

EXPERT OPINION

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MF101: a multi-component botanical selective estrogen receptor beta modulator for the treatment of menopausal vasomotor symptoms

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Introduction: The Women's Health Initiative Estrogen Plus Progestin clinical trial demonstrated the risks exceeded the benefits which have led to a decline in menopausal hormone therapy (MHT) by greater than 50%. MHT use was initiated long before there was a significant understanding of the molecular mechanisms of estrogens. It has become clear that the problem with the current estrogens in MHT is they act non-selectively as an agonist in all tissues that contain estrogen receptors. MF101 is an oral, botanically derived extract that was designed to selectively regulate estrogen receptor beta (ER β) because the increased risk of breast and endometrial cancer is due to the activation of estrogen receptor alpha (ER α) by estrogens. Preclinical and clinical data support a role for selective ER β agonists, such as MF101, for vasomotor symptoms without increasing cancer risks.

Areas covered: The review covers the biological, pharmacological and clinical advantages of MF101, and the unique ability of MF101 to selectively target the ER β pathway for the treatment of hot flashes (HF).

Expert opinion: Preclinical and clinical studies indicate that MF101, a selective estrogen receptor beta agonist, represents a new class of drugs that is safe and effective for treating HF and nighttime awakenings.

Keywords: botanical, estrogen receptor, estrogen receptor beta, hot flash, menopausal hormone therapy, menopause, MF101, vasomotor symptoms

Expert Opin. Investig. Drugs [Early Online]

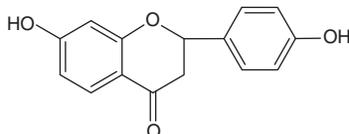
1. Introduction

Currently, over 40 million women in the USA and 37 million women in Europe are transitioning through menopause. Approximately 75% of them are experiencing vasomotor symptoms, including hot flashes (HF), night sweats and associated insomnia [1]. On average, HF persist for 5 – 10 years after their onset. The menopausal transition is a genetically programmed cessation of reproductive capacity [2,3] that occurs at an average age of 51 years [4-6]. While most mammals experience a decrease in the frequency of gestation and smaller, less healthy litter sizes, a few primates and humans go through menopause, which is due to atresia of ovarian follicles and cessation of ovarian production of estrogens [7-10]. Interestingly, as a result of the dramatic increase in life span of women since the early 20th century, women spend on average 38% of their life in menopause [11], while other primates who undergo menopause spend 0 – 13% of their life span in menopause [12-15]. The transition to menopause leads to anatomical, physiological and biochemical changes related to the aging process, which result in significant increased risks for aging-related disorders such as cancer, cardiovascular disease, diabetes, obesity,

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Box 1. Drug summary.

Drug name	MF101
Phase	Phase III clinical trial
Indication	Menopausal symptoms
Pharmacology description	Estrogen receptor beta agonist
Route of administration	Oral
Chemical structure	Chemical name: 4H-1-benzopyran-4-one, 2,3-dihydro-7-hydroxy-2-(4-hydroxyphenyl)-, (S)-(liquiritigenin)



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osteoporosis, urogenital atrophy, immune suppression, cognitive decline and symptoms such as insomnia, depression, decreased libido and urinary incontinence.

HF are a brief experience of body surface temperature irregularity resulting in fever like redness, sweating, and a prickly sensation that lasts from 4 to 10 min. The experience may be accompanied by other symptoms such as anxiety, irritability, palpitations, blushing, panic and a loss of sensation, along with significant physical and emotional distress [16,17]. A modest, 0.9°C, change in core body temperature occurs 7 – 20 min prior to the sensation of the HF [18]. The change in temperature increases energy expenditure and respiratory quotient. Generalized peripheral vasodilatation appears in the first few seconds of the experience and then sweating and an increase in skin electric conductance are observed [19-24]. Oophorectomy or hormonal deprivation agents such as gonadotropin-releasing hormone (GnRH) analogs [25], selective estrogen receptor modulators (SERMs), aromatase inhibitors [26,27] and chemotherapeutic agents, which result in ovarian failure, can lead to HF in both men and women [28]. Although directly associated with female reproductive senescence and decline in circulating estrogen, it is unclear how estrogen deficiency causes HF. The frequency and severity of the experience vary among women. Approximately 30% of women experience severe symptoms (> 50 moderate to severe HF per week). The autonomic nervous system may play a role in HF, but a neurological basis is lacking. Heart rate variability significantly decreased during HF relative to periods preceding and following physiologic HF [29,30]. A role for α_2 -adrenergic receptors in noradrenergic receptors [31] and a decline in glucose transport across the blood-brain barrier resulting in energy fluctuations and loss of temperature control

has been suggested to be involved in the formation of HF [32]. The precise mechanisms that cause HF have been difficult to elucidate because the loss and maintenance of homeothermic control results in activation and adaptation of multiple physical (breathing rate, cardiac output, vasodilation, sweating, mineral flux, ion channels), chemical (hormones, neurotransmitters, ATP, glucose) and biological (metabolic rate, receptor proteins, transport proteins, enzymes and genes) feedback mechanisms attempting to restore equilibrium [22,33,34]. These changes are interdependent on multiple variable parameters which differ among species and individuals.

Recently, it has been suggested that women experiencing HF are at higher risk for cardiovascular disease, type 2 diabetes, osteoporosis and early dementia [35,36], while at lower risk for breast cancer [37,38]. Although not life threatening, HF should be viewed as a marker of health decline and a risk factor for age-associated diseases in women. Menopausal hormone therapy (MHT), which is the only Food and Drug Administration (FDA) approved treatment for HF has been used since the 1940s to treat menopausal symptoms and later to prevent osteoporosis. Menopausal women with an intact uterus are most often prescribed a combination of estrogen and progestin, as unopposed estrogen therapy increases the risk of endometrial cancer. The National Institutes of Health (NIH) established the Women's Health Initiative (WHI) in 1991 to address the most common causes of death, disability and impaired quality of life in postmenopausal women. The WHI trials were designed to evaluate the risks of cardiovascular disease, cancer and osteoporosis in postmenopausal women. The WHI was a 15-year, multi-million dollar endeavor, and the aggregate of the studies have become the largest US prevention studies in women. After 60 years of physician's prescribing MHT for menopausal symptoms, the WHI trial evaluated two different regimens of MHT, conjugated equine estrogen (CEE) plus medroxyprogesterone acetate (MPA) and CEE alone in two separate randomized, double-blind, placebo-controlled trials to assess the preventive effects of these therapies. Since the primary risk reduction to be evaluated in the WHI hormone therapy studies was cardiovascular disease, the trial designed called for an older postmenopausal patient population in order to observe an adequate number of cardiovascular events. The average age of the cohort of women in the WHI trial evaluating CEE plus MPA treatment was 63. Women on average become postmenopausal at age 51 which is also the age when women commonly seek treatment for vasomotor symptoms. In 2002, the original results of the WHI trial demonstrated that combination MHT increases the risks for breast cancer, cardiovascular disease, stroke, venous thromboembolic events and early dementia [39,40]. In a second WHI study, the administration of CEE-only-based MHT among a cohort of women with hysterectomies in the WHI trial led to an increased risk for stroke and venous thromboembolism [41]. Both WHI studies were stopped early on the recommendation of the independent Data Safety Monitoring Board (DSMB) which

judged that the risks of MHT outweighed its benefits. Following publication of these results, the FDA and European Medicines Agency (EMA) recommended using MHT in the lowest possible dose, for the shortest possible time period. Despite the fact that the WHI CEE plus MPA data showed that the increased risks for cardiovascular disease were limited to older women and not younger postmenopausal women [40], the seven serious black box warnings imposed by the FDA on all MHT packet inserts have made clinicians reluctant to prescribe MHT and women fearful of the serious side effects. After the publication of the WHI data in July 2002, patient use of hormone therapy has dropped 52% [42]. The decline in MHT use among menopausal women has been associated with an 8.6% decrease in age-adjusted breast cancer incidence in the USA [43-46].

Two other non-hormonal therapies in Phase III clinical testing (Pristiq and Serada) have been found to be unacceptable treatments for vasomotor symptoms. On 16 September 2011, the FDA reviewed Pfizer's new drug application (NDA) for Pristiq and the Agency did not approve the antidepressant (serotonin and norepinephrine reuptake inhibitor (SNRI)) for the treatment of postmenopausal vasomotor symptoms. Depomed announced on 13 October 2011 that Serada (gabapentin in an extended-release formulation) did not meet the end points of its FDA Special Protocol Assessment for a third Phase III trial. Depomed's previous two Phase III trials did not confirm gabapentin's superiority when compared with placebo. Bionovo's MF101 and Noven's paroxetine (Paxil, selective serotonin reuptake inhibitor (SSRI)) are the only non-hormonal drugs currently in Phase III clinical testing for the treatment of menopausal HF. MF101 is a multi-component botanical selective estrogen receptor beta (ER β) agonist (Box 1). MF101's mechanism of action to potentially decrease HF is by selectively regulating the ER β pathway while leaving the estrogen receptor alpha (ER α) pathway unaffected which is a major distinction between estrogens currently used in MHT that act as non-selective ER α and ER β agonists. MF101's unique ability to selectively regulate the ER β pathway gives it a more favorable safety profile than MHT because the activation of ER α by estrogens used in MHT results in elevated risks for breast and uterine cancer and possibly blood clots.

2. Herbal medications, alternative therapies and botanical drugs

Botanical remedies have been in medical use for millennia. Most cultures employed the use of botanical preparations throughout human history. Recently, the World Health Organization estimated that 70 – 80% of the world population relies on traditional botanical medicine to treat all of their illnesses and diseases (WHO, 2008). Botanical medicine can be divided into two categories: shamanistic/experiential approaches and medical systems such as Greek, Arabic, Indian and Chinese medicines. Systems of medicine

have long-standing histories with an extensive clinical practice, substantial literature, internal criticism as well as academic and apprentice-based programs. The advent of modern biomedical medicine did not eliminate the use of these alternative modalities [47]. In recent surveys, it has been estimated that Americans pay more visits to alternative practitioners than to medical doctors with 629 million visits per year [48]. It has been reported that 50 – 80% of women use botanical alternative therapies for the treatment of menopausal symptoms [49-51]. The most common therapies for menopausal symptoms are soy phytoestrogens, black cohosh, evening primrose oil, red clover, progesterone creams made from the Mexican yam and vitamin E oil [49]. Several randomized placebo-controlled trials were conducted with most of the above-mentioned therapies in multiple doses and preparations. Although the short-term safety is clearly superior to MHT, none of the studies showed efficacy over placebo. It is important to note that these studies were mostly opportunistic and used commercially marketed nutritional supplements. Serious investigation into the pharmacology and dosing of these products has never appropriately tested.

In 2004, the FDA issued a Guidance to Industry for the development of botanical drug products. The guidance allows sponsors to enter Phase II clinical testing with minimal preclinical toxicology studies due to the fact that these products were used in human for centuries. Prior to initiation of pivotal Phase III studies, the botanical drug has to complete and comply with all FDA requirements for preclinical and clinical toxicology. The clinical development path for botanical drugs is similar to that of a new chemical entity (NCE) and biologic agent. Bionovo's MF101 has been developed under the botanical drug guidance and thus, is setting precedence for this new FDA regulatory path for drug development. The most significant difference between NCE, biologics and botanical drugs lies in the manufacturing and characterization of such drugs. Botanicals are manufactured from agricultural plants which are inherently variable and contain multiple chemical substances. Controlling the sources and chemical variability as well as sufficiently characterizing the final drug product requires complex analytical tools. The MF101 drug development strategy embraces traditional knowledge from Chinese medicine, which was the primary form of medical intervention over many centuries, by very large populations across Asia. Their approach is to harness modern biological rationale and analytical technologies to develop a new form of therapy exploiting and integrating both traditional and modern forms of knowledge, under regulatory and scientific scrutiny. This approach differs from traditional pharmacognosy and opportunistic testing of existing nutritional supplements. It also provides a bridge to the medical community and regulators when addressing women who wish to take safer therapies for a non-life-threatening symptom such as menopausal HF.

3. MF101 drug product

MF101 is a lyophilized powder of purified aqueous extracts of botanical raw materials. MF101 is manufactured from 22 botanical raw materials (Table 1), consisting of 20 botanical species, 1 fungus and a mineral. The lyophilized powder is blended with flavoring ingredients to mask the bitter taste of the active components. Bionovo claims that many, but not all the active ingredients in MF101 have been identified. MF101 and its active chemical ingredients characterized so far are selective ER β agonists. The company claims that the structures of the actives are varied, yet none are steroidal-like estrogens made in humans. Moreover, MF101 does not interact with other steroid nuclear receptors such as the thyroid receptor, glucocorticoid receptor, androgen receptor and the progesterone receptors [52]. The only published structure isolated from one of the species, the root of *Glycyrrhiza uralensis*, liquiritigenin, shows high selectivity to the ER β [53].

4. Mechanism of action of estrogen receptors

Estrogen effects are mediated through estrogen receptor ER α or ER β , which are members of the steroid nuclear receptor superfamily [54]. ER α contains 595 amino acids, whereas ER β contains 530 amino acids. Both ERs are modular proteins made up of three distinct domains. The amino-terminus domain (A/B domain) is the least conserved region, exhibiting only a 15% homology between ER α and ER β . This domain harbors an activation function (AF-1) that can activate gene transcription in the absence of estradiol or presence of SERMs, such as tamoxifen [55]. The central region, known as DNA-binding domain (DBD) contains two zinc finger motifs that allow ERs to bind directly to DNA in target genes. The DBD in ER α and ER β are virtually identical, exhibiting 95% homology. The carboxy-terminus domain contains the ligand-binding domain (LBD), which carries out several essential functions. The LBD forms a large hydrophobic cavity where estrogenic compounds bind. The LBD also contains a second activation function (AF-2) that serves as a binding site for coregulatory proteins [56] that are required for estrogen activation [57] and repression [58] of gene transcription. The LBDs of ER α and ER β are only about 55% homologous. The striking differences in the amino acid composition of the ER α and ER β LBDs may have evolved to create ERs that have distinct transcriptional roles. This would permit ER α and ER β to regulate the activity of different genes and to elicit different physiological effects. The notion that ER α and ER β evolved to produce different biological roles is supported by studies with ER α and ER β knockout mice. For example, the ER α knockout mice have primitive mammary and uterine development [59], whereas the ER β knockout mice develop normal mammary glands and uterus [60].

ERs regulate physiological processes by two major pathways. The best characterized mechanism of action for ERs is known as the genomic pathway. In the genomic pathway, estrogens

act by binding to ERs in the nucleus of cells which then regulate the transcription of target genes. The change in target gene mRNA levels by estrogens leads to an increase or decrease in the production of specific proteins that ultimately alter the activity of the cells. For example, after estrogens bind to ERs, the estrogen/ER complex can activate growth-promoting genes in mammary gland and endometrial cells, which causes cells to proliferate thereby increasing the risk of breast and endometrial cancer. Estrogens regulate gene transcription by promoting a series of steps required for gene regulation. The first step is initiated by the binding of an estrogenic ligand to the ER. This produces a conformational change in ER that allows it to bind to a specific site on target genes known as a regulatory element. The best characterized regulatory element is known as the estrogen response element (ERE), which consists of a 13-nucleotide inverted palindromic sequence. Once the liganded ER complex is bound to the ERE, then helix 12 in the LBD moves to a position that creates a docking site for the binding of coregulatory proteins [61], which mediate the regulation of target genes. There are two major classes of coregulatory proteins. Coactivator proteins bind to ERs and activate the target gene, whereas corepressor proteins lead to the repression of target genes [61]. Coregulatory proteins work by altering the chromatin structure surrounding the target gene leading to a change in the recruitment of additional factors required for gene transcription.

The second pathway for the mechanism of action of estrogens is the non-genomic pathway [62,63]. In this pathway, rather than binding to ERs in the nucleus, estrogens bind to ERs in the membrane. Once estrogens bind to membrane ERs, it can cause non-genomic effects by regulating ion transport channels or signal transduction pathways, such as the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/AKT, alpha-Ca²⁺/calmodulin-dependent kinase II and protein kinase C (PKC) pathways [62,63]. The regulation of these pathways leads to a change in the intracellular levels of ions or second messengers that ultimately promote a functional change in cells.

4.1 MF101: preclinical studies

Studies detailing the mechanism of action and ER subtype selectivity of MF101 have been conducted in cancer cell lines, neurons derived from embryonic stem cells and mouse models. These studies demonstrated that MF101 is a unique drug because it acts as a selective ER β agonist, whereas all forms of estrogens used in MHT regulate both ER α and ER β .

4.2 MF101 is a selective ER β agonist in transfection studies

In transfection assays, U2OS, HeLa, MDA-MB-453 or Ishikawa cell lines were cotransfected with a classical ERE upstream of a minimal thymidine kinase (tk) promoter (ERE-tk-Luc) and expression vectors for human ER α or ER β . MF101 produced a dose-dependent activation of

Table 1. Composition of MF101.

Pin Yin Name	Family	Botanical name	Plant part	% in formula
Ban Zhi Lian	Lamiaceae	<i>Scutellaria barbata</i>	Aerial parts	11.2
Bai Zhu	Asteraceae	<i>Atractylodes macrocephala</i>	Rhizome	3.7
Da Huang	Polygonaceae	<i>Rheum palmatum</i>	Rhizome	3.0
Fu Ling	Polyporaceae	<i>Wolfiporia extensa</i>	Sclerotium	3.7
Fu Xiao Mai	Poaceae	<i>Triticum aestivum</i>	Fruit	5.6
Gan Cao	Fabaceae	<i>Glycyrrhiza uralensis</i> , <i>G. glabra</i> (and interspecific hybrids)	Root	3.0
Ge Gen	Fabaceae	<i>Pueraria montana</i> var. <i>lobata</i>	Root	3.7
Hei Dou	Fabaceae	<i>Glycine max</i>	Seed	7.5
Huai Niu Xi	Amaranthaceae	<i>Achyranthes bidentata</i>	Root	3.7
Huang Qi	Fabaceae	<i>Astragalus mongholicus</i>	Root	4.5
Lian Zi Xin	Nelumbonaceae	<i>Nelumbo nucifera</i>	Plumule	3.7
Mu Dan Pi	Ranunculaceae	<i>Paeonia suffruticosa</i>	Root bark	3.0
Mu Li	Ostreidae	<i>Crassostrea gigas</i>	Shell	4.5
Nu Zhen Zi	Oleaceae	<i>Ligustrum lucidum</i>	Fruit	5.6
Sheng Di Huang	Schrophulariaceae	<i>Rehmannia glutinosa</i>	Root	4.5
Shan Dou Gen	Fabaceae	<i>Sophora tonkinensis</i> var. <i>tonkinensis</i>	Root	5.6
Shan Zhu Yu	Cornaceae	<i>Cornus officinalis</i>	Fruit	3.7
Suan Zao Ren	Rhamnaceae	<i>Ziziphus jujuba</i>	Seed	3.7
Tian Men Dong	Asparagaceae	<i>Asparagus cochinchinensis</i>	Root	4.5
Yin Yang Huo	Berberidaceae	<i>Epimedium</i> spp. (in <i>E. brevicornu</i> clade)	Aerial parts	3.0
Zhi Mu	Liliaceae	<i>Anemarrhena asphodeloides</i>	Rhizome	4.5
Ze Xie	Alismataceae	<i>Alisma plantago-aquatica</i> var. <i>orientale</i>	Rhizome	3.7
				100

ERE-tk-Luc with ER β to the same magnitude as E₂ treatment, whereas no activation was observed with ER α [52]. The ER antagonists, ICI 182,780, raloxifene and tamoxifen, blocked the activation ERE-tk-Luc by MF101. These studies indicated that the transcriptional effect of MF101 is mediated through ER β .

4.3 MF101 is a selective ER β agonist on endogenous target genes

The effect of MF101 on gene transcription was also examined on endogenous genes using U2OS cells stably transfected with a doxycycline-inducible ER α or ER β [52]. Doxycycline was used to induce the production of ER α or ER β . The *keratin 19* gene is a known estrogen target gene, which was used to investigate the ER subtype selectivity of MF101. E₂ activated the *keratin 19* gene in both U2OS-ER β and U2OS-ER α cells demonstrating that it non-selectively activates both ER α and ER β [52]. By contrast, MF101 produced a dose-dependent increase in keratin 19 mRNA only in the U2OS-ER β cells. In addition to activating genes, estrogens exert anti-inflammatory properties by repressing the expression of inflammatory genes, such as *TNF α* and *IL-6*. Similar to the findings with activated genes, MF101 also repressed the *TNF α* and *IL-6* genes in the U2OS-ER β cells but not in the U2OS-ER α cells [52]. These studies demonstrate that MF101 selectively activates and represses several genes through ER β . However, it is important to demonstrate that MF101 exhibits ER β selectivity on a wide range of genes. To investigate the effects of MF101 on the regulation of

numerous genes, U2OS ER α or U2OS ER β cells were treated with MF101 and then the number of regulated genes was determined using microarrays. E₂ regulated 489 specific genes in the U2OS-ER α cells and 200 genes in U2OS-ER β cells compared with untreated cells, demonstrating that it acts as a non-ER selective agonist [64]. MF101 weakly activated only 13 genes in U2OS-ER α cells whereas it regulated 382 genes in U2OS-ER β cells [64]. These studies demonstrated that MF101 is a highly ER β selective regulator of gene transcription.

4.4 MF101 binds equally to ER α and ER β

The findings from transfection assays and gene regulation studies demonstrate that MF101 is a selective ER β agonist on gene regulation, despite being a complex, crude plant extract. Most drugs exhibit receptor subtype selectivity by binding to one receptor with a greater affinity. In fact, several synthetic ER β selective agonists have been discovered that bind to ER β with a much greater affinity to ER β compared with ER α [65,66]. Surprisingly, MF101 binds to both ER α and ER β with a similar affinity, demonstrating that its ER β selectivity does not occur through the selective binding to ER β [52].

4.5 MF101 induces an active conformational change only with ER β that leads to the selective recruitment of coregulatory proteins

Another possible explanation for the ER β selectivity of MF101 is that it produces different conformations in ER α and ER β , such that only the conformation in ER β is

Table 2. MF101 doses used in Bionovo's clinical program.

Clinical trial	Phase of clinical testing	Doses used in trial	Equivalent dose to Phase III drug product
MF101-001	Phase I	5 g/day	1.25 g/day
MF101-002	Phase II	Dose 1: 5 g/day Dose 2: 10 g/day	2.5 g/day 5.0 g/day
MF101-003	Single ascending dose pharmacokinetics	Dose 1: Single 5 g dose Dose 2: Single 10 g dose Dose 3: Single 20 g dose	Single 2.5 g dose Single 5 g dose Single 10 g dose
MF101-008	Phase I tolerability	Dose 1: 10 g/day Dose 2: 15 g/day	Dose 1: 10 g/day Dose 2: 15 g/day
MF101-009	Phase I tolerability	Dose 1: 5 g/day Dose 2: 10 g/day	Dose 1: 5 g/day Dose 2: 10 g/day
MF101-004	Phase III		Dose 1: 5 g/day Dose 2: 10 g/day

functional. Fluorescence resonance energy transfer and protease digestion are two different methods that are used to assess the conformational change of a protein that occurs after ligand binding. The formation of an active conformation of ER is required for transcriptional regulation, because a proper conformation is necessary for ER to bind to the regulatory element in a target gene and promote the subsequent recruitment of coregulatory proteins. Both techniques demonstrated that MF101 produces an active conformational change in ER β , but not ER α even though it binds to both ERs [52]. To further demonstrate that MF101 was capable of producing an active conformation, a chromatin immunoprecipitation assay found that MF101 stimulated the binding of ER β but not ER α and the recruitment the coactivators, GRIP1 and CBP to the *keratin 19* gene with ER β [52]. By contrast, MF101 did not recruit ER α or recruit coactivators in U2OS-ER α cells [52]. These results demonstrate that MF101 produces a conformation in ER β but not in ER α that allows the MF101-ER β complex to bind to regulatory elements in target genes and recruit coregulatory proteins that activate target genes. Therefore, the ER β selective activity of MF101 results from its capacity to produce an active conformational change in ER β that permits the recruitment of coregulatory proteins. When MF101 binds to ER α , the conformational change that occurs is inactive.

4.6 MF101 does not stimulate MCF-7 cell tumor formation or uterine growth in mouse xenograft models

Another way to assess the ER β selectivity that is clinically relevant is to determine if MF101 increases proliferation of ER positive breast cancer cells and uterine cells. The MCF-7 breast cancer cell line is an excellent cell model to assess the proliferative effects in response to estrogens. It is known that estrogens promote proliferation of MCF-7 cells by binding to ER α , because MCF-7 cells that express only ER α proliferate in response to estrogen. Furthermore, if ER β is expressed along with ER α , the proliferative response to estrogens is

abolished [67], demonstrating that ER β blocks the proliferative effects of ER α . ER α causes MCF-7 cells to proliferate by activating key proliferative genes, such as *c-myc* and *cyclin D1* [67]. This leads to the increased production of *c-myc* and cyclin D1 which cause the cells to progress through the cell cycle. Unlike E₂, MF101 does not stimulate cell proliferation of MCF-7 cells or activate the *c-myc* or *cyclin D1* genes [52]. In addition, MF101 did not increase tumor formation of MCF-7 cells grafted in nude mice [52]. Another known process that is mediated by ER α is the stimulation of the mouse uterine size, which does not occur in ER α knockout mice treated with E₂ because of the absence of ER α . MF101 did not increase uterine size in mice treated for 1 month [52]. These results demonstrated that MF101 did not promote proliferation of MCF-7 or uterine cells, which are known adverse effects mediated by ER α , and provide additional evidence that MF101 is an ER β selective agonist.

4.7 MF101 acts as an ER β selective agonist in neurons

To develop alternative estrogens for treating HF, it is important to demonstrate that they can act directly on neurons because the beneficial effects of estrogens are likely mediated through a direct action on neurons involved in thermoregulation. Because of the difficulty in obtaining neurons from humans, the effects of MF101 were examined on neurons derived from human and mouse embryonic stem cells [68]. Immunoprecipitation and western blot analyses demonstrated neurons derived from human and mouse embryonic stem cells express ER β . The addition of MF101 to the neurons produced a rapid increase in the frequency of calcium oscillations similar to E₂ [68]. These results demonstrate that MF101 can act directly with ER β in neurons derived from human and mouse embryonic stem cells to elicit non-genomic effects. Based on the findings from transcription studies in cancer cells, animal models and neuronal studies, MF101 was shown to act as an ER β selective agonist of genomic and non-genomic ER pathways.

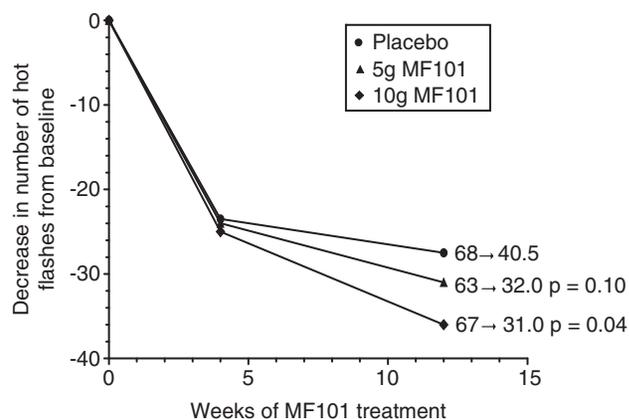


Figure 1. Median change in number of hot flashes per day after 12 weeks of treatment.

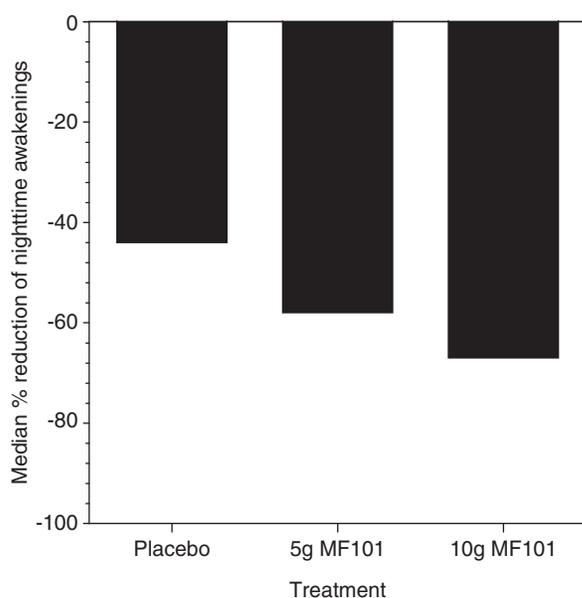


Figure 2. Median percent reduction in number of nighttime awakenings after 12 weeks of treatment.

5. MF101: pharmacokinetics

Since MF101 contains multiple active ingredients and other chemical constituents covering a wide concentration range, a comprehensive assessment of the drug's pharmacokinetic profile is difficult. It is interesting to note that several metabolites of liquiritigenin were isolated from *G. uralensis* root [69], mostly different sugar conjugates, and these had longer T_{max} and varying C_{max} in rodent pharmacokinetic studies. Such sugar conjugates showed lower to no activity on ER β in *in vitro* models suggesting that enzymatic cleavage of the sugar moieties is required. This would suggest that these natural analogs are pro-drugs and exert pharmacological benefit

only after simple deconjugation of the sugar moieties. It is reasonable to expect that such compounds will mainly be cleared from the system via Phase II conjugation reactions.

5.1 MF101: summary of clinical trial results

Five well-controlled human clinical trials evaluating MF101 have been conducted and all have demonstrated excellent safety, tolerability and efficacy (Table 2). A multi-center, randomized, double-blind, placebo-controlled Phase III clinical trial evaluating MF101 for postmenopausal vasomotor symptoms among 1200 healthy women was launched in October 2011. This review will summarize the clinical data from the Phase II clinical trial only.

5.2 Phase II clinical trial design

The Phase II clinical trial was designed to evaluate the safety and efficacy of two doses of MF101 versus placebo and was conducted under the direction of Dr. Deborah Grady at the University of California, San Francisco, CA, USA. The trial was a randomized, double-blind, placebo-controlled study that enrolled 217 healthy postmenopausal women at 6 clinical sites in the USA who reported at least 7 moderate to severe HF per day or 50 moderate to severe HF per week. Participants were randomized to receive 5 g/day of MF101, 10 g/day of MF101 or identical placebo for 12 weeks. The complete results of the trial were previously reported [70].

5.3 Phase II baseline data, adherence and compliance

The mean age of participants was 54 years and 80% were white subjects. A total of four participants stopped the trial early; two randomized to the placebo arm, one randomized to 5 g/day of MF101 and one randomized to 10 g/day of MF101. The trial was completed by 98% of participants and 91% of participants took at least 75% of assigned study medication based on packet counts after 12 weeks of treatment.

5.4 Phase II efficacy analysis

Since the publication of the original data, a re-analysis of the efficacy data was performed. The initial analysis used a Poisson regression model which was an inappropriate statistical model as the data did not follow a Poisson distribution. Most of the FDA pivotal Phase III trials for evaluating new treatments for postmenopausal HF have used an analysis of covariance (ANCOVA) model to assess the primary efficacy outcome. To this end, the ANCOVA linear model for rank transformation of HF frequency, controlling for site and years since menopause, was used for the re-analysis of the Phase II efficacy data. As shown in Figure 1, the median reduction in the number of all HF per week from baseline to 12 weeks of treatment was as follows: 68 – 41 in the placebo group, 63 – 32 in the MF101 low-dose group and 67 – 31 in the MF101 high-dose group. The difference in the median reduction in HF per week after 12 weeks of treatment in the MF101 high-dose group compared with placebo was statistically significant ($p = 0.04$). In addition, the difference in ranks of percent change of moderate

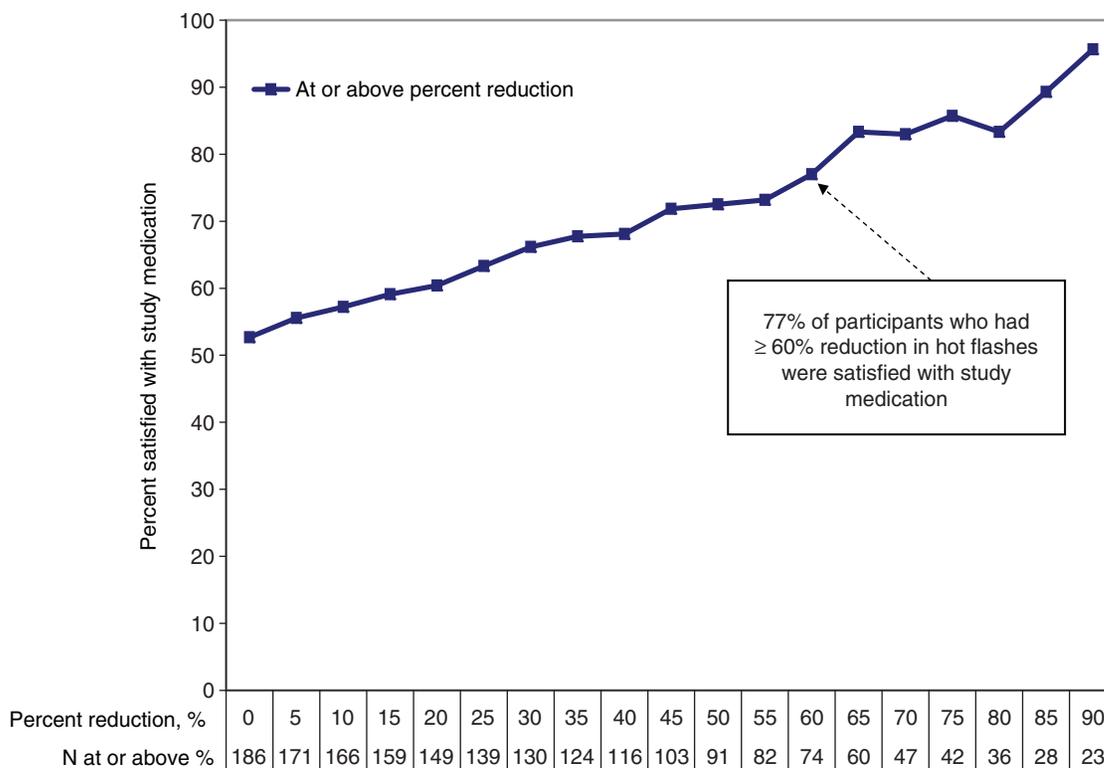


Figure 3. Proportion of women willing to continue treatment having $\geq 55, 60, 65, 70, 75$ and 80% reduction in hot flashes.

Table 3. Effect of MF101 on HF in postmenopausal women.

Phase II efficacy MF101 10 g/day vs placebo	OR (95% CI)	p-Value
50% reduction in HF at 12 weeks	2.3 (1.1 – 4.7)	0.03
60% reduction in HF at 12 weeks	2.4 (1.1 – 5.3)	0.02

CI: Confidence interval; HF: Hot flashes; OR: Odds ratio.

to severe HF at baseline to 12 weeks were 50% on placebo, 58% on the 5 g/day dose and 62% on the 10 g/day dose. These were not statistically significant.

5.5 Phase II secondary outcome measurements

Another important clinical result was the observation that MF101 reduced the number of times women woke up from sleep due to HF. As shown in Figure 2, the median percent reduction in nighttime awakenings from HF for women randomized to the higher dose of MF101 was 67%, and this reduction was statistically superior compared with placebo ($p = 0.05$).

5.6 Clinically meaningful efficacy of MF101 from the Phase II clinical trial

To determine what level of clinical efficacy would satisfy symptomatic postmenopausal women with HF and lead to

continuation of MF101, Bionovo analyzed the Phase II data to assess the degree of efficacy of MF101 that correlates with a willingness of postmenopausal women to continue using MF101 for the treatment of HF [71]. As shown in Figure 3, 77% of women with at least a 60% reduction in HF were willing to continue treatment after 12 weeks on study medication. There was no linear relationship between more improvement and greater willingness to continue therapy from 60 to 80% reduction of HF (test for linear trend, $p = 0.51$). In the responder analysis, compared with placebo, participants in the MF101 10 g/day group were 2.3- and 2.4-fold more likely to have at least a 50 or 60% reduction in all HF after 12 weeks of treatment (odds ratio (OR) 2.3, $p = 0.03$ or OR 2.4, $p = 0.02$), respectively (Table 3).

5.7 Adverse events of MF101

MF101 was very well tolerated and the only statistically significant side effect was transient loose stools (12% on MF101 vs 3% on placebo; $p = 0.03$). This single, minimal side effect was most likely due to the presence of soluble fiber in MF101.

5.8 Uterine safety of MF101

Safety analyses showed no cases of endometrial hyperplasia or uterine cancer during the 12-week trial and there were no differences in incidents of vaginal bleeding between the placebo group and the two cohorts treated with MF101. There were

19 participants who had vaginal bleeding, 7 on placebo, 4 on MF101 5 g/day and 8 on MF101 10 g/day.

6. Conclusion

The estrogens currently used in MHT to treat menopausal symptoms were introduced many years before there was an understanding of the mechanism of action of estrogens. Estrogen signaling is now known to be highly complex, involving multiple estrogen receptors that have different biological roles, different types of DNA regulatory elements that estrogen receptors bind to and numerous coregulatory proteins and transcription factors that interact with ERs to modify chromatin structure to regulate gene transcription [72]. Estrogens also can produce physiological effects through non-genomic mechanisms [62]. These fundamental discoveries have created a new opportunity for future drug discovery to redesign MHT to make it more selective and safer. Although current estrogens are very effective at treating menopausal symptoms, they act non-selectively by binding similarly to ER α and ER β and acting as agonists in all tissues that contain ERs. These actions produce beneficial effects in the bone, brain and adipose tissue, but can lead to adverse effects in the mammary gland, uterus and liver. One promising strategy to overcome the adverse effects of estrogens is to develop selective estrogens. MF101 was found to be a highly ER β agonist in cell lines, neurons and mice, despite being a complex botanical mixture. Because of its ER β selectivity, preclinical studies showed that it did not mimic the proliferative effects of estrogens used in MHT on human breast cancer cells or mouse uterine tissue, which are mediated by ER α . After using non-selective estrogens for over 50 years, preclinical and clinical studies with MF101 have provided evidence that selective estrogen receptor agonists might lead to a new therapeutic era for preventing and treating menopausal symptoms.

7. Expert opinion

MF101 is the first selective ER β agonist to undergo clinical testing in women for HF. Multiple clinical trials using MF101 for HF have produced several exciting positive findings. First, MF101 decreased the frequency of HF and nighttime awakenings in healthy postmenopausal women with moderate to severe symptoms [70,71]. These observations provide the first evidence in humans that indicate ER β has an important role in thermoregulation, and is consistent with the findings that ER β is present in neurons in thermoregulatory regions in the hypothalamus [73]. As described above, MF101 acts directly on neurons to regulate calcium fluxes [68]. In addition, MF101 had a favorable safety profile. The only adverse effect associated with use of MF101 was loose stools, which is likely due to the relatively high carbohydrate content with soluble fiber in MF101. For the Phase III clinical program, a manufacturing process to reduce the non-active mass of the extract by 50% was implemented, which will

likely reduce this side effect. Another important finding in the Phase II study was that MF101 did not exert adverse effects on the endometrium. In a dose-ranging clinical trial evaluating unopposed oral 17 β -estradiol in a cohort of postmenopausal women being treated for vasomotor symptoms, Notelovitz and Mattox showed that endometrial hyperplasia can develop after 12 weeks of treatment. In a randomized, placebo-controlled clinical trial, 15% of postmenopausal women on 1 mg of 17 β -estradiol were diagnosed with endometrial hyperplasia and 47% reported abnormal uterine bleeding after 12 weeks of therapy [74]. These results indicate that unopposed estrogen therapy can lead to rapid proliferative changes in the endometrium consistent with cancer after 3 months of treatment. If MF101 continues to demonstrate safety on the endometrium in the Phase III trial, this will be a very exciting result because there will be no need to co-treat women with progestins which further increase the risks of breast cancer and cardiovascular disease. As suggested from the large number of women who take supplements for HF, the Phase II study provided evidence that postmenopausal women have great enthusiasm for a plant-based product as demonstrated by the high retention rate, as well as the excellent adherence to study medication and no evidence of unblinding. Furthermore, 77% of women are willing to continue MF101 treatment if they experience a 60% reduction in HF [71]. By contrast, studies with other non-hormonal treatments for HF have reported higher discontinuation rates.

These positive findings propelled Bionovo to launch the first of two Phase III clinical trials in October 2011. The Phase III randomized, placebo-controlled trial is currently underway at approximately 50 clinical sites across the USA and total enrollment will include 1200 patients. Two doses of MF101 (5 and 10 g/day – the higher dose tested in the Phase II trial and a dose that is twofold higher) will be compared with placebo. The primary aim of the study is to measure the safety and efficacy of MF101 compared with placebo in reducing the frequency of moderate to severe HF among healthy postmenopausal women after 12 weeks of treatment. Topline safety and efficacy data from the Phase III trial will be available in 2013. The trial is powered to detect a difference in reduction of one moderate to severe HF per day between the placebo and each of the treatment groups. Bionovo has implemented several procedural and statistical measures to control the placebo effect as well as early dropouts.

In conclusion, preclinical and clinical studies indicate that ER β selective agonists represent a new class of drugs that are safe and effective for treating menopausal symptoms. While Bionovo has only tested MF101 for HF, ER β selective agonists might also be indicated for other conditions associated with menopause, such as vaginal dryness. ER β selective agonists, like MF101, are likely to be clinically important alternatives to MHT for HF, and possibly other menopausal symptoms if the results of the Phase II clinical trial are reproduced in the Phase III study.

Declaration of interest

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