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ORIGINAL STUDY

A multicenter, randomized study to select the minimum effective dose of estetrol (E4) in postmenopausal women (E4Relief): part 1. Vasomotor symptoms and overall safety

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Abstract

Objective: The aim of this study was to select the minimum effective dose of estetrol (E4) for the treatment of vasomotor symptoms in postmenopausal women.

Methods: This was a multicenter, randomized, double-blind, placebo-controlled study. Postmenopausal women ($n = 257$, of whom 32 were hysterectomized) aged 40 to 65 years, with ≥ 7 moderate to severe hot flushes (HFs) per day, or 50 or more moderate to severe HFs weekly, received 2.5, 5, 10, or 15 mg E4, or placebo once-daily for a period of 12 weeks. Efficacy was assessed by recording the frequency and severity of HFs. Overall safety was assessed by recording adverse events, measuring endometrial thickness, and monitoring bleeding patterns. Treatment groups were compared using analysis of covariance.

Results: The frequency of moderate to severe HFs decreased with all E4 doses. The difference in the percentage change of weekly HF frequency was significant for 15 mg E4 versus placebo at both W4 (−66% vs −49%, $P = 0.032$) and W12 (−82% vs −65%, $P = 0.022$). The decrease in severity of HFs was significantly more pronounced for 15 mg E4 than for placebo at both W4 (−0.59 vs −0.33, $P = 0.049$) and W12 (−1.04 vs −0.66, $P = 0.049$); the other doses failed to achieve statistical significance. In nonhysterectomized women, endometrial thickness increased during treatment and normalized following progestin treatment at study completion. No endometrial hyperplasia was observed.

Conclusions: Estetrol 15 mg is considered to be the minimum effective daily oral dose for treatment of vasomotor symptoms. Its current seemingly favorable safety profile is further to be confirmed in phase 3 clinical development.

Key Words: E4 – Estetrol – Hormone therapy – Menopause – Vasomotor symptoms.

Video Summary: http://links.lww.com/MENO/A591.

Menopause may be associated with a cluster of symptoms (vasomotor, genitourinary, etc) that have a negative impact on physical, sexual, and psycho-social well-being, and as a consequence, on the overall quality of life of many women.[1] Vasomotor symptoms (VMS), in particular hot flushes (HFs) and night sweats, are reported to be the most bothersome of all.[2] They occur in the late menopausal transition and early postmenopause, and are a prominent reason why women may seek medical care for menopausal complaints. Estimates suggest that about 75% of women who are more than 50 years old will suffer from HFs.[3] Most menopausal women experience HFs for about 4 to 5

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years,[4] although women whose HFs started near entry into the menopause transition may suffer for more than 12 years.[5]

Estetrol (E4) is a native estrogen with selective action in tissues.[6-8] It acts as an estrogen in the nucleus by activating the nuclear estrogen receptor α (ERα) and recruiting the same coregulator activators and repressors as estradiol (E2), or estriol (E3) in a pattern very different from the selective estrogen receptor modulators, tamoxifen, or raloxifene.[9,10] In bone, vagina, endometrium, brain, and the vascular system, E4 acts in synergy with the endogenous estrogens and provides similar effects. E4, however, exerts much less effects on the liver and the breast than E2; this may reduce some unwanted side effects of endogenous estrogens.[8,11,12]

Estetrol has a high oral availability of more than 70%.[13] Its elimination half-life is approximately 28 hours, which is an important prerequisite for its development as a once-daily oral drug. Moreover, in contrast to E2, E4 is not metabolized to other active estrogen metabolites.[14,15] In a multiple rising dose study in postmenopausal women, 2 to 40 mg E4 once-daily improved vaginal cytology and VMS (only evaluated at 2 and 10 mg E4), and a dose-dependent estrogenic effect was observed on endocrine parameters, bone turnover markers, lipids, and lipoproteins, together with only a small increase in triglycerides and almost neutral for hemostatic parameters.[16,17]

The present phase 2 study (E4Relief) is part of the clinical development program of E4 and was designed to define the minimum effective oral daily dose of E4 for the treatment of VMS in postmenopausal women. In this article we report on the change in the frequency and severity of HFs as primary efficacy variable, and the outcome on overall safety. Both hysterectomized and nonhysterectomized women were allowed to participate. In nonhysterectomized women, safety assessments included the measurement of endometrial thickness and bleeding pattern. In our companion paper on the outcome of secondary variables, we will present the effects on genitourinary syndrome of menopause and health-related quality of life. The outcome on lipid and glucose metabolism, hemostatic, and bone laboratory parameters will be reported separately.

METHODS

Study design and objectives

This was a multicenter, randomized, double-blind, placebo-controlled, dose-finding study in postmenopausal hysterectomized and nonhysterectomized women, performed in six European countries (Clinicaltrials.gov NCT02834312, EudraCT 2015-004018-44). The primary efficacy objective was to select the minimum effective oral dose of E4, by evaluating absolute and relative changes in frequency and severity of moderate to severe HFs in postmenopausal women. The VMS-weighted score (see section ''Assessments''), and a responder analysis, served as secondary efficacy endpoints. Overall safety was evaluated by recording changes in endometrial thickness by transvaginal ultrasound (TVUS) in nonhysterectomized women, and by adverse event monitoring and routine laboratory testing in all women. The effects on genitourinary syndrome of menopause, health-related quality of life, and the outcome on hemostatic, lipid, and glucose metabolism, and bone laboratory parameters served as additional secondary parameters, but are not the topic of the present paper.

The study was approved by independent ethics committees of the participating centers, and conducted in accordance with the ethical principles established by the Declaration of Helsinki and the International Conference on Harmonization – Good Clinical Practice guidelines. All participants provided written informed consent before study entry, and had the right to withdraw at any time. Reasonable, documented, travel expenses were reimbursed for each participant. For some countries, an additional modest payment for study inconvenience was provided as financial compensation.

Treatments

Eligible women were randomly allocated (1:1:1:1:1) to one of the five study treatments: 2.5, 5, 10, or 15 mg E4 (SEQENS VLG CHEM, Villeneuve-la-Garenne, France), or placebo. Randomization was stratified by center, and codes were generated by means of the PLAN procedure in SAS (Version 9.4; SAS Institute Inc., Cary, NC). Treatments (blinded encapsulated tablets) were administered orally, once-daily, during 12 consecutive weeks. All nonhysterectomized women received 10 mg dydrogesterone (Duphaston, first batch: Abbott Healthcare Products, Weesp, The Netherlands; second batch: BGP Products, Hoofddorp, The Netherlands) once-daily posttreatment for 14 days.

Participant selection

Postmenopausal women, aged 40 to 65 years (inclusive) with a body mass between 18.0 and 35.0 kg/m^2 (inclusive) were eligible when presenting with at least 7 moderate to severe HFs per day, or at least 50 moderate to severe HFs in the week preceding randomization. Postmenopausal status was defined as level of follicle stimulating hormone (FSH) more than 40 IU/L and amenorrhea for at least 12 consecutive months, *or* amenorrhea for at least 6 months with estradiol (E2) level less than 20 pg/mL, *or* at least 6 weeks after surgical bilateral oophorectomy with or without hysterectomy. Participants with an intact uterus were eligible when TVUS showed a bilayer endometrial thickness of 5 mm or lesser. The main inclusion and exclusion criteria have been summarized in Table 1.

Assessments

The primary efficacy endpoint of this study was the change in e-diary recorded weekly frequency and severity of moderate to severe VMS from baseline to week 4 (W4), and from baseline to week 12 (W12). The weekly frequency of moderate to severe VMS at baseline, W4, and W12 was defined as the total number (sum) of all recorded moderate to severe VMS experienced during the 7-day periods preceding the time point, that is, days -7 through -1 (baseline), days 22 through 28

TABLE 1. *Main inclusion and exclusion criteria*

Inclusion criteria	Exclusion criteria
All participants • Age 40-65 years (inclusive) • BMI 18.0-35.0 kg/m^2 (inclusive) • 7 Moderate to severe HFs per day, or ≥50 moderate to severe HFs in the week preceding randomization Postmenopausal status • FSH > 40 IU/L, and amenorrhea for ≥12 consecutive months, or ≥6 months with E2 levels <20 pg/mL, or • ≥6 Weeks postsurgical bilateral oophorectomy Nonhysterectomized participants • Bilayer endometrial thickness ≤5 mm (TVUS)	All participants • Use of estrogen and/or progestin therapy (oral, vaginal, transdermal, intrauterine) within 4 weeks, within 3 months for estrogen injectable or progestin implants, and within 6 months for estrogen pellet therapy or progestin injectable • History of malignancy, thromboembolism or coagulopathy, coronary heart disease or stroke, diabetes with poor glycemic control, and/or breast cancer Nonhysterectomized participants • Uterine disease, defined as bilayer endometrial thickness >5 mm • History or presence of uterine cancer, presence of fibroids, endometrial hyperplasia, or a polyp with hyperplastic or malignant epithelium.

BMI, body mass index; E2, estradiol; FSH, follicle stimulating hormone; HFs, hot flushes; TVUS, transvaginal ultrasound.

(W4), and days 78 through 84 (W12). Severity of VMS was to be scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The mean severity score of VMS was defined as the arithmetic mean of the severity score values of VMS (moderate to severe) observed during the 7-day periods days -7 to -1 at baseline, and the arithmetic mean of the severity score values of VMS (mild, moderate, or severe) observed during the days 22 to 28 and days 78 to 84 postbaseline for W4 and W12, respectively. At baseline, the daily severity score is computed as [((2 × number of moderate VMS) + (3 × number of severe VMS))/(total number of moderate + severe VMS)] if at least one moderate to severe VMS was recorded during the day. Postbaseline (days 22-28 [week 4] and days 78-84 [week 12]), the severity score is computed as [((1 × number of mild VMS) + (2 × number of moderate VMS) + (3 × number of severe VMS))/(total number of mild + moderate + severe VMS)] if at least one mild to severe VMS was recorded during the day. In case of documented absence of VMS during the day, the severity was set to zero. In addition, the VMS weekly weighted score, which takes into account both the frequency and the severity of VMS, was computed based on the e-diary data as, at baseline [(2 × number of moderate VMS) + (3 × number of severe VMS)]; postbaseline as [(1 × number of mild VMS) + (2 × number of moderate VMS) + (3 × number of severe VMS)]. In case of documented absence of VMS during the day, the weighted score was set to zero.

For nonhysterectomized participants, bilayer endometrial thickness was evaluated by TVUS (change in endometrial thickness) at baseline, W4, and W12; and vaginal bleeding pattern by daily e-diary recording. In the case of finding an endometrial thickness of 15 mm or more, or abnormal uterine bleeding occurred, the participant underwent endometrial biopsy and received dydrogesterone 10 mg once daily for 14 days.

Other safety parameters, including treatment emerging adverse event (TEAE) monitoring, physical and gynecological examination (including vital signs at W4 and W12, and breast examination at W12), electrocardiogram (ECG) (at Screening and W12), and routine clinical laboratory tests (eg, hematology and chemistry at screening and W12), were evaluated in both hysterectomized and nonhysterectomized participants. At screening (and at baseline in case of a hormonal wash-out), FSH and E2 levels were assessed centrally by means of a fluoroimmunoassay and liquid chromatography-tandem mass spectrometry assay, respectively. E2 levels were also assessed at W12.

Statistical analysis

The estimation of the needed sample size was based on the expected change from baseline in weekly frequency of moderate to severe VMS at W12. Based on historical data, a difference between placebo and active treatment of 30 weekly HFs (in the change from baseline) and an SD of 50 weekly HFs was assumed. A two-sided *t* test of superiority at level of 0.05 achieved a power of 80% if the sample size was set to 45 per treatment group. Hence, at least 225 participants were planned to be randomized (ie, at least 45 per treatment group).

Statistical analyses were based on the safety population for the demographic and safety analyses, and on the intent-to-treat (last observation carried forward) population (primary analysis) for the efficacy analyses. The intent-to-treat set included women who took at least one dose of the study treatment and completed their VMS baseline e-diary for at least 4 days in the week preceding randomization, and their VMS e-diary for at least 1 week while on treatment.

Descriptive statistics were calculated for baseline characteristics, all primary and secondary endpoints, and included the arithmetic mean, SD, minimum value, median, maximum value for continuous data; and counts and percentages for categorical data. Homogeneity across the treatment groups was evaluated using one-way analysis of variance for age, body mass index (BMI), and FSH levels. The Kruskal-Wallis test was used for number of pregnancies, duration of amenorrhea, and the chi-square test for hysterectomy and smoking. The analysis of E2 plasma levels at baseline and at W12 was performed post hoc. For E2 levels, homogeneity across the treatment groups was evaluated by a Kruskal-Wallis test at baseline and W12. Pairwise comparisons were tested using the Dwass-Steel-Critchlow-Fligner method.

Treatment groups were compared using an analysis of covariance (ANCOVA) with respect to the relative change in weekly frequency and to the change in severity of moderate to severe VMS from baseline to W4 and W12. The ANCOVA model included treatment as a fixed effect and baseline as a covariate. A Dunnett adjustment for multiplicity test was applied for the pairwise comparisons of each E4 dose to the placebo group. For weeks with missing data up to 2 days, the weekly frequency and severity total were imputed by the mean values of the remaining days of the respective 7-day period. If data were missing for more than 2 days in any week, the weekly total was classified as missing, and the nonmissing data of the preceding week were carried forward. For dropouts, the last available weekly value was carried forward to W4 or W12 (last observation carried forward approach).

A responder analysis was used to assess the change in VMS frequency from baseline by treatment group. Responder rates were defined as the percentage of participants after W4 and W12 of treatment who exhibited a reduction of symptoms by at least 50% or 75% as compared to baseline. A chi-square test was performed on each of the two responder types to check for independence, starting with an overall test. Then, if a significant effect was detected, pairwise comparisons between the treatment groups and placebo were implemented and corrected for multiplicity (Bonferroni correction).

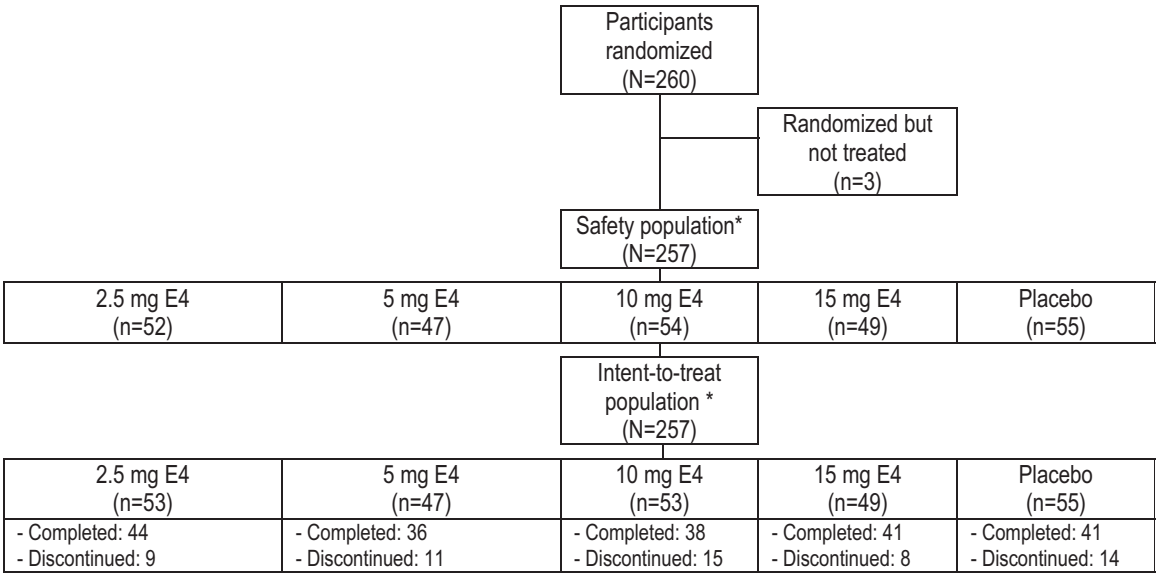
That the E2 level may be a possible confounding factor on the effect of E4 was assessed post hoc by adding the endogenous E2 level as an additional covariate in the ANCOVA model, instead of excluding participants who had E2 levels above a fixed threshold. This was thought to better reflect what occurs in ''real life'' conditions.

All statistical tests and confidence intervals (CIs) were two sided, alpha was set at 0.05. For safety variables, no formal inferential statistical tests were performed. All data were analyzed using SAS software Version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Participant disposition and baseline characteristics

The study was performed from May 2016 until January 2018. In total, 609 women were screened, of whom 349 were considered as not eligible, by not meeting the inclusion criteria for sufficient HFs, or any of the other eligibility criteria (Table 1). The resulting 260 women were randomized, the majority came from Poland, Czech Republic, and Belgium (65% [168/260], 17% [45/260], and 11% [28/260], respectively). Three women did not receive study treatment; thus, a total of 257 participated of whom 32 were hysterectomized (Fig. 1, Table 2). At baseline, the mean weekly frequency of moderate to severe HFs varied from 60.2 (15 mg E4) to 76.1 (2.5 mg E4), and the mean severity score was similar among treatment groups (ie, 2.4-2.5) (Tables 3 and 4). In the Safety population, the mean age was 54.2 ± 4.4 years, mean BMI was 26.0 ± 3.9 kg/m^2, and the median duration of amenorrhea was 3 years (range 1-28 years) (Table 2). Mean FSH levels were indicative of postmenopausal status and similar among groups (90.2 ± 27.8 IU/L). The proportion of hysterectomized women was 12.5%, ranging from 8.5% (5 mg E4) to 18.4% (15 mg E4). Overall, 12.1% were smokers ($\leq$10 cigarettes/day), ranging from 4.1% (15 mg E4) to 20.4% (10 mg E4). No statistical differences were observed among the five treatment groups in any of the baseline characteristics (Table 2). A low



*Two participants accidentally received also another dose than planned. One participant was randomized to E4 15 mg, but temporarily took E4 2.5 mg and one participant was randomized to E4 2.5 mg, but temporarily took E4 10 mg. The first participant was allocated to the (planned) 15 mg group for the ITT and the safety analysis. The second participant was included in the (planned) 2.5 mg group for ITT analysis and included in the 10 mg group for the safety analysis.

FIG. 1. Disposition of participants.

TABLE 2. *Baseline characteristics of postmenopausal women randomized (safety population)*

	2.5 mg E4 $n=52$	5 mg E4 $n=47$	10 mg E4 $n=54$	15 mg E4 $n=49$	Placebo $n=55$	Total $N=257$	P
Mean age, y (SD)	54.0 (4.4)	53.8 (4.8)	54.3 (4.4)	55.2 (4.0)	53.7 (4.4)	54.2 (4.4)	*0.45*[a]
Mean BMI, kg/m^2 (SD)	25.4 (3.7)	26.1 (4.3)	26.0 (3.7)	26.2 (3.9)	26.6 (3.9)	26.0 (3.8)	*0.64*[a]
Mean FSH, IU/mL (SD)	86.1 (29.4)	88.0 (28.3)	92.2 (25.4)	93.8 (26.1)	91.0 (29.9)	90.2 (27.8)	*0.63*[b]
Median no. of pregnancies (range)	2 (0-5)	2 (0-5)	2 (0-5)	2 (0-8)	2 (0-6)	2 (0-8)	*0.16*[b]
Median duration of amenorrhea, years (range)	3 (1-28)	3 (1-22)	3.5 (1-22)	3 (1-22)	4 (1-21)	3 (1-28)	*>0.99*[b]
Hysterectomized, *n* (%)	8 (15.4)	4 (8.5)	5 (9.3)	9 (18.4)	6 (10.9)	32 (12.5)	*0.51*[c]
Smoking, *n* (%)	6 (11.5)	4 (8.5)	11 (20.4)	2 (4.1)	8 (14.5)	31 (12.1)	*0.12*[c]

P value: associated with the null hypothesis of homogeneous treatment group.
BMI, body mass index; E2, estradiol; E4, estetrol; FSH, follicle stimulating hormone; SD, standard deviation.
[a]One-way analysis of variance (ANOVA).
[b]Kruskal-Wallis test.
[c]Chi-square test.

number of women (10.9%) used various medications to treat VMS (eg, antidepressant drugs such as selective serotonin reuptake inhibitors), and were washed-out before the start of study treatment. There were no major differences in this usage between the treatment groups. Fifty-seven women (22%) did not complete the 12-week treatment period (Fig. 1), mainly because of a protocol deviation or self-withdrawal. The number of completers was comparable between treatment groups.

Primary efficacy endpoint: changes in vasomotor symptoms

Absolute changes

Table 3 displays the numerical differences between the treatment and placebo groups in the mean absolute frequency of moderate to severe HFs at W4 and W12. The difference in the least square adjusted mean of the absolute HF frequency between the 15 mg E4 and placebo groups was the largest, which was borderline significant at W4 (−44 HFs vs −34 HFs, $P=0.068$) and at W12 (−55 HFs vs −44 HFs, $P=0.071$).

The mean absolute decrease in the severity scoring of HFs was the highest in the 15 mg E4 group (Table 4, Fig. 2B). The absolute change for 15 mg E4 was significant versus placebo at both W4 (−0.59 vs −0.33, $P<0.05$) and at W12 (−1.04 vs −0.66, $P<0.05$).

Relative changes

The relative reduction, expressed as the difference in the percentage change of weekly HF frequency, varied from −43% (5 mg E4) to −66% (15 mg E4) at W4, and from −62% (2.5 mg E4) to −82% (15 mg E4) at W12. Statistical significance was reached for 15 mg E4 at both time points (Table 3, Fig. 2A).

The relative change in severity scoring of HFs for 15 mg E4 was borderline significant (W4: −24.3% vs −14.1%, $P=0.070$; W12: −43.8% vs −28.0%, $P=0.057$).

Confounding factors

As a majority (65%) of participants were enrolled from a single country (Poland), statistical analyses by nested

TABLE 3. *Weekly change and percentage of change from baseline in the frequency of moderate to severe hot flushes at weeks 4 and 12 (intent-to-treat population, last observation carried forward)*

	2.5 mg E4 ($n=53$)	5 mg E4 ($n=47$)	10 mg E4 ($n=53$)	15 mg E4 ($n=49$)	Placebo ($n=55$)
Baseline					
Mean number (SD)[a]	76.1 (32.6)	67.0 (22.4)	69.1 (19.9)	60.2 (11.7)	65.9 (15.5)
W4					
Mean number (SD)[a]	40.2 (32.2)	39.4 (30.7)	32.7 (28.8)	18.8 (16.6)	33.0 (22.1)
Change from baseline, LS Mean (95% CI)	−32.68 (−39.06 to −26.30)	−27.87 (−34.54 to −21.20)	−35.87 (−42.16 to −29.59)	−44.36 (−50.97 to −37.75)	−33.65 (−39.82 to −27.48)
P value *vs placebo*	*>0.99*	*0.59*	*0.97*	*0.068*	
Percentage change from baseline, LS mean (95% CI)	−49.53 (−58.55 to −40.52)	−43.13 (−52.56 to −33.70)	−55.50 (−64.39 to −46.62)	−66.01 (−75.36 to −56.67)	−48.99 (−57.71 to −40.26)
P value *vs placebo*	*>0.99*	*0.73*	*0.70*	*0.032*	
W12					
Mean number (SD)[a]	31.1 (35.2)	26.4 (26.6)	21.9 (27.5)	9.3 (13.6)	23 (21.8)
Change from baseline, LS mean (95% CI)	−40.68 (−47.26 to −34.11)	−41.01 (−47.89 to −34.13)	−46.53 (−53.01 to −40.06)	−54.91 (−61.72 to −48.09)	−43.93 (−50.29 to −37.57)
P value *vs placebo*	*0.90*	*0.93*	*0.95*	*0.071*	
Percentage change from baseline, LS Mean (95% CI)	−62.21 (−70.90 to −53.53)	−61.44 (−70.52 to −52.35)	−71.73 (−80.29 to −63.18)	−82.34 (−91.34 to −73.34)	−65.11 (−73.51 to −56.70)
P value *vs placebo*	*0.97*	*0.94*	*0.66*	*0.022*	

Treatment groups were compared group using ANCOVA (analysis of covariance).
P values vs placebo were obtained using pairwise comparison between the placebo group and each active treatment.
CI, confidence interval; E4, estetrol; LS, least square; SD, standard deviation; W12, week 12; W4, week 4.
[a]Arithmetic mean.

TABLE 4. *Weekly change and percentage of change from baseline for the severity scores of hot flushes at weeks 4 and 12 (intent-to-treat population, last observation carried forward)*

	2.5 mg E4 ($n=53$)	5 mg E4 ($n=47$)	10 mg E4 ($n=53$)	15 mg E4 ($n=49$)	Placebo ($n=55$)
Baseline					
Mean score (SD)[a]	2.4 (0.3)	2.4 (0.3)	2.4 (0.3)	2.4 (0.3)	2.5 (0.3)
W4					
Mean score (SD)[a]	2.0 (0.7)	2.1 (0.5)	1.9 (0.7)	1.8 (0.7)	2.1 (0.5)
Change from baseline,	−0.34	−0.24	−0.48	−0.59	−0.33
LS mean (95% CI)	(−0.49 to −0.20)	(−0.39 to −0.08)	(−0.63 to −0.34)	(−0.74 to −0.44)	(−0.47 to −0.18)
P value *vs placebo*	*>0.99*	*0.83*	*0.38*	*0.049*	
Percentage change from baseline,	−14.53	−9.56	−20.16	−24.27	−14.10
LS mean (95% CI)	(−20.66 to −8.51)	(−15.96 to −3.15)	(−26.17 to −14.14)	(−30.53 to −18.01)	(−20.02 to −8.18)
P value *vs placebo*	*>0.99*	*0.70*	*0.43*	*0.070*	
W12					
Mean score (SD)[a]	1.7 (0.9)	2.0 (0.7)	1.7 (0.8)	1.4 (0.9)	1.8 (0.9)
Change from baseline,	−0.63	−0.40	−0.69	−1.04	−0.66
LS mean (95% CI)	(−0.84 to −0.42)	(−0.63 to −0.18)	(−0.91 to −0.48)	(−1.26 to −0.82)	(−0.87 to −0.45)
P value *vs placebo*	*>0.99*	*0.31*	*>0.99*	*0.049*	
Percentage change from baseline,	−27.50	−16.46	−28.62	−43.75	−27.97
LS mean (95% CI)	(−36.52 to −18.49)	(−26.06 to −6.91)	(−37.62 to −19.62)	(−53.11 to 34.39)	(−36.83 to −19.12)
P value *vs placebo*	*>0.99*	*0.25*	*>0.99*	*0.057*	

Treatment groups were compared group using ANCOVA (analysis of covariance); *P* values vs placebo were obtained using pairwise comparison between the placebo group and each active treatment; mean severity score was defined as the arithmetic mean of the severity score values (0, 1, 2, or 3) of hot flushes observed during the 7-day periods before baseline, day 28 (W4), and day 84 (W12), respectively. The higher the score the more severe.
CI, confidence interval; E4, estetrol; LS, least square; SD, standard deviation; W12, week 12; W4, week 4.
[a]Arithmetic mean.

ANCOVA were performed to establish whether there was any significant heterogeneity across countries. The difference between each dose and placebo groups was not found to be statistically dependent on the country nor to the study center (data not shown). Therefore, there was no indication that the outcome of the present study was driven by a particular country or center.

Mean E2 levels varied at baseline between 6.1 ± 2.4 pg/mL (15 mg E4) and 13.2 ± 23.1 pg/mL (5 mg E4), and was 11.4 ± 16.5 pg/mL in the placebo group (Table 5), illustrating a somewhat marked intersubject variability. Interestingly, in the 15 mg E4 group, the E2 plasma levels at baseline were all in the range expected for postmenopausal women (<20 pg/mL) and remained within that range during the course of the study. In the other groups (including placebo), although most E2 levels recorded at baseline were in the range expected for postmenopausal women, there were unexpectedly higher values observed in about 5% of the samples at W12. These E2 levels, as depicted in Table 5, could reach outlying levels up to 245 pg/mL. Although the post hoc analysis on E2 plasma levels showed no statistical differences in E2 levels among the five treatment groups at baseline ($P=0.73$), a significant difference was found at week 12 ($P=0.046$). Pairwise comparisons versus placebo, however, did not show any statistically significant difference (Table 5). A covariance analysis was performed to adjust the potential effect of E2 plasma levels on the frequency and severity of HFs at baseline and W12. The ANCOVAs identified E2 level as a significant covariate on the primary efficacy endpoints (relative frequency: $P=0.041$; severity: $P=0.0002$) and confirmed a statistically significant difference among the five treatment groups (frequency: $P=0.01$; severity: $P=0.0008$). Specifically, when the E2 level is included as a covariate in the model, the decrease in HF frequency between W12 and baseline was less pronounced in the placebo group (−43.39 vs −43.93 HFs), whereas this decrease became more prominent in the 15 mg E4 group (−55.79 vs −54.91 HFs). Consequently, the difference in HF frequency between the 15 mg E4 and placebo groups at W12 was found to be larger (−12.4 vs −11.0 HFs), statistically more significant ($P=0.041$ instead of 0.071) than without controlling for the E2 effect. A similar effect of E2 level was observed in terms of HF severity. A reduced difference in HF severity between baseline and W12 was also observed in the placebo group (−0.60 vs −0.66) leading to a more significant reduction of HF severity between the 15 mg E4 and placebo group when adjusted for E2 level ($P=0.013$ instead of 0.049).

Secondary efficacy endpoints

VMS weighted score

At W4, but not at W12, the absolute mean VMS weighted score was significantly different between 15 mg E4 and placebo (W4: −108.5 vs −80.4, $P=0.048$; W12: −132.7 vs −106.8, $P=0.094$). The mean percentage change versus baseline in the VMS-weighted score was significantly different at both time points (W4: −65.9% vs −47.9%, $P=0.019$; W12: −81.8% vs −64.7%; $P=0.021$).

Responder analysis

An overall significant difference was found between the five experimental groups when considering both responder types (50% and 75%) at W4 and W12 (all $P<0.03$). Pairwise comparisons between the treatment groups and placebo revealed statistically significant effects at W12 with the 15 mg E4 group only. Notably, 92% of the women treated with 15 mg E4 experienced a 50% reduction in frequency of moderate to severe HFs from baseline to W12 compared to 65% with placebo ($P=0.0048$), whereas 78% experienced a

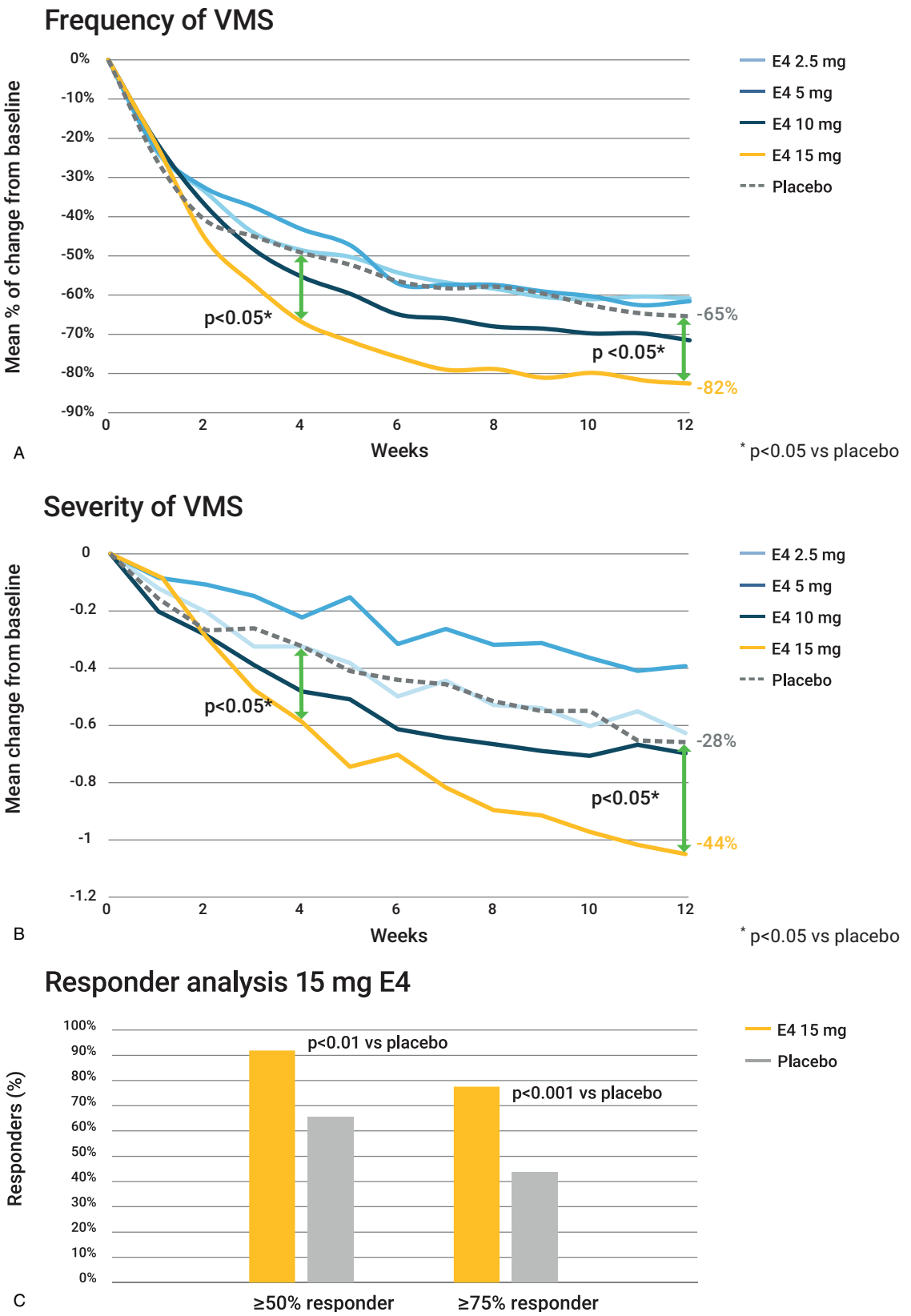


FIG. 2. Changes versus baseline in moderate to severe weekly VMS (least squares adjusted arithmetic mean, intent-to-treat population, last observation carried forward). (**A**) Frequency of VMS. (**B**) Severity of VMS. (**C**) Responder analysis 15 mg E4. E4, estetrol; VMS, vasomotor symptoms. **A** and **B** are a visualization of the weekly changes in frequency and severity of hot flushes, presented in Tables 3 and 4, respectively.

TABLE 5. *Mean E2 plasma levels (pg/mL) at baseline and at week 12 (safety population)*

	2.5 mg E4	5 mg E4	10 mg E4	15 mg E4	Placebo	P^a
Baseline						
Mean (SD)	12.0 (20.6)	13.2 (23.1)	7.9 (9.1)	6.1 (2.4)	11.4 (16.5)	*0.73*
Min-max	<5-128	<5-106	<5-54	<5-15	<5-86	
N	50	44	49	46	48	
W12						
Mean (SD)	11.9 (20.1)	11.0 (18.6)	15.0 (43.3)	5.7 (2.2)	11.6 (27.8)	*0.046*
Min-max	<5-119	<5-99	<5-245	<5-19	<5-180	
N	49	44	48	43	48	
P value vs placebo[b]	*0.61*	*0.82*	*0.97*	*0.78*		

E2, estradiol; E4, estetrol; SD, standard deviation; W12, week 12.
[a]*P* value associated with the null hypothesis of homogeneous treatment group (Kruskal-Wallis test).
[b]*P* values vs placebo were obtained using pairwise comparison (Dwass-Steel-Critchlow-Fligner method) between the placebo group and each active treatment.

75% reduction with 15 mg E4 compared to 44% with placebo ($P = 0.0017$) (Fig. 2C). Similarly, nearly significant effects were also observed at W4. Seventy-one percent of the women treated with 15 mg E4, versus 47% of women receiving placebo, experienced a reduction of at least 50% in the frequency of moderate to severe HFs from baseline to W4 ($P = 0.0501$); 49% experienced a 75% reduction versus 25% for placebo ($P = 0.052$).

Overall safety

In total, 142 (55.3%) of the women reported 372 TEAEs (Table 6). The frequency of women experiencing one or more TEAEs was comparable between the 2.5, 5, and 10 mg E4 groups (53.2%-57.7%) and slightly higher than in the placebo group (47.3%). In the 15 mg E4 group this frequency was 63.3%. Overall, 21 women (8.2%) experienced one or more severe TEAEs, with frequencies ranging from 6.1% in the 15 mg E4 group to 10.6% in the 5 mg E4 group; the frequency in the placebo group was 9.1%. Serious adverse events were reported for two women in the 15 mg E4 group (abnormal uterine bleeding and intervertebral disc protrusion), and for one in the placebo group (intervertebral disc protrusion). In the woman with abnormal bleeding, TVUS showed an endometrial thickness of 14.3 mm, and study treatment was stopped. An endometrial biopsy did not demonstrate endometrial hyperplasia, and the participant had no sequelae and had a normal endometrial thickness (2.7 mm) after progestin treatment at follow-up.

During the study, no major changes were observed in vital signs, ECG parameters, physical and gynecological examinations, and routine laboratory tests.

Mean endometrial thickness at screening and baseline was 2.5 mm (SD 1.0) in all nonhysterectomized women ($n = 225$) and was comparable between groups. At W4, mean endometrial thickness increased with increasing doses from 3.9 mm (SD 1.9) to 6.2 mm (SD 3.9) in the 2.5 mg E4 and 15 mg E4 groups, respectively. At W12, the mean endometrial thickness further increased to 7.9 mm (SD 4.0) for 15 mg E4, and remained stable in the other groups. At follow-up, following progestin therapy, mean endometrial thickness dropped to baseline levels (3.2 mm, $n = 205$) and was comparable between all groups (range 3.0-3.6 mm). Endometrial biopsy was performed throughout the study for 34 women, mainly because of abnormal bleeding. During the whole study period, the number of women in the 2.5, 5, 10, and 15 mg E4 groups who had an endometrial tissue sample taken was 4, 5, 12, and 9, respectively; in the placebo group this number was 4. No endometrial hyperplasia was observed in any of these women, and endometrial thickness returned to baseline after progestin therapy. Before treatment start, the percentage of nonhysterectomized women who reported no spotting or bleeding in their e-diary ranged between 98% and 100%. During treatment this percentage dropped to around 80% at W12. The change was dose dependent, and most pronounced in the 10 mg E4 and 15 mg groups.

TABLE 6. *Overview of treatment emergent adverse events (safety population)*

	2.5 mg E4 ($N = 52$) N (%) N_e	5 mg E4 ($N = 47$) N (%) N_e	10 mg E4 ($N = 54$) N (%) N_e	15 mg E4 ($N = 49$) N (%) N_e	Placebo ($N = 55$) N (%) N_e	Total ($N = 257$) N (%) N_e
Any AE	30 (57.7) 61	25 (53.2) 63	30 (55.6) 95	31 (63.3) 82	26 (47.3) 71	142 (55.3) 372
Severe AE	4 (7.7) 5	5 (10.6) 8	4 (7.4) 7	3 (6.1) 5	5 (9.1) 8	21 (8.2) 33
AE related to study drug	14 (26.9) 27	12 (25.5) 31	21 (38.9) 54	25 (51.0) 51	13 (23.6) 24	85 (33.1) 187
Serious AE	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	2 (4.1) 2	1 (1.8) 1	3 (1.2) 3
AE leading to death	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
AE leading to study discontinuation	1 (1.9) 2	3 (6.4) 6	3 (5.6) 4	2 (4.1) 3	2 (3.6) 4	11 (4.3) 19

AE, adverse event; E4, estetrol; N, number of participants affected; N_e, number of AEs.

There were no obvious changes from baseline to W12 in any of the routine hematology, biochemistry, and urinalysis parameters investigated.

DISCUSSION

Estrogen therapy is the most consistently effective treatment used in the United States and in Europe for menopausal VMS.[18,19] Following the safety issues reported in the primary Women's Health Initiative publications, and with continued participant requests for treatment, a challenge to clinicians has been to identify the lowest effective dose of new and safer estrogens for alleviating menopausal symptoms.[20] The recent long-term Women's Health Initiative findings are a reason for less concern when prescribing hormonal based therapies,[21,22] but still there is a need for an estrogen that is safer than those currently available.

In this study, the reduction in VMS at weeks 4 and 12 was the most pronounced in the 15 mg E4 group. The other doses failed to achieve statistical significance in comparison to placebo. Statistically significant effects versus placebo were observed for the percentage of change in the weekly frequency of HFs, the responder analysis of 50% and 75% reduction of frequency of HFs, the absolute decrease of the severity of HFs, and the percentage of change in the VMS-weighted score. Absolute changes versus placebo in frequency of HFs and percentage of change of the severity of HFs were borderline significant.

The relative reduction versus placebo in VMS (frequency and severity of HFs) following E4 treatment reached statistical significance for 15 mg E4 only. This could at least in part be attributable to the pronounced placebo effect observed in this study as discussed below. It should, however, be noted that for 15 mg E4, the reduction of 82% in frequency of HFs at week 12 is a large one. This reduction is higher than achieved at week 12 with the 1 mg E2/100 mg P4 combination (approximately 74%) in a recently completed VMS treatment study using estradiol/progesterone (E2/P4) oral capsules.[23] In terms of efficacy, it is noteworthy that responder analysis showed 50% and 75% reductions in frequency of HFs at week 12 for approximately 90% and 80% of the participants receiving 15 mg E4.

For the sake of comparison, the Cochrane review of 24 randomized, placebo-controlled studies (3,329 participants in total) showed a reduction of frequency of VMS of 75.0% (95% CI, 64.3- 82.3) for menopausal hormone therapy, and of 57.7% (95% CI, 45.1-67.7) for placebo.[24] Our results of −82% for 15 mg E4 and −65% for placebo are both at the upper limit of the ranges in the Cochrane review, showing a high efficacy level of E4, at least comparable to those of the conjugated equine estrogens and E2 reported.

No clear explanation can be given for the observed large placebo effect in the present study, although this has also been described by others in trials using classic estrogens.[25] The relatively high E2 levels at baseline and also at week 12 (Table 5) in the placebo group (mean ± SD 11.4 ± 16.5 pg/mL with min-max values of <5 and 86 at baseline, and 11.6 ± 27.8 pg/mL with min-max values of <5 and 180 at Week 12, respectively), were higher than the E2 levels recorded in the 15 mg E4 group (6.1 ± 2.4 pg/mL, min-max values of <5-15 at baseline, and 5.7 ± 2.2 pg/mL, min-max values of <5-19 pg/mL at week 12). This large difference in E2 levels observed between the placebo and the 15 mg E4 groups may have played a role (as observed in the post hoc analysis using E2 levels as a covariate), and suggests that some women may have been perimenopausal with some fluctuating, unpredictable increases in residual ovarian activity.[26] Some women in this group may not have been compliant with the protocol and may have used other compounds. In addition, there is the issue of the subjective element inherent to self-scoring the frequency as well as the severity of HFs. To our knowledge, the present study is the first using an e-diary with, therefore, the risk of over-reporting of symptoms. Moreover, considerable variability in the placebo response may occur and is influenced by numerous nonspecific factors. These include a higher response rate in trials of hormonal versus nonhormonal drugs,[25,27] severity of symptoms, anxiety, mood changes, treatment expectation both by clinician and patient, suggestibility, ethnicity, current smoking, or BMI more than 30. All these factors may contribute to a greater placebo response with a decrease in HF frequency more than 30% versus baseline, not only transiently, but also for a sustained period of time.[27] Recently, in the 12-month VMS treatment study using E2/P4 oral capsules, the placebo effect was smaller than in our study, and approximately −55% for frequency and −22% for severity.[23]

Limitations of this study include the small sample size of this exploratory phase 2 study, which adds to the chance of an increase in variation of results which in particular can affect the statistical analysis of subjective measures.

Although endometrial thickness increased in a dose-dependent way with the administration of E4 *alone*, and a biopsy was warranted in 34 out of 257 women (13%), no endometrial hyperplasia was observed in any of the treatment groups. Altogether, the current findings are also indicative of a similar efficacy and safety profile as observed for low-dose oral or transdermal estrogens.[28]

CONCLUSION

Estetrol is effective for the treatment of VMS, and this phase 2 study suggests that daily 15 mg orally is the minimum effective dose. Estetrol was well tolerated. Although there were no apparent concerns regarding endometrial safety and treatment-emergent adverse events, this seemingly favorable safety profile is further to be confirmed in phase 3 clinical development.

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