

MF101, a selective estrogen receptor β modulator for the treatment of menopausal hot flashes: a phase II clinical trial

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Abstract

Objective: To determine the optimal dose, safety, and efficacy of an estrogen receptor β selective Chinese herbal extract, menopausal formula 101 (MF101), for treating hot flashes.

Methods: A randomized, blinded trial in 217 postmenopausal women with hot flashes randomized to 5 or 10 g/day of MF101 or placebo for 12 weeks.

Results: The effects of 5 g/day of MF101 did not differ from those of placebo. After 12 weeks, the mean percent decrease in frequency of hot flashes in the 10 g/day group was 12.9% greater than that in the placebo group ($P = 0.15$), the median percent decrease was 11.7% greater than that in the placebo group ($P = 0.05$), and the proportion of women with at least a 50% reduction in hot flashes was 16.2% greater than that in the placebo group ($P = 0.03$).

Conclusions: Treatment with 10 g/day of MF101 reduces the frequency of hot flashes. Trials with higher doses are planned.

Key Words: Menopause – Hot flush – Estrogen receptor β selective estrogen.

More than half of women in the United States report hot flashes during the menopausal transition,¹ and about 20% report that flushes are severe enough to warrant treatment.² Although estrogen is an effective treatment for vasomotor symptoms,³ many women are concerned about

the increased risk of uterine cancer,⁴ breast cancer, and cardiovascular events associated with use of postmenopausal hormone therapy.^{5,6} Selective serotonin reuptake inhibitors and gabapentin have modest efficacy for treatment of hot flashes, but these drugs have adverse effects that limit their use.⁷ Treatments that are both effective and safe would be welcome. One approach to attaining this goal is to develop selective estrogen-receptor modulators (SERMs) that are effective for relief of vasomotor symptoms but that do not cause the adverse effects associated with current postmenopausal estrogen therapies.

Menopausal formula 101 (MF101) is a combination of 22 herbs used in traditional Chinese medicine to treat vasomotor symptoms. MF101 is a SERM that has been shown to bind equally to estrogen receptor (ER)- α and to ER- β but to activate only ER- β transcriptional pathways.⁸ In addition, MF101 does not stimulate MCF-7 breast cancer cell proliferation or increase breast cancer tumor size or uterine weight in mouse xenograft models.⁸ Preliminary data in a small, uncontrolled phase I trial showed that MF101 reduced the frequency of hot flashes without causing adverse effects.

We performed a multicenter, randomized, blinded, placebo-controlled, phase II trial to investigate whether two doses of MF101 were safe and effective in reducing the frequency and severity of hot flashes in generally healthy postmenopausal women with moderate to severe hot flashes.

METHODS

Design and setting

The Chinese Herbs in Menopausal Symptoms (CHIMES) study was a multicenter, randomized, blinded,

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placebo-controlled trial designed to determine whether two doses of MF101 were safe and effective in reducing the frequency and severity of hot flushes. The trial was coordinated at the University of California, San Francisco, and participants were recruited at six clinical sites in the United States. The institutional review boards of the coordinating center and of each clinical site approved the study protocol.

Participants

Eligible participants were generally healthy postmenopausal women 40 to 60 years old who reported at least seven moderate to severe hot flushes per day or 50 per week. We excluded women with a history of breast, uterine, or ovarian cancer; melanoma; venous thromboembolism; cardiovascular disease; or severe food or medicine allergies. We also excluded women reporting active liver or gallbladder disease, abnormal uterine bleeding, and pregnancy or lactation and those with mammogram, breast examination, Papanicolaou test, or pelvic examination results suggestive of cancer. Women with endometrial thickness exceeding 5 mm measured by transvaginal ultrasound and those using medications known or suspected to affect hot flushes (estrogens, tamoxifen, raloxifene, progestins, selective serotonin reuptake inhibitors, or gabapentin) were also excluded.

From February to October 2006, women were recruited by direct, community-based media efforts. At screening, placebo medication and diaries to record hot flushes, bleeding, and medication adherence were provided for a 1-week run-in period. Participants who correctly completed their diaries, took at least 80% of the placebo medication, and remained eligible after screening physical, sonographic, and laboratory examinations were randomized.

A data safety and monitoring board convened once before the trial began and three times during the trial to review data on recruitment and retention, adherence, data quality, outcomes, and safety. At the conclusion of each meeting, the board recommended that the study proceed.

Role of the funding source

Bionovo (Emeryville, CA), maker of MF101 (US Food and Drug Administration [FDA] investigational new drug application no. 58,267), participated in the development of the protocol, provided study medications, and funded the study. Data were collected, cleaned, and analyzed by the coordinating center at the University of California, San Francisco. The investigators wrote the manuscript with nonbinding input from the sponsor.

Randomization

Randomization was stratified by time since last menstrual period (<24 vs >24 months) and by clinical site; within strata, treatment was randomly assigned in randomly permuted blocks of three and six in a 1:1:1 ratio to MF101 5g/day, MF101 10g/day, or placebo. A research pharmacist at the University of California, San Francisco, received the study medication from Bionovo, applied labels with treatment identification numbers generated by the coordinating center

statistician, and shipped study medication to each clinical site. Study medication was allocated to eligible participants sequentially according to the randomization scheme.

Study medications and blinding

MF101 is a combination of 22 herbs described in the Appendix. A water-based extract of the formulation was decanted, pressure filtered to remove insoluble materials, and freeze dried. Carmel coloring and food dyes approved by the FDA were added to the dry powder to reach a uniform color, and citrus flavorings and sweeteners were added to mask the bitter taste of the herbs. Similar coloring and taste excipients were added to maltodextrin and cornstarch to produce a placebo powder with the same look, taste, and granularity as the active medication. MF101 (US patent 60/667,887, pending) is not licensed or registered as an herbal product in the United States and is not approved for clinical use.

Participants received placebo or one of the two doses of MF101 packaged as a powder and were instructed to dissolve the contents of the packet in at least 3 oz of noncitrus fluid and drink the beverage twice daily. All investigators, study staff, laboratory personnel, and participants were blinded to study medication status.

Measurements

At baseline, participants completed questionnaires regarding demographics, medical history, medications, quality of life (SF-36⁹), menopausal symptoms (Menopausal Quality of Life Scale¹⁰), and sexual function (Female Sexual Function Index¹¹). All participants had a physical examination, including blood pressure and heart rate, a breast and pelvic examination, and, in women without a hysterectomy, a transvaginal ultrasound to measure endometrial double wall thickness. To evaluate safety, we measured serum hematology, creatinine, urea nitrogen, liver function, and estradiol (double-antibody extraction radioimmunoassay using estradiol antiserum from Diagnostic Products Corporation, Los Angeles, CA) and performed a urine analysis (Covance Central Laboratory Services, Indianapolis, IN). All baseline measures were repeated after 12 weeks of treatment or at the final study visit.

Hot flush frequency and severity were recorded on a diary modeled after a diary widely used in prior studies.¹² The 7-day diary was completed before randomization and during weeks 4 and 12 on study medication. For each hot flush, severity was rated as 1 (mild), 2 (moderate), or 3 (severe). A hot flush score was calculated by adding the severity rating for each hot flush. In addition, participants noted on the diary if the hot flush awoke them from sleep.

While taking the study medication, participants were contacted by telephone (or came to the clinic) at 2 and 8 weeks and had a clinic visit at 4 and 12 weeks to monitor adherence and adverse events. Medication packets were counted to assess adherence; adverse events were elicited and recorded.

Four weeks after discontinuing study medication, each participant was contacted by telephone to ascertain information

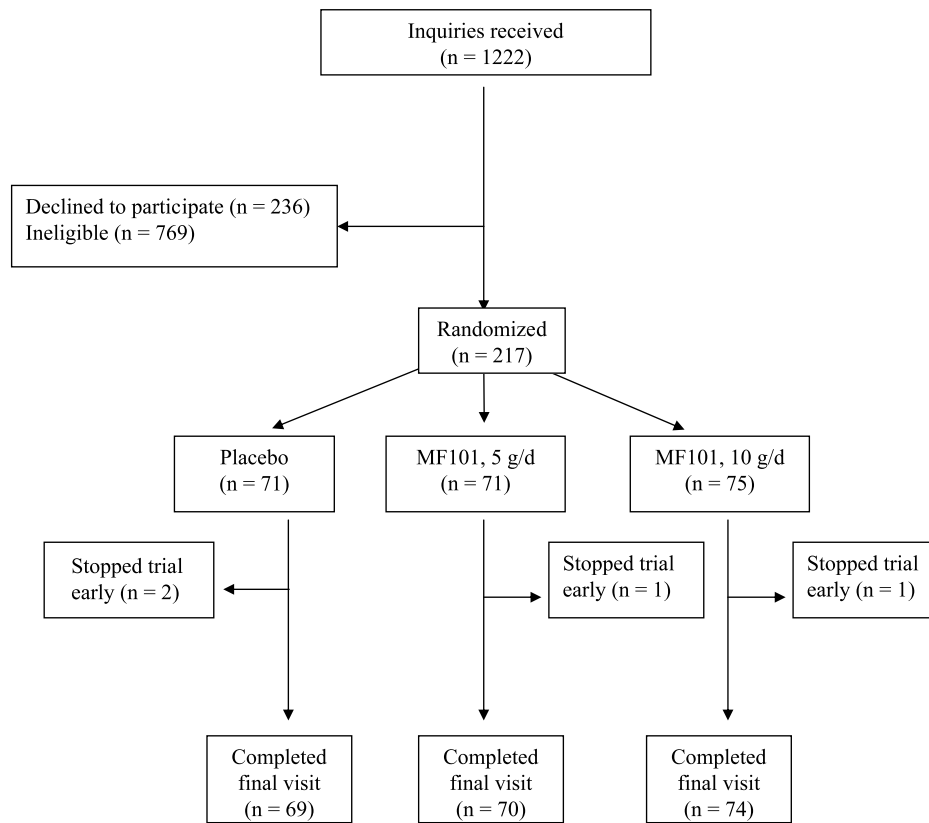


FIG. 1. Enrollment and follow-up in the Chinese Herbs in Menopausal Symptoms trial. MF101, menopausal formula 101.

on adverse events. Self-reported adverse events were classified using the Medical Dictionary for Regulatory Activities system.

Diagnostic endometrial biopsies were performed during the study if a participant reported vaginal spotting or bleeding, or if the final endometrial wall thickness measured by transvaginal sonography was more than 5 mm or had increased by 2 mm or more from baseline. Two blinded pathologists at the University of California, San Francisco, evaluated biopsy specimens independently. If the pathologists disagreed regarding histology, a third blinded pathologist reviewed the slide and made the final diagnosis. Adjudication by a third pathologist was required in 1 of the 24 pathology specimens.

Statistical analysis

The planned sample of 180 participants was estimated to provide 80% power to detect a between-group difference of 20 percentage points in the percent change in hot flush frequency from baseline to 12 weeks.

All analyses were by intention to treat, according to randomized assignment, without regard to adherence and without imputing or carrying forward missing values. No adjustment was made for multiple testing. Baseline characteristics of the participants were compared using *t* tests if the data were normally distributed or Wilcoxon tests if not.

Primary analyses compared changes from baseline to 4 and 12 weeks in frequency of hot flushes and hot flush score between each of the MF101 groups and the placebo group.

Because the outcomes were right-skewed, we used repeated-measures Poisson models with log link and terms for time (4 or 12 weeks vs baseline), treatment, and a time-by-treatment interaction, as well as clinical center and stratum.¹³ Primary analyses of secondary outcomes (quality of life and sexual function scores) used analogous methods.

In secondary analyses, we used analysis of variance controlling for site and stratum to compare rank-transformed percent change in number of hot flushes between the treated and placebo groups. We also used logistic regression models adjusted for clinical site and stratum to compare the proportions in each treatment group with a reduction in frequency of hot flushes of 50% or greater from baseline to 12 weeks.

The frequency of adverse events that occurred in more than 2% of any of the treatment groups was compared between treatment groups using a χ^2 test and exact methods as appropriate, stratified by clinical center and stratum.

In prespecified exploratory analyses, we used interaction terms to determine differences in the treatment effect (percent change in hot flushes at 12 wk) in subgroups, including age (45 - <50, 50 - <55, 55 - 60 ys), ethnicity (white, other), years since menopause (<2, \geq 2 ys), bilateral oophorectomy (yes, no), history of estrogen use (yes, no), smoking (current, former, or never), current alcohol use (yes, no), body mass index (tertiles), baseline serum estradiol level (\leq 5, >5 pg/mL), and baseline frequency of hot flushes (tertiles). All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Between February and October 2006, 217 eligible women were randomized: 71 to placebo, 71 to 5 g/day MF101, and 75 to 10 g/day MF101 (Fig. 1). The trial was completed by 98% of participants, and 91% took at least 75% of assigned study medication based on packet counts. There were no differences between groups in completion rate or adherence.

Participants were, on average, 54 years old, and 80% were white. At baseline, the mean (SD) daily frequency of hot flushes was 9.8 (3.7) and the mean (SD) daily hot flush score was 18.4 (8.7) (Table 1). The treatment groups were comparable at baseline ($P > 0.05$ for all between-group comparisons).

Hot flushes improved more in the treated groups than in the placebo group, but the differences were small and not statistically significant (Fig. 2). After 12 weeks of treatment, the mean percent decrease in the frequency of all hot flushes was 9.7% greater in the low-dose MF101 group ($P = 0.29$) and 12.9% greater in the high-dose MF101 group ($P = 0.15$) compared with that in the placebo group (Table 2). After 12 weeks of treatment, the mean percent decrease in the frequency of mild hot flushes was 27% greater in the low-dose MF101 group ($P = 0.06$) and 33% greater in the high-dose MF101 group ($P = 0.02$) compared with that of the placebo group. The mean percent decrease in the number of

moderate to severe hot flushes was also greater at 12 weeks in both the low-dose and high-dose MF101 groups compared with that in the placebo group, but the differences were small.

The median percent reduction in the number of hot flushes per week from baseline to 12 weeks of treatment was 37% in the placebo group, 37% in the 5 g/day MF101 group ($P = 0.25$ vs placebo), and 48% in the 10 g/day MF101 group ($P = 0.05$ vs placebo; Table 3). At 12 weeks of treatment, the median percent reduction in the number of hot flushes per week that awoke participants from sleep was 44% in the placebo group, 58% in the 5 g/day MF101 group ($P = 0.10$ vs placebo), and 67% in the 10 g/day MF101 group ($P = 0.05$ vs placebo). At 12 weeks of treatment, 31% of women in the placebo group had at least 50% reduction in frequency of hot flushes, compared with 39% in the 5 g/day MF101 group ($P = 0.29$ vs placebo) and 47% in the 10 g/day MF101 group ($P = 0.03$ vs placebo; Table 4).

At 12 weeks of treatment, there was greater improvement in the hot flush score in both treated groups (10.9% greater decrease in the 5 g/day MF101 group and 9.5% greater decrease in the 10 g/day MF101 group) compared with the placebo group, but these differences were small and not statistically significant (Table 2). There were no differences in the effectiveness of MF101 across subgroups defined at baseline by age, race, smoking, alcohol consumption, time since menopause, body mass index, baseline estradiol level, or prior use of estrogen therapy. The findings were unchanged when analyses were restricted to women who were at least 75% adherent to study medication.

The effects of treatment with MF101 on measures of quality of life and sexual function did not differ from those of placebo. Treatment with MF101 had no effect on change in estradiol levels from baseline to 12 weeks (increase of 1.1% in the placebo, 0.5% in the 5 g/d MF101, and 1.5% in the 10 g/d MF101 groups).

Of the 217 participants in the CHIMES study, 164 had a uterus. All of these women had transvaginal ultrasound at baseline, and all but three had this test repeated at the end of the trial. Mean (SD) endometrial thickness was 2.8 (1) mm at baseline and 3.1 (1.6) mm at 12 weeks; there were no differences between treatment groups in change in mean endometrial thickness. Of the 161 women who had transvaginal ultrasound at the end of the study, 21 were found to have a double-wall endometrial thickness of 5 mm or greater or to have endometrial thickness that increased by 2 mm or greater from the baseline measurement—3 women in the placebo group (6%), 7 (13%) in the 5 g/day MF101 group, and 11 (18%) in the 10 g/day MF101 group ($P = 0.05$ for comparison of 10 g/day vs placebo; Table 5). Endometrial biopsy was completed in 17 of these women; in 3 women, a biopsy was attempted but was unsuccessful (final endometrial thickness was 5.0, 5.4, and 5.6 mm in these women), and 1 woman refused biopsy (final endometrial thickness, 4.0 mm). During the study, 19 women reported vaginal bleeding or spotting. Endometrial biopsy was completed in 15 of these women; in the remaining 4 women,

TABLE 1. Baseline characteristics of participants in the Chinese Herbs in Menopausal Symptoms trial

Characteristic	Placebo (n = 71)	MF101	
		5 g/day (n = 71)	10 g/day (n = 75)
Age, mean (SD), y	53.7 (2.8)	53.9 (2.6)	53.6 (3.0)
Race/ethnicity, n (%)			
White	54 (76)	60 (85)	60 (80)
African American	9 (13)	7 (10)	12 (16)
Other	8 (11)	4 (5)	3 (4)
Education level, n (%)			
High school or less	12 (17)	11 (16)	15 (20)
Some college or college graduate	42 (59)	39 (55)	44 (59)
Graduate education	17 (24)	21 (30)	16 (21)
Time since menopause, mean (SD), y	4.6 (5.0)	4.4 (4.1)	4.1 (3.6)
Time since menopause <24 months, n (%)	24 (34)	26 (37)	26 (35)
Alcohol consumption, n (%)			
None	15 (21)	19 (27)	14 (19)
Less than once per week	27 (38)	17 (24)	24 (32)
One or more per week	29 (41)	35 (49)	37 (49)
Cigarette smoking, n (%)			
Never	40 (56)	45 (63)	38 (51)
Former	19 (27)	21 (30)	32 (43)
Current	12 (17)	5 (7)	5 (7)
Hysterectomy, n (%)	19 (27)	19 (27)	15 (20)
Bilateral oophorectomy, n (%)	10 (14)	7 (10)	6 (8)
Prior estrogen use, mean (SD), y	1.6 (4.1)	1.9 (4.0)	1.5 (3.3)
Hot flushes at baseline, n (SD)			
All hot flushes	10.0 (3.6)	9.6 (3.8)	9.7 (3.8)
Moderate to severe hot flushes	7.7 (3.8)	6.9 (4.4)	7.0 (3.6)
Hot flush score at baseline, mean (SD)	19.4 (8.4)	18.0 (9.7)	17.9 (7.8)

P values for comparison of 5 and 10 g/day to placebo were all >0.05 . MF101, menopausal formula 101.

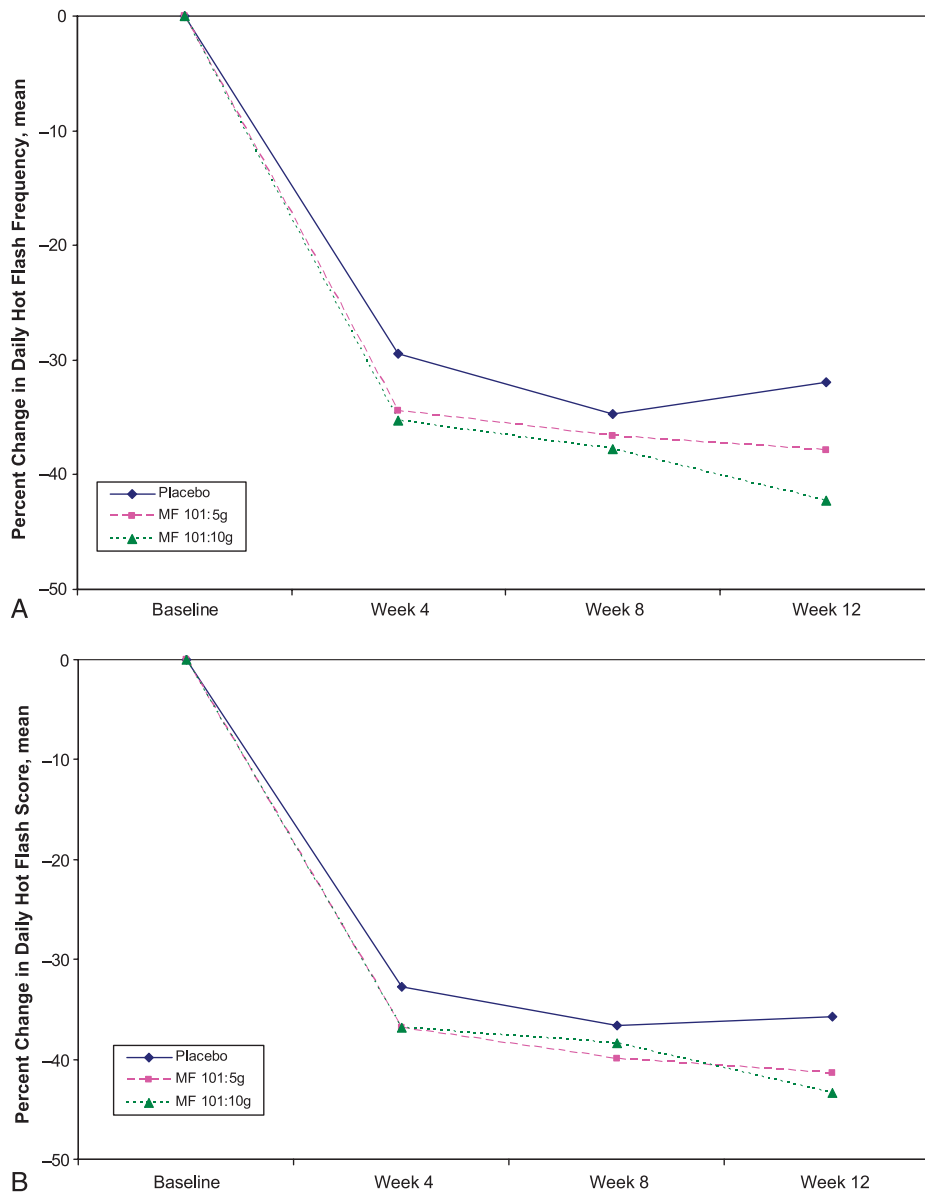


FIG. 2. **A:** Mean percentage change in number of hot flushes per day. **B:** Mean percentage change in hot flush score. MF101, menopausal formula101.

the clinical site gynecologist felt that biopsy was unnecessary given that final endometrial thickness was 3.0, 3.0, 3.7, and 4.0 mm.

Eight participants reported *both* vaginal bleeding and endometrial thickening and underwent only one biopsy for evaluation of both abnormalities. Thus, a total of 24 women underwent endometrial biopsy (7 in the placebo group, 6 in the 5 g/day MF101 group, and 11 in the 10 g/day MF101 group), and all had benign histologic findings (Table 6).

Three serious adverse events occurred during the trial: one participant in the placebo group was hospitalized for cellulitis; one participant in the 10 g/day MF101 group for idiopathic pancreatitis; and one in the 10 g/day MF101 group for axillary adenocarcinoma of the skin or subcutaneous tissue that was diagnosed on the biopsy of an axillary mass that the participant noted before starting study medication. There were no differ-

ences in the incidence of adverse events in the study groups, with the exception that women in both MF101 groups were more likely to report loose stools than were women in the placebo group (11.3% of women in the 5 g/d MF101 group, 12.0% of women in the 10 g/d MF101 group, and 2.8% of women in the placebo group; $P < 0.05$ for comparison of each MF101 group to the placebo group).

DISCUSSION

Treatment with the higher dose of MF101 reduced the median frequency of hot flushes and was more likely to result in at least a 50% reduction in the frequency of hot flushes compared with placebo. Treatment with MF101 did not improve quality of life, perhaps because the improvement in hot flushes was not adequate to have an impact on quality of

TABLE 2. Effect of treatment with MF101 compared with placebo on frequency and severity of hot flashes: percentage change from baseline to follow-up

Difference between placebo and MF101 ^a	5 g/day MF101 (n = 71), %	P ^b	10 g/day MF101 (n = 75), %	P ^b
All hot flashes				
Week 4	-10.0	0.21	-4.9	0.53
Week 12	-9.7	0.29	-12.9	0.15
Moderate to severe hot flashes				
Week 4	-9.8	0.41	0.0	1.00
Week 12	-3.6	0.81	-4.2	0.78
Mild hot flashes				
Week 4	-16.0	0.22	-19.2	0.13
Week 12	-26.9	0.06	-32.9	0.02
Daily hot flush score				
Week 4	-10.9	0.25	-2.0	0.84
Week 12	-10.9	0.35	-9.5	0.42
Hot flashes that awoke participant				
Week 4	-10.0	0.46	-6.8	0.58
Week 12	-17.6	0.24	-20.1	0.14

MF101, menopausal formula 101.

^a Percent change from baseline to follow-up in number of hot flashes or hot flush score, comparing change in the MF101 groups to that of the placebo group (change in placebo group minus change in MF101 groups) derived from repeated-measures, log-link generalized linear models adjusted for clinical center and years since menopause.

^b P value from repeated-measures, log-link Poisson generalized linear models with terms for time (4 or 12 wk vs baseline), treatment, clinical center, and years since menopause.

life. Given these findings, we believe that higher doses of MF101 warrant further investigation.

Strengths of this study include the facts that recruitment exceeded the estimated sample size, that retention in the study and adherence to study medication were excellent, and that there was no evidence of unblinding. Study power, however, may have been limited by assigning one third of participants to a dose of MF101 that seems to be too low to achieve an adequate clinical effect.

Although estradiol is an effective treatment for menopausal hot flashes,¹⁴ the currently approved SERMs tamoxifen and raloxifene increase the incidence of menopausal hot flashes.^{15,16} Because neither estradiol nor the SERMs are ER subtype selective, it is unclear which ER, ER- α or ER- β , mediates these effects. It has been shown that activation of ER- α by estrogen in human breast cancer cells results in proliferation and tumor formation, whereas activation of ER- β results in growth inhibition and no tumor formation.¹⁷

TABLE 3. Effect of treatment with MF101 compared with placebo on frequency of hot flashes: median percent change at 12 weeks of treatment

Treatment group	Median percent change ^a (IQR)	P ^b
Placebo	36.7 (56.4-6.1)	
5 g/day MF101	37.1 (67.7-15.1)	0.25
10 g/day MF101	48.4 (68.7-10.9)	0.05

MF101, menopausal formula 101; IQR, interquartile range (25th-75th percentile).

^a Median percent change (frequency of hot flashes per day at 12 wk of treatment minus frequency at baseline divided by frequency at baseline).

^b P value from analysis of variance controlling for site and stratum comparing rank-transformed percentage change in number of hot flashes between the treated and the placebo groups.

TABLE 4. Effect of treatment with MF101 compared with placebo on proportion of participants with at least 50% improvement in frequency of hot flashes

Treatment group	Frequency of hot flashes reduced by $\geq 50\%$	P ^a
Placebo	30.9%	
5 g/day MF101	39.1%	0.29
10 g/day MF101	47.1%	0.03

MF101, menopausal formula 101.

^a P value from logistic regression models comparing the proportion improved in the MF101 groups to that in the placebo group adjusted for clinical site and stratum.

Additional evidence is clearly needed, but this study provides the first data to suggest that hot flashes are relieved by ER- β -selective compounds that might be safer for the breast than unselective hormone therapies.

At the end of the trial, double-wall endometrial thickness was more likely to exceed 5 mm or to have increased at least 2 mm from baseline in the 10 g/day MF101 group compared with the placebo group ($P = 0.05$). The incidence of vaginal bleeding was the same among the three treatment groups, and no participant was found to have abnormal endometrial histology. Low levels of ER- β receptors have been identified in the endometrium,^{18,19} but the functional role of these receptors is unknown. In an ER- β mouse knockout model, no changes in uterine development were observed,²⁰ and treatment with MF101 does not increase uterine weight in mouse xenograft models.⁸ In our study, an increase in endometrial thickness was associated with higher baseline estradiol level (odds ratio, 1.12 per pg/mL increase, $P = 0.0025$) and an increase in serum estradiol level from baseline to week 12 (odds ratio, 1.10 per pg/mL, $P > 0.0001$), but treatment with MF101 had no effect on change in estradiol levels. Longer duration clinical trials that include endometrial sampling will be required to document the endometrial safety of MF101.

TABLE 5. Number of participants with vaginal bleeding or endometrial abnormalities in the Chinese Herbs in Menopausal Symptoms trial

Abnormality ^a	Placebo (n = 52 ^b)	5 g/day MF101 (n = 52 ^b)	10 g/day MF101 (n = 60 ^b)
Vaginal bleeding or spotting ^c	6	5	8
Endometrial biopsy completed	4	4	7
Thickened endometrium ^d	3	7	11 ^e
Endometrial biopsy completed	3	4	10

MF101, menopausal formula 101.

^a Thirty-two participants underwent endometrial biopsy for reported vaginal bleeding or thickened endometrium; 8 of these participants reported both vaginal bleeding and had thickened endometrium and had only one biopsy performed for evaluation of both abnormalities. Thus, a total of 24 biopsies were completed (7 in the placebo group, 6 in the 5 g/d MF101 group, and 11 in the 10 g/d MF101 group).

^b Number of participants with a uterus.

^c Vaginal bleeding or spotting reported on a daily diary at any time during follow-up.

^d Double-wall endometrial thickness > 5 mm at study end and/or thickness increased by ≥ 2 mm from baseline.

^e $P = 0.05$ compared with placebo using Fisher's exact test.

TABLE 6. Endometrial histology among women in the Chinese Herbs in Menopausal Symptoms trial

Histology on biopsy, n	MF101		
	Placebo (n = 7)	5 g/day (n = 6)	10 g/day (n = 11)
Insufficient tissue	1	0	0
Normal/benign	6	6	11
Inactive/atrophic	4	5	8
Proliferative	2	1	2
Secretory	0	0	1
Endometrial hyperplasia or cancer	0	0	0

MF101, menopausal formula 101.

The only other adverse effect associated with use of MF101 was loose stools, which may be due to the presence of soluble fiber.

CONCLUSIONS

This trial provides evidence that treatment with 10 g/day MF101 reduces the frequency of hot flushes in healthy postmenopausal women. This study provides the first evidence that the ER- β pathway may play a role in the treatment of hot flushes. Additional studies with higher doses are planned.

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APPENDIX

1. Composition of MF101

The herbal composition of MF101 in the amount required to prepare a 10-g dose and the percentages of the herbs in the formula are listed in Appendix Table. The 10-g dose is diluted with maltodextrin to obtain the 5-g dose.

2. Method of authentication

All botanical products are shipped to Bionovo's manufacturing site in Taiwan, and each herb is assigned a batch number. All herbs arrive with a certificate of authenticity from the provincial agricultural authorities of China. A photograph is taken of each herb and a professional botanist determines morphological identification.

Each individual herb of the formulation is extracted in water, ethyl acetate, or ethanol and identified against a taxonomic marker using thin layer chromatography. The taxonomic markers for each of the botanical agents in MF101 are established by either the manufacturer or by an industry-accepted chemical. For example, the taxonomic marker for *Herba Scutellaria Barbata* D. Don is Scutellarein (C15 H10 O6, molecular weight: 286.24), one of the chemical constituents found in the plant. For the herb to be accepted for inclusion in the formulation, the ID band of the taxonomic marker obtained from the plant extract was required to match the pure compound from the library with greater than 90% accuracy. In addition, the taxonomic marker was analyzed using high-performance liquid chromatography. A certificate of analysis for each individual herb was generated.

After aqueous extraction of the entire 22-herb formulation, additional analytical testing is performed using high-performance liquid chromatography and mass spectrometry to prepare a fingerprint of the final product and to follow the peaks of two known active compounds. Confirmation of the biological activity of the drug product is verified through a biological assay for ER- β activity. The final formulation is tested for the presence of microbiological organisms, heavy metals, and aflatoxins by the manufacturer and an independent FDA-approved laboratory to comply with regulatory standards.

All chemistry, manufacturing, and control documentation for MF101 has been submitted by Bionovo to the FDA

APPENDIX TABLE. Composition of MF101

Pin Yin ^a	Botanical name ^b	Family ^b	Pharmaceutical name ^c	Daily dose, g ^d	Percentage in formula ^e
Ban Zhi Lian	<i>Scutellaria barbata</i> D. Don	Lamiaceae	Herba Scutellaria Barbata	30	11.2
Shan Dou Gen	Sophorae Subprostratae or Tokinensis Gapnep	Leguminosae	Radix Sophora Subprostratae	15	5.6
Zhi Mu	<i>Anemarrhena asphodeloides</i> Bunge	Liliaceae	Radix Anemarrhena	12	4.5
Hei Dou	<i>Glycine soja</i> Sieb. Et Zucc.	Leguminosae	Semen Glycine Sojae	20	7.5
Gan Cao	<i>Glycyrrhizae uralensis</i> Fisch.	Leguminosae	Radix Glycyrrhiza	8	3
Da Huang	<i>Rheum palmatum</i> L.	Polygonaceae	Rhizoma Rhei	8	3
Fu Xiao Mai	<i>Triticum sativum</i> L.	Gramineae	Fructus Triticis Levis	15	5.6
Huang Qi	<i>Astragalus membranaceus</i> Fisch. Bge. Var. mongolicus Bge.	Leguminosae	Radix Astragali	12	4.5
Sheng Di Huang	<i>Rehmannia glutinosa</i> Libosch.	Scrophulariaceae	Radix Rehmannia	12	4.5
Nu Zhen Zi	<i>Ligustrum lucidum</i> Ait.	Oleaceae	Fructus Ligustri Lucidi	15	5.6
Suan Zao Ren	<i>Ziziphus jujuba</i> Mill. Var spinosa Bunge Hu ex H.F. Chou	Rhamnaceae	Semen Zyziphi Spinozae	10	3.7
Lian Zi Xin	<i>Nelumbo nucifera</i> Gaertner	Nymphaeaceae	Plumula Nelumbinis	10	3.7
Fu Ling	<i>Poria cocos</i> Schw. Wolf	Polyporaceae	Poria Cocos	10	3.7
Ze Xie	<i>Alisma orientalis</i> Sam. Juzep.	Alismataceae	Rhizoma Alismatis	10	3.7
Mu Dan Pi	<i>Paeonia suffruticosa</i> Andr.	Ranunculaceae	Cortex Moutan Radicis	8	3
Shan Zhu Yu	<i>Cornus officinalis</i> Sieb. Et Zucc.	Cornaceae	Fructus Corni	10	3.7
Huai Niu Xi	<i>Achyranthes bidentata</i> Bl.	Amaranthaceae	Radix Achyranthis	10	3.7
Mu Li	<i>Ostrea gigas</i> Thunberg	Osteridae	Concha Ostrea	12	4.5
Tian Men Dong	<i>Asparagus cochinchinensis</i> Lour. Merr.	Liliaceae	Radix Asparagi	12	4.5
Ge Gen	<i>Pueraria lobata</i> Willd. Ohwi	Leguminosae	Radix Pueraria	10	3.7
Bai Zhu	<i>Atractylodes macrocephala</i> Koidz	Compositae	Radix Atractylodis Macrocephala	10	3.7
Yin Yang Huo	<i>Epimedium brevicornum</i> Maxim.	Berberidaceae	Herba Epimedi	8	3
Total				267	100

For medicinal use, plant parts are selected for their particular pharmaceutical applications. The English translations of the plant parts are the following: *herba*, whole aerial part of the plant; *radix*, root; *semen*, seed; *rhizome*, tuber; *fructus*, fruit; *plumula*, flower bud; *cortex*, bark; *concha*, shell.

^a Pin Yin is the Romanization system accepted by the People's Republic of China.

^b Herb names are written in accordance with the international code for botanical nomenclature (Tokyo Code, 1994).

^c Herbs are sold under their pharmaceutical name, which indicates plant part and genus but not always the particular species and never the family.

^d Dose of dry plant before extraction to extract the 10-g dose of MF101. This is diluted by 50% to reach the 5-g dose.

^e Percentage of the dry plant material before extraction.

Division of Reproductive and Urologic Drug Products as part of the investigational new drug application number 58,267 and is held in a confidential drug master file. The final high-performance liquid chromatography fingerprint of MF101 is part of Bionovo's proprietary information and a critical component of the company's intellectual property included in a pending patent application to the US Patent and Trademark Office.

3. Method of preparation

The formulation is mixed with distilled water in a ratio of 1 part herb to 10 parts water (1:10 herb/H₂O) and boiled for

60 minutes at 70°C to 75°C in an airtight, stainless-steel tank. The liquid extract is decanted to remove insoluble material and then is pressure filtered through a 20-mesh filter three times to remove any residual insoluble material. As a final step, the extract is freeze dried and FDA-approved caramel coloring and food dyes are added to the dry powder to reach a uniform color, and citrus flavorings and sweeteners are added to mask the bitter taste of the herbs. The powder is then packaged in a heat-sealed, polyethylene-lined aluminum pouch.

A retention sample of MF101 is stored at Bionovo, Suite 375, 5858 Horton St, Emeryville, CA 95608.