

## Short communication

# Oestrogen is bad for patients with breast cancer?

V Craig Jordan<sup>1</sup>, Eric A Ariazi<sup>1</sup>, Joan S Lewis-Wambi<sup>1</sup>, Ramona R Swaby<sup>1</sup>, Heather E Cunliffe<sup>2</sup>, Anna T Riegel<sup>3</sup> and Anton Wellstein<sup>3</sup>

<sup>1</sup>Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA

<sup>2</sup>Translational Genomics Research Institute, Phoenix, Arizona 85004, USA

<sup>3</sup>Vincent T Lombardi Cancer Center, Georgetown University, Washington, District of Columbia 20057, USA

Corresponding author: V Craig Jordan, [v.craig.jordan@fccc.edu](mailto:v.craig.jordan@fccc.edu)

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Breast cancer is a hormone-dependent disease, and a proportion of patients with oestrogen receptors (ERs) will respond to ovarian ablation [1-3]. For this reason, oestrogen is considered bad for patients with breast cancer. This short communication presents our evolving understanding of oestrogen's role as a survival signal in breast cancer and new emerging knowledge of the apoptotic actions of oestrogen [4].

Synthetic oestrogens based either on the structure of triphenylethylene or the very potent but shorter acting diethylstilboestrol [5,6] were described more than 60 years ago. This proved to be a cheap source of new medicines. High-dose synthetic oestrogen administration was found to be effective in the treatment of breast and prostate cancer [7], but low-dose synthetic oestrogens never really became accepted as hormone replacement therapy in postmenopausal women. Indeed, diethylstilboestrol subsequently achieved notoriety as an oestrogen supplement to prevent recurrent abortion. Children of treated mothers had a high incidence of clear cell carcinoma of the vagina [8,9]. In contrast, the synthetic oestrogens based on triphenylethylenes were subsequently to undergo a metamorphosis and be transformed into anti-oestrogens used for the treatment of breast cancer [10].

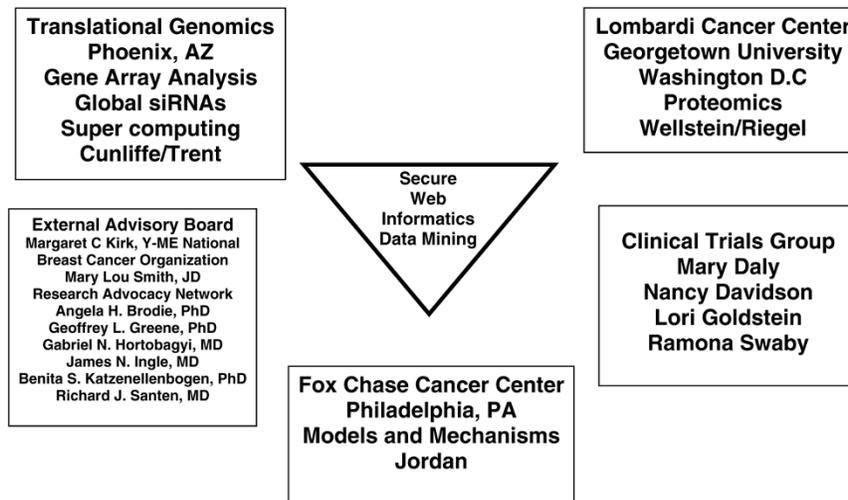
Based on the link identified between oestrogen and the development and growth of some breast cancers, the current strategy for the treatment and prevention of ER-positive breast cancer is the application of long-term antihormonal therapy [11]. The use of long-term tamoxifen therapy [12] has had a profound effect on survival, but in addition the wide distribution of tamoxifen has resulted in a declining death rate from breast cancer over the past few years. Currently, the aromatase inhibitors [13-15] are proving to represent a modest improvement over tamoxifen therapy, especially for the postmenopausal woman with concerns about endometrial cancer and blood clots. However, tamoxifen remains the

treatment of choice for the premenopausal woman with ER-positive breast cancer.

The past 30 years have seen dramatic advances in the practical prospects for the chemoprevention of breast cancer. Studies in the laboratory with tamoxifen [16,17] and raloxifene [18,19] have now translated into clinical practice for either chemoprevention of breast cancer in high-risk women with tamoxifen [20,21] or treatment of osteoporosis with prevention of breast cancer with raloxifene [22,23]. However, widespread use of long-term antihormonal therapies for the treatment and prevention of breast cancer creates consequences for the tumour in the form of antihormonal drug resistance. Nevertheless, laboratory study of antihormonal drug resistance has revealed an unanticipated vulnerability of breast cancer cells.

It has been known for about 20 years that long-term oestrogen treatment of athymic mice inoculated with the ER-positive breast cancer cell line MCF7 will result in transplantable ER-positive tumours [24]. Tamoxifen will initially prevent tumour growth, but long-term tamoxifen therapy causes tumours to become drug-resistant, which is expressed as tamoxifen-stimulated growth [25]. This model system replicates the clinical situation for the treatment of advanced breast cancer, and second-line therapies in the clinic are usually an aromatase inhibitor or the pure anti-oestrogen fulvestrant [26,27]. However, the process of developing tamoxifen-stimulated tumour growth in the laboratory, which takes 1 to 2 years, does not replicate adjuvant therapy with tamoxifen, which has a duration of 5 years. To address this issue, tamoxifen-stimulated tumours were serially transplanted into successive generations of athymic mice and a novel form of drug resistance was recognized. Tamoxifen and other selective ER modulators (SERMs) such as raloxifene stimulate tumour growth [28], but remarkably oestrogen now does not support tumour growth but causes rapid tumour regression [29,30].

Figure 1



Organization of Department of Defense Center of Excellence Grant. Shown is the organization of our Department of Defense Center of Excellence Grant entitled 'A new therapeutic paradigm for breast cancer exploiting low-dose oestrogen-induced apoptosis'. The model systems to study the survival and apoptosis induced with oestrogen are being used in time course experiments at the Fox Chase Cancer Center. The materials are distributed to Translational Genomics for genomic analysis using comparative genomic hybridization, small interfering (si)RNA analysis or Agilent gene array analysis, and the Vincent T Lombardi Cancer Center is involved in conducting proteomics analysis. All results are uploaded into a shared secure web for data processing and target identification by our informatics and biostatistical group. Each laboratory is able to validate emerging pathways and study individual genes of interest. Our programme is integrated with a clinical trials programme that provides patient samples for validation of apoptotic or survival pathways. We are grateful to our external advisory board of patient advocates and professional colleagues for their continuing advice and support.

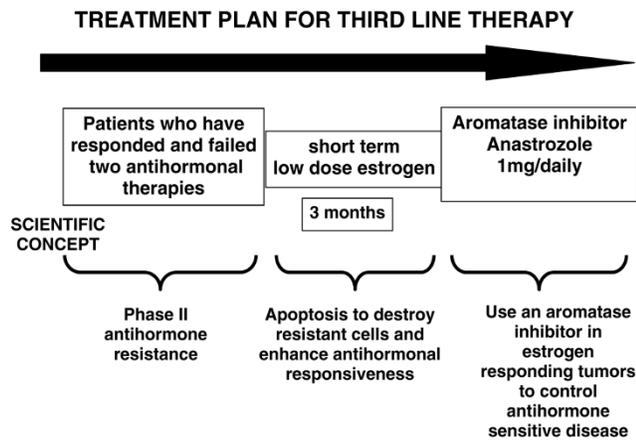
This action of oestrogen after 5 or more years of tamoxifen therapy demonstrates that there is an evolution of drug resistance in breast cancer cells. This was recently classified [31]. The early phases of drug resistance with tamoxifen are referred to as phase I resistance. This is indicated by a tumour growing with either tamoxifen or oestrogen treatment. In contrast, phase II resistant tumours grow only with tamoxifen, and oestrogen kills tumour cells. Similar studies are now being conducted using long-term oestrogen deprivation to replicate what will occur with the aromatase inhibitors [32].

Early studies growing MCF7 breast cancer cells in oestrogen-free media identified increased intracellular ER levels and spontaneous cell growth [33,34]. Several oestrogen-independent clones were isolated for study [35,36] and the idea was proposed that MCF7 cells are hypersensitized to grow in extremely low levels of oestrogen (below the level that can be detected or further reduced) [37]. However, Song and coworkers [38] observed that increasing concentrations of oestradiol could increase apoptosis in oestrogen-deprived cells by increasing the concentration of Fas ligand that activates death receptor pathways. Thus, the original observations that phase II tamoxifen resistant tumours could be treated with physiological oestrogen [29,30] were extended to aromatase inhibitor resistant cells. However, in contrast to the study

conducted by Song and coworkers [38], phase II tamoxifen resistant tumours respond to increasing oestrogen treatment by increasing the Fas receptor, and decreasing HER2/neu and nuclear factor- $\kappa$ , which is associated with tumour regression [39]. Furthermore, MCF7 cells kept for many years under oestrogen-depleted conditions using medium containing stripped foetal bovine serum produce rapid apoptosis via an intrinsic mechanism directed at the mitochondrion [40,41]. However, both Lewis and coworkers [41] and Song and Santen [42] found that apoptosis is modulated through bcl-2 or bcl-2XL.

It is also perhaps important to note that the new knowledge about oestrogen action emerged through re-examination of existing cell lines. In early reports on the effects of oestrogen withdrawal, no oestrogen-induced apoptosis was noted [35,36], but by altering culture conditions or extending the period of oestrogen exposure, apoptosis occurs [40,41,43]. Overall, the phenomenon observed with long term oestrogen withdrawal is similar to the phase II resistance of the model described for SERMs [32].

Lonning and coworkers [44] addressed the hypothesis that patients with ER-positive breast cancers who have been treated exhaustively with antihormonal therapy could potentially respond to high-dose oestrogen therapy. Thirty-

**Figure 2**

Anticipated treatment plan for third-line endocrine therapy. Patients must have responded and failed two successive antihormonal therapies to be eligible for a course of low-dose oestradiol therapy for 3 months. The anticipated response rate is 30% [44] and responding patients will be treated with anastrozole until relapse. Validation of the treatment plan via the Center of Excellence Grant (Figure 1) will establish a platform to enhance response rates with apoptotic oestrogen by integrating known inhibitors of tumour survival pathways into the 3-month low-dose oestrogen debulking treatment plan. The overall goal is to increase response rates and maintain patients for longer on antihormonal strategies before chemotherapy is required.

two patients with advanced breast cancer previously exposed to between two and ten (median four) endocrine treatments were treated with diethylstilboestrol 5 mg three times daily. Therapy was well tolerated but four patients terminated treatment within 2 weeks of starting and another two stopped treatment before progression. One of these patients had stable disease for 15 weeks and one a partial response for 39 weeks. Of the remainder, four patients obtained a complete response and six patients a partial response. Two patients had stable disease for 6 months and one for more than 1 year. Overall, these extremely encouraging preliminary studies with high-dose oestrogen therapy are complemented by anecdotal reports of the effectiveness of low-dose oestrogen treatment for those women with endocrine refractory breast cancer after exhaustive antihormonal therapy (Ingle J, Dixon M, personal communication). As a result, several clinical studies are currently underway (Ellis M, Santen R, personal communications).

Based on preclinical laboratory modelling, we have translated the new biology of oestrogen action into a Department of Defense Center of Excellence grant with laboratory and clinical collaborators illustrated in Figure 1. Our goal is to define the pathways for oestrogen-induced survival and apoptosis in endocrine responsive breast and endometrial cancer, and to use the emerging database to guide the interpretation and development of a series of clinical trials.

The ultimate goal of our clinical trial design is illustrated in Figure 2 and currently consists of two separate but interconnected therapeutic oestrogen studies, designed to determine the lowest dose of a 12-week course of oestrogen that causes a positive therapeutic effect.

In summary, the development and extensive clinical application of long-term antihormonal therapy [11] has had consequences for the patient with the development of antihormonal drug resistance in some breast cancers [31]. However, with the development of drug resistance to exhaustive antihormonal therapy, a vulnerability of the cancer has been exposed. The recognition of the new biology of oestrogen action that causes apoptosis in sensitive breast tumours now opens an unanticipated door of opportunity to exploit the findings to aid patients. Although the actual clinical responses may not be profound in unselected patient populations or in populations whose tumours do not have the correct (stage II) form of breast cancer drug resistance, our ability to decipher apoptotic mechanisms from laboratory models, and eventually to target patients appropriately, may have profound and positive effects for some patients. The translational knowledge gained over the coming few years may again provide unanticipated opportunities to exploit the discovery of 'apoptotic triggers' for other forms of cancer.

It is perhaps pertinent to restate that for 70 years there has been an 'ebb and flow' relationship for the role of oestrogen in breast tumour homeostasis. We have illustrated in this article many of the changing fashions that have occurred in the perception of oestrogen as either hero or villain with respect to women's health. The effects of modulating the ER system in the breast, at one time or another, have been dismissed because the effects are small or believed to be of no major consequence. Nevertheless, the number of events becomes accumulative. By way of example, it is important to recall that initial use of tamoxifen, a failed contraceptive, to treat unselected populations yielded only modest responses for some patients with metastatic breast cancer [45]. Years later, after deciphering the target populations and translating the appropriate treatment strategies from the laboratory to the clinic, the drug became the 'gold standard' for endocrine therapy [45] and was credited with improving the survival of hundreds of thousands of women [12]. The challenge for the future is to exploit the profound apoptotic action of oestradiol as a lead to develop innovative new therapies for cancer.

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