Tissue Selective Estrogen Complexes (TSECs) Differentially Modulate Markers of Proliferation and Differentiation in Endometrial Cells

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Abstract

Selective estrogen receptor modulators (SERMs) have tissue-specific estrogen receptor (ER) modulating properties. Combining an SERM with one or more estrogens to form a tissue selective estrogen complex (TSEC) can provide an improved blend of tissue-specific ER agonist and antagonist effects. While both estrogens and SERMs affect the uterine endometrium, not all TSECs reverse the endometrial effects of estrogens preventing endometrial proliferation and hyperplasia. Their action in uterine cells is not completely understood. *HOXA 10*, leukemia inhibitory factor (*LIF*), progesterone receptor (*PR*), and *EMX2* are genes known to regulate endometrial proliferation and differentiation. The expression of these genes was used to assess endometrial effects of SERMs and TSECs. We evaluated the effects of raloxifene (RAL), tamoxifen (TAM), lasofoxifene (LAS), bazedoxifene acetate (BZA), and progesterone (P) alone and in combination with estradiol (E2) in Ishikawa cells. Increased *HOXA 10*, *LIF*, *PR*, and *EMX2* messenger RNA (mRNA) expression was noted in E2-treated cells compared with vehicle-treated controls. All TSECs maintained E2-induced *PR* expression and all except TAM prevented estrogen-induced *LIF* expression. The TSEC containing BZA uniquely decreased *HOXA 10* expression and increased *EMX2* expression. The TSECs alter endometrial cell proliferation by selective modulation of estrogen responsive genes, maintaining the antiproliferative effects mediated by *PR* and inhibiting *LIF*. The differential effect of TSECs on endometrial gene expression suggests a mechanism by which they manifest differential effects on endometrial safety against the risk of estrogen-induced endometrial hyperplasia.

Keywords

TSECs, SERMs, *HOXA 10*, leukemia inhibitory factor (*LIF*), progesterone receptor, *EMX2*, ishikawa

Introduction

As the population of the United States ages, the number of postmenopausal women is increasing.¹ When estrogen levels decline at menopause, many women experience vasomotor instability and vaginal atrophy; further, they are at increased risk for developing osteoporosis.²⁻⁴

Hormone therapy can address many of the consequences of reduced ovarian sex steroid production, however not all menopausal women are inclined to use hormone therapy due to the widely publicized controversies surrounding its benefits and risks.⁵⁻⁷

In reality, conjugated estrogens (CEs) have been well studied and have a favorable benefit /risk profile.⁸ Treatment with CE was recently shown to have minimal risks, especially in younger menopausal women.^{8,9} However, in patients with a uterus, estrogens must be administered concomitantly with a progestin to prevent endometrial hyperplasia.¹⁰ The results of the Women's Health Initiative (WHI) demonstrate few risks with the use of CE, especially in younger postmenopausal

women,⁸ however some of the benefits were attenuated by the addition of a progestin.¹¹

In the WHI, CE treatment reduced net cardiovascular events in the 50- to 59-year-old age group, however no such beneficial effect was observed with combined conjugated equine

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estrogens (CEE) and medroxyprogesterone acetate (MPA) therapy.¹¹ Similarly, CEE reduced breast cancer incidence in the WHI, an effect that became statistically significant in the postintervention phase.^{8,9} In contrast, a small increase in breast cancer and breast cancer mortality was seen in women using CEE/MPA,¹¹ that did not persist in the postintervention phase.¹² It is likely that the risk of breast cancer associated with hormone therapy is predominantly attributable to the progestin. However, in a woman with a uterus, use of estrogens without progestins can lead to endometrial hyperplasia and therefore estrogens cannot be used alone.

Progestins are used in combination with estrogens solely for endometrial protection.^{10,11} Clearly, alternatives to progestins that counteract estrogen effects in the uterus are needed. Tissue selective estrogen complexes (TSECs) are a new class of compounds that pair a selective estrogen receptor modulator (SERM) with 1 or more estrogens in order to attain unique ER actions that are tissue specific.¹³ An ideal TSEC should allow estrogen action where it is beneficial (ie, central nervous system to reduce vasomotor symptoms and bone) while counteracting the effects of estrogens in the uterus, eliminating the risk of endometrial hyperplasia. While 1 TSEC consisting of raloxifene and estradiol (RAL/E2) has been implicated in endometrial hyperplasia, another containing bazedoxifene acetate and conjugated estrogens (BZA/CE) has demonstrated endometrial safety.^{13,14}

Classically, estrogens function via binding to 1 of 2 ERs (ER α and ER β).¹⁵ The molecular basis of estrogen activity involves liganded ER, undergoing conformational changes that facilitate interactions with coactivator or corepressor proteins. These complexes subsequently enhance or suppress transcription of target genes.¹⁶ Tissue response to estrogens is dependent on the individual ligand or combination of ligands, levels of ER α and ER β expressed as well as the availability of coactivators and/or corepressors.¹⁷ Each ligand or a blend of ligands can therefore induce a distinct set of responses that are also tissue specific.

Selective estrogen receptor modulators comprise a group of compounds that are capable of ER agonist and antagonist effects in a tissue-specific manner.¹³ The activity of SERM is intrinsic to each compound that accomplishes a differential tissue-specific profile by formation of distinct transcriptional complexes with unique combinations of coactivators and corepressors. The cell-type-specific action of this class of compounds is clinically useful; however each SERM's pharmacology is different and needs to be evaluated individually when considering its use clinically.

Since the development of tamoxifen (TAM), other triphenylethylene SERMs have been studied for breast cancer prevention, including droloxifene, idoxifene, toremifene, and ospemifene.¹⁸ Recently other SERMs have entered clinical development including benzothiophenes (raloxifene and arzoxifene), benzopyrans (ormeloxifene, levormeloxifene, and EM-800), lasofoxifene (LAS), pipendoxifene, bazedoxifene, HMR-3339, and fulvestrant, an antiestrogen which is approved for breast cancer treatment.^{19,20} Differences in SERM activity suggest that

any clinical end point must be evaluated individually, and conclusions about any particular SERM may vary by tissue. The mechanisms responsible for differential SERM activity are poorly understood but known to be cell type and gene promoter dependent.¹⁸ The effects of TSECs on target tissues are not well characterized and large clinical trials have thus far been conducted on only a single TSEC containing BZA.²¹ This TSEC relieves vasomotor symptoms and improves bone mineral density while avoiding endometrial hyperplasia.^{21,22}

An essential prerequisite for the clinical use of a TSEC is the lack of endometrial stimulation and hyperplasia. While administration of estrogens alone leads to endometrial hyperplasia, use of the TSEC containing BZA/CE did not result in hyperplasia in clinical trials.¹³ While all estrogen, SERM, and TSEC functions are mediated through ER, not all result in the same pattern of gene activation.^{16,23} We hypothesized that the mechanism of TSEC action varies depending on the individual TSECs ability to differentially activate downstream target genes. Currently there are no studies that identify endometrial target genes preferentially activated or repressed by a TSEC. Some of those target genes regulate pathways essential for endometrial proliferation and therefore have a propensity to promote hyperplasia. The aim of this study was to compare the effect of several TSECs on well-characterized markers of proliferation and differentiation in endometrial cells.

Methods

Cell Culture and In Vitro Treatment Exposure

Ishikawa cells, a well-differentiated uterine epithelial cancer cell line known to express HOXA10, leukemia inhibitory factor (LIF), EMX2, and progesterone receptor (PR), were obtained from the American Type Collection (Rockville, Maryland).^{24,25} Ishikawa cells were cultured in phenol-free Eagle MEM (Life Technologies, Inc, Gaithersburg, Maryland) containing 10% (v/v) charcoal-stripped fetal bovine serum and supplemented with penicillin/streptomycin (100 μ g/mL), L-glutamine (2 mmol/L), and sodium pyruvate (1 mmol/L). Cells were grown to confluence in plastic flasks (75 cm², Falcon, Franklin Lakes, New Jersey) and maintained at 37°C in a humidified atmosphere (5% CO₂ in air). The 70% to 80% confluent monolayers were harvested by trypsinization, seeded in a 6-well plate, and maintained in serum-free medium for 24 hours. The cells were subsequently treated for 24 hours with E2, RAL, RAL + E2, TAM, TAM + E2, LAS, LAS + E2, BZA, or BZA + E2 each at 0.1 μ mol/L concentration, a physiologic dose of estrogen and an equimolar concentration of each SERM. Both E2 and TAM were obtained from Sigma, St Louis, Missouri. All RAL, LAS, and BZA were obtained from Pfizer, Collegeville, Pennsylvania. RNA was isolated using the RNEasy Kit (Qiagen, Valencia, California) according to the manufacturer's protocol. Adequate RNA quality was determined by an A260-280 ratio of >1.7. RNA samples were stored at -80°C until use. Each experiment was repeated 3 times and performed in triplicate.

Quantitative Reverse Transcription–Polymerase Chain Reaction

Total RNA (500 ng) was reverse transcribed in 20 μ L of reaction mixture using the iScript complementary DNA (cDNA) synthesis kit (Bio-Rad, Hercules, California). The reaction mix was incubated for 5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C using the Eppendorf Mastercycler (Eppendorf North America). Quantitative real-time reverse transcription–polymerase chain reactions (RT-PCRs) were prepared using the iQ SYBR Green Supermix (Bio-Rad). Each PCR reaction consisted of the following: 1 μ L of cDNA template, 1 μ L of forward primer (1 μ mol/L), 1 μ L of reverse primer (1 μ mol/L), 9.5 μ L of nuclease-free H₂O, and 12.5 μ L of iQ SYBR Green Supermix. β -Actin was used as a housekeeping gene. β -Actin primers have been described previously.²⁶ The PCR amplification of *HOXA10*, *LIF*, and *PR* was performed for 45 cycles of 95°C for 2 seconds; 65°C for 5 seconds; 72°C for 18 seconds. Polymerase chain reaction amplifying β -actin was performed for 45 cycles of 95°C for 2 seconds; 61°C for 5 seconds; 72°C for 18 seconds. Analysis of variance (ANOVA) was used to determine statistically significant differential expression.

Primers

HOXA10

Sense AGGTGGACGCTGCGGCTAATCTCTA
Antisense GCCCCTTCCGAGAGCAGCAAAG

LIF

Sense TGAACCAGATCAGGAGCCAACT
Antisense CCACATAGCTTGTCAGGTTGTT

PR

Sense TGGAAGAAATGACTGCATCG
Antisense TAGGGCTTGGCTTTCATTTG

EMX2

Sense ACTAGCCCCGAGAGTTTCATTTTG
Antisense CTCCAGCTTCTGCCTTTTGAACCTT

β -Actin

Sense CGTACCACTGGCATCGTGAT
Antisense GTGTTGGCGTACAGGTCCTTG

Statistics

Gene expression was confirmed to be normally distributed. Analysis of variance with post hoc Bonferroni correction was used to determine the significance of differential gene expression. A corrected *P* value of less than .05 was considered significant.

Results

Preclinical studies have shown that SERMs can antagonize 17- β E2-mediated stimulation of breast cancer cell proliferation.^{16,18,26} Recently, BZA was shown to effectively antagonize the E2-stimulated proliferation of MCF-7 cells.²⁷ However, SERMs have not been evaluated for their antagonistic effect on E2-treated endometrial epithelial cells. Therefore, we used Ishikawa cells to study the effect of E2, RAL, TAM, LAS, and BZA alone and in combination with E2 forming a TSEC. Estradiol and each SERM were used at 0.1 μ mol/L concentration. While the potency of each SERM may vary with the gene studied, we initially screened for differences in target gene expression when each SERM and E2 were used at equimolar concentrations. The expression of 4 genes that are well-characterized markers of endometrial proliferation and differentiation were used to evaluate the effect of each SERM/TSEC in Ishikawa cells. As shown in Figures 1 to 4, *HOXA10*, *LIF*, *PR*, and *EMX2* were each regulated in Ishikawa endometrial cells by E2: *P* = .03, *P* = .06, *P* = .001, and *P* = .001, respectively. Gene expression in response to E2, SERMs, and TSECs compared to vehicle control is described subsequent paragraphs.

HOXA10 was used as a well-characterized marker of estrogen response in endometrial cells.^{28–31} *HOXA10* mediates both endometrial cell proliferation and differentiation.^{28,32–34} Compared to treatment with vehicle control, E2 treatment significantly increased *HOXA10* messenger RNA (mRNA) expression, as previously reported.^{30,31,35} *HOXA10* gene expression was not significantly changed by treatment with any of the SERMs in Ishikawa cells (Figure 1A). Estradiol was used in combination with each SERM and the resultant *HOXA10* expression regulated by each TSEC was evaluated. While each TSEC appeared to counter the effect of E2 (BZA + E2 > LAS + E2 > TAM + E2 > RAL + E2), BZA was the only SERM that significantly blocked the E2 effect on *HOXA10* gene expression. (*P* = .03; Figure 1B).

Leukemia inhibitory factor was used as a second marker of estrogen effect on endometrial cells. Leukemia inhibitory factor is known to lead to increased proliferation of endometrial cells.^{36,37} There were no significant changes in *LIF* expression induced by RAL, TAM, or LAS. Treatment with BZA led to a statistically significant decrease in *LIF* expression as compared to control (*P* = .02; Figure 2A). In response to concomitant administration of E2, all TSECs significantly prevented estrogen-induced *LIF* expression except for the TSEC that included TAM (Figure 2B).

Estradiol-induced expression of *PR* is required to mediate the differentiating effects of progesterone.³⁸ Here, as expected, E2 treatment led to increased *PR* mRNA expression. Both TAM and LAS were also able to induce *PR* gene expression (*P* = .0004 and *P* = .01) respectively, whereas no change in *PR* expression was seen after RAL or BZA treatment (Figure 3A). All TSECs maintained the E2 effect on *PR*. A 3- to 15-fold increase in *PR* was seen in TSEC-treated cells compared with SERM treatment alone: RAL + E2 versus RAL

Figure 1. A, *HOXA10* gene expression was measured by qPCR after treatment of Ishikawa cells with an SERM or vehicle control. All values were normalized to beta-actin expression. No significant changes were seen in *HOXA10* mRNA gene expression after SERM treatment. B, The TSECs were used to treat Ishikawa cells. *HOXA10* expression measured by qPCR. The TSEC combination containing BZA + E2 showed significant attenuation of E2-related increase in *HOXA10* mRNA gene expression. None of the other TSECs showed a significant effect. ^a $P = .03$; ^b $P = .03$. qPCR indicates quantitative polymerase chain reaction; SERM, selective estrogen receptor modulator; mRNA, messenger RNA; TSECs, tissue selective estrogen complexes; BZA, bazedoxifene acetate; E2, estradiol.

($P = .005$); TAM + E2 versus TAM ($P = .0002$); LAS + E2 versus LAS ($P = .0008$); BZA + E2 versus BZA ($P < .00001$; Figure 3B). Addition of an SERM did not lead to antagonism of estrogen action on PR expression.

EMX2 has been demonstrated to inhibit the proliferation of endometrial cells.¹⁷ Expression of *EMX2* gene in cells treated with TAM and BZA was significantly increased compared to control ($P < .001$; Figure 4A). Estradiol-treated cells showed low *EMX2* gene expression; its repression by E2 is consistent with the need to repress EMX2-mediated suppression of endometrial cell proliferation ($P < .001$). The combination BZA + E2 significantly increased *EMX2* gene expression in

comparison to E2 alone (Figure 4B; $P = .03$). The TSEC containing RAL did not increase EMX2 to a significant degree, consistent with the risk of endometrial hyperplasia reported in women using this combination of estrogen and SERM.³⁹

Discussion

As a class of compounds, SERMS act on ERs, demonstrating mixed function depending on the particular SERM as well as the target gene and tissue.¹⁸ The SERMs when used alone typically prevent uterine cell proliferation, however one (TAM) may have a stimulatory effect.^{27,40} Their ability to counteract

Figure 2. A, Expression of *LIF* mRNA in SERM-treated cells was measured by qPCR. No difference in *LIF* mRNA expression was seen after treatment with RAL, TAM, or LAZ compared to CTL. Treatment with BZA significantly decreased *LIF* expression in comparison to control. $*P = .02$. B, The qPCR results showing *LIF* mRNA gene expression in TSECs-treated cells. a versus b ($P = .06$); b versus c ($P = .04$); b versus d ($P = NS$); b versus e ($P = .02$); b versus f ($P = .04$). qPCR indicates quantitative polymerase chain reaction; SERM, selective estrogen receptor modulator; mRNA, messenger RNA; TSECs, tissue selective estrogen complexes; BZA, bazedoxifene acetate; E2, estradiol; RAL, raloxifene; TAM, tamoxifen; LAZ, lasofoxifene; LIF, leukemia inhibitory factor; CTL, control.

the effect of E2 on endometrial cells has not been previously tested. In this study, we have shown that proliferation and differentiation markers can be affected differentially by the combination of E2 and SERMs. The TSECs vary depending on the specific SERM used. Therefore, SERMs that antagonize estrogen effects in the endometrium may be better suited for inclusion in TSECs.

HOXA10 encodes an evolutionarily conserved transcription factor that is essential to endometrial development and endometrial receptivity.^{28,29} *HOXA10* expression is apparent in endometrial stroma and glands, where it is regulated by sex steroid hormones.^{28,30} We have previously shown that *HOXA10* expression is significantly upregulated in response to E2 in Ishikawa cells³⁰ as also demonstrated in this study. All TSECs showed some degree of attenuation of this E2-mediated increase in *HOXA10* in Ishikawa cells, however the only combination that led to a statistically significant decrease was BZA + E2. The BZA displayed a significant ER antagonist effect on the expression of this gene. While the data reported here were determined in an endometrial cell line, the findings may help to explain the clinical endometrial effects of the BZA/E2

combination in postmenopausal patients.^{13,41} A difference in *HOXA10* expression alters cell proliferation and differentiation, likely contributing to the differential effect of BZA compared to other SERMs in a TSEC. The CE/BZA does not induce endometrial hyperplasia while other TSECs have been demonstrated to do so.^{13,21}

Leukemia inhibitory factor is an essential cytokine in the reproductive tract, regulating epithelial development, proliferation, and gland formation.^{42,43} It is expressed in the glandular epithelium of the endometrium where it is essential for implantation in humans.^{44,45} The LIF is a mediator of endometrial cell proliferation.^{44,46} Tamoxifen was the only SERM that did not block E2 action on LIF expression. The data suggest that TAM is a poor choice for inclusion in a TSEC. Consistent with this conclusion, multiple studies have reported the development of endometrial cancer in TAM-treated breast cancer patients.^{24,25} Several investigators prospectively followed patients on TAM and detected a variable incidence of endometrial pathologies. The incidence of hyperplasia was reportedly up to 20%, while carcinoma developed in 0% to 8%,⁴⁷⁻⁴⁹ showing that TAM has a partial agonistic action on the endometrium.

Figure 3. A, Expression of PR was measured after treating Ishikawa cells with each SERM. Both TAM and LAS significantly increased PR mRNA gene expression; a versus b ($P = .0004$); a versus c ($P = .01$). RAL and BZA did not alter PR gene expression. B, Expression of PR mRNA was increased by E2; a versus b ($P = .001$). All TSECs tested maintained the E2-induced increase in PR; b versus c ($P = \text{NS}$). SERM indicates selective estrogen receptor modulator; mRNA, messenger RNA; TSECs, tissue selective estrogen complexes; PR, progesterone receptor; E2, estradiol; RAL, raloxifene; BZA, bazedoxifene acetate; TAM, tamoxifen; LAS, lasofoxifene.

Progesterone receptor is a target gene for estrogen action⁵⁰ and is of particular physiological importance in the uterus. Progesterone receptor mediates the response to progesterone that occurs during the luteal phase of the ovarian cycle and is essential for endometrial differentiation that leads to endometrial receptivity and embryo implantation. Here, as expected, E2 treatment led to increased PR expression. The addition of an SERM did not change this effect, showing that the potential antiproliferative mechanisms mediated by PR are maintained when TSECs are used.⁵¹ The effect on PR may be an important mediator of differentiation and contribute to the prevention of an estrogenic effect when the appropriate TSEC is used. However, all TSECs maintained PR expression, suggesting that this effect is not helpful in selection of SERMs for inclusion in clinically useful TSECs. Additionally the expression of PR may only become clinically relevant in the presence of a progestin, making this pathway of unlikely relevance in postmenopausal women administered a TSEC.

The *EMX2* is expressed in Ishikawa cells and adult uterine endometrium.⁵² The *EMX2* suppresses endometrial epithelial proliferation^{53,54} and is a candidate tumor suppressor gene based on its location within a consensus region of allelic loss in uterine endometrial adenocarcinoma.⁵⁵ BZA/E2 was the only TSEC that significantly increased *EMX2* gene expression when comparison to E2 alone, thus demonstrating an antiproliferative effect of this TSEC. The effects on *EMX2* suggest that, among those tested, BZA may be the optimal SERM for use in a TSEC, inducing the antiproliferative and tumor-suppressing effects of *EMX2*.

The combination of an SERM and an estrogen produced differential effects on endometrial cell gene expression. The specific response depends on the SERM used as well as the target gene studied. This study showed that distinct molecular signals underlie the action of TSECs in the regulation of gene expression in Ishikawa cells. The differential gene expression induced by each TSEC indicates that these combinations may show

Figure 4. A, *EMX2* gene expression after treatment with SERMs. An increase in *EMX2* mRNA gene expression was induced by TAM and BZA; a versus b ($P < .001$). B, *EMX2* gene expression was significantly increased by the combination BZA + E2 in comparison to E2 alone ($*P < .03$). None of the other TSECs significantly alter *EMX2* expression. SERM indicates selective estrogen receptor modulator; mRNA, messenger RNA; TSECs, tissue selective estrogen complexes; E2, estradiol; BZA, bazedoxifene acetate.

differential clinical activity in the uteri of postmenopausal women using TSECs. Future studies will determine whether these findings in Ishikawa cells accurately model the uterus of postmenopausal women as well as explore the effects of differential dose and timing of TSEC administration.

Bazedoxifene is a novel SERM undergoing clinical development for the prevention and treatment of postmenopausal osteoporosis.^{56,57} Results of preclinical studies using rodent models suggest that BZA alone has little to no endometrial stimulation and potentially antagonizes CE-induced stimulation of the endometrium when the 2 are coadministered.^{40,58} Similarly in women, CE/BZA treatment does not induce endometrial

hyperplasia.^{13,41,59} The effects of BZA on markers of endometrial proliferation and differentiation demonstrated here suggest mechanisms by which BZA prevents endometrial proliferation leading to hyperplasia.

In conclusion, we demonstrate that the 4 SERMs analyzed here induced significantly different patterns of gene expression in an endometrial cancer cell line and showed differences in their abilities to antagonize E2-induced gene expression. Our data identify that BZA is more potent than RAL, LAS, and TAM in inhibiting the E2-mediated expression of proliferative genes (*LIF* and *HOXA10*) and at the same time maintains the expression of genes that have antiproliferative implications

(*PR* and *EMX2*) in endometrial cells. Based on these data, a TSEC containing an estrogen and BZA offers optimal potential for endometrial protection, antagonizing the effects of E2.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Komm is an employee of Pfizer. Yale University has received a grant from Pfizer to support Dr. Taylor's research.

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References

- US Census Bureau. Table 1: annual estimates of the population by sex and five year age groups for the United States: April 1st, 2007. NC-EST20070-01. May 2008.
- Gallagher JC, Levine JP. Preventing osteoporosis in symptomatic postmenopausal women. *Menopause*. 2011;18(1):109-118.
- Bachmann GA. Menopausal vasomotor symptoms: a review of causes, effects and evidence-based treatment options. *J Reprod Med*. 2005;50(3):155-165.
- Panay N, Maamari R. Treatment of postmenopausal vaginal atrophy with 10-mug estradiol vaginal tablets. *Menopause Int*. 2012;18:15-19.
- Taylor HS, Manson JE. Update in hormone therapy use in menopause. *J Clin Endocrinol Metab*. 2011;96(2):255-264.
- Hardie C, Bain C, Walters M. Hormone replacement therapy: the risks and benefits of treatment. *J R Coll Physicians Edinb*. 2009;39(4):324-326.
- Buick DL, Crook D, Horne R. Women's perceptions of hormone replacement therapy: risks and benefits (1980-2002). A literature review. *Climacteric*. 2005;8(1):24-35.
- Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA*. 2004;291(14):1701-1712.
- LaCroix AZ, Chlebowski RT, Manson JE, et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. *JAMA*. 2011;305(13):1305-1314.
- Lethaby A, Farquar C, Sakris A, Roberts H, Jepson R, Barlow D. Hormone replacement therapy in postmenopausal women: endometrial hyperplasia and irregular bleeding. *Cochrane Database Syst Rev*. 2000;(2):CD000402.
- Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;288(3):321-333.
- Chlebowski RT, Anderson GL, Gass M, et al. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. *JAMA*. 2010;304(15):1684-1692.
- Pickar JH, Mirkin S. Tissue-selective agents: selective estrogen receptor modulators and the tissue-selective estrogen complex. *Menopause Int*. 2010;16(3):121-128.
- Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. *JAMA*. 1999;281(23):2189-2197.
- Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J, Nilsson S. Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. *Mol Pharmacol*. 1998;54(1):105-112.
- Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. *Science*. 2002;295(5564):2465-2468.
- Klinge CM. Estrogen receptor interaction with co-activators and co-repressors. *Steroids*. 2000;65(5):227-251.
- Miller CP. SERMs: evolutionary chemistry, revolutionary biology. *Curr Pharm Des*. 2002;8(23):2089-2111.
- Chia S, Gradishar W. Fulvestrant: expanding the endocrine treatment options for patients with hormone receptor-positive advanced breast cancer. *Breast*. 2008;17(suppl 3):S16-S21.
- Valachis A, Mauri D, Polyzos NP, Mavroudis D, Georgoulas V, Casazza G. Fulvestrant in the treatment of advanced breast cancer: a systematic review and meta-analysis of randomized controlled trials. *Crit Rev Oncol Hematol*. 2010;73(3):220-227.
- Stovall DW, Tanner-Kurtz K, Pinkerton JV. Tissue-selective estrogen complex bazedoxifene and conjugated estrogens for the treatment of menopausal vasomotor symptoms. *Drugs*. 2011;71(13):1649-1657.
- Gennari L, Merlotti D, De Paola V, Martini G, Nuti R. Bazedoxifene for the prevention of postmenopausal osteoporosis. *Ther Clin Risk Manag*. 2008;4(6):1229-1242.
- Berrodin TJ, Chang KC, Komm BS, Freedman LP, Nagpal S. Differential biochemical and cellular actions of Premarin estrogens: distinct pharmacology of bazedoxifene-conjugated estrogens combination. *Mol Endocrinol*. 2009;23(1):74-85.
- Lessey BA, Ilesanmi AO, Castelbaum AJ, et al. Characterization of the functional progesterone receptor in an endometrial adenocarcinoma cell line (Ishikawa): progesterone-induced expression of the alpha1 integrin. *J Steroid Biochem Mol Biol*. 1996;59(1):31-39.
- Ishiwata I, Ishiwata C, Nagayama T, Ishikawa H. Histogenesis and culture of human uterine carcinosarcoma. *Cancer Res*. 1981;41(5):1978-1983.
- Smith CC, Taylor HS. Xenoestrogen exposure imprints expression of genes (Hoxa10) required for normal uterine development. *FASEB J*. 2007;21(1):239-246.
- Komm BS, Kharode YP, Bodine PV, Harris HA, Miller CP, Lyttle CR. Bazedoxifene acetate: a selective estrogen receptor modulator with improved selectivity. *Endocrinology*. 2005;146(9):3999-4008.
- Daftary GS, Taylor HS. Endocrine regulation of HOX genes. *Endocr Rev*. 2006;27(4):331-355.
- Vitiello D, Pinard R, Taylor HS. Gene expression profiling reveals putative HOXA10 downstream targets in the periimplantation mouse uterus. *Reprod Sci*. 2008;15(5):529-535.
- Taylor HS, Arici A, Olive D, Igarashi P. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest*. 1998;101(7):1379-1384.

31. Taylor HS, Daftary GS, Selam B. Endometrial HOXA10 expression after controlled ovarian hyperstimulation with recombinant follicle-stimulating hormone. *Fertil Steril*. 2003;80(suppl 2):839-843.
32. Daftary GS, Taylor HS. Implantation in the human: the role of HOX genes. *Semin Reprod Med*. 2000;18(3):311-320.
33. Das SK. Regional development of uterine decidualization: molecular signaling by Hoxa-10. *Mol Reprod Dev*. 2010;77(5):387-396.
34. Daftary GS, Taylor HS. Pleiotropic effects of Hoxa10 on the functional development of peri-implantation endometrium. *Mol Reprod Dev*. 2004;67(1):8-14.
35. Zanatta A, Rocha AM, Carvalho FM, et al. The role of the Hoxa10/HOXA10 gene in the etiology of endometriosis and its related infertility: a review. *J Assist Reprod Genet*. 2010;27(12):701-710.
36. Salamonsen LA, Nie G, Dimitriadis E, Robb L. Genes involved in implantation. *Reprod Fertil Dev*. 2001;13(1):41-49.
37. Senturk LM, Arici A. Leukemia inhibitory factor in human reproduction. *Am J Reprod Immunol*. 1998;39(2):144-151.
38. O'Malley BW. Steroid hormone receptors as transactivators of gene expression. *Breast Cancer Res Treat*. 1991;18(2):67-71.
39. Williams-Brown MY, Salih SM, Xu X, et al. The effect of tamoxifen and raloxifene on estrogen metabolism and endometrial cancer risk. *J Steroid Biochem Mol Biol*. 2011;126(3-5):78-86.
40. Kharode Y, Bodine PV, Miller CP, Lyttle CR, Komm BS. The pairing of a selective estrogen receptor modulator, bazedoxifene, with conjugated estrogens as a new paradigm for the treatment of menopausal symptoms and osteoporosis prevention. *Endocrinology*. 2008;149(12):6084-6091.
41. Archer DF, Lewis V, Carr BR, Olivier S, Pickar JH. Bazedoxifene/conjugated estrogens (BZA/CE): incidence of uterine bleeding in postmenopausal women. *Fertil Steril*. 2009;92(3):1039-1044.
42. Lass A, Weiser W, Munafo A, Loumaye E. Leukemia inhibitory factor in human reproduction. *Fertil Steril*. 2001;76(6):1091-1096.
43. Salamonsen LA, Menkhorst EED. Leukemia inhibitory factor and human endometrial receptivity. *Indian J Physiol Pharmacol*. 2010;54:17-26.
44. Kimber SJ. Leukaemia inhibitory factor in implantation and uterine biology. *Reproduction*. 2005;130(2):131-145.
45. Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. *Proc Natl Acad Sci U S A*. 1996;93(7):3115-3200.
46. Sawai K, Matsuzaki N, Okada T, et al. Human decidual cell biosynthesis of leukemia inhibitory factor: regulation by decidual cytokines and steroid hormones. *Biol Reprod*. 1997;56(5):1274-1280.
47. Barakat RR. The effect of tamoxifen on the endometrium. *Oncology (Williston Park)*. 1995;9(2):129-134.
48. Kedar RP, Bourne TH, Powles TJ, et al. Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. *Lancet*. 1994;343(8909):1318-1321.
49. Lahti E, Blanco G, Kauppila A, Apaja-Sarkkinen M, Taskinen PJ, Laatikainen T. Endometrial changes in postmenopausal breast cancer patients receiving tamoxifen. *Obstet Gynecol*. 1993;81(5 pt 1):660-664.
50. Allan GF, Hutchins A, Liu X, Clancy J. Induction of the progesterone receptor gene in estrogen target cells monitored by branched DNA signal amplification. *Steroids*. 2001;66(9):663-671.
51. Bramlett KS, Burris TP. Target specificity of selective estrogen receptor modulators within human endometrial cancer cells. *J Steroid Biochem Mol Biol*. 2003;86(1):27-34.
52. Troy PJ, Daftary GS, Bagot CN, Taylor HS. Transcriptional repression of peri-implantation EMX2 expression in mammalian reproduction by HOXA10. *Mol Cell Biol*. 2003;23(1):1-13.
53. Noonan FC, Mutch DG, Ann Mallon M, Goodfellow PJ. Characterization of the homeodomain gene EMX2: sequence conservation, expression analysis, and a search for mutations in endometrial cancers. *Genomics*. 2001;76(1-3):37-44.
54. Taylor HS, Fei X. Emx2 regulates mammalian reproduction by altering endometrial cell proliferation. *Mol Endocrinol*. 2005;19(11):2839-2846.
55. Peiffer-Schneider S, Noonan FC, Mutch DG, et al. Mapping an endometrial cancer tumor suppressor gene at 10q25 and development of a bacterial clone contig for the consensus deletion interval. *Genomics*. 1998;52(1):9-16.
56. Duggan ST, McKeage K. Bazedoxifene: a review of its use in the treatment of postmenopausal osteoporosis. *Drugs*. 2011;71(16):2193-2212.
57. Silverman S, Christiansen C. Individualizing osteoporosis therapy. *Osteoporos Int*. 2012;23(3):797-809.
58. Taylor HS. Using bazedoxifene plus conjugated estrogens for treating postmenopausal women: a comprehensive review. *Menopause*. 2012;19(4):479-485.
59. Archer DF, Pinkerton JV, Utian WH, et al. Bazedoxifene, a selective estrogen receptor modulator: effects on the endometrium, ovaries, and breast from a randomized controlled trial in osteoporotic postmenopausal women. *Menopause*. 2009;16(6):1109-1115.