A Novel Strategy to Improve Women's Health: Selective Estrogen Receptor Modulators

Balkees Abderrahman and V. Craig Jordan

Abstract Tamoxifen is the first selective estrogen receptor modulator. The extensive clinical and laboratory testing during the 1980s and 1990s raised questions about why there is target site specificity of tamoxifen in different species, i.e., tamoxifen is an estrogen in mice but a complete anti-estrogen in chicks. Additionally, tamoxifen has estrogen-like effects to lower circulating cholesterol, build postmenopausal bone in women, and stimulate the uterus and endometrial cancer growth but paradoxically prevents breast tumor growth. These observations lead to the SERM solution to prevent osteoporosis with a safe SERM but to prevent breast cancer at the same time. Raloxifene is the result with no increase in endometrial cancer incidence. There are now five FDA-approved SERMS available for use: tamoxifen, raloxifene, bazedoxifene, toremifene, and ospemifene. All have connections with discovery and basic research in Jordan's laboratory.

Keywords Selective estrogen receptor modulators · Women's health · Bazedoxifene · Ospemifene · Toremifene · Raloxifene · Lasofoxifene

1 Introduction

The clinical evaluation of tamoxifen in the 1970s for the treatment of metastatic breast cancer (MBC) and during the 1980s for the long-term adjuvant therapy of breast cancer [1] created a therapeutic benchmark to be improved. Numerous new nonsteroidal anti-estrogens were evaluated to treat MBC (Fig. 1), but only one toremifene was successful in achieving a market. By a lucky set of circumstances, tamoxifen, a nonsteroidal anti-estrogen, was to dominate the endocrine therapy and prevention of breast cancer for 35 years.

During the 1970s and early 1980s, the unusually species-specific pharmacology of tamoxifen and indeed other nonsteroidal anti-estrogens [2] was perplexing.

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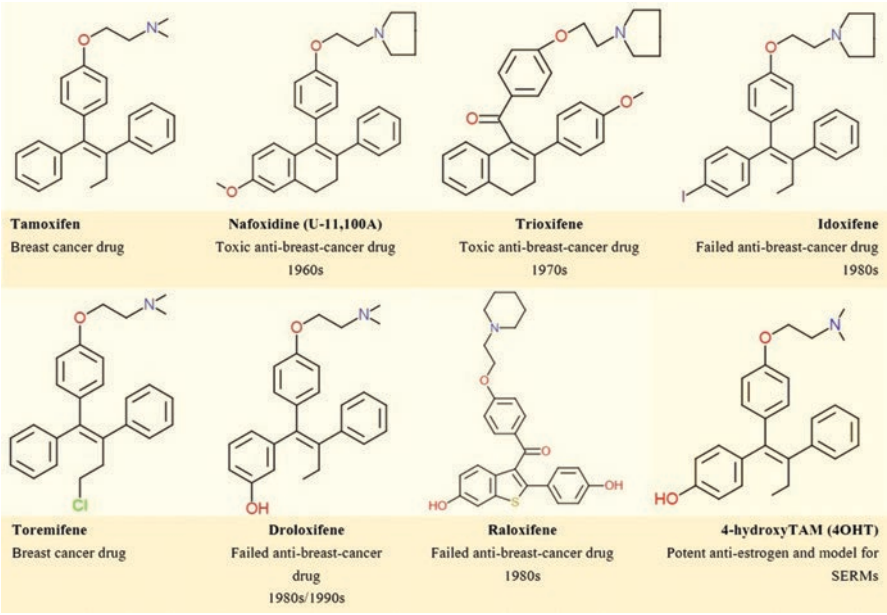

Fig. 1 Compounds that were evaluated in clinical trials but failed as competitors for tamoxifen 1960s–1990s. The exception was toremifene that is used for MBC. The tamoxifen metabolite, 4OHT, became the key drug discovery with high binding affinity for the ER. This was the new model for SERM discoveries (Fig. 5)

Tamoxifen was classified as an estrogen in short-term mouse uterine weight or vaginal cornification assays [2]. Tamoxifen was a partial agonist in the rat uterine weight test but an anti-estrogen in rat vaginal cornification assays [3]. Tamoxifen was a complete anti-estrogen in the chick oviduct [4]. In the dog, tamoxifen is an estrogen, and metabolite E, tamoxifen without the dimethylamine-ethane anti-estrogenic side chain, is observed [5]. One possible explanation considered for the species-specific estrogen target site, estrogenic actions of tamoxifen was the species-specific metabolism of tamoxifen to nonsteroidal estrogens.

This hypothesis was addressed in vivo and in vitro [6] with liver microsomes using [^{3}H] tamoxifen. It was first proven that tamoxifen was metabolically activated to the more potent uterotrophic agent, 4-hydroxytamoxifen, in ovariectomized mice. This was achieved by using 4-chlorotamoxifen, which cannot be hydroxylated at the four position [7]; the tamoxifen derivative was a weak uterotrophic agent. Tamoxifen was ten times more potent as a uterotrophic agent than 4-chlorotamoxifen [6]. 4-Hydroxytamoxifen is more potent than tamoxifen in the ovariectomized mouse uterus [6]. This confirmed previous work in the immature rat [7] where 4-hydroxytamoxifen and 4-chlorotamoxifen exhibited potent and weak partial uterotrophic effects, respectively. The candidate nonsteroidal estrogenic metabolites of tamoxifen as estrogen are metabolite E (tamoxifen with the dimethylaminoethyl side chain severed at the ether link) and bisphenol, the equivalent metabolite

of 4-hydroxytamoxifen. The principal metabolite of tamoxifen 4-hydroxytamoxifen comprised 27%, 14%, and 17% of radio activity from mouse, rat, and chicken livers [6]. Bisphenol and metabolite E were not detected. A similar conclusion was reported in a comparison of athymic mice and human sera [8].

In 1980, Sutherland and coworkers [9] reported a new class of binding sites in uterine tissue that bound anti-estrogens with high affinity and specificity but did not bind estradiol. This was called the anti-estrogen-binding protein (AEBP). It was proposed that the AEBP independently binds nonsteroidal anti-estrogens and blocks estrogen action [9]. This hypothesis was addressed by using mouse uterine weight assays to compare and contrast tamoxifen, an estrogen in the mouse, with MER25, a complete anti-estrogen, which binds to ER but has little interaction AEBS. Additionally, a broad range of ligands that bound with different binding affinities for AEBS were used to explore the modulation of anti-uterotrophic action in the mouse [10]. The study by Lyman reported that (1) MER25 completely inhibits the uterotrophic response of tamoxifen and 4-hydroxytamoxifen as well as estradiol and (2) the panel AEBS ligands did not correlate with biological properties in mice [10]. In related studies, the Katzenellenbogen group found no evidence for the role of the AEBS in anti-estrogen action [11, 12]. Simultaneously, Lieberman and coworkers [13] demonstrated that estrogen/anti-estrogen action was dependent upon a direct and reversible interaction of nonsteroidal anti-estrogens with the ER. The shape of the resulting ER complexes resulted in agonist, partial agonist, and antagonist actions [14–17]. However, it was the biology of nonsteroidal anti-estrogens in vivo that was to result in the new group of medicines referred to as SERMs. A recognition of the unusual species-specific actions of tamoxifen at estrogen target tissues was essential to advance therapeutics.

2 The Athymic Rodent Model in Breast Cancer Research

The description of the immune-deficient mouse [18] and its development for the hetero-transplantation and growth of human cancer cells [19] was an important new laboratory model to investigate human cancer therapeutics. The MCF-7 cell line grows into solid tumors if inoculated into estrogen-treated athymic mice [20]. This is necessary because athymic mice have a hypothalamus pituitary lesion [21] resulting in very low estradiol levels and no estrous cycles.

3 Pharmacology of Tamoxifen in the Athymic Mouse Model Transplanted with ER-Positive Tumors

The fact that estrogen-stimulated growth of human breast tumor MCF-7 is blocked by tamoxifen in athymic mice was unexplained because the mouse uterus was simultaneously stimulated to grow [22, 23]. Administration of [^{3}H] tamoxifen to

investigate the radiolabeled metabolites of tamoxifen in either human ER-positive breast tumors or mouse uterus revealed no differences [23]. The conclusion was "these studies strongly support the concept that the drug can selectively stimulate or inhibit events in the target tissues of different species without metabolic intervention. We propose that the species differences observed with tamoxifen are the result of differences in the interpretation of the drug-ER complex by the cell. The drug ER complex is perceived as either a stimulatory or an inhibitory signal in the different target tissue from different species."

Taken one step further, Satyaswaroop and coworkers [24] first described that a transplanted human ER-positive endometrial cancer could be stimulated to grow with tamoxifen. This laboratory report received little clinical attention, as it was not focused on the clinical community and patient care. In a later collaborative study, athymic mice were bitransplanted with either a human endometrial tumor or a breast cancer and the two anterior axillae [25]. The results demonstrated that tamoxifen blocked the growth of estrogen-stimulated breast cancer, but endometrial cancer grew with tamoxifen, estrogen, or the combination (Fig. 2). These data were presented at a symposium to celebrate the 800th anniversary of the University of Bologna. There was an immediate but unexpected response from the clinical community through letters to the Lancet [26–28]. However, proof was needed that there was a correlation between tamoxifen treatment and an increased incidence of

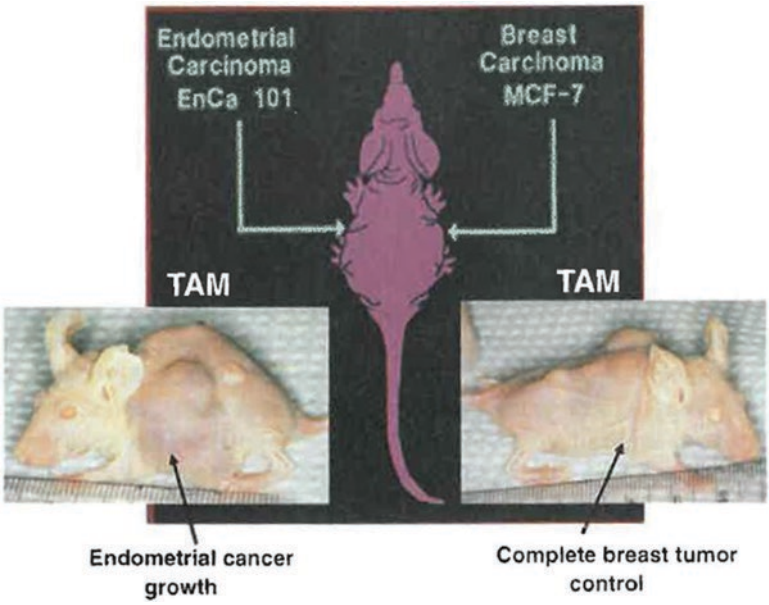

Fig. 2 The pioneering bitransplantation study by Gottardis et al. [25] with an ER-positive breast tumor MCF-7 implanted in one axilla and an ER-positive endometrial tumor (EnCa101) in the other axilla. Tamoxifen blocks estrogen-stimulated growth of the breast tumor, but tamoxifen encourages the growth of the endometrial cancer

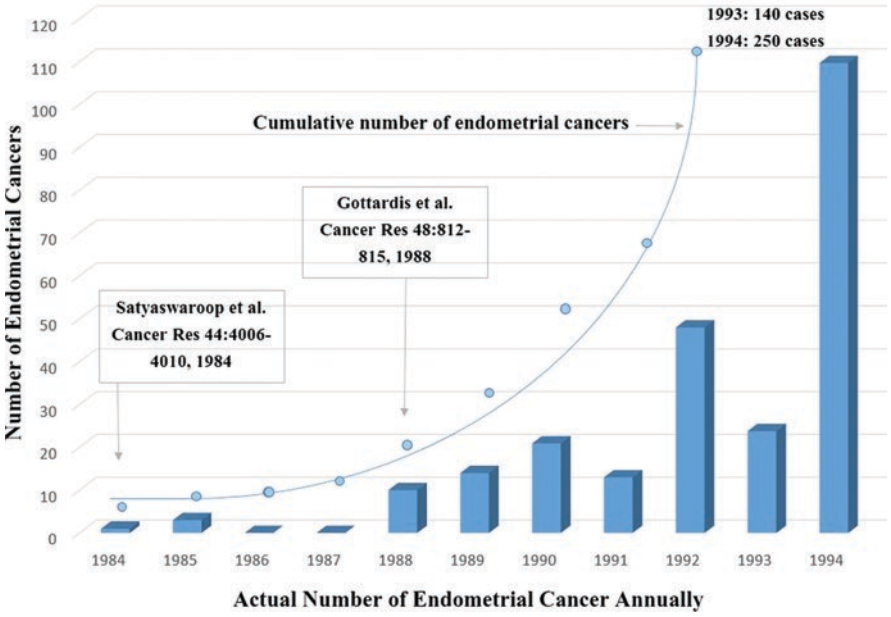

Fig. 3 Reporting of endometrial carcinomas in tamoxifen-treated patients per annum. Cumulative number of cases is plotted; two major studies [24, 25] that led the medical community to focus on the issue are highlighted (Reproduced with permission from [30])

endometrial cancer in randomized clinical trials. Proof was provided by Fornander and coworkers [29], who compared a contrasted 2 and 5 years of adjuvant tamoxifen therapy vs placebo in postmenopausal patients following surgery. Longer adjuvant tamoxifen treatment caused an increase in the detection of endometrial cancer. This chain of events triggered enormous interest by the clinical community summarized in Fig. 3 [31]. Standards of clinical care were changed based on the target tissue-specific actions of tamoxifen around a patient's body. However, the question was subsequently posed: "If tamoxifen is an estrogen, in mouse estrogen target tissues, would tamoxifen be an antitumor agent during mouse mammary carcinogenesis in high incidence strains?" Results of 2-year experiments showed that long-term tamoxifen therapy was superior to ovariectomy in causing mammary tumor chemoprevention in mice. There was tissue site modulation of target tissues in mice where tamoxifen is classified as an estrogen! [32, 33].

4 The Target Site Specificity of Tamoxifen and Keoxifene (Now Known as Raloxifene) in Rats and Humans

The clinical utilization of tamoxifen expanded in the 1980s from its original focus on the treatment of early breast cancer by extending adjuvant therapy [34] to a strategic application to prevent the development of breast cancer in high-risk

women [35]. However, not only is estrogen action necessary to cause the development and growth of breast cancer [36] but also to maintain bone density and reduce circulating low-density lipoprotein cholesterol. Presaged by animal studies which showed that tamoxifen and keoxifene (a failed breast cancer drug from Eli Lilly that became raloxifene) could maintain bone density in ovariectomized rats [37] and lower circulating cholesterol [3], tamoxifen was shown to do the same in postmenopausal women [38, 39]. These laboratory and clinical data with tamoxifen and laboratory data with keoxifene were used as evidence to develop the SERM solution. This was simply stated [40] as a roadmap for the pharmaceutical industry to follow. This they did! "Is this the end of the possible applications for anti-estrogens? 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 postmenopausal women in general, thereby avoiding the requirement to select a high-risk group to prevent breast cancer."

Subsequent work with tamoxifen [41] and raloxifene [42] in laboratory animals confirmed the bone-sparing properties of these "anti-estrogens" as did the clinical studies published subsequently. The SERM solution was validated by effective translation to clinical practice. A new group of medicines was created and new applications of SERMs advanced.

The development of SERMs not only promised to improve on tamoxifen to prevent breast cancer but also provided the first multifunctional medicines to prevent multiple diseases in aging women. Hormone replacement therapy (HRT) had promised eternal youth for women but numerous problems occurred (Fig. 4), and potentially there was a better way to prevent multiple diseases in postmenopausal women. Indeed, as we will show, SERMs and HRT coalesced after 20 years of trial and error with a SERM plus conjugated equine estrogen (CEE) as an HRT.

5 The Development of SERMs from Laboratory Leads

Interestingly enough, the new SERMs all had origins in earlier publications in the refereed literature. The discovery that tamoxifen is metabolically activated as a prodrug to 4-hydroxytamoxifen [7, 43] was important to create the new group of medicines now referred to as SERMs. Although 4-hydroxytamoxifen was not developed itself as a SERM because the tamoxifen metabolite is rapidly excreted [44], the structural modification and high binding characteristics for the ER were key to future SERM design [45]. The SERMs that were evaluated in clinical trial with a strategic hydroxyl group are raloxifene, bazedoxifene, arzoxifene, and lasofoxifene (Fig. 5).

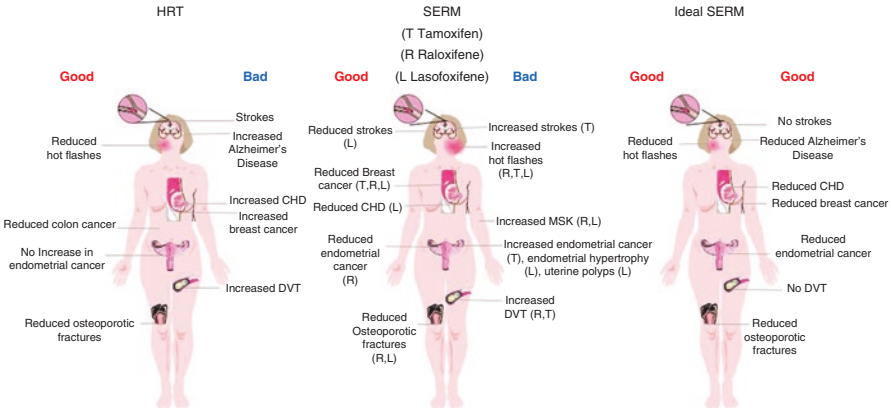

Fig. 4 Current status of available SERMs and progress toward an ideal SERM. The overall good or bad aspects of administering hormone replacement therapy to postmenopausal women compared with the observed site-specific actions of the selective estrogen receptor modulators tamoxifen and raloxifene. The known beneficial or negative actions of SERMs have opened the door for drug discovery to create the ideal SERM or targeted SERMs to either improve quality of life or prevent diseases associated with aging in women. *CHD* cardiovascular heart diseases, *DVT* deep vein thrombosis, *MSK* musculoskeletal symptoms

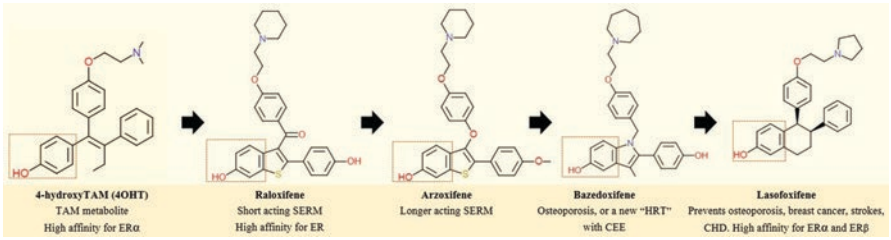

Fig. 5 The value of the early clue from 4OHT that the strategically placed phenolic hydroxyl will result in high binding affinity for prospective SERMs

The SERMs arzoxifene and lasofoxifene were not advanced successfully for FDA approval. Arzoxifene was designed as a "longer-acting raloxifene derivative." In this way it was predicted that arzoxifene would prove to be useful for the treatment of breast cancer [46]. However, the phase III breast cancer trial was stopped because "arzoxifene was statistically significantly inferior to tamoxifen with regard to progression-free survival and other time-to-event parameters, although tumor response was comparable between the treatments" [46]. Nevertheless, in a phase III trial [47], arzoxifene treatment increased spine and hip bone density in postmenopausal women. Other trials support the conclusion that arzoxifene is effective only at preventing vertebral fractures. Further development of arzoxifene was abandoned.

Lasofoxifene is a remarkable molecule and a miracle of medicinal chemistry. The molecule uses the core structure of nafoxidine, the failed contraceptive and failed breast cancer drug (Fig. 6). In laboratory test, lasofoxifene exhibits no uterotrophic actions in either immature or aged female rats [48]. Additionally lasofoxifene preserves bone density and lowers serum cholesterol in ovariectomized rats [49, 50]. There are no stimulatory effects of lasofoxifene on the growth of estrogen-deprived MCF-7 cells in vitro [48]. Lasofoxifene prevents rat mammary carcinogenesis induced by N-nitrosomethylurea [51]. Drug excretion of lasofoxifene is reported [52] to be 95% via the biliary route as a glucuronidated conjugate.

Lasofoxifene is the levorotatory (*l*) enantiomer which is more potent at binding to the ER than the dextrorotatory (*d*) isomer. The (*l*) enantiomer is also resistant to glucuronidation thereby improving bioavailability [48]. Increased potency was confirmed in humans using 0.017, 0.05, 0.15, and 0.5 mg/day which was shown to be effective at maintaining lumbar bone density over a 1-year period [53].

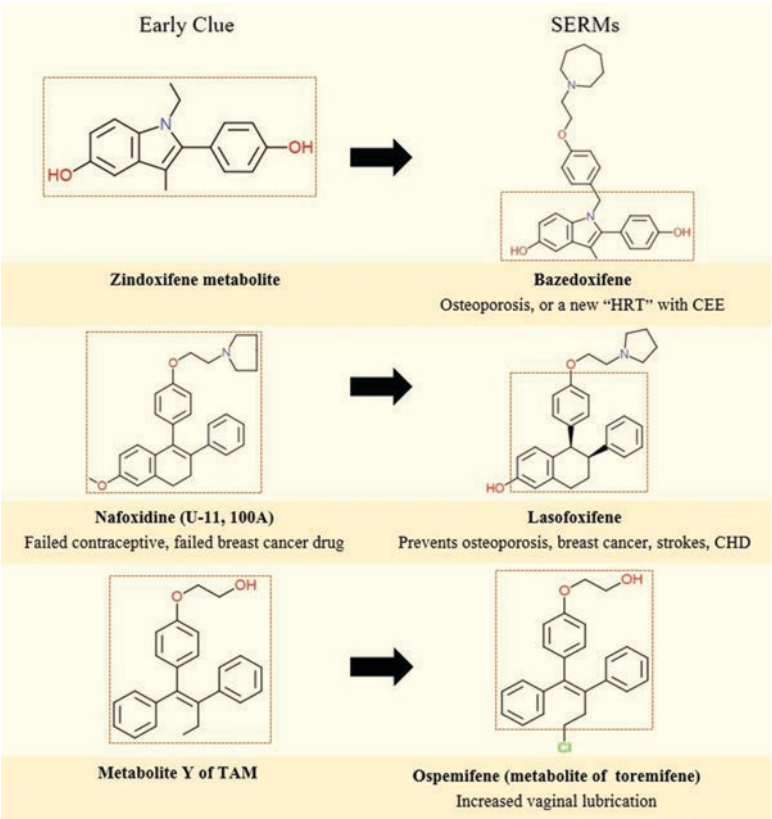

Fig. 6 The laboratory or literature clues that provided the rationale for the development of new SERMs

There are results from three phase III clinical trials with lasofoxifene: (1) Postmenopausal Evaluation and Risk Reduction with Lasofoxifene (PEARL), (2) Osteoporosis Prevention and Lipid Lowering (OPAL), and (3) the study and the Comparison of Raloxifene and Lasofoxifene (CORAL). The PEARL Study noted that lumbar spine and femoral neck bone mineral density were increased at 3 years [54]. The OPAL trial tested three doses of lasofoxifene versus placebo [55, 56]. All three doses showed improved lumbar spine and hip bone mineral density compared to placebo. CORAL noted that lasofoxifene maintained bone mineral density in the lumbar spine better than raloxifene and lowered cholesterol better than raloxifene [56].

Overall lasofoxifene is not only the most potent SERM to date, being a 100 times more potent than raloxifene used at 60 mg daily, but also comes the closest to the therapeutics properties of the ideal SERM [40]. Lasofoxifene reduces breast cancer incidence, producing no increase in endometrial cancer, reduces lumbar fractures, reduces strokes, and reduces coronary heart disease. The medicine is approved in the European Union, and plans are in place for a European marketing plan.

Bazedoxifene has its origins in the failed breast cancer drug zindoxifene (Fig. 6). A study of the metabolites of zindoxifene [57] found them to be estrogens, one of which was extremely potent at the ER. This metabolite, with an appropriately positioned anti-estrogenic side chain, became bazedoxifene [58].

Bazedoxifene has been successfully tested as an agent to improve bone density and bone turnover [59] without negative effects upon the reproductive track [60]. Vertebral fractures are reduced compared to placebo, and in high-risk women, bazedoxifene lowers the risk of non-vertebral fractures significantly relative to placebo and raloxifene [61]. Nevertheless, bazedoxifene is not available in the United States as a treatment for osteoporosis. The innovation that is preferred is to substitute bazedoxifene in HRT instead of medroxyprogesterone acetate (MPA).

Women have used HRT in the United States for the past 40 years. Originally, CEE was used alone, but a small but significant increase in endometrial cancer was noted in the mid-1970s [62, 63]. It was reasoned that a combination of CEE with a progestin would prevent unopposed estrogen-stimulated uterine proliferation that resulted in endometrial cancer. Despite the conviction that HRT would not only create a strong skeleton but also reduce the risk of coronary disease following menopause, the Women's Health Initiative (WHI) put the theory to the test by selecting women over their 60s to enter trials of HRT versus placebo in women with a uterus and CEE versus placebo in hysterectomized women. The results were interesting. The trial of HRT versus placebo was stopped once a predetermined incidence of breast cancer was observed in the HRT arm [64]. This was anticipated. However, the CEE versus placebo was stopped not for an increased risk of breast cancer but an increase incidence of strokes [65]. There was an unanticipated persistent decrease in breast cancer in the CEE group. Though surprising to the medical community, these data for CEE in long-term estrogen-deprived women followed biological rules established through clinical trials over the previous 60 years. The first therapy to treat any cancer successfully was high-dose synthetic estrogen treatment of metastatic breast cancer in postmenopausal women [66, 67]. No mechanisms were

known at the time (1950–1975); the biology of estrogen-killing breast cancer cells in patients was established by experimental medicine and observations. However, this was a paradox as oophorectomy with estrogen withdrawal was standard of care for premenopausal patients with MBC. Response rates were about 30%. Paradoxically high-dose estrogen therapy was only effective in 30% of patients if administered 5 years after menopause [67]. If estrogen was administered earlier, breast tumors grew. It is interesting to note that 5 years of adjuvant tamoxifen therapy became the standard of care for 20 years at the end of the twentieth century. However, there was an unexplained phenomenon, the "carryover effect."

Tamoxifen is a competitive inhibitor of estrogen action at the ER. It is a rule of pharmacology that if tamoxifen was not being given to block the ER, a woman's own estrogen would reactivate tumor growth. In the case of 5 years adjuvant tamoxifen therapy, this did not occur, and in fact mortality decreased after the drug was stopped! [68]. The key was the consistent 5-year rule of LTED in breast cancer. It is proposed that there is clonal selection pressure for survival of breast cancer cells in micrometastases during LTED [69]. Discovery of mechanisms started with the finding that the serial transplantation of MCF-7 breast tumors with acquired tamoxifen resistance into new generations of athymic mice treated with tamoxifen actually sensitized the tumors to the tumoricidal actions of low-dose estrogen [70, 71]. Estrogen-induced apoptosis has been noted to occur [72, 73], and this experimental biology has been advanced as the reason for the "carryover effect" after adjuvant antihormone therapy is stopped [69] and for the tumoricidal action of CEE alone in hysterectomized woman in the WHI [74].

The idea of combining CEE with a SERM was first proposed in 1998 [75]. To paraphrase, "there are concerns that site-specific anti-estrogens used for the long-term treatment of postmenopausal women may not produce estrogenic effects in the CNS." In fact, the main problem with long-term anti-estrogen therapy is menopausal side effects. "Indeed, the combination of an appropriate compound with Premarin would provide the benefits in the CNS and the benefits of a targeted anti-estrogen in the periphery" [75]. The combination of CEE and bazedoxifene is available for the amelioration of postmenopausal symptoms. The anti-estrogen bazedoxifene blocks breast and endometrial tumor ER, thereby preventing an increase in breast and endometrial cancer [76].

The question has to be asked: "If an anti-estrogen prevents estrogen-stimulated breast cancer growth with a bazedoxifene/CEE combination, why does MPA increase breast cancer when combined with CEE?" The answer lies in the modulation of estrogen-induced apoptosis by glucocorticoids [77–79]. It is well known that MPA has glucocorticoid activity at the glucocorticoid receptor and has been proven to block estrogen-induced apoptosis in LTED breast cancer cells [78]. As a result, microscopic early breast cancer has the potential to grow into invasive breast cancer during HRT. By contrast, CEE alone causes apoptosis in LTED breast cancer thereby reducing the incidence of breast cancer.

Finally, there is the interesting application of ospemifene, a known metabolite of the tamoxifen derivative of toremifene (Fig. 6). The discovery of metabolite Y of tamoxifen [80, 81] was found to be a step during the systematic metabolism of the

anti-estrogenic side chain of tamoxifen. The molecule has a low binding affinity for the ER, but the innovation was the identification of the equivalent metabolite for toremifene and the use of the metabolite for dyspareunia. The trick was knowing that tamoxifen causes increase vaginal secretions in women [82]. The metabolite now called ospemifene does the same.

The extensive investigation of tamoxifen and related nonsteroidal anti-estrogens in the 1980s [40, 83] created the incentive to commercialize the new group of medicines now referred to as SERMs [45]. This resulted in multiple advances in women's health [84]. In the next section, we will describe the new knowledge pertaining to the molecular biology of estrogen action that provides an insight into the mechanisms of SERM action.

6 Mechanism of SERM Action

Studies of the pharmacology of the metabolites of tamoxifen provided the laboratory tools to explore mechanisms of action of anti-estrogens in modulating prolactin synthesis in normal cells [13, 14, 16, 17] and the replication of breast cancer cells in culture [85, 86]. The resulting hypothetical "crocodile model" [17, 87] informed what was occurring inside of the ER and led to the identification of the "anti-estrogen region" [17] that is required by anti-estrogenic action for both 4-hydroxytamoxifen and raloxifene to create an anti-estrogenic mechanism. The target for the bulky anti-estrogen side chain is asp351 [88–91]. The subsequent x-ray crystallography of raloxifene and 4-hydroxytamoxifen demonstrated that the anti-estrogenic side chain of raloxifene neutralized and shielded asp351 [92], whereas the anti-estrogenic side chain of 4-hydroxytamoxifen was positioned further away from asp 351 which was not adequately shielded [93]. Indeed, these data illustrated the reason for the more promiscuous estrogen-like action of tamoxifen compared to raloxifene. This mechanism can be traced back to the imperfect closing of helix 12 in the tamoxifen ER complex. Subsequent, structure-activity relationships of asp351 and the anti-estrogens side chain of either tamoxifen or raloxifene confirmed the pivotal role of asp351 as an anchor to helix 12 closure [94–99].

Hypothetical models were advanced to aid in the explanation of agonist (crocodile jaws closed), antagonist (crocodile jaws open by the anti-estrogenic side chain), and partial agonist (a proportional mixture of estrogen/anti-estrogen complexes) [83]. However, advances in technology and the molecular biology of estrogen action facilitated an understanding of SERM action.

Differences between estrogen and anti-estrogen actions are based on the change in the conformation of the ER complex (Fig. 7). There are three complementary mechanisms that modulate the ligand ER complex:

(1) The ligand shape alters the shape of the external surface of the ER complex [101, 102]. Selective estrogen receptor modulators each induce distinct conformational changes in ER alpha and ER beta [102, 103] that attract either coactivators or

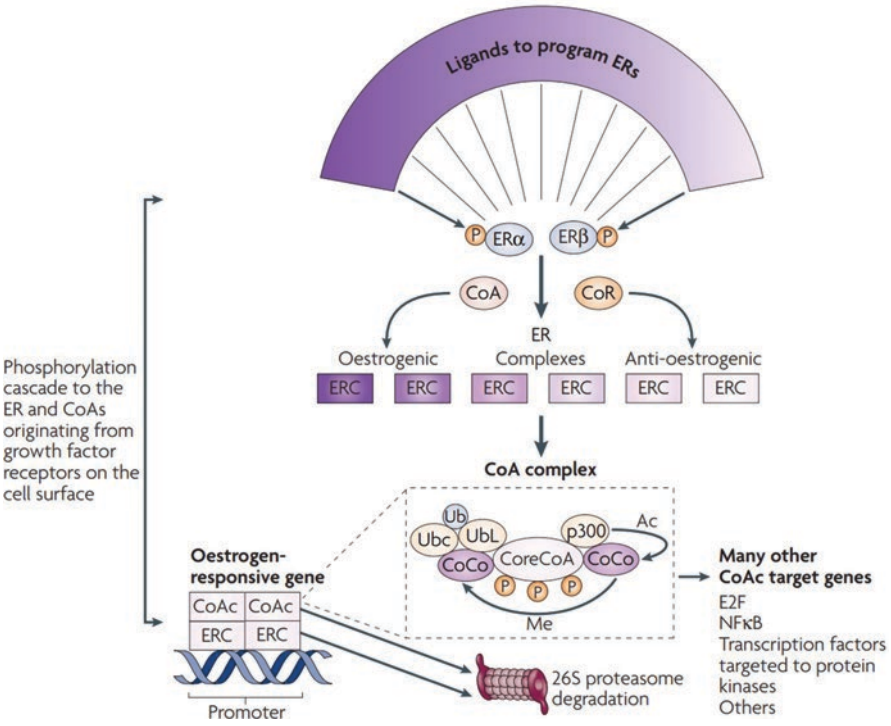

Fig. 7 Molecular networks potentially influence the expression of SERM action in a target tissue. The shape of the ligands that bind to the estrogen receptors (ERs)α and β programs the complex to become an estrogenic or anti-estrogenic signal. The context of the ER complex (ERC) can influence the expression of the response through the numbers of corepressors (CoR) or coactivators (CoA). In simple terms, a site with few CoAs or high levels of CoRs might be a dominant anti-estrogenic site. However, the expression of estrogenic action is not simply the binding of the receptor complex to the promoter of the estrogen-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-coactivators (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 estrogen-responsive gene promoter to switch on transcription. The CoCo proteins then perform methylation (Me) or acetylation (Ac) to activate dissociation of the complex. Simultaneously, ubiquitylation 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 coactivator, specifically SRC3, has ubiquitous action and can further modulate or amplify the ligand-activated trigger through many modulating genes 101 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 estrogen action and anti-estrogen action on the basis of the shape of the ligand and the sophistication of the tissue-modulating network. *NFκB* nuclear factor κB (Reproduced with permission from [100])

corepressors. Indeed, peptide antagonist of the human estrogen receptor can block SERM actions [104, 105].

(2) Allosteric regulation of ER structure and function by different estrogen response elements [106].

(3) The turnover of the SERM ER complex whose stability is regulated by agonist, antagonist, and SERM complexes by the ubiquitinylation and destruction of the complex via the 26S proteasome.

The discovery of coactivators [107] that bind to the estrogen ER complex and corepressors that bind to the anti-estrogen ER complex provided a new dimension in the understanding of the complexities of SERM action [108, 109]. Now it is documented that not only is the ER complex with the SERM modulated through destruction, but also the coactivators are independently destroyed thereby emasculating signal transduction pathway. These events are all summarized in Fig. 7.

7 Summary and Selective Nuclear Receptor Modulators

Blockbuster success, i.e., revenues over a billion dollars annually with tamoxifen, the first SERM, and raloxifene, the first truly multifunctional SERM, has naturally raised the possibility that modulators for all members of the nuclear receptor super family can be discovered. This prospect could result in the treatment of diseases never before believed to be possible. Additionally, steroid therapies could be safer, e.g., a selective glucocorticoid could be found with anti-inflammatory action but without the problem of promoting bone resorption.

The full range of experimental compounds is selective ER modulators (SERMs), selective androgen receptor modulators (SARMs), selective progesterone receptor modulators (SPRMs), selective glucocorticoid receptor modulators (SGRMs), selective mineralocorticoid receptor modulators (SMRMs), selective thyroid receptor modulators (STRMs), and selective peroxisome proliferator-activated receptor modulators (SPPARMs), which has recently been reviewed [110].

At the beginning of the journey to SERMs in the 1980s, once the foundation had been laid with the first SERM tamoxifen in the 1970s, one could not have immediately predicted the success of the concept with the enhancement of women's health nor the survival of millions of women with breast cancer or, in the case of raloxifene for the treatment of osteoporosis, fewer breast cancer. Nevertheless, search continues for the ideal SERM. This search is achieving successes (Fig. 4) and especially if the plans to market lasofoxifene in Europe come to fruition.

Personal Postscript of V. Craig Jordan

In 1980, I was recruited to the University of Wisconsin Clinical Cancer Center (UWCCC), Madison. At this one campus, there was excellence in basic and clinical cancer research. The University of Wisconsin-Madison was perfect for me.

The University of Wisconsin was then unique in America as it had two cancer centers. The McArdle Laboratory for Cancer Research focused on basic research

for the causes of cancer. This remarkable cancer research unit was created by its inaugural Director Harold Rusch, MD. Harold went on to create the UWCCC following the signing of the National Cancer Act in 1971. It was Harold that recruited Paul P. Carbone, MD, from the National Cancer Institute in Washington to be his successor upon his retirement. The goal was to build a team of staff to translate discoveries in the laboratory that would accelerate progress in cancer care.

The UWCCC, at that time, had no graduate program, but the faculty at the McArdle laboratory generously permitted Anna C. Tate, a Fulbright Hays Scholar (see Fig. 8b Anna Riegel (née Tate)), to be my PhD student in their program. I was permitted to be her PhD supervisor for a McArdle PhD but with her work conducted in the Department of Human Oncology, UWCCC. She had already been awarded a BSc with first class honors in Pharmacology and a Master's of Science degree with distinction in Steroid Endocrinology, both from the University of Leeds. She successfully defended her thesis at the end of the 3 years in my laboratory at UWCCC. Her thesis work built upon my earlier connections with Elwood Jensen in Chicago who introduced me to his postdoctoral fellow Geoffrey Greene (see Fig. 8c). We became collaborators then, and we have an active collaboration today. During his fellowship with Elwood Jensen, Geoff created the first polyclonal antibodies to the human ER [111] and the first monoclonal antibodies [112, 113]. This work was critical to make the subsequent cloning and sequencing of the human ER possible [114]. However, I had a different idea: to understand the molecular interaction of anti-estrogens with the ligand binding domain of the ER.

My collaboration with Geoff Greene and Elwood Jensen (who then had moved to Switzerland) resulted in numerous publications using polyclonal and monoclonal antibodies to the human ER. The hypothesis was that there would be differences in the estradiol and 4-hydroxytamoxifen ER complexes. The fact that I had received the first synthetic [^{3}H]4-hydroxytamoxifen (4OHT) from ICI pharmaceuticals in Cheshire was a plus [115]. A difference in antibody binding to reconfigured epitope might be informative for estrogen and anti-estrogen ER complexes. The monoclonal antibodies did not detect differences in the estrogen or anti-estrogen receptor complex from rat pituitary tumor GH3 cells, MCF-7 breast cancer cells, or breast tumor cytosols. By contrast, the polyclonal goat antibody raised to the calf uterine ER [116] discriminated radiolabeled E_2 or radiolabeled 4-OHT binding to human breast cancer ER. Preincubation of the unoccupied human ER with the polyclonal antibodies prevented radiolabel E_2 from being locked into the ER. Estrogen rapidly dissociated from the paralyzed ER. The "crocodile jaws" remained open with polyclonal antibody binding to the ER. By contrast, the [^{3}H] 4-OHT wedged into the ER complex whether the polyclonal antibody was preincubated with ER or the complex was incubated subsequently [116]. Anti-estrogens worked by preventing the jaws from closing. Subsequently, the jaws would be identified as helix 12, a dozen years later.

During my 3-month recruitment visit to Madison in 1977, I was lucky to become friends with Jack Gorski. This was during the worst winter in living memory as snow started the week we arrived in October and continued for 4 months after we left in January 1978. Jack introduced me to Mara Lieberman in his laboratory who showed me her unpublished data on estradiol-stimulated prolactin synthesis in

Fig. 8 Multiple photographs from the 2 days of celebrations in 1999 for Dr. Jordan's investiture as the Inaugural Diana, Princess of Wales, Professor of Cancer Research at Northwestern University in Chicago. (**a**) Dr. Jordan with Diana, Princess of Wales, at a private reception given by the President of Northwestern University. Dr. Jordan had organized the program of a symposium on women's health. Diana, Princess of Wales, had agreed to deliver the keynote address and accepting the invitation to visit Chicago's Northwestern University June 4–6, 1996. (**b**) Elwood V. Jensen keynote speaker in the symposium honoring Dr. Jordan during the celebrations surrounding his investiture as the Inaugural Diana, the Princess of Wales, Professor for Cancer Research. Their positions in the tamoxifen teams when they were in training followed by their current positions. From left to right clockwise: Anna T. Riegel (née Tate), PhD student, today Cecilia Fisher Rudman Professor Department of Oncology and Pharmacology Director of Research Education. William H. Catherino, MD PhD student, today Professor and Research Head, Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences Associate Program Director. Anna Levenson, MD, PhD, postdoctoral fellow, today Associate Dean for Research and Graduate Studies. Eun-Sook Lee, visiting Faculty, today Director of the Cancer Research Center of South Korea. Dr. Elwood Jensen. Dr. Debra Tonetti, postdoctoral fellow, today Department Head, Professor of Pharmacology at the University of Illinois, Chicago. (**c**) Dr. Geoffrey Greene and Dr. Elwood Jensen, participants in the symposium honoring Dr. Jordan during the 2-day celebration with his investiture as the Diana, Princess of Wales, Professor of Cancer Research. (**d**) The symposium speakers at the event to honor Dr. Jordan upon his investiture as the Diana, Princess of Wales, Professor of Cancer Research. Dr. Marco Gottardis, who was Dr. Jordan's PhD student at Wisconsin in the Tamoxifen Team, is receiving his commemorative plaque from Dr. Steven Rosen, the Director of the Robert H. Lurie Cancer Center in Chicago

isolated immature rat pituitary cells in short-term culture. She published it a year later in the Proceedings of the National Academy of Sciences [117]. Here was a new and, at the time, the only model we could use to address the structure function relationships of nonsteroidal anti-estrogens! This was an essential first step toward our discovery of the pharmacology of SERMs, as we had just published our work on the metabolic activation of tamoxifen to 4-hydroxytamoxifen [43].

The discovery that an anti-estrogen could have this same affinity for the ER as an estrogen was revolutionary. Up to that point, it was reasoned that since anti-estrogens had only weak binding affinity to the ER, the complex would easily dissociate and full estrogen action would be impossible [118]. If low affinity to the ER did not predict anti-estrogen action, the hypothesis evolved to become the shape of the complex that predicted pharmacological activity. Based upon the shape of the resulting ligand, ER complex partial agonist or complete antagonist could be predicted [83].

The modulation of estrogen-stimulated synthesis of prolactin in vitro resulted in the publication of the "crocodile model": the bulky alkyl aminoethoxy side chain of 4OHT (or later raloxifene) needs to interact with an anti-estrogen binding region [17] to prevent closure of the crocodile's jaw (now known to be helix 12). This was deciphered and validated by the UWCCC Tamoxifen Team. This advance was told in our companion chapter: the Tamoxifen Tale. It required a multifaceted team of PhD students to create models to decipher mechanisms that paralleled the essential work of others to crystallize the ligand binding domain of the human ER with estrogens and the SERMs 4-hydroxytamoxifen and raloxifene.

An understanding of the species differences of the pharmacology of nonsteroidal anti-estrogens was pivotal for progress in human therapeutics with tamoxifen. The development of athymic animals that were immune deficient now allowed human breast cancer cell lives to be inoculated and therapy evaluated in vivo. We were fortunate in the new facilities at UWCCC to have state-of-the-art athymic animal suites. Marco Gottardis (Fig. 8d) and Doug Wolf, both PhD students on the UWCCC T32 training grant (Doug was also a recipient of a Komen scholarship), deserve credit for their skill using the new research model. Marco was the pioneer. He developed the model of acquired resistance to tamoxifen using MCF-7 breast cancer cells inoculated into athymic mice. He proved that acquired resistance to tamoxifen in athymic mice was tamoxifen-stimulated growth [119]. He used athymic rats to demonstrate that metabolism was not critical for tamoxifen-stimulated tumor growth [120]. Furthermore, he was the first to test a new pure steroidal anti-estrogen in the mouse model to show that a "pure anti-estrogen" would be suitable second-line therapy in patients when acquired resistance to tamoxifen occurred [121]. Today fulvestrant is used routinely in the treatment of MBC.

Most importantly, Marco was an essential investigator in the SERM story. He used the athymic mouse model bitransplanted with a human ER-positive endometrial cancer with a human ER-positive breast cancer transplanted in the contralateral axilla. The question to be addressed was: "Is tamoxifen an anti-estrogen in both target tissue tumors from women?" The answer was that tamoxifen prevented breast tumor growth, but the endometrial cancer grew robustly [25]. Following much correspondence in the Lancet [26–28], a link was established between long-term adjuvant tamoxifen use to treat breast cancer, and a small but significant increase in an increased incidence in endometrial cancer was noted. As a result, gynecologists were involved in screening women for occult endometrial cancer prior to tamoxifen therapy. Patient care was changed.

Most importantly, tamoxifen and, a failed breast cancer drug, keoxifene were tested in the laboratory to determine the extent of bone loss in ovariectomized old

breeder rats [37]. This line of research came about by accident but was an essential step to progress with chemoprevention in healthy women. Without this study [37], there would not have been the SERM solution, as it would not have been proposed in 1990 [40]. If estrogen is essential to build bones in women, it would be a disaster to prevent breast cancer in planned chemoprevention trials but at the same time increase the incidence of osteoporosis for all who took the anti-estrogen tamoxifen. If estrogen prevents osteoporosis, then an anti-estrogen by definition would make osteoporosis worse. This was the pivotal laboratory clue that drove all subsequent clinical work at Wisconsin on tamoxifen, and, eventually, after several years, the baton in the relay race for SERMs was picked up by Eli Lilly.

Dr. Urban Lindgren was a visiting scientist from the Karlinska Institute in Stockholm who was now working at the University of Wisconsin, Madison, in the Biochemistry Department. He approached me to consider using nonsteroidal anti-estrogens to create an enhanced laboratory model of osteoporosis. He wanted to test vitamin D analogs to prevent osteoporosis. He reasoned if estrogen is good to build bone, then an anti-estrogen would make osteoporosis worse. This did not seem like an unreasonable hypothesis. We used as our study design a prior study by Beall and coworkers [122], who determined the action of the nonsteroidal anti-estrogen clomiphene on bone density of ovariectomized retired breeder rats. The study [122] paradoxically showed that clomiphene maintained bone density. But I noticed the authors had made a fatal error with their choice of an anti-estrogen. Clomiphene is a mixture of estrogenic and anti-estrogenic geometric isomers. I reasoned that the estrogenic zuclomiphene might be bone specific and that could prevent osteoporosis. By contrast, our study tested tamoxifen, the pure anti-estrogenic *trans* isomer, and keoxifene (LY156758), a high-affinity anti-estrogen that had been abandoned by Eli Lilly as a breast cancer drug. Fortunately, they had allowed me to keep a large quantity of their anti-estrogen keoxifene. Eric Phelps a summer student in our Wisconsin Tamoxifen Team analyzed the data. He showed that both tamoxifen and keoxifene were estrogen-like on ovariectomized rat bone density. All osteoporosis journals rejected our manuscript with the opinion that our data could not be correct, as an anti-estrogen cannot build bone! However, Breast Cancer Research and Treatment and Bill McGuire, the editor, embraced our findings. The bone density data in rats with tamoxifen was confirmed by others [41], but no animal bone studies with keoxifene were published after the original Jordan study in 1987 [37]. This study [37] became the translational research foundation for the whole of the Wisconsin tamoxifen study [38, 39]. In 1987, my Cancer Center Director Paul Carbone appointed me to be the Director of his Breast Cancer Research and Treatment Breast Program (1987–1993). As a full professor, appointed in 1985, I was a member of Dr. Richard Love's promotion committee along with Drs. Ernest Borden and Tom Davis. Dick was an assistant professor, but Paul was keen to ensure that Dick appropriately advanced up the professional ladder. However, up to this point, Dick's strong academic suit was teaching rather than translational research. He was, however, enthusiastic about chemoprevention and had previously struck up a collaboration with Dr. Ray Brown, who was enthusiastic about the application of retinoids, as chemopreventive agents. Much clinical work in this area was then

being conducted by Professor Umberto Veronesi's group in Milan. However, this strategy changed very rapidly after Paul and I attended a meeting in New York that was planning to address chemoprevention. This meeting was held because of the announcement by Dr. Trevor Powles in the United Kingdom that he was about to start a pilot clinical trial of tamoxifen. These preliminary data were published in 1989 [123]. We now saw tamoxifen as top of the international clinical agenda. Indeed, it was of such importance that my mother rang me up and declared that "somebody in England is planning to use your drug to prevent breast cancer – do something about it!" So, at Wisconsin we did.

I became the Head of the Breast Cancer Research and Treatment program, and Dick started to plan clinical studies with tamoxifen. Dick's assets at Wisconsin were Polly Newcome, an excellent epidemiologist; Dave DeMets, an exceptional biostatistician from NIH; and my Tamoxifen Team which was considered the world's center of tamoxifen research at that time. This was a most fortuitous mix of talent at one place. Dick advanced the laboratory data on tamoxifen and bone density we had produced [37]. He threw the dice to get a quick positive paper comparing and contrasting patients taking tamoxifen, but this was, regrettably, a negative finding [124]. He, then, chose to go the clinical trial route with funding for him from the American Cancer Society and support for our program from AstraZeneca. The rat bone data with tamoxifen became the translational rationale for the Wisconsin Tamoxifen Study of bones and lipids in node-negative patients treated with tamoxifen or placebo for 2 years.

Keoxifene was reinvented as raloxifene by changing the salt of the compound from LY156758 mesylate (keoxifene) to LY139481 HCl (raloxifene). There were no patents for the use of either keoxifene or raloxifene in the late 1980s for osteoporosis, only a breast cancer indication, and that was abandoned after 1987. Black and coworkers [42] confirmed that the molecule keoxifene (raloxifene) was able to reduce circulating cholesterol and build bone in laboratory rats. Patents were awarded [125], and raloxifene went forward to become a blockbuster medicine (i.e., a billion dollar a year sales).

Raloxifene went to clinical trials to test the hypothesis that the compound would prevent osteoporosis and reduce the incidence of breast cancer at the same time. Marco had already demonstrated that tamoxifen and keoxifene would prevent rat mammary carcinogenesis. However, keoxifene was less effective [126].

The Multiple Outcome of Raloxifene Evaluation (MORE) trial demonstrated that raloxifene reduced spinal fractures by 50% compared to placebo. A separate analysis of breast cancer incidence demonstrated a 76% decrease in the incidence of ER-positive breast cancer over a 3-year period [127]. At the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, I was the Director of the Breast Cancer Research Program and because of my previous interactions with Diana, Princess of Wales, I was appointed the Diana, Princess of Wales, Professor of Cancer Research (Fig. 8a) after her untimely death. I (VCJ) was the chair of the breast cancer adjudication committee of the MORE trial. This translational work was an exceptional, though unconventional, team effort between a university (Wisconsin/Northwestern) investigator and the pharmaceutical industry. The SERM concept [40] worked!

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