

Antihormonal Drug Resistance in Breast and Endometrial Cancer

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During the past 30 years, antihormonal therapy targeted to the estrogen receptor (ER) has become the standard of care for the treatment of breast cancer. Tamoxifen, (V.C. Jordan, *Nature Rev. Drug Dev.* 2:205, 2003), a nonsteroidal antiestrogen that blocks estrogen binding to the ER, is effective in the treatment of all stages of ER positive breast cancer and is credited with saving the lives of a half million women worldwide. Tamoxifen also reduces the risk of breast cancer in high-risk pre and postmenopausal women. However, the toxicological standards necessary for chemoprevention are much more stringent than those accepted for treatment. Tamoxifen is a selective ER modulator (SERM) (V.C. Jordan, S. Gapstur and M. Morrow, *J. Natl. Cancer Inst.* 93(19):1449, 2001), which means that the medicine exhibits antiestrogenic action in the breast but also beneficial estrogen-like action to increase bone density and reduce circulating cholesterol. Tamoxifen also produces estrogen action that increases the thromboembolic events and causes a modest but significant increase in endometrial cancer incidence in postmenopausal women. The related SERM raloxifene is currently being tested as a chemopreventive for breast cancer in high risk women. Aromatase inhibitors, which block the synthesis of estrogen in post menopausal women, are proving to be superior to tamoxifen in the treatment of breast cancer and are being evaluated as chemopreventives. Both aromatase inhibitors and raloxifene do not increase the incidence of endometrial cancer.



The success of long-term (5 years) antihormonal treatment is encouraging the evaluation of extended antihormonal therapies with non-cross resistant agents. This approach is proving to be an important long-term maintenance strategy but there are consequences with the development of different types of antihormonal drug resistance (V.C. Jordan, *Cancer Cell* 5:207, 2004). Antihormonal resistance will eventually develop and render a successful targeted therapy ineffective. The goal of our laboratory is to develop models of antihormone-resistant breast and endometrial cancer, study molecular mechanisms, and translate findings through clinical trials to improve the treatment of breast cancer.

Models of antihormonal resistance. Jordan, Ariazi, Cordera

Although it is possible to develop antihormone resistant breast or endometrial cancer cells in a

few months, these experimental techniques do not replicate clinical treatment schedules. We have made the strategic decision to create clinically relevant models of antihormone treatment

to study the consequences of long-term (5–10 years) therapy. Two approaches have been taken: 1) ER positive cell lines are inoculated into ovariectomized immune deficient (athymic) mice and exposed to oral SERMs (tamoxifen or raloxifene) daily with growing tumors being retransplanted into successive generations of SERM treated mice; 2) cells in culture are exposed to SERMs or estrogen deprivation and passaged for years before biological evaluation in athymic mice. A series of SERM or estrogen withdrawn (to replicate resistance to aromatase inhibitors) MCF-7 breast cancer cells are being characterized as well as ECC-1 endometrial cancer cells. The general conclusion following two decades of model development is that antihormonal resistance evolves through a series of phases (V.C. Jordan, *Cancer Cell* 5:207, 2004). Phase I SERM resistant tumors can grow with either a SERM or estrogen. This phase mimics current clinical experience where treatment with an aromatase inhibitor, (to reduce a woman's own estrogen), or fulvestrant, (an agent that binds to and subsequently destroys the ER), are the therapeutic options. However, if treatment with a SERM is continued, then phase II antihormone resistance occurs; the tumors require the SERM to grow but a vulnerability of the drug resistant cancer cells is exposed. Estrogen, rather than acting as a survival signal, now induces rapid apoptosis in sensitive cells. Remarkably, any tumors that re-grow now regain sensitivity to antihormone treatment to prevent estrogen stimulated growth. The tumor models maintained in athymic mice also provide a rich resource for the study of angiogenic pathways that must expand as tumors evolve from antihormone sensitive, to antihormone dependent and back again.

Steroid receptor signal transduction. Jordan, Ariazi, in collaboration with Levin^a

The ER has proved to be an important therapeutic target but it is clear that there are interactions of the ER pathway with the HER2 cell signaling pathway as well as other members of the steroid receptor super family, e.g., androgen receptor (AR). The complex decision making network is particularly important as breast cancer cells evolve through different phases of resistance. In other words, dependence on the ER may switch to AR under the correct circum-

stances to allow cells to survive. Additionally, a group of ER-related receptors (alpha, beta, gamma) that usually do not bind ligands but which interact with estrogen responsive genes can enhance the prospects of survival. Our goal is to understand receptor interaction and modulation during the process of antihormone resistance. This knowledge will potentially provide an important insight into cellular survival mechanisms that can subsequently be targeted.

Estrogen-induced apoptosis. Jordan, Lewis, in collaboration with Cunliffe^b

The finding that estrogen-deprivation can result in spontaneous cell growth both *in vitro* and *in vivo* and that some cell lines can be killed by low concentrations of estrogen provides a unique opportunity to study the mechanisms of spontaneous growth and the apoptotic action of estrogen. A series of MCF-7 cell lines have been developed that are either responsive to antihormones or are resistant to SERMs or estrogen deprivation (aromatase inhibitors). Additionally, since the ER is also the critical signal transduction pathway for survival, a fulvestrant resistant cell line that has no ER is available as a comparator. The approach being taken is to compare and contrast the survival of apoptotic pathways in the cells' time courses using Affymetrix U133. Gene arrays and analysis are by the supercomputer at Translational Genomics (TGen, AZ). Creation of survival and apoptotic maps will allow subsequent targeted interrogation so that shifts in the survival pathways can create predictable apoptosis. These studies will identify possible therapeutic strategies to test in clinical trial.

Clinical translation. Jordan, Ariazi, in collaboration with Daly,[§] Swaby[§]

The current fashion for the antihormonal treatment of ER positive breast cancer is to employ an exhaustive cascade of therapies until combination cytotoxic chemotherapy is the only option. Preliminary studies demonstrate that the use of standard dose (15 mg daily) diethylstilbestrol (a synthetic estrogen) will produce a 30% response rate in patients treated exhaustively with a succession of antihormone therapies. We are currently replicating these findings but with the goal of identifying select gene profiles that identify tumors that respond or are resistant to high dose estrogen therapy. Patients

are also monitored for apoptotic products in serum during the 12-week course of estrogen. Our aim is to determine the earliest possible indication of tumor response. Creation of our database for response rate will enable us to address dose de-escalation, thereby establishing the lowest dose of estrogen necessary to pro-

duce an optimal response with reduced estrogen-related side effects. Completion of this program of establishing a targeted apoptotic mechanism to aid specific patients will create a platform to test new anti-survival agents and enhance the prospects of tumor remissions in advanced breast cancer.

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V. Craig Jordan (center) with Professors Anna Lydia Pinho do Amaral and Nilson Gomes. Jordan was awarded an Honorary Professorship and Medal of Merit from the University of Iguacu, Rio de Janeiro, Brazil.