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ORIGINAL STUDY

A phase 1/2, open-label, parallel group study to evaluate the safety and pharmacokinetics of DARE-HRT1 (80 μg estradiol/4 mg progesterone and 160 μg estradiol/8 mg progesterone intravaginal rings) over 12 weeks in healthy postmenopausal women

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Abstract

Objectives: Primary objectives were to evaluate the safety and systemic pharmacokinetics (PK) of DARE-HRT1, an intravaginal ring (IVR), which releases 17β2-Estradiol (E2) with progesterone (P4) for 28 days in healthy postmenopausal women.

Methods: This was a randomized, open-label, 2-arm, parallel group study in 21 healthy postmenopausal women with an intact uterus. Women were randomized (1:1) to either DARE-HRT1 IVR1 (E2 80 μg/d with P4 4 mg/d) or DARE-HRT1 IVR2 (E2 160 μg/d with P4 8 mg/d). They used the IVR for three 28-day cycles, inserting a new IVR monthly. Safety was measured by treatment emergent adverse events and changes in systemic laboratories and the endometrial bilayer width. Baseline adjusted plasma PK of E2, P4, and estrone (E1) was described.

Results: Both DARE-HRT1 IVR were safe. All treatment emergent adverse events were mild or moderate and were distributed similarly among IVR1 versus IVR2 users. Month 3 median maximum plasma (C_{max}) P4 concentrations were 2.81 and 3.51 ng/mL and C_{max} E2 was 42.95 and 77.27 pg/mL for IVR1 and IVR2 groups, respectively. Month 3 median steady state (C_{ss}) plasma P4 concentrations were 1.19 and 1.89 ng/mL, and C_{ss} E2 was 20.73 and 38.16 pg/mL for IVR1 and IVR2 users, respectively.

Conclusions: Both DARE-HRT1 IVRs were safe and released E2 in systemic concentrations, which were in the low, normal premenopausal range. Systemic P4 concentrations predict endometrial protection. Data from this study support further development of DARE-HRT1 for the treatment of menopausal symptoms.

Key Words: Intravaginal ring – Menopausal symptoms – Pharmacokinetics – Safety – Vasomotor symptoms.

Menopause is defined by the Endocrine Society as the "clinical status after the final menstrual period, diagnosed retrospectively after cessation of menses for 12 months in a previously cycling woman and reflecting complete or nearly complete permanent cessation of ovarian function and fertility,"[1] and occurs at a median age of 51 years in North America.[2] Vasomotor symptoms (VMS), including hot flashes or flushes and night sweats, are the most frequently

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observed symptoms during menopause, although these are not experienced by all women. Vasomotor symptoms, especially drenching night sweats, disrupt the sleep of menopausal women, and can lead to fatigue, irritability, anxiety, and other symptoms associated with chronic sleep deprivation.

Hormone therapy (HT) is recommended as a first-line, effective treatment for the management of VMS among healthy postmenopausal women by the American College of Obstetricians and Gynecologists,[2] the International Menopause Society,[3] and The North American Menopause Society (NAMS).[4] Specifically, the 2022 NAMS guidelines note that HT remains the most effective treatment for VMS and the genitourinary syndrome of menopause and has been shown to prevent bone loss and fracture. The risks of E2 HT differ depending on type, dose, duration of use, route of administration, timing of initiation, and whether a progestogen is used.[4] Nonoral routes of administration (eg, transdermal, vaginal) avoid first pass hepatic metabolism and are often preferred because of potentially lower rates of adverse effects.[5-7] Treatment should be individualized using the best available evidence to maximize benefits and minimize risks, with periodic re-evaluation of the benefits and risks of continuing therapy.[4]

DARE-HRT1 is an ethylene vinyl acetate copolymer intravaginal ring (IVR) containing 17β estradiol (E2) and progesterone (P4). It is being developed for the treatment of moderate-to-severe VMS associated with menopause in women with an intact uterus. This would be the first vaginally delivered combination (E2/P4) VMS product.

The primary objectives of this study were to evaluate the safety and systemic plasma pharmacokinetics (PK) of DARE-HRT1, in two doses: DARE-HRT1 IVR1 that delivers E2 80 μg/d with P4 4 mg/d versus DARE-HRT1 IVR2 that delivers E2 160 μg/d with P4 8 mg/d.

METHODS

Clinical study

This was a randomized, open-label, 2-arm, parallel group, dose-finding study in 21 healthy postmenopausal women with an intact uterus at two centers in Australia. The study was approved by the ethics committees at each study site (Central Adelaide Local Health Network Human Research Ethics Committee 2021HRE00421) and registered at ClinicalTrials.gov (NCT05367973). Participants provided written informed consent prior to the performance of any study procedures. We enrolled healthy postmenopausal women who did not use tobacco products and who were not taking exogenous hormones. Menopause was defined by 12 months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with a plasma follicle stimulating hormone concentration of 40 mIU/mL or higher. Participants had to have a body mass index of ≥18 and ≤38 kg/m^2, an endometrial thickness ≤4 mm on transvaginal ultrasound (TVUS), a normal mammogram within 2 years of the screening visit, and normal cervical cytology cancer screening. Baseline laboratories had to be either within normal limits or accepted by the investigator and medical monitor as not clinically significant. Participants with an endometrial thickness of 4 to 6 mm on TVUS at screening underwent an endometrial biopsy, which had to show benign histology (eg, no hyperplasia, no polyps, no atypia, no carcinoma). Participants were excluded if they had any postmenopausal bleeding, a sexually transmitted infection, a history of endometrial hyperplasia, and/or significant cardiovascular, renal, pulmonary, neurological, or hepatic diseases preventing compliance with the study or impacting data quality. Eligible participants taking exogenous HT at study entry were required to undergo an appropriate washout period.[8]

Participants used the study product for 12 weeks, across three 28-day cycles, with a new IVR administered on day 1 of each cycle. For each cycle, day 1 was defined as the first day of treatment, that is, the day the IVR was self-administered by the participant. Participants were instructed to leave the IVR in place for 28 days and used a diary to document insertion and removal of the IVR and any instances of IVR expulsion, as well as any adverse events (AEs) or concomitant medication use.

Randomization

In this open label, parallel-group, randomized study, participants who met study entry criteria were randomly assigned in a 1:1 ratio, to either DARE-HRT1 IVR1 (E2 80 μg/d with P4 4 mg/d) or DARE-HRT1 IVR2 (E2 160 μg/d with P4 8 mg/d). The randomization schedule was computer-generated using a permuted block algorithm. The study center was not a blocking factor in the randomization schedule. The randomization numbers were assigned sequentially by a member of the data management team who was not otherwise involved in the study.

Study product

The IVR component of DARE-HRT1 is an ethylene vinyl acetate copolymer ring. DARE-HRT1 IVRs were supplied as either IVR1 (E2 80 μg/d with P4 4 mg/d) or IVR2 (E2 160 μg/d with P4 8 mg/d) by Sever Pharma Solutions (Malmö, Sweden). All study products were at refrigerated temperatures (2°C-8°C).

Safety assessments

Safety was assessed primarily through treatment emergent adverse events (TEAEs), which were graded as to their severity and relationship to product use. Any vaginal or vulvar irritation, vaginal discharge, or pelvic pain was reported as an AE using the standardized Medical Dictionary for Regulatory Activities codes and graded for severity. Adverse drug reactions (ADR) were defined as all noxious and unintended responses to a medicinal product, for which a causal relationship between a medicinal product and an AE is at least a reasonable possibility, that is, the relationship cannot be ruled out. All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to an investigational product qualified as ADR. An unexpected ADR was defined as an ADR for which the nature or severity is not consistent with the applicable product information (eg, investigator brochure for an unapproved investigational product).

Safety was also measured by changes from screening in clinical laboratory assessments, vital signs, 12-lead electrocardiograms, physical examinations, and endometrial thickness width measurements by TVUS.

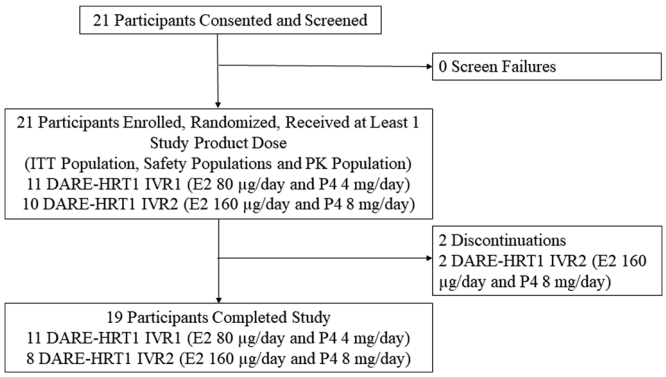

FIG. 1. Disposition of the participants.

Pharmacokinetic evaluation

As illustrated in Supplemental Table 1, http://links.lww.com/MENO/B140, participants were seen over three, 28-d cycles. Pharmacokinetic analytes measured included estrone (E1), E2, and P4. During the first IVR use cycle, baseline PK sampling for E1, E2, and P4 occurred before a new IVR insertion on day 1. These baseline values of E1, E2, and P4 were added to postdosing plasma concentrations of the analytes to adjust for each woman's endogenous hormonal state. On day 1, plasma PK sampling was repeated at 0.5, 1, 2, 4, and 8 hours after IVR insertion. Participants also returned to the clinic 24 and 48 hours after a new IVR insertion for a single PK blood draw in each cycle. They also returned on days 8, 15, and 22 of each treatment cycle for a single PK blood draw and safety assessments, which included monitoring of AEs and concomitant medications, clinical laboratory findings, physical examinations, vital signs, speculum examinations, and TVUS (and endometrial biopsies, if required).

In cycle 3, day 29, which was the end of the 12-week study treatment, participants underwent safety assessments. Pharmacokinetic blood sampling was done before removal of the last IVR and then was repeated at 0.5, 1, 2, 4, 8, and 24 hours after removal.

Plasma concentrations of E1, E2, and P4 were analyzed by Agilex Biolabs (Thebarton, South Australia) using dual tandem liquid chromatography, mass spectrometry. The lower limit of quantification (LLOQ) for each analyte was as follows: E1 5 pg/mL, E2 2 pg/mL, and P4 0.025 ng/mL. For PK characterization, concentrations of analytes for each IVR dose were calculated using noncompartmental analysis. Concentrations that were below the LLOQ were imputed as 0.5 × LLOQ. The PK parameter estimates were completed using WinNonlin (Pharsight Corporation). Actual sampling time was used for all parameter estimation. Standard PK parameters assessed included measures of the extent of absorption using estimates of the area under the plasma concentration-time curve (AUC), the maximum observed drug concentration (C_{max}), the time to reach maximum drug concentration (T_{max}), and the steady state concentration (C_{ss}).

Sample size and statistical analysis

A total of 20 healthy, postmenopausal female participants (N = 10 per arm) were planned for inclusion in this study. The sample size for this study was based on feasibility and the goal to accurately assess the safety and PK of the DARE-HRT1 IVR over a 12-week period. As such, the data are primarily descriptive in nature. The defined analysis populations for the primary objectives included (1) the safety population, made up of all enrolled participants who received active treatment, that is, inserted an IVR (and thus were exposed to at least one of the IVR), and (2) the PK population, which was all participants who received a full course of study treatment for their dispensed treatment and who had sufficient concentration data for determination of PK parameters (primary endpoint).

Duration of exposure was calculated as the number of treatment days between first IVR insertion and last IVR removal. Total exposure to E2 and P4 was calculated as the analyte dose × duration of exposure. Finally, treatment compliance was calculated as 100% × (Total Exposure E2 [in micrograms] / Total Required E2 [in micrograms]; where total required = Planned Dose [in micrograms] × 84.

Summary statistics were described for each dosing cohort. Continuous variables included number of participants, mean, standard deviation (SD), median, minimum, and maximum.

TABLE 1. *Summary of demographic data and baseline characteristics (randomized population)*

Parameter	DARE-HRT1 IVR dose 1 E2 80 µg/d + P4 4 mg/d (N = 11)	DARE-HRT1 IVR dose 2 E2 160 µg/d + P4 8 mg/d (N = 10)	Overall (N = 21)
Age, y			
Mean (SD)	58.6 (3.41)	59.0 (4.74)	58.8 (4.00)
Median (min–max)	59.0 (53-64)	59.5 (51-65)	59.0 (51-65)
Ethnicity, n (%)			
Not Hispanic or Latino	11 (100.0)	10 (100.0)	21 (100.0)
Race, n (%)			
White	11 (100.0)	9 (90.0)	20 (95.2)
Mixed race	0 (0.0)	1 (10.0)	1 (4.8)
Weight, kg			
Mean (SD)	69.5 (10.4)	76.7 (13.6)	72.9 (12.3)
Median (min–max)	68.5 (51.7-85.9)	73.6 (59.9-99.6)	72.8 (51.7-99.6)
Height, cm			
Mean (SD)	164 (4.02)	163 (6.93)	163 (5.47)
Median (min–max)	164 (157-172)	161 (153-175)	163 (153-175)
Body mass index, kg/m^2			
Mean (SD)	25.9 (3.53)	28.9 (4.62)	27.3 (4.26)
Median (min–max)	25.0 (19.7-30.8)	27.2 (24.4-37.5)	26.5 (19.7-37.5)

TABLE 2. *Frequency of treatment emergent AEs in both dosing groups*

TEAEs	Treatment: DARE-HRT1-IVR dose 1 E2 80 μg/d + P4 4 mg/d (N = 11)		Treatment: DARE-HRT1-IVR dose 2 E2 160 μg/d + P4 8 mg/d (N = 10)		Fisher exact *P*
TEAE description	No. (%) participants reporting	No. TEAEs	No. (%) participants reporting	No. TEAEs	Fisher exact *P*
Any TEAE	11 (100.0)	35	10 (100.0)	62	0.39
Any grade 3 or higher TEAE	0	0	0	0	
Any treatment-related TEAEs (TEAEs with causality possibly related or related)[a]	10 (90.9)	20	9 (90.0)	34	
TEAEs leading to study discontinuation	0	0	2 (20.0)	4	
TEAEs leading to study drug discontinuation	0	0	2 (20.0)	3	

AE, adverse events; IVR, intravaginal ring; TEAE, treatment emergent adverse events.
[a]System organ class preferred term of these TEAEs listed in Supplemental Table 2, http://links.lww.com/MENO/B140.

For categorical variables, we present the number and percentage of participants in each category. The denominator for percentage was based on the number of participants appropriate for the purpose of analysis. The study was not powered to demonstrate differences in the proportion of participants in each dosing group reporting various TEAEs, and therefore, comparison of categorical variables for the safety analysis was done by Fisher exact test. Although this statistical test is not dependent on sample size, this comparison must be interpreted with caution based on the small sample size. *P* values less than 0.05 were considered significant.

RESULTS

Participant population

As shown in Figure 1, 21 volunteers were screened and randomized to either DARE-HRT1-IVR1 (n = 11) or DARE-HRT1-IVR2 (n = 10). There were no screen failures. Table 1 displays the demographics of both dosing cohorts. All participants were menopausal based on 12 months of spontaneous amenorrhea.

Safety assessments

Table 2 demonstrates TEAEs, severity, and relatedness to study product. Overall, 11/11 participants (100.0%) reported 35 TEAEs in the IVR1 group and 10/10 participants (100.0%) reported 62 TEAEs in the IVR2 group, with the proportion of participants in each dosing group reporting various categories of TEAEs being similar (Fisher exact *P* value = 0.39) (Table 2). All TEAEs were graded as either mild or moderate. No serious AEs were reported.

Two participants in the IVR2 dose group had TEAEs (continued breakthrough bleeding, nipple tenderness, and depressed mood), considered related to study drug that led to study drug discontinuation and discontinuation from the study.

At baseline, the mean (SD) endometrial thickness width was 2.40 (1.26) and 2.11 (0.71) mm for IVR1 and IVR2, respectively. At the end of treatment, the mean (SD) endometrial thickness width was 3.03 (1.86) and 2.50 (0.98) for IVR1 and IVR2 respectively. All endometrial thickness measurements were ≤4.8 mm at the end of treatment.

Supplemental Table 2, http://links.lww.com/MENO/B140, illustrates duration of treatment, calculated total exposure to E2 and P4, self-reported treatment adherence, and the most common TEAEs by system/organ class. Some compliance numbers exceeded 100% because the end of the 12-week study treatment occurred after day 84 because of appointment scheduling logistics.

Pharmacokinetic evaluations

Tables 3 to 5 and Figures 2A to F demonstrate the baseline adjusted plasma PK parameters and the mean (SD) baseline adjusted plasma concentrations versus time during each cycle of study product use for P4, E2, and E1. Table 3 and Figures 2A and B demonstrate that both IVR resulted in baseline adjusted plasma P4 concentrations >1 ng/mL throughout the 12-week treatment. Table 4 and Figures 2C and D demonstrate that the

TABLE 3. *Summary of baseline adjusted plasma pharmacokinetic parameters for progesterone (in nanograms per milliliter)*

Parameter (unit)	Statistic	Cycle 1		Cycle 2		Cycle 3	
		IVR dose 1	IVR dose 2	IVR dose 1	IVR dose 2	IVR dose 1	IVR dose 2
Progesterone, ng/mL	n (unless specified)	11	10	11	9	10	8
C_{ss}, ng/mL	N	11	9	11	8	10	7
	Mean	1.10	1.89	1.12	1.70	1.25	1.80
	SD	0.28	0.49	0.31	0.23	0.34	0.28
	Median	1.10	1.88	1.14	1.72	1.19	1.89
C_{max}, ng/mL	Mean	5.57	8.95	2.56	3.50	2.83	3.40
	SD	2.44	3.38	0.94	0.55	0.87	0.34
	Median	4.62	8.01	2.52	3.30	2.81	3.51
t_{max}, h	Median	23.23	23.25	24.08	23.67	0.50	4.00
Time to C_{max}	Min–max	7.85-24.2	23.00-24.82	0.50-48.30	1.00-47.95	0.50-23.03	0.48-48.17
$AUC_{D1–D29}$, h*ng/mL	Mean	1,090	1,654	860	1,293	951	1,290
area under the curve between	SD	279	499	243	336	243	375
day 1 and day 29	Median	1,164	1,585	888	1,309	878	1,491

AUC, area under the curve; IVR, intravaginal ring.

TABLE 4. *Summary of baseline adjusted plasma pharmacokinetic parameters for estradiol (in picograms per milliliter)*

Parameter (unit)	Statistic	Cycle 1		Cycle 2		Cycle 3	
		IVR dose 1	IVR dose 2	IVR dose 1	IVR dose 2	IVR dose 1	IVR dose 2
Estradiol, pg/mL	n	11	10	11	9	10	8
C_{ss}, pg/mL	Mean	23.00	38.11	23.10	37.35	22.17	38.97
	SD	8.49	8.45	5.273	8.964	4.468	10.79
	Median	22.28	35.28	23.93	36.26	20.73	38.16
C_{max}, pg/mL	Mean	70.90	113.98	43.59	70.10	45.33	75.37
	SD	19.35	38.95	15.66	22.194	9.52	25.99
	Median	74.00	113.70	40.80	64.90	42.95	77.27
t_{max}, h	Median	24.20	47.09	48.48	47.95	47.26	47.26
	Min–max	22.07-48.05	23.07-48.85	23.28-167.08	23.23-170.87	23.03-48.43	23.07-48.23
$AUC_{D1–D29}$, h*pg/mL	Mean	19,921	32,077	17,534	30,649	17,328	31,511
	SD	5,865	7,634	5,167	8,316	2,981	9,225
	Median	19,690	31,255	17,519	28,723	15,798	29,932

AUC, area under the curve; IVR, intravaginal ring.

baseline adjusted mean (SD) steady state (C_{ss}) plasma E2 concentrations achieved with the 160 μg/d E2 dose IVR was at least 37.35 ± 8.96 pg/mL, with a range of 37.35-38.97 pg/mL over the 12-week treatment. The mean (SD) baseline adjusted, C_{ss} plasma E2 concentrations achieved with the lower 80 μg/d E2 dose IVR over each of the treatment cycles ranged from 22.17 ± 4.47 pg/mL to 23.10 ± 5.27 pg/mL.

DISCUSSION

This 3-month study of two strengths of DARE-HRT1 demonstrated that both IVR were safe over the treatment duration and released plasma E2 concentrations in the general range of the normal premenopausal follicular phase,[9] approximately 20 to 80 pg/mL. Plasma P4 concentrations at steady state for both IVR were in the range of the normal, postovulatory, luteal phase, >1 ng/mL, which supports that the in vivo release of P4 would protect the endometrium from the proliferative effects of the exogenous E2.[10]

While all of the TEAEs were mild or moderate in severity, two participants in the IVR2 group elected to discontinue study product because they experienced known hormone-related adverse effects. Product-related TEAEs and severity of TEAEs were similar among the two dosing groups.

To effectively treat VMS in postmenopausal women, who typically have plasma E2 concentrations of <20 pg/mL, exogenous E2 regimens should increase plasma E2 concentrations into the normal premenopausal range. In healthy premenopausal women, plasma E2 concentrations are approximately 20 to 80 pg/mL in the early follicular phase of the menstrual cycle and increase to 150 to 500 pg/mL at ovulation.[11] Our PK data demonstrate that the steady state plasma E2 concentrations achieved with the 160 μg/d E2 dose IVR put all participants in the normal, premenopausal, early follicular phase range for E2 (approximately 20-80 pg/mL), a level which is optimal to effectively treat VMS in menopause. On the contrary, the mean (SD) steady state plasma E2 concentrations resulting from use of the 80 μg/d E2 IVR left some IVR1 users in the menopausal range for plasma E2 (<20 pg/mL).

We measured the highest systemic absorption of E2 and P4 in the first 2 days of the first month of DARE-HRT1 IVR use. This is likely due to the fact that the study was in postmenopausal women, with a median age of 59 years. Although the participants did not have to meet Food and Drug Administration-defined criteria for vulvo-vaginal atrophy (VVA),[8] baseline vaginal maturation index parameters, to be published elsewhere, demonstrated few superficial cells and many parabasal cells, consistent with VVA. We have previously reported that menopausal women absorb vaginally applied pharmaceuticals more efficiently and rapidly than premenopausal women,[12] and after 1 month of vaginal estrogen use, vaginal absorption of topically applied drug is

TABLE 5. *Summary of baseline adjusted plasma pharmacokinetic parameters for estrone (in picograms per milliliter)*

Parameter (unit)	Statistic	Cycle 1		Cycle 2		Cycle 3	
		IVR dose 1	IVR dose 2	IVR dose 1	IVR dose 2	IVR dose 1	IVR dose 2
Estrone (E1), pg/mL	n (unless Specified)	11	9	11	8	10	8
C_{ss}, pg/mL	N	11	9	11	8	10	7
	Mean	19.89	31.95	20.07	28.78	17.77	29.45
	SD	13.82	8.76	7.57	7.43	6.52	6.88
	Median	15.15	33.20	19.70	30.19	18.12	31.76
C_{max}, pg/mL	Mean	183.12	286.62	88.44	99.03	90.78	120.98
	SD	70.40	114.22	41.86	32.68	25.98	40.40
	Median	161.13	270.75	81.80	95.70	81.43	125.91
t_{max}, h	Median	7.92	15.57	23.23	23.73	15.52	15.46
	Min–max	2.00-23.75	4.00-47.02	2.00-24.10	7.93-47.47	1.98-24.82	2.00-24.82
$AUC_{D1–D29}$, h*pg/mL	Mean	24,611	36,707	19,355	26,814	18,696	25,083
	SD	10,047	12,135	7,742	10,970	7,414	8,906
	Median	21,525	38,216	20,155	30,019	17,389	26,352

AUC, area under the curve; IVR, intravaginal ring.

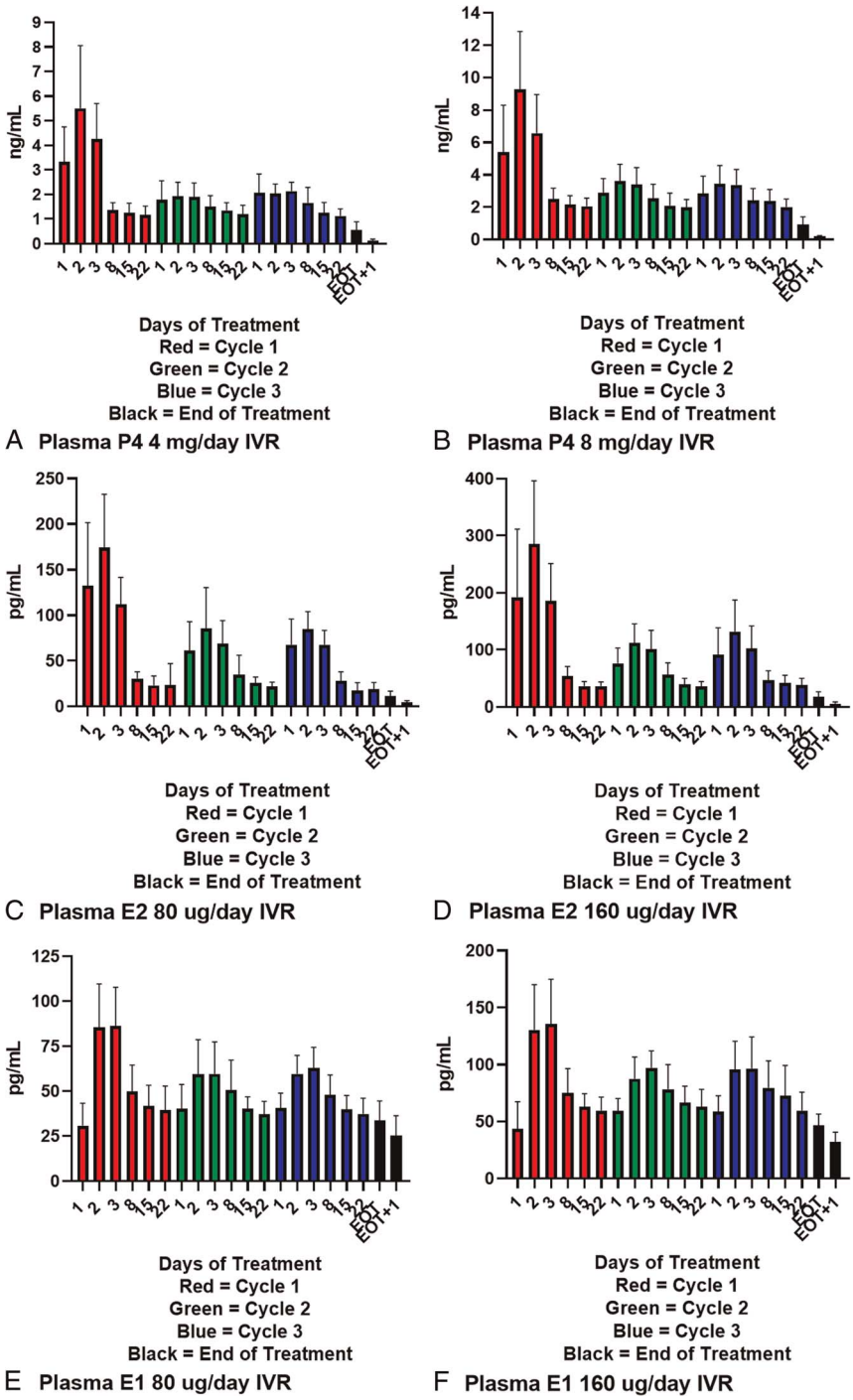

FIG. 2. **(A)** Plasma E2 80 µg/d IVR. **(B)** Plasma E2 160 µg/d IVR. **(C)** Plasma P4 4 mg/d IVR. **(D)** Plasma P4 8 mg/d IVR. **(E)** Plasma E1 80 µg/d IVR. **(F)** Plasma E1 160 µg/d IVR.

reduced and is similar to that of premenopausal women.[12] Although we demonstrated higher absorption in the first few days of each cycle, this was most pronounced in cycle 1.

In terms of systemic safety, the DARE-HRT1-002 PK data also support that both the 160 and 80 µg/d E2 IVR are low-dose products.[10] Systemic safety of an exogenous E2 product is improved by the use of low-dose formulations, which result in low, normal, premenopausal, early follicular phase E2 plasma concentrations (20-80 pg/mL E2; eg, conjugated equine estrogens 0.3 or 0.45 mg daily), rather than higher-dose exogenous E2 products (eg, conjugated equine estrogens 2.5 mg/d), which result in plasma E2 concentrations >150 pg/mL, consistent with ovulation.[10,11] E2-related systemic severe AEs (eg, pulmonary embolus, venous thromboembolism) and adverse effects (eg, nausea and mastalgia) are directly related to steady state plasma E2 concentrations and participant age.[13-15] Because

this study was only a 12-week exposure, longer duration studies will be required to verify our hypothesis regarding systemic safety.

The DARE-HRT1-002 PK data also support that both DARE-HRT1 IVR deliver P4 concentrations at a level expected to protect the endometrium from endometrial hyperplasia. Specifically, in menopause, plasma P4 concentrations are <1 ng/mL.[11] In the normal, postovulatory luteal phase of the menstrual cycle, plasma P4 concentrations rise above 1 ng/mL, causing secretory, progesterone dominant and atrophic changes in the endometrium.[16] Unopposed exogenous estrogen use in menopause is associated with endometrial hyperplasia because of the very low concentrations of P4, due to anovulation. Our P4 PK data demonstrate that the mean (SD) and median steady state plasma P4 concentrations achieved during either DARE-HRT1 IVR1 or DARE-HRT1 IVR2 use during the 12-week treatment period were all >1 ng/mL. Thus, both combination IVR deliver P4 resulting in plasma concentrations, which are likely sufficient to counteract the proliferative effects of E2 to protect the endometrium from hyperplasia. Although these initial PK data are reassuring, a study of more than 12-week exposure will be required to confirm the endometrial safety of the DARE-HRT1 IVR.[8]

We did not perform endometrial biopsies in the present study but note that all TVUS endometrial thickness measurements were ≤4.8 mm at screening and end of treatment. We plan to perform endometrial biopsies during any additional studies of DARE-HRT1 to confirm endometrial safety.[8] However, the fact that this IVR is a combination E2 and P4 product and that steady state plasma P4 concentrations were in the normal, postovulatory range (>1 ng/mL) support that the dose of P4 will offset any proliferative impacts of the E2 on the endometrium.[10]

The strengths of this study were that we achieved the primary objectives of measuring safety and systemic PK of two strengths of DARE-HRT1 in a healthy postmenopausal population. Although the sample size was small, consistent with early PK and safety studies, we obtained systemic PK data, which was consistent with other recently approved oral[17] and IVR[18] VMS treatments. Participants used the study products over a 12-week study period, which is the normal period required to measure VMS treatment efficacy in placebo controlled studies,[8] but not long enough to confirm systemic and endometrial safety.[8] DARE-HRT1 is the first vaginal combination IVR of E2 and P4 hormones for the treatment of VMS in healthy postmenopausal women with an intact uterus. We believe that this long-acting, nonoral VMS treatment regimen will fill an important gap in the options for treatment of this common menopause-related condition.

CONCLUSIONS

These data demonstrate that the study products were safe over a 12-week period of use and delivered E2 in a range consistent with the normal, early follicular phase. The combination IVR also delivered P4 at concentrations expected to provide endometrial safety. We will use these data to move forward with the clinical development of DARE-HRT1.

REFERENCES

1. Stuenkel CA, Davis SR, Gompel A, et al. Treatment of symptoms of the menopause: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2015;100:3975-4011. doi: 10.1210/jc.2015-2236
2. ACOG Practice Bulletin No. 141: management of menopausal symptoms. *Obstet Gynecol* 2014;123:202-216. doi: 10.1097/01.AOG.0000441353.20693.78
3. de Villiers TJ, Hall JE, Pinkerton JV, et al. Revised global consensus statement on menopausal hormone therapy. *Climacteric* 2016;19:313-315. doi: 10.1080/13697137.2016.1196047
4. "The 2022 Hormone Therapy Position Statement of The North American Menopause Society" Advisory Panel. The 2022 hormone therapy position statement of the North American Menopause Society. *Menopause* 2022;29:767-794. doi: 10.1097/GME.0000000000002028
5. Black D. The safety of oral versus transdermal estrogen. *Menopause* 2020;27:1328-1329. doi: 10.1097/GME.0000000000001636
6. Laliberte F, Dea K, Duh MS, Kahler KH, Rolli M, Lefebvre P. Does the route of administration for estrogen hormone therapy impact the risk of venous thromboembolism? Estradiol transdermal system versus oral estrogen-only hormone therapy. *Menopause* 2018;25:1297-1305. doi: 10.1097/GME.0000000000001232
7. Crandall CJ, Hovey KM, Andrews C, et al. Comparison of clinical outcomes among users of oral and transdermal estrogen therapy in the Women's Health Initiative Observational Study. *Menopause* 2017;24:1145-1153. doi: 10.1097/GME.0000000000000899
8. FDA. FDA guidance document estrogen and estrogen/progestin drug products to treat vasomotor symptoms and vulvar and vaginal atrophy symptoms—recommendations for clinical evaluation. Available at: https://wwwfdagov/regulatory-information/search-fda-guidance-documents/estrogen-and-estrogenprogestin-drug-products-treat-vasomotor-symptoms-and-vulvar-and-vaginal-atrophy. 2003. Accessed February 15, 2023.
9. Speroff L, Glass RH, Kase NG. Steroid metabolism. In: Speroff L (ed). *Clinical Endocrinology and Infertility*. Philadelphia: Lippincott Williams and Wilkins; 1989:629.
10. Furness S, Roberts H, Marjoribanks J, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. *Cochrane Database Syst Rev* 2012;2012:CD000402. doi: 10.1002/14651858.CD000402.pub4
11. Verdonk SJE, Vesper HW, Martens F, Sluss PM, Hillebrand JJ, Heijboer AC. Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method. *Clin Chim Acta* 2019;495:198-204. doi: 10.1016/j.cca.2019.04.062
12. Thurman AR, Chandra N, Yousefieh N, et al. Differences in local and systemic TFV PK among premenopausal versus postmenopausal women exposed to TFV 1% vaginal gel. *J Acquir Immune Defic Syndr* 2018;78:82-92. doi: 10.1097/QAI.0000000000001648
13. Dumanski SM, Ramesh S, James MT, et al. The effect and safety of postmenopausal hormone therapy and selective estrogen receptor modulators on kidney outcomes in women: a protocol for systematic review and meta-analysis. *Syst Rev* 2017;6:134. doi: 10.1186/s13643-017-0519-2
14. Manson JE, Hsia J, Johnson KC, et al. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med* 2003;349:523-534. doi: 10.1056/NEJMoa030808
15. Whitehead MI, Fraser D. Controversies concerning the safety of estrogen replacement therapy. *Am J Obstet Gynecol* 1987;156:1313-1322. doi: 10.1016/0002-9378(87)90170-0
16. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Am J Obstet Gynecol* 1975;122:262-263. doi: 10.1016/s0002-9378(16)33500-1
17. Therapeutics MD. Bijuva package insert. Available at: https://wwwaccessdatafdagov/drugsatfda_docs/label/2018/210132s000lblpdf. 2018. Accessed February 15, 2023.
18. Chilcott W. Estradiol acetate vaginal ring (Femring) package insert. Available at: https://wwwaccessdatafdagov/drugsatfda_docs/label/2009/021367s009lblpdf. 2009. Accessed February 15, 2023.