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TITLE: Phase I/II Pilot Study to Assess Toxicity and Efficacy of Chinese Herbs to Treat Hot Flashes and Menopausal Symptoms for Women with a History of Breast Cancer

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Fort Detrick, Maryland 21702-5012

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## INTRODUCTION

The management of menopausal symptoms in women with a history of breast cancer poses a major therapeutic challenge. Hot flashes are a significantly bothersome symptom for many post-menopausal women and patients receiving drugs such as tamoxifen. The lack of effective agents for menopausal symptoms and the burgeoning interest in alternative modalities has led to an increasing use of herbal remedies despite a lack of objective data on safety and efficacy. Our originally proposal was for a clinical trial to assess feasibility, toxicity and preliminary efficacy analyses of an herbal regimen to ameliorate menopausal symptoms in individuals with a past history of breast cancer who are menopausal and experiencing hot flashes. The FDA required a preliminary phase I clinical trial in a group of 20 healthy women with no previous history of breast cancer to ensure there were no adverse estrogenic effects from the herbal regimen prior to commencing the clinical trial in women with a previous history of breast cancer. We have completed the phase I trial and enrolled a total of 30 women with no previous history of breast cancer.

## BODY

Our hypothesis was that the herbal menopause formula (MF101) consisting of 22 botanical agents could be administered safely and with high compliance to women in order to alleviate hot flashes and the undesirable effects of menopause among women with a history of breast cancer. Per the FDA, we had to revise our statement of work to complete a phase I/II preliminary clinical trial in a cohort of healthy women prior to proceeding with a clinical trial involving women with a history of breast cancer. We were successful in obtaining an Investigational New Drug (IND) license from the FDA. Since January 14, 2002, a total of 31 women enrolled in the study. The FDA required a preliminary phase I trial of 20 patients. We have completed analysis of the patients completing the trial and enclosed below is a list of adverse events. There were no grade 3 or 4 adverse events. The primary complaints on study were the undesirable taste of the herbs and minimal gastrointestinal side effects.

### All Reported Adverse Events for MF101 as Reported to the Investigator

Reporting Period: 01/14/02-7/29/03
Total Patients Recruited: 31
Total Patients Who Completed the Study: 22
Total Patients Who Were Lost to Follow Up: 4

Total Patients Who Dropped Out Prior To Taking MF101: 1
Total Patients Who Stopped Taking MF101 Prior To Study Termination: 2

| Patient No. | Adverse Event | Start Date | End Date | Grade CTC | Related to IND Agent | Related to Non-IND | Related to Menopause | Category | Action Taken | Therapy Required |
|---|---|---|---|---|---|---|---|---|---|---|
| 001 | None | | | | | | | | | |
| 002 | None No F/U | | | | | | | | | |
| 003 | Poor Taste | Not recorded | | 1 | 4-Probable | 1-Unrelated | 1-Unrelated | 3-Expected | 2-Dose Reduction | 1-None |
| 004 | None | | | | | | | | | |
| 005 | None | | | | | | | | | |
| 006 | None No F/U | | | | | | | | | |
| 007 | None | | | | | | | | | |
| 008 | Loss to F/U | | | | | | | | | |
| 009 | None | | | | | | | | | |
| 010 | Nausea | 08/04/02 | 08/13/02 | 2 | 4-Probable | 2-Unlikely | 2-Unlikely | 3-Expected | 2-Dose Reduction | 1-None |
| | Headache | 08/04/02 | 08/07/02 | 2 | 4-Probable | 2-Unlikely | 2-Unlikely | 2-Unexpected | 2-Dose Reduction | 1-None |
| 011 | None | | | | | | | | | |

| Patient No. | Adverse Event | Start Date | End Date | Grade CTC | Related to IND Agent | Related to Non-IND | Related to Menopause | Category | Action Taken | Therapy Require |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Marked Lethargy | 08/10/02 | 08/16/02 | 2 | 3-Possible | 2-Unlikely | 3-Possible | 2-Unexpected | 2-Dose Reduction 3-Agent Discontinued | 1-None |
| | Depression | 8/10/02 | 08/16/02 | 2 | 3-Possible | 2-Unlikely | 3-Possible | 2-Unexpected | 2-Dose Reduction 3-Agent Discontinued | 1-None |
| 13 | Migraine headache | Intermittent | | 2 | 2-Unlikely | 2-Unlikely | 2-Unlikely | 2-Unexpected | 1-None | 1-None |
| | Heart racing | 1 day | | 1 | 2-Unlikely | 2-Unlikely | 2-Unlikely | 2-Unexpected | 1-None | 1-None |
| 14 | Flatulence | Not recorded | Not recorded | 1 | 4-Probable | 2-Unlikely | 2-Unlikely | 3-Expected | 2-Dose reduction | 1-None |
| | Abdominal bloating | Not recorded | Not recorded | 1 | 4-Probable | 2-Unlikely | 2-Unlikely | 3-Expected | 2-Dose reduction | 1-None |
| 15 | Mood changes | Not recorded | Con't at end of study | 1 | 3-Possible | 3-Possible | 3-Possible | 2-Unexpected | 2-Dose reduction | 1-None |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Nausea | Not recorded | Not recorded | 1 | 4-Probable | 2-Unlikely | 2-Unlikely | 3-Expected | 2-Dose reduction | 1-None |
| | Stomachache/"Ovaries Tweaking" | Not recorded | Not recorded | 1 | 4-Probable | 2-Unlikely | 2-Unlikely | 3-Expected | 2-Dose reduction | 1-None |
| 16 | No F/U | | | | | | | | | |
| 17 | None | | | | | | | | | |
| 18 | None | | | | | | | | | |
| 19 | Elevated Blood pressure | 10/14/02 | | 1 | 3-Possible | 2-Unlikely | 2-Unlikely | 2-Unexpected | 1-None | 1-None |
| 20 | Never started study medication | | | | | | | | | |
| 21 | None | | | | | | | | | |
| 22 | None | | | | | | | | | |
| 23 | None | | | | | | | | | |
| **atient o.** | **Adverse Event** | **Start Date** | **End Date** | **Grade CTC** | **Related to IND Agent** | **Related to Non-IND** | **Related to Menopause** | **Category** | **Action Taken** | **Therapy Require** |
| 24 | None | | | | | | | | | |
| 25 | None | | | | | | | | | |
| 26 | None | | | | | | | | | |
| 27 | None | | | | | | | | | |
| 28 | Stomache Burning | 03/25/03 | 03/29/03 | 1 | 2-Unlikely | 2-Unlikely | 1-Unrelated | 2-Unexpected | 2-Dose reduction 3-Agent Discontinued | 1-None |
| | Nausea | 03/25/03 | 03/29/03 | 1 | 4-Probable | 3-Possible | 1-Unrelated | 3-Expected | 2-Dose reduction 3-Agent Discontinued | 1-None |
| 29 | None | | | | | | | | | |
| 30 | None | | | | | | | | | |

Below is an abstract accepted for a poster presentation at the National Menopause Society (NAMS) meeting in Oct 2004.

A Pilot Clinical Trial of an Estrogen Receptor β Selective Drug for Hot Flashes

**Objective**: Estrogen plus progestin hormone therapy (HT) has been the standard therapy for menopausal symptoms, but HT is associated with serious adverse effects. To develop safer estrogens for hot flashes, it is important to determine the role of two estrogen receptors (ERα and ERβ) in hot flash prevention. We found that an herbal formula (MF101) selectively activates ERβ transcription, but does not stimulate the proliferation of breast cancer cells unlike estrogens used in HT. We

hypothesized that ERβ-selective drugs will not promote breast cancer, but may prevent hot flashes. In this study, we assessed the feasibility, toxicity and preliminary efficacy of MF101 to reduce hot flashes.

**Methods:** The study was a prospective, single-arm, pilot clinical trial conducted of 31 post-menopausal women. After a 30-day run-in period, women were treated with the estrogen receptor beta selective drug for 30 days. Outcome measures included the patients' daily diary of hot flash frequency and severity as well as laboratory work.

**Results:** There were no statistically significant changes in any of the laboratory values and no serious adverse events were reported. A statistically significant reduction in both the frequency and severity of hot flashes was found after 30 days of treatment.

**Conclusion:** Our results show ERβ may be involved in preventing hot flashes and that short-term use of an ERβ selective drug is safe and well tolerated for the treatment of hot flashes. A more definitive double-blind, placebo-controlled clinical trial approved by the FDA is underway to elucidate the efficacy and toxicity of MF101.

# A Pilot Clinical Trial of a Traditional Chinese Herbal Extract for the Treatment of Hot Flashes

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A Pilot Clinical Trial of a Traditional Chinese Herbal Extract for the Treatment of Hot Flashes

Tagliaferri M, Cohen I, Tripathy D, Goldman M, Moore D, Leitman D

## Abstract

*Objective:* To assess feasibility, tolerability and preliminary efficacy of a Chinese herbal extract, MF101, to reduce hot flashes in women with moderate to severe hot flashes.

*Methods:* The study was a prospective, single-arm, pilot clinical trial conducted between March 2002 and June 2003 of 31 post-menopausal women between the ages of 20-60 who reported ≥7 moderate to severe hot flashes per day. Outcome measures included the patients' daily diary of hot flashes and laboratory work assessing hematology, blood chemistry, hepatic function, liver function and hormonal status.

*Results:* There were no statistically significant changes in any of the laboratory values and no serious adverse events were reported. The mean frequency of hot flashes reported by study participants was 57.3 per week at baseline and 44.9 after 30 days of treatment (p=0.003). The hot flash score (frequency multiplied by severity) decreased from 98.0 at baseline to 81.7 after treatment (p=0.03). There was a 22% reduction in the frequency of hot flashes experienced over a 7-day period measured at baseline as compared to the last 7 days on the study medication (p=0.0035).

*Conclusion:* MF101 is safe and well tolerated for the treatment of hot flashes and can be feasibly administered with good compliance. After 30 days of treatment with MF101, women reported a moderate clinical benefit in the reduction of both the frequency and severity of hot flashes. A more definitive double-blind, placebo-controlled clinical trial is necessary to more clearly elucidate the efficacy and toxicity of MF101 among menopausal women with hot flashes.

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## Introduction

Epidemiological data reporting the incidence of hot flashes among menopausal women throughout the world varies widely. In the United States, data from the longitudinal, Nurses' Health Study show that 75% of women experienced hot flashes for an average duration of 3.8 years between the peri-menopausal and post-menopausal period (Avis, 1997). Up to 90% of women who have undergone a bilateral oophorectomy experience a period of hot flashes (Feldman, 1985) (Chakravarti, 1977) indicating a more acute decline in estrogen levels and accounting for a more pronounced initial period of vasomotor symptoms. Both hot flashes and associated symptoms of menopause such as insomnia, night sweats and mood changes can be extremely debilitating. Until recently, vasomotor symptoms were commonly treated with estrogens, which are effective in 80-90% of women who initiate treatment with hormonal therapy (HT) (Rabin, 1999) (Notelovitz 2000). However, data from the Women's Health Initiative have shown that postmenopausal combination estrogen-progestin hormone therapy increases a woman's risk for coronary events, stroke, breast cancer, venous thromboembolic events and dementia [was dementia statistically worse?]. Moreover, treatment with standard dose hormone therapy did not improve quality of life indices such as emotional and sexual functioning and vitality (Hays, 2003). Of 670 women regularly taking HT between July 1, 2001 and June 30, 2002, 93% reported hearing about the WHI results and 56% attempted to discontinue HT in the 6-8 months after the original WHI results were published in July 2002 (Ettinger, 2003). Given the increased risks for serious diseases, the lack of benefit in quality of life and other common side effects such as uterine bleeding and breast tenderness (Greendale, 1998), many women opt to avoid using hormone therapy for treatment of hot flashes.

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In addition to hormone replacement therapy, numerous approaches have been tested to treat menopausal symptoms and most of these have used hot flash-related indices as the primary endpoint (Hoda, 2003). One example is Vitamin E, which demonstrated a statistically significant but a clinically minor reduction of one hot flash per day (Barton, 1998). The progestin megestrol acetate has also been found to be effective, but is associated with weight gain and vaginal bleeding (Loprinzi, 1994). Clonidine administered as a patch did lower hot flashes (20% over baseline) but the authors concluded that the side effects outweighed the small clinical benefit (Goldberg, 1994). Gabapentin lowered hot flashes by 45%, however 50% of patients experienced an adverse event including somnolence and dizziness (Guttuso, 2003). Newer generation anti-depressants including venlafaxine can be effective for the management of hot flashes although these also are associated with side effects such as mouth dryness, nausea and constipation (Loprinzi, 2000; Stearns, 2003).

Complementary and alternative (CAM) approaches to medicine are widely used in the United States and throughout the world to treat a broad spectrum of conditions as well as to maintain wellness. It has been reported that Americans make 629 million visits to alternative medicine practitioners each year, exceeding all visits to primary care providers (Eisenberg, 1998). The general public spends more money out of pocket on

complementary and alternative treatments than they do for all hospitalizations on an annual basis. Despite widespread use, alternative and complementary medical approaches have not been subjected to rigorous clinical trials to measure outcomes. Consequently, while more patients are seeking alternative medicine, there is little information in the medical literature to determine if these therapies can be used safely to improve one's quality of life or provide a therapeutic benefit.

Over the past two decades, the dietary supplement and herbal medicine industry in the US has grown exponentially, with current annual sales capping $18 billion (Marcus, 2002). Although a number of dietary supplement companies are making claims about their products based on "clinical trials", these have generally not been designed with proper controls and outcome measures. Today, the entire dietary supplement and herbal medicine industry falls under the legislative protection of the Dietary and Supplement Health Education Act (DSHEA) of 1994, which exempts companies from any requirement to prove that their products are safe and effective. Even though the FDA has no direct role in the regulation of the herbal medicine industry, the agency has been asked to issue warnings about the nephrotoxic, hepatotoxic and carcinogenic effects of botanical products containing commonly used herbal agents such as kava, comfrey and aristolochic acid (Kessler, 2000). Because of convincing data regarding toxicity, the sale of ephedra has now been banned in the US (FDA, 2003).

Despite an increased awareness of known adverse effects of dietary supplements and herbal remedies, the American public continues to use these products in ever-increasing amounts. In fact, black cohosh, commonly used by women to treat hot flashes had $59 million in sales in 2002, a more than fivefold increase from 1998, while the sales of soy supplements, often purported to reduce menopausal symptoms, have quadrupled in five years, to $102 million (Berger, 2003). Yet, while CAM use is widespread, Americans understand little about the regulation of the herbal medicine industry. In a recent survey, 59% of respondents believed that dietary supplements are approved by a government agency, such as the FDA (Taylor, 2003). Additionally, the general public assumes that manufacturers of dietary supplements and herbal remedies are restricted from making claims about a product's safety and efficacy unless there is sound, scientific evidence (55% of respondents) and warning labels detailing potential side effects are required (68% of respondents) (Taylor, 2003). Given this level of confusion among the general population, there is a compelling need to address the lack of evidence for dietary supplements and to quickly move forward by testing, in controlled clinical trials, those herbal approaches already widely used.

Traditional Chinese Medicine (TCM) includes the use of individualized and complex formulae of herbs based on centuries of adaptation and empiric testing. TCM is widely used to control menopausal symptoms; however, unlike phytoestrogen therapy, combination TCM herbal formulae have yet to be tested in a randomized, placebo-controlled clinical trial. In TCM, it is standard practice to provide individualized care to treat the underlying imbalance of a patient after a thorough medical history and physical exam. Although individualized therapy is the gold standard for treatment according to the principles of TCM, there are specific time periods in a woman's life when the TCM

diagnosis is largely uniform. One study analyzed the diagnostic practices of nine licensed practitioners of TCM who were treating a cohort of post-menopausal women experiencing hot flashes. All nine practitioners diagnosed the women on the same day and there was a high degree of concordance among the TCM practitioners about the specific diagnosis; in 168 of the 207 visits (81%), the practitioners concurred that kidney yin deficiency was the TCM diagnosis for this cohort of post menopausal women (Zell, 2000). Because this specific deficiency is extremely common among menopausal women, it is possible to design a clinical trial with one standardized TCM formula to address this imbalance.

In a pilot clinical trial our group evaluated the feasibility, safety and preliminary efficacy a traditional Chinese herbal therapy for menopausal hot flashes. The formulation studied, MF101, was packaged as a granulated powder that was made from the extracts of 22 Chinese herbs. This particular formulation is based on both empirical data from prior practice as well as preliminary laboratory data showing that components of MF101 bind only to the estrogen receptor beta (ERβ). Our group has previously shown that ERβ activation represses growth-promoting genes and inhibits estrogen receptor alpha (ERα) mediated proliferation of human breast cancer cells (Paruthiyil, 2004). The ERβ receptor is prevalent in non-reproductive tissues such as the brain and bone and agonists of the ERβ receptor may function by decreasing central nervous system activation that causes vasomotor symptoms. Importantly, components of MF101 *do not* bind to ERα. We believe that this differential ER binding is very important, and may identify selective estrogen receptor modulators (SERMs) that relieve hot flashes and prevent bone loss, but do not increase a woman's risk for breast cancer. In this paper, we present the results of the first pilot study of an herbal agent used for the treatment of hot flashes that has received an Investigational New Drug (IND) license from the Food and Drug Administration.

**Materials and Methods**

In this pilot study, eligible women between the ages of 20-60 were enrolled who had been amenorrheic for at least 12 months due to natural menopause or who had been amenorrheic for 6 months with an FSH level >40 mIU/ml and estradiol level <20 pg/dl. Study participants had to report ≥7 moderate to severe hot flashes per day, for at least one month, coupled with 3 of the following additional signs and symptoms: night sweats, mood changes, depression, irritability, insomnia, vaginal dryness, arthralgia, decreased libido, tiredness, lethargy, anxiety, nervousness, inability to concentrate and general sweating. Women were excluded from the study if there was a history of multiple or severe food or medicine allergies. Other exclusion criteria included: women who had used oral estrogen and/or progestin therapy within 8 weeks, transdermal hormonal products within 4 weeks, projestational implants or estrogen/progestational injectable therapy within 3 months or vaginal hormonal products within 7 days. Participants could not use any concurrent herbal medicine purchased over the counter nor could they use medication for either depression or hypertension. Women were not eligible if they had an abnormal PAP smear within the past year or a history of breast, endometrial or breast cancer.

The Institutional Review Board of the University of California, San Francisco approved the pilot clinical trial. The Food and Drug Administration, Division of Reproductive and Urologic Drugs issued an Investigational New Drug license for the 22 herbal extract formulation, MF101, manufactured by Bionovo, Inc. Written informed consent was obtained from all of the study participants.

Women were recruited from the gynecology practice at the University of California, San Francisco (UCSF). Advertisements approved by the UCSF Committee on Human Research were place in one of the daily local newspapers as well as in specified areas of the UCSF campus.

The length of the study was 60 days. At study initiation, participants were asked to record both the frequency and severity of their daily hot flashes on a preprinted diary for 30 days prior to starting MF101 for a treatment period of 30 days. During the first 30 days prior to treatment, the participants had a physical examination by a board certified OBGYN, a PAP smear if one had not been completed within the last 12 months, a vaginal scraping, and laboratory work including: a complete blood count, platelet count with differential, serum electrolytes and chemistry, hormonal panel, liver panel and urinalysis.

After the initial 30-day run-in period, all participants were treated with 5 grams of granulated MF101 as a powder mixed with warm water, taken orally twice a day for 30 days. The primary efficacy outcome measure was change in the frequency of hot flashes. The primary outcome measure to assess toxicity from MF101 included the patients' reported adverse events according to general organ system and the panel of blood work. Secondary outcomes included: change in the serum estradiol, vaginal maturity and bone resorption markers.

**Results**

Between January 14, 2002 and June 8, 2003, there were 31 patients consented to the trial. Two patients dropped out of the study prior to starting treatment with MF101. Four patients were lost to follow up, two patients dropped out of the study due to side effects and one patient dropped out as a result of the unpleasant taste of the liquid extract. Refer to Table 1 for a summary of the study participants included in the analysis, those excluded and the reasons for exclusion. A total of 22 women completed the study. Table 2 shows the baseline characteristics of the women who participated in the trial.

Table 1: Summary of the Study Participants Included in the Analysis

| | |
|---|---|
| Study Participants Consented | 31 |
| Consented but not treated with MF101 | 2 |
| Included in Safety Analysis | 25 |
| Completed Study | 22 |
| Discontinued Treatment: | 7 |
| Loss to Follow Up* | 4 |
| Adverse Effects | 2 |

| | |
|---|---|
| Unpleasant Taste | 1 |

Table 2 Baseline Characteristics of Patient Population

| **Characteristic** | **Value** |
|---|---|
| **Age** | |
| Mean | 54.0 years |
| Median | 52.0 years |
| Range | 46-72 years |
| **Height** | |
| Mean | 64.9 inches |
| Median | 65.0 inches |
| Range | 59.0-71 inches |
| **Weight** | |
| Mean | 150.2 pounds |
| Median | 145 pounds |
| Range | 108-248 pounds |
| **Race** | |
| Caucasian | 24 participants (77%) |
| Hispanic | 4 participants (13%) |
| Asian | 2 participants (7%) |
| Unknown | 1 participant (3%) |

On average, the study participants who completed the trial took 89.5% of the prescribed dose of MF101 over the 30-day treatment period.

There were no reported Grade III or IV adverse events measured by NCI common toxicity criteria for the 25 patients with available toxicity data. There were a total of 9 adverse events reported by the study participants to the investigative physician and categorized as possibly, probably or definitely related to MF101. Five of the adverse events were categorized as Grade I and four were recorded as Grade II adverse events according to the NCI common toxicity criteria. The most common toxicities reported were slight nausea or stomach bloating (4/25 women). The other five adverse events were for the following reasons: headache, lethargy, depression, mood changes and elevated blood pressure. None of the study participants required treatment for any reported side effects nor were there any hospitalizations.

All 22 patients who had baseline and study termination blood and urine laboratory tests were included in the second analysis for toxicity. There were no statistically significant changes in any of the laboratory values for the complete blood count, chemistry, liver panel, serum hormonal levels or urine test. Despite a statistically significant decrease in luteinizing hormone according to the paired t-test, this decrease was not statistically significant by the more conservative nonparametric Mann-Whitney test.

In terms of efficacy, there was a statistically significant reduction in both the frequency and severity of hot flashes after 30 days of treatment. The mean frequency of hot flashes was 57.3 per 7 days at baseline and 44.9 after treatment (p=0.003). The hot flash score (frequency multiplied by severity) decreased from 98.0 at baseline to 81.7 after treatment (p=0.03). Of the 22 women who completed the study, 18 (82%) had fewer hot flashes after 30 days of study medication while only 4 (18%) had more. There was a 22% reduction in the frequency of hot flashes experienced over a 7-day period measured at baseline as compared to the last 7 days on the study medication (p=0.0035). There was also a 17% reduction in hot flash score (frequency multiplied by severity) after 30 days of treatment (p=0.031).

In summary, our preliminary clinical trial data indicate MF101 is safe and well tolerated for the treatment of hot flashes and can be feasibly administered with good compliance. MF101 reduced the both the frequency and severity of hot flashes. The traditional Chinese herbal formula does not adversely alter serum reproductive hormones, suggesting that MF101 will not increase the risk of either breast or uterine cancer.

**Discussion**

In the new millennium, it is clear that hormone therapy has emerged as a leading issue in women's health, largely due to the longevity of women. For at least the past several centuries, the average age of menopause has remained constant at 51 years. In 1900, the average life expectancy of women was 48 years. Therefore, just one hundred years ago, most women never experienced the changes associated with menopause. Today, the life expectancy of American women exceeds 80 years of age. Not only will a majority of women reach menopause, but they will also live approximately one-third of their life after menopause. Since there are currently 36 million women between the ages of 45 and 65, a number that will soar to 50 million in 25 years, it is imperative that we develop better strategies to identify more selective therapies to prevent menopause without causing deleterious side effects.

There is a rapidly growing interest in using natural plant estrogens (phytoestrogens) as an alternative to standard estrogen/progestin therapies. Interest in phytoestrogens has been fueled by studies that show a lower incidence of menopausal symptoms, osteoporosis, cardiovascular disease and breast, colon and prostate cancer in Asian populations that consume large quantities of phytoestrogens. Despite compelling evidence that estrogens cause breast cancer, observational studies paradoxically show that women in Asian countries have the lowest incidence of breast cancer even though they consume large quantities of plant estrogens. Likewise, Asian women report minimal symptoms during menopause and are far less prone to experience hot flashes at the time of cessation of ovarian function. These findings have encouraged many menopausal women in the United States to take phytoestrogens present in soybeans or herbal therapies as an alternative to estrogen, hoping to alleviate hot flashes without increasing their risk of developing breast cancer. The paradoxical finding that estrogens promote breast cancer while phytoestrogens may prevent breast cancer may lie in the differential binding of the two known estrogen receptors, leading to distinct effects on transcriptional activation and repression of genes involved in proliferation of breast cells.

Estrogenic compounds elicit their clinical effects by interacting with two distinct estrogen receptors. Estrogen receptor alpha (ERα) is a 595 amino acid protein that was cloned in 1986. Ten years later, a second estrogen receptor termed beta (ERβ), with 530 amino acids, was discovered. The reason for the existence of two ERs remains an enigma. Like other nuclear receptors, ERα and ERβ are modular proteins that exhibit a highly conserved DNA-binding domain (DBD) that enables them to bind to a specific DNA sequence in the promoter region of regulated genes, known as the estrogen response element (ERE). On the other hand, the ligand binding domain (LBD), which allows estrogen receptors to bind to estrogenic compounds is less conserved between ERα and ERβ, exhibiting only 55% homology. The LBD has other critical functions, including receptor dimerization and association with co-activators and co-regulators. The third domain of the ERs consists of the amino-terminus, referred to as the A/B domain; this domain is the least conserved with only 25% homology. The A/B domain is involved in transcriptional activation in the absence of a ligand or in response to estrogen antagonists such as tamoxifen. Refer to Figure 1 for an illustration of the three domains of the ER. Based on studies previously reported (Paruthiyil, 2004), we hypothesize that estrogens promote breast cancer by interacting with estrogen receptor alpha (ERα) whereas the phytoestrogens found in MF101 may prevent breast cancer and menopausal symptoms such as hot flashes by selectively interacting with estrogen receptor beta (ERβ). In our previous work, we have shown that ERβ represses growth-promoting genes and inhibits ERα mediated proliferation of breast cells.

Studies show that the tissue distribution, physiological effects and transcriptional activities are quite different between ERα and ERβ. ERβ is more ubiquitous, and is expressed in many non-reproductive tissues, such as bone, brain, urinary tract, vascular system, and prostate gland, in addition to reproductive tissues, such as the ovary and testis. ERα is expressed mainly in the uterus, liver, breast and kidney. The different physiological roles of ERα and ERβ have been definitely demonstrated in ERα or ERβ knockout mice. The ERα knockout mice develop major defects, such as primitive mammary glands and uterus and are infertile (Hewitt, 2003). In contrast, the effects observed in the ERβ knockout mice have been more subtle, including subfertility, with decreased litter size, thickening of female cortical bone and prostate hyperplasia. It has been shown that estrogens, selective estrogen receptor modulators (SERMS) and phytoestrogens bind to the same hydrophobic binding pocket, however, they each induce different conformations in ERα and ERβ as demonstrated by the X-ray crystal structure and phage libraries. Different conformations of ERα and ERβ induced by different ligands may lead to the recruitment of distinct co-regulators, which ultimately result in the regulation of different genes that mediate clinical responses. We have found that isoflavones (the phytoestrogens in soybeans) are 10-300 fold more potent at triggering ERβ-mediated repression activation and recruiting co-regulators to ERβ. The ERβ-selective recruitment of co-regulators and transcriptional activity, especially repression, by phytoestrogens may be partially responsible for the lower incidence of menopausal symptoms and other conditions observed in postmenopausal Asian women who consume large quantities of phytoestrogens. To this end, we believe the phytoestrogens in MF101

will also elicit their clinical effect through the ERβ pathway to selectively effect vasomotor symptoms without causing an increase in breast cancer.

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