**Femarelle® Introduction**

With the gradually increasing life expectancies of the world's populations, the populations of menopausal women are growing dramatically. As a result, most women will live approximately one-third of their life span after menopause. This fact highlights the importance of menopause management that provides patients with better health and a higher quality of life.

The burden on physicians who care for this population has become significantly more complicated, because women not only live longer, but in modern societies they also remain active for much of their older life.

With individuals now living longer, qualitative changes in their lives become much more relevant. This means that healthcare providers and physicians who treat peri- and post-menopausal women have to cope with the challenging concept of healthy aging.

The uniqueness of Femarelle® derives from its origin as a plant-based SERM (Selective Estrogen receptor Modulator) that targets specific tissue sites, while having no effect on others.

Femarelle® relieves menopausal symptoms both in the short and long term and increases bone mineral density, while having no effect on the uterus or on breast tissue. This tissue-selective mechanism of activity has been demonstrated and confirmed in clinical and pre-clinical studies.

Femarelle® represents the new generation of treatment for menopause management, providing a safe and effective solution for the relief of vasomotor symptoms, vulvo-vaginal atrophy and bone health.

**Scientific Review of Femarelle® Published Studies**

**Clinical study: menopausal symptoms**

The effects of Femarelle® were assessed on a wide variety of menopause-related symptoms. In a clinical trial, healthy post-menopausal women ($n=80$) were randomly allocated to receive either the standard dose (SD, 644 mg/day) or a low dose (LD, 344 mg/day) of Femarelle®. A detailed medical history was taken from each participant upon enrollment, and this was followed by a physical examination, pelvic ultrasound, and recording of sex hormone and lipid profiles. Each patient completed a detailed questionnaire comprising a Kupperman index plus six additional parameters. The hormonal blood profile, endometrial thickness, and breast tissue in all patients were monitored. These procedures were repeated every 3 months during the 12 months of the study.

**Results:**

Both groups achieved a significant reduction in menopausal symptoms, which was sustained throughout the 12 months of treatment.

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There were no changes in mean levels of thyroid-stimulating hormone or in the sex hormones estradiol (E$_2$) or follicle-stimulating hormone, nor were any changes perceived in mean endometrial thickness.

**Safety of Femarelle® Treatment**

### Effect of Femarelle on Endometrial Thickness

<table>
<thead>
<tr>
<th></th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femarelle</td>
<td>3.22</td>
<td>2.42</td>
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### Effect of Femarelle on TSH Levels

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<tr>
<td>Femarelle</td>
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### Effect of Femarelle on Serum E$_2$

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Femarelle</td>
<td>40.00</td>
<td>40.47</td>
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</tbody>
</table>

### Effect of Femarelle on Serum FSH

<table>
<thead>
<tr>
<th></th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
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</thead>
<tbody>
<tr>
<td>Femarelle</td>
<td>73.66</td>
<td>65.59</td>
</tr>
</tbody>
</table>

**Conclusion:**

Femarelle® significantly alleviated menopausal symptoms in about 75% of the patients without affecting endometrial thickness or hormonal blood profiles. The lack of change in hormonal levels showed that the body does not recognize Femarelle® as estrogen, despite the fact that it affects estrogen receptors in designated sites.

**Clinical study: menopausal symptoms$^2$**

Hormone therapy is the treatment of choice for the alleviation of menopausal symptoms; concerns, however, about its concomitant long term health risks have limited its use. DT56a (Femarelle) is a unique enzymatic isolate of soybeans. The purpose of our study was to evaluate the efficacy and safety of DT56a, compared to hormone therapy (HT), in symptomatic postmenopausal women.

89 postmenopausal women were studied prospectively. Women with climacteric symptoms were randomly assigned to receive either DT56a (n=27) or oral low dose continuous combined HT (17β-estradiol 1 mg plus norethisterone acetate 0.5mg, Activelle, Novo-

$^2$ Labos G., Trakakis E. et al Efficacy and safety of DT56a (Femarelle) compared to hormone therapy in Greek postmenopausal women. *J Endocrinol. Invest.* 2013; e-pub ahead of print.
Nordisk, Copenhagen, Denmark) (n=26). Symptomatic women not wishing to receive any treatment served as controls (n=36). Menopausal symptoms as assessed through the Kupperman index, serum lipids and lipoproteins, calcium, as well as bone mineral density, endometrial thickness, and mammography were assessed at baseline and at 12 months.

Results:
Patients receiving HT and DT56a showed a significant and independent decrease in menopausal symptoms (mean difference in Kupperman score, DT56a group: -3.98, HT group -5.601, no treatment group +1.76, p-value < 0.001). Lumbar spine bone mineral density T-score was significantly lower in women receiving no treatment, as opposed to the two treatment arms which showed no significant change (No treatment, baseline: -0.85, final: -0.85, p=0.001; HT, baseline:-84, final -0.99, p=0.79; DT56a, baseline -0.51, final:-0.76, p=0.75). No differences in femoral bone density, endometrial thickness or mammography classification were detected in any of the treatment arms. Likewise, serum lipids or lipoproteins did not differ between the three groups.

Conclusions:
DT56a decreased menopausal symptoms significantly and in the same degree as HT.

Pre-clinical mechanism of action: brain region responsiveness to Femarelle

It has been well established that certain neurotransmitters have a positive effect on various vasomotor symptoms. Allopregnanolone reduces anxiety and has the ability to create calm and beta-Endorphins act as an analgesic in the body, numbing or dulling pain. Beta-Endorphins also increase relaxation and promote overall well-being. Levels of Allopregnanolone and beta-Endorphins decrease in ovariectomized (OVX) rats, and in postmenopausal women. Estrogen therapy is well known for its beneficial effect on brain related vasomotor symptoms, such as hot flushes, sleep disturbances and mood changes. Estrogen has also been found to enhance allopregnanolone and improves beta-Endorphin activity in the brain. Based on this previous research, Femarelle® was examined to determine if it would have similar effects on these neurotransmitters as did estrogen.

Five groups of ovariectomized (OVX) rats received one of the following treatments for 14 days:
Femarelle® at a dose of 6 mg/kg/day, 12 mg/kg/day, 60 mg/kg/day, 120 mg/kg/day (the equivalent to the recommended human dosage); E2 at a dose of 0.05 mg/kg/day; 2 control groups, one consisting of young rats, and the second one, OVX rats. Allopregnanolone concentration was assessed in the frontal and parietal lobes, hippocampus, hypothalamus, anterior pituitary and serum. The beta-endorphin content was evaluated in the frontal and parietal lobes, hippocampus, hypothalamus, neurointermediate lobe, anterior pituitary and plasma.

Results:
Femarelle® increased Allopregnanolone levels (compared to placebo) in all tested areas of the brain of OVX rats showing a classical dose-range curve, with the optimal effect at the dose of 120mg/kg/day. In some brain areas, levels of Allopregnanolone attained were comparable to those of ovariectomized rats treated with E2. Femarelle® was also found to increase beta-Endorphin levels in selected areas of the brains of OVX rats. These areas

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included the hypothalamus, hippocampus, anterior pituitary and the neurointermediate lobe. Treatment with Femarelle® raised beta-Endorphin levels in these areas within the range of those rats treated with E₂.

Conclusion:
This study demonstrated that Femarelle® positively affects brain neurosteroidogenesis and the opiatergic system: Femarelle® exerts an estrogen-like effect on selective areas related to mood, cognition and homeostasis control, presenting a specific pattern of interaction with the brain function. These findings may, in part, explain the clinical effect of Femarelle® on menopausal symptoms.

Post Marketing Study: Menopausal Symptoms

A Post Marketing Menopausal Symptoms International Survey (POMMSIS) was launched in 5 countries: India, Norway, Lithuania, Spain and Sweden. These countries represent 3 races: Indian, North-European and Mediterranean-European.

2,022 women participated in this survey, the mean age of menopause was 51 (SD=4.97). The survey monitored selected menopausal symptoms- hot flushes, quality of sleep, quality of life, night sweats, headaches and joint and muscle pains before and following 4 weeks of treatment with Femarelle.

A detailed questionnaire regarding the main menopausal symptoms was filled at entry of the study and following 4 weeks of treatment. A daily hot flushes diary looking at the number and intensity of hot flushes was filled for 5 weeks, one week without treatment as baseline followed by 4 weeks of treatment.

Results:
In this survey hot flushes and night sweats were the prominent symptoms in all 5 countries. Cultural differences could be seen when looking at smoking habits, weight and even how hot flushes were perceived in different countries. European women were significantly heavier than Indian women and smoking was in higher prevalence in Europe. The perception of hot flushes was different in warmer climates, such as Spain and India than in colder climates, such as Lithuania and Norway. And the percentage of natural vs. surgical induced menopause was also seen to differ between India and Europe.

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4 Genazzani A., Nachtigall L., Panay N. & Yoles I. Symposium: 2 Continents, 3 cultures, 4 countries, 2,000 women and Femarelle®; 13th World Congress on Menopause. Rome, Italy June 2011
Femarelle® was found to significantly relieve menopausal symptoms within the first two weeks of treatment and this trend continued following 4 weeks of treatment in all countries surveyed. A statistically significant reduction was found at each week of treatment.

**Hot Flushes at Baseline & Following 4 Weeks of Treatment with Femarelle®**

![Graph showing the percentage of women experiencing hot flushes at different levels before and after treatment with Femarelle®. Pre-treatment (mean 6.17 HF/day; SD=4.38) and post-treatment (mean 2.88 HF/day; SD=3.23).]

Overall 84.4% reported their hot flushes were better or much better after 4 weeks of treatment with Femarelle®.

**Relief of symptoms following 4 weeks of treatment with Femarelle® (N=1,833)**

![Graph showing the percentage of women experiencing relief of symptoms after 4 weeks of treatment with Femarelle®.]

Femarelle® had the highest effect on severe symptoms (7+ HF/day) with 92.2% experiencing a reduction of hot flushes after 4 weeks of treatment.

**Numerical Reduction of Hot Flushes 7+ hot flushes at baseline (n=577)**

![Graph showing the numerical reduction of hot flushes at baseline with Femarelle®.]

-5 or more: 65%
-4: 11.3%
-3: 6.6%
-2: 5.3%
-1: 3.8%
No change: 4.7%
Worse: 3%
Conclusion:
Menopausal bothersome symptoms were found to be a cross cultural phenomenon although symptoms may vary in different countries. Treatment with Femarelle resulted in a significant improvement in all symptoms within the month of treatment. Interestingly, the beneficial effect of Femarelle was perceived as beneficial even beyond the improvement of the specific symptoms.

Clinical Study: vaginal dryness

Vaginal atrophy is due to low estrogen and affects 10-40% of post-menopausal women. It may manifest as vaginal soreness, dryness, pruritus, dyspareunia, or bleeding on contact. Unlike symptoms such as hot flushes, vaginal atrophy does not diminish over time and often progresses in severity.

To investigate the effect of Femarelle® on vaginal atrophy, 12 post-menopausal women with vaginal atrophy (<5% superficial cells on cervical cytology) with at least one moderate-to-severe symptom (dryness-2, irritation-1, soreness-3, dysuria-0, Dyspareunia-5, or bleeding with coitus-1) were recruited for a 12-week open-label pilot study. Femarelle capsules were given 2X/day for 12 weeks. At each visit (0, 4 and 12 weeks) subjects had a vaginal atrophy assessment (speculum exam, maturation index, vaginal pH) and completed questionnaires on atrophy symptoms and quality of life (Utian Quality of Life- UQoL). At weeks 0 and 12, physical exams, pap smears, vaginal cultures, and blood work were also performed.

Results:
All the 12 patients, who were extremely atrophic, reported a significant improvement in their most bothersome symptom. In all women a significant reduction in vaginal pH was measured from baseline of 7.7 ± 2.2 at baseline to 4.9 ± 1.4 at week 12 (p< 0.0001).

Reduction in Vaginal pH, following 12 weeks of treatment with Femarelle

A significant improvement was also found in the maturation index. At enrolment showed an average predominance of Parabasal cells (about 85%), some intermediate cells (15%) and no superficial cells. Following 12 weeks of treatment there was a significant shift in cell type distribution: about 51% were Parabasal, 46% intermediate and about 3% were superficial cells. This trend continued and was significantly increased at 6 months.

A significant improvement was found in UQoL index from mean pre-treatment of 75.4± 22.7 points to mean post-treatment of 88.9± 26.8 (p< 0.001). In the sexual domains of the UQoL

there was a significant improvement from 6.5 ± 2 points (mean pre-treatment) to 10.6± 3.2 (mean post-treatment) p< 0.001.

**Conclusion:**
Femarelle® is a good candidate for the long term use during and after menopause; it provides both vasomotor symptom relief for the early menopausal women and relief of vaginal atrophy for the post menopausal women, even for very atrophic conditions.

**Clinical study: bone health**

The effect of Femarelle® was assessed on human bone through dual-energy x-ray absorptiometry (DEXA) scan of the hip and the spine.

In a clinical trial, healthy post-menopausal women (n=98) were randomly allocated, on a double-blind basis, to receive either the recommended dosage of Femarelle® (644 mg/day) or a low dosage of DT56a (344 mg/day), supplemented with calcium, for twelve months. A comprehensive health questionnaire, and physical, laboratory and pelvic sonography examinations were performed at the start of the trial and every 3 months thereafter. Bone mineral density (BMD) was assessed by DEXA of the lumbar spine and femoral neck at enrollment and after twelve months of treatment.

**Results**

Following twelve months of treatment, BMD in the Femarelle® group was increased by 3.6% in the lumbar spine and by 2.0% in the femoral neck. In the low-dose group, BMD was decreased by 0.6% both in the femoral neck and in the lumbar spine.

![Effect of Femarelle® on BMD following 12 months of treatment](image)

Neither group showed any change in endometrial thickness or in hormonal levels (FSH and E$_2$). There were no reported side effects.

**Conclusion**

Femarelle® selectively affects estrogen receptors in the bone, increasing BMD without affecting the uterus. Since the hormonal blood profile was left unchanged this means that although the estrogen receptors were affected in target tissues, the body does not perceive Femarelle® as estrogen.

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Pre-clinical study: mechanism of action in skeletal tissues and uterus

An in-vivo study in rats investigated the mechanism of action of Femarelle®, on bone buildup and its safety in the uterus.

The study measured Creatine Kinase (CK) activity, an assay of estrogenic response, in the skeletal tissues and in the uterus. The activity of CK in bone tissue serves as an indirect marker of estrogen participation in the processes of cell growth and bone formation, and a direct indicator of mediation of these processes through estrogen receptors. In this study measurement of the specific activity of CK was used to compare the effects of Femarelle®, 17β-estradiol (E₂), and a vehicle (control) on bone and cartilage in non-ovariectomized (non-OVX) and ovariectomized (OVX) female rats.

OVX rats were fed by placebo (control), E₂ or Femarelle®, and CK activity was measured in the epiphyseal cartilage and the diaphyseal bone (the tissue-building sites in the bone) as well as in the uterus. Raloxifene, which acts as an estrogen receptor blocker, was added in order to determine whether the mechanism of action of Femarelle® is through estrogen receptors.

Results:
As early as after several days of treatment with Femarelle®, an increase in CK activity was observed in both the diaphysis and the epiphysis of the.

Effects of treatment with Femarelle®, E₂ and a vehicle (control) on creatine kinase activity

Contrary to estrogen Femarelle® did not induce any effect on the uterus.

The effect on bone growth was lost when the estrogen receptors were blocked with Raloxifene.

7 Somjen D, Yoles I. DT56a (Tofupill/Femarelle), selectively stimulates creatine kinase specific activity in skeletal tissues of rats but not in the uterus. J. of Steroid Biochemistry & Molecular Biology 2003; 86(1):93-98
Effect after blockage of estrogen receptors with raloxifene

Conclusion:
Relative to the control, both Femarelle® and E₂ significantly stimulated bone structure. However, whereas E₂ stimulates estrogen receptors in the uterus, Femarelle® does not. The raloxifene-induced inhibition of both E₂ and Femarelle® activity points to their common mechanism of action through estrogen receptors. These findings identify Femarelle® as a SERM, capable of selectively activating bone formation via estrogen receptors without inducing any estrogenic activity in the uterus.

Pre-clinical study: histology of skeletal tissues

Histological techniques and histomorphological measurements, by enabling direct observation of the tissues, yield highly accurate data on the effects of a product on bone. We compared the effects of long-term daily treatment with Femarelle® (DT56a), E₂, and placebo on the skeletal histology of ovariectomized (OVX) and non-OVX rats in order to study the dynamics of their effects on the rebuilding process in different parts of the bone.

Thirty rats were divided into four groups. Rats in the control group were left with intact ovaries and those in the other groups underwent ovariectomy. The OVX rats were treated for 2 months with Femarelle®, E₂, or placebo (control). Histomorphometry of the trabecular bone volume (TBV) in each rat (expressed as a percentage of the total bone volume), as well as cortical thickness and growth plate width, were recorded by a computerized system. In addition, CK-specific activity, a marker of estrogen receptor activation, was analyzed in the skeletal tissues and in the uterus.

Results:
The OVX rats showed substantial losses of TBV, cortical thickness and growth plate width, with the result that they were markedly osteoporotic relative to non-OVX rats.

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Sample histology slides showing the effects of treatment with Femarelle®, E₂ and a vehicle on bone

Treatment with Femarelle® and with E₂ prevented the bone loss following OVX, maintaining all parameters to non-OVX control levels.

The effects of E₂ and Femarelle® on bone histomorphological parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Trabecular bone volume (%)</th>
<th>Cortical bone width (μm)</th>
<th>Growth plate width (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Intact (control)</td>
<td>62±1.5</td>
<td>458±13</td>
<td>177±6</td>
</tr>
<tr>
<td>b. OVX (control)</td>
<td>43±2</td>
<td>363±15</td>
<td>117±1</td>
</tr>
<tr>
<td>c. E₂-treated</td>
<td>57±4*</td>
<td>472±24*</td>
<td>157±4*</td>
</tr>
<tr>
<td>d. Femarelle®-treated</td>
<td>53±3*</td>
<td>502±16*</td>
<td>176±5*</td>
</tr>
</tbody>
</table>

* P< 0.01

A significant increase in CK activity was found following E₂ and Femarelle® treatment in the skeletal tissues (Epiphysis and Diaphysis). However, while E₂ produced significant CK activation in the uterus, Femarelle® had no stimulatory effect in that site.

Conclusion:
Since peak bone mass in women is reached around the age of 28, comparing the bone structure of a young rat to that of an OVX one following treatment with Femarelle® provides the best indication regarding the properties of Femarelle® on bone build-up and bone health preservation.
This study serves as further proof of the beneficial effect of Femarelle® on the bone without affecting the uterus.

Pre-clinical testing: mechanism of action in human bone- osteoblast activation

The products in current use for the treatment of osteoporosis are anti-resorptive compounds, which slow osteoclast activity. New bone tissue, however, is formed through the stimulation of osteoblast activity.

To investigate the mechanism of action of Femarelle® (DT56a) in promoting bone mineral density, osteoblast activation was examined in human bone cultures of pre- and post menopause females by the use of three markers:

- **DNA synthesis**: a marker of increased activity of osteoblasts through cell proliferation;
- **Alkaline phosphatase (ALP)**: a marker of osteoblast activation;
- **CK activity**: a marker of estrogen receptor activation, which induces osteoblast activity.

Bone cells from pre- and post-menopausal women were prepared from bone explants by a non-enzymatic method. DNA synthesis, CK stimulation and alkaline phosphatase specific activity were assessed in human bone cells treated either with E₂ or with Femarelle®. In order to examine the mechanism of action of Femarelle®, the estrogen-receptor inhibitor raloxifene was added in order to allow examination of the effect of Femarelle® after blocking of the estrogen receptors.

**Results:**
Femarelle® stimulated CK activity, DNA synthesis and ALP in both in pre- and post menopause female human bone cells; the optimal dose was seen at equivalent dosage of 2 capsules of Femarelle/day. Raloxifene inhibited Femarelle’s effect in both groups, proving that Femarelle® works through estrogen receptors.

**Activation of osteoblasts in human bone tissue culture**

**Conclusion:**
Femarelle® stimulates osteoblast activity, indicating that, through its bone-forming properties, Femarelle® is a unique agent for prevention of post-menopausal bone loss.
Pre-Clinical Study: Mechanism of Action in Bone Marrow Content

Osteoporosis is associated with atrophy of the spongy bone due to reduction in bone formation, thus affecting bone strength to the extent that fractures occur after minimal trauma. At menopause, an accelerated loss of bone mass (3%/yr) takes place during the first five years, along with an increased volume of bone marrow adipocytes.

Previous histomorphometric studies showed an association between osteopenia and increased adipose tissue in bone marrow with aging. Such a phenomenon was observed also in OVX rats.

In humans, osteoporosis and age related osteopenia were shown to be associated with an increase in marrow fat tissue. It was shown that the number of osteoblasts were negatively correlated with the numbers of adipocytes, suggesting that adipocytes were generated at the expense of osteoblasts. Following ovariectomy, bone volume in rat metaphysis decreased and the space is filled with hemopoietic and adipose tissue. Marrow fat content increases with time after ovariectomy, with a reciprocal relationship between marrow fat content and bone formation rate.

The aim of the present study was to evaluate the effects of Femarelle DT56a) different estrogenic compounds as well as vitamin D less-calcemic analogs on bone marrow adipocytes volume (%MAV) in bone marrow of long bones from OVX female rats.

OVX rats were injected with treatments known to affect bone formation, 5 days per week for 2.5 month for analysis of fat content in bone marrow.

Results:
In OVX young adults there is a decreased bone formation and a 10 folds increase in fat cells content in bone marrow. Treatment with Femarelle (DT56a), E2 and raloxifene (RAL) resulted in abolishment of fat cells content. (Table 2)

These changes in fat cells content are inversely correlated with changes in bone formation.

The effects of Femarelle® and different compounds on fat volume in bone marrow

<table>
<thead>
<tr>
<th></th>
<th>% of fat volume in bone marrow (% FV±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Intact)</td>
<td>0.28 ± 0.17</td>
</tr>
<tr>
<td>OVX (Control)</td>
<td>27.10 ± 1.80**</td>
</tr>
<tr>
<td>OVX + E2</td>
<td>0.03 ± 0.002**</td>
</tr>
<tr>
<td>OVX + Femarelle (DT56a)</td>
<td>0.00-0.00**</td>
</tr>
<tr>
<td>OVX + RAL</td>
<td>0.20 ± 0.02**</td>
</tr>
<tr>
<td>OVX + Daidzein</td>
<td>5.37 ± 0.30*</td>
</tr>
<tr>
<td>OVX + Genistein</td>
<td>23.00 ± 2.10</td>
</tr>
</tbody>
</table>

* P <0.05, ** P < 0.01 from the corresponding mean values of OVX control rats.

Conclusion:
The results demonstrate that adipogenesis caused by ovariectomy is a reversible process, which can be corrected and may rejuvenate bone marrow by the treatment with Femarelle® (DT56a).

The awareness of a relationship between fat and bone at the marrow level provide a better understanding of the pathophysiology of bone-loss as well as a novel approach to diagnosis and treatment of post-menopausal osteoporosis.

Somjen D. et al. The Effects of Native and Synthetic Estrogenic Compounds as well as Vitamin D Less-Calcemic Analogs on Adipocytes Content in Rat Bone Marrow. J Endocrinol Invest. 2011;34(2):106-10
Pre-clinical safety testing: human breast cancer tissue

Estrogen-positive tumors account for 70% of all breast cancers. A common model to examine the estrogentic activity of compounds is through MCF-7 breast cancer cell line. MCF-7 are estrogen dependent breast cancer cells.

Using this cell line, a study was carried out to determine whether Femarelle® (DT56a) has any estrogenic effect on the breast tissue. MCF-7 cells were seeded and incubated for 96 hours in estrogen-depleted medium, and were then incubated with Femarelle® or E₂ for 72 hours. The pharmacological dose of E₂ was added to the cultured cells. Femarelle® was added in incremental doses, starting with the calculated pharmacological dose and increasing to 66,000 times the pharmacological dose.

Results:
Unlike E₂, Femarelle® did not stimulate estrogen receptors in the breast tissue, and therefore did not induce proliferation of cancer cells.

Effect of Femarelle® on growth of MCF-7 breast cancer cells

Conclusion:
Femarelle® does not trigger breast cancer cell growth. This finding further supports the accumulating data verifying the activity of Femarelle® as a SERM.²⁵

Clinical study: blood clotting¹¹

HT & ET increases clotting which can lead to venous thromboembolism (VTE) and stroke and thus cannot be given to women with shortened clotting time. The frequency of inherited Factor V Leiden and other risk factors for VTE in the general population is estimated at 5-10%. This population has a 5-21 fold greater risk to develop VTE.

To investigate the effect of Femarelle® on the coagulation system, a platelet adhesion and aggregation device (PFA-100) was used. The PFA-100 measures the time for blood drawn

through fine capillary to block membrane coated with collagen and epinephrine (CEPI) or collagen and ADP (CADP). This time is referred to as the closure time (CT), measured in seconds the clotting time of blood under flow.

In a clinical trial, a platelet function test was performed at enrollment, after 8 weeks and 12 months.

Out of 121 women 96 women were found to have normal clotting times (CEPI 85-165 sec. and CADP 71-118 sec.). From this group 29 women were placed on oral estrogen, 42 women on transdermal estrogen and 25 women with normal clotting times were treated with Femarelle®. 13 women were found to have borderline clotting times (CEPI 70-84 and CADP 56-70 sec.); 5 were placed on oral estrogen and 8 on transdermal estrogen for 8 weeks.

13 women were found to have significantly decreased clotting times (CEPI ≤ 60 sec and CADP ≤ 56 sec) were tested for inherited or acquired thrombophilia and were placed on Femarelle® for 12 months.

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**CADP Closure Times with Oral or Transdermal Estrogen, in Women with Borderline, Clotting Times**

7 women found to have significantly decreased clotting times (CEPI ≤ 60 sec and CADP ≤ 56 sec) were tested for inherited or acquired thrombophilia and were placed on Femarelle® for 12 months.

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Results:
It was found that women with normal clotting time, who were placed on either oral or transdermal estrogen, showed no change in CT after 8 weeks of treatment. However, women who were borderline and treated with oral estrogen had a significant decrease in clotting time, while those treated with transdermal estrogen showed no change in CT. In both groups of women who were treated with Femarelle®, those with normal clotting times and those with significantly decreased clotting times, showed no change in CT following 12 months of treatment.
All women in the shortened clotting group were found to have clotting pathologies: Factor V Leiden (n=4), Mutant Prothrombin (n=1), Protein S Deficiency (n=1), and Elevated Anticardiolipin Antibodies (n=1).

Conclusion:
A major problem in ET and HT is increased clotting leading to VTEs and stroke. It is expensive and difficult to screen women before they begin estrogen therapy. Femarelle® was shown to have no effect on the coagulation system in either normal or thrombophilic women. This information helps place Femarelle® as the first line treatment for menopause, providing a therapeutic option that does not increase risk factors.

Pre-clinical study: toxicology
A toxicity study failed to show any secondary estrogenic effects of Femarelle® on the estrogen-dependent characteristics of the female reproductive tract in a post-menopausal model of OVX rats. In that study, OVX adult female rats were randomly treated for 14 days with a placebo or with a low or high dose of E2 or of Femarelle®. The low dose was calculated as 3 times the recommended dose (RD) and the high dose was 9 times the RD.
Uterine weight, keratinization of the vaginal lining, and sensitivity of the uterine smooth muscle to serotonin were monitored.

**Results:**
Unlike $E_2$, Femarelle® did not display any estrogenic effect on the uterine weight or any sensitivity to serotonin. Compared to rats treated with $E_2$, the vaginal lining of Femarelle-treated rats showed a small degree of keratinization.

**Wet uterine weight from OVX rats treated with low and high dose conjugated equine estrogens and Femarelle®**

* P<0.05 ; ** P<0.01 as compared with conjugated equine estrogens

**Conclusion:**
The findings show that Femarelle® does not induce any of the typical and problematic estrogenic effects in the female reproductive tract.

**Femarelle® as a SERM**

In order for a substance to be defined as a Selective Estrogen Receptor Modulator, the following criteria proving this mode of action need to be shown:

- *Estrogen receptor agonistic or antagonist activity in various tissues* - in this case the effect on menopausal symptoms, the bone, breast tissue, and on the uterus;
- *Blocking of the activity by an estrogen receptor blocker* (such as raloxifene) - in order to determine whether the effect is through estrogen receptors.
- *Blocking the bondage of estrogen to the estrogen receptors* - when Femarelle® and $E_2$ are administered simultaneously, Femarelle® blocks the bondage of $E_2$ in all tissues, proving Femarelle's antagonistic properties.

In clinical studies, Femarelle® relieved menopausal symptoms and increased bone mineral density (agonistic activity) while not affecting sex steroid hormone levels or endometrial thickness (antagonistic activity). Studies in vivo showed that Femarelle® stimulates the activity of creatine kinase (CK), a marker of estrogen receptor activation, in skeletal tissues of female rats (agonistic activity). In the uterus, however, CK was activated only by $E_2$ and not by Femarelle® (antagonistic activity). Furthermore, Femarelle® was shown to activate osteoblast activity, promoting bone build-up through estrogen receptor activation (agonistic activity).

In the present study, CK specific activity was monitored in various tissues following the simultaneous administration of Femarelle® and $E_2$:

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14 Somjen D, Yoles I. DT56a (Femarelle): a Natural Selective Estrogen Receptor Modulator (SERM). *J. of Steroid Biochemistry & Molecular Biology* 2007; 104:252-58
• **In vivo:** Epiphyseal cartilage, diaphyseal bone, thymus, pituitary gland and in the uterus were monitored in intact and ovariectomized female rats.

• **In vitro:** Human cultured osteoblasts and human umbilical artery smooth muscle cells were monitored.

**Results:**
The results showed that the estrogen receptors bound preferentially with Femarelle® in the bone tissue, thymus and the pituitary gland. In the uterus Femarelle® blocked the effect of E2, demonstrating its antagonistic effect on estrogen receptors in this organ.

**Conclusion:**
Femarelle® bonds to all estrogen receptors; it displays either agonistic or antagonistic activity, depending on the tissue. In the uterus, Femarelle’s antagonistic activity was demonstrated following its blockage of the estrogen receptors to the effect of E2. This study provides additional proof that Femarelle® works as a Selective Estrogen Receptor Modulator (SERM).

**Pre-Clinical Study: The Effect of Femarelle in High Glucose Environment\(^\text{15}\)**

Since the skeletal protective effect of estrogen is significantly lessened in diabetic women, we sought to look at the effect of DT56a vs. estradiol-17β (E2) on estrogen receptor activation in female-derived cultured osteoblasts.

Human female osteoblasts were obtained from biopsies of patients undergoing corrective surgery following accidental injury, hip or knee replacement. The cultures consisted of osteoblast-like cells (with no fibroblasts) showing the characteristic of osteoblasts: high alkaline phosphatase (ALP) activity, dose-dependent increase of ALP by 1,25-(OH)2D3, high levels of parathyroid hormone (PTH)-induced cyclic AMP, and 1,25-(OH)2D3-induced osteocalcin.

To obtain “high glucose” (HG) conditions, the medium was supplemented with glucose up to a final concentration of 44mM (9 gm/liter). Glucose concentration in the regular medium (NG) was 22mM (4.5gm /liter).

**Results:**
In normal glucose environment, ER responsiveness to Femarelle was similar to the responsiveness of ER to E2.

In high glucose environment, ER responsiveness to E2, as measured by CK activity and DNA synthesis, was significantly reduced, while, ER responsiveness to DT56a was not changed and was similar to Femarelle’s effect in normal glucose environment.

\(^{15}\) Somjen D. et al. DT56a (Femarelle); contrary to estradiol-17β; is effective in human derived female osteoblasts in hyperglycemic condition. *J Steroid Biochem Mol Biol*. 2011; 123:25-29
Stimulation of DNA synthesis, by E₂ or Femarelle® (DT56a), in primary bone derived cells from pre & post-menopausal women in normal medium (NG) or high glucose medium (HG).

*, P <0.05; **, P < 0.01.

Conclusion:
Femarelle®, contrary to E₂, was found to activate estrogen receptors in the bone even in high glucose environment; thus, Femarelle® may be an effective treatment for bone health in diabetic post-menopausal women.

The pros and cons of plant estrogens for menopause

In a review on the pros and cons of plant estrogens for menopause, Femarelle® was cited numerous times under the different categories showing that it may provide a safe and effective alternative to HT.

Abstract of article: Concerns pertaining to the risk of estrogen exposure through HT have prompted an increase in the use of natural alternatives. Phytoestrogens may provide postmenopausal women with a practical alternative and many women have already begun to utilize phytoestrogen supplements. However, research regarding the efficacy of phytoestrogens as a hormone therapy alternative has been previously pessimistic or questionable at best. This review scrutinizes the most current research regarding the efficacy of three types of phytoestrogens, isoflavones, lignans and coumestans, and their specific effect on the reduction of climacteric symptoms, specifically vasomotor symptoms, vaginal atrophy, insomnia and osteoporosis. A discussion of the research pertaining to the relative safety of each phytoestrogen in terms of breast and endometrial health is also included. Overall, current research demonstrates that phytoestrogens are effective in reducing the intensity of hot flushes, and some phytoestrogen combinations result in a decreased frequency. Certain phytoestrogens have also been shown to decrease vaginal atrophy, improve sleep and cognition, and positively affect bone health. Even though initial research was generally unconvincing, the more recent evidence reviewed here is rather

positive. In terms of safety and reports of adverse reactions, trials have not shown an increase in breast cancer risk or increase in endometrial hyperplasia following phytoestrogen use, but trials explicitly designed to find neoplasia have not been reported. Moreover, unlike hormone therapy, lignans may not increase clotting risk in postmenopausal women, thus supplements may serve as a treatment option for patients who have contraindications to hormone therapy. Phytoestrogens may provide a safe and partially effective alternative to HT. However, because research regarding phytoestrogens is relatively new, pharmacovigilance is still required, as these products are not yet FDA-approved.

References:
10. Somjen D. et al. The Effects of Native and Synthetic Estrogentic Compounds as well as Vitamin D Less-Calcemic Analogs on Adipocytes Content in Rat Bone Marrow. J Endocrinol Invest. 2011;34(2):106-10
15. Somjen D. et al. DT56a (Femarelle); contrary to estradiol-17β; is effective in human derived female osteoblasts in hyperglycemic condition. J Steroid Biochem Mol Biol. 2011; 123:25-29