

Paradoxical Clinical Effect of Estrogen on Breast Cancer Risk: A "New" Biology of Estrogen-induced Apoptosis

V. Craig Jordan¹ and Leslie G. Ford²

Abstract

Administration of estrogen replacement therapy (ERT) decreases the incidence of breast cancer, as shown in a double-blind, placebo-controlled randomized trial of the *Women's Health Initiative* (WHI) in 10,739 postmenopausal women with a prior hysterectomy. Although paradoxical because estrogen is recognized to stimulate breast cancer growth, laboratory data support a mechanism of estrogen-induced apoptosis under the correct environmental circumstances. Long-term antiestrogen treatment or estrogen deprivation causes the eventual development and evolution of antihormone resistance. Cell populations emerge with a vulnerability, as estrogen is no longer a survival signal but is an apoptotic trigger. The antitumor effect of ERT in estrogen-deprived postmenopausal women is consistent with laboratory models. *Cancer Prev Res*; 4(5); 633–7. ©2011 AACR.

Introduction

It is widely held that estrogen can be carcinogenic in breast tissue (1) and is the "fuel for the fire" to stimulate the growth of estrogen receptor (ER)-positive breast cancer cells (2). This knowledge, supported by an enormous body of laboratory data, provides the conceptual basis for the successful development of antihormonal strategies to treat breast cancer (3). Selective ER modulators (SERMs), for example, tamoxifen, block estrogen-stimulated tumor growth at the ER, and aromatase inhibitors prevent peripheral estrogen synthesis in postmenopausal patients, thereby creating estrogen deprivation to stop tumor growth (3). The successful treatment strategy for breast cancer with SERMs was subsequently translated into reducing the risk of breast cancer in high-risk women. SERMs are available to reduce the incidence of breast cancer in pre- and postmenopausal (tamoxifen) or postmenopausal (raloxifene) women (4–6). As predicted by the mechanism of action of SERMs as anticancer agents, only ER-positive breast cancer is reduced. In practice, preventing estrogen action prevents breast tumor initiation and growth. Paradoxically, the recent analysis of estrogen replacement therapy (ERT) in the *Women's Health Initiative* (WHI) double-blind, placebo-controlled randomized trial in 10,739 postmenopausal women with a prior hysterectomy (ages 50–79; ref. 7) actually showed a decrease in invasive breast cancer, which

was sustained for 5 years after ERT was stopped. This result seems to run counter to the perceived wisdom of the role of estrogen in breast carcinogenesis, was significant in women of all ages, and was similar in every age group.

When the WHI was initiated in 1993, their present clinical result of a reduction in breast cancer was unanticipated (7) but is consistent nevertheless with parallel laboratory studies completed over the past 20 years. Estrogen-induced apoptosis is a plausible molecular mechanism to support an antitumor action of physiologic estrogen (8). The key to our understanding of estrogen-induced apoptosis is the finding that breast cancer cell populations adapt to estrogen deprivation, but these populations are dynamic, and resistance to estrogen deprivation evolves over time (5 years). This evolution of resistance to estrogen deprivation causes a reconfiguration of cellular survival pathways, which in turn exposes a vulnerability of breast cancer cell survival. Physiologic estrogen causes apoptosis and does not act as a survival signal (8).

We will weigh the laboratory and clinical evidence to support the proposition that physiologic estrogen can cause apoptosis in breast cancer cells following long-term estrogen deprivation. Our objective is to make a case based on scientific observations to support our proposition that nascent breast cancer cells could have the same apoptotic response to ERT after estrogen deprivation caused by menopause. We will present the evidence in chronological order (Box 1).

Evidence from the Historical Use of Estrogens to Treat Metastatic Breast Cancer

The application of high-dose estrogen therapy for the treatment of metastatic breast cancer was the first use of a chemical therapy to treat any cancer successfully (9). Estrogen therapy became the standard of care to treat metastatic breast cancer in postmenopausal patients until

Authors' Affiliations: ¹Georgetown Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, District of Columbia; and ²Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Corresponding Author: V. Craig Jordan, Georgetown University Medical Center, 3970 Reservoir Rd NW, Research Building, Suite E501, Washington, DC 20057. Phone: 202-687-2897; Fax: 202-687-6402; E-mail: vcj2@georgetown.edu

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BOX 1. Cumulative evidence to support low dose estrogen-induced apoptosis in long-term estrogen-deprived nascent breast cancer?

1. Historical use of estrogens to treat breast cancer.
2. Physiologic estrogen as an antitumor agent in SERM-resistant breast cancer models *in vivo*.
3. Estrogen-induced apoptosis in estrogen-deprived ER-positive cell lines *in vitro*.
4. A current evaluation of estrogen to treat acquired antihormone resistance in metastatic breast cancer.
5. The extrapolation of the concept that physiologic estrogen kills breast cancer cells to adjuvant anti-hormone therapy.

the introduction of tamoxifen (late 1970s in the United States), a nonsteroidal antiestrogen (10). Tamoxifen became the "gold standard" for the treatment of ER-positive (estrogen stimulated) breast cancer for the next 20 years. Estrogen was all but abandoned as a treatment option, but Ingle and colleagues completed a provocative trial of tamoxifen versus the synthetic estrogen diethylstilbestrol (DES; high-dose) in metastatic breast cancer (11). Responses were equivalent with fewer side effects with tamoxifen, but a re-analysis years later demonstrated that survival was significantly improved with DES (12).

Towards the end of his distinguished career, Professor Sir Alexander Haddow FRS reflected (during the inaugural Karnofsky Memorial Lecture; ref. 13) on the remarkable responses noted with estrogen in some tumors, often when treatment was more than a decade past menopause: "*The extraordinary extent of tumour regression observed in perhaps 1% of post-menopausal cases (with oestrogen) has always been regarded as of major theoretical importance, and it is a matter for some disappointment that so much of the underlying mechanisms continues to elude us.*"

Although laboratory research to address Haddow's estrogen paradox essentially ceased for the next 20 years, at least 1 animal model transplanted with a human breast tumor replicated the antitumor action of high-dose estrogen therapy for breast cancer (14, 15). The question could have been addressed. However, the breakthrough in our understanding of a mechanism for estrogen-induced apoptosis came with a study of continuous long-term SERM treatment in transplantable SERM-resistant breast cancer in athymic mice. As often happens in science, a discovery in an apparently unrelated area becomes the required breakthrough to create transparency in nature.

Physiologic Estrogen Is an Antitumor Agent in SERM-Resistant Breast Cancer *In Vivo*

In the 1980s, the first athymic animal models of tamoxifen-induced antihormone resistance were reported, but

the acquired resistance surfaced within 2 years as tamoxifen-stimulated growth (2). This replicated the use of tamoxifen in the treatment of metastatic ER-positive breast cancer but did not explain the astonishing success of 5 years of adjuvant tamoxifen therapy in reducing recurrences by 50% and mortality by 30%. Most important, the gains obtained during therapy are maintained (and mortality further reduced) for the next 15 years. We were missing a vital clue about the evolution of antihormone resistance in micrometastatic breast cancer.

Five years of re-transplantation of tumors into tamoxifen-treated athymic mice revealed a vulnerability in breast cancer that would subsequently be exploited in clinical trial. Physiologic estradiol does not promote tumor growth, but small tumors undergo rapid and complete regression (16). It was suggested (16) that following the cessation of adjuvant tamoxifen, a woman's own estrogen would exert an antitumor action and enhance survivorship. Further studies (17) subsequently demonstrated that following tumor regression with physiologic estradiol, any remaining tumor that re-grows in the estrogen environment is again responsive to tamoxifen as an antitumor agent. Continuing studies demonstrated that the principle of physiological estrogen therapy causing apoptosis in SERM-resistant disease was also true for raloxifene (18, 19). These data provided a scientific rationale for subsequent clinical studies.

Estrogen Induces Apoptosis in Estrogen-deprived ER-positive Breast Cancer Cell Lines

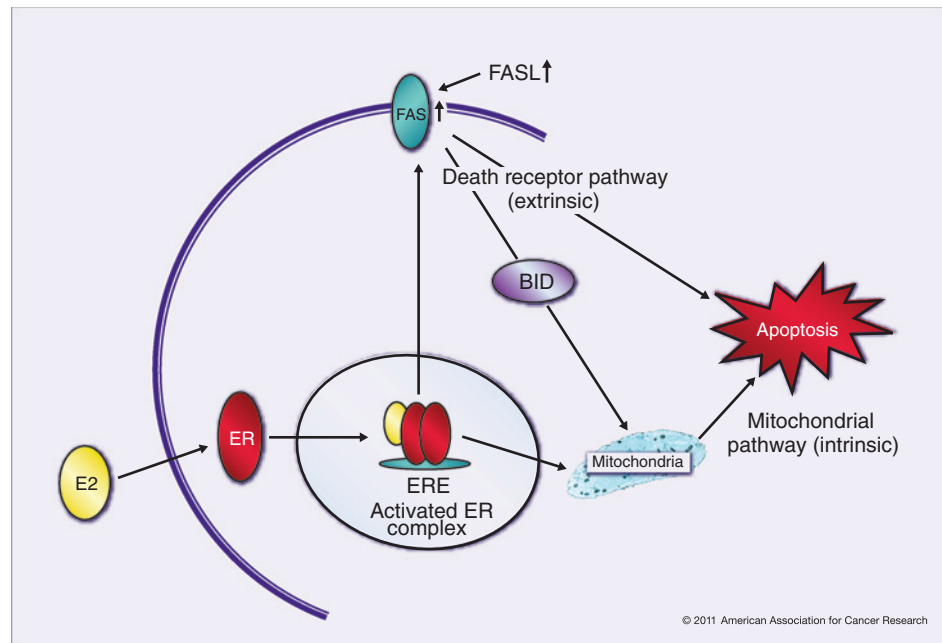
Song and colleagues (20) first showed in cell culture that high concentrations of estrogen could induce cellular apoptosis directly through a FAS/FASL pathway. However, the discovery that physiologic concentrations of estradiol could induce apoptosis (21) in both cell culture and animal models was the advance pertinent to the clinical observation that ERT reduces the incidence of breast cancer in postmenopausal women (7). This is now a consistent experimental observation with new knowledge emerging about the molecular mechanisms of estrogen-induced apoptosis. Figure 1 summarizes much of the current data on molecular mechanisms of estrogen-induced apoptosis, the topic of a forthcoming mini-review in *Cancer Prevention Research* later this year.

Despite the significant body of laboratory data to support the proposition that physiologic estrogen can induce apoptosis in long-term estrogen-deprived breast cancer cells, only the translation to patients tests the veracity of the experimental approach as a conversation with nature and a general principle.

Current Evaluation of Estrogen to Treat Acquired Antihormone Resistance in Metastatic Breast Cancer

Lonning and colleagues (22) studied the efficacy of high dose of DES on the responsiveness of metastatic breast

Figure 1. The 2 main pathways involved in estrogen-induced apoptosis regulation. This apoptosis can be triggered either through the extrinsic death-receptor pathway with an increase in Fas ligand (20) or Fas (27) or via the intrinsic pathway of mitochondrial disruption and release of cytochrome C (28). E2, estradiol (the most potent estrogen in women); ER, estrogen response element; BID, Bcl-2–interacting domain.



cancer following exhaustive treatment with antihormone therapies (tamoxifen, aromatase inhibitors, etcetera). A remarkable 4 of 32 patients had complete responses (22), and 1 patient, who was treated for 5 years, had no recurrence of her disease 6 years after stopping DES (23). The question, however, is whether physiologic estrogen has efficacy as an antitumor agent in the appropriately prepared estrogen-deprived breast tumor. Ellis and colleagues (24) addressed this question and found an equivalent clinical benefit for high (30 mg daily) and low (6 mg daily) dose of estradiol in metastatic breast cancer patients who had failed aromatase inhibitor therapy, that is, long-term estrogen deprivation. Their clinical advance was that low-dose estrogen was as efficacious as high-dose estrogen for antitumor therapy in breast cancer (for the appropriate tumor that had been estrogen deprived), but there were fewer side effects with low-dose therapy. The target, estrogen-deprived breast cancer, is vulnerable to physiologic estrogen.

The Extrapolation of the Concept that Physiologic Estrogen Kills Breast Cancer to Adjuvant Antihormone Therapy

The result from the WHI Trial of ERT in hysterectomized women (7), which showed a sustained reduction in the incidence of breast cancer, provides additional evidence that the strategy to decipher the mechanism of physiologic estrogen to induce apoptosis (8, 25, 26) has significance for both treatment and prevention. Indeed, the idea that a woman's own estrogen was responsible for enhanced survivorship by causing apoptosis of the appropriately

prepared and vulnerable micrometastases (16) followed the completion of long-term adjuvant tamoxifen therapy and now is incorporated into the Study of Letrozole Extension (SOLE) Trial. This extended adjuvant antihormone treatment study (Fig. 2) is addressing the question of whether regular drug holidays will decrease recurrence rates compared with continuous therapy. For initial safety reasons, a women's own estrogen during the drug holiday is hypothesized to be adequate as an apoptotic trigger because rigorous prior antihormone therapy will have selected vulnerable cell populations as the waiting target. Subsequent trials may have to use ERT for a few weeks to trigger apoptosis.

We have presented an integrated approach to support the proposition that ERT could induce apoptosis and reduce the incidence of breast cancer. The important issue for the decision of breast cancer cells to survive or die in response to estradiol depends entirely on the cell populations present in an estrogenized environment or following estrogen deprivation. Based on laboratory data, the decision is survival or death, respectively. The role of estrogen deprivation, either pharmacologic with antihormones or physiologic with menopause, is to select populations of cells that can survive without physiologic estrogen. These cells choose to die through a natural process when re-exposed to pharmacologic or physiologic estrogen. The genetics are the same, but different epigenetic events based on the well-established property of cancer cells to be able to adapt to any environment and survive remains true. As the WHI study of ERT shows (7), physiologic estrogen has delivered what the scientific database would now predict.

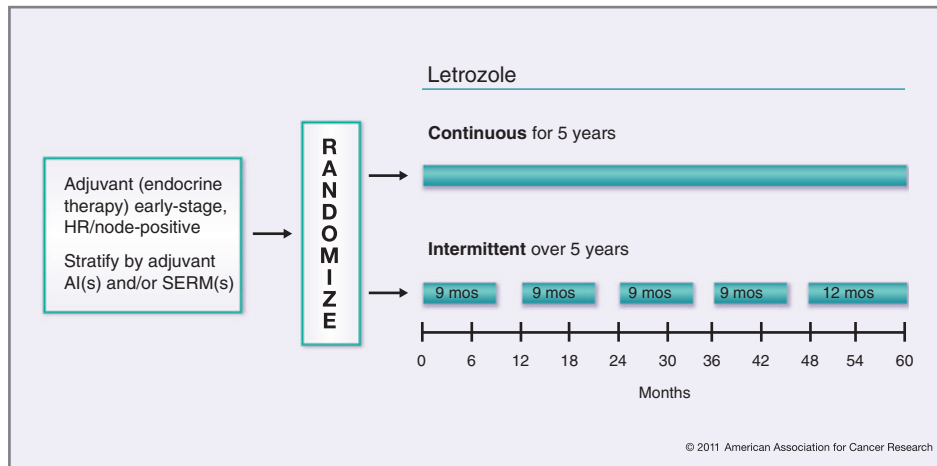


Figure 2. Schema for the Study of Letrozole Extension (SOLE; IBCSG 35-07) conducted by the International Breast Cancer Study Group (IBCSG). Upon completing 4 to 6 years of prior adjuvant endocrine therapy with a SERM(s) and/or aromatase inhibitor(s) (AI), patients were randomly assigned to continuous or intermittent letrozole (3-month drug holidays per year) for 5 years. The rationale for this approach was that the woman's own estrogen in the intermittent arm would trigger apoptosis in long-term estrogen-deprived breast cancer and reduce recurrence rates. Adapted from *International Breast Cancer Study Group - Study of Letrozole Extension* (www.ibcsg.org).

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the NIH. The views and opinions of the author(s) do not reflect those of the U.S. Army or the Department of Defense. No conflicts of interest were reported.

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