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Historical Perspective

Proven value of translational research with appropriate animal models to advance breast cancer treatment and save lives: the tamoxifen tale

V. Craig Jordan

Departments of Oncology and Pharmacology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA

Correspondnece
Professor V. Craig Jordan OBE PhD DSc FMedSc, Departments of Oncology and Pharmacology, Vincent T. Lombardi Professor of Translational Cancer Research, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, 3970 Reservoir Road NW, Research Building, Suite E501, Washington, DC, 20057, USA.
Tel.: +1 202 687 2897
Fax: +1 202 687 6402
E-mail: vcj2@georgetown.edu

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Introduction

Our current healthcare system is the beneficiary of the landmark successes of earlier pioneers who struggled, but persevered, to save lives. In the 19th century, two individuals stand out. Dr Louis Pasteur, a PhD basic scientist who luckily was 'encouraged' to conduct applied research and saved a life. Professor Paul Erhlich, a medically qualified research pathologist and winner of the Nobel Prize for antitoxin research, would create a model for successful synthetic drug development that would save thousands of lives. In my generation, it was my friend and supporter Sir James Black, Nobel laureate, who would advance the selectivity implied by receptor theory to treat patients for long periods with pathological conditions. Infectious diseases were cured within months but chronic heart disease, elevated blood pressure and gastric acid secretion were stabilized for years. Lives were saved and the practice of medicine changed to become evidence based. The key to success throughout was the creation and use of appropriate animal models.

In this article, I will focus on the essential aspects of animal models in the unanticipated development of an orphan medicine tamoxifen, used initially to treat late stage breast cancer. The results from the animal models taught the medical profession how to use tamoxifen effectively to save lives, how to detect life-threatening side effects, or provided clues about a new group of medicines that now have multiple applications in women's health. But first, what did our pioneers do and how did they do it?

A perspective on pioneers

Dr Louis Pasteur had already had a prestigious career studying crystal structure, inventing 'pasteurization' for milk and wine to stop spoilage and a vaccine to protect sheep from anthrax, when he turned his attention to the fatal disease rabies. He used a rabbit model to attenuate the rabies virus and a dog model to test the vaccine [1]. His initial goal was to develop an experimental vaccine for study in animals until the fateful day the mother of 9-year-old Joseph Meister pleaded with Pasteur to save her son from a slow and painful death. He had been severely bitten by a rabid dog and death was assured. The unexpected arrival of the young Joseph Meister at that moment was critical, as Pasteur had recently revised his method to prepare attenuated rabies virus and the strategy to treat dogs to protect them from rabies. Pasteur found through his earlier experiments that passing the virus through monkeys, was not optimal and he selected passage through rabbits and collected the infected spinal cords. He fixed them by drying inside flasks protected from moisture. Two weeks of drying reduced the extracted virus to

become harmless to dogs who were now immune to rabies once inoculated with preparations of increasing virulence based on less dessication time. The young Meister was injected over a period of 11 days with a total of 13 injections of increasing rabies virulence. He escaped certain death. Following Pasteur's death and burial in the crypt of the Pasteur Institute in Paris, Joseph Meister became the faithful custodian to this medical pioneer's memory until the German occupation of Paris in 1940. It is said he chose suicide rather than surrender the keys to the crypt and the memorial to the scientist who saved his life and changed medicine.

Professor Paul Ehrlich devised the drug discovery and development system used today [2]. Earlier in his career as a pathologist he was fascinated to find that organic dyes would specifically bind to bacterial and not human tissue. This gave him the clue to devise chemical therapy. Ehrlich's primary interest was vaccines and antitoxins for which he received the Nobel Prize. Ehrlich believed in the fidelity of the immune system to neutralize and destroy infectious disease. However with the expansion of European colonial interests into Africa came new challenges. It became obvious that the immune system could not kill tropical diseases such as malaria and sleeping sickness whose cause was protozoal. The immune system was overwhelmed by the sheer bulk of the infectious agent. Ehrlich stated 'an attempt must be made to kill the parasites within the body by chemical agents. In other words chemical agents must be used when serum therapy is impossible. French scientists Alphonse Laurier (awarded the Nobel prize for the discovery of the causative agent of malaria) and Mesnel found they could transfer trypanasomes from mouse to mouse to replicate human disease. Progression of the disease could be monitored through blood tests.

Ehrlich used the model to show that dyes could be 'parasitotropic' in mice. Trypan red could cure infected mice. However, when Ehrlich identified the nitrogen-containing azo group in trypan red this brought him to organic arsenicals. An arsenical para-aminophenyl arsenic acid (atoxyl) was marketed already but the compound was ineffective in their model. They had discovered arsenical resistance. A fortunate series of scientific advances in microbiology in 1905 occurred with the chance observation by others, that syphilis was associated with spirochetes that occupied a position between protozoans and bacteria. This was followed by the validation of animal models by scientists in Italy in 1906. At this point Ehrlich appears to have integrated a study of syphilis and a study of resistance to trypanosomes to arsenicals into his laboratory strategic plan. The key to success for the eventual discovery of compound 606 (Salvarsan), through methodical structure activity relationship, was the recruitment of Sahachiro Hata from Tokyo to screen all the compounds in the appropriate models of human disease. Salvarsan was discovered in June 1909. Following toxicology in animals, clinical trials were conducted with the drug manufactured by the Hoechst Company in Germany. Another deadly infectious disease was cured and thousands lived.

Sir James Black (of β-adrenoceptor blocker fame) [3] worked in the laboratories of Imperial Chemical Industries (ICI) Pharmaceuticals Division, Alderley Park, near Macclesfield, Cheshire. He had left ICI by the time I was a summer student at ICI in 1967. Alderley Park was 10 miles from my home and I was then an undergraduate in the Pharmacology Department at Leeds University, keen to do research in cancer drug discovery. There was none of significance then at ICI but the cell biologist Dr Steven Carter (of cytochalasin B fame) [4] was looking at the effects of compounds on mouse cancer cells in culture. It was a start! Coincidentally, the Head of the Fertility control programme, Dr Arthur Walpole had his laboratory opposite Dr Carter's. He had just published a paper [5] on the effects of ICI 46 474 as a 'morning after pill' in rats – but nobody cared! We will meet ICI 46 474 (tamoxifen) later.

Although this was a prescient meeting with Dr Walpole as he would later be the examiner of my PhD on 'failed morning after pills' at Leeds in 1972, the critical players at the start of our tale were being assembled. I met Dr Michael Barrett (of atenolol fame) [6] whose laboratory was next to Dr Carter's at ICI. He had taken over the β-adrenoceptor blocker programme after Jim Black left. Dr Barrett was to talent spot me for a faculty position at Leeds when he became the Professor of Pharmacology in 1970.

Also at ICI in the summer of 1967, I had the privilege to meet Dr James Raventos who was studying gastric acid secretion in dogs with histamine. Jim later told me that the known antihistamines did not block histamine stimulated gastric acid secretion in the dog model. Based on Jim's pioneering studies on the regulation of accelerated cardiac function and arrhythmias in the dog model with his new β-adrenoceptor blockers, he reasoned that the 'antihistamine anomaly' must be because there was a second subtle histamine receptor modulating mechanism [3] – and so it was. The H_2-receptor blockers were born at Smith, Kline and French and long term treatment with H_2–receptor blocker 'antacids' was possible as was β-adrenoceptor blocker treatment for heart conditions before.

Regulations for the safety of medicines

Pasteur, Ehrlich and Black each chose not to conform to the dogma that disease and death were inevitable. Each chose to question Nature through experimental animal models. Their persistence was translated to patient care. However, success in one area of therapeutics demands regulations imposed by society on claims in other areas thereby preventing Charlatans peddling 'cures' that are neither evidenced based nor safe. The elected representatives of the people in our democratic society are charged

with the responsibility to enact laws and regulations that ensure the safety of any new medicine. A strict protocol of appropriate animal toxicology is enforced by acts of government to prevent unanticipated injury or deaths.

It is not necessary to expand further as the concepts of safety and the documented worth of a medicine for patient care should be obvious to all. Nevertheless, a couple of examples will be given to illustrate instances when an inadequate system of protection can fail or a warning model appears to do so.

It is axiomatic that one should always err on the side of caution with safety and side effects of medicines. Thalidomide taught us that lesson so why was there no caution? The reason that the tragedy occurred was that there was no legal requirement to test for teratogenicity in the 1950s [7]. The toxicology concern was first raised by observation in humans [8]. Tragically, the value of thalidomide was seen to be in the control of nausea in pregnant women during the first trimester [9], exactly when limb development is occurring in the foetus. It is now known that thalidomide can stop blood vessel formation and limb formation is particularly vulnerable. Now there is rigorous teratogenesis testing of medicines to be used in women of childbearing age. It is important to note that thalidomide used in a cancer context, to treat a fatal disease, can produce improvements in multiple myeloma deployed as an anti-angiogenic agent.

The thalidomide tragedy and introduction of teratogenic testing is why women taking the anti-oestrogen tamoxifen during their childbearing years to treat breast cancer, must use barrier contraception to prevent pregnancy. However, there was an apparent anomaly in the toxicology testing of tamoxifen when it transitioned from cancer therapy with a requirement for only liberal toxicity testing for a fatal disease, to a chemopreventive in disease-free women only at risk for breast cancer. This toxicological surprise in rats given tamoxifen for years was hepatocellular carcinoma that was first reported [10] nearly 20 years after tamoxifen had been used by perhaps a million women worldwide.

Tamoxifen was discovered to be a rat liver carcinogen at high doses given for a lifetime [10] but increases in human hepatocellular carcinoma were not noted either in the 1990s [11, 12] when the toxicological issue was raised initially or indeed now [13]. Millions of women have benefited from tamoxifen with its long term use. However, tamoxifen would not have been knowingly developed by any company had the toxicological knowledge been available at the beginning of the tamoxifen tale in 1973 [14]. Without the success of tamoxifen as a lifesaving medicine there were no agents waiting as the 'first reserve' anti-oestrogen – nobody cared. Without the success of tamoxifen, there would have been no financial incentive to develop aromatase inhibitors [15] and there would have been no selective oestrogen receptor modulators (SERMs) [16, 17]. So this would seem to argue against animal testing? Certainly not. The toxicological requirements for an anticancer therapy to delay a fatal disease are rightly less draconian than for the treatment of a subject with an infection or no life-threatening disease. The fact the rat liver carcinogenesis was discovered after 20 years of tamoxifen use, allowed the clinical and toxicological community also to evaluate 'real world' experience in women [11, 12] No increase in liver cancer was noted. Scientists were able to determine that the rat is particularly susceptible with its metabolism of tamoxifen to producing a carcinogen but the human rapidly repairs DNA damage [18]. The system for protecting human safety for the introduction of an unknown drug to prevent a disease worked with appropriate toxicology testing in animals. Cancer patients lived because of appropriate testing and risk management for treatment of a fatal disease.

The early years of the tamoxifen tale

Cancer therapeutics and cancer prevention are a particular challenge as we seek to destroy renegade cells that are 'self'. Ehrlich chose to explore the development of appropriate animal models of human disease to address cancer chemical therapy (chemotherapy) at the dawn of the 20th century. In the year before he died in 1916 he declared 'I have wasted 15 years of my life on experimental cancer research' [19].

The banner of progress in therapeutics was picked up in the 1940s using a process of translational research i.e. first validation of an antitumour response in animal models and then a clinical trial. Sir Alexander Haddow FRS discovered [20] that high dose synthetic oestrogen treatment could produce a 30% response rate in breast cancer patients more than 5 years after their menopause [21]. This was the first chemical therapy to treat any cancer successfully and was proven in clinical trials. However, high dose oestrogen treatment is a paradox as all other approaches before the Haddow breakthrough caused regression of breast cancer by endocrine ablation (oophorectomy, adrenelectomy), i.e. taking away oestrogen just as tamoxifen blocks oestrogen from stimulating tumour growth. High dose oestrogen therapy remained the treatment of choice for breast cancer after the menopause for the next 30 years until the introduction of tamoxifen (1973 UK, 1977 USA) with fewer side effects [22, 23]. The only randomized trial [23] of high dose oestrogen *vs*. tamoxifen in unselected (no oestrogen receptor (ER) selection) postmenopausal patients with metastatic breast cancer was quite small with 74 patients and 69 patients, respectively. Response rates were both about 30% and disease control was similar over a 2 year period. Only the increased side effects noted with high dose oestrogen led the authors to recommend tamoxifen [23].

It is interesting to note that all the early events in the story of breast cancer 'chemical therapy' are actually connected. Haddow's experimental oestrogens were synthesized by ICI [20]. Walpole was specifically interested in cancer research. He tried unsuccessfully to discover why only some tumours responded to oestrogen therapy [24] and successfully developed an early 'chemotherapy' [25] but was directed to create a safer 'morning after pill'. The discovery by scientists in America that synthetic oestrogens could be converted to anti-oestrogens through the skill of the medicinal chemist [26] that were also effective 'morning after pills' in the rat which could potentially now create another 'blockbuster' in the wake of the success of oral contraceptives. My connection with the anti-oestrogen research team at ICI throughout the 1970s has recently been told [27] and the clinical development of tamoxifen explained [28, 29]. However, tamoxifen is not about a single medicine but the pioneer in a group of medicines now called SERMs.

Forty years ago there were no SERMs, today there are five but with a sixth, lasofoxifene, approved in the European Union a few years ago. This approval has lapsed (Figure 1). The SERMs were predicted to treat multiple diseases in post-menopausal women simultaneously [26]. The currently approved SERMs treat breast cancer, prevent breast cancer, prevent osteoporosis and preparations prevent menopausal symptoms including dyspareunia. The general outline of the development of the two principal SERMs, tamoxifen and raloxifene, are illustrated and explained in Figures 2 and 3 and a current view of the molecular mechanism of action illustrated for target sites in Figure 4. These stories have been explained recently in detail [30, 31]. However, none of the SERM story would have occurred but for the appropriate use of animal models to guide clinical trials, to understand patient safety and finally to define a new biology of oestrogen-induced apoptosis. This cascade of knowledge answered the question 'how can oestrogen stimulate breast cancer growth (which is the basis of all successful anti-oestrogenic therapy for the past 40 years [32]) but also cause apoptosis as a breast cancer therapy [22, 23]'. It is animal models that aided the understanding of 'Haddow's paradox' [21] that oestrogen can kill correctly prepared breast cancer cells. That knowledge and the molecular mechanism again have clinical significance.

The role of appropriate animal models in breast cancer research to save lives

In 1972, just 2 months after my successful PhD examination with Dr Arthur Walpole on the topic of 'failed morning after pills' entitled *A study of the oestrogenic and anti-oestrogenic activities of some substituted triphenylethylenes and triphenylethanes*, I found myself at the Worcester

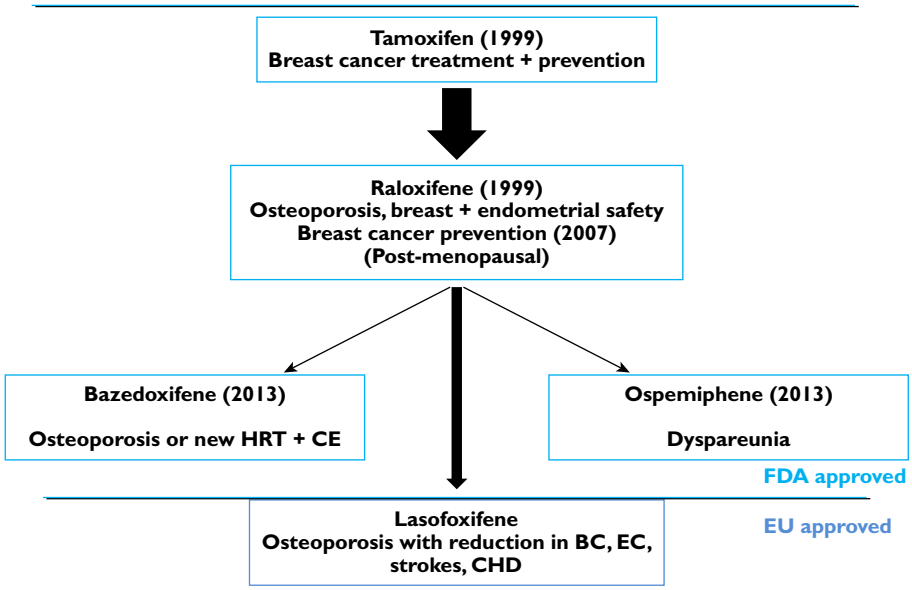

Figure 1

The approvals of individual selective oestrogens receptor modulators (SERMs) in the United States of America through the evaluation system of the Food and Drug Adminisration (FDA). Approvals were specifically for indications at the highest level of toxicologic safety for women without disease but as a new hormone replacement therapy with conjugated oestrogen (HRT + CE) to prevent disease i.e. chemoprevention of osteoporosis, breast cancer (BC), menopausal symptoms or dyspareunia. One SERM, lasofoxifene, was approved for use in the European Union (EU) but was never launched or marketed despite the fact that clinical trials demonstrated a reduction in breast cancer (BC), osteoporosis fracture, strokes, endometrial cancer (EC) and coronary heart disease (CHD) [92]

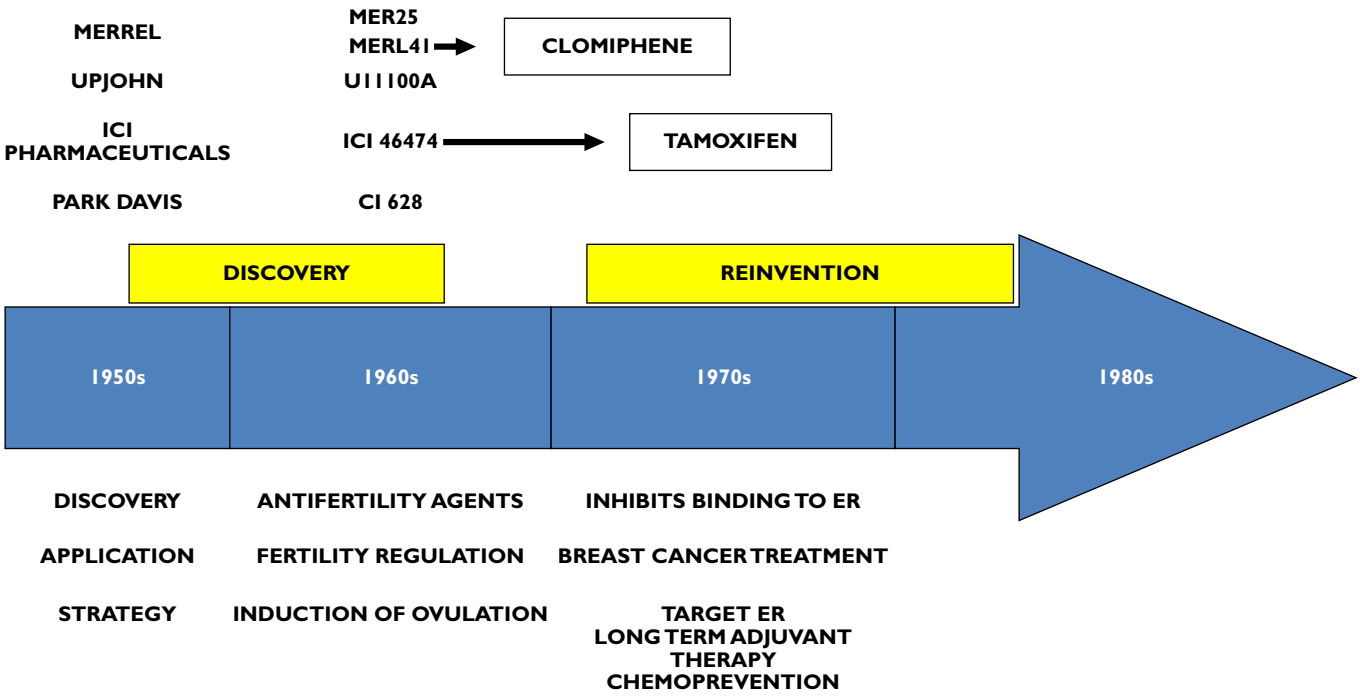

Figure 2

A trickle to tamoxifen (ICI 46 474). During the 1960s, a number of triphenylethylene derivatives were discovered that were excellent novel post-coital antifertility agents in rats but induced ovulation in subfertile women (clomiphene and tamoxifen) [26]. Tamoxifen moved forward as a palliative treatment for metastatic breast cancer, only after being all but abandoned as a commercially viable enterprise. It was then rescued as an orphan drug in 1972 [93]. Laboratory models informed about possible applications as a long term adjuvant therapy or as a chemopreventive agent [27]. Clinical trials demonstrated major survival advantages for women with ER positive breast cancer who received long term (>5 years) tamoxifen therapy and tamoxifen was tested and was the first medicine to be approved for the reduction of breast cancer in high risk women [93]

Foundation for Experimental Biology in Shrewsbury, Massachusetts, USA. I discovered I was to be an independent investigator working in the 'home of the oral contraceptive' but I chose to explore the possibility with ICI of contributing to the development of their orphan drug ICI 46 474 (but not yet tamoxifen). During the time I was at the Worcester Foundation (1972–1974) there were only two clinical reports [22, 33] of the use of tamoxifen to treat breast cancer, but these were not randomized trials, there was no correlation between tumour ER and endocrine ablation, that was to be published in 1975 [34], and there was no mention of tamoxifen as it was not used in this context. A correlation between response and tumour ER was noted later [35, 36]. Adjuvant tamoxifen therapy and chemoprevention were not on the clinical landscape. There was much to do at the beginning to develop a rationale to advance a 'failed morning after pill'. They funded my research proposal but how to start. I needed to train myself to find a model to evaluate and quantify the antitumour effect of ICI 46 474. No laboratory antitumour studies had been done by ICI (or anyone else) but as Ehrlich had taught an 'appropriate animal model of human disease was necessary' to convince the clinical cancer community to conduct clinical trials. The prowess of ICI 46 474 as an effective 'morning after pill in rats' would not suffice!

I found my model in Chicago at the Ben May Cancer Research Laboratories of the University of Chicago. I visited at the invitation of the Director, the late Dr Elwood V. Jensen in the spring of 1973. I learned the 'Jensen method' of measuring the tumour ER, an enormous improvement over my 'Heath Robinson' approach alone in the basement of Leeds University Old Medical School during my PhD. I learned the dimethylbenzanthracene (DMBA)-induced rat mammary carcinoma model [37] and had the good fortune to meet and talk with Professor Charles Huggins, the former director of the Ben May laboratory for Cancer Research and Nobel Laureate for his work on hormone dependent prostate cancer. This readily reproducible mammary tumour model is hormone (ovarian) dependent for growth and the tumours contained the ER [38]. It was the only appropriate model. For the next decade this model would be my medium to propose targeting the ER positive tumour [39] with long term adjuvant tamoxifen therapy [40–42] or use the animal model in the first step towards chemoprevention of breast cancer [43, 44]. All of this would occur at the

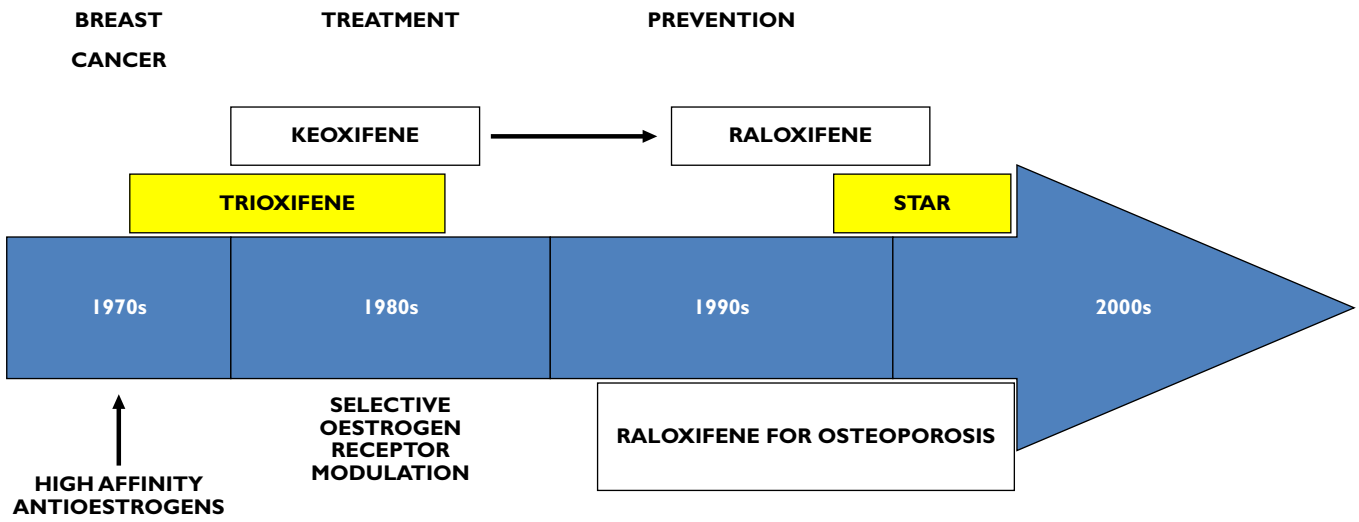

Figure 3

Rush to raloxifene. The success of tamoxifen for the treatment of breast cancer created potential opportunities to develop drugs to correct toxicological issues of concern i.e. the increase in endometrial cancer. Trioxifene was developed as a potential competitor for tamoxifen but failed to demonstrate either increased efficacy in the treatment of metastatic breast cancer or decrease in serious side effects. In the wake of the discovery that tamoxifen was metabolically activated to 4-hydroxytamoxifen with high binding affinity for the ER (Figure 2) [70, 94] a compound LY156758 or keoxifene was developed that had high binding affinity for the ER and did not have oestrogen-like activity in the uterus [95]. Keoxifene failed to become a breast cancer therapy and was abandoned in 1987. However, the discovery that keoxifene prevented bone loss and mammary cancer in rats [51, 75] ultimately resulted in the resurrection of the molecule as raloxifene. The clinical testing resulted in the approval of raloxifene to treat and prevent osteoporosis in post-menopausal women in 1997 and for the reduction of the incidence in breast cancer in high risk post-menopausal women in 2006. This was the Study of Tamoxifen and Raloxifene (STAR). Unlike tamoxifen, raloxifene does not increase the incidence of endometrial cancer [78]

Worcester Foundation (1972–1974) and at the Department of Pharmacology of the University of Leeds (1974–1979). The next dimension in discovery for therapeutics would occur in the 1980s at the University of Wisconsin Clinical Cancer Center (Madison) (1980–1993) in the United States.

The nu/nu athymic mouse model was found to be immune deficient so human tumours could be transplanted and therapies studied to seek cures for cancer [45]. Of particular interest to my new embryonic tamoxifen team in the Department of Human Oncology at the Clinical Cancer Center were the observations that the ER positive human breast cancer cell line MCF-7 [46, 47] inoculated into the axillary mammary fat pad was able to grow into tumours with oestrogen treatment [48, 49]. Furthermore, tamoxifen prevented oestrogenic-stimulated tumour growth [50]. Here was the new model we needed.

Marco Gottardis was an extremely talented technician conducting animal studies in the Department of Human Oncology in the Cancer Center. He accepted my invitation to become a graduate student in my laboratory. His work and publications changed therapeutics and medical care multiple times as he expertly used carcinogen-induced rat mammary tumour models [51] and established our colony of MCF-7 tumour bearing athymic mice [52]. The latter model revolutionized our understanding of acquired resistance to long term tamoxifen therapy [53] and what to do about it in the clinic [54]. The athymic mouse model would provide the leads to the target site specificity of 'non-steroidal anti-oestrogens' [55, 56]. Harper & Walpole [57] had discovered the unusual species specificity to ICI 46 474. The triphenylethylene was classified as an oestrogen in the mouse vagina and this classification was confirmed by Terenius in immature micee with uterine weight tests [58]. ICI 46 474 was classified as an anti-oestrogen in the rat with partial agonist uterine action [5]. However the fact that ICI 46 474 (tamoxifen) acted as an anti-oestrogen to block oestrogen stimulated tumour cell growth in athymic mice [55] was a first clue that tamoxifen was tissue, not species, specific. The development of this observation in different target tissues would give the insight into a new group of medicines in women's health, the SERMs that switch on or switch off oestrogen target sites around the body [59]. This is a fascinating story in molecular pharmacology as the interpretation of the two known ERs, i.e. α and β with different coregulators and receptor processing at different gene promoters, can produce agonist or antagonist action. This multifaceted decision network is summarized in Figure 4. Marco is now the Vice President and Prostate Cancer Disease Area

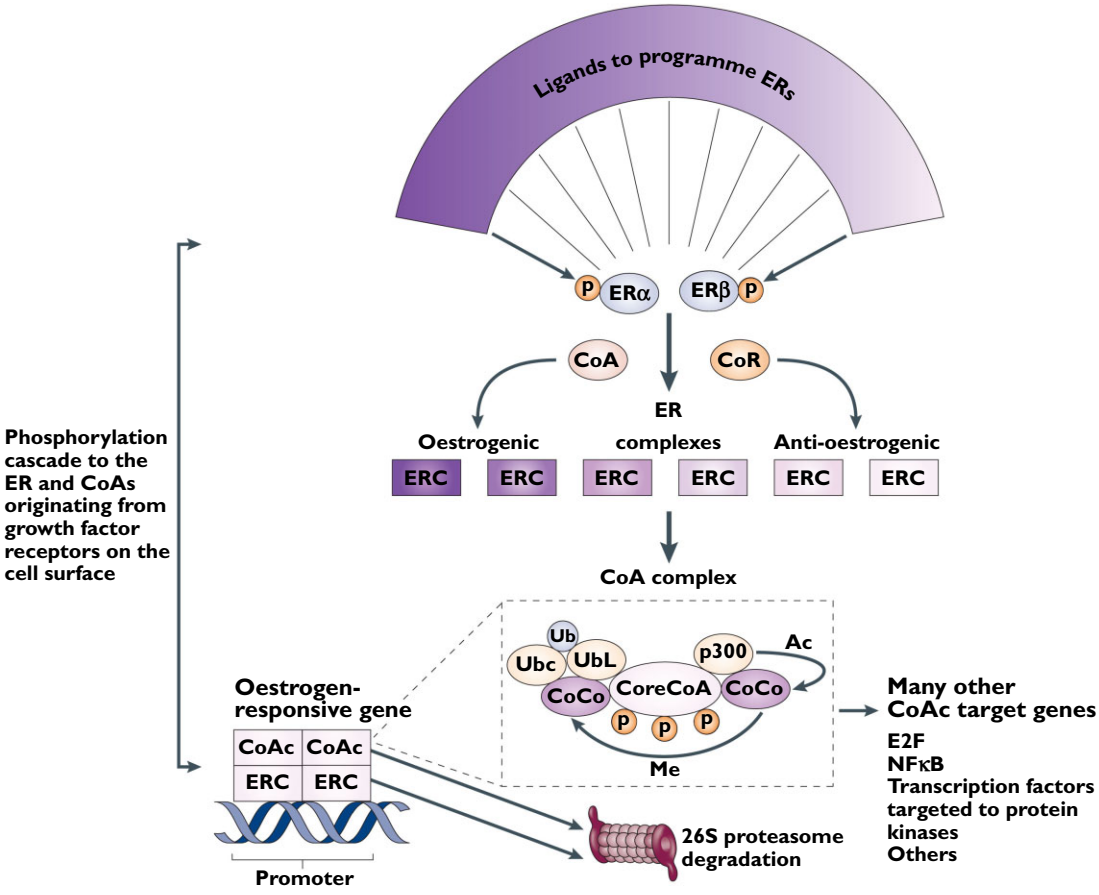

Figure 4

The oestrogen target tissue decision network for selective oestrogen receptor modulation. The shape of the ligands that bind to the oestrogen receptors (ERs) α and β programmes the complex to become an oestrogenic or anti-oestrogenic signal. The context of the ER complex (ERC) can influence the expression of the response through the numbers of co-repressors (CoR) or co-activators (CoA). In simple terms, a site with few CoAs or high levels of CoRs might be a dominant anti-oestrogenic site. However, the expression of oestrogenic action is not simply the binding of the receptor complex to the promoter of the oestrogen-responsive gene, but a dynamic process of CoA complex assembly and destruction. A core CoA, for example, steroid receptor coactivator protein 3 (SRC3), and the ERC are influenced by phosphorylation cascades that phosphorylate target sites on both complexes. The core CoA then assembles an activated multiprotein complex containing specific co-co-activators (CoCo) that might include p300, each of which has a specific enzymatic activity to be activated later. The CoA complex (CoAc) binds to the ERC at the oestrogen-responsive gene promoter to switch on transcription. The CoCo proteins then perform methylation (Me) or acetylation (Ac) to activate dissociation of the complex. Simultaneously, ubiquitiylation by the bound ubiquitin-conjugating enzyme (Ubc) targets ubiquitin ligase (UbL) destruction of protein members of the complex through the 26S proteasome. The ERs are also ubiquitylated and destroyed in the 26S proteasome. Therefore, a regimented cycle of assembly, activation and destruction occurs on the basis of the preprogrammed ER complex. However, the co-activator, specifically SRC3, has ubiquitous action and can further modulate or amplify the ligand-activated trigger through many modulating genes that can consolidate and increase the stimulatory response of the ERC in a tissue. Therefore, the target tissue is programmed to express a spectrum of responses between full oestrogen action and anti-oestrogen action on the basis of the shape of the ligand and the sophistication of the tissue-modulating network. NFkB, nuclear factor kB. Reprinted with permission from the Nature Publishing Group, Jordan [96]

Stronghold Leader for the Oncology Therapeutic Area at Janssen Research and Development, LLC in New York.

It would be another graduate student, Doug Wolf who would have the transplantable model of acquired resistance to tamoxifen passed to him! He would discover that after retransplantation of the tumours for years into successive generations of tamoxifen-treated athymic mice, that physiological oestrogen could make tumours melt away [60]. This serendipitous discovery at Wisconsin would be developed fully [61] at the Robert H. Lurie Comprehensive Cancer Center at Northwestern University, Chicago (1993–2005) by surgical residents, medical oncology fellows or scientists: Kathy Yao, Gale England, Eun-Sook Lee, David Bentrem, Ruth O'Regan, Rita Dardes, Jennifer MacGregor, Hong Liu, Clodia Osipo, Debra Tonnetti and Joan Lewis all co-operated and achieved successes [61–67]. Our tamoxifen teams have remained an essential balance of clinical and laboratory expertise to ensure we never lose sight of the goal – improving

cancer care. Doug Wolf is now the Senior Director Oncology regional medical research specialist at Pfizer.

I will illustrate the translational aspects of our tamoxifen tale by our tamoxifen teams over the decades with the following examples of successful translational research outcomes.

An appropriate strategy for the adjuvant antihormone treatment of breast cancer

Laboratory model

The DMBA-induced rat mammary carcinoma model has been examined extensively by hundreds of investigators [38] but the main hypothesis to be tested in our studies was that longer treatment starting when animals had only occult disease following DMBA administration was superior to short term therapy [40–42]. The secondary hypothesis to be addressed was that only ER positive disease would respond as tamoxifen and metabolites blocked the binding of [^{3}H]-oestradiol to tumour ER [39, 68–70].

Clinical translation

The overviews of clinical trials conducted every 5 years at Oxford demonstrated that only patients with an ER positive primary tumour responded to adjuvant tamoxifen and longer therapy (5 years) was superior to either 1 or 2 years of adjuvant tamoxifen [12, 13]. There was a 50% decrease in recurrence rates and a 30% decrease in mortality. Maybe a million lives were saved.

Tamoxifen and target site specific anticancer action

Laboratory model

Athymic mice were transplanted with an ER positive breast cancer and an ER positive endometrial cancer and treated with oestrogen to stimulate growth. Tamoxifen was administered to determine whether the anti-oestrogen controlled the growth of both breast and endometrial cancer. Breast cancer was controlled but endometrial cancers grew dramatically [56].

Clinical translation

Marco Gottardis and I presented these data prior to publication to staff at ICI Pharmaceuticals Division, Alderley Park. In 1987, I presented the results and my concerns at a medical conference organized during the celebration of the 900th anniversary of the first university in the world, the University of Bologna, Italy. As a result of my lecture, Dr Leonard Hardell wrote a letter to the Lancet [71] and I replied appealing for results from a large prospective clinical trial [72]. The database from Fornander and colleagues [73] demonstrated that longer tamoxifen (5 years) caused the detection of more endometrial cancer than shorter (2 years) of adjuvant tamoxifen. The report also confirmed that the incidence of new primary breast cancers was reduced by tamoxifen but endometrial cancer incidence went up. I replied [74]. Medical practice changed with new package inserts and gynaecologists became involved as part of the breast cancer patient care team. The whole process of translational research to clinical practice took 2 years and almost certainly saved lives.

The discovery of SERM action

Laboratory model

In the 1980s, as a prelude to chemoprevention, we rigorously investigated the fascinating target site specific actions of tamoxifen. Human breast tumours implanted in athymic mice did not grow [55] with tamoxifen despite the fact that tamoxifen is an oestrogen in the mouse [5]. But oestrogen is needed to maintain bone, so what would the value be of preventing a few breast cancers in a thousand post-menopausal women per year if hundreds of women subsequently developed osteoporosis? To our surprise both tamoxifen and raloxifene (an abandoned breast cancer drug called keoxifene) both maintained ovariectomized rat bone density [75] and prevented carcinogen-induced mammary cancers in a rat model [51]. Tamoxifen was better than raloxifene at suppressing mammary tumour appearance [51]. This is because tamoxifen has a long biological half-life producing optimal tumour suppression whereas raloxifene is a polyphenolic compound that is rapidly cleared and short acting.

The SERM concept applied to clinical practice was proposed in the last paragraph of the Cain Memorial Lecture in 1990 [26]. This roadmap for industry is reproduced in the last section, Retrospective and conclusions.

Clinical translation

The animal study of rat bone density with tamoxifen translated to building bone in post-menopausal women [76]. Raloxifene was approved to prevent osteoporosis but prevented breast cancer at the same time [77]. The chemoprevention trial Study of Tamoxifen and Raloxifene (STAR) showed that both SERMs were able to prevent breast cancer in high risk post-menopausal women by 50% during treatment [78] but after therapy stopped at 5 years tamoxifen maintained chemoprevention of breast cancer but raloxifene did not [79]. These clinical results echoed our laboratory study in animals 20 years earlier [51]. Raloxifene is recommended to be taken indefinitely to maintain chemoprevention of breast cancer. Perhaps hundreds of thousands of lives have been improved.

The evolution of acquired resistance to tamoxifen

Laboratory model

The serial retransplantation of MCF-7 breast tumours with acquired resistance to tamoxifen into tamoxifen treated mice passes through two phases: Phase I acquired resistance occurs in the ER+ tumour within 1–2 years of tamoxifen treatment. Acquired resistant tumours are characterized as being stimulated to grow with either physiologic oestrogen or tamoxifen [53]. No oestrogen or tamoxifen treatment or treatment with a pure anti-oestrogen stops tumour growth [54]. Phase II acquired resistance develops with retransplantation after 3–5 years, but now tamoxifen stimulates tumour growth but physiological oestrogen causes tumour regression [61].

Clinical outcome

Low dose oestrogen causes a 30% benefit rate after a woman's tumour becomes resistant to long term adjuvant aromatase inhibitor treatment [80]. Most provocatively, the new science of oestrogen-induced apoptosis could be the reason for dramatic decreases in mortality after adjuvant tamoxifen is stopped. Recent data demonstrate that 10 years of tamoxifen is superior to 5 years of tamoxifen [81] but mortality is decreased by 50% compared with historical no treatment data but only in the decade after 10 years of tamoxifen is stopped. Oestrogen-induced apoptosis is also offered as the reason [82] mortality decreases with oestrogen alone treatment as hormone replacement therapy in 60 year old post-menopausal women following a decade of oestrogen deprivation following menopause. It may be that this research strategy leads to new and safer hormone replacement therapy for post-menopausal women.

Retrospective and conclusions

Looking back at this point in our tale, it can be predicted that this will not be the end at all, but the beginning of a new phase of a conversation with Nature. The outcomes of that conversation may determine the next advance in therapeutics.

What started out with a desire to contribute to the development of a medicine to treat cancer seemed, on reflection now, a forlorn hope 40 years ago [27] but I did not realize that at the time (fortunately)! The formula for a successful outcome in my quest to contribute, depended on two principal factors: a willingness to learn new laboratory techniques using relevant animal models that turned out to have significance for translational research in therapeutics and the willingness of innovative and committed individuals in industry and Yorkshire Cancer Research to invest in a young investigator [27]. This was followed by the generosity of a philanthropic organization, the Lynn Sage Breast Cancer Foundation in Chicago, who raised a million dollars a year for a decade for my tamoxifen team to define and understand the new science oestrogen-induced apoptosis [61–67].

As a pharmacologist, I defined my goal – use models to discover mechanisms and develop new medicines. Animal models were the key to that success. At the start, the use of long term adjuvant tamoxifen therapy was counterintuitive to the clinical community. Tamoxifen was only effective in controlling the growth of metastatic breast cancer for a year or two [22, 23] so it would be dangerous at worst, and unwise at best, to extend adjuvant tamoxifen beyond a year. But micrometastases are clearly different from larger metastatic lesions and a different pharmacology pertains. Perhaps millions of women benefited. There was no clinical understanding of the relevance of the mixed oestrogenic/anti-oestrogenic effects of tamoxifen. In the clinical lectures, I called it the 'oestrogenic tickle of tamoxifen'. The laboratory finding that tamoxifen selectively blocks oestrogen stimulated breast tumour growth but enhances the growth of pre-existing occult endometrial cancer changed all that [56]. Medical practice changed, gynaecologists were involved in breast cancer patient care and major medical problems were avoided that could have killed the patient without appropriate pre-emptive action. A cluster of consistent findings [51, 52, 55, 56, 75] by my tamoxifen team at Wisconsin (1980–1993) resulted in the group of medicines, the SERMs.

The idea that a 'non-steroidal anti-oestrogen' could switch on or switch off oestrogen target sites around the body could not have been anticipated without animal models to demonstrate antitumour action in the rat mammary gland [43, 44, 51] but oestrogen-like activity in ovariectomized rat bone [75]. This led to a road map for industry [26] as stated in my Bruce F. Cain Award and Memorial Lecture in 1989:

'Is this the end of the possible applications for anti-oestrogens? Certainly not. We have obtained valuable clinical information about this group of drugs that can be applied in other disease states. Research does not travel in straight lines and observations in one field of science often become major discoveries in another. Important clues have been garnered about the effects of tamoxifen on bone and lipids so it is possible that derivatives could find targeted applications to retard osteoporosis or atherosclerosis. The ubiquitous application of novel compounds to prevent diseases associated with the progressive changes after menopause may, as a side effect, significantly retard the development of breast cancer. The target population would be post-menopausal women in general, thereby avoiding

the requirement to select a high risk group to prevent breast cancer'.

The declaration resulted in a whole new drug group that overall, aids women's health. Millions of women continue to benefit.

Lastly, the creation of an animal model of acquired tamoxifen resistance of breast cancer informed us about the unique nature of tamoxifen-stimulated tumour growth. However, the then disheartening fact that this tumour model could not be transferred to cell culture, but demanded constant retransplantation into subsequent generations of tamoxifen treated athymic mice, opened the door to a discovery. Little did we suspect at the beginning, that this routine, labour-intensive procedure, would cause the tumours to evolve through continuing selection pressure over the years. Acquired resistance changed after a couple of years. Tamoxifen treatment caused acquired resistance with either tamoxifen or oestogen-stimulated growth. At 3–5 years of transplantation now the new tamoxifen resistant cell population responded to physiologic oestrogen with tumour regression. It is possible that a woman's own oestrogen does exactly the same to execute prepared micropopulations of tamoxifen resistant cells after 5 years of adjuvant tamoxifen stops [60]. Based on the original animal studies demonstrating the evolution of acquired resistance to tamoxifen, subsequent cellular models were used to decipher the molecular events involved in oestrogen-induced apoptosis [65, 83–86]. This knowledge became pre-positioned in the refereed literature so that the paradoxical finding of fewer breast cancers reported in the oestrogen alone clinical trial of the WHI with a reduction of mortality were understood. Select women lived [82] but the finding that a combination of oestrogen plus a synthetic progestin, which causes an increase in breast cancer incidence, now demands understanding. Resolution of mechanisms and the creation of a safer hormone replacement therapy that prevents breast cancer may indeed be the next chapter of the tamoxifen tale that affects the lives of millions of women worldwide.

However, it would be, perhaps, misleading to imply that human disease can always be modelled successfully with animal equivalents. There is, for example, no animal model for human breast cancer that faithfully replicates outcomes. Focusing on the pharmacology of tamoxifen, but bearing in mind this is just the tip of the iceberg of all medicines, a number of uncertainties and problems persist. To be successful as a therapeutic agent, the medicine must be taken for perhaps a decade or more as a treatment or as a chemopreventive agent in high risk women. Regrettably, and predictably, one of the major side effects of tamoxifen that reduces compliance is menopausal symptoms, particularly hot flushes. Decreases in compliance result in lives lost [87]. These are no satisfactory laboratory models to predict this in the clinic. Nevertheless, changes in patient care may be possible. A new combination of the SERM bazedoxifene plus conjugated oestrogen has recently been approved by the Food and Drug Administration in America for the treatment of osteoporosis or menopausal symptoms [88]. It seems that the oestrogen can win in the brain to ameliorate menopausal symptoms, but the SERM prevents oestrogen induced breast and endometrial cancer. The combination has an additive effect in bone, an effect first noted with both tamoxifen and raloxifene in animals [75]! Metabolism and pharmacodynamics remain a challenge in the two way conversation between laboratory animal results and clinical trials. Although algorithms are available, to model dosage modifications in animals is often not precise. Additionally blood concentrations and metabolites are not consistent between human and other species [89]. One long running controversy has been the genotyping of patients for CYP2D6 that governs the available levels of endoxifen in tamoxifen treated patients. The technical issues have recently been reviewed [90] but the simple theory that only higher levels of metabolically produced endoxifen will produce optimal results, can really only be addressed in cell culture. Animal modelling is not possible [89]. However, cell culture only provides data on a transient moment in the life of tumour cells and not the shifting adaptive populations that evolve over years of treatment.

As a science, our exploration evolves by trial and error as we meet each new challenge in selective toxicity. In cancer research there has been in the past decade, a huge shift to genetically engineered mice to answer the question 'is this gene significant?' At the other extreme is the continuous sequencing of human tumour types to discover patterns and vulnerabilities. However, human tumour data are a single 'snapshot' but what human cancer is, is a relentless journey of immense possibilities to overwhelm the human host. This remains hard to model if we subscribe to the mantra that every tumour is different and that only personalized medicine is the way of the future. Tamoxifen with its target of the tumour ER was the first personalized medicine in cancer. Now we have the challenge of navigating out of the Pandora's box we opened.

Professor Paul Ehrlich chose to view the selective targeting of a chemical therapy to cure disease as the search for the 'Magic Bullet'. Tamoxifen can, in retrospect, be viewed as the discovery of a 'Magic Machine Gun', as no other chemical therapy for cancer is used to treat all stages of breast cancer, ductal carcinoma *in situ* (DCIS), male breast cancer and can be used for the prevention of breast cancer, all by targeting the ER [91], but the ER target around a patient's body can be switched on or switched off selectively by tamoxifen. So, a search for new medicines gave us SERMs. Broad improvements in women's health by selective modulation of the same target in different tissues was an unanticipated consequence of 'anti-oestrogenic' treatment. Appropriate animal models significantly

advanced health for millions of women to live longer and healthier lives. Mothers see their children grow up, children experience the affection of a grandmother.

Competing Interests

There are no competing interests to declare.

This article is dedicated to my late friend and supporter Sir James Black FRS. The inspiration to create this article occurred when the American Society of Clinical Oncology chose to select my contributions in translational research to be one of the 50 Oncology Luminaries http://cancerprogress.net/node/2086 and, simultaneously, Ms Elodie Picard, a veterinary student from Brussels enquired about my views on the use of animal models in medical research. This is the result. I thank my generations of members of my tamoxifen teams who used laboratory models to transform ideas into lives saved. I thank Fadeke Agboke and Russell McDaniel for their invaluable assistance with this manuscript. This work (VCJ) was supported by the Department of Defense Breast Program under Award number W81XWH-06-1-0590 Center of Excellence; the Susan G Komen For The Cure Foundation under Award number SAC100009; GHUCCTS CTSA (Grant # UL1RR031975) and the Lombardi Comprehensive Cancer Center Support Grant (CCSG) Core Grant NIH P30 CA051008. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. Additionally, the views and opinions of the author(s) do not reflect those of the US Army or the Department of Defense.

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