *in vivo* (Fig. 3). The model for the treatment phase of breast cancer was developed by injecting ER α -positive MCF-7 cells into athymic mice and supplementing them with post-menopausal doses of oestradiol (E2) (86–93 pg/ml) [92]. These MCF-7 tumours were oestradiol (E2)-stimulated and tamoxifen (TAM)-inhibited. Phase I TAM-resistant breast tumours developed with short term treatment (<2 years) with tamoxifen and were stimulated to grow by both E2 and tamoxifen [70,93]. The novel model of Phase II resistance to tamoxifen was observed when breast tumours were treated long-term with tamoxifen for more than 5 years (MCF-7TAMLT). These MCF-7TAMLT tumours were stimulated to grow with tamoxifen but paradoxically inhibited by E2 [85,94,95] (Fig. (3)). Phase III resistance developed when all known therapies failed and only E2-inhibited growth [96]. However, during the progression from the treatment phase to Phase III resistance, a cyclic phenomenon was observed where initially E2-inhibited growth of Phase II TAM-resistant tumours followed by re-sensitization to E2 as a growth stimulant [94]. These E2 re-stimulated MCF-7 tumours from Phase II tamoxifen-resistant tumours were growth inhibited by no treatment, TAM, and fulvestrant demonstrating complete reversal of drug resistance to tamoxifen. In addition to tamoxifen-resistant tumours, oestradiol, at physiologic concentrations, has also been shown to induce apoptosis in long term oestrogen deprived (LTED) breast cancer cells *in vitro* and *in vivo*. It should be noted that in the past, pharmacologic oestrogen was a routinely employed therapy that resulted in durable responses with regression of disease [5]. Oestrogen therapy has yielded as high as 40% response rate as first-line treatment in patients with hormonally sensitive breast cancer with metastatic disease [97] and approximately 31% in patients heavily pre-treated with previous endocrine therapies [98] (Table 1A). What is still unclear, however, is the mechanism of oestradiol-induced apoptosis in breast cancer cells that are stimulated by tamoxifen or that grow spontaneously when deprived of oestradiol for a long time (> 1 year).

MECHANISMS OF OESTROGEN INDUCED APOPTOSIS

Apoptosis is a form of programmed cell death that is executed by a family of proteases called caspases, which can be activated either by cell-surface death receptors (i.e., the extrinsic pathway) or by perturbation of the mitochondrial membrane (i.e., the intrinsic pathway) [99] (Fig. (4)). Components of the extrinsic pathway include the death receptors FasR/FasL, DR4/DR5, and tumour necrosis factor (TNFR), whereas the intrinsic pathway centers on the mitochondria, which contain key apoptogenic factors such as cytochrome *c* and apoptosis inducing factor (AIF) [99] (Fig. (4)). In the intrinsic pathway, the integrity of mitochondrial membranes is controlled primarily by a balance between the antagonistic actions of the pro-apoptotic and anti-apoptotic members of the Bcl-2 family. Bcl-2-family proteins comprise three principal subfamilies: (1) anti-apoptotic members, including Bcl-2/Bcl-x_L, which possess the Bcl-2 homology (BH) domains BH1, BH2, BH3, and BH4; (2) pro-apoptotic members, such as Bax, Bak, and Bok, which have the BH1, BH2, and BH3 domains; and (3) BH3-only proteins, such as Bid, Bim, Bad, Bik, and Puma, which generally possess only the BH3 domain [100]. The Bcl-2 family of proteins regulate apoptosis by altering mitochondrial membrane permeabilization and controlling the release of cytochrome *c*.

Mechanistic studies have used either SERM-stimulated models [88,95] or long-term oestrogen deprived MCF-7 breast cancer cell lines [90,91,101] to demonstrate a link between oestradiol-induced apoptosis and activation of the FasR/FasL death-signaling pathway. Osipo and coworkers [95,101] reported that physiologic levels of oestradiol induced regression of tamoxifen-stimulated breast cancer tumours by inducing the death receptor Fas and suppressing the antiapoptotic/prosurvival factors NF-κB and HER2/neu. A similar finding was reported by Liu and coworkers [88] for raloxifene (Ral)-resistant tumours. These investigators reported that the growth of Ral-resistant MCF-7/Ral cells *in vitro* and *in vivo* was repressed by oestradiol by a mechanism involving increased Fas expression and decreased NF-κB activity. Furthermore, Song and coworkers [90] showed that MCF-7 cells deprived of oestrogen for up to 24 months (MCF-7LTED) *in vitro* expressed high levels of Fas compared to the parental MCF-7 cells, which do not express Fas and treatment of the MCF-7/LTED cells with oestradiol resulted in a marked increase in Fas ligand (FasL) in these cells. Apart from the death receptor pathway, there is also evidence that the mitochondrial pathway is involved in oestradiol induced apoptosis. Oestradiol induced apoptosis occurs in a LTED breast cancer cell line named MCF-7:5C by activating the Bcl-2 family proteins (Fig. (4)). In MCF-7:5C cells the expression of several pro-apoptotic proteins-including Bax, Bak, Bim, Noxa, Puma, and p53- are markedly increased with oestradiol treatment and blockade of Bax and Bim expression using siRNAs almost completely reversed the apoptotic effect of oestradiol. Oestradiol treatment also led to a loss of mitochondrial potential and a dramatic increase in the release of cytochrome *c* from the mitochondria, which resulted in activation of caspases and cleavage of PARP. Furthermore, overexpression of anti-apoptotic Bcl-x_L was able to protect MCF-7:5C cells from oestradiol-induced apoptosis. This particular study was one of the first to show a link between oestradiol-induced cell death and activation of the mitochondrial apoptotic pathway using a breast cancer cell model resistant to oestrogen withdrawal. It is worth noting that Song and coworkers [102] have also demonstrated the importance of Bcl-2 in mediating oestradiol-induced apoptosis in LTED cells. These investigators reported that basal bcl-2 level was markedly elevated in LTED cells and that knockdown of bcl-2 expression with siRNA dramatically sensitized these cells to the apoptotic action of oestradiol. At present, there is renewed interest in developing small molecule inhibitors of bcl-2 as anticancer cell and antiangiogenic agents [103] (Table 1B).

Apart from its action on the mitochondria, there is evidence that Bcl-2 also possesses antioxidant property. Bcl-2 overexpression increases cellular glutathione (GSH) level which is associated with increased resistance to chemotherapy-induced apoptosis [104]. GSH is a

water-soluble tripeptide composed of glutamine, cysteine, and glycine. It is the most abundant intracellular small molecule thiol present in mammalian cells and it serves as a potent intracellular antioxidant protecting cells from toxins such free radicals [105,106]. Changes in GSH homeostasis have been implicated in the etiology and progression of a variety of human diseases, including breast cancer [107] and studies have shown that elevated levels of GSH prevent apoptotic cell death whereas depletion of GSH facilitates apoptosis [108]. Recently, our laboratory has found evidence which suggests that glutathione participates in retarding apoptosis in antihormone-resistant MCF-7:2A human breast cancer cells and that depletion of this molecule by L-buthionine sulfoximine (BSO), a potent inhibitor of glutathione biosynthesis, sensitizes these resistant cells to oestradiol-induced apoptosis [109]. GSH levels were elevated ~60% in antihormone-resistant MCF-7:2A cells compared to wild-type MCF-7 cells and unlike MCF-7:5C cells, the MCF-7:2A cells failed to undergo apoptosis following 1 week of treatment with physiological concentrations of oestradiol. In the presence of BSO (100 μ M), however, 1 nM oestradiol caused a dramatic increase in apoptosis which was observed as early as 48 hours with maximum induction observed at day 7. It is worth noting that the concentration of BSO (100 μ M) used in this study is clinically achievable [110]. Furthermore, early phase clinical trials of BSO at doses resulting in both peripheral and tumour GSH depletion show that BSO can be safely administered to patients with refractory disease [111]. Thus it is possible that future clinical studies of BSO infusions combined with low dose oestrogen hold the promise of improving disease control for patients with antihormone resistant ER positive metastatic breast cancer.

NONGENOMIC OESTROGEN ACTION

There is also evidence that E2 has “nongenomic or membrane-initiated” effects, that is, independent of ER-mediated transcription, that occur within minutes after E2 administration in a G-protein-coupled manner [112,113]. Since ER α lacks a transmembrane domain, how it gets to the plasma membrane is somewhat controversial but it appears to require palmitoylation [114]. ER α interacts with a number of proteins, including c-Src, the p85 subunit of PI3K, caveolin-1, and modulator of nongenomic activity of ER (MNAR) [115]. ER α has also been shown to interact with the epidermal growth factor receptor (EGFR), IGFR1, and HER2 in the plasma membrane of breast cancer cells [116]. In MCF-7 human breast cancer cells, E2 rapidly increased PIP2-phospholipase C activity [117], mobilized intracellular Ca²⁺, and activated the MAPK [118] and PI3K/Akt pathways [119]. Since E2 activation of the “nongenomic” pathway occurs within minutes, it is unlikely that this pathway plays a major role in E2-induced apoptosis in our SERM-resistant or LTED-breast cancer cells which undergo apoptosis after hours of treatment.

CLINICAL EXPLOITATION OF OESTROGEN-INDUCED APOPTOSIS

Laboratory studies uniformly demonstrate that low concentrations of oestrogen can cause apoptotic tumour cell death following profound oestrogen deprivation with antihormones. This can be viewed as an enhanced vulnerability to oestrogen when Phase II antihormone resistance is developed consistent with the earlier use of high dose oestrogen to treat breast cancer in women 2–3 decades after menopause [5]. The question that needs now to be answered is how can this new laboratory knowledge be translated into patient care?

Several clinical trial groups are currently addressing this issue. In a recent study presented at the 31st annual San Antonio Breast Cancer Symposium, Ellis and coworkers [120] reported that a daily dose of 6 mg estradiol could stop the growth of tumors or even cause them to shrink in about 25% of women with metastatic breast cancer that had developed resistance to standard antihormonal therapy (Table 1A). In our own case, we are recruiting patients with metastatic breast cancer who have succeeded and experienced treatment failure with at least two

successive endocrine therapies and we are determining the efficacy of a 12-week purge of high-dose oestradiol (30 mg daily) therapy (Fig. (5)). The goal is to confirm and extend the previous study published by Lonning and colleagues [98] and then to determine the minimum dose of oestradiol necessary to induce the anticipated 30% response rate [98]. Based on our previous laboratory studies [94] we propose to re-treat responding patients with the aromatase inhibitor anastrozole to determine efficacy. Overall, our clinical program is part of a multi-institutional Center of Excellence grant BCO50277 entitled “A New Therapeutic Paradigm for Breast Cancer Exploiting Low-Dose Oestrogen-Induced Apoptosis” that will map the survival and death pathways of our models and integrate clinical material to determine the validity of the laboratory-derived molecular mechanisms and, ultimately, to address the issue of why the majority of tumours do not respond to oestrogen alone. We reason that knowledge of the new apoptotic biology of oestrogen could be enhanced in the future in much the same way as the modest responses of tamoxifen and raloxifene were enhanced to benefit patients. The philosophy is to deploy the right treatment at the right time, for the right patient.

PERSPECTIVES

Our proposed model clinical trial now provides opportunities to test compounds in associations with oestrogen as an apoptotic trigger. Pre-clinical data from our laboratory [109] clearly show that it is possible to enhance the apoptotic effect of low dose oestradiol by combining it with BSO. We propose that the combination of BSO and oestradiol could be used to improve the efficacy of oestradiol as an apoptotic agent if glutathione depletion is fundamental to tumour cell survival. Phase I clinical trials of BSO at doses resulting in both peripheral and tumour GSH depletion show that BSO can be safely administered to patients with refractory disease. BSO was administered intravenously twice daily either alone or together with chemotherapy to cancer patients whose disease had progressed despite multiple lines of previous chemotherapy [111,121].

We propose that inhibitors of survival pathways will enhance the apoptotic/growth inhibitory effects of oestrogen. Bcl-2 (B-cell lymphoma/leukemia-2) is a low molecular weight protein that is localized to the mitochondria and endoplasmic reticulum that acts as a key inhibitor of apoptosis. Expression of Bcl-2 is essential for growth of certain tumour cell lines *in vitro* and has been found to be upregulated in a variety of tumour types *in vivo* [122,123]. It is widely believed that some cancers evade apoptosis and obtain a survival advantage through aberrant expression of Bcl-2. To date, several independent groups have developed small-molecule inhibitors of Bcl-2 as antitumour agents [103]. These inhibitors encompass various drugs that bind the anti-apoptotic Bcl-2 family members with more or less efficacy. Oblimersen (Genasense; G3139; Genta Inc, Berkeley Heights, NJ) is an anti-Bcl-2 antisense oligonucleotide which has reached phase III clinical trials in combination therapy [124]. Peptide-based drugs have also been shown to attenuate Bcl-2 activity [125] and to activate Bax [126]. There are also natural inhibitors of Bcl-2 which include tea polyphenols such as catechins and theaflavins [127,128] and the natural polyphenol derivative gossypol [129]. Inspired by the potential of natural Bcl-2 inhibitors, several research groups have developed specific inhibitors of Bcl-2. HA14-1 was the first Bcl-2 binding ligand to be discovered using computer-based screening strategies using the predicted structure of Bcl-2 [130]. Other small-molecule inhibitors of Bcl-2 include TW-37 [130,131] and ABT-737 [132], both of which have better efficacy than HA14-1 [133] (Table 1B). Overall, the small-molecule inhibitors of Bcl-2, although they are not magic bullets, have great therapeutic potential and are proving to be an important investigative tool for understanding the function of Bcl-2.

There is strong clinical evidence that trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor (HER) two tyrosine kinase receptor, is an important component of first-line treatment of patients with HER-2 positive metastatic breast cancer

[134–136] (Table 1C). In particular, the combination with taxanes and vinorelbine has been established [137]. In the preoperative setting inclusion of trastuzumab has significantly increased the pathological complete response rate. Results from large phase III trials evaluating adjuvant therapy in HER-2 positive early breast cancer indicate that the addition of trastuzumab to chemotherapy improves disease-free and overall survival [138]. Based on our preclinical studies [88,95], we have found that HER-2 is an important target of oestradiol-induced apoptosis, hence, the possibility exist that the combination of oestradiol therapy with that of trastuzumab might have beneficial effects. In addition to trastuzumab, there is also pertuzumab (2C4, Omnitarg®) (Genentech Inc. San Francisco, CA, USA), a monoclonal antibody directed against HER-2 that sterically blocks dimerization of HER-2 with HER-1 and HER-3 [139, 140]. It is currently under early clinical evaluation, phase I data have shown that the drug is well tolerated and clinically active [141]. Ertumaxomab (Rexomun®) (Fresenius Biotech GmbH, Munich, Germany) is a novel trifunctional, bispecific antibody that targets HER-2 and CD3. A phase I study among 17 patients with HER-2 positive metastatic breast cancer has demonstrated strong immunologic responses with this antibody [142]. In addition, recent studies have reported that ertumaxomab induces cellular cytotoxicity against various tumour cell lines including cells with low expression of HER-2 [143]. Thus, this antibody may provide a new therapeutic option for breast cancer patients with low expression of HER-2.

Apart from monoclonal antibodies, the use of tyrosine kinase inhibitors to target HER-2 has also shown great promise. Lapatinib (Tyverb®, GW572016) (GlaxoSmithKline, Middlesex, UK) is a dual tyrosine kinase inhibitor of both HER-1 and HER-2, and of Akt and mitogen activated protein kinase (MAPK). Preclinical studies have demonstrated that this compound inhibits growth and induces apoptosis in breast cancer cell lines [144]. Results from phase I/II trials suggest that the compound has activity against several tumour types, in particularly breast cancer [144–147] (Table 1C). In addition, xenograph studies have shown that lapatinib may be able to restore tamoxifen sensitivity [148]. The compound has also been evaluated in combination with aromatase inhibitors in preclinical and clinical studies [149,150]. However, increased ER signaling has been demonstrated in biopsies from HER-2 positive breast tumours treated with lapatinib. This finding might indicate that ER signaling could be involved in lapatinib-resistance [151]. A phase I study of lapatinib in combination with the aromatase inhibitor letrozole in patients with solid tumours showed a positive response [149]. A phase III study comparing letrozole with letrozole plus lapatinib in patients with ER/PR-positive metastatic breast cancer has recently completed enrolment [152]. Data have not yet been published.

In recent years, compelling evidence suggests that increased growth factor signaling, in particular the EGFR/HER2 pathway, contributes to tamoxifen resistance [153]. Thus it is possible that exogenous inhibitors of the HER-signaling network and other mitogenic pathways can abrogate or improve the response rate of breast cancer with acquired phase II resistance during oestrogen therapy.

CONCLUSION

The discovery of a new biology of oestradiol-induced apoptosis provides a unique signal transduction pathway to exploit in the treatment of metastatic breast cancer that has become refractory to exhaustive antihormone therapy. The clinical clues with the use of high-dose oestrogen therapy [5,98,154] have now been supported by a wealth of laboratory data defining apoptotic mechanisms. It is plausible to consider that the methodical evaluation of monoclonal antibodies and small molecule tyrosine kinase inhibitors to prevent breast cancer survival could amplify the apoptotic actions of oestradiol in a select group of patients. Indeed, if a study of the molecular biology of oestrogen-induced apoptosis can precisely define the mechanism then the molecules involved will become the target for a new drug group. These new drugs may be

able to precipitate apoptosis in ER-negative breast tumours or indeed be used universally to treat cancers other than breast cancer. All will depend on tissue selectivity.

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Fig. 1.

ERα and ERβ functional domains. ERα and ERβ proteins both are members of nuclear hormone receptor superfamily and bind oestrogens and regulate transcription of oestrogen responsive genes. They share the highest degree of amino acid sequence, 61 and 97% in LBD and DBD respectively, however A/B and D domains have only 27% and 26% amino acid homology respectively, however with lack of homology of A/B domain between ERα and ERβ, functional studies have indicated that ERβ lacks AF-1 activity.

Fig. 2.
Coregulators and Oestrogen receptor action.

A: Molecular interactions between oestradiol, 4-hydroxytamoxifen and ER α . using the X-ray crystallographic structures. Oestrogen is aligned in the cavity by hydrogen bonds at both ends of the ligand, particularly the 3-OH group at the A-ring end of E2 forms a hydrogen bond network with Glu353 and Arg394, while E2's 17 β -OH group at the D-ring end of the ligand forms a hydrogen bond with ER's His524. This results in sealing of the ligand-binding cavity by H12, and exposes the AF-2 surface for interaction with coactivators to promote transcriptional transactivation. In contrast, 4-hydroxytamoxifen binding to ER's LBD blocks the closure process by relocating H12 away from the binding pocket, thus preventing coactivator molecules from binding to the appropriate site on the external surface of the complex.

B: Structurally different ligands change the conformation of the oestrogen receptor and thus program them to bind either to coactivators with different enzymatic activities or co-repressors. Coactivators are necessary for the receptor to bind to the promotor regions of oestrogen responsive genes and then subsequently be degraded by the 26S proteasome complex, which is necessary for sustaining gene transcription. In the case of antioestrogen binding, co-repressors prevent activation of oestrogen responsive gene transcription.

Fig. 3.

Evolution of antihormone resistance. Acquired resistance of breast cancer cells occurs during long-term treatment with SERMs and is evidenced by SERM-stimulated growth of these cells. Tumours still exploit oestrogen for growth when the SERM treatment is stopped, meaning that a promiscuous transduction pathway has developed. At this point aromatase inhibitors are effective as they destroy the ER. This phase of drug resistance is called phase I. Continued exposure to SERMs eventually leads to oestrogen-independent autonomous growth of breast cancer cells, continuing to be SERM-stimulated. However, these cells now respond to oestradiol by triggering apoptosis rather than growth. This phase of drug resistance is called phase II.

Fig. 4.

The proposed subcellular mechanism for oestrogen induced apoptosis of SERM resistant (Phase II) and long term oestrogen deprived (aromatase inhibitor-resistant) breast cancer. In SERM-resistant breast cancer, there is an induction of the Fas receptor/Fas ligand resulting in activation of caspase 8 and induction of apoptosis (programmed cell death). In long term oestrogen deprived breast cancer, the oestradiol ER complex alters Bcl-2 family protein expression which then activates the mitochondria causing cytochrome *c* release, caspase 9 activation, and PARP cleavage, ultimately resulting in cell death.

Fig. 5.

Clinical protocol to investigate the efficacy of oestradiol treatment to induce apoptosis in long-term endocrine refractory breast cancer. An anticipated treatment plan for third-line endocrine therapy. Patients must have responded and experience treatment failure with two successive antihormone therapies to be eligible for a course of low-dose oestradiol therapy for 3 months. The anticipated response rate is 30% and responding patients will be treated with anastrozole until relapse. The overall goal is to increase response rates and maintain patients for longer on antihormone strategies before chemotherapy is required.

Results from Previous Clinical Trials and Ongoing Trials with Oestrogen and Other Targeted Therapies

A: Results of clinical trials of low dose oestrogen (DES and oestradiol) treatment in patients with advanced breast cancer. CR- complete response, PR- partial response, CI- confidence interval, SD- stable disease. B: Results of preclinical trials of Bcl-2 inhibitors [-]-glossypol and TW-37 in diffuse large cell lymphoma xenograft model. T/C- reduction of tumour weight in treated group of mice relative to control in percents, T-C- time in days to weight equivalence of tumours in treated group of mice with tumour weight in control group of mice. C: Results of clinical trials of HER2 inhibitor antibodies in patients with advanced breast cancer. ORR- overall response rate.

Table 1

A.					
Oestrogen/mg	CR	PR	CI, %	SD	Reference
DES/15mg	4/32	6/32	N/A	2/32	Lonning ^a
Oestradiol/6mg	N/A	1/32	15-37	7/32	Ellis ^b
Oestradiol/30mg	N/A	3/34	19-42	7/34	Ellis ^b

B.				
Bcl-2 Inhibitor	T/C, %	T-C, Days	No. of Animals	Reference
[-]-Glossypol	56	3	5	Mohammad ^c
TW-37	57	4	5	Mohammad ^d

C.				
HER2 Inhibitors		ORR	Phase	Reference
Trastuzumab+paclitaxel or doxorubicin+cyclophosphamide		50% vs. 32%	Phase III	Slamon ^e
Trastuzumab+docetaxel		61% vs. 34%	Phase II	Marty ^f
Lapatinib		24%	Phase III	Gomez ^g
Lapatinib+capecitabine		24% vs. 36%	Phase III	Cameron ^h

^a reference [98]
^b reference [120]
^c reference [129]
^d reference [133]
^e reference [134]
^f reference [135]
^g reference [145]

$h_{\text{reference}}$ [147]