Review Article

Laboratory Models of Breast and Endometrial Cancer to Develop Strategies for Antiestrogen Therapy

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Tamoxifen (Nolvadex®) (Fig 1), has revolutionized the treatment of breast cancer. In 1973, the antiestrogen was approved by the committee on the Safety of Medicines for breast cancer therapy in the United Kingdom and this was followed by approvals in more than 100 countries around the world. Tamoxifen was approved in Japan in 1981.

Tamoxifen has become one of the most investigated drugs for cancer therapy but its proven efficacy resulted in it being listed by the World Health Organization as an essential drug for the treatment breast cancer. It is estimated that tens of thousands of women are alive today because of long-term adjuvant tamoxifen therapy. Nevertheless, the major limitations of adjuvant tamoxifen therapy are the potential to develop drug resistance and the possibility of developing second cancers, especially endometrial cancer.

In this review we describe the animal model systems that have been essential to plan an effective and safe strategy to treat human disease. Most importantly we will point out the appropriate, and the inappropriate, extrapolation of laboratory data for the safe treatment of human disease. Since there has been some concern about the development of second cancers during tamoxifen therapy it is important to focus on this aspect of drug safety and to place these concerns in perspective. The International Agency for Research on Cancer (IARC) conducted a rigorous review of the clinical data base and concluded that no patient being treated for breast cancer should stop therapy because of concerns about endometrial cancer.

Drug Resistance to Tamoxifen

A decade ago, the perceived wisdom was that drug resistance to tamoxifen developed when estrogen receptor (ER)-positive disease converted to ER-negative disease. It was known that tamoxifen was more effective in ER-positive disease so the hypothesis was logical.

In the laboratory, the dimethylbenzanthracene (DMBA)-induced rat mammary carcinoma model was used to define the optimal strategy for adjuvant therapy. In the mid 1970's the clinical

![Fig 1. Structures of tamoxifen, toremifene, ICI 182,780 and raloxifene.](image-url)
plan was to use tamoxifen for one year because one year, on average, was effective in controlling advanced disease and there was also a concern that longer treatment would cause premature development of drug resistance. It was believed that the early development of ER-negative disease would be a catastrophic outcome of extending adjuvant tamoxifen therapy. In 1977, we proposed that a strategy of longer rather than shorter therapy would be more beneficial to patients. Simply stated, we demonstrated that short-term tamoxifen therapy given to rats with subclinical disease produced by DMBA did not control the development of mammary tumors as well as continuous therapy. If therapy was stopped tumors developed. However, the tumors that developed were responsive to a second endocrine modality. Tumors induced by DMBA are predominantly ER-positive and we did not detect a significant incidence of ER-negative tumors or drug resistance after months of therapy. We therefore proposed that long-term tamoxifen therapy should be tested in clinical trials. The clinical studies subsequently demonstrated that two years and five years of adjuvant tamoxifen therapy produced a significant survival advantage for node-positive breast cancer patients. These conclusions were supported by the overview of clinical trials and we now know that 5 years of adjuvant tamoxifen is superior to 2 years of tamoxifen.

Since we were acutely aware of the fact that tamoxifen was not a cure for breast cancer, and that drug resistance would eventually occur, we decided to address the question by establishing a model of human disease. Our strategy was to establish a model system to evaluate new agents to use when tamoxifen failed. Additionally, we believed it was important to understand the molecular events that occurred so that new agents could be rationally designed.

The ER-positive MCF-7 breast cancer cell line requires estradiol to grow into tumors when inoculated into the mammary fat pads of athymic mice. Cell lines that are ER-negative grow without estrogen supplementation. Dr Kent Osborne's group in San Antonio demonstrated that tamoxifen controlled estrogen-stimulated MCF-7 tumor growth for prolonged periods, but was ineffective against ER-negative tumor growth. We replicated these findings but took the studies a step further by developing a transplantable model of tamoxifen-stimulated tumor growth. Remarkably, the tumors remain ER-positive and grow in response to estradiol or tamoxifen in athymic rats or mice. Since there was the possibility that species-specific metabolism of tamoxifen might be causing enhanced growth of tumors in mice (where the drug is classified as an estrogen in mouse target tissues), these studies were critical. We subsequently demonstrated that the direct, estrogen-like properties of tamoxifen cause selection of a tamoxifen-stimulated tumor and that local metabolism to estrogens does not play a role in the support of the tamoxifen-stimulated phenotype.

We had, therefore, developed a unique model of drug resistance in which tamoxifen stimulated growth. Indeed, if tamoxifen was withdrawn tumor growth slowed but we found that estradiol could then re-activate the ER-positive tumors. It was clear therefore that an additional therapy was necessary, after tamoxifen, to block re-activation of tumor growth by estrogen through the ER. Currently two alternatives are available: peripheral aromatase inhibitors, like anastrozole (Arimidex) or pure antiestrogens, like ICI 182780 (Faslodex) (Fig 1). Specific inhibitors of aromatase prevent the synthesis of estradiol or estrone thereby denying the tumor a growth.