Clinical Study Protocol

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

Protocol Number: DARE-HRT1-002

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1 PROTOCOL SUMMARY

Protocol Title:	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
Product Name/Number	DARE-HRT1
Protocol Number	DARE-HRT1-002
Study Phase:	1/2
Objectives:	Primary: • To assess the safety and tolerability of DARE-HRT1 intravaginal rings (IVRs) over 12 weeks of use • To describe the pharmacokinetic (PK) parameters of 2 different dose combinations of DARE-HRT1 over 12 weeks: o Estradiol 80 µg/progesterone 4 mg/day IVR o Estradiol 160 µg/progesterone 8 mg/day IVR Exploratory: • To assess usability and participant tolerability of the DARE-HRT1 IVR • To conduct a preliminary evaluation of the effect of the DARE-HRT1 IVR on vasomotor symptoms (VMS) and on vulvovaginal atrophy (VVA) signs and symptoms of menopause
Endpoints:	Primary: • Incidence and severity of adverse events • Changes from baseline in clinical laboratory findings • Changes from baseline in physical examination findings • Changes from baseline in vital signs results • Changes from baseline in speculum examination findings • Changes from baseline in transvaginal ultrasound findings • Steady-state concentration (C_{ss}) and maximum observed plasma concentration (C_{max}) in each cycle for estradiol, estrone, and progesterone Exploratory: • Responses to the Usability and Tolerability questionnaire • Change from baseline to Week 12 in responses to the Menopause-specific Quality of Life (MENQOL) questionnaire • Mean change from baseline to Week 12 in severity of the participant's VVA signs and symptoms • Mean change from baseline to Week 12 in the severity of the participant's most bothersome VVA symptom • Mean change from baseline to Week 12 in vaginal pH • Mean change from baseline to Week 12 in vaginal maturation index (parabasal, intermediate, and superficial cells)

Study Design:

This is a randomized, open-label, 2-arm, parallel group study in approximately 20 healthy postmenopausal women with an intact uterus. This study is designed to assess the safety of DARE-HRT1 IVRs at two dose strengths, and the PK of progesterone, estradiol, and its metabolite estrone, from the IVRs. A preliminary assessment of the effect of DARE-HRT1 on VMS and on VVA signs and symptoms of menopause will be included. Screening procedures will ensure that participants are eligible, and will include a transvaginal ultrasound and, if needed, an endometrial biopsy and/or cervical screening test. Eligible participants with ongoing hormone replacement therapy at study entry will be required to undergo an appropriate washout period.

Randomization will occur prior to initiation of treatment and participants will be randomized in a 1:1 ratio to the following treatment groups.

Treatment Group and Number of Participants	**Treatment**
IVR Dose 1 (N = 10)	12-week IVR 80/4 (estradiol 80 µg/day + progesterone 4 mg/day), replaced every 28 days
IVR Dose 2 (N = 10)	12-week IVR 160/8 (estradiol 160 µg/day + progesterone 8 mg/day), replaced every 28 days

Participants will use DARE-HRT1 IVRs for 12 weeks, across three 28-day cycles, with a new IVR administered on Day 1 of each cycle. For each cycle, Day 1 is defined as the first day of treatment, i.e., the day the IVR is self-administered by the participant. Correct placement of the IVR will be confirmed by the study staff. The same IVR is to remain in position for 28 days, with removal of the IVR by the participant in the clinical research unit (CRU) on the morning of Day 1 of the next cycle. Participants will use a paper diary to document insertion and removal of the IVR and any instances of the IVR falling out, as well as any concomitant medication use.

In Cycle 1:

- Baseline PK sampling will occur predose on Day 1 at -1, -0.5 and 0 (within 10 minutes) hours prior to insertion of the first IVR. Repeated blood sampling for PK will occur at 0.5, 1, 2, 4, and 8 hours following insertion.

In Cycles 2 and 3:

- Participants will return to the CRU on Day 1. During that visit, the IVR will be removed, blood will be drawn for PK (0 hours), and a new IVR inserted immediately (within 10 minutes) after blood was drawn. Repeated blood sampling for PK will occur at 0.5, 1, 2, 4, and 8 hours following insertion.

In Cycles 1, 2, and 3:

- On the mornings of Days 2 and 3, participants will return to the CRU for a single PK blood draw (24 and 48 hours following IVR insertion, respectively)
- On the morning of Days 8, 15, and 22, participants will return to the CRU for a single PK blood draw and safety assessments

In Cycle 3 only:

- On the morning of Cycle 3, Day 29 (EOS), participants will return to the CRU for removal of the IVR. Repeated blood sampling will be performed before removal of the IVR (0 hours), and at 0.5, 1, 2, 4, and 8 hours after removal
- Participants will return to the CRU the following day (Day 30) to provide a single PK blood sample 24 hours after IVR removal

	A follow-up telephone call will be made from the CRU to each participant to monitor any ongoing or emergent AEs approximately 7 days after the removal of the third IVR.
Participant Selection Criteria:	Healthy postmenopausal women with body mass index between 18 and 38 kg/m^2, inclusive, normal cervix, vagina, uterus, and adnexa, based upon pelvic examination (speculum and bimanual), normal transvaginal ultrasound and acceptable result from endometrial biopsy if needed at screening, normal cervical screening test and normal mammogram within 24 months before screening.
Study Product, Dose, and Route of Administration:	DARE-HRT1 is an IVR that delivers estradiol and progesterone. It is self-administered by intravaginal insertion once and remains in place for 28 days. Two formulations will be studied, providing different doses: • Estradiol 80 µg/progesterone 4 mg/day IVR • Estradiol 160 µg/progesterone 8 mg/day IVR Participants will self-administer DARE-HRT1 in the CRU on Cycle 1, Day 1. A replacement IVR will be self-administered every 28 days, on Day 1 of Cycles 2 and 3.
Planned Study Sites	Approximately two study sites in Australia.
Planned Sample Size:	Approximately 20 participants are planned to be enrolled, with 10 participants randomly allocated to each treatment arm. Participants who terminate early from the trial may be replaced to ensure an adequate number of participants contributing to the PK analysis in each treatment arm.

Table 1-1: Schedule of Events

Study Procedure	**Screening**	**Cycle 1**			**Cycle 2**					**Cycle 3**			**EOS / ET**		**Follow-Up Phone Call**
Study Day	**Days -28 to -2**	**1**	**2, 3**	**8, 15, 22**	**1**	**2, 3**	**8**	**15**	**22**	**1**	**2, 3**	**8, 15, 22**	**29**	**30**	**+7**
Informed consent[a]	X														
Assign Participant ID	X														
Demographics	X														
Medical and surgical history	X	X													
Eligibility assessment	X	X													
Physical examination[b]	X												X		
Cervical screening test (if needed)	X														
Speculum examination[c]	X	X			X					X			X		
Bimanual examination	X														
Vaginal pH	X	X			X					X			X		
Vaginal cytology	X	X			X					X			X		
Transvaginal ultrasound[d]	X							X					X		
Endometrial biopsy[d]	X[e]														
Height (cm), weight (kg), and BMI[f]	X	X			X					X			X		
12-lead ECG	X														
Vital signs (BP and PR), RR, oral temperature	X	X		X	X		X	X	X	X		X	X		
Hematology and blood chemistry	X	X		X	X		X	X	X	X		X	X		
Urine dipstick	X	X			X					X			X		
FSH	X														

Study Procedure	**Screening**	**Cycle 1**			**Cycle 2**					**Cycle 3**			**EOS / ET**		**Follow-Up Phone Call**
Study Day	**Days -28 to -2**	**1**	**2, 3**	**8, 15, 22**	**1**	**2, 3**	**8**	**15**	**22**	**1**	**2, 3**	**8, 15, 22**	**29**	**30**	**+7**
Serology tests[g]	X														
Drug and alcohol urine screen	X	X													
Training on use of IVR	X	X													
Randomization		X[h]													
Dispense IVR(s)		X			X					X					
Insert new IVR (after predose PK sample)[i]		X			X					X					
Remove used vaginal ring/return IVR(s)					X					X			X		
Confirm IVR placement		X[j]	X	X	X[j]	X	X	X	X	X[j]	X	X			
PK blood samples[k]	X	X[l]	X	X	X[l]	X	X	X	X	X[l]	X	X	X[m]	X[m]	
Usability and Tolerability questionnaire					X					X			X		
Vulvovaginal atrophy symptoms including most bothersome symptom		X											X		
MENQOL questionnaire		X											X		
Dispensing / Review of daily diary	X				X					X			X		
Adverse events, including serious adverse events	Adverse events and serious adverse events collected beginning at ICF signing and in an ongoing manner														
TEAEs		TEAEs collected beginning at IVR placement and in an ongoing manner as appropriate													
Prior/concomitant medications	X	Concomitant medications collected in ongoing manner, as appropriate													
Follow-up telephone call[n]															X

Abbreviations: AE = adverse event; BMI = body mass index; BP = blood pressure; ECG = electrocardiogram; EOS = end of study; ET = early termination; FSH = follicle-stimulating hormone; HCV = hepatitis C virus; HIV = human immunodeficiency virus; ICF = informed consent form; ID = identification;

IVR = intravaginal ring; MENQOL = Menopause-specific Quality of Life; PK = pharmacokinetic; PR = pulse rate; RR = respiratory rate; SAE = serious adverse event; TEAE = treatment-emergent adverse events; temp = temperature.

a. Participants undergoing washout of prior hormone replacement therapy will be required to sign the ICF before starting washout. Where washout of HRT will take approximately 8 weeks, screening procedures should not commence until 28 days prior to Cycle 1, Day 1.

b. Complete physical examination at Screening and EOS/ET visit. Additional symptom-directed examinations can be performed at all other visits for participants exhibiting signs or reporting symptoms since their last visit.

c. The purpose of the speculum examination on Cycle 1 Day 1 is to identify vaginal abnormalities at baseline, to allow differentiation of baseline abnormalities from AEs related to the IVR.

d. In addition to scheduled transvaginal ultrasounds, any participant experiencing postmenopausal bleeding during study will undergo a transvaginal ultrasound and possible endometrial biopsy as an unscheduled visit, to have this evaluated histologically.

e. Upon transvaginal ultrasound:
   - if endometrial thickness is ≤ 4.0 mm in a participant without postmenopausal vaginal bleeding, an endometrial biopsy is not indicated for the purposes of screening
   - if endometrial thickness is > 4.0 mm ≤ 6.0 mm in a participant without postmenopausal vaginal bleeding, an acceptable result from an evaluable screening endometrial biopsy, evaluated by a pathologist, is required for inclusion into the study. Tissue must be read as benign, inactive, or atrophic endometrium by at least 1 pathologist

f. Height will be measured at Screening only.

g. Serology tests include HIV-1 and HIV-2 antibodies, hepatitis B surface antigen, and HCV antibodies.

h. Randomization will occur after screening procedures are completed and eligibility is verified.

i. Participants will self-administer the IVR in the clinical research unit on Day 1 of each cycle. Insertion should take place after the 0-hour PK sample has been collected. At the end of each cycle, participants will remove their own IVR.

j. In each cycle, investigators will confirm correct placement of the IVR prior to the first postdose PK sample collection.

k. Repeated blood sampling for PK will be performed on Day 1 of each cycle, with single samples collected on Day 2 (24 hours postdose), Day 3 (48 hours postdose), and on Days 8, 15, and 22 of each cycle.

l. On Day 1 of each cycle, repeated blood sampling for PK will be performed, with samples collected at the following time points: 0 (within 10 minutes) hours prior to insertion of the IVR, then after insertion of the IVR at 0.5, 1, 2, 4, and 8 hours following insertion. On Day 1 of Cycle 1, baseline sampling will occur predose at -1, -0.5, and 0 (within 10 minutes) hours prior to insertion of the first IVR.

m. Repeated blood sampling for PK will be performed on Day 29 of Cycle 3, with samples collected at the following time points: before removal of the IVR (0 hours), then after removal of the IVR at times 0.5, 1, 2, 4, and 8 hours, with a final sample on Day 30 of Cycle 3, 24 hours after removal of the IVR.

n. The purpose of the call is to follow up on any ongoing or emergent AEs after the participant's treatment has been completed (approximately 7 days after removal of the third IVR.

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
AUC_{D1-D29}	area under the plasma concentration-time curve from time 0 (Day 1) to the time of the last quantifiable concentration on Day 29; calculated using the linear/log trapezoid rule
BMI	body mass index
BP	blood pressure
CFR	Code of Federal Regulations
C_{max}	maximum observed plasma concentration
C_{ss}	steady-state concentration
CRU	clinical research unit
ECG	electrocardiogram
eCRF	electronic case report form
EOS	end-of-study
EVA	ethylene vinyl acetate
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HREC	Human Research Ethics Committee
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Council for Harmonisation
ID	identification
IP	investigational product
IVR	intravaginal ring
mITT	modified intent-to-treat (population)
OTC	over-the-counter
PK	pharmacokinetic(s)
PR	pulse rate
RR	respiration rate
SAE	serious adverse event
SOP	standard operating procedure
SRM	study reference manual

Abbreviation	Definition
t_{max}	time at which the maximum plasma concentration was observed
TEAE	treatment-emergent adverse event
temp	temperature
VMS	vasomotor symptoms
VA	vinyl acetate
VVA	vulvovaginal atrophy

STUDY ADMINISTRATIVE STRUCTURE

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2 INTRODUCTION AND BACKGROUND

2.1 Introduction

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" (Stuenkel CA, et al., 2015), and occurs at a median age of 51 years in North America (American College of Obstetricians and Gynecologists, 2014). Vasomotor symptoms (VMS), including hot flushes and night sweats are the most frequently observed symptoms during menopause, although these are not experienced by all women. Additional symptoms may include vaginal symptoms such as dryness and dyspareunia, sleep disturbances, and arthralgia (Stuenkel CA, et al., 2015).

The pathophysiology of genitourinary symptoms is caused by a reduction in estrogen levels. Lower levels of estrogen cause a thinning of the vaginal epithelium, the elasticity of the vagina is reduced, and there is an increase in connective tissue with eventual fibrotic change in some women. Decreased estrogen levels are also associated with a reduction in vaginal blood flow and in vaginal lubrication (Mac Bride MB, et al., 2010). These physiological changes lead to "vulvovaginal atrophy" (VVA) and are responsible for the range of symptoms observed in many menopausal women, including vaginal dryness, vaginal and/or vulvar irritation/itching, dysuria, vaginal pain associated with sexual activity, and vaginal bleeding associated with sexual activity.

Hormone therapy is accepted as an effective treatment for the management of both VMS and symptomatic VVA, with both local and systemic treatments widely used. The use of estrogen for the treatment of symptoms of menopause is advocated by professional medical organizations (American College of Obstetricians and Gynecologists, 2014).

Although estrogen is the most effective treatment for VMS, unopposed treatment (estrogen alone without progesterone) is associated with an increased risk of endometrial hyperplasia and carcinoma in women with an intact uterus, with a meta-analysis of 30 studies showing a relative risk of 2.3 (95% confidence interval: 2.1-2.5) among women using estrogen and those not using it (Grady D, et al., 1995).

This risk is reduced by the addition of progestogens, with the incidence of endometrial cancer under combined treatment being no different from that in untreated women (Anderson GL, et al., 2003). Furthermore, a Cochrane Review noted a greater effect on reducing hot flash severity following treatment with estrogen and progestogens than with estrogen alone (MacLennan AH, et al., 2004).

DARE-HRT1 is an ethylene vinyl acetate (EVA) copolymer intravaginal ring (IVR) containing 17β-estradiol and progesterone. It is being developed for use in the treatment of menopause, with the following indications:

- Treatment of moderate-to-severe VMS associated with menopause in women with an intact uterus.
- Reduction in the incidence of symptomatic VVA in women requiring treatment for VMS due to menopause.

2.2 Investigational Product Background

The DARE-HRT1 IVR is being developed to release 17β-estradiol at an average rate of 80 µg or 160 µg/day, and progesterone at an average rate of 4 mg or 8 mg/day, for a period of up to 28 days, as long as the ring is present intravaginally.

Estradiol and progesterone are the two major steroid reproductive hormones in females and are produced by the ovaries. Estradiol is approved for a range of indications, including the treatment of VMS of menopause, VVA, postmenopausal osteoporosis prevention, hypoestrogenism, and the palliative treatment of breast cancer and androgen-dependent carcinoma of the prostate. Dose formulations include oral tablets (10 µg to 2 mg), vaginal cream (0.01%), vaginal inserts (0.004 mg to 0.01 mg), transdermal film release (0.025 to 0.1 mg/24 hr), and transdermal spray (1.53 mg). Progesterone is also approved for a wide range of indications, including amenorrhea, hormone replacement therapy (HRT), and infertility as part of assisted reproductive technology treatment. Dose formulations include oral capsules (100 mg to 200 mg), vaginal gels (4% to 8%) and inserts (100 mg), and injections (50 mg/mL).

2.3 Summary of Findings to Date

The IVR component of DARE-HRT1 is an EVA copolymer ring. Subject exposure to the vinyl acetate (VA) monomer within the EVA is not expected due to the extremely low amount of residual VA in the EVA. The product's specification for residual vinyl acetate monomer (100 ppm) falls below the qualification limit as detailed in the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidance Q3B(R2). The safety of the EVA copolymer has been established from nonclinical and clinical investigations described in the literature, and IVRs made from EVA have been widely approved for use as a vaginal insert for drug delivery, including approvals in the United States, the European Union, and Australia.

The DARE-HRT1 IVR has been evaluated in a nonclinical study conducted in ovariectomized sheep to evaluate the pharmacokinetics (PK) of the 17β-estradiol and progesterone and the safety and tolerability of the ring. In this study, IVRs were placed in sheep on Day 1 and remained in place through Day 29. The IVRs were well retained over the 28-day period. Pharmacokinetic analysis showed sustained release of 17β-estradiol and progesterone over a 28-day period. Results of histological assessments of the vagina and cervix showed minimal to mild irritation consistent with the expected pharmacodynamic response and a foreign object placement.

A Phase 1 human factors study was conducted with 4 mm and 6 mm cross sectional diameter placebo IVRs. These rings had the same EVA composition as the DARE-HRT1 IVR but did not

include any active hormone components. In addition to evaluating participants' comprehension of the instructions for use, participants were also evaluated on their ability to properly insert and remove the rings and were asked to report on the acceptability and tolerability of the IVR. The study demonstrated that the placebo IVRs were safe and well tolerated, in terms of reported adverse events (AEs) and physicians' assessments and evaluation of the vaginal mucosa, vaginal sections, epithelial integrity, epithelial surface thickness, and vaginal color. The most frequently reported AE was vulvovaginal discomfort in 8 of 44 (18.2%) treated participants. Subjects generally found the instructions provided for the IVR easy to understand, and 32 of 44 (73.7%) participants were able to successfully insert and remove the IVR. The IVRs were reported to be comfortable by the majority of participants, with most participants reporting that the IVR was convenient to use and worked with their lifestyle.

2.3.1 Phase 1 Study: DARE-HRT1

A Phase 1 open-label study of DARE-HRT1 has been conducted to evaluate the PK and safety of the two DARE-HRT1 formulations in healthy post-menopausal women. Participants were randomized in a 1:1:1 ratio to receive DARE-HRT1 Dose 1 (80 µg estradiol/4 mg progesterone), DARE-HRT1 Dose 2 (160 µg/8 mg), or oral treatment with daily estradiol 1 mg tablet and a progesterone 100 mg capsule. Participants in the DARE-HRT1 groups used an IVR for 28 days, while those in the oral group received treatment every day for 29 days. In addition to PK sampling, participants underwent similar assessments to those conducted in the present study, including speculum examination, transvaginal ultrasounds, safety laboratory tests, and vital signs; participants also completed a Usability and Tolerability questionnaire at the end of the study.

A total of 34 women were randomized to treatment, and 33 received the allocated treatment: 10 participants received DARE-HRT1 Dose 1, 12 received Dose 2, and 11 received oral treatment. Of these, all 10 participants in the Dose 1 group completed the scheduled treatment, 10 participants (83.3%) in the Dose 2 group completed treatment, and all 11 participants receiving oral estradiol/progesterone completed treatment.

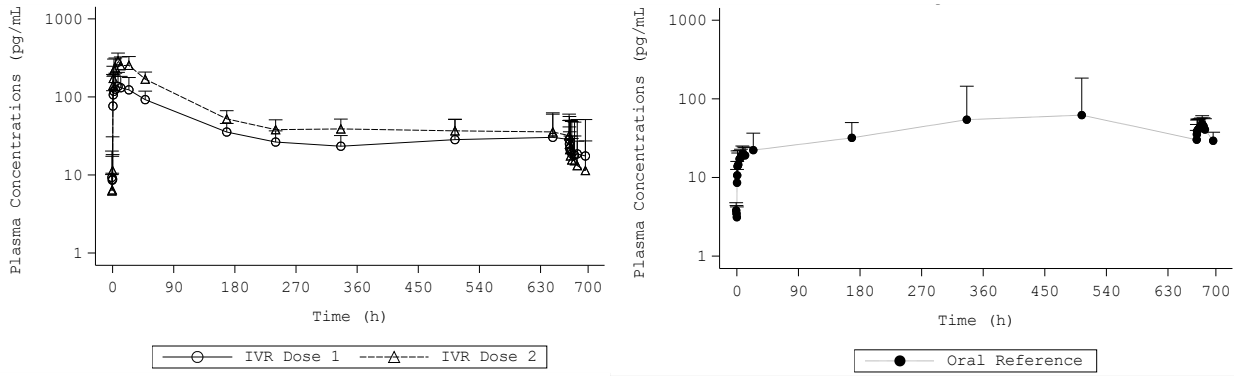
2.3.1.1 Pharmacokinetics

Following the insertion of the IVR on Day 1, plasma concentrations of estradiol, estrone, and progesterone followed a similar pattern: an initial increase in concentrations, peaking within 1-2 days after IVR insertion, followed by a decline in concentrations up to approximately Day 8 (192 hours), after which concentrations were maintained until the removal of the IVR on the morning of Day 29 (Figure 2-1). Post-removal of the IVR, concentrations declined towards baseline levels. Concentrations of estradiol, estrone, and progesterone were generally higher for IVR Dose 2 compared to IVR Dose 1, consistent with the increased dose of both estradiol and progesterone in IVR Dose 2, with the exception of the steady-state concentrations of estrone that were very similar between Day 8 and the morning of Day 29 for both Dose 1 and Dose 2.

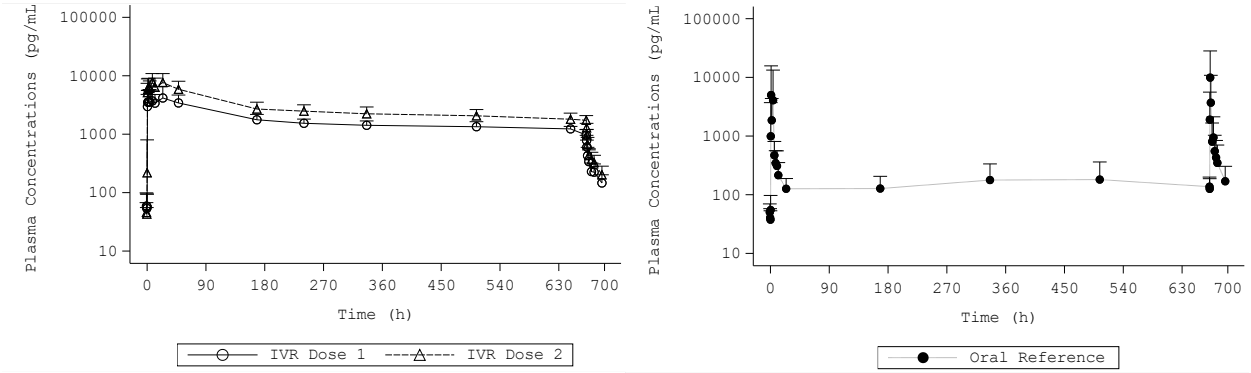
For the oral arm, there was the expected increase in estradiol, estrone, and progesterone concentrations on both Day 1 and Day 30, that had returned close to baseline/predose levels within 24 hours (Figure 2-1). For both estrone and progesterone, steady-state concentrations appeared to have been achieved by Day 8 and were maintained for the duration of the 29 days of dosing. Trough concentrations of estrone were more variable than either estradiol or progesterone, with steady-state appearing to have been achieved between Days 8 and 15.

Figure 2-1: Mean (±SD) Unadjusted Plasma Concentrations (pg/mL) Over Time by Treatment Arm

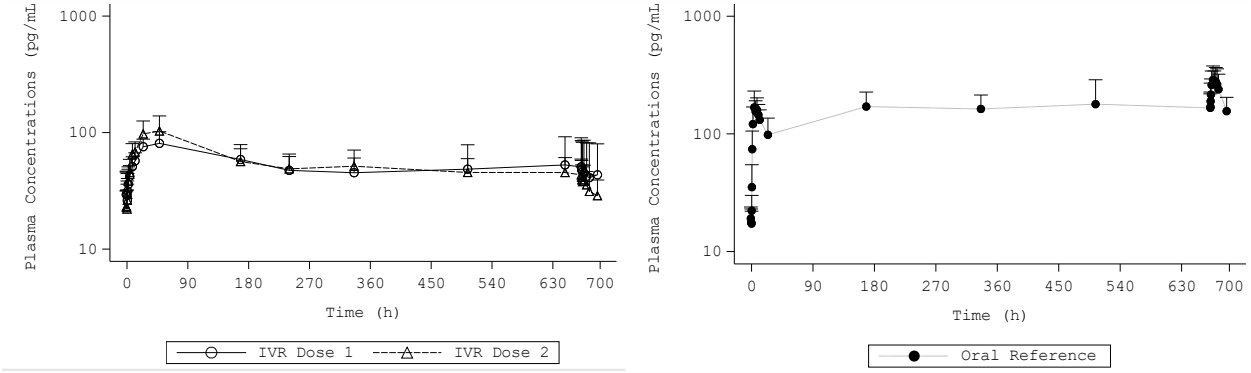
Estradiol



Progesterone



Estrone



Selected summary PK parameters are shown for the IVR treatments in Table 2-1 and for the oral treatment group in Table 2-2.

IVR Treatment Arms: For all 3 analytes, there was an increase in exposure associated with IVR Dose 2 compared to IVR Dose 1 for baseline-adjusted (and unadjusted, data not shown) C_{max} and $AUC_{D1\text{-}D30}$, although the increase for estrone was generally less than the 2-fold increase in the amount of both estradiol and progesterone in Dose 2 compared to Dose 1 (Table 2-1). For both estradiol and progesterone, higher C_{ss} was also associated with Dose 2 but for estrone, C_{ss} was more similar for Dose 1 and Dose 2.

For both estradiol and progesterone, peak concentrations (t_{max}) were observed within 24 hours of insertion, whereas peak estrone concentrations were observed 48 hours after insertion.

For both unadjusted and baseline-corrected estradiol C_{max} and C_{ss}, there was a less than dose-proportional increase, as indicated by slope estimates being less than 1. For uncorrected and baseline-corrected progesterone C_{max}, the slope estimate was closer to 1 and the 90% CI for the estimate contained 1, indicating an approximate dose-proportional increase in exposure after treatment by IVR. There was a less than dose-proportional increase in both unadjusted and baseline-corrected progesterone C_{ss}.

Table 2-1: Summary of Plasma Pharmacokinetic Parameters by IVR Treatment Arm

		Estradiol		Progesterone		Estrone	
Parameter (unit)	**Statistic**	**IVR Dose 1 N = 10**	**IVR Dose 2 N = 11**	**IVR Dose 1 N = 10**	**IVR Dose 2 N = 11**	**IVR Dose 1 N = 10**	**IVR Dose 2 N = 11**
C_{max} (pg/mL or ng/mL)[a] Baseline-adjusted conc.	Mean	143.655	294.154	4.527	8.984	57.213	82.961
t_{max} (h) Baseline-adjusted conc.	Median	12.000	8.000	18.015	8.000	47.990	48.000
$AUC_{D1\text{-}D30}$ (h*pg/mL or h*ng/mL)[a] Baseline-adjusted conc.	Mean	20793.725	37263.109	1142.290	1884.718	16942.583	22085.960
C_{ss} (pg/mL or ng/mL)[a] Baseline-adjusted conc.	Mean	20.379	30.920	1.322	2.084	22.148	25.248

Abbreviations: $AUC_{D1\text{-}D30}$ = area under the plasma concentration-time curve from time 0 (Day 1) to the time of the last quantifiable concentration on Day 30; calculated using the linear/log trapezoid rule; C_{max} = maximum observed plasma concentration; conc. = concentration, C_{ss} = steady-state concentration; h = hour(s); IVR = intravaginal ring; N = number of participants; t_{max} = time at which the maximum plasma concentration was observed

a. Estradiol and estrone parameters reported in pg/mL; progesterone parameters reported in ng/mL.

Oral Treatment Arm: For both unadjusted and baseline-corrected estradiol and estrone, both peak and overall exposure were higher on Day 29 compared to Day 1 of oral treatment (Table 2-2). Unadjusted and baseline-corrected progesterone exposures were also higher on Day 29 compared to Day 1, but not to the same extent as for either estradiol or estrone. On both Day 1 and Day 29,

peak progesterone levels occurred earlier than for either estradiol or estrone. The estimated effective $t_{1/2}$ on Day 29 for both estradiol and estrone was much longer than the corresponding estimate for progesterone.

Table 2-2: Summary of Plasma Pharmacokinetic Parameters for Oral Treatment Arm

		Estradiol		Progesterone		Estrone	
Parameter (unit)	**Statistic**	**Day 1 N = 11**	**Day 29 N = 11**	**Day 1 N = 11**	**Day 29 N = 11**	**Day 1 N = 11**	**Day 29 N = 11**
C_{max} (pg/mL or ng/mL)[a] Baseline-adjusted conc.	Mean	18.903	47.848	10.217	13.209	167.878	296.424
t_{max} (h) Baseline-adjusted conc.	Median	10.000	6.000	2.000	1.020	6.000	4.000
AUC_{0-24H} (h*pg/mL or h*ng/mL)[a] Baseline-adjusted conc.	Mean	366.453	849.479	17.245	19.010	2568.370	5012.178
C_{avg} (pg/mL or ng/mL)[a] Baseline-adjusted conc.	Mean	NA	35.4	NA	0.792	NA	209
$t_{1/2, eff}$ (h) Baseline-adjusted conc.	Median	NA	30.775	NA	5.736	NA	22.672

Abbreviations: AUC_{0-24H} = area under the plasma concentration-time curve from time 0 (Day 1) to the time of the last quantifiable concentration 24 hours; calculated using the linear/log trapezoid rule; C_{avg} = time-weighted average observed plasma concentration over 24 hours; C_{max} = maximum observed plasma concentration; conc. = concentration, h = hour(s); IVR = intravaginal ring; N = number of participants; NA = not applicable; $t_{1/2, eff}$ = effective half-life

a. Estradiol and estrone parameters reported in pg/mL; progesterone parameters reported in ng/mL.

2.3.1.2 Safety

Adverse events (AEs) in the DARE-HRT1-001 study were assessed by the investigator regarding the relationship to study product but no distinction was made regarding AEs that may have been related to the IVR device itself rather than the drugs released from the IVR. Therefore, it is possible that some AEs were related to the intravaginal presence of an EVA copolymer ring rather than either combination of hormones released from the IVR.

No serious AEs (SAEs) were reported, 1 treatment-emergent AE (TEAE) led to the discontinuation of 1 participant in the Dose 2 group (upper respiratory tract infection, unrelated, participant completed treatment), and all TEAEs were mild or moderate in severity.

Product-related TEAEs were reported in 70% of participants in the Dose 1 group (18 events), 58.3% of participants in the Dose 2 group (37 events), and 45.5% of participants in the oral reference arm (8 events). The most frequent TEAEs were recorded in the SOC of reproductive systems and breast disorders: 60% of participants in the Dose 1 group experienced 14 TEAEs,

58.3% of participants in the Dose 2 group experienced 24 TEAEs, and 45.5% of participants in the oral reference group experienced 6 TEAEs. The most frequently experienced TEAEs (> 10% of participants in at least 1 dosage group) included abdominal pain, vaginal hemorrhage, vulvovaginal pruritus, vulvovaginal burning sensation, vaginal discharge, vulvovaginal candidiasis, pelvic discomfort, breast tenderness, and headache.

There were no clinically meaningful abnormalities or trends identified in observed values or changes from baseline in clinical laboratory data, vital signs, physical examination findings, ECGs, speculum examination findings, or transvaginal ultrasound results.

There was a minimally increased incidence in positive ultrasound results at Day 30 compared to screening in both IVR treatment groups but not the oral reference group. New onset positive ultrasound results were reported for 2 participants in each IVR dose group and included hydrosalpinx, increased endometrial thickness (8 mm), 2 small fibroids, and a small cyst. Abnormal postdose endometrial biopsy results showed no evidence of plasma cells, endometritis, atypia, hyperplasia, or malignancy.

The majority of participants in both IVR treatment groups responded favorably for each category related to ease of insertion/removal, comfort when wearing, convenience to use, fit with lifestyle, and likelihood to use an IVR for future health conditions.

2.4 Study Rationale

Daré Biosciences Inc. is developing an IVR to provide local administration of bioidentical 17β-estradiol and progesterone to treat VMS in women with an intact uterus, while aiming to reduce the likelihood of the development of genitourinary symptoms. Non-oral routes such as vaginal administration should bypass the first-pass hepatic effect, although this has not been demonstrated conclusively. Among the various vaginal dosing treatment modalities, evidence suggests a patient preference for the use of IVRs over cream or tablets, with an improvement in adherence to treatment (Suckling J, et al., 2006). With low-dose estradiol IVRs already approved in the United States for the treatment of VMS due to menopause, and vaginal progesterone products also on the market, coadministration of the two hormones together in the same IVR is a convenient proposition for the treatment of VMS and urogenital symptoms of menopause.

2.4.1 Dose Rationale

Estradiol and progesterone have been administered via IVR in previous studies (Hamada AL, et al., 2003). The DARE-HRT1 17β-estradiol and progesterone IVR has been investigated in a recently completed first-in-human study in approximately 34 healthy postmenopausal women with an intact uterus. Study DARE-HRT1-001 was a 28-day study designed to assess the PK and safety of progesterone, estradiol, and its metabolite estrone, from DARE-HRT1 IVRs at two dose strengths, and included a treatment group receiving oral estradiol 1 mg/progesterone 100 mg as an active reference (see Section 2.3.1). Treatment with the two IVR dose strengths was

considered to be safe and well tolerated, and suitable for further investigation. The estradiol and progesterone doses in the present study are the same as those used in DARE-HRT1-001.

The rationale for the initial selection of these doses is provided here. In the present study, the treatment period will be 12 weeks, to allow evaluation of safety and tolerability of DARE-HRT1 over a longer period, since treatment is intended to be for longer than 28 days. In addition, the longer treatment period will allow a preliminary evaluation of the effect of the DARE-HRT1 IVR on VMS and on VVA signs and symptoms of menopause.

Estradiol – Pharmacokinetics of Vaginal Route

In a randomized clinical study with a novel vaginal capsule containing solubilised 17β-estradiol, the bioavailability and PK of the vaginal capsule in healthy postmenopausal women were compared to those of an approved vaginal estradiol tablet (Vagifem®) (Pickar JH, et al., 2016). A crossover design was used, with participants randomized to receive a single dose of the novel capsule then the Vagifem tablet, or the Vagifem tablet followed by the novel capsule. Treatments were administered by an investigator. Blood samples were collected at intervals over 24 hours after treatment. Two separate studies investigated 10 µg and 25 µg doses.

Mean values of PK parameters observed for both estradiol doses following single administration of the two treatments are provided in Table 2-3. The C_{max} values for estradiol differed significantly between the two different formulations, with greater C_{max} values observed with the Vagifem tablets than the novel capsule. Similarly, AUC_{0-24} values were greater with the Vagifem tablet. This study also showed that the time to reach the highest observed (peak) concentration in plasma following administration (t_{max}) for vaginally administered estradiol is greatly dependent on the formulation.

Table 2-3 Mean Pharmacokinetic Values for Estradiol

	AUC_{0-24} (pg•h/mL)			C_{max} (pg/mL)			t_{max} (h)	
Dose (µg)	**Test**	**Vagifem®**	**p**	**Test**	**Vagifem®**	**p**	**Test**	**Vagifem®**
10	49.62	132.92	<0.0001	14.38	20.38	0.0194	1.75	9.28
25	89.21	292.1	<0.0001	23.08	42.70	<0.0001	1.85	11.18

p-values indicate statistical significance at <0.05 between the test products and the Vagifem products.
Source: Pickar JH, et al., 2016

Prescribing information for the FDA-approved IVR Femring reports PK data for estradiol (FEMRING Package Insert, 2018). Femring is a cured silicone elastomer IVR that releases estradiol acetate at two strengths: 0.05 mg/day and 0.10 mg/day, for 3 months. With the 0.05 mg/day IVR, the reported C_{max} was 1129 pg/mL, C_{avg} was 40.6 pg/mL, and the t_{max} was 0.9 hours. With the 0.10 mg/day IVR, the reported C_{max} was 1665 pg/mL, C_{avg} was 76.0 pg/mL, and the t_{max} was 0.7 hours.

Since the DARE-HRT1 IVR comprises an EVA polymer compared to Femring's silicone elastomer, differences in the kinetics of drug release might be expected; thus the reported C_{max}

and t_{max} values for Femring are very different from those observed for DARE-HRT1 in the previous Phase 1 study (see Section 2.3.1.1). Furthermore, the doses of estradiol in the Femring (50 and 100 µg/day) are not the same as those in the DARE-HRT1 IVR (80 and 160 µg/day). However, the estradiol PK results from DARE-HRT1 in the Phase 1 study showed that C_{ss} concentrations were comparable to the C_{avg} reported for Femring (Table 2-4).

Table 2-4: Estradiol Pharmacokinetics: Femring Versus DARE-HRT1

Parameter	Femring 0.05 mg/day	Femring 0.10 mg/day	DARE Dose 1[a] Estradiol 80 µg/day / Progesterone 4 mg/day	DARE Dose 2[a] Estradiol 160 µg/day / Progesterone 8 mg/day
C_{max}	1129 pg/mL	1665 pg/mL	144 pg/mL	294 pg/mL
C_{avg}	40.6 pg/mL	76.0 pg/mL	C_{ss} = 20.4 pg/mL	C_{ss} = 30.9 pg/mL
t_{max}	0.9 h	0.7 h	12.0 h	8.0 h

Abbreviations: C_{avg} = time-weighted average observed plasma concentration over 24 hours; C_{max} = maximum observed plasma concentration; C_{ss} = steady-state concentration; t_{max} = time at which the maximum plasma concentration was observed

a Values for DARE-HRT1 Dose 1 and Dose 2 are baseline-adjusted values.

Source: FEMRING Package Insert, 2018

Progesterone – Pharmacokinetics of Vaginal Route

In a study comparing the PK of progesterone from a vaginal progesterone gel (Crinone 8%, 90 mg) with that of orally administered progesterone (Prometrium 100 mg), 12 healthy postmenopausal women were randomized in a 1:1 fashion to receive a single dose of the allocated progesterone treatment. The vaginally administered progesterone had greater bioavailability than the oral progesterone in terms of AUC_{0-24} (1.48 ng•h/mL per mg versus 0.035 ng•h/mL per mg), with a correspondingly greater C_{max} (10.51 ng/mL versus 2.20 ng/mL). However, t_{max} was observed earlier with oral progesterone (1.00 hours) than with the vaginal gel (7.67 hours) (Levine H, et al., 2000).

The PK of vaginally administered progesterone has also been compared to that of intramuscular progesterone administration (Miles RA, et al., 1994) in a study with functionally agonadal women aged 25 to 54 years. Subjects were randomized to receive a cycle of estrogen and progesterone replacement, comprising oral ethinyl estradiol with either vaginally administered micronized progesterone capsules (200 mg every 6 hours) or intramuscular progesterone (50 mg twice daily) from Day 15 of the cycle. For the PK assessments, samples were collected for 6 hours after the first progesterone dose, with samples for steady-state PK collected on Day 21. Single-dose exposure was significantly greater with the intramuscular treatment (C_{max} 16.06 ng/mL) than with the vaginal capsule (C_{max} 6.64 ng/mL), with a similar difference observed in steady -state concentrations (69.80 versus 11.90 ng/mL). Serum concentrations increased more rapidly with the intramuscular treatment than with the vaginal treatment. As

expected, evaluation of progesterone levels in endometrial samples collected on Day 21 showed higher progesterone concentrations with vaginal treatment (11.50 ng/mL) than with intramuscular treatment (1.40 ng/mL).

An alternative vaginal progesterone formulation, using an effervescent delivery system rather than micronized progesterone capsules, showed similar results. Treatment with the effervescent delivery system was able to produce greater endometrial progesterone concentrations than the intramuscular treatment, while measured systemic exposure was lower (Paulson RJ, et al., 2014).

Another study compared progesterone PK following vaginal administration of two different formulations, both of which contained 100 mg progesterone. Single administrations of vaginal tablets (Endometrin®) and vaginal capsules (Utrogestan®) in postmenopausal women showed largely similar results for the two formulations. The AUC was 379.99 nmol/h/L with the tablet and 325.89 nmol/h/L with the capsule. A significant difference was observed for C_{max} of the two formulations, with values of 31.53 nmol/L reported for the tablet and 23.85 nmol/L for the capsule (Table 2-5). The authors also reported an increase in systemic progesterone levels when participants were "primed" with estrogen (3 mg/day ethinyl estradiol for 2 weeks) before receiving the progesterone treatment (Levy T, et al., 2000).

Table 2-5: Progesterone Pharmacokinetics: Endometrin and Utrogestan Versus DARE-HRT1

Parameter	Endometrin 100 mg Tablets	Utrogestan 100 mg Capsules	DARE Dose 1[a] Estradiol 80 µg/day / Progesterone 4 mg/day	DARE Dose 2[a] Estradiol 160 µg/day / Progesterone 8 mg/day
AUC	380 nmol/h/L	326 nmol/h/L	NA	NA
C_{max}	31.53 nmol/L (9.915 ng/mL)	23.85 nmol/L (7.500 ng/mL)	4.527 ng/mL	8.984 ng/mL

Abbreviations: AUC = area under the plasma concentration-time curve; C_{avg} = time-weighted average observed plasma concentration over 24 hours; C_{max} = maximum observed plasma concentration; C_{ss} = steady-state concentration; NA = not applicable

a Values for DARE-HRT1 Dose 1 and Dose 2 are baseline-adjusted values.

Source: Levy T et al, 2000

Administration via the vaginal route should avoid a first-pass effect of liver metabolism, (Levy T, et al., 2000), which is supported by the comparison of the oral and vaginal routes described by Levine H, et al (2000). Based on the PK findings from DARE-HRT1-001, the baseline-adjusted progesterone C_{ss} concentrations associated with both IVR dose 1 and IVR dose 2 should be sufficient to suppress estrogen-induced endometrial hyperplasia.

2.4.2 Study Design Rationale

A parallel group study of DARE-HRT1 in healthy postmenopausal women is an appropriate design to assess PK and safety over an extended treatment period of 12 weeks. No control group is included; in the first-in-human study, DARE-HRT1-001, an oral treatment group was included

to allow a comparison of the PK of estradiol and progesterone by the 2 different administration routes; it is not considered necessary to duplicate this comparison here. The present study will include an evaluation of the effect of DARE-HRT1 on VVA signs and symptoms (based on vaginal pH, vaginal cytology, and most bothersome symptom) and VMS (based on relevant Menopause-specific Quality of Life [MENQOL] questions). However, the present study is not designed to demonstrate the efficacy of the investigational product (IP) compared to a placebo control or active comparator.

3 OBJECTIVES

3.1 Primary Objectives

- To assess the safety and tolerability of DARE-HRT1 IVRs over 12 weeks of use
- To describe the PK parameters of two different dose combinations of DARE-HRT1 over 12 weeks:
  - Estradiol 80 µg/progesterone 4 mg/day IVR
  - Estradiol 160 µg/progesterone 8 mg/day IVR

3.2 Exploratory Objectives

- To assess usability and participant tolerability of the DARE-HRT1 IVR
- To conduct a preliminary evaluation of the effect of the DARE-HRT1 IVR on VMS and on VVA signs and symptoms of menopause

4 STUDY DESIGN

4.1 Study Design and Overview

This is a randomized, open-label, 2-arm, parallel group study in approximately 20 healthy postmenopausal women with an intact uterus.

This study is designed to assess the safety and tolerability of DARE-HRT1 IVRs at two dose strengths over 12 weeks of use, and to evaluate the PK of progesterone, estradiol, and its metabolite estrone, from the IVRs over this extended period. The study also includes a preliminary evaluation of the effect of DARE-HRT1 on VVA signs and symptoms (based on vaginal pH, vaginal cytology, and most bothersome symptom) and VMS (based on VMS questions in MENQOL). In addition, usability and tolerability will be assessed via a questionnaire.

Two treatment groups will be assessed in this study as shown in Table 4-1.

Table 4-1: Treatment Allocations

Treatment Group	Number of Participants	Treatment
IVR Dose 1	10	12-week IVR 80/4 (estradiol 80 µg/day + progesterone 4 mg/day), replaced every 28 days
IVR Dose 2	10	12-week IVR 160/8 (estradiol 160 µg/day + progesterone 8 mg/day), replaced every 28 days

Participants who have been on prior HRT therapy will be required to complete the following washout period prior to randomization (for participants actively using HRT, washout may begin only after they have provided informed consent):

- 4 weeks or longer for prior transdermal estrogen alone or estrogen/progestogen products
- 8 weeks or longer for prior oral estrogen, oral estrogen/progestogen, or intrauterine progestogen therapy

Due to study enrollment goals and the length of the required washout periods, patients who are currently on progestogen implants or estrogen alone injectable drug therapy (requiring 3 months' washout) or estrogen pellet therapy or progestogen injectable drug therapy (requiring 6 months' washout) at the time of screening will not be enrolled.

Where washout of HRT will take approximately 8 weeks, screening procedures should not commence until 28 days prior to Day 1.

Upon completion of their screening procedures and confirmation of their eligibility, eligible participants will attend the clinical research unit (CRU) on the morning of Day 1.

Randomization will occur prior to initiation of treatment. Participants will be randomized in a 1:1 ratio to the two treatment groups (Table 4-1).

Participants will use DARE-HRT1 IVRs for 12 weeks, across three 28-day cycles, with a new IVR administered on Day 1 of each cycle. For each cycle, Day 1 is defined as the first day of treatment, i.e., the day the IVR is self-administered by the participant. Correct placement of the IVR will be confirmed by the study staff. The same IVR is to remain in position for 28 days, with removal of the IVR by the participant in the CRU on the morning of Day 1 of the next cycle. Participants will use a paper diary to document insertion and removal of the IVR and any instances of the IVR falling out, as well as any concomitant medication use.

In Cycle 1:

- Baseline PK sampling will occur predose on Day 1 at -1, -0.5 and 0 (within 10 minutes) hours prior to insertion of the first IVR. Repeated blood sampling for PK will occur at 0.5, 1, 2, 4, and 8 hours following insertion.

In Cycles 2 and 3:

- Participants will return to the CRU on Day 1. During that visit, the IVR will be removed, blood will be drawn for PK (0 hours), and a new IVR inserted immediately (within 10 minutes) after blood was drawn. Repeated blood sampling for PK will occur at 0.5, 1, 2, 4, and 8 hours following insertion.

In Cycles 1, 2, and 3:

- On the mornings of Days 2 and 3, participants will return to the CRU for a single PK blood draw (24 and 48 hours following IVR insertion, respectively).
- On the morning of Days 8, 15, and 22, participants will return to the CRU for a single PK blood draw and safety assessments.

In Cycle 3 only:

- On the morning of Cycle 3, Day 29 (EOS), participants will return to the CRU for safety assessments, to complete study questionnaires, and for removal of the IVR. Repeated blood sampling will be performed before removal of the IVR (0 hours), and at 0.5, 1, 2, 4, and 8 hours after removal
- Participants will return to the CRU the following day (Day 30) to provide a single PK blood sample 24 hours after IVR removal

A follow-up telephone call will be made from the CRU to each participant to monitor any ongoing or emergent AEs approximately 7 days after the removal of the third IVR.

Safety assessments will include monitoring of AEs and concomitant medications, clinical laboratory findings, physical examinations, vital signs, speculum examinations, and transvaginal ultrasounds (and endometrial biopsies, if required).

Exploratory assessments will include completion of a Usability and Tolerability questionnaire. Exploratory assessments will also be conducted to evaluate VVA signs and symptoms (including

vaginal cytology, vaginal pH, and most bothersome symptom) and VMS (based on relevant MENQOL questions).

Refer to the schedule of events in Table 1-1 for specific details.

4.1.1 Duration of Study

Participant participation is expected to last up to 121 days, including a 28-day screening period, an 86-day on-study period (consisting of an 84-day treatment period and additional sample collections), and a follow-up telephone call 7 days after the removal of the third IP).

4.1.2 Definition of Study Completion

End-of-study procedures will be performed as specified in the schedule of events (Table 1-1). Participants who terminate from the study early will undergo the same procedures at the time of discontinuation as those scheduled for EOS (early termination assessments). Participants with ongoing significant clinical or laboratory findings at the time of EOS/early termination will be followed until the finding is resolved or medically stable. All reasonable attempts will be made to follow-up with participants. Participation in the study will end once all study assessments and follow-up have been completed.

4.1.3 End of Study

The end of the study is defined as the date when the last participant has completed all study procedures up to and including the EOS/early termination visit as specified in the schedule of events (Table 1-1).

5 SELECTION AND WITHDRAWAL OF PARTICIPANTS

Participants must meet all the following criteria in order to be enrolled in the study.

5.1 Inclusion Criteria

Participants must meet all inclusion criteria to be eligible for study participation.

1. Postmenopausal women with a body mass index (BMI) ≥ 18 and ≤ 38 kg/m^2.

   $$\text{BMI} = \text{weight (kg)}/(\text{height [m]})^2$$

   Postmenopausal is defined as 12-months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with serum follicle-stimulating hormone (FSH) levels > 40 mIU/mL or 6 weeks postsurgical bilateral oophorectomy with or without hysterectomy (although participants who have had a hysterectomy are not eligible for this study).

   The investigator will need to determine if a participant's BMI falling within the obese-severely obese range could potentially interfere with the protocol-required procedures, specifically the pelvic examinations described in Inclusion Criterion #2. Any participant whose BMI is determined to fall into this category should be excluded from trial participation.

2. Normal cervix, vagina, uterus, and adnexa based on speculum examination and bimanual examination.

3. Normal transvaginal ultrasound, and endometrial biopsy results as follows:

   - If endometrial thickness is ≤ 4.0 mm in a participant without postmenopausal vaginal bleeding, an endometrial biopsy is not indicated for the purposes of screening,
   - If endometrial thickness is > 4.0 mm ≤ 6.0 mm in a participant without postmenopausal vaginal bleeding, an acceptable result from an evaluable screening endometrial biopsy, evaluated by a pathologist, is required for inclusion into the study. Tissue must be read as benign, inactive, or atrophic endometrium by at least 1 pathologist,

4. Current on all Australian screening requirements for cervical cancer.

5. Able and willing to correctly and independently complete all study procedures.

6. Able and willing to stop any ongoing HRT in accordance with the appropriate washout periods (see Section 4.1 for washout requirements). Participants who are using HRT that requires more than 8 weeks to wash out (e.g., progestogen implants or progestogen injectable drug therapy, estrogen alone injectable drug therapy or estrogen pellet therapy) will not be eligible.

7. Able to read, understand, and provide written informed consent after the nature of the study has been fully explained and must be willing to comply with all study requirements and procedures.
8. Normal mammogram report within 24 months of screening.

5.2 Exclusion Criteria

Participants will not be eligible for study participation if they meet any of the exclusion criteria, or will be discontinued at the discretion of the investigator in consultation with the medical monitor if they develop any of the exclusion criteria during the study.

1. Prior abnormal cervical screening test or Papanicolaou result within 2 years of screening. Participant can have atypical squamous cells of undetermined significance, if human papillomavirus negative.
2. Participants with any self-reported active sexually transmitted disease and/or evidence of infection based on vaginal visual examination by the investigator.
3. Participants with a urinary tract infection during screening as assessed by urine dipstick test with abnormal test findings (any positive result for leukocytes AND any positive result for nitrites).
4. Have a history of endometrial hyperplasia or cervical or uterine carcinoma.
5. Participants with indwelling catheters or requiring intermittent catheterization.
6. Participants with multiple or unsuccessful (e.g., still having symptoms) pelvic reconstructive surgery, or who suffer from pelvic relaxation.
7. Participants who have had a hysterectomy.
8. Participants taking any estrogen and/or progesterone products who are not willing to stop this treatment during their participation in this trial (see Section 4.1 for washout requirements). Participants who are using HRT that requires more than 8 weeks to wash out (e.g., progestogen implants or progestogen injectable drug therapy, estrogen alone injectable drug therapy or estrogen pellet therapy) will not be eligible.
9. Participants with concomitant use of personal lubricants (water-based lubricants are allowed) or any intravaginal product or medication, either by prescription or over-the-counter (OTC) (e.g., Femring [estradiol acetate vaginal ring], ESTRING® [estradiol vaginal ring]) with the exception of those who agree not to use these products during the IVR use period.
10. Self-reported or observed vaginal irritation unrelated to VVA; vaginal, vulvar, or cervical lesions, undiagnosed vaginal bleeding; or tenderness.
11. Participants with a finding of clinically significant uterine fibroids at screening.

12. Participants with a known hypersensitivity to progesterone, estradiol, Femring, or the components of the IVR (e.g., ethylene vinyl acetate).
13. Participants with prior pelvic malignancies.
14. Participants with a history of any severe acute or chronic medical or psychiatric condition or laboratory abnormality that could increase the risk associated with trial participation or study treatment administration or could interfere with the interpretation of trial results and, in the judgment of the investigator, would make the participant inappropriate for entry into the trial. This includes but is not limited to the following:
    a. Human immunodeficiency virus (HIV) infection (confirmed by medical history/ serology testing),
    b. Active chronic hepatitis B or hepatitis C infection including hepatitis B surface antigen and hepatitis C antibody positive participants with or without abnormal liver enzymes (confirmed by medical history/serology testing),
    c. Concurrent neurodegenerative disease,
    d. Cardiovascular: uncontrolled hypertension, unstable angina, myocardial infarction or symptomatic congestive heart failure within the past 6 months, serious uncontrolled cardiac arrhythmia, use of Class 1 antiarrhythmic medications, or history of venous thromboembolism or stroke,
    e. Dementia or significantly altered mental status that would prohibit the understanding or rendering of informed consent and compliance with the requirements of the protocol,
    f. Participants with known thrombophilias may not participate in this study because estrogen-based products are contraindicated for them,
    g. Symptomatic bacterial vaginosis.
15. Fasting triglyceride of > 3.39 mmol/L and/or total cholesterol of > 7.77 mmol/L.
16. Aspartate aminotransferase or alanine aminotransferase > 1.5 times the upper limit of normal.
17. Fasting glucose > 6.94 mmol/L.
18. Evidence of current alcohol or drug abuse in the past 60 days including a positive result from the urine drugs of abuse or alcohol screen, or history of drug or alcohol dependence in the last 2 years, as assessed by principal investigator. Alcohol abuse is defined as greater than 14 standard units/week for females and drug abuse is defined as known psychiatric or substance abuse disorder that would interfere with participation with the requirements of this study, including current use of any illicit drugs. Use of medical cannabis is not exclusionary.
19. Participation in any other investigational drug or device trial in which administration of an investigational study drug/device occurred within 30 days or placement of a non-drug eluting medical device within 15 days prior to screening.

5.3 Participant Re-enrollment

Participants who screen-fail from the study prior to randomization and study product administration may be rescreened and re-enrolled as long as the participant was not screen-failed due to noncompliance with the protocol (i.e., positive urine drugs of abuse screen, etc.). The medical monitor must be consulted prior to rescreening any participant. If the participant is rescreened, she must be reconsented and a new participant number must be used.

5.4 Participant Withdrawal

Participants are free to discontinue the study at any time, for any reason, and without prejudice to further treatment. The investigator may remove a participant if, in the investigator's judgment, continued participation would pose unacceptable risk to the participant or to the integrity of the study data. All procedures for early termination must be completed. Reasons for removal or withdrawal may include:

- Withdrawal of consent
- Administrative decision by the investigator or sponsor
- Ineligibility
- Significant protocol deviation
- Participant noncompliance
- Safety concern by the investigator or sponsor
- Lost to follow-up

Participants who are withdrawn prior to completing all study visits may be replaced.

In the event of a participant's withdrawal, the investigator will promptly notify the sponsor and medical monitor and will make every effort to complete the EOS assessments. All withdrawn participants with ongoing clinically significant clinical or laboratory findings will be followed until the finding is resolved or medically stable; reasonable attempts will be made to follow-up with participants.

5.5 Early Termination of Study

The study may be terminated at any time by the sponsor if serious side effects occur, if potential risks to study participants are identified, if the investigator does not adhere to the protocol, or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In the event that the clinical development of the study product is discontinued, the sponsor shall inform all investigators/institutions and regulatory authorities.

6 TREATMENT OF PARTICIPANTS

6.1 Identity of Study Products

A description of the study products is presented in Table 6-1.

Table 6-1: Study Products

Treatment Group and Number of Participants	Treatment
IVR Dose 1 (N=10)	12-week IVR 80/4 (estradiol 80 µg/day + progesterone 4 mg/day), replaced every 28 days
IVR Dose 2 (N=10)	12-week IVR 160/8 (estradiol 160 µg/day + progesterone 8 mg/day), replaced every 28 days

DARE-HRT1 IVRs will be sourced by Daré Bioscience Australia Pty LTD.

6.2 Treatments Administered

Each participant will be assigned to receive an IVR (Dose 1 or Dose 2) for 12 weeks, across three 28-day cycles, with a new IVR administered on Day 1 of each cycle. In Cycles 2 and 3, the new IVR should be self-administered as soon as possible after the previous IVR has been removed.

For each cycle, Day 1 is defined as the first day of treatment, i.e., the day the IVR is self-administered by the participant. Correct placement of the IVR will be confirmed by the study staff. The same IVR is to remain in position for 28 days, with removal of the IVR by the participant in the CRU on the morning of Day 1 of the next cycle.

If a participant cannot self-insert the IVR, the site physician will insert the IVR.

At the end of each cycle, participants will remove their own IVR.

6.3 Method of Assigning Participants to Treatment Groups

ICON will prepare the randomization scheme in accordance with its standard operating procedures (SOPs) and the randomization plan, which reflect Good Clinical Practice (GCP) standards. Refer to Section 9.3 for a description of randomization methods. Eligible participants will be assigned to a treatment group according to the list of participant randomization assignments.

6.4 Measurements of Treatment Compliance

Treatment compliance of study product administration will be confirmed by PK evaluation and participant daily diary.

6.5 Investigational Product Storage, Accountability, and Retention

6.5.1 Storage Conditions

The IP is to be stored at refrigerated temperature (2°C to 8°C). The product does not need protection from light. Additional information can be found in the Investigator's Brochure.

The investigator will ensure that all the study products are stored and dispensed in accordance with local regulations concerning the storage and administration of investigational drugs.

6.5.2 Study Product Accountability and Retention

The investigator must ensure that all study product supplies are kept in a secure locked area with access limited to those authorized by the investigator. The investigator must maintain accurate records of the receipt of all study products shipped by Daré Bioscience Australia Pty LTD or their representative, including but not limited to the date received, lot number, expiration date, amount received, and the disposition of all study products. Current dispensing records will also be maintained including the date and number of study products dispensed and the participant receiving the product. All remaining study products not required by regulations to be held by the CRU must be returned to Daré Bioscience Australia Pty LTD or their representative immediately after the study is completed.

It is the responsibility of Daré Bioscience Australia Pty LTD to ship a sufficient number of dosage units to allow the CRU to maintain an appropriate sampling on-site as per applicable regulatory requirements. Each reserve sample shall consist of a sufficient quantity to permit the local regulatory agency to perform 5 times all of the release tests required in the application or supplemental application.

6.6 Packaging and Labeling

6.6.1 Investigational Product

Details regarding IP packaging, labeling, and use instructions will be provided in a separate Study Reference Manual (SRM).

6.6.2 Blinding of Treatment Assignment

Not applicable. This is an open-label study.

6.7 Concomitant Medications and Procedures and Other Restrictions

6.7.1 Concomitant Medications and Procedures

No concomitant medications (prescription, OTC, and herbal), with the exception of paracetamol up to 650 mg/day (not to exceed 2000 mg total in the 7 days before the first treatment with DARE-HRT1), no personal lubricants (with the exception of water-based lubricants), or intravaginal products may be administered during the study unless they are prescribed by the investigator for treatment of specific clinical events. No concomitant procedures will be performed during the study unless approved by the investigator. No vaccinations are allowed

other than for coronavirus disease 2019 (COVID-19) unless approved by the investigator. All medications (prescription and OTC), vitamin and mineral supplements, and herbal remedies taken during the study will be documented on the concomitant medication electronic case report form (eCRF). Information recorded will include: start and stop dates and times, dose and route of administration, and indication. Medications taken for a procedure will also be included, as well as the procedure itself.

6.7.2 Other Restrictions

Participants will be instructed to adhere to the following restrictions:

- Participants must comply with the CRU smoking policy, if applicable
- Participants are not permitted to participate in any other clinical trial or donate blood or plasma while participating in this clinical trial.

7 STUDY ASSESSMENTS AND PROCEDURES

Participants will undergo study procedures and assessments at time points specified in the schedule of events (Table 1-1).

7.1 Medical and Surgical History

The investigator or designee will collect a complete medical and surgical history at screening. Medical and surgical history will be collected on Cycle 1 Day 1 to determine if any changes have occurred since screening.

7.2 Demographic Characteristics

Demographic characteristics including sex, age, race, and ethnicity will be recorded.

7.3 Physical Measurements

Height (cm) and body weight (kg) without shoes will be recorded and BMI will be calculated.

7.4 Daily Diary

Participants will use a paper diary to document insertion and removal of the IVR and any instances of the IVR falling out, as well as any concomitant medication use (see Appendix C). Diaries will be dispensed at screening, and participants should bring the diary to study visits in accordance with the schedule of events (Table 1-1) for review.

7.5 Training

Participants will be trained how to use the IP in accordance with the schedule of events (Table 1-1).

7.6 Pharmacokinetic Assessments

7.6.1 Drug Concentration Measurements

Plasma PK samples will be collected at time points specified in the PK sampling schedule within the schedule of events (Table 1-1). Blood sample collection, processing, and shipping details will be outlined in a separate SRM.

Plasma concentrations of estradiol, estrone, and progesterone will be determined using a validated method.

7.6.2 Pharmacokinetic Parameters

Pharmacokinetic variables will be calculated from the plasma concentration data using noncompartmental methods (Phoenix™ WinNonlin®, Version 6.4 or later; Certara LP, Princeton, New Jersey, USA) and actual sampling times.

The following PK parameters will be determined:

C_{ss}	Steady-state concentration

C_{max}	Maximum observed plasma concentration
t_{max}	The time that C_{max} was observed
AUC_{D1-D29}	Area under the plasma concentration-time curve from time 0 (Day 1) to the time of the last quantifiable concentration on Day 29; calculated using the linear/log trapezoid rule

Baseline correction, using the average of the 3 predose concentrations on Day 1, may be performed prior to the calculation of PK parameters by noncompartmental methods. The C_{max} (and t_{max}) will be computed over each 24-hour PK profile (Day 1 of each cycle) and over the entire 28-day treatment interval. Additional PK parameters may be calculated as appropriate.

7.7 Safety Assessments

7.7.1 Adverse Events

Participants will be monitored for AEs from the time of consent. Treatment-emergent AEs (TEAEs) will be those with an onset after insertion of the first IVR through the follow-up telephone call. Serious adverse events will be collected beginning at ICF signing and in an ongoing manner through the follow-up telephone call. Refer to Section 8 for additional details.

7.7.2 Laboratory Tests

A certified laboratory will be utilized to process and provide results for the clinical laboratory tests listed in Table 7-1. The baseline laboratory test results for clinical assessment for a particular test will be defined as the last measurement prior to the first administration of study product.

Participants will fast for a minimum of 8 hours prior to clinical laboratory sample collection at screening. For subsequent clinical laboratory sample collections, participants will not be required to be fasted.

During screening, if a participant has an out-of-range value for a clinical laboratory parameter that the investigator believes is not clinically significant or the investigator does not believe is correct (e.g., laboratory or specimen processing error), but the investigator wants to confirm with a repeat laboratory test, a single repeat is allowed to confirm the initial result.

Additional safety laboratory tests may be conducted as needed by the investigator to evaluate participant safety.

Table 7-1: Clinical Laboratory Tests

Hematology	Chemistry	Urine Dipstick
Hematocrit	Albumin	Specific gravity
Hemoglobin	Alkaline phosphatase	Ketones
Red blood cell count	Alanine aminotransferase	pH
White blood cell count	Aspartate aminotransferase	Protein
Neutrophils (absolute)	Gamma glutamyl transferase	Blood
Lymphocytes (absolute)	Direct bilirubin	Glucose
Monocytes (absolute)	Total bilirubin	Leukocyte esterase
Basophils (absolute)	Lactate dehydrogenase	Microscopic analysis (performed if blood, leukocytes, or protein are present)
Eosinophils (absolute)	Total protein	Nitrites
Platelet count (estimate not acceptable)	Blood urea nitrogen	
	Creatinine	
	Calcium	
	Phosphate	
	Sodium	
	Potassium	
	Bicarbonate	
	Chloride	
	Glucose [a]	
	Total cholesterol	
	Triglycerides [a]	

a. Fasting, at screening visit

For any laboratory test value outside the reference range that the investigator considers to be clinically significant during the on-study period (i.e., following dose administration), the investigator will:

- Repeat the test to verify the out-of-range value and clinical significance
- Follow the out-of-range value until the value returns to normal or baseline, or until the value is deemed stable and not clinically significant by the investigator
- Record as an AE any laboratory test value that is confirmed by repeat and which:
  - the investigator considers clinically significant
  - requires a participant to be discontinued from the study
  - requires a participant to receive treatment, or
  - requires a change or discontinuation of the study product (if applicable)

7.7.3 Other Assessments

The following assessments will be performed:

- Urine drugs of abuse according to the local standard, including (at a minimum, cocaine, tetrahydrocannabinol, amphetamines and opiates), and alcohol screens via urinalysis or a commercially available urine dipstick.
    - During the screening period, urine drugs of abuse, and alcohol screens may not be repeated for eligibility unless the investigator believes that a positive result is possibly attributable to a concomitant medication; a repeat test will be allowed under this circumstance.
- Serology tests (i.e., HIV-1 and HIV-2 antibodies, hepatitis B surface antigen, and HCV antibody, and any confirmatory tests performed at the discretion of the investigator)
- FSH (as needed to confirm postmenopausal status)
- Transvaginal ultrasound
- Cervical screening test (if needed)
- Speculum examination to identify vaginal abnormalities, with a bimanual examination at Screening
- Endometrial biopsy (if needed): any participant experiencing bleeding during the study will undergo a transvaginal ultrasound and possible endometrial biopsy.
- Record prior and concomitant medications (Section 6.7)

7.7.4 Vital Signs

Vital signs assessments will include oral temperature (°C), respiratory rate (breaths per minute), systolic and diastolic blood pressure (mmHg) and pulse rate (beats per minute). Blood pressure and pulse rate will be measured after the participant has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes. Any clinically significant abnormal vital sign assessment requires at least one repeat measurement.

Vital signs abnormalities that are considered clinically significant initially and on confirmation, require a participant to be discontinued from the study, require a participant to receive treatment, or require a change or discontinuation from the study product (if applicable) will be recorded as AEs.

7.7.5 Physical Examination

Comprehensive physical examinations (excluding rectal and breast examinations [unless indicated]) will be performed, and abnormal findings will be documented in the participant's eCRF as shown in the schedule of events (Table 1-1). Additional symptom-directed

examinations can be performed at all other visits for participants exhibiting signs or reporting symptoms since their last visit.

An abnormal physical examination finding that is considered clinically significant and requires the participant to be discontinued from the study, requires the participant to receive treatment, or requires a change or discontinuation of the study product (if applicable) will be recorded as an AE.

7.7.6 Electrocardiograms

Electrocardiogram assessments will be conducted as a standard check to ensure overall stable health prior to trial entry.

7.7.7 Appropriateness of Safety Assessments

Safety evaluations selected for this study are typical of those for this participant population and utilize widely accepted measures.

7.8 Exploratory Assessments

7.8.1 Usability and Tolerability Questionnaire

A Usability and Tolerability questionnaire (see Appendix A) will be administered to all participants in accordance with the schedule of events (Table 1-1).

7.8.2 Vaginal Cytology

Samples for vaginal cytology will be collected at the time points specified in the schedule of events (Table 1-1) to determine the maturation index. Investigators should collect a swab of the lateral vaginal wall to be submitted for determination of the vaginal maturation index.

The maturation index is determined by categorizing the ratio of the 3 types of vaginal epithelial cell (parabasal, intermediate, and superficial).

7.8.3 Vaginal pH

Vaginal swabs for determination of pH will be collected in accordance with the schedule of events (Table 1-1). The pH of vaginal secretions will be measured using pH paper. Additional details and instructions will be provided in the SRM.

7.8.4 Most Bothersome Symptom

At the Cycle 1 Day 1 Visit, participants will rate the first 4 items in the list of VVA symptoms in Table 7-2 as either not present (none), mild, moderate, or severe, and will indicate whether vaginal bleeding associated with vaginal activity is present or absent. They will then select 1 of the 5 symptoms as their most bothersome symptom. All 5 symptoms will be evaluated again during later study visits (see Table 1-1), and the change in severity from baseline will be determined. Changes from baseline in each participant's most bothersome symptom will be evaluated.

Table 7-2: Assessment of Most Bothersome Symptom

Symptom	Assessment
Vaginal dryness	None, mild, moderate, or severe
Vaginal and/or vulvar irritation/itching	None, mild, moderate, or severe
Dysuria	None, mild, moderate, or severe
Vaginal pain associated with sexual activity (dyspareunia)	None, mild, moderate, or severe
Vaginal bleeding associated with sexual activity	Presence versus absence

7.8.5 Menopause-specific Quality of Life

Participants will complete the MENQOL questionnaire (see Appendix B) on Cycle 1 Day 1 and at the end of the study (see Table 1-1). Developed in 1996, the MENQOL is a self-administered, 29-item questionnaire with 4 domains: vasomotor, physical, psychosocial, and sexual. Participants record whether they have experienced the listed problems in the past month, and rate the severity on a Likert scale from 0 (Not at all bothered) to 6 (Extremely bothered) (Hilditch JR, et al., 1996; Lewis JE, et al., 2005).

Assessment of the severity of VMS at baseline and at the end of the study will be based on the relevant questions from MENQOL.

8 ADVERSE EVENTS

An AE is defined as any untoward medical occurrence in a participant administered a pharmaceutical product during the course of a clinical investigation. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a study drug/product, whether or not thought to be related to the study drug/product.

Participants will be monitored for AEs throughout the study from the time of consent. Treatment-emergent AEs (TEAEs) will be those with an onset after insertion of the first IVR through the follow-up telephone call. Adverse events that are identified at the last assessment visit (or the early termination visit) as specified in the protocol must be recorded on the AE eCRF with the status of the AE noted. All events that are ongoing at this time will be recorded as ongoing on the eCRF. All (both serious and nonserious) AEs must be followed until they are resolved or stabilized, or until reasonable attempts to determine resolution of the event are exhausted. The investigator should use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events.

The procedures specified in Section 8.4 are to be followed for reporting SAEs.

8.1 Recording Adverse Events

Adverse events are to be recorded on the AE page of the eCRF. The following information will be recorded:

- Assessment of whether or not the AE is an SAE (Section 8.2.1)
- Assessment of AE intensity (Section 8.2.2)
- Assessment of AE relationship to study product (Section 8.2.3)
- Action taken - categorized as dose increased, dose not changed, treatment interrupted, treatment withdrawn, not applicable, or unknown, as applicable
- Outcome - recorded as fatal, not recovered/not resolved, recovered/resolved, recovered/resolved with sequelae, recovering/resolving, or unknown, as applicable

8.2 Assessment of Adverse Events

The investigator will assess each AE for seriousness, intensity, and relationship to study product.

8.2.1 Serious Adverse Events

The investigator is responsible for determining whether an AE meets the definition of an SAE. An SAE is any AE occurring from ICF signing through the follow-up telephone call that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- An important medical event*

*Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent any of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Note: SAEs require immediate reporting to the sponsor and medical monitor. Refer to Section 8.4 for details.

8.2.2 Intensity

The intensity of an AE will be graded according to the following definitions:

- Grade 1: Mild; the participant experiences awareness of signs or symptoms but these are easily tolerated or managed without specific treatment
- Grade 2: Moderate; the participant experiences discomfort enough to cause interference with usual activity, and/or the condition requires specific treatment
- Grade 3: Severe; the participant is incapacitated with inability to work or do usual activity, and/or the event requires significant treatment measures
- Grade 4: Disabling or with life threatening consequences, urgent intervention indicated
- Grade 5: Death

8.2.3 Relationship to Study Product

The relationship of an AE to the study product should be determined by the investigator according to the following criteria:

- Not related: The event is most likely produced by other factors such as the participant's clinical condition, intercurrent illness, or concomitant drugs, and does not follow a known response pattern to the study product, or the temporal relationship of the event to study product administration makes a causal relationship unlikely
- Unlikely related: The event is most likely produced by other factors such as the participant's clinical condition, therapeutic interventions, or concomitant drugs administered to the participant and it does not follow a known response pattern to the study product
- Possibly related: The event follows a reasonable temporal sequence from the time of study product administration, and/or follows a known response pattern to the study product, but could have been produced by other factors such as the participant's clinical condition, intercurrent illness, or concomitant drugs
- Related: The event follows a reasonable temporal sequence from the time of study product administration, and/or follows a known response pattern to the study product, and cannot be reasonably explained by other factors such as the participant's clinical condition, intercurrent illness, or concomitant drugs

8.3 Discontinuation due to Adverse Events

Any participant who experiences an AE may be withdrawn at any time from the study at the discretion of the investigator. Participants withdrawn from the study due to an AE, whether serious or nonserious, may be followed by the investigator until the clinical outcome of the AE is determined. The AE(s) should be noted on the appropriate eCRFs and the participant's progress should be followed until the AE is resolved or stabilized as determined by the investigator. The sponsor and medical monitor must be notified. If the AE relates to overdose of study treatment, the Investigator's Brochure should be consulted for details of any specific actions to be taken.

8.4 Reporting Serious Adverse Events

Serious adverse events will be collected beginning at ICF signing and in an ongoing manner through the follow-up telephone call. In the event of any SAE reported or observed during the study, whether or not attributable to the study product, site personnel will report it immediately by telephone to the sponsor, medical monitor, and SAE hotline at ICON (ICON plc Pharmacovigilance) in accordance with procedures described in the SRM and/or SAE study-specific procedure. Site personnel will follow up with a written report to the sponsor on the next working day.

SAE Report Forms will be provided to the CRU to assist in collecting, organizing, and reporting SAEs and follow-up information.

All SAEs should be followed to their resolution, with documentation provided to the sponsor, medical monitor, and ICON on a follow-up SAE Report Form.

9 STATISTICAL CONSIDERATIONS

The statistical analysis will be conducted following the principles as specified in ICH Topic E9 (CPMP/ICH/363/96).

All statistical analyses will be described in a separate statistical analysis plan.

9.1 Sample Size Calculation

A total of 20 healthy, postmenopausal female participants (N = 10 per arm) are planned for inclusion in this study. The sample size for this study is based on the desire to accurately assess the safety, tolerability, and PK of the DARE-HRT1 IVR over a 12-week period. The sample sizes provided are deemed sufficient to allow for adequate assessment of the IVR. Dropouts may be replaced. Participants who terminate early from the trial may be replaced to ensure an adequate number of participants contributing safety data from extended treatment with the IVR for each treatment arm.

9.2 Analysis Populations

Safety Population: all enrolled participants who received active treatment, i.e., inserted an IVR (and thus who were exposed to one of the IVRs).

All safety analyses (primary endpoints) will be conducted in the Safety Population.

Pharmacokinetic Population: all participants who received a full course of study treatment for their dispensed treatment and who have sufficient concentration data for determination of PK parameters (primary endpoint); data will be analyzed according to the actual study treatment received.

All PK analyses will be conducted in the Pharmacokinetic Population.

Modified Intent-to-treat (mITT) Population: all enrolled participants who received active treatment and who underwent a baseline assessment and at least 1 post-treatment assessment of at least 1 of the following exploratory assessments: Usability and Tolerability questionnaire, vaginal cytology, vaginal pH, most bothersome symptom, or VMS (MENQOL)

All exploratory analyses will be conducted in the mITT population.

9.3 Randomization

Upon completion of their screening procedures and confirmation of their eligibility (including a transvaginal ultrasound and, if needed, an endometrial biopsy and/or cervical screening test), eligible participants will return to the CRU on Cycle 1 Day 1 and will be randomized. Randomization will occur prior to initiation of treatment. Participants will be randomized in a 1:1 ratio to the two treatment groups.

9.4 Endpoints

9.4.1 Primary Endpoints

The primary endpoints are as follows:

- Incidence and severity of AEs
- Changes from baseline in clinical laboratory findings
- Changes from baseline in physical examination findings
- Changes from baseline in vital signs results
- Changes from baseline in speculum examination findings
- Changes from baseline in transvaginal ultrasound findings
- Steady-state concentration (C_{ss}) and maximum observed plasma concentration (C_{max}) in each cycle for estradiol, estrone, and progesterone

9.4.2 Exploratory Endpoints

Exploratory endpoints are as follows:

- Responses to the Usability and Tolerability questionnaire
- Change from baseline to Week 12 in responses to the MENQOL questionnaire
- Mean change from baseline to Week 12 in the severity of the participant's VVA signs and symptoms
- Mean change from baseline to Week 12 in the severity of the participant's most bothersome VVA symptom
- Mean change from baseline to Week 12 in vaginal pH
- Mean change from baseline to Week 12 in vaginal maturation index (parabasal, intermediate, and superficial cells)

9.5 Pharmacokinetic Statistical Analysis

The PK analysis will be performed for each IVR dose. Graphics will be provided to allow for visual inspection of the mean and individual participant concentration-time profiles.

The PK parameters C_{ss}, C_{max}, t_{max}, and $AUC_{D1\text{-}D29}$ will be determined from the plasma concentration-time profiles, where possible. Geometric mean values and coefficients of variation (CV%) will be estimated and reported.

If possible, the dose proportionality for the DARE-HRT1 IVR estradiol and progesterone doses will be assessed.

Other details of the PK analysis will be described in a separate statistical analysis plan.

9.6 Safety Analysis

Adverse events, concomitant medications, clinical laboratory findings, physical examinations, speculum examination findings, transvaginal ultrasound results (and endometrial biopsy findings if required), and vital signs for each participant will be tabulated or summarized descriptively, where appropriate.

Vital signs data (observed and change from baseline) will be summarized by time point and treatment using appropriate descriptive statistics. Using the safety population, changes from baseline at each post-randomization visit and end of treatment will be compared between treatment arms. Qualitative analysis of laboratory tests in terms of abnormality will be performed. Findings on physical examination at screening and end of treatment will be reported and compared.

The number and percentage of participants reporting any TEAE will be tabulated by system organ class and preferred term for each treatment (coded using Medical Dictionary for Regulatory Activities [MedDRA] version 24.1 or later). All TEAEs will be further classified by severity and relationship to treatment.

10 ACCESS TO SOURCE DATA/DOCUMENTS

The investigator will provide direct access to source data and documents for individuals conducting study-related monitoring, audits, Human Research Ethics Committee (HREC) review, and regulatory review. The investigator must inform the study participant that their study-related records may be reviewed by the above individuals without violating the participant's privacy of personal health information in compliance with the Australian Privacy Act 1988 regulations.

By signing this protocol, the investigator affirms to the sponsor that the investigator will maintain, in confidence, information furnished to them by the sponsor and will divulge such information to the HREC under an appropriate understanding of confidentiality with such committee.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Daré Bioscience Australia Pty LTD/ICON will implement and maintain quality control and quality assurance procedures with written SOPs to ensure the study is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements.

11.1 Conduct of Study

This study will be conducted in accordance with the provisions of the Declaration of Helsinki and all revisions thereof (Tokyo 2004), and in accordance with the ICH E6 Guidelines on GCP (CPMP/ICH/135/95). Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by an HREC; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the participants will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each participant will give their written, informed consent before any protocol-driven tests or evaluations are performed.

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate HREC, except when necessary to eliminate immediate hazards to the participant or when the change(s) involve only logistical or administrative aspects of the study and are approved by the medical monitor and/or Daré Bioscience Australia Pty LTD. Any deviation may result in the participant having to be withdrawn from the study, and may render that participant nonevaluable.

11.1.1 Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the participant, investigator, or site staff. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications, food, drink, herbal remedies, or supplements that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Study product not administered within the time frame specified in the protocol

- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. – either tests not done, incorrect tests done, or tests not done within the time frame specified in the protocol
- Procedural deviations such as incorrect storage of IP, failure to update the ICF when new risks become known, failure to obtain HREC approvals for the protocol and ICF revisions

At the outset of the study, a process for defining and handling protocol deviations will be established. This will include determining which violations will be designated "key," requiring immediate notification to the medical monitor and Daré Bioscience Australia Pty LTD. The investigator is responsible for seeing that any known protocol deviations are recorded and handled as agreed.

11.2 Protocol Amendments

Only Daré Bioscience Australia Pty LTD may modify the protocol. Amendments to the protocol will be made only after consultation and agreement between Daré Bioscience Australia Pty LTD, the medical monitor, and the investigator. All amendments that have an impact on participant risk or the study objectives, or require revision of the ICF, must receive approval from the HREC prior to their implementation.

11.3 Monitoring of Study

The investigator will permit the site monitor to review study data as frequently as is deemed necessary to ensure data are being recorded in an adequate manner and protocol adherence is satisfactory.

The investigator will provide access to medical records for the monitor to verify eCRF entries. The investigator is expected to cooperate with Daré Bioscience Australia Pty LTD/designee in ensuring the study adheres to GCP requirements.

The investigator may not recruit participants into the study until Daré Bioscience Australia Pty LTD or a designee has conducted a visit at the site to conduct a detailed review of the protocol and eCRF. With agreement of Daré Bioscience Australia Pty LTD, attendance at an investigator meeting may fulfil this requirement.

12 ETHICS

12.1 Human Research Ethics Committee Approval

12.1.1 Ethics Review Prior to Study

The investigator will ensure that the protocol and consent form are reviewed and approved by the appropriate HREC prior to the start of any study procedures. The HREC will be appropriately constituted and will perform its functions in accordance with ICH GCP guidelines and local requirements as applicable.

12.1.2 Ethics Review of Other Documents

In addition, the HREC will approve all protocol amendments (except for Daré Bioscience Australia Pty LTD-approved logistical or administrative changes), written informed consent documents and document updates, participant recruitment procedures, written information to be provided to the participants, available safety information, information about payment and compensation available to participants, the investigator's curriculum vitae and/or other evidence of qualifications, and any other documents requested by the HREC and regulatory authority as applicable.

12.2 Written Informed Consent

The nature and purpose of the study will be fully explained to each participant (or the participant's legally responsible guardian). The participants must be given ample time and opportunity to inquire about details of the study, to have questions answered to their satisfaction, and to decide whether to participate. Written informed consent must be obtained from each participant (or guardian) prior to any study procedures being performed.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Reporting and Case Report Forms

13.1.1 Case Report Forms

The investigator will be provided with eCRFs, and will ensure all data from participant visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign the eCRFs to verify the integrity of the data recorded.

13.1.2 Laboratory Data

All safety samples will be analyzed by the CRU and all bioanalytical samples will be analyzed by Agilex Biolabs Pty Ltd, Adelaide, South Australia. A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. The investigator must maintain source documents such as laboratory reports and complete history and physical examination reports.

13.1.3 Retention of Source Documents

The investigator must maintain source documents such as laboratory reports, x-rays, ECGs, consultation reports, and complete history and physical examination reports.

13.2 Retention of Essential Documents

The study essential documents must be maintained as specified in the ICH guidelines for GCP and the applicable regulatory requirements. The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with Daré Bioscience Australia Pty LTD. It is the responsibility of the Daré Bioscience Australia Pty LTD to inform the investigator/institution as to when these documents no longer need to be retained.

14 ADMINISTRATIVE INFORMATION

14.1 Financing and Insurance

Financing and insurance will be addressed in a separate agreement between Daré Bioscience Australia Pty LTD and the investigator.

14.2 Publication Policy

Daré Bioscience Australia Pty LTD will retain ownership of all data. All proposed publications based on this study will be subject to Daré Bioscience Australia Pty LTD's approval requirements.

15 REFERENCES

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16 SIGNATURES

Protocol Number: DARE-HRT1-002

Protocol Title: 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

Daré Bioscience Australia Pty LTD Signatures

This clinical study Protocol Version 1.0, dated 06 January 2022, has been reviewed and approved by Daré Bioscience Australia Pty LTD.

Nadene Zack, MS Vice President, Clinical Operations	Date

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David Friend, PhD Chief Scientific Officer	Date

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Version 1.0: 06 January 2022

Investigator Signature

I agree to conduct the aforementioned study according to the terms and conditions of the protocol, GCP guidelines, and all other applicable local and regulatory requirements. All information pertaining to the study will be treated in a confidential manner.

Site Name	
Print Name	
Signature	Date

17 APPENDICES

APPENDIX A Usability and Tolerability Questionnaire

"Usability and Tolerability Questionnaire for Subjects Assigned to an Intravaginal Ring (IVR)" (Version 1, 18 December 2021) is provided here.

Usability and Tolerability Questionnaire for Subjects Assigned to an Intravaginal Ring (IVR)

EASE OF USE

	1	2	3	4	5
	Very easy	Somewhat easy	Neutral; not easy or difficult	Somewhat difficult	Very difficult
Ease of insertion					
Ease of removal					

COMFORT AND CONVENIENCE

	1	2	3	4	5
	Strongly agree	Agree	Neither agree or disagree	Disagree	Strongly disagree
IVR is comfortable when worn					
IVR is convenient to use					
IVR works with my lifestyle					

Please rate the overall comfort of the IVR (circle one):				
1	2	3	4	5
Very Comfortable	Comfortable	Neither Comfortable nor Uncomfortable	Uncomfortable	Very Uncomfortable

Question (circle the number that best represents how you feel)	**Very likely**	**Somewhat likely**	**Neither likely nor unlikely**	**Somewhat unlikely**	**Very Unlikely**
How likely would you be to use the intravaginal ring for a condition or disease that is related to women's health (e.g., hormone replacement, overactive bladder, uterine fibroids)?	1	2	3	4	5
How likely would you be to use the intravaginal ring for a condition or disease that is not related to women's health (e.g., high blood pressure, diabetes)?	1	2	3	4	5

APPENDIX B MENQOL Instrument

The 1-month recall MENQOL questionnaire is provided here.

1

2

3

4

5

6

7

APPENDIX C Participant Daily Diary

"Daily Diary Questions for Subjects Assigned to an Intravaginal Ring (IVR)" (Version 1, 18 December 2021) is provided here.

Daily Diary Questions for Subjects Assigned to an Intravaginal Ring (IVR)

Date: _____

Did you take any medications today? YES NO <circle one>

If yes, record medication name, time, reason, and dose below. If no, continue to next question.

Medication Name	Time Taken	Reason Taken	Dose
1.			
2.			
3.			
4.			
5.			

Did the ring fall out today? YES NO <circle one>

Complete one line for **EACH** time the ring falls out:

If yes, time ring fell out: _____Time of reinsertion: _____
Activity at time ring fell out (running, walking, lifting, restroom use, sitting, sleeping, other, <specify>):_____

If yes, time ring fell out: _____Time of reinsertion: _____
Activity at time ring fell out (running, walking, lifting, restroom use, sitting, sleeping, other, <specify>):_____

If yes, time ring fell out: _____Time of reinsertion: _____
Activity at time ring fell out (running, walking, lifting, restroom use, sitting, sleeping, other, <specify>):_____

Did you remove the ring today? YES NO <circle one>

Complete one line for **EACH** time the ring was removed:

If yes, time ring removed: _____Time of reinsertion: _____
Removal reason (discomfort, other, <specify>):_____

If yes, time ring removed: _____Time of reinsertion: _____
Removal reason (discomfort, other, <specify>):_____

If yes, time ring removed: _____Time of reinsertion: _____
Removal reason (discomfort, other, <specify>):_____

Did you experience any of the following today?

	Circle one per question	
Vaginal pain	Yes	No
Vaginal burning	Yes	No
Vaginal itching	Yes	No
Discomfort during physical activity	Yes	No
Discomfort while sitting	Yes	No
Discomfort while standing	Yes	No
Discomfort while lifting an object	Yes	No
Discomfort while using the restroom	Yes	No
Discomfort during sexual activity	Yes	No
Intravaginal ring didn't feel like it was in the right place	Yes	No
Intravaginal ring felt like it was slipping out	Yes	No