Belgian Menopause Society  
Saturday, November 14, 2015

"New and alternative regimens for hot flushes."

UMC Sint-Pieter – CHU Saint-Pierre  
Rue Haute 326 Hoogstraat  
Bâtiment FORUM Gébouw  
1000 BRUXELLES

08.45  Coffee and registration

09.00  Chairs: Serge Rozenberg (ULB/VUB)

09.10  Non medical alternative treatment of vasomotor symptoms  
Raffaella Votino (UCL)

09.30  Non hormonal medical treatment of vasomotor symptoms  
Caroline Antoine (Brussels, UMC Saint-Pierre)

09.50  Cognitive behavior and vasomotor symptoms  
Eleonor Mann (United Kingdom)

10.20  Coffee break, visit of the Booths

10.50  New medication for menopausal symptoms:  
Conjugated Estrogens/Bazedoxifene (CE/BZA)*  
Chairs: Herman Depypere (UG)

10.55  What is a tissue selective estrogen complex (TSEO)? How does it work?  
Axelle Pintiaux (ULG)

11.15  Clinical overview of conjugated estrogens/bazedoxifene (CE/BZA)  
Serge Rozenberg (ULB/VUB)

11.35  Poster Prices

12.00  General discussion and Conclusion

* With an unrestricted grant of Pfizer

Price: free for members and assistants / 30€ for non members  
A request of accreditation has been made
Tissue-selective estrogen complexes for postmenopausal women

BMS
Satellite Symposium
14 nov 2015
A Pintiaux ULg
Hazard Ratios for Clinical Outcomes

Global index
CHD
Stroke
Venous Thromboembolism
Breast Cancer
Colorectal cancer
Hip fracture

Effects of CEE in Post Menopausal Women with Hysterectomy

- **CHD**: 0.91 (0.75 – 1.12)
- **BC**: 0.77 (0.59 – 1.01)
- **Stroke**: 1.39 (1.10 – 1.77)
- **PE**: 1.34 (0.87 – 2.06)
- **Colorectal Cancer**: 1.08 (0.75 – 1.55)
- **Hip Fracture**: 0.61 (0.41 – 0.91)

*JAMA* April 14, 2004
Progestin intolerance is one of the main factors for reduced compliance

• About 20% of women receiving progestin-containing HT have significant progestin intolerance, and half of these experience serious effects that prevent treatment continuation\(^1\)

• The 2013 British Menopause Society & Women’s Health Concern recommendations on hormone replacement therapy do recognise progestin intolerance as one of the main factors for reduced compliance with HT \(^2\)

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1. Panay N & Studd J. Human Reprod Update 1997;
2. Panay N et al. on behalf of the British Menopause Society and Women's Health Concern. Menopause Int 2013;
Unmet need with the treatment of postmenopausal symptoms

- Alternatives to progestin are needed that will protect the endometrium while avoiding other progestin-associated effects and preserving the desired effects of estrogens in postmenopausal women.¹³

**Most common adverse events leading to discontinuation are related to progestins¹,²:**

- Breakthrough bleeding
  - Increase in the number of uterine procedures (i.e., unnecessary endometrial biopsies)

- Breast pain/tenderness
  - Increase in the number of breast interventions

**Other progestin-related intolerance issues⁴:**

- Nausea
- Depressive mood
- Poor concentration
- Hirsutism
- Headache
- Dizziness
- Fluid retention
- Weight gain

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Medical conditions that may be exacerbated by treatment with progestins

History of the following conditions may make progestin inappropriate:

- Depression
- PMS*/PMDD**
- High breast density
- Diabetes and metabolic syndrome

*PMS: premenstrual syndrome
**PMD: premenstrual dysphoric disorder
TSEC

• The tissue selective estrogen complex (TSEC) = a selective estrogen receptor modulator (SERM) + one or more estrogens

• represents a new approach to menopausal therapy designed to improve tolerability
Duavee™
(conjugated estrogens/bazedoxifene)
tablets

0.45 mg/20 mg*

After opening foil pouch, product must be used within 60 days.
Dispense and store product in original package to protect from moisture.

7 tablets  Rx only

Package includes 1 blister card containing 7 tablets.
SERMs, such as raloxifene (RLX), lasofoxifene (LAS), and bazedoxifene (BZA), are compounds that exhibit tissue-selective estrogen receptor (ER) agonist and antagonist activities.
SERMs

• SERMs are compounds acting as an estrogen on some tissues (for example, bone tissue) and as an antiestrogen on other tissues (for example, breast, uterus), based on relative distribution of subtypes of ER (alpha and beta) and on ER-associated co-regulatory proteins (co-activators or co-repressors) activity depending on interested tissue.
ER Ligands: mechanism of action

Agonist/antagonist

Agonist

Antagonist

ERE

COACTIVATORS

COREPRESSORS

Pol II

Transcription activation

Stop transcription
The activation of different DNA sequences, the so-called Estrogen Response Elements (EREs), imply a different response, based on involved ligand (for example, estradiol, or a SERM, as BZA), also due to a different conformational changes of ligand-receptor complex, with a differentiated, subsequent, and tissue-selective agonist or antagonist effect.
The overall conformation with an antagonist could favour the recruitment of co-repressors and resemble misfolded conformation of ERα, thus promoting the degradation of the protein.

The antagonist action of tamoxifen is mainly attributed to its effect on Helix 12 and is highly dependent on absolute and relative level of co-repressor and co-activator proteins expressed in particular tissues.
Figure 6.
Predicted “non-classical” co-activator binding pocket using QSiteFinder. Pocket volume is rendered as red. A. Antagonist ERα structure in presence of bound 4-OHT (starting structure of MD simulation). B. Agonist like ERα conformation in presence of bound 4-OHT observed ~ 7 ns of MD simulation.
Bone

A

Antagonist

Fulvestrant

Agonist

Reference estrogen (17βestradiol, ethinyl estradiol)

Bazedoxifene

Raloxifene

Lasofoxifene

Tamoxifen

Ospemifene

B

Antagonist

Fulvestrant

Raloxifene, Ospemifene

Agonist

Reference estrogen (17βestradiol, ethinyl estradiol)

Bazedoxifene

C

Antagonist

Fulvestrant

Tamoxifen

Lasofoxifene

Ospemifene

Razedoxifene

Agonist

Reference estrogen (17βestradiol, ethinyl estradiol)

Fig. 1. Activity of SERMs in different target tissues. (A) Bone [4–8]; (B) endometrium [4–6, 8, 9]; and (C) breast [4, 9–13].

Adapted with permission of Informa Healthcare [3].
## Impacts of tamoxifen on metabolism.

<table>
<thead>
<tr>
<th>Results</th>
<th>Study subjects</th>
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</thead>
<tbody>
<tr>
<td><strong>Tamoxifen</strong></td>
<td><strong>Diabetes</strong></td>
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<td><strong>Fasting blood glucose</strong></td>
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<td><strong>Insulin sensitivity</strong></td>
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<tr>
<td><strong>Lipid profile</strong></td>
<td><strong>Hypertriglyceridemia</strong> [20–23]</td>
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<tr>
<td><strong>Steatosis</strong></td>
<td>Increased incidence of fatty liver [24–28]</td>
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<td>Elevated liver triglyceride level due to increased synthesis of triglyceride [29]</td>
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<td>Increased de novo fatty acid synthesis through decreased AMPK kinase activity [30]</td>
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<td>Impaired fatty acid β-oxidation and hepatic triglyceride accumulation [31]</td>
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<td>Increased intracellular triglyceride concentration [32]</td>
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<td></td>
<td>No effect on steatosis in the absence of high fat diet [33]</td>
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<td>Diminished inflammatory responses [34]</td>
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<td></td>
<td>Upregulated hepatic Mmrd2 mRNA [35]</td>
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<td>Upregulated expression of two fatty acid β-oxidation key regulators in the liver, Lcn13 and PPARγ [36]</td>
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<tr>
<td><strong>Weight gain and obesity</strong></td>
<td>Decreased food intake, body weight and body fat content [37]</td>
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<td>Suppressed weight gain although it was not achieved only via suppression of food intake [38]</td>
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<td>Higher serum leptin level [40]</td>
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<td>Higher serum leptin level in patients who developed fatty liver during short-term (3 month) tamoxifen treatment than those without fatty liver [41]</td>
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<td>Lower weight gain in obese women (free of cancer) [42]</td>
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<tr>
<td></td>
<td>Lower weight gain and decreased food intake in rats receiving tamoxifen [42]</td>
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</tbody>
</table>
### Impacts of raloxifene on metabolism.

<table>
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<th><strong>Results</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Raloxifene</strong></td>
<td><strong>Ovariectomized rat</strong></td>
</tr>
<tr>
<td><strong>Body weight and fat mass</strong></td>
<td><strong>Inhibited weight gain caused by estrogen deficiency [44,45]</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Prevented body weight gain and abdominal adiposity: fat distribution pattern shifted from android fat distribution to gynoid fat distribution [46]</strong></td>
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<td></td>
<td><strong>No effect on body weight, but increased fat-free mass and total body water [47]</strong></td>
</tr>
<tr>
<td></td>
<td><strong>No effect on exercise-induced weight loss and fat-mass loss [48]</strong></td>
</tr>
</tbody>
</table>

**Fasting blood glucose** | **Unchanged after 3-month, 6-month [49–52] or 12-month treatment [53]** | **Postmenopausal women without T2D [49,50,53]** |

**Fasting insulin level** | **Unchanged after 3-month or 6-month treatment [49,50,52]** | **Postmenopausal women without [49–51] or with T2D [52]** |

**Glucose tolerance** | **No effect after 3-month, 6-month [49,50] or 12-month treatment [53]** | **Postmenopausal women without [49–51] or with T2D [52]** |

**Insulin sensitivity** | **Unchanged after 3-month or 6-month treatment [50–52], but in a subgroup of subjects with hyperinsulinemia, plasma insulin were significantly reduced [49]** | **Postmenopausal women without T2D** |
| | **Decreased insulin sensitivity after 12-month treatment [53]** | **postmenopausal women** |

**Lipid profile** | **Downregulated LDL cholesterol level [50,51,53,55]** | **Postmenopausal women** |
| | **Unchanged HDL cholesterol level [50,51]** | **Postmenopausal women** |
| | **Unchanged triglyceride level [32,50,51,56,57]** | **HepG2 cells [32]** |
| | | **INS-1 cells [58]** |

**Leptin level** | **Prevented triglyceride accumulation under lipogenic conditions** | **Ovariectomized rats** |
| | **Reversed estrogen deficiency-induced hyperleptinemia [45]** | **Postmenopausal women** |
| | **Increased serum leptin level compared to the basal level [59,60]** | **Postmenopausal women** |
| | **Unchanged serum leptin levels compared to the basal level, although the value was significantly lower compared to the subjects receiving no treatment [61]** | **Postmenopausal women undergoing oophorectomy surgery** |
| | **Serum leptin level were maintained as low as basal level [62]** | |
TSEC concept

• An optimal TSEC would combine the desired ER agonist activities of estrogens (on vasomotor symptoms, vulvar/vaginal atrophy, and bone) with the tissue selectivity of a SERM (specific ER neutral or antagonist activity in endometrium and breast)
BZA alone

• has been shown to prevent postmenopausal osteoporosis
• reduce fracture risk in postmenopausal women with osteoporosis
• bazedoxifene is already approved as a monotherapy for osteoporosis
• potent antagonistic effect in uterine tissue
Bazedoxifene acetate (BZA) is a third-generation Selective Estrogen Receptor Modulator (SERM). Its molecule was developed based on raloxifene’s model, replacing benzothiophenic core with an indolic ring. BZA can interact with estrogen receptors (ERs) alpha and beta, in all tissues that contains them, as bone, breast, uterus, vessels, and liver; The binding affinity is slightly greater for ER-alpha versus ER-beta. Mean half-life is 28 hours and maximum concentration (Cmax) is reached in 1–2 hours after oral administration; BZA is mainly excreted by feces (84.7 %), while renal excretion is negligible. The main metabolic pathway is glucurono-conjugation; steady-state is reached after about 2 weeks of oral administration.


Ethun KF, Wood CE, Cline JM, Register TC, Appt SE, Clarkson TB. Endometrial profile of bazedoxifene acetate alone and in combination with conjugated equine estrogens in a primate model. Menopause 2013.


BZA+ CE: decrease in ER α expression and activity;
BZA+CE reduced CE induced epithelial density, ductal proliferation

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**Preclinical studies of BZA/CE in breast tissue.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Song 2012 [20]</td>
<td>MCF-7 breast cancer cells</td>
<td>• BZA blocked CE-induced stimulatory effects, including DNA synthesis, reduction of apoptosis, and expression of cMyc, pS2, and WNT1 inducible signaling pathway protein 2</td>
</tr>
<tr>
<td>Wardell 2012 [21]</td>
<td>MCF-7 breast cancer cells</td>
<td>• BZA displayed inverse agonist activity on many estradiol-regulated genes</td>
</tr>
<tr>
<td>Chang 2010 [65]</td>
<td>MCF-7 breast cancer cells and microarray studies</td>
<td>• BZA, RLX, and LAS inhibited CE-induced breast cancer cell proliferation, with an order of antagonism of BZA &gt; RLX &gt; LAS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• BZA inhibited a group of genes regulated by CE; this profile is different from those of RLX and LAS</td>
</tr>
<tr>
<td>Peano 2009 [18]</td>
<td>Ovariectomized mice</td>
<td>• Stimulatory effects of CE on mammary gland amphiregulin expression (a marker of ductal proliferation) was antagonized by BZA &gt; RLX &gt; LAS</td>
</tr>
<tr>
<td>Song 2012 [19]</td>
<td>Ovariectomized mice</td>
<td>• BZA was more effective than RLX and LAS at reducing ductal branching</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• BZA blocked CE-induced gene expression and mammary gland ductal growth and terminal end bud growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• BZA blocked estrogen-stimulated tumor growth and gene expression in mice with MCF-7 xenografts</td>
</tr>
<tr>
<td>Ethun 2012 [22]</td>
<td>Ovariectomized cynomolgus macaques</td>
<td>• BZA/CE for 6 months significantly reduced CE-induced increases in epithelial density, lobular enlargement, and ductal proliferation (all P &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• BZA/CE treatment resulted in a decrease in ERα protein expression and a reduction in markers of ERα activity</td>
</tr>
</tbody>
</table>

BZA, bazedoxifene; CE, conjugated estrogens; MCF-7, Michigan Cancer Foundation-7; DNA, deoxyribonucleic acid; RLX, raloxifene; LAS, lasofoxifene; ER, estrogen receptor.

_S. Mirkin, B.S. Komm / Maturitas (2013)_
BZA antagonized CE induced increases in uterine weight, and proliferation

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<tr>
<td>Peano 2009</td>
<td>Ovariectomized mice</td>
<td>• BZA, but not RLX or LAS, antagonized CE-induced increases in uterine wet weight to levels similar to vehicle control</td>
</tr>
<tr>
<td>Oliva 2012</td>
<td>Ovariectomized MITO-luc mice</td>
<td>• BZA paired with CE resulted in complete inhibition of the proliferative effects of CE in the uterus</td>
</tr>
<tr>
<td>Kharode 2008</td>
<td>Ovariectomized rats</td>
<td>• BZA paired with CE blocked CE-induced uterine stimulation</td>
</tr>
<tr>
<td>Komm 2011</td>
<td>Ovariectomized rats</td>
<td>• BZA paired with CE reduced CE-induced increases in uterine wet weight in a dose-dependent manner</td>
</tr>
<tr>
<td>Ethun 2013</td>
<td>Ovariectomized cynomolgus monkeys</td>
<td>• Endometrial epithelial area and proliferation were significantly reduced with BZA/CE compared with CE alone ($P&lt;0.001$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Rates of endometrial hyperplasia with BZA/CE were comparable to controls</td>
</tr>
</tbody>
</table>

BZA, bazedoxifene; CE, conjugated estrogens; RLX, raloxifene; LAS, lasofoxifene.

S. Mirkin, B.S. Komm / Maturitas (2013)
Fig. 2. Total breast epithelial density in ovariectomized cynomolgus macaques treated with BZA, CEE, BZA + CEE, or no treatment for 6 months [22]. Reproduced from Ethun KF, Wood CE, Register TC, Cline JM, Appt SE, Clarkson TB. Effects of bazedoxifene acetate with and without conjugated equine estrogens on the breast of postmenopausal monkeys. Menopause 2012;19(11):1242–52, with permission from the publisher. Mean percent (95% CI) total breast epithelial density (evaluated in hematoxylin and eosin-stained breast biopsy tissues) plotted for each group. Treatment groups not connected by the same letter (a, b) are significantly different (P<0.05 by ANOVA). BZA, bazedoxifene; CEE, conjugated equine estrogens; CI, confidence interval; ANOVA, analysis of variance.
Protective effects of bazedoxifene paired with conjugated estrogens on pancreatic β-cell dysfunction.

- *In vivo* administration of BZA, alone or paired with CE, reduced hyperglycemia and diabetic incidence and improved glucose tolerance, without causing uterine hypertrophy in streptozotocin-treated OVX mice.
- Serum insulin/glucose ratio significantly increased in the BZA/CE combination group compared to the OVX vehicle, although there were no changes in pancreatic insulin content.
- BZA could enhance the protective effects of estrogen on β-cell survival, potentially mediated via an ERE-independent mechanism suggesting a novel therapeutic application of tissue-selective estrogen complex with BZA.

Metabolic benefits of BZA alone were lost in ER-α knockout mice, indicating that BZA exhibits estrogen-mimetic action in metabolic regulation.

*Kim JH, Mol. Metab., 3, 177-190 (2014).*
CONCLUSIONS

– A new approach for menopausal women
– Complex and non completely elucidated mechanism of TSEC
– Preclinical studies : reassuring, hopeful
– But ...mice are not women : clinical studies, the SMARTS