29 January 2026
EMA/39397/2026
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

FYLREVY

International non-proprietary name: Estetrol

Procedure No. EMEA/H/C/006213/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

Abbreviation	Definition
ADME	absorption, distribution, metabolism and excretion
AE	adverse event
AESI	adverse event of special interest
aGFR	absolute glomerular filtration rate
APCsr ETP	endogenous thrombin potential based activated protein C sensitivity ratio
AR	Argentina
ASMF	Active Substance Master File
ATC	anatomical therapeutic chemical (classification system)
ATR	Attenuated total reflectance
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-inf}}$	area under the curve from time 0 to infinity
$AUC_{0\text{-last}}$	area under the curve from time 0 to the last measured time point
$AUC_{0\text{-}\tau}$	area under the curve during a dosage interval
AUC_{τ}	AUC of a single dosing period
BCRP	breast cancer resistance protein
BMI	body mass index
BR	Brazil
CA	Canada
CAD	coronary artery disease
C_{av}	average steady state concentration
CEE	conjugate equine estrogen
CGI	clinical global impression
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CID	clinically important difference
CLr	renal clearance
C_{max}	maximum plasma concentration
COSY	Correlated Spectroscopy
COVID-19	Coronavirus disease: infectious disease caused by the SARS-CoV-2 virus
CQA	Critical quality attribute
CSR	clinical study report
CTX-1	C-terminal telopeptide
CV	cardiovascular
CVD	cardiovascular disease
DDI	drug-drug interaction
DPE	disordered proliferative endometrium
DRSP	drospirenone
DSC	Differential scanning calorimetry
DSG	desogestrel
DVT	deep vein thrombosis
E2	estradiol
E2V	estradiol valerate
E4[1]	estetrol
E4 0.1 mg[1]	estetrol monohydrate 0.1 mg (equivalent to estetrol 0.0944 mg)
E4 1 mg[1]	estetrol monohydrate 1 mg (equivalent to estetrol 0.944 mg)
E4 1.25 mg[1]	estetrol monohydrate 1.25 mg (equivalent to estetrol 1.18 mg)
E4 2 mg[1]	estetrol monohydrate 2 mg (equivalent to estetrol 1.89 mg)
E4 2.5 mg[1]	estetrol monohydrate 2.5 mg (equivalent to estetrol 2.36 mg)
E4 5 mg[1]	estetrol monohydrate 5 mg (equivalent to estetrol 4.72 mg)
E4 10 mg[1]	estetrol monohydrate 10 mg (equivalent to estetrol 9.44 mg)
E4 15 mg[1]	estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg)
E4 20 mg[1]	estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg)
E4 30 mg[1]	estetrol monohydrate 30 mg (equivalent to estetrol 28.3 mg)

[1] E4 is used as abbreviation of "estetrol", the native/natural/circulating form of the hormone, while when referring to the dose (E4 X mg), it stands for "estetrol monohydrate", the specific chemical solid active substance of the drug product, as it was used in this form in the clinical study reports (CSRs) (see the detailed list of abbreviations of the different estetrol monohydrate doses with corresponding estetrol dose equivalence above).

Abbreviation	Definition
E4 40 mg[1]	estetrol monohydrate 40 mg (equivalent to estetrol 37.8 mg)
E4 45 mg[1]	estetrol monohydrate 45 mg (equivalent to estetrol 42.5 mg)
E4 100 mg[1]	estetrol monohydrate 100 mg (equivalent to estetrol 94.4 mg)
ECG	electrocardiogram
EE	ethinyl estradiol
eGFR	estimated glomerular filtration rate
ELITE	Early Versus Late Intervention Study with Estradiol
EMA	European Medicines Agency
EMAS	European Menopause and Andropause Society
EOS	end of study
EOT	end of treatment
ESP	Efficacy Study Part
EU	European Union
F	bioavailability of E4
FCT	film-coated tablet
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GC	Gas chromatography
GCP	Good Clinical Practice
GM	geometric mean
GMP	Good Manufacturing Practice
H	hysterectomized
HbA1c	hemoglobin A1c
HDL	high-density lipoprotein
HERS	Heart and Estrogen/Progestin Replacement Study
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High performance liquid chromatography
HOMA-IR	homeostatic model assessment of insulin resistance
HR-MS	High Resolution Mass Spectrometry
HRQoL	health-related quality of life
HRT	hormone replacement therapy
HSQC	Heteronuclear single quantum coherence spectroscopy
ICH	International Conference on Harmonization
IR	Infrared spectroscopy
ISS	integrated summary of safety
ITT	intent to treat
KEEPS	Kronos Early Estrogen Prevention Study
LDL	low-density lipoprotein
LH	luteinizing hormone
LP(a)	lipoprotein a
LS	least squares
MATE	multidrug and toxin extrusion
Max	maximum
MD	Multiple dose
MED	minimum effective dose
MedRA	Medical Dictionary for Regulatory Activities
MENQOL	Menopause specific Quality of Life
Min	minimum
NMR	Nuclear magnetic resonance
MMRM	mixed-effect models for repeated-measures
MPA	medroxyprogesterone-acetate
N	number of subjects
n	number of subjects in category of interest
NAMS	North American Menopause Society
NH	non-hysterectomized
NK3	neurokinin 3
NMR	Nuclear magnetic resonance
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter

Abbreviation	Definition
P1NP	procollagen type I N-propeptide
P4	progesterone
PD	pharmacodynamics
P-gp	P-glycoprotein
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetics
PT	preferred term
QoL	quality of life
QTc	corrected QT
QTcF	corrected QT using Fridericia's formula
QTTP	quality target product profile
RCT	randomized control study
ROESY	Rotating frame Overhauser Spectroscopy
SAE	serious adverse event
SD	standard deviation
SEM	standard error of the mean
SHBG	sex hormone binding globulin
SmPC	Summary of product characteristics
SNRI	serotonin and norepinephrine reuptake inhibitor
SOC	System Organ Class
SSP	Safety Study Part
SSRI	selective serotonin reuptake inhibitor
$t_{1/2}$	terminal elimination half-life
T2D	type-2 diabetes mellitus
TGA	Thermogravimetric analysis
TIA	transient ischaemic attack
T_{max}	time to maximum plasma concentration
TQT	thorough QT
TVUS	transvaginal ultrasound
UGT	uridine diphosphate glucuronosyltransferase
UK	United Kingdom
US	United States
UV	Ultraviolet
V	volume of distribution
VMS	vasomotor symptoms
VTE	venous thromboembolism
VVA	vulvovaginal atrophy
WHI	Women's Health Initiative
WHO	World Health Organization
WHO-DD	WHO drug dictionary
XRPD	X-ray powder diffraction

1. Administrative/regulatory information and recommendations on the procedure

1.1. Information on the product

Product data	
Product name	FYLREVY
Active substance	Estetrol monohydrate
INN or common name	Estetrol
Applicant	Gedeon Richter Plc. Gyömrői út 19-21 X Kerület 1103 Budapest HUNGARY
EMA Product Number	EMEA/H/C/006213
ATC code and Pharmacotherapeutic group	G03CA10 Sex hormones and modulators of the genital system, natural and semisynthetic oestrogens, plain
Pharmaceutical form(s) and strength (s)	Film-coated tablet 14.2 mg and 18.9 mg
Packaging	blister (PVC/alu)
Package size(s)	168 tablets + 1 storage bag, 28 tablets + 1 storage bag and 84 tablets + 1 storage bag
Route of administration	Oral use
Device or diagnostic	Not applicable
Orphan designation	No
Orphan indication status confirmed	Not applicable
PRIME scheme	Not applied for
Type of marketing authorisation granted at opinion	Standard
Legal basis	Article 8.3 of Directive 2001/83/EC
Final indication	Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in hysterectomised postmenopausal women. Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in non-hysterectomised postmenopausal women with at least 12 months since last menses.
New active substance status	Not applied for

1.2. Scientific advice

Table 1: Scientific advice and protocol assistance

Date	Topic (quality/ non-clinical/ clinical)	Reference number / Coordinator(s)	Brief summary of the advice

13 December 2018	Non-clinical and clinical	EMA/CHMP/SAWP/844981/2018
		Prof. Minne Casteels
		Dr Peter Mol
			• Overall non-clinical strategy
			• Need for renal impairment study
			• PK characterisation
			• Phase 3 design:
			  ○ Dose
			  ○ Sample size
			  ○ Patient population
			  ○ Efficacy endpoints
			  ○ Statistics
			• Safety database
			• E4 only HRT in hysterectomised women

19 March 2019	Non-clinical and clinical	EMA/171260/2019 Prof Minne Casteels Dr Carine Berquist	Scientific advice pertained to further developments and new formulations

24 March 2022	Clinical	EMA/SA/0000078164
		Carin Bergquist
		Dina Apele-Freimane
			• Design of a vulvar/vaginal atrophy study for explicit 4.1 statement
			  ○ Dose
			  ○ Sample size
			  ○ Study population
			  ○ Efficacy endpoints
			  ○ Statistics
			• Safety database
			• Exploratory endpoints for FSAD

22 April 2022	Non-Clinical	EMA/SA/0000079803 Carin Bergquist Markku Pasanen	• ERA: NOEC for E4 • Proposal for a new MEOGRT study
23 February 2023	Clinical	EMA/SA/0000122140 Carin Bergquist Livia Puljak	• Evidentiary plans and strategy for an initial MAA as hormone replacement therapy for oestrogen deficiency symptoms in hysterectomised women only
17 October 2024	Clinical	EMA/SA/0000220993 Minne Casteels Viktoriia Starokozhko	• Re-reading of endometrial biopsy slides from Phase 3 studies and analytical approach considering the initial reading and analysis plan to inform endometrial safety assessment

1.3. Eligibility to the centralised procedure

The applicant Gedeon Richter Plc. submitted on 30 January 2025 an application for Marketing Authorisation to the European Medicines Agency (EMA) for FYLREVY (estetrol), through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised

procedure was agreed upon by the EMA/CHMP on 13 October 2022.

The applicant applied for the following indication: Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women.

1.4. Legal basis and dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies.

1.5. Information on paediatrics

Pursuant to Articles 7 and 8 of Regulation (EC) No 1901/2006, the application included an EMA Decision (CW/0001/2015) on the granting of a class waiver.

1.6. Information on orphan market exclusivity

Not applicable.

1.6.1. Similarity with authorised orphan medicinal products

Pursuant to Article 8 of Regulation (EC) No 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products from the start of the procedure because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.7. Applicant's requests for consideration

Not applicable.

1.8. Patient experience data

Table 2: Patient experience data relevant to the application

Patient experience data submitted with this application				*Section where discussed (if applicable)*
X	Patient experience data submitted by the applicant:			
	X	Clinical outcome assessments (COAs) such as		
		X	Patient-reported outcomes (PRO)	5.3 Clinical efficacy
		☐	Other	
	☐	Patient preference studies		
	☐	Observational studies/RWD designed to capture patient experience data		

Patient experience data submitted with this application			Section where discussed (if applicable)
	☐	Qualitative information or studies (e.g. summaries/analysis from patient engagement activities such as individual patient/caregiver interviews, focus group interviews, expert interviews, etc)	
	☐	Other (please specify)	
☐	Other patient experience data not submitted by the applicant but considered in this evaluation:		
	☐	Input informed from participation in meetings or public hearings with patient stakeholders	
	☐	CHMP early dialogue with patient organisations	
	☐	Third party interventions from patients and patient groups	
	☐	Other (such as medical literature, summaries/analysis from patient engagement activities - please specify)	

1.9. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur:	Patrick Vrijlandt
Co-Rapporteur:	Ewa Balkowiec Iskra

The application was received by the EMA on	30 January 2025
The procedure started on	20 February 2025
The CHMP Rapporteur's first Assessment Report was received on	12 May 2025
The CHMP Co-Rapporteur's first Assessment Report was added to the Rapporteur's report on	14 May 2025
The PRAC Rapporteur's first Assessment Report was added to the Rapporteurs' report and circulated to all PRAC and CHMP members on	26 May 2025
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	19 June 2025
The applicant submitted the responses to the CHMP consolidated List of Questions on	10 September 2025
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP and PRAC members on	21 October 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	30 October 2025
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	13 November 2025
The applicant submitted the responses to the CHMP List of Outstanding Issues on	10 December 2025
The CHMP Rapporteur circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP and PRAC members on	14 January 2026

The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to FYLREVY on	29 January 2026

1.10. Final CHMP outcome

1.10.1. Considerations related to paediatrics

The requirements for the submitted dossier in relation to paediatrics are described in section 1.5 of this report.

1.10.2. Considerations related to orphan market exclusivity

The requirements of the submitted dossier in relation to orphan market exclusivity are described in section 1.6 of this report.

1.10.3. Final opinion

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of FYLREVY is favourable in the following indication(s):

Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in hysterectomised postmenopausal women

Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in non-hysterectomised postmenopausal women with at least 12 months since last menses.

1.10.4. Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

1.10.5. Other conditions and requirements of the marketing authorisation

1.10.5.1. Periodic safety update reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

1.10.6. Conditions or restrictions with regard to the safe and effective use of the medicinal product

1.10.6.1. Risk management plan (RMP)

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

2. Introduction

2.1. Therapeutic Context

Disease definition

Postmenopause, also called "menopause" in international several guidelines, is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 months of amenorrhea without any other obvious pathologic or physiologic cause. Natural menopause typically occurs between 45 and 55 years old (median age of 54 years in Europe and 51.4 years in the United States [US] [*Palacios et al. 2010*]) and is a reflection of complete, or near complete, ovarian follicular depletion, with resulting very low estradiol levels and high follicle-stimulating hormone (FSH) concentrations.

Menopause is characterized by decreased estradiol levels due to decreased ovarian function, and a resultant increase in gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus leading to high luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations (*Freeman et al. 2005*). Decreased estrogen levels, thought to be a main cause for vasomotor symptoms (VMS), occur in natural menopause and also in situations when estrogen levels are suppressed by medical intervention such as bilateral oophorectomy or adjuvant endocrine (anti-estrogen) therapy, for instance in hormone receptor positive breast cancer (*Rance et al. 2013, Zhang et al. 2021*).

Symptoms

Menopausal women may experience multiple symptoms of estrogen deficiency, including vasomotor symptoms (VMS) such as hot flushes and night sweats, and vulvovaginal atrophy (VVA) such as vaginal dryness, itching, dyspareunia, and urinary tract problems. Other menopausal symptoms include mood changes such as depression and anxiety, sleep, and cognitive disturbances such as insomnia, fatigue, irritability and difficulties with short-term memory and concentration, muscle and joint discomfort, increased bone turnover that may lead to bone loss and increased risk of osteoporosis, and increased risk for cardiovascular disease (*El Khoudary et al. 2020; Rees et al. 2022*).

The most common and bothersome menopausal complaints are VMS, which occur most often in the late menopausal transition and the early postmenopause. VMS are transient (lasting between 1-5 minutes) episodes of flushing and intense heat sensation (*Bansal and Aggarwal 2019*). Up to 80% of menopausal women suffer from VMS, which last a mean duration of 7 to 9 years. In one-third of women, VMS can last more than 10 years (NAMS 2023). VMS can contribute towards physical and psycho-social impairment, with a consequent reduction in health-related quality of life (HRQoL) and are one of the main reasons why women may seek medical care for the menopause (*Santoro 2008*). VMS have also been linked to an adverse lipid profile, insulin resistance, and greater risk for hypertension (*El Khoudary et al. 2020*).

Vulvovaginal atrophy (VVA) is another predominant menopause-related health concern with 50-70% of postmenopausal women suffering from symptoms such as vaginal dryness, irritation, and pain during sexual intercourse. Unlike VMS, which tend to improve with time, VVA typically worsens with aging if left untreated, and lasts years beyond the menopause transition (*El Khoudary et al. 2020*). VVA not only causes physical symptoms such as irritation and pain, but also significantly alters sexual health, with important consequences on women's quality of life (QoL).

Current standard therapies

Hormonal treatment

Hormone replacement therapy (HRT) is the broad term used to describe the use of estrogen only (unopposed estrogen) in hysterectomized women or combined estrogen-progestogen therapy in women with a uterus to relieve vasomotor symptoms associated with estrogen deficiency during menopause. Currently the estrogen component consists of estradiol (E2) in a dose of 0.5, 1 mg, and 2 mg, and estradiol valerate 1 and 2 mg. E2 and E2V can be increased or decreased based on clinical response. The addition of a progestogen to the estrogen regimen in women with a uterus is aimed at preventing the increase in endometrial cancer risk associated with estrogen therapy. In Europe, the following progestogens are approved for this indication i.e. dydrogesterone, norethisterone, progesterone, and medroxyprogesterone.

Recent international menopause guidelines such as the *European Menopause and Andropause Society* (EMAS) 2022 and *North American Menopause Society* (NAMS) position statement 2022 (hormone therapy) and 2023 (non-hormone therapy), identify HRT as the first-line treatment for VMS in menopausal women. They recommend an individualized approach, considering each patient's symptoms, health status, and preferences. A systemic estrogen or combined estrogen-progestogen therapy (depending on the hysterectomized status) is advised and is the preferred first line-treatment, in menopausal women aged younger than 60 years, and particularly within 10 years of menopause onset for optimal benefits (NAMS 2022, 2023).

Non-hormonal treatment

A variety of non-hormonal therapies can be offered to women who cannot use HRT because of contraindications or personal preference. As presented in the EMAS position statement, several non-hormonal agents can be used for hot flushes, including clonidine, selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), gabapentin, although these are less effective than systemic estrogens (*Rees et al. 2022*). SSRIs and SNRIs are not approved for the treatment of VMS and would therefore be used off-label. Fezolinetant, a non-hormonal selective neurokinin 3 (NK3) receptor antagonist, has recently been approved in the US and in Europe for the treatment of moderate-severe VMS.

Over-the-counter-remedies

Natural over-the-counter remedies, including herbal/non-prescription products, have not been approved for VMS treatment, and their safety and efficacy are not well established. Furthermore, these products do not address the burden of disease considering the very limited evidence to support their use and limited data to support their mechanisms of action.

2.2. Aspects of development

The safety profile, tolerability, and pharmacokinetics (PK) of oral administration of E4 alone have been evaluated in 11 phase 1 clinical studies performed in healthy pre- and postmenopausal women.

A phase 2 dose finding study in healthy H and non-hysterectomized (NH) postmenopausal women to select the minimum effective dose (MED) of E4 for the treatment of VMS after multiple dose (MD) administration of E4 2.5 mg, E4 5 mg, E4 10 mg or E4 15 mg for 12 weeks (MIT-Do0001-C201).

The applicant initiated 2 pivotal phase 3 studies in postmenopausal women to evaluate efficacy for the relief of VMS with E4 alone, safety with E4 alone up to 12 months, and endometrial protection when combined with a commercially available progestogen (progesterone [P4]) in NH women.

2.3. Description of the product

The medicinal product consists of film-coated tablets containing estetrol monohydrate 15 mg and 20 mg (E4 15 mg and E4 20 mg) equivalent to estetrol (E4) 14.2 mg and 18.9 mg, respectively. In the documents, except if explicitly mentioned, doses refer to the dose of estetrol monohydrate. E4 belongs to the pharmacotherapeutic group sex hormones and modulators of the genital system, with anatomical therapeutic chemical (ATC) code G03CA10.

E4 is a naturally occurring estrogen produced by the human foetal liver. Pregnant women are exposed to E4 as it reaches the maternal circulation through the placenta. The physiological function of E4 during pregnancy remains unclear. Since early 2000, E4 has been extensively studied in several women's health care therapeutic indications such as contraception, VMS and VVA related to menopause, osteoporosis and breast cancer.

E4, in combination with drospirenone (DRSP), has obtained regulatory approval for the indication of oral contraception in more than 50 countries worldwide, including the US and in the European Union (EU) (19 May 2021) with duplicate marketing authorizations under the brand names LYDISILKA and DROVELIS.

Proposed indication

Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women.

Proposed posology

One tablet should be taken orally once daily at about the same time with or without food. Continuous administration is recommended. For initiation and continuation of treatment of postmenopausal symptoms, the lowest effective dose for the shortest duration should be used. The treatment should be initiated with FYLREVY 14.2 mg and, if symptoms are not adequately controlled, the dose could be increased to FYLREVY 18.9 mg.

In women with a uterus, a progestogen approved for addition to oestrogen treatment should be added continuously or for at least 12-14 consecutive days each month/28-day cycle.

Unless there is a previous diagnosis of endometriosis, it is not recommended to add a progestogen in hysterectomised women.

2.4. Inspection issues

2.4.1. Good manufacturing practice (GMP) inspection(s)

No inspection required.

2.4.2. Good laboratory practice (GLP) inspection(s)

No inspection required.

2.4.3. Good clinical practice (GCP) inspection(s)

No inspection required.

3. Quality aspects

3.1. Introduction

The finished product is presented as film-coated tablets containing 14.2 mg or 18.9 mg of estetrol (as estetrol monohydrate) as active substance.

Other ingredients are:

Tablet core: Lactose monohydrate, sodium starch glycolate (Type A), maize starch, povidone K30, and magnesium stearate (E572)

Tablet coating: Hypromellose (E464), hydroxypropylcellulose (E463), talc (E553b), hydrogenated cottonseed oil, titanium dioxide (E171), iron oxide yellow (E172), and iron oxide red (E172).

The product is available in transparent PVC/aluminium blister containing 28 film-coated tablets in a carton an etui storage case.

3.2. Active substance

3.2.1 General information

The chemical name of estetrol monohydrate is (8R,9S,13S,14S,15R,16R,17R)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,15,16,17-tetrol monohydrate corresponding to the molecular formula $C_{18}H_{24}O_4.H_2O$. It has a molecular mass of 322.40 g/mol and the following structure:

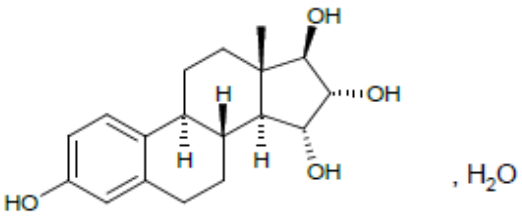


Figure 1: Estetrol monohydrate active substance structure

The chemical structure of estetrol monohydrate was elucidated by a combination of elemental analysis, IR, ATR-IR, 1H-NMR, 13C-NMR (including NMR spectra: 1H, 13C{1H}, H-H COSY, HSQC (multiplicity edited), HMBC (double low pass filter) and 2D ROESY.), HR/MS and UV. The solid state properties of the active substance were measured by single crystal X-ray diffraction analysis and TGA-DSC/MS.

Estetrol monohydrate is a slightly hygroscopic white to off-white crystalline solid, poorly soluble in water and aqueous solutions.

Estetrol monohydrate exhibits stereoisomerism due to the presence of seven chiral centres. Estetrol monohydrate crystallises as a pure enantiomer. Enantiomeric purity is controlled routinely by specific optical rotation. Adequate information has been provided by the ASMF holder confirming that interconversion of the stereo-centres present in the active substance does not take place during storage.

Polymorphism has been observed for estetrol monohydrate. A detailed discussion of the polymorphic forms has been provided, substantiating that only one polymorph is manufactured by the ASMF holder. Polymorphism is controlled in the active substance specification by high resolution XRPD. During the procedure data has been provided demonstrating that the polymorph of the active substance is stable also during storage. Estetrol monohydrate is micronised by the ASMF holder and the particle size distribution (PSD) is controlled in the active substance specification.

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

Changes in the synthesis route have been presented in sufficient detail and have been justified. The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised. The information presented regarding potential impurities/degradation products is considered sufficient, including the specification limits for impurities/degradation products and residual solvents, which meet the requirements of the ICH-guidelines Q3A, Q3C, Q3D, M7.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program.

Estetrol monohydrate is packaged in in double polyethylene bags, which comply with the EC directive 2002/72/EC and EC 10/2011 as amended, and placed in a polyethylene drum.

3.2.3 Specification

The active substance specification includes tests for: characters (visual), specific optical rotation (anhydrous (Ph. Eur.)), particle size distribution (laser diffraction); identification (IR (Ph. Eur.), HPLC, XRPD), water content (KF), sulphated ash (Ph. Eur.), metal residue (palladium and osmium content (ICP)), related substances (HPLC), residual solvents (ethanol and methanol, GC), microbiological purity (Ph. Eur.), and assay (HPLC).

The limits are in line with batch data and are compliant with regulatory requirements.
During the procedure, upon request of the CHMP, limits for identified impurities have been tightened to the identification threshold, in line with stability data.
The limit for elemental impurities and limits for potentially genotoxic impurities, set in line with ICH Q3D and ICH M7 respectively, are considered safe and accepted. The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data from three commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

3.2.4 Stability

Stability data from six commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial container closure system for up to 24 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The stability parameters tested are: characters, specific optical rotation, particle size distribution; identification, water content, related substances microbiological purity, and assay. This is acceptable as the other parameters are not affected by storage. The analytical procedures used were the same as for release and were stability indicating. No significant changes were observed in any of the monitored parameters and all tested parameters were within the specifications.

Photostability testing following the ICH guideline Q1B was performed on samples of the active substance. Results on stress conditions (heat and heat-humidity on the dry active substance and acidic, alkaline and oxidative conditions on the active substance in solution) were also provided on samples of the active substance. No significant degradation was detected under heat, heat-humidity, acidic and light induced photodegradation.

The stability results justify the proposed retest period of 36 months with no special storage precautions, when stored in the proposed container.

3.3. Finished medicinal product (FYLREVY)

3.3.1 Description of the product and Pharmaceutical development

FYLREVY 14.2 mg film-coated tablets are orange, 6 mm diameter, round, biconvex with a drop-shaped debossing on one side. FYLREVY 18.9 mg film-coated tablets are yellow, 6 mm diameter, round, biconvex with a drop-shaped debossing on one side.

During the procedure a major objection (MO1) was raised by the CHMP regarding the distinguishability of the two strengths by the end user. In order to demonstrate that the two tablet strengths are sufficiently visually distinguishable by colour only, the applicant has provided photographs of the products. From these, it can be observed that the 15 mg strength is pale yellow, while the 20 mg strength is brown/orange. Additionally, the contrast between the two colours is also adequate to allow an end user with colour deficiency to distinguish between the two strengths. Based on the submitted images, it can be concluded that the two strengths are distinguishable by colour. The quality target product profile (QTPP) and critical quality attributes (CQAs) were identified.

A risk assessment (RA) of the active substance attributes has been performed on the finished product CQAs. It was concluded that none of the active substance attributes have a significant impact on the CQAs of the product, when used at the proposed specification.

All excipients are well-known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. standards, with the exception of iron oxide yellow, iron oxide red and the colouring Aquapolish (D Orange 034.23 MS used in the 14.2 mg strength and Aquapolish Yellow 024.15 MS used in the 18.9 mg strength) which comply with in-house specifications. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 1.1.1. of this report.

The compatibility of the active substances with the excipients has been demonstrated. No incompatibilities were observed. Functionality Related Characteristics (FRC) of the excipients having a potential impact on the CQAs have been assessed through a RA.

A RA of manufacturing process development with relevant risk mitigation has been provided and the critical process steps have been identified and justified during the procedure.

Two manufacturers of the finished product are proposed. The manufacturing process initially developed at one manufacturer has been transferred to the second manufacturer: small adaptations were made. The quality of the finished product manufactured across manufacturing sites, as supported by batch and stability data.

The formulation used during the phase 3 clinical studies, is the same as that intended for marketing, with the exception of the colouring.

The proposed dissolution method was developed in line with Ph. Eur. recommendation (Ph. Eur. 5.17.1). The discriminatory power could not be demonstrated. This was accepted as the active substance estetrol monohydrate is a BCS class I molecule. During the procedure a MO (MO2) was raised by the CHMP requesting to tighten the proposed dissolution limit. The applicant resolved MO2 justifying a QC dissolution limit based on clinical batch data.

The primary packaging is transparent PVC/aluminium blister. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

3.3.2 Manufacture of the product and process controls

The finished product is manufactured at two different sites,. Satisfactory evidence of GMP compliance has been provided for all sites involved in the manufacturing, testing and batch release of the finished product.

The manufacturing process consists of seven main steps: sieving, dry blending, granulation, final blending, compression, film-coating and packaging. This is the manufacturing process performed by one manufacturer, which is also representative of the process used by the second manufacturer. The process is considered to be a standard manufacturing process.

During the procedure, upon request of the CHMP, the applicant has provided additional information on the method used by one manufacturer (inclusion of ICPs and CPPs), the description of the method is now considered adequate. Process validation data has been provided on at least three consecutive batches from each strength from both sites. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

3.3.4 Product specification

The finished product release and end of shelf-life specifications for the two strengths include appropriate tests for this kind of dosage form: characters (visual), average mass (in-house), identity (HPLC, UV and DAD), assay (HPLC), uniformity of dosage units (Ph. Eur.), dissolution (Ph. Eur.), related substances (HPLC) and microbiological purity (Ph. Eur.).

Tests and limits have been justified in line with current regulatory guidance and pharmacopoeial requirements. The limits for impurities are set in accordance with ICH Q3B.

The dissolution limits have been fully justified during the procedure as discussed in the pharmaceutical development section.

The limit for total degradation products has been tightened.

Based on a RA on residual solvent, the control of residual solvents at the level of the active substance only is justified.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the RA it can be concluded that it is not necessary to include any elemental impurity controls. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

The finished product is released on the market based on the above release specifications, through traditional final product release testing,

The analytical methods used in both sites have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for three full scale commercial batches, for each manufacturing site, confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

3.3.5 Stability of the product

For the 14.2 mg strength, stability data from four commercial batches from Haupt Pharma and three commercial batches from Gedeon Richter have been provided; for the 18.9 mg strength, stability data from five commercial batches from Haupt Pharma and three commercial batches from Gedeon Richter have been provided. For the manufacture of the stability batches, active substance from both manufacturers was used. The batches of finished product were stored for up to 48 months under long term conditions, for up to 6 months under accelerated conditions according to the ICH guidelines were provided. Stability data was also provided for the same batches stored under intermediate conditions, however, they were not assessed. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing. Samples were tested in line with the specifications using stability-indicating analytical procedures, as demonstrated in a the compatibility study performed under increased temperature and humidity stress conditions.

For all attributes, all batches comply with the defined specifications at all conditions, and no significant changes have been observed in long-term and accelerated data. The batches manufactured by Haupt Pharma or Gedeon Richter show the same stability behaviour.

In addition, one commercial batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products, confirming that the finished product is not light sensitive.

Furthermore, hold-time studies have been conducted for 12 months on the film-coated tablets from one site and 6 months on the film-coated tablets from the other site.

Hold-time stability data from two batches of each strength manufactured by both manufacturers have been provided. All results are within the specified limits, confirming that the compressible mixture stored in the proposed container is stable for up to 1 month, the bulk uncoated tablets are stable for up to 3 months and bulk film-coated tablets are stable for up to 12 months under warehouse conditions.

Based on available stability data, the proposed shelf-life of 3 years and with storage conditions "This medicinal product does not require any special storage conditions" as stated in the SmPC (section 6.3) are acceptable.

3.3.6 Adventitious agents

It is confirmed that the lactose is produced from milk from healthy animals in the same condition as those used to collect milk for human consumption and that the lactose has been prepared without the use of ruminant material other than calf rennet according to the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and veterinary medicinal products.

3.4. Discussion and conclusion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The quality of the active substance has been documented by ASMF – the currently assessed versions are RP Version 1.1/date September-2025 and AP Version Version 2.0/date December-2025. During the procedure a major objection (MO1) was raised regarding the distinguishability of the two strengths by the end user. The applicant demonstrated that the two strengths are distinguishable by colour and by packaging, as the two strengths are not given simultaneously, this was accepted. A second MO (MO2) was raised requesting to tighten the proposed dissolution limit. The applicant resolved MO2 by justifying the QC dissolution limit based on clinical batch data. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The quality of this product is acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on TSE safety.

3.5. Recommendations for future quality development

Not applicable.

4. Non-clinical aspects

4.1. Introduction

Estetrol (E4), first described in 1965, is a naturally occurring estrogen produced by the human foetal liver. It is only produced during pregnancy and reaches the maternal circulation through the placenta.

E4 has been isolated in maternal urine as early as week 9 of gestation. At term, the hormone is found at concentrations of about 1 ng/mL (3 nM) in maternal plasma and over ten times higher in foetal plasma. The physiological function of E4 in pregnancy is unknown.

The pharmacological profile of E4 is that of a weak estrogen. E4 displays a highly selective binding to the human estrogen receptors (ER) and binds to both ERα and ERβ. The binding affinity of E4 for ERα is 25- to 60-fold lower compared to estradiol (E2) and the gene transactivation ~300-fold lower than E2. The *in vivo* potency of E4 regarding its estrogenic activity is generally about 20 times lower than the potency of ethinyl estradiol (EE) and about 5-fold lower than the potency of E2.

The non-clinical program for the currently proposed HRT is largely based on the non-clinical program conducted for the development of the COC E4/DRSP 15/3 mg. Therefore, most non-clinical studies with E4 alone have been previously assessed in procedures EMEA/H/C/005382/00001 and EMEA/H/C/005336/00002. The following new studies were included in the current procedure:

- A 13-week repeat-dose oral toxicity study with the E4/progesterone (P4) combination in female monkeys with supportive toxicokinetic analysis (Do001-NC-01; GLP) - See Section 4.3.2
- Effect on HUVEC cell migration (*Dama et al, 2023*; non-GLP) - See Section 4.3.1.2
- Effect on endothelial healing in ovariectomized mice (*Davezac et al, 2023*; non-GLP) - See Section 4.3.1.2
- *In vitro* pharmacokinetic drug interaction studies Es0001-NC-018, Es0001-NC-019 Es0001-NC-020, and Es0001-NC-021 - See Sections 4.3.2.5 and 5.2

4.2. Analytical methods

Estrogenic properties and potency of E4 were evaluated in a range of *in vitro* and *in vivo* primary pharmacodynamic studies. Secondary pharmacodynamic studies with E4 comprised an assessment of potential off-target activities in an *in vitro* Panlabs screen (approximately 130 targets), studies in relation to breast cancer risk, studies in relation to coagulation risks, studies on vascular effects, studies in relation to metabolic disorders risks and studies in animal models for other indications (arthritis, osteoporosis, multiple sclerosis). Safety pharmacology studies with E4 included Functional Observation Battery in rat, in vitro hERG assay, cardiovascular function in telemetered monkey and whole-body plethysmography in rat.

The program of pharmacokinetic studies performed with E4 included single- and repeat-dose toxicokinetics (toxicology species), tissue distribution (rat), *in vitro* plasma protein binding (toxicology species and human), *in vitro* blood distribution (human), *in vitro* metabolism in hepatocytes (toxicology species and human), and *in vivo* metabolism/excretion studies (mouse, rat, interspecies comparison).

The safety of E4 was evaluated in a range of repeat-dose toxicity studies in rat or monkey with E4 given by oral gavage up to 26 weeks in rat and 39 weeks in monkey. A 13-week repeat-dose toxicity study with E4 given in combination with progesterone (P4) was conducted in female monkeys. Genotoxicity studies include the core battery option 1 defined in ICH S2(R1) (*in vitro* Ames test and mouse lymphoma assay, *in vivo* rat bone marrow micronucleus test). In view of a weakly positive and not clearly reproducible mutagenic activity of E4 in the Ames test in *Salmonella typhimurium* strain TA102, a second *in vivo* test (rat Comet assay) was added to the program. In addition, an *in vitro* study on the potential of E4 to induce the formation of reactive oxygen species was performed in an effort to explain the mechanism behind the findings in strain TA102. Two-year carcinogenicity studies were completed in mouse and rat. Reproductive and developmental toxicity studies comprise return-

to-fertility studies in rat, embryo-foetal development studies in rat and rabbit and a pre-/postnatal development study in rat.

4.3. Pharmacology

4.3.1. Pharmacodynamics

4.3.1.1. Primary pharmacodynamics

The relative binding affinity (RBA) of E4 for human oestrogen receptors ERα and ERβ versus estradiol (E2) was between 1% and 4%, and in a transactivation assay around 0.4%. The RBA versus ethinylestradiol (EE) in the transactivation assay was 0.14% and 2.1%, respectively. E4 showed no antagonistic activity on either receptor in this assay. Activation of gene transcription by E4 was shown to be mediated via the oestrogen-responsive element (ERE).

Crystal structures of ERα-ligand binding domain complexed with E4, E2 and E3 (estriol) were reported to be similar and the binding affinity, determined by time-resolved fluorescence resonance transfer, of SRC3 for ERα-ligand complex was in the same order of magnitude for the three oestrogens. The pattern of ERα coregulators recruitment induced by E4 was highly similar to that elicited by E2, but E4 potency was lower. Data indicate that E4 has lower binding affinity for ERα as compared to E2 but still forms a complex with this receptor that binds to SRC3, almost as well as does the complex with E2.

The metabolites E4-3-glucuronide and E4-16-glucuronide showed weak oestrogenic activity with a potency approximately 3800 and 600 times lower than the potency of E4 in the ERα transactivation bioassay, respectively, and 400 times less in the ERβ bioassay for the E4-16-glucuronide (the E4-3-glucuronide did not produce a full dose response in the ERβ bioassay).

E2 (8 or 80 µg/kg/day) and E4 (1 or 6 mg/kg/day) given by the subcutaneous route for 3 weeks caused comparable increases in vaginal weight, epithelial height, epithelial proliferation, and lubrication in ovariectomized C57BL/6J mice. These vaginal responses to E4 were completely abolished in ERαAF2^{0}mice, lacking the Erα activation function AF2, indicating that the effects were entirely mediated by nuclear ERα activation.

In ovariectomized C57BL/6J mice, E4 (1 mg/kg) increased uterine luminal epithelial and stromal height and induced uterine epithelial proliferation. Its potency was ~1% of the potency of E2. E2-responsive genes in the uterus also responded to E4, with a potency 1/100 for most upregulated genes and 1/3 to 1/10 for most downregulated genes, as compared to E2.

E4 stimulated the growth of mammary gland epithelial ducts in prepubertal ovariectomized mice with a lower potency than E2. Progesterone receptor (PGR) proteins were expressed in the mammary epithelium of E4-treated mice, but not in OVX untreated mice and PGR mRNA was shown to be overexpressed under E4 treatment. In addition, the pure ERα antagonist ICI 182 780 completely blocked the elongation of the ductal tree induced by E4. These findings suggest that the epithelial growth stimulation was ERα-mediated. E4 was also shown to partially antagonize the effect of E2.

In a modified Allen-Doisy test in ovariectomized Sprague-Dawley rats, E4 caused a dose-related vaginal cornification, reduced terminal body weight and enhanced uterus weight. Its oestrogenic potency was higher after oral than after subcutaneous administration and was estimated to be 1/20th of the potency of EE.

In a hot flush model, E4 (3 mg/kg/day) given orally for 8 days, completely attenuated, like EE, the tail-skin temperature rise induced by naloxone treatment in morphine-dependent ovariectomized Sprague-Dawley rats, while lower doses were partially active.

When administered by oral gavage over the 4-day period of the oestrus cycle to female Sprague-Dawley rats, E4 inhibited ovulation with an ED50 of approximately 0.2 mg/kg bid. The anti-ovulatory potency of E4 was about 1/20th of that of EE and was also lower than the potency of E2. In New Zealand White rabbits, E4 inhibited ovulation and implantation with respective oral ED50s of 0.7 mg/kg bid and 0.06 mg/kg bid.

4.3.1.2. Secondary pharmacodynamics

Off-target receptor interactions

E4, at 10 µM, showed no interactions with a panel of 130 drug targets (receptors, transporters, ion channels) other than with its primary targets, the ERα and ERβ receptors. It might be noted that E4 did not bind to the progesterone, androgen or glucocorticoid receptors.

Studies in relation to breast cancer risk

E4 enhanced proliferation of normal human breast cells and MCF-7 breast cancer cells. Its potency towards normal breast cell proliferation was 1% of the potency of E2. Both nuclear (transcriptional activation) ERα activation and extra-nuclear (membrane-initiated steroid signalling (MISS)) pathways were involved. E4 also enhanced T47-D breast cancer cell migration and invasion, but with less potency than E2. The effect of E4 on breast cell proliferation and migration could be blocked with the anti-oestrogens ICI 182 780 and/or tamoxifen. E4 was also shown to attenuate the effects induced by E2 on breast cell proliferation, migration and invasion.

In vivo, E4 stimulated tumour growth in immunodeficient ovariectomized mice subcutaneously implanted with MCF-7 breast cancer cells with a ~3 times lower potency than E2 (growth at a dose of 10 mg E4/kg/day was similar to growth at 3 mg/kg/day E2), and partially antagonized E2-induced tumour growth in a dose-dependent manner. E4 inhibited the development of chemically induced mammary tumours in the dimethylbenz(a)anthracene (DMBA)-mammary tumour model in female Sprague-Dawley rats and seemed to inhibit tumour development as effectively as ovariectomy and the anti-oestrogen tamoxifen at high doses. E4 at 10 mg/kg/day even caused regression of existing tumours in this model.

Studies in relation to coagulation risks

The level of Sex Hormone Binding Globulin (SHBG) has been suggested to be a predictive marker for the risk of venous thromboembolism. E4 showed no affinity for human Sex Hormone-Binding Globulin (SHBG) and did not induce SHBG or corticosteroid-binding protein (CBG) production in ERα-expressing Hep89 cells. Both E4 and E2 caused a dose-dependent increase of the expression of tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and tissue plasminogen activator inhibitor 1 (PAI-1) in HUVEC cells. E4 had a lower potency than E2 and antagonized the effects induced by high concentrations of E2 but did not impair the effects induced by low concentrations of E2.

Following chronic treatment, ovariectomized female mice exhibited a prolonged tail-bleeding time and were protected from arterial and venous thrombosis. In addition, E4 treatment decreased *ex vivo* thrombus growth on collagen under arterial flow conditions. In hematopoietic chimera mice with implanted bone marrow cells deficient for nuclear ERα, E4-induced protection against thromboembolism was significantly reduced, while the increased tail-bleeding time was not impacted

by this deletion. Indicating that nuclear ERα activation contributes to the protective action of E4 against thromboembolism but it is not involved in the effect of E4 on primary haemostasis.

Studies on vascular effects

E4 induced rapid nitric oxide (NO) release, and endothelial nitric oxide synthase (eNOS) activation and expression in HUVEC cells with less potency compared with E2. The effect was inhibited by the ER antagonist ICI 182 780. E4 also attenuated E2-induced NO synthesis. E4 did not affect eNOS activation or NO production in mouse aorta *in vitro* but did antagonize E2-induced stimulation in this model. E4 was thus found, in rat, mouse and human tissue, to have the capacity to inhibit the E2-induced vascular eNOS expression and NO release, which has a protective function in the cardiovascular system. Based on available literature, clinical safety concerns related to an inhibition of estrogen-induced eNOS activity and NO release in the presence of E4 are not expected since estrogens are not the major drivers of NO release supporting vasodilation and endothelial function, which is more stringently regulated by shear stress, temperature lowering and neurohumoral mediators. In addition, E4 is also shown to be able to induce vasodilation via an ER-dependent and NO-independent mechanism.

E4 (10^{-9} to 10^{-7} M for 6 hours) enhanced the migration of HUVEC in scratch and Boyden chamber assays. The effect of E4 was comparable to the effects of EE, suggesting comparable vascular remodelling and regeneration capacity. The data indicated that G-protein coupled estrogen receptor 1 (GPER) was involved in E4- or EE-mediated endothelial cell migration.

E4 caused vasorelaxation of rat arteries with a lower potency than E2. The effect of E4 on vasorelaxation was mediated by both an endothelium-dependent (involving ERs) cGMP-mediated mechanism and an endothelium-independent mechanism involving inhibition of smooth muscle cell Ca2+ entry and contraction. E4 also reduced atheroma deposits in the aortic sinus in ERα+/+/LDLr /- ovariectomized mice, possibly via an ERα-dependent mechanism but had no effect on endothelial healing in ovariectomized C67BL/6J mice following carotid electric injury. However, E4 abolished the accelerated endothelial healing induced by E2 in this model. In contrast, in a newly submitted second study using a carotid endovascular injury model, E4 stimulated endothelial healing to the same extent as E2 or a combination of E2 and E4. The effect of E4 was shown to be mediated by ERα in smooth muscle cells. In an experimental model of femoral artery injury, E4 prevented neointimal hyperplasia to the same extent as E2.

E4, like E2, protected female ovariectomized mice against AngII-induced hypertension. The hypertensive effect of Ang II was significantly more exacerbated in ovariectomized mice or in mice lacking ERα, showing that the beneficial effect of endogenous oestrogen is ERα-dependent. Taken together, these data suggest potential vaso-regulatory properties of E4, albeit to a lower extent than E2.

Studies in models for other indications

E4 suppressed the development and severity of collagen-induced arthritis in DBA/1 mice (model for arthritis) and reduced the incidence and severity of experimental autoimmune encephalomyelitis in SJL/J mice (model for multiple sclerosis).

4.3.1.3. Safety pharmacology

E4 was devoid of effects in a Functional Observation Battery in conscious female Sprague-Dawley rats up to and including a single oral dose of 15 mg/kg (~30 times clinical AUC). A slight decrease of rectal temperature at 1- and 2-hours post-dose was noted at 150 mg/kg, possibly explained by a slight increase in this parameter at these time points in the control group.

Compared to vehicle control, E4 at a concentration of 28.17 µM (~500x clinical Cmax) decreased hERG tail current amplitude by 7.1%, while the positive control E-4031 decreased hERG tail current amplitude by 83.6%. The effect on hERG is not considered to be of any toxicological significance.

E4 had no effect on heart rate, blood pressure or electrocardiogram parameters and did not induce arrhythmia in conscious telemetered female cynomolgus monkeys, at single oral dose levels up to and including the highest tested dose of 100 mg/kg (with 1 mg/kg giving an exposure somewhat higher than clinical AUC).

E4 did not affect respiratory parameters in conscious female Sprague-Dawley rats at single oral dose levels up to and including the highest tested dose of 150 mg/kg (more than 600 times clinical AUC).

The non-clinical pharmacological and safety-pharmacological characterization of estetrol is sufficient.

4.3.1.4. Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies with E4 have not been performed. That was considered acceptable.

4.3.2. Pharmacokinetics

Studies have been performed in female mouse, rat, monkey and rabbit (absorption and metabolism) to characterize the pharmacokinetic properties of E4, using validated LC-MS/MS assays for E4 and a validated UPLC-MS/MS method for P4/progesterone in a 13-week repeat-dose toxicity study with the combination of E4 and Progesterone.

4.3.2.1. Absorption

Oral bioavailability of E4 relative to the subcutaneous route of administration was estimated to be 70% or more in rats.

Tmax was 0.5 hour in mice and rats, and between 0.5 and 1.5 hours in cynomolgus monkeys. Both Cmax and AUC0-last increased with an increase in dose. Although there was some variability between studies within the same species, systemic exposure to E4 showed dose proportionality up to dose levels of 30 mg/kg/day in mice, 50 mg/kg/day in rats and 30 mg/kg/day in monkeys. At comparable dose levels, systemic exposure to E4 in Wistar rats after 13 weeks of dosing tended to be slightly lower (in terms of AUC) than that in Sprague-Dawley rats.

In mice and rats at dose levels up to 10 mg/kg/day and 15 mg/kg/day, respectively, AUC0-last was generally similar after single and multiple dosing, suggesting a lack of accumulation in plasma. Above these dose levels, AUC0-last was slightly lower after repeated administration compared to the first dose. In monkeys, AUC0-last tended to be slightly higher after repeated administration than after the first dose.

Plasma half-life of elimination (T1/2) was 1.5 – 3 hours in mice, 2 – 6 hours in rats and 10 – 19 hours in monkeys. In monkeys, plasma-concentrations-versus-time curves showed evidence of a second peak, suggesting the E4 undergoes enterohepatic circulation.

In mated/pregnant Wistar rats and pregnant New Zealand White rabbits, Tmax was 0.5 hour and between 0.5 and 3.0 hours respectively. Exposure to E4 was essentially dose-proportional up to 10 mg/kg/day in rats and 0.45 mg/kg/day in rabbits. At dose levels of 10 mg/kg/day and higher in rats and at 0.45 mg/kg/day in rabbits, Cmax was lower after multiple dosing than after the first

administration. At these dose levels, AUC0-last was lower after repeated administration in rats but slightly higher in rabbits compared to the first dose.

In a new 13-week oral toxicity study in monkeys, it was shown that co-administration of estetrol with progesterone did not affect estetrol exposure compared to the control group exposed to estetrol alone. However, the exposure to progesterone was increased when co-administered with estetrol compared to the control group exposed to progesterone alone.

4.3.2.2. Distribution

Radioactivity was extensively distributed in tissues of female non-pigmented Sprague-Dawley rats and partially pigmented Lister Hooded rats after a single oral dose of [14C]-E4 at 15 mg/kg. The highest radioactivity concentration was found in the liver at all time points post-dose. Tissue levels of radioactivity were below the limit of quantification in all tissues except liver and thyroid gland by 48 hours in non-pigmented rats (last sampling time point) and by 7 days post-dose (liver only) in partially pigmented rats (including melanin-containing tissues). No binding to melanin was indicated.

Plasma protein binding of E4 was 45 – 67% in mouse, rat, monkey and human. There was no indication of species-related differences or of concentration dependence up to 1000 ng/mL in animal plasma and 50 ng/mL in human plasma. E4 showed no binding to human sex hormone binding globulin (SHBG) and distributed equally between plasma and blood cells in human blood at concentrations up to 1 µM.

No placental transfer or milk excretion study has been performed as such studies were considered not relevant in view of the indication and the target population. Furthermore, regarding foetal transfer, the applicant assumes that, since transfer from foetus to the mother occurs, there will also not be a barrier in the other direction. Data suggest that E4 is already produced by the foetal liver as early as in the 9th week of pregnancy and increases with increasing pregnancy duration. In the first trimester, maternal-foetal exchanges are limited. Furthermore, foetal E4 levels at term birth are higher than maternal, and highly variable, indicating that the transport of E4 from foetal to maternal circulation largely exceeds possible transport in the other direction.

4.3.2.3. Metabolism

E4 was extensively metabolized in hepatocytes from mouse, rat, rabbit, monkey and human. Metabolic pathways included direct glucuronidation and sulfation, hydroxylation combined with sulfation, hydroxylation combined with methylation, and hydroxylation combined with methylation and glucuronidation or sulfation. With the exception of the 3 glucuronide (A-ring), the exact metabolite structures could not be determined. Glucuronidation and sulfation were predominant reactions in hepatocytes from all species. Only a direct glucuronide (D-ring) and a direct sulfate (unconfirmed site) were found in human hepatocytes. These metabolites were also formed by the other species, notably rat and monkey. Methylation was an important metabolic route in rat hepatocytes. Hepatocytes from three mouse strains showed no strain differences in metabolism of E4.

The major metabolites observed in human plasma were the E4-3-glucuronide and E4-16-glucuronide (17 and 62% of radioactivity at Tmax of 0.25-0.5 hour) and an E4-glucuronide-sulfate conjugate (9% of radioactivity). In an interspecies comparison study, the two glucuronides were identified in mouse and rat. The E4-3-glucuronide was a significant metabolite in rat and mouse plasma and in mouse urine and rat bile. The E4-16-glucuronide was not found at significant levels in mouse samples or rat plasma but was an important metabolite in rat urine and rat bile. The E4-glucuronide-sulfate conjugate was only noted at minor amounts in rat bile. *In vivo* metabolic pathways have not been studied in

monkeys. No data on the levels of the two major human metabolites in plasma, the E4-3- and E4-16-glucuronide, is thus available. Since the two glucorinides have low pharmacological activity and glucorinidation and sulfation seem to be formed via direct reactions, they are of no toxicological concern.

4.3.2.4. Excretion

After a single oral dose of 15 mg/kg to mice or rats, excretion was complete by 7 days post-administration, with the main part of the dose recovered in faeces (67% in mouse, 87% in rat).

4.3.2.5. Pharmacokinetic drug interactions

Enzyme induction and inhibition

In vitro studies were performed to evaluate the potential of E4 to induce human CYP450 enzymes (0031-100, Es0001-NC-019) and to inhibit human CYP450 enzymes (PR3038, 0031-101) or UGT (0031-105). A study was also performed to evaluate the inhibitory potential of E4 metabolites on CYP450 enzymes (Es0001-NC-017).

These studies are presented and discussed in detail in Section 5.2.2.11.

Interactions with transporters

In vitro studies were performed to investigate bidirectional transport in human Caco-2 cells (0031-NC-017), and the interaction of E4 as substrate or inhibitor with ABC efflux transporters or SLC transporters (0031-103, Es0001-NC-013, 0031-107, Es0001-NC-020, Es0001-NC-021). A study was also performed to evaluate the potential of E4 metabolites to inhibit ABC and SLC transporters (Es0001-NC-018).

These studies are presented and discussed in detail in Section 5.2.2.11.

4.4. Toxicology

4.4.1. Single-dose toxicity

A single escalating dose study in female cynomolgus monkey identified a NOAEL of 1000 mg/kg. The only findings observed were emesis in one female at the 1000 mg/kg dose and decreased levels of alkaline phosphatase reaching significance at a dose level of 1000 mg/kg.

4.4.2. Repeat-dose toxicity

Repeated-dose toxicity has been evaluated in Sprague-Dawley rat and Cynomolgus monkey in studies of up to 6 and 9 months respectively. Further, as carcinogenicity studies were performed in CD1-mouse and Wistar rats, repeated-dose toxicity was also evaluated up to 13 weeks in these strains.

Mortalities: In the 26-week study in SD rats, one mortality occurred in the 15mg/kg/day group for reasons of poor clinical condition. The cause of moribund condition was considered by the Study Pathologist to be related to gavage procedure and a relationship to the treatment was excluded.

Bodyweight: Oestrogens exert anti-obesity effects in women and female mammals, and oestradiol replacement in rodents prevents obesity by decreasing food intake and increasing energy expenditure. It was therefore surprising to note that the CD-1 mice in the 4- and 13-week studies consistently

showed dose-relatedly increased body weights with E4 treatment. There is no reasonable explanation (food consumption included) except to conclude that oestrogens (E4 included) administered to experimental animals yields considerable interspecies variation in physiological responses. In rats and monkeys, expected reductions in bodyweight were consistently noted down to adverse levels. No data is available on monkey food-consumption which makes the relation between bodyweight and food-consumption speculative.

Reproductive organs and genitals: E4 induces oestrous cycle arrest in all species evaluated, including effects associated with exaggerated estrogenic effects. Changes in weights of reproductive tissues, generally accompanied by macroscopic and/or microscopic observations, were found in all species evaluated. Ovaries, oviducts, uterus, vagina, mammary gland and pituitary gland displayed microscopic changes reflecting estrogenic responses. The findings were overall considered non-adverse and showed recovery. However, in the 39-week study in cynomolgus monkey, treatment-related changes in the cervix, including chronic inflammation in the lamina propria is considered adverse at 3 and 10mg/kg/day. This finding was not evident in the 13-week combination study at any dose-level.

Lymphoid organs: Lymphoid atrophy was noted in all species evaluated. Thymus weight was reduced in all species, associated with minimal to severe lymphoid atrophy. Whereas thymic atrophy is a known effect of oestrogens, the finding was considered adverse in some studies where marked lymphoid atrophy was evident. No evaluations of immune function have been undertaken. While acceptable, such evaluations would have improved the possibility to make a functional evaluation of the lymphoid atrophy. However, no clear effects on immune function have been reported from the clinical studies.

Adrenals: In the mouse 13-week study, subcapsular spindle cell hyperplasia including increased vacuolation/degeneration of the x zone was noted dose-dependently from 0.3 mg/kg/day. In the rat, adrenal weights were increased in all studies. In the 4-week study, dose-related congestion of the cortex and zona reticularis cell hypertrophy (minimal-marked) was noted associated with blood vessel expansion/dilation. This correlated with increased adrenal weights. At recovery, congestion (minimal-slight) was still noted in one female each from the 50 and 150 mg/kg/day groups. In the 26-week rat study 26-week at doses at and above 1.5 mg/kg/day, dose-related cortical hypertrophy (primarily seen in the reticular layer of the adrenal glands) was evident, along with capillary blood vessel dilation and cystic degeneration. At recovery, cortical hypertrophy and minimal cystic degeneration remained in 1/6 and 2/6 of treated animals. These effects were considered adverse from 5mg/kg/day in the rat. In monkey, complex changes consisting of dose-dependent but variable association of hypertrophy/vacuolation of the zona glomerulosa (minimal-slight) and zona fasciculata (minimal-slight) and atrophy of the zona reticularis (minimal-marked) evidenced that adrenals are target organs of toxicity in all species tested. Since cells in the zona reticularis synthesize oestrogens, negative feedback by E4 may have caused atrophy and lower weights.

Liver: In mice and rats, increased liver weights and hepatocellular hypertrophy was evident, and histopathology findings included hepatocellular hypertrophy (minimal-moderate) in both species. In the rat, hepatocellular microvacuolation (minimal to moderate) was seen in the 26-week study without clear dose-relation. However, the rats administered the highest dose had slightly higher incidence and severity compared to the lower dose groups. However, given that no degeneration was reported, this change may reflect a cellular adaptation, rather than a degenerative change. In the monkey, liver-weights were increased, and a decreased hepatic glycogen content was seen. This may be related to reduced food-intake and bodyweight. Liver is a known target organ of oestrogen toxicity, and oestrogens modulate liver lipid metabolism so liver involvement after oestrogen administration is expected.

Pituitary: The pituitary gland shows increased weight hyperplasia/hypertrophy of eosinophil cells across studies. This is likely an oestrogen dependent effect, as prolactin cell hyperplasia has previously been shown to result from oestrogen exposure.

Cardiotoxicity: Microscopic cardiac abnormalities were not observed (or at least not evaluated with specific histological staining) in mice and rats. When assessing the estetrol-related microscopic data of NHP in the repeat-dose toxicity studies, it was noted that immune cell infiltrates and interstitial fibrosis at the apex occurred in all treatment groups, including controls, and thus are likely a background finding in (Mauritian-origin) cynomolgus monkeys.

4.4.3. Genotoxicity

The genotoxicity testing of E4 was not straightforward. In the first Ames test, E4 was positive, as increased revertants were seen in the TA102 strain. A follow-up test in TA102 using the same batch, but with ethanol as solvent (instead of DMSO) was undertaken. A dose-related increase in revertants was noted (in both preliminary assay and confirmatory assay), with and without S9 mix, exceeding the 2-fold threshold at and above 2500 µg/plate, the conclusion was that E4 is mutagenic. A third Ames test was performed with E4, in which two assays were performed. One assay used the direct plate incorporation method, and the other was performed according to the pre-incubation method. The first assay did not pass the study requirements and was therefore not considered valid. The second study showed no increase in revertants in E4 treated plates compared to vehicle control. It was unclear why this study was negative whereas two previous GLP-compliant studies showed that E4 is mutagenic both in absence and presence of S9-mix. Differences in TA102 strain sensitivity may be a possible explanation for the different results noted in the three tests.

Two mouse lymphoma assays were performed to evaluate the potential of estetrol to induce gene mutations in mammalian cells. Both studies showed increased mutation frequencies in cells treated with E4. In the first study, increased frequencies were only noted in the highest dose without S9 mix and it was not considered positive due to lack of dose-response. In the second study, mutation frequency was dose-relatedly increased with and without S9 but did not exceed the GEF threshold and was therefore not considered positive.

Three *in vivo* studies have been performed with E4, one bone-marrow micronucleus test and two comet assays evaluating effects in liver and duodenum. While the micronucleus test is a standard *in vivo* study in accordance with ICH S2, it is assumed that the comet assays were performed in response to the positive results resulting from 2 of the three Ames tests conducted. The liver was chosen as it is the major site of metabolism of E4, and the duodenum is an early site of contact with E4 after oral exposure. All studies were performed in SD rats at doses up to 2000 mg/kg, which is the top dose suggested in the current ICH S2 guideline.

In the micronucleus test, the mean values of MPCE and PCE/NCE ratio were not statistically different between the groups. The first comet assay performed at Institut Pasteur De Lille (Lille Cedex, France) showed a significant increase in DNA strand breaks, but only at the lowest dose tested of 2 x 500 mg/kg/day. The second study was performed at another CRO (Covance Laboratories, England), was more focused on doses at and around 500 mg/kg and used a slightly different experimental set-up which included parameters for tissue toxicity and ROS evaluations. Tail moment and tail intensity are relatively stable among groups. It is however noted that the 500 mg/kg group (which was positive in duodenum in the first study) showed more clouds. This suggests more cytotoxicity (or possibly lower sample quality) rather than genotoxicity. Collectively, the *in vivo* studies do not suggest that E4 is a genotoxic substance.

4.4.4. Carcinogenicity

Two long-term (two-year) carcinogenicity studies with E4 have been performed, one using CD-1 mice and one with Wistar rats. Wistar rats were used since this strain has a lower incidence of spontaneous mammary neoplasia as compared to S.D rats. In the mouse study, the HD was set based on weight-increase in the 13-week pre-study in CD-1 mice, and it is evident that weight was a sensitive parameter with up to 33% increase in the 1mg/kg group. Neoplastic findings (tumours) were evident in uterus and cervix (from 0.25 mg/kg/day) and in mammary gland and pituitary (at the 1 mg/kg/day dose). The overall impression of the neoplasms in reproductive organs is that they are consistent with an E4 related estrogenic effect and therefore expected. Further, increased mammary hyaline duct content, galactoceles, hyperplasia and neoplasia are consistent with direct effects of estetrol and/ or secondary to increased prolactin secretion. The increased neoplasias noted in the pituitary (increase in hyperplasia, adenoma and carcinoma of the pituitary pars distalis) have been shown to be prolactinomas suggesting that they too are related to an estrogenic effect. The dose-level of 0.125 mg/kg/day was not carcinogenic in this study.

In the rat, E4-induced proliferative and non-proliferative effects were noted in genital tract, liver, spleen, mammary gland, adrenal cortex, mesenteric lymph node and thymus. Although there were no differences in overall mortality between treated and control groups, the percentage deaths or premature sacrifices caused by *pars distalis* adenomas was higher in treated groups compared with controls. These adenomas correlated with pituitary enlargements noted macroscopically and with increased ventricular dilatation and compression of the brain. It has previously been shown in SERM-studies that this tumour is driven by oestrogens.

The only neoplastic observation presented is an increase in mammary adenocarcinoma noted at 0.8 mg/kg which was accompanied by mammary gland acinar cell hyperplasia already from the lowest dose. Oestrogen and prolactin are known as important drivers of these tumours in the rat. It is also noted that pituitary tumours were also noted in the mouse carcinogenicity study. Given the increased pituitaries it is possible that prolactin levels were increased. It is to this end strange that such an apparent factor has not been evaluated within the present study, as it could have given more clear data on this. Overall small effects were noted on haematology and blood biochemistry parameters with effects mainly on increases in reticulocytes count, triglyceride levels and alkaline phosphatase and decreased cholesterol. The blood biochemistry changes are similar to the findings in the 13-week study, but there doses up to 6 mg/kg were used.

Except for the mammary gland hyperplasia noted already at 0.08mg/kg, non-neoplastic proliferative findings (i.e. hyperplasia) were noted from 0.27mg/kg/day uterus, ovaries and liver. Non-proliferative findings reported are overall involving lymphoid and oestrogen sensitive organs and are reflective of the findings already noted in the 13-week study in Wistar rats. From this study, it can be concluded that E4 is carcinogenic and produced mammary gland adenocarcinoma at 0.8 mg/kg/day.

Overall, it can be concluded that E4 is carcinogenic in mice and rats.

4.4.5. Developmental and reproductive toxicity

Modified segment I study: The E4 exposure of rat females led to a reversible decrease in oestrus-cycling females from 0.5mg/kg/d. There were no clear signs of reduced fertility or disturbed early embryogenesis (e.g. fertility index, gestation index). Exposure to E4 led to an overall decrease in food intake (-10%, 1.5mg/kg/d) and body weight (-4% to -7% from 0.50mg/kg/d) that was most evident during the first 1-2w of exposure. The food intake and body weight reduction effects were not evident during the recovery phase (although there was a +50% to 90% body weight gain from 0.17mg/kg/d) but seemed to become manifest again during pregnancy in both pivotal studies (reduced food intake -

4.5% to -12.5% across doses and studies, reduced body weight of -6.6% to -7.2% and a trend of reduced body weight gain at ≥0.50mg/kg/d in one of the pivotal studies). Overall, the general NOAEL across studies was set to 0.17mg/kg/d and the LOAEL to 0.50mg/kg/d based on the reduction of oestrus cycling during the exposure period (first pivotal study) and reduced food intake/body weight gain (second pivotal study).

Segment II (EFD): In dose-range finding rat studies, there was total embryofoetal loss at ≥10mg/kg/d. In the pivotal rat study (exposure period Gd6-Gd17, 0.30 to 3.0mg/kg), several animals (n=4/24) were sacrificed early at the high dose (3.0mg/kg/d) due to abortions/poor clinical conditions. At the high dose, there was also an overall clear reduction of dams with live foetuses (42%). There was a significant increase of external limb malformations (severe flexed or malrotated ankle joint, shortened limbs), skeletal malformations (bent radius, thickened forelimb humerus, bent pectoral scapula) and variations (nodulated or kinked ribs, un-ossified sternebrae) and a general increase of number of litters with malformed foetuses from the middle dose (1.0mg/kg/d). There was also a trend (statistically non-significant) of increased mean of pre- and post-implantation loss and number of foetuses per female with increasing dose. There was a reduced body weight gain (+16.5 to +17.5% compared to control +20.5%) from the middle dose (1.0mg/kg) which correlates with trends of reduced food intake and mean body weight. At Gd20, there was a significant mean body weight (corrected for uterus weight) reduction and enlarged and thickened placenta at the highest dose. The overall maternal and embryofoetal NOAEL was 0.30mg/kg/d and the LOAEL was 1.0mg/kg. No toxicokinetic measurements were conducted in the pivotal rat EFD study, but a non-pivotal Rat EFD study covered the same doses and gave a NOAEL Cmax (Gd17) of 6825pg/mL, a NOAEL AUC0-t (Gd17) of 9330pg x h/mL, a LOAEL Cmax (Gd17) of 20878pg/mL and an LOAEL AUC0-t (Gd17) of 37656pg x h/mL.

In the rabbit pivotal study (exposure period Gd6-Gd18; 0.05 to 0.45mg/kg/d), there were some minor adverse clinical signs (hair loss) in the females from the middle dose (0.15mg/kg/d) and more adverse signs at the high dose of 0.45mg/kg/d (emaciated appearance, absence of faeces). There was a general reduction of main study females with live foetuses from the middle dose (n=9/20 at 0.15mg/kg/d and n=2/20 at 0.45mg/kg/d) due to abortion/total resorption (with an stat. sign. increase of late gestation resorptions at the high dose). There was also an increase of post-implantation loss (+37.4%) at the high dose. There was no clear exposure-dependent increase in embryo-foetal anomalies (malformations/variations) except an increase in supernumerary 13th ribs (a variation) from the middle dose (around +65% to +71%) although the teratological assessment is complicated by there being less females with live foetuses at the middle dose and especially the high dose. The 13th rib observation is of unlikely relevance for human extrapolation. There was a general reduced maternal food intake from the middle dose (also transiently before Gd10 at the low dose of 0.05mg/kg/d) spanning -36% to -74%, a reduction in mean body weight (-7% at 0.15mg/kg/d and -13% at 0.45mg/kg/d) and a reduced body weight gain during the exposure period (Gd6-Gd18) followed by an increased gain during the recovery period (Gd19-Gd24). There was a non-significant trend of reduced gravid uterus weight from the middle dose (-12% to 24%). The stroma of the endometrium was slightly more prominent plus minimal atrophy of the endometrial epithelium at the high dose (n=4-5/8 observed animals) plus some minimal focal haemorrhagic ovary cysts (n=2/8 observed animals) at the high dose. There was weak dose-accumulation of E4 during the exposure period at the lowest dose (Cmax 1.2x, AUC0-t 1.63x) that was reduced with increasing doses. The overall maternal and embryofoetal NOAEL is 0.05mg/kg/d (Cmax 925pg/mL, AUC0-t 6835pg x h/mL) and LOAEL 0.15mg/kg/d (Cmax 3816pg/mL, AUC0-t 24477pg x h/mL).

Based on a clinical AUC of 59100pg x h/mL (clinical study MIT-Es0001-C103), the exposure margins to the EFD NOAELs are: I) rat EFD NOAEL AUC exposure margin between 0.15x (the fraction between animal AUC0-6h and human AUC0-24h and likely misrepresentative) and 0.63x (the fraction between

4xAUC0-6h and human AUC0-24h), II) rabbit EFD NOAEL AUC exposure margin 0.41x (rabbit AUC0-t from 24h post-dose sampling vs human AUC0-24h. The applicant has not provided any discussion on exposure margins for the EFD data, but using these rough estimates, there is no human exposure margin for the non-clinical observations.

Segment II+III: In the modified segment III rat study (exposure period Gd6-Gd18+PND1-PND21 with recovery up to PND116 in males, doses 0.17 to 1.5mg/kg/d), there was an increase of F0 female mortality/pre-termination deaths partly due to reduced survival during delivery, partly to the finding of dead litters after delivery. At the high dose (1.5mg/kg/d), there was several adverse clinical signs among females after delivery (e.g. cold to the touch, pallor of extremities, hypoactivity, prostration, blood in the bedding). At the high dose, there was a clear reduction of females with live born offspring (control n=20 compared to high dose n=11, total live control pups n=197 vs high dose pups n=54) and the gestation index was reduced to 64.7%. The increase in F0 female and F1 offspring mortality was greatest around delivery and the first days after delivery (more cannibalized pups or intact pups found dead at PND4) but not afterwards (no difference in lactation index).

Exposed F0 females demonstrated clearly reduced food intake (-15% to -26%) from the middle dose (0.50mg/kg) during pregnancy and in the lactation phase at the high dose (-21% to -28% at 1.5mg/kg/d). This corresponded to a reduced body weight gain (>10%) during pregnancy (but without clear difference in mean body weights) but not during the lactation period (yet with a reduced mean body weight of -9% to -10%) from the middle dose (0.50mg/kg/d). Pre-weaning F1 offspring did not demonstrate any clear change in mean body weight or body weight gain. Instead, this was observed post-weaning in males with a reduction in food intake (-6% to -9%) from the middle dose, a reduced mean body weight (-6% to -10%) from the middle dose throughout recovery and a reduced body weight gain primarily between PND36 to PND57 from the middle dose (the overall weight gain across the recovery period was still reduced compared to controls). No food intake/body weight differences were observed in F1 pregnant females (termination at Gd15).

There were no exposure generated differences in morphological landmarks and behavioural development (motoric and reflex development, cognitive development). With regards to F1 animal sexual development, there were no E4 effects on prenatal, pre-weaning or post-weaning sexual maturation landmarks (sex-ratio, anogenital distance, balanopreputial separation or vaginal opening) nor on F1 female pairing, mating or fertility (e.g. mean number of corpora lutea, pre- and post-implantation loss). The overall F0 maternal and F1 offspring NOAEL is 0.17 mg/kg/d and the LOAEL is 0.50 mg/kg/d.

4.4.6. Toxicokinetics and exposure margins

NO(A)Els, systemic exposures at the NO(A)ELs and associated animal/human dose and exposure ratios are summarized in the below table.

For the calculation of animal/human dose ratios, dose levels were normalized to Body Surface Area according to FDA Guidance for Industry (2005). A dose of 15 mg E4/3 mg DRSP per day corresponds to 9.25/1.85 mg/m2/day in a 60-kg woman.

Exposure ratios have been calculated versus steady-state human exposure (AUC_{0-24}: 59.1 ng.hr/mL; Cmax: 17.9). Taking into account that exposures in female subjects tend to be slightly higher under steady-state conditions than after the first dose, this result is the most conservative ratios also for potential acute concerns.

Table 3: Exposures, Animal/Human Dose Ratios and Animal/Human Exposure Ratios at No-Observed-(Adverse-) Effect-Levels

Species	NO(A)EL (mg/kg/day)	$AUC_{0\text{-last}}$ (ng·hr/mL)	C_{max} (ng/mL)	Dose Ratio	AUC Ratio	C_{max} Ratio
Safety Pharmacology						
CNS, rat	15	1968[1]	1039[1]	7.3	23	53
hERG, *in vitro*	28.17 µM	NA	8574	NA	NA	437
CV, monkey	100	6816[1]	2282[1]	97	81	116
Respiratory, rat	150	48990[1]	14394[1]	73	583	734
Repeat-Dose Toxicity						
Rat	5	736	238	2.4	8.8	12
Monkey	3	328	97.2	2.9	3.9	4.9
Genotoxicity						
Micronucleus test	2000	NA	42400	973	NA	2163
Comet Assay	2000	NA	22800	973	NA	1163
Carcinogenicity						
Mouse	0.125	4.60	3.75	0.03	0.05	0.19
Rat	0.27	18.8	9.99	0.13	0.22	0.51
Return-to-Fertility						
Rat	1.5	118[2]	61.2[2]	0.73	1.4	3.1
Embryo-Fetal Development						
Rat	1	37.7[3]	20.9[3]	0.49	0.45	1.07
Rabbit	0.05	6.84	0.925	0.05	0.08	0.05
Pre-/Postnatal Development						
Rat	0.17[4]	13.4[2]	6.94[2]	0.08	0.16	0.35

[1] Data on Day 1 in the 4-week toxicity study in rat and the maximum tolerated dose study in monkey
[2] Calculated with average normalized $AUC_{0\text{-last}}$ (78.7) and C_{max} (40.8) under steady-state conditions in female Wistar rats
[3] Determined in the dose-finding study ES-T02
[4] NOAEL concerning parturition difficulties and neonatal survival until Day 4 post-partum
Abbreviations: $AUC_{0\text{-last}}$ = area-under-the-concentration-versus-time-curve up to the last measurable concentration, CNS = central nervous system, C_{max} = maximum plasma concentration, CV = cardiovascular, hERG = human ether-a-go-go-related gene, NA = not applicable, NO(A)EL = No-Observed-(Adverse-)Effect-Level

4.4.7. Local tolerance

Not applicable

4.4.8. Other toxicity studies

Induction of reactive oxygen species

E4 was evaluated for the potential to induce the formation of ROS in L5178Y TK+/- mouse lymphoma cells.

Three independent experiments were performed. L5178Y TK+/- mouse lymphoma cells were exposed to E4 at concentrations of 1, 10, 50, 100, 1000 and 3000 µM for 3 hours in a 37°C, 5% CO2 humidified incubator. Concentrations of 1000 and 3000 µM (highest feasible concentration) were applied in the third experiment only. Lactate (up to 100 µM) was used as a negative control for ROS induction and hydrogen peroxide (up to 100 µM) as a positive control. The vehicle control consisted of 0.1% (v/v) ethanol in phosphate-buffered saline.

Following exposure, cells were incubated with ROS reagent for 1.5 hours, background fluorescence was quenched with 0.1% (w/v) Trypan Blue and fluorescence was read at λexcitation 492 nm, λemission 520 nm. Readings were corrected for cellular autofluorescence. The study was not conducted in compliance with GLP.

The negative control lactate did not result in ROS induction in any experiment. The positive control hydrogen peroxide increased cellular ROS levels, but the increase at the highest concentration (100 µM) was very inconsistent between experiments (122%, 400% and 125%, respectively). E4 caused a dose-dependent decrease of ROS levels in all experiments with a maximum effect (approximately 50%) at 3000 µM.

Although the data should be interpreted with caution in view of the variable results with the positive control, the results suggest that E4 acted as an antioxidant under the experimental conditions of this study, rather than as a pro-oxidant.

4.4.9. Ecotoxicity/environmental risk assessment

Table 4: Identity and PBT/vPvB assessment

Substance (INN/Invented Name):	estetrol monohydrate		
CAS-number (if available):	2055649-81-3		
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	1.65	Potential PBT: N
PBT-statement:	estetrol is not PBT, nor vPvB		

Table 5: Phase I

Calculation	Value	Unit	Conclusion
PEC_{sw}, default	0.095	$\mu g \cdot L^{-1}$	≥ 0.01 threshold: Y
Other concerns	endocrine active substance		Y

Table 6: Phase II – Physical-chemical properties and fate

Study type	Test protocol	Results	Remarks
Adsorption-Desorption Soil 1 clay loam Soil 2 = loam Soil 3 = sand Sludge 1 = Burley-M Sludge 2 = Pooll	OECD 106	$K_{oc,\ soil\ 1}$ = 88 L·kg$_{oc}^{-1}$ $K_{oc,\ soil\ 2}$ = 130 L·kg$_{oc}^{-1}$ $K_{oc,\ soil\ 3}$ = 147 L·kg$_{oc}^{-1}$ $K_{oc,\ sludge\ 1}$ = 35 L·kg$_{oc}^{-1}$ $K_{oc,\ sludge\ 2}$ = 36 L·kg$_{oc}^{-1}$	
Ready Biodegradability Test	OECD 301B	not readily biodegradable	
Aerobic and Anaerobic Transformation in Aquatic Sediment systems Sediment 1 = silt loam Sediment 2 = sand	OECD 308	$DT_{50,\ water}$ = 4.3/3.3 d $DT_{50,\ sediment}$ = 7.2/15 d $DT_{50,\ whole\ system}$ = 7.3/4.0 d CO_2 = 15/1 8% NER = 52/44 % Transformation products >10% =Y	DT50s at 20±2°C sediment 1/2 at day 101 at day 101 Two transformation products >10% appeared nearly identical to parent after structural elucidation.

Table 7: Phase IIA effect studies

Study type	Test protocol	Result	Value*	Unit	Remarks
Algae, Growth Inhibition Test/*P. subcapitata*	OECD 201	NOEC	≥94	mg·L^{-1}	growth rate
Daphnia magna, Reproduction Test	OECD 211	NOEC	10	mg·L^{-1}	mortality, reproduction, growth, time to 1st brood
Fish, Short Term Reproduction Assay/*D. rerio*	OECD 229	NOEC NOEC	2.4 24	µg·L^{-1} µg·L^{-1}	vtg males survival, fecundity, fertility, vtg females, hatching
Fish full life cycle study, MEOGRT/*O. latipes*	OECD 240	NOEC	0.69	µg·L^{-1}	F1 hatching
Activated Sludge, Respiration Inhibition Test	OECD 209	NOEC	≥994	mg·L^{-1}	respiration

Table 8: Phase IIB effect studies

Study type	Test protocol	Result	Value*	Unit	Remarks
Sediment dwelling organism/*C. riparius*	OECD 218	NOEC	90.9	$mg \cdot kg_{dw}^{-1}$	emergence, NOEC normalised to 10% organic carbon

* values are given for the active substance estetrol.

Table 9: Risk characterisation

Compartment	PEC	PNEC	RQ	Conclusion
STP	$0.95\ \mu g \cdot L^{-1}$	$\geq 9.4x10^{4}\ \mu g \cdot L^{-1}$	$\leq 1.0x10^{-5}$	no risk
Surface water	$0.095\ \mu g \cdot L^{-1}$	$0.069\ \mu g \cdot L^{-1}$	1.4	risk
Groundwater	$0.024\ \mu g \cdot L^{-1}$	$0.0069\ \mu g \cdot L^{-1}$	3.4	risk
Sediment	$1.73x10^{-3}\ mg \cdot kg_{dw}^{-1}$	$0.91\ mg \cdot kg_{dw}^{-1}$	$1.9x10^{-3}$	no risk

The ERA dossier is complete. Considering the data of the definitive hazard assessment, estetrol is not PBT nor vPvB. Considering the above data, estetrol is not expected to pose a risk to the sewage treatment plant and sediment compartment. A potential risk of estetrol is identified for the surface water and groundwater compartment.

Estetrol should be used according to the precautions stated in the SmPC, to minimize any potential risks to the environment.

4.5. Overall discussion and conclusions on non-clinical aspects

4.5.1. Discussion

The pharmacological profile of estetrol is similar to the oestrogenic responses of the two reference compounds used, oestradiol and ethinyl oestradiol, but has a weaker response. The difference seen *in vitro* is larger than that seen in *in vivo* studies. Estetrol displays a selective binding to the human oestrogen receptors (ER) and binds to both ERα and ERβ, with a higher affinity for ERα compared to ERβ. The potency of estetrol *in vivo* is generally 10 to 20 times lower than the potency of ethinyl oestradiol (EE) and is also lower than the potency of E2. In both ERα and ERβ transactivation assays, E4 metabolites E4-3-glucuronide and E4-16-glucuronide showed weak estrogenic activity. In general, the difference in potency seems to be similar for both wanted and unwanted effects. The lower potency of oestradiol seems to be reflected also by the clinical dose chosen (15 mg), which is at least 500 times higher compared to the dose of ethinyl oestradiol (0.02 – 0.03 mg) used in combined oral contraceptives already on the market.

Estetrol is a naturally occurring oestrogen produced by the human foetal liver and reaches the maternal circulation through the placenta. It should be noted that the physiological function of estetrol in pregnancy is unknown but the transfer through the placenta to the maternal circulation would strongly indicate that externally administrated estetrol is also likely to reach the foetus. Based on the conditionality of its developmental expression, it seems likely that estetrol has a specific function during pregnancy in comparison to other endogenous oestrogens and it is therefore possible that the focus on comparing the pharmacological effects of estetrol using established assays for known oestrogens might be somewhat misleading. However, without further knowledge regarding the

physiological function of estetrol, the performed non-clinical pharmacological programme is considered acceptable and sufficient.

No concerning effects have been observed in safety pharmacology studies. No effects on the CNS, heart rate, blood pressure or electrocardiogram parameters as well as respiratory parameters were reported. The non-clinical pharmacokinetic characterisation of estetrol is in general considered acceptable and sufficient. In brief, rapid absorption of E4 after oral administration was reported in three animal models (mice, rats, monkeys). Up to 30 mg/kg/day dose proportional exposure has been observed. No or limited accumulation was reported. The only metabolic routes observed in human hepatocytes are glucuronidation and sulfation, two main metabolites - E4-3-glucuronide and E4-16-glucuronide have been detected in plasma and urine. As these metabolites are also relevant for conversion of E4 observed in mouse and rat, it was agreed that any risks related to these metabolites have been covered by the conducted repeat-dose toxicity studies.

As was also indicated in several of the scientific advice, information on placental transfer as well as transfer into breast milk is absent. This is agreed as such studies are considered not relevant in view of the indication and the target population.

The toxicology studies performed with estetrol include single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, and reproductive toxicity. Additional studies investigated if estetrol induced ROS. The toxicity profile of E4 is mainly driven by the estrogenic properties of the substance with changes in reproductive tissues, lymphoid tissues, liver (rats), adrenals and pituitary (monkey).

In the 26-week study in SD rats, one mortality occurred in the 15 mg/kg/day group for reasons of poor clinical condition. The cause of moribund condition was considered by the study pathologist to be related to gavage procedure and a relationship to the treatment was excluded.

The genotoxicity testing of E4 was not straightforward, with different test giving contradictory or unclear results.

According to the applicant, and in accordance with the expert statement by *Kirkland 2013*, differences in TA102 strain sensitivity might be a possible explanation for the different results noted in the three tests. As TA102 carries the *his* mutation on the multicopy plasmid pAQ1, it was agreed that different copy numbers can potentially give a change in sensitivity to mutagens. However, there were no data available to show that there were in fact differences in pAQ1 copies between the tester strains in the studies. It was therefore not possible to further evaluate the hypothesis. It was agreed that ROS induction is less likely to cause the effects, and thus also the differences in effects. Therefore, the Ames test studies suggest a not fully reproducible mutagenic activity in the strain TA102 perhaps via a cross-linking mode of action.

Two-year carcinogenicity studies in rats and mice showed that estetrol is carcinogenic.

Toxicokinetic data in (pregnant) rats and rabbits in the reprotoxicity and repeat-dose toxicity studies showed that the exposure in these animal species was comparable or -considerably- lower than the AUC level in humans at 15 mg/day estetrol.

Nevertheless, it is expected that further increases in the doses in the animal species used would not be feasible because of e.g. maternal toxicity, total embryofoetal loss and parturition failure. Based on the pharmacological activity of estetrol, effects on pregnancy, lactation and fertility are to be expected in humans. However, the applicant indicated that estetrol HRT is indicated for postmenopausal women and not intended for use during pregnancy and therefore the risk of embryofoetal effects of estetrol HRT is considered low. Nevertheless, if pregnancy accidentally occurs during the use of estetrol, the treatment should be withdrawn immediately as covered in SmPC 4.6. This was agreed.

Because effects on fertility are anticipated with estetrol, return-to-fertility with subsequent pregnancy was evaluated in rats with estetrol doses of 0.17-0.5 mg/kg/day PO for 4 weeks. Although return to cycling after cessation of the treatment already occurred at the lowest dose tested, the NOAEL for maternal tolerance was set at 0.5 mg/kg/day because of reduction in body weight (gain) at the highest estetrol dose. The NOAEL for fertility and early embryonic development (i.e. when mothers are only treated before mating) was 1.5 mg/kg/day, because no effects on gestation index and implantation were found. This was agreed.

Estetrol at doses ≥0.1 mg/kg PO twice daily prevented implantation in rabbits, thus an early embryonic development study in rats was not considered feasible. Although estetrol exposure and related effects in rats and rabbits may be different, the absence of early embryonic development studies in which rats are specifically treated during the first days of pregnancy can be endorsed.

Oestrogens are known to induce embryo-foetal abnormalities when used in high doses. Still, the applicant performed EFD studies in rats (0.3-3 mg/kg/day PO, G6-G17) and rabbits (0.05-0.45 mg/kg/day PO, G6-G18). In both species, considerable maternal toxicity (body weight loss) was noted, even at low doses. Increase in total embryo-foetal loss (rat) and delay in foetal development (rabbit) were observed at all doses, while reduced foetal weight (rat), abortion/resorption (rabbit) and increased post-implantation loss were associated with the highest doses used in the pivotal studies (rat 3 mg/kg/day, rabbit ≥0.15 mg/kg/day). The applicant attributes these effects to maternal toxicity and the known effects of highly dosed oestrogens on embryo and foetal development.

Foetal malformations with skeletal abnormalities were found in mothers treated with ≥1 or ≥0.15 mg/kg/day (rat and rabbit, respectively), but the applicant reasoned that these abnormalities were likely maternal toxicity-related and reversible. Therefore, these malformations are not considered related to teratogenic properties of estetrol. This rationale is plausible.

The applicant has conducted a pre- and post-natal development study (preceded by two preliminary/DRF studies) in the rat, as suggested by ICH 5 (R3). The pivotal and DRF studies showed that estetrol-related toxicity at doses ≥0.5 mg/kg/day occurred primarily due to maternal toxicity, leading to abortion and death of pups around parturition, even when treatment was temporarily discontinued at the time of parturition. The applicant sufficiently explained the oestrogen-related mechanisms behind the observed toxicities. The proposed maternal NOAELs for gestation and lactation (0.5 and 0.17 mg/kg/day, respectively) was endorsed.

F0 pups surviving the first days of life and the F1 generation did not show considerable abnormalities, and the NOAELs proposed for viability, survival and development of F0 offspring and F1 generation can be accepted (0.17, 1.5 and 1.5 mg/kg/day, respectively).

The applicant performed a GLP 13-week toxicity study in female cynomolgus monkeys aimed to evaluate E4 in combination with P4 at dose levels up to 10/100 mg/kg/day. There was no mortality related to the test articles during the study. Minimal to mild non-adverse and reversible changes in clinical pathology parameters were reported. At histopathological examination, non-adverse effects, related to the pharmacological activity of the compounds, were observed in the female genital organs and mammary gland at all dose levels of the combination and with E4 or P4 alone. The NOAEL for the combination E4/P4 was set at 10/100 mg/kg/day.

Considering the data of the definitive hazard assessment, estetrol is not considered PBT, nor vPvB.

Considering the above data, estetrol is not expected to pose a risk to the sewage treatment plant and sediment compartment. A potential risk of estetrol is identified for the surface water and groundwater compartment.

Estetrol should be used according to the precautions stated in the SmPC in order to minimize any potential risks to the environment.

4.5.2. Conclusions

The non-clinical development programme is overall adequate to support the FYLREVY MAA.

5. Clinical aspects

5.1. Introduction

5.1.1. GCP aspects

The clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Based on the review of clinical data, CHMP did not identify the need for a GCP inspection of the clinical trials included in this dossier (see section 2.4.3).

5.1.2. Tabular overview of clinical trials

The efficacy of estetrol as hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women is based on data from the two pivotal phase 3 studies MIT-Do001-C301 and MIT-Do001-C302 (hereafter named as C301 and C302) with E4 alone, safety with E4 alone up to 12 months, and endometrial protection when E4 20 mg is combined with a commercially available progesterone (P4) in a dose of 100 mg in non-hysterectomized (NH) women up to 12 months.

These studies which both included an efficacy study part (ESP) and a safety study part (SSP), are supported by the dose-finding study MIT-Do0001-C201 (hereafter named as C201). An overview of the one phase 2 and the two phase 3 clinical development is available in the table below.

Table 10: Clinical studies

Study ID	Enrolment status Start and end date* Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
Phase 2: MIT-Do0001-**C201**	Completed, 12 May 2016-22 January 2018 N screened: 609 N randomized: 260 N treated: 257 N completed: 200	Randomized, multicenter, double-blind, placebo-controlled	E4 2.5 mg: n=53 E4 5 mg: n=47 E4 10 mg: n=53 E4 15 mg: n=49 Placebo: n=55 Total=257 (ITT) (NH=225, H=32)	Postmenopausal NH and H women (≥40 to ≤65 years) with ≥7 moderate to severe VMS per day or ≥50 moderate to severe VMS

			Oral/ Once daily/ 12 weeks (91 days maximum) After completion of E4 or placebo treatment, all NH subjects received dydrogesterone 10 mg once daily for 14 days[2]	
Phase 3 studies:				
MIT-Do001-**C301 ESP**	Completed, 30 December 2019- 07 December 2021 N screened: 3496 N randomized: 641 N completed: 504	Randomized, multicenter, double-blind, placebo-controlled	E4 15 mg: n=213 (NH=103, H=110) E4 20 mg: n=213 (NH=103, H=110) Placebo: n=214 (NH=104, H=110) Total=640 (ITT) Oral/ Once daily/ 12 weeks After completion or discontinuation of E4 or placebo treatment, all NH subjects received P4 200 mg once daily for 14 days	Postmenopausal NH and H women (≥40 to ≤65 years) with ≥7 moderate to severe VMS per day or ≥50 moderate to severe VMS per week
MIT-Do001-**C301 SSP** *(Endometrial and General Safety Study Part; **no** primary efficacy endpoint)*	Completed, 30 December 2019- 13 May 2024 N screened: unknown N randomized: 929 N completed: 402	Multicenter, open label	E4 20 mg + **P4 100** mg: n=922 (SAF[3]) Oral/ Once daily/ 12 months After discontinuation of E4 due to an adverse event of bleeding or an endometrial event (DPE, hyperplasia or worse), subjects could receive P4 200 mg once daily for 14 days if deemed necessary by the Investigator	NH postmenopausal women (≥40 to ≤65 years) with ≥1 moderate to severe VMS per week
MIT-Do001-**C302 ESP** *US and CA*	Completed, 27 September 2019- 23 November 2022 N screened (ESP and SSP combined): 3974 N randomized : 584 N completed: 289	Randomized, multicenter, double-blind, placebo-controlled	E4 15 mg: n=192 (NH=93, H=99) E4 20 mg: n=193 (NH=93, H=100) Placebo: n=194 (NH=95, H=99) Total=579 (ITT) Oral/ Once daily/ 12 months (3 months for efficacy evaluation followed by 9-month ***safety*** extension) After completion or discontinuation of E4 or placebo treatment, all NH subjects received P4 200 mg once daily for 14 days	Postmenopausal NH and H women (≥40 to ≤65 years) with ≥7 moderate to severe VMS per day or ≥50 moderate to severe VMS per week

* start date is Study Initiation Date for all studies, end date is Study Completion Date (last subject, last visit) for C201 and database lock date for C301 and C302

[2] If a bi-layer endometrial thickness ≥15 mm was detected or abnormal uterine bleeding occurred in a NH woman during E4/placebo treatment, the subject received dydrogesterone 10 mg once daily until the end of Week 11 (14 days on, 14 days off).

[3] No ITT set was defined in MIT-Do001-C301 SSP. SAF was used for the efficacy evaluation.

MIT-Do001-**C302 SSP** *(Safety Study Part; **no** primary efficacy endpoint)*	Completed, 17 September 2019- 23 November 2022 N screened (ESP and SSP combined): 3974 N randomized: 431 N completed: 149	Multicenter, open label	E4 20 mg: n=430 (SAF[4]) (NH=229, H=201) Oral/ Once daily/ 12 months After completion or discontinuation of E4 treatment, all NH subjects received P4 200 mg once daily for 14 days	Postmenopausal NH and H women (≥40 to ≤65 years) with ≥1 moderate to severe VMS per week

5.2. Clinical pharmacology

Altogether, the PK and PD properties of estetrol were assessed in 11 phase 1 and 2 phase 2 clinical trials, including one phase 1 study investigating potential DDIs.

It should be noted that several studies supporting the development of estetrol in HRT and presented in this document were initially submitted and assessed for the development of the COC estetrol/drospirenone (DRSP) 15/3 mg (EMEA/H/C/5336/0000 and EMEA/H/C/005382/0000). This includes studies PR3050, PR3054, PR3081, MIT-Es0001-C102, MIT-Es0001-C105, MIT-Es0001-C109 and MIT-Es0001-C110. For this reason, several studies presented in this module were conducted to assess the contraceptive effect of estetrol in premenopausal women and were conducted with estetrol in combination with progestins like DRSP or desogestrel (DSG). Additional supportive studies in this dossier are MIT-Do001-C101, MIT-Do001-C102, MIT-Do001-C103, MIT-Do001-C104, MIT-Do001-C106 and MIT-Do0001-C201.

An overview of these clinical pharmacology studies conducted with estetrol is provided in the table below.

[4] No ITT set was defined in MIT-Do001-C302 SSP. SAF was used for the efficacy evaluation.

Table 11: Overview of the clinical pharmacology trials conducted with estetrol alone or in combination with DRSP, DSG and P4

Study	Description	Dosing regimen
PK studies in healthy female subjects		
MIT-Es0001-C102	PK and safety	Single oral dose estetrol 5 mg, 15 mg and 45 mg Multiple oral dose of estetrol 15 mg once daily for 14 days
MIT-Es0001-C105	Mass balance, safety	Single oral dose of [^{14}C]-estetrol monohydrate 15 mg
PR3081	PK, PD and safety	Multiple oral dose of estetrol 10 mg and 20 mg, estetrol/DSG 20/0.15 mg and estetrol/P4 20/0.2 mg for 28 days
MIT-Do001-C104	PK and safety	Single oral dose of estetrol 20 mg and 100 mg
PK studies in healthy postmenopausal female subjects		
PR3050	PK, PD and safety	Single oral dose estetrol 0.1 mg, 1 mg, 10 mg and 100 mg
PR3054	PK,PD and safety	Multiple oral dose of estetrol 2 mg, 10 mg, 20 mg and 40 mg once daily for 28 days
MIT-Do001-C101	PK, food effect and safety	Single oral dose of 30 mg estetrol
MIT-Do001-C106	PK and safety	Multiple oral dose of estetrol 20 mg once daily for 8 days
PK studies in patients		
MIT-Do0001-C201	PK, efficacy and safety	Multiple dose of estetrol 2.5 mg, 5 mg, 10 mg and 15 mg once daily for 12 weeks
PK studies in special populations		
MIT-Es0001-C109	PK in Japanese and Caucasian populations	Single oral dose of estetrol 15 mg, or single oral dose of estetrol/DRSP 5/3 mg, 15/3 mg and 20/3 mg
MIT-Do001-C102	Hepatic impairment	Single oral dose of estetrol 20 mg
MIT-Do001-C103	Renal impairment	Single oral dose of estetrol 20 mg
DDI studies		
MIT-Es0001-C110	effect of valproic acid on estetrol/drosperidone	Single oral dose of estetrol/DSRP 15/3 mg Multiple oral dose of valproic acid 500 mg twice daily for 11 days

In 2 early clinical trials (Studies PR3050 and PR3054), postmenopausal women received single or repeated doses of estetrol over a dose range from estetrol 0.1 mg to E4 100 mg. Pre- and postmenopausal women received a single ascending dose of estetrol (5 mg, 15 mg and 45 mg) and multiple doses of 15 mg as a tablet in a further clinical trial (Study MIT-Es0001-C102). Postmenopausal women received a single oral dose of [^{14}C]-estetrol 15 mg as a solution to assess mass balance recovery, PK, metabolite profiling and identification in a mass balance study (Study MIT-Es0001-C105).

The PK of estetrol 15 mg alone or estetrol 5,15 and 20 mg in combination with DRSP 3 mg as tablets in Japanese and Caucasian women was characterized in study MIT-Es0001-C109. Estetrol PK was further studied in healthy women in a Phase 2a feasibility study of the contraceptive effect of estetrol alone (10 mg or 20 mg) or combined with progesterone or desogestrel (DSG) (Study PR3081). A clinical DDI study with valproic acid was also conducted (study MIT-Es0001-C110).

The effect of high fat food on estetrol (30 mg) bioavailability was studied in postmenopausal women in Study MIT-Do001-C101. PK of estetrol 20 mg was also evaluated in women with normal and impaired hepatic function (Study MIT-Do001-C102) and with normal and impaired renal function (Study MIT-Do001-C103). PK of estetrol was studied in a crossover study evaluating the effects of estetrol 20 mg and 100 mg on QTc interval in healthy postmenopausal women (Study MIT-Do001-C104). Also, PK of estetrol 20 mg was evaluated after multiple oral doses in healthy postmenopausal women (Study MIT-Do001-C106).

Additionally, to support human pharmacokinetics the following *in vitro* pharmacokinetic studies using human biomaterials were performed:

Table 12: Overview of the in vitro pharmacokinetic studies using human biomaterials

Study ID	Type of study
Permeability and interaction with uptake and efflux transporters	
0031-NC-017	Bidirectional transport study in Caco-2 cells
0031-107	Human SLC transporters inhibition/substrate study
0031-103	Human ABC transporters inhibition/substrate study
Es0001-NC-013	Human ABC transporters inhibition/substrate study
Es0001-NC-020	OATP1B drug transporters substrate study
Es0001-NC-021	OATP1B drug transporters inhibition study
Es0001-NC-018	Human ABC and SLC transporters inhibition study (with E4 metabolites)
Distribution	
J&J Preclinical Studies of Estetrol	Binding to human plasma proteins
ES-T33b	Binding to human plasma proteins
0031-104	Plasma/blood cell partitioning in human blood
PR3025	Binding affinity to human SHBG
Metabolism	
PR3041	Metabolism study in human hepatocytes
ES-T06	Metabolism study in human hepatocytes
Es0001-NC-012	Confirmation of glucuronide conjugate position in the two major human metabolites
Es0001-NC-015	Review of data and human samples for hydroxylated metabolites
PR3100	Human CYP450 reaction phenotyping study

Study ID	Type of study
0031-102	Human CYP450 reaction phenotyping study
Es0001-NC-011	Human UGT reaction phenotyping, identification of the glucuronide formed by human UGT2B7
0031-106	
PR3099	Human SULT reaction phenotyping study
Drug-drug interaction	
PR3038	Human CYP450 inhibition study
0031-101	Human CYP450 inhibition study
0031-105	Human UGT inhibition study
0031-100	Human CYP450 induction study
Es0001-NC-019	Human CYP450 induction study
Es0001-NC-017	Human CYP450 inhibition study (with E4 metabolites)

5.2.1. Methods

The concentration of estetrol in human plasma and urine were determined using validated liquid chromatography methods with tandem mass spectrometry (LC-MS/MS) or ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Overall, the applied bioanalytical techniques appear adequate and validated accordingly. The submitted documentation concerning the bioanalytical methods (i.e. validation reports and bioanalytical reports) is adequate.

Furthermore, a short overview was presented of the analytical methods used to the standard assessment of PD biomarkers. FSH, LH, prolactin, progesterone, and E2 were measured in accredited labs with reference ranges. This is acceptable for supportive PD use.

5.2.2. Pharmacokinetics

An overview of the pharmacokinetics of E4 15 mg and 20 mg tablets is given in the table below.

Table 13: Pharmacokinetics of E4 15 mg and 20 mg tablets

Parameters	**15 mg tablet**	**20 mg tablet**
Oral bioavailability	≥69%	
$t_{max,SD}$ (median)	0.34-0.50 hours	0.48-0.63 hours
$C_{max,SD}$ (GM mean)	14.7-18.6 ng/mL	17.7-20.8 ng/mL
$AUC_{0\text{-}inf,SD}$ (GM mean)	69.0-87.3 ng × h/mL	76.5-104 ng × h/mL
$t_{max,ss}$	0.50 hours	0.50 hours
$C_{max,ss}$	16.7 ng/mL	19.6 ng/mL
$AUC_{0\text{-}tau,ss}$	73.8 ng × h/mL	84.0 ng × h/mL
Steady state	within 7 days	
Food effect	C_{max}: 47% reduction AUC: no effect	
Plasma protein binding	45.4-50.4%	

Blood/plasma ratio	1 (e.g. equal distribution)	
Distribution (V_Z/F)	4507-8278	
Metabolising enzymes	UGT2B7, SULT1E1	
Substrate for transporters	P-gp, BCRP	
Excretion	Renal: 69% as glucuronide metabolites Faeces: 22% as parent compound	
$t_{1/2}$	18.5-23.8 hours	18.6-21.7 hours

5.2.2.1. Introduction

Overall, the clinical pharmacokinetics of estetrol has been described and substantiated adequately with the submitted clinical package. The role of the pharmacokinetics for estetrol is mainly descriptive and further used for SmPC recommendations on special populations (e.g. renal and hepatic impairment) and drug-drug interactions (e.g. UGT2B7 inhibitors).

5.2.2.2. Evaluation and qualification of models

5.2.2.2.1. Population Pharmacokinetics

No population pharmacokinetics modelling was submitted by the Applicant in support of this dossier. This is acceptable.

5.2.2.2.2. Physiology based pharmacokinetic model

No physiology based pharmacokinetic modelling was submitted by the Applicant in support of this dossier. This is acceptable.

5.2.2.3. Absorption

The pharmacokinetics of estetrol after a single oral dose of the mono-component tablet formulation in healthy female subjects was investigated in the dose range of 5 to 100 mg of estetrol monohydrate.

The pharmacokinetics of estetrol is characterized by the following phases: a short and rapid absorption phase, a rapid distribution phase, secondary peaks of absorption, and finally the terminal elimination phase. After the initial absorption phase, lower secondary reabsorption peaks are observed in the concentration-time profile of estetrol, which are most likely due to the process of enterohepatic recirculation.

After a single oral dose of 15 mg under fasting conditions, estetrol is absorbed with a median t_{max} of 0.34-0.50 hours, with a mean C_{max} of 14.7-18.6 ng/mL and mean $AUC_{0\text{-}inf}$ of 69.0-87.3 ng × h/mL. After a single oral dose of 20 mg under fasting conditions, estetrol is absorbed with a median t_{max} of 0.48-0.63 hours, with a mean C_{max} of 17.7-20.8 ng/mL and mean $AUC_{0\text{-}inf}$ of 76.5-104 ng × h/mL. After the initial absorption phase, lower secondary reabsorption peaks are observed in the concentration-time profile of estetrol, which are most likely due to the process of enterohepatic recirculation.

Multiple dose pharmacokinetics of estetrol in healthy female subjects were investigated in the dose

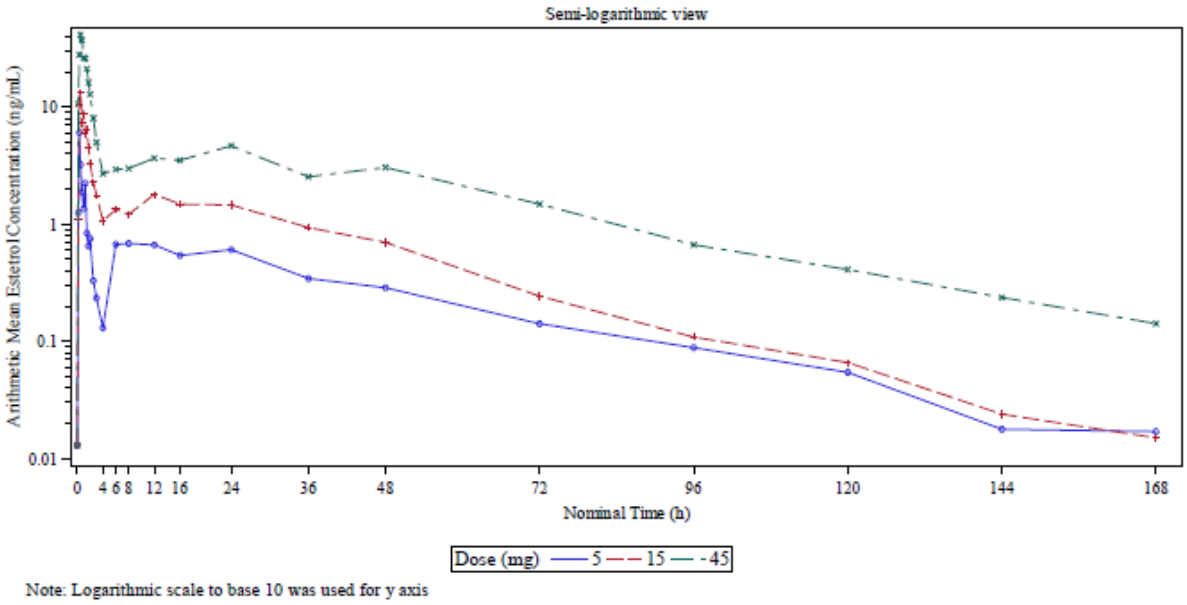
range of 2 to 40 mg once daily.

After multiple oral doses of 15 mg once daily of the tablet formulation, estetrol is absorbed with a median $t_{max,ss}$ of 0.50 hours, with a mean $C_{max,ss}$ of 16.7 ng/mL and mean $AUC_{0\text{-}tau}$ of 73.8 ng × h/mL. After multiple oral doses of 20 mg once daily of the tablet formulation, estetrol is absorbed with a median $t_{max,ss}$ of 0.50 hours, with a mean $C_{max,ss}$ of 19.6 ng/mL and mean $AUC_{0\text{-}tau}$ of 84.0 ng × h/mL.

Geometric mean CV% for estetrol C_{max} and estetrol AUC was high (17%-84% for C_{max} and 15%-74% for $AUC_{0\text{-}inf}$), suggesting a high intra-individual variability.

Typical concentration-time curves of estetrol after an oral dose are illustrated in Figure 2 below.

Figure 2: Study MIT-Es0001-C102. Overall arithmetic mean concentrations (log-linear scale) Period 1 (PK Population)



Note: Logarithmic scale to base 10 was used for y axis

Bioavailability

In vitro

No relevant pH dependency of solubility was observed for estetrol in aqueous media with a pH range of 1 to 6.8 (pH 1.0, pH 4.5, pH 6.8) and water. More than 20 mg of estetrol is soluble in 250 mL of aqueous media over this pH range. Therefore, estetrol is considered highly soluble. The *in vitro* permeability of estetrol (2-200 µM) was investigated in Caco-2 cells. The results of the *in vitro* study indicate that estetrol is a medium to highly permeable compound.

In vivo

The absolute bioavailability of estetrol was not determined following intravenous administration. In the mass balance study 69% of administered radioactivity was recovered from urine after administration of [^{14}C]-estetrol 15 mg. Therefore, the absolute bioavailability is at least 69%.

Influence of food

The food effect study was conducted with estetrol 30 mg tablets instead of the to-be-marketed 15 mg and 20 mg tablets, but this is not expected to result in a different outcome. The mean peak plasma concentration (C_{max}) of estetrol is reduced with 47% after single dose of 30 mg tablets after a high fat high calorie meal. No significant difference in the rate of absorption was observed, as the ranges in T_{max} after fasting and fed conditions overlap. The median T_{max} (min-max) is 0.5 (0.17-2.5) and 1.0

(0.33-4.0) hours after fasting and fed conditions, respectively. The extent of exposure ($AUC_{0\text{-tlast}}$) is similar after fasting and fed conditions, as the 90%-CI for the ratio of $AUC_{0\text{-tlast}}$ is 0.85-0.99 and falls within boundaries for equivalence. The difference in C_{max} is not considered to be clinically meaningful because for steroid activity, but the exposure to the steroid receptor is the important parameter, which is related to the AUC. Therefore, it is acceptable that estetrol can be administered with and without food.

5.2.2.4. Bioequivalence

The final dosing form is a film-coated tablet containing E4 15 mg or estetrol 20 mg. No bioequivalence or comparative bioavailability studies were conducted during development. During clinical development either oral solutions or tablet formulations of estetrol were used. The rate of absorption was a bit slower with the tablet formulation compared to the oral solution, which can be expected. The pharmacokinetics of the tablet formulations are comparable, as seen across the different studies.

5.2.2.5. Distribution

The *in vitro* plasma protein binding of estetrol was investigated over a concentration range of 1 to 50 ng/mL using the equilibrium dialysis method. The blood plasma protein binding varied between 45.4% and 50.4% in human plasma. Estetrol equally distributes between plasma and blood cells in human blood.

Estetrol has an apparent volume of distribution of between 4507 and 8278 L across clinical studies which indicates that estetrol has an extensive extravascular distribution.

5.2.2.6. Metabolism

Estetrol is extensively metabolised *in vitro* in human liver hepatocytes (5% of 1 µM remaining after 4 hours). Human CYP450 enzymes do not play a major role in the biotransformation of estetrol. The major metabolites are formed by direct glucuronidation or direct sulfation. The major metabolites are estetrol-16-glucuronide mainly catalysed by UGT2B7 and estetrol-3-sulfate mainly catalysed by SULT1E1.

Estetrol undergoes extensive metabolism after an oral dose, as the circulating amount of estetrol is only 7.5 to 17.2% in plasma at $T_{max,1}$ and $T_{max,2}$, respectively. The two major metabolites circulating in plasma are formed by direct conjugation of estetrol with glucuronic acid to estetrol-3-glucuronide and estetrol-16-glucuronide, which account for 78.7% to 75.8 of the circulating radioactivity in plasma. at $T_{max,1}$ and $T_{max,2}$, respectively. In urine, estetrol-16-glucuronide (46.8%) and estetrol-3-glucuronide (20.2%) accounted also for the most radioactivity. In faeces, estetrol was only detected as parent compound (21.8%).

Inter-conversion

Estetrol molecule is chiral due to the presence of seven asymmetric carbons in its structure (C8 R, C9 S, C13 S, C14 S, C15 R, C16 R, C17 R). Considering the *in vitro* and *in vivo* metabolic profile of estetrol in humans, the lack of detection of epimers and/or keto-derivatives of estetrol, together with considerations about feasibility of the chemical process, it seems unlikely that inter-conversion at specific chiral centres of estetrol in the D-ring would be of any clinical significance.

Pharmacokinetics of metabolites

The major metabolites observed in plasma were two estetrol-glucuronides. Following a single oral administration of E4/DRSP 15/3 mg alone to healthy female subjects, median T_{max} for E4-3-glucuronide and E4-16-glucuronide was 0.8 hour. Plasma concentrations were significantly higher than parent estetrol. Neither metabolite was found to be active and further studies with these metabolites are not necessary.

Consequences of possible genetic polymorphism

Estetrol is a substrate for UGT2B7 which is a highly polymorphic enzyme. Contribution of UGT polymorphism can be considered of no clinical relevance. However, it cannot be excluded that the UGT2B7 polymorphism may contribute to the variability of the PK of estetrol.

5.2.2.7. Elimination

In the mass balance study (Study MIT-Es0001-C105) six female subjects received a single oral solution dose of [14C]-estetrol 15 mg (2.8 MBq). Blood, urine and faecal samples were taken at specified time points throughout the study for the analysis of total radioactivity in whole blood, plasma, urine and faeces, and E4 and metabolite profiling in plasma, urine and faeces.

After a sampling period of 312 hours (13 days) 91 % of the total administered radioactivity was recovered, of which was 69% and 22% found in urine and faeces, respectively. In urine, only the estetrol-3-glucuronide and estetrol-16-glucuronide metabolites were identified. In faeces, estetrol was only excreted as parent compound.

An overview of the excretion pathway is given in the table below:

Table 14: Elimination pathways and metabolite profile of estetrol from mass balance study (MIT-Es0001-C105)

Compound	Plasma*	Urine	faeces
	% of sample radioactivity	% of radioactive dose	% of radioactive dose
Estetrol	7.5;17.2	ND	21.8
Estetrol-3-glucuronide	17.0;32.8	20.2	ND
Estetrol-glucuronide-sulfate	9.4; ND	ND	ND
Estetrol-16-glucuronide	61.7; 43.0	46.8	ND

*: Values in plasma are presented as measurements at $t_{max,1}$;$t_{max,2}$

5.2.2.8. Dose proportionality and time dependency

Dose proportionality

The pharmacokinetics of estetrol mono-component tablets were assessed after a single dose in the dose range 5 mg to 100 mg. Estetrol shows dose proportional exposure increase over the dose range 5 mg to 100 mg of estetrol monohydrate for the C_{max} and AUC.

Accumulation

After multiple doses of E4 15 mg once daily for 14 days in healthy subjects, an accumulation ratio (R_{AC}) of 1.8 was observed, indicating a limited accumulation (R_{AC}<2). This is in line with the observed $t_{1/2}$ across the studies, which is around 24 hours.

Time dependency

A slight tendency towards time-dependent pharmacokinetics is observed for estetrol, as $AUC_{0\text{-}tau}$ at steady-state was 15% lower than $AUC_{0\text{-}inf}$ after single dose administration of estetrol. The observed

trend for time-dependency is not expected to have a clinical relevance for estetrol itself, when considering the relatively low decrease in its exposure (i.e. less than 20%).

Time to reach steady state

Based on C_{trough} levels, steady state of estetrol is achieved within 7 days after once daily dosing. This is in line with the observed $t_{1/2}$ of estetrol of 18.5-26.9 hours.

5.2.2.9. Pharmacokinetics in the target population

No pharmacokinetic parameters were calculated for the phase 2 and 3 studies, as the pharmacokinetic sampling was limited. However, in the phase 1 studies, premenopausal women and postmenopausal women were both included. The pharmacokinetic parameters C_{max} and AUC_{0-24h} were comparable between both groups. Therefore, the pharmacokinetics in the target population can be considered comparable with that of healthy female volunteers.

Efficacy/safety/PK data related to long-term administration of supra-therapeutic doses are not available. E4 15 mg once daily was defined as the minimal effective dose. Single doses up to estetrol 100 mg estetrol and multiple doses up to E4 40 mg once daily for 28 days were well tolerated. Based on these considerations, the therapeutic exposure window after multiple doses of 15-40 mg of estetrol monohydrate once daily, is 17-70 ng/mL for $C_{max,ss}$ and 75-180 ng.h/ml for $AUC_{0-24,ss}$.

5.2.2.10. Special populations

Renal impairment

The effects of renal impairment on the pharmacokinetics of estetrol and metabolites after a single dose of E4 20 mg in female subjects was investigated in study MIT-Do001-C103. Renal function was categorised as normal renal function (eGFR ≥90 mL/min/1.73 m2) and mild (eGFR ≥60 to <90 mL/min/1.73 m^2), moderate (eGFR ≥30 to <60 mL/min/1.73 m^2) or severe (eGFR <30 mL/min/1.73 m^2) renal impairment. Another *post hoc* analysis with categories based on absolute GFR (aGFR: not normalised to body surface area) was submitted by the applicant. The following results are based on the categories based on aGFR.

C_{max} of estetrol increased 1.1-fold, 1.8-fold and 1.5-fold for subjects with mild, moderate and severe renal impairment, respectively. AUC_{0-inf} of estetrol increased 1.7-fold, 2.3-fold and 2.3-fold for subjects with mild, moderate and severe renal impairment, respectively. Furthermore, the 90%-CI for the ratio's completely exceeds the upper limit for bioequivalence of 125% for AUC_{0-inf} in all renal impairment groups.

Overall, a trend is observed between lower aGFR and higher exposure and lower clearance for the two metabolites estetrol-3-glucuronide and estetrol-16-glucuronide. Increase in exposure to estetrol-3-glucuronide between 1.2 and 1.9-fold for C_{max} and between 1.7 and 9.0-fold for AUC_{0-inf} was observed with increasing severity of renal impairment. Increase in exposure to estetrol-16-glucuronide between 1.9 and 2.7 fold for C_{max} and between 1.9 and 5.8-fold for AUC_{0-inf} was observed with increasing severity of renal impairment.

Based on the therapeutic window and the known short-term safety data up to E4 40 mg once daily, the increase in AUC_{0-inf} of >2-fold for estetrol in the groups with moderate and severe impairment could be of clinical relevance, and use of estetrol in moderate and severe renal impairment is not recommended in section 4.2 of the SmPC.

Hepatic impairment

The effects of hepatic impairment on the pharmacokinetics of estetrol and metabolites after a single dose of E4 20 mg in female subjects was investigated in study MIT-Do001-C102. Hepatic function was categorised as normal hepatic function and mild (Child-Pugh Grade A [5–6 points]), moderate (Child-Pugh Grade B [7–9 points]) or severe (Child-Pugh Grade C [10–14 points]) hepatic impairment.

C_{max} of estetrol increased 1.7-fold, 1.9-fold and 5.4-fold for subjects with mild, moderate and severe hepatic impairment, respectively. $AUC_{0\text{-}inf}$ of estetrol did not increase in female subjects with mild and moderate hepatic impairment. In subjects with severe hepatic impairment $AUC_{0\text{-}inf}$ increased 1.9-fold and the 90%-CI for the ratio completely exceeds the upper limit for bioequivalence of 125%.

Increase in exposure to estetrol-3-glucuronide of 1.7-fold, 1.6-fold and 1.4-fold for C_{max} was observed for mild, moderate and severe hepatic impairment subjects, respectively. The $AUC_{0\text{-}inf}$ for estetrol-3-glucuronide increased by 1.1-fold in the mild hepatic impairment subjects and decreased by 20% and 30% for the moderate and severe hepatic impairment subjects, respectively. Increase in exposure to estetrol-16-glucuronide of 2.0-fold, 2.1-fold and 2.6-fold for C_{max} was observed for mild, moderate and severe hepatic impairment subjects, respectively. The $AUC_{0\text{-}inf}$ for estetrol-16-glucuronide increased by 1.5-fold, 1.6-fold and 2.3-fold for mild, moderate and severe hepatic impairment subjects, respectively.

In severe hepatic impairment patients, the C_{max} increased 5.3-fold to 111.2 ng/mL, which falls outside the therapeutic window for C_{max}. Furthermore, the 90% CI for $AUC_{0\text{-}inf}$ completely shifted above the usual bioequivalence limits. Therefore, estetrol is contraindicated in patients with severe hepatic impairment.

Ethnic factors

Overall, the data indicate that, after body weight adjustment, there are no relevant pharmacokinetic differences between Japanese and Caucasian subjects and that the estetrol component has similar exposure in Japanese and Caucasian subjects in terms of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}inf}$ and C_{max}. A *post hoc* analysis performed on data from phase 3 studies showed that the race comparison Black/African American v.s. White, Black/African American v.s. Other and Other v.s. White populations, had no significant effect on the estetrol concentration. Furthermore, the fact that metabolites do not produce pharmacological effects indicates that any observed racial differences in metabolite levels would not affect clinical outcomes.

Weight

Comparison of the estetrol concentrations from the two phase 3 studies between three BMI-categories <25 kg/m^2 (low), 25-30 kg/m^2 (intermediate) and ≥30 kg/m^2 (high) showed that estetrol concentrations were significant lower ($p<0.05$) between the low BMI group (< 25 kg/m^2) and intermediate BMI group (25-30 kg/m^2), and between the low BMI group (< 25 kg/m^2) and high BMI group (≥30 kg/m^2). Compared to the low BMI group, the estetrol concentrations in the intermediate BMI group were 14% lower (95% CI: 0 to 26%). Compared to the low BMI group, the estetrol concentrations in the high BMI group were 18% lower (95% CI: 4 to 30%). The difference of pharmacokinetics between BMI categories is not considered clinically relevant.

Elderly

Altogether, 52 older subjects, all between age of 65 and 74 years, were exposed to any dose of estetrol during the clinical development programme with estetrol for postmenopausal women. Pharmacokinetic data was available for 30 out of 52 elderly subjects. No significant effect of age group was observed for estetrol concentrations from the phase 3 studies when comparing patients of ≥55 years versus <55 years. Furthermore, no difference in pharmacokinetics between premenopausal

women and postmenopausal women, which is also related to age, was found (see target population section).

Gender

In this application, the effect of sex on the pharmacokinetics of estetrol has not been assessed, as estetrol is only indicated for postmenopausal women. This is acceptable.

Paediatric population

No data of the pharmacokinetics of estetrol in de paediatric population was provided. This is acceptable, as estetrol-based HRT is indicated for postmenopausal women only.

5.2.2.11. Pharmacokinetic interaction studies

In vitro DDI studies

Estetrol as object

In vitro studies indicated that estetrol is substrate for UGT2B7 and SULT1E1 enzymes. The applicant has conducted an *in vivo* DDI study with valproic acid, an UGT2B7 inhibitor. No *in vivo* study with SULT1E1 is warranted, as the data from the mass balance study showed only a small contribution of this enzyme, e.g. <10% of the estetrol was found as the sulfate metabolite, and only in plasma.

According to *in vitro* studies with transfected MDCKII and HEK293 cells, estetrol is substrate for ABC efflux transporters P-glycoprotein (P-gp) and BCRP, indicating that estetrol can be actively excreted in the intestine and urine and actively secreted from the brain. Estetrol is not a substrate for OAT1, OATP1B1, OATP1B3, OCT2, MATE1 and MATE2-K. The DDI potential is however considered to be low, because estetrol is a medium to high permeable drug with a fast t_{max} (0.5 hours), which limits impact of BCRP and P-gp dependent transport on estetrol absorption.

Estetrol as precipitant

In vitro studies suggested that estetrol is a direct inhibitor of CYP3A4 at maximal intestinal concentrations ($K_i = IC_{50}/2$ = 19.3 µM). No time dependent inhibition of CYP enzymes was observed at relevant concentrations. The applicant reasons that clinically relevant DDI are unlikely to occur, because estetrol weakly inhibited human CYP3A4 with a calculated risk marginally above the intestinal cut-off, and only when using testosterone as substrate. This is acceptable.

In vitro induction experiments showed no positive signal for induction of CYP enzymes.

In vitro studies indicated that estetrol is not a clinically relevant inhibitor of UGTs at maximal systemic concentrations.

In vitro studies with transporters indicated that estetrol is an inhibitor of OAT3 with an IC_{50} of 16.5 µM. However, at the maximal systemic concentration, which is clinically relevant for OAT3 present in the kidneys, estetrol is not an inhibitor and no further clinical studies are warranted.

Metabolites estetrol-3-glucuronide and estetrol-16-glucuronide as precipitant

The applicant investigated the DDI potential of the two glucuronide metabolites of estetrol with CYP enzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4. Both metabolites were not direct or time dependent inhibitors of CYP enzymes. The applicant has also investigated the DDI potential of the two glucuronide metabolites of estetrol with ABC efflux transporters P-gp and BCRP and SLC uptake transporters MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2. Results showed that estetrol-3-glucuronide is not an inhibitor of these transporters. Estetrol-16-glucuronide is an inhibitor of OAT3

with an IC_{50} of 16.01 µM. This is below 50*$C_{max,u}$ = 50*0.5*0.72 = 18 µM, therefore not considered clinically relevant.

In vivo DDI studies

A clinical DDI study was conducted to investigate the effect of UGT2B7 inhibition on the PK of estetrol. Compared to the oral administration of 15 mg estetrol/3 mg DRSP (Treatment A), co-administration with valproic acid (Treatment B), an UGT2B7 inhibitor, resulted in a statistically significant increase in the exposure of estetrol, with a 1.36-fold increase in geometric mean C_{max} for estetrol, and 1.13-fold increase in $AUC_{0\text{-}inf}$. However, the observed increase in estetrol concentrations was not considered to be of clinical relevance.

5.2.3. Pharmacodynamics

5.2.3.1. Mechanism of action

Estetrol (E4) is an estrogen that is only produced by the foetal liver during pregnancy. It is developed as an estrogen in combination with the progestogen drospirenone for the indication of oral contraception approved by centralized procedure in 2021 (Drovelis, Lydisilka), and in the current MAA as an estrogen for hormone replacement therapy (HRT) in postmenopausal women with symptoms of estrogen deficiency. All current HRTs consist of the estrogen estradiol (E2).

In Vitro Estrogen Receptor Binding - Estrogenic properties

In vitro, E4 displays a highly selective binding to both human estrogen receptor (ER)α and ERβ, with a 4 to 5-fold binding preference for ERα. The binding affinity of E4 for ERα is at least 25-fold lower compared to estradiol (E2) (Study PR3019) (*Visser, Foidart et al. 2008*). The potency of E4 is lower than the potency of E2 or ethinylestradiol (EE). E4 metabolites E4-3-glucuronide and E4-16-glucuronide show weak estrogenic activity on both ERα and ERβ. The potency of these metabolites is several hundred-fold lower than the potency of E4 (3793 and 586 times less potent than E4, respectively, for ERα) (Study Es0001-NC-014).

5.2.3.2. Primary and secondary pharmacology

Primary pharmacology

Two early phase 1 clinical trials (studies PR3050, PR3054) were performed in postmenopausal women.

Single Rising Dose of E4 0.1, 1, 10, or 100 mg in Healthy Postmenopausal Women (Study PR3050)

First-in-human double-blind, placebo-controlled study to investigate safety/tolerability of single oral administration of escalating doses E4 0.1, 1, 10 and 100 mg as an oral solution to reach plasma E4 levels known from late foetal life, and to investigate the PD effect of E4 on luteinizing hormone (LH). In the 100 mg dose group only, FSH, SHBG, CBG, plasminogen, Factor XII, triglycerides, cholesterol, high density lipoproteins (HDL), cholesterol, cholesterol/HDL ratio, low density lipoproteins (LDL)-cholesterol, apolipoprotein B-100, osteocalcin, and c-terminal telopeptide of type I collagen (CTX-1) were evaluated.

For safety reasons, subjects in the 100 mg dose group were treated with 10 mg dydrogesterone for 10 days starting 14 days after dosing to counteract any stimulatory effect on the endometrium.

Pharmacodynamic results

LH:

No effect on LH levels was visible after dosing with 0.1 and 1.0 mg E4. LH was slightly decreased after 10 mg in all subjects as compared to placebo. Levels were back to baseline after approximately 24 hours. In the 100-mg group, an immediate decrease (mean decrease of 18 IU/L) was observed in all subjects as compared to placebo and returning to baseline at approximately 72 h post dose.

FSH SD 100 mg:
A profound inhibition of FSH levels over 48 h could also be established in the E4 100 mg group, denoting a strong central inhibiting potency of the compound.

PD biological outcomes in the SD 100-mg dose group only:
A slight increase in triglycerides and a slight decrease in LDL-cholesterol and apolipoprotein-B100 were noticed. Minimal changes were observed in the bone parameters osteocalcin and C-telopeptide. No relevant changes were observed in the clotting parameters Factor XII and plasminogen. Slight increases in SHBG and CBG were observed.

Multiple Oral Dosing of E4 2, 10, 20, or 40 mg in Healthy Postmenopausal Women (Study PR3054)

Phase I, partly randomized, open-label, multiple dose study to evaluate safety, tolerability, PK, and PD of 3 dosages of estetrol, the lowest dose of 2 mg estetrol compared with estradiol valerate 2 mg (E2V), after daily oral administration for 28 days in 49 healthy postmenopausal women. The subjects of the E4 2 mg group and the E2V 2 mg group were randomly assigned to one of these 2 groups, while women in the E4 10, 20 and 40 mg groups were consecutively allocated, after safety of the preceding E4-dose had been established, see below:

Figure 3: Study PR3054. Subject disposition

	Treatment	Days 1-28	After study discontinu-ation	No. of subjects	Subject condition
Group 1	E4 2 mg	Once daily for 28 days	5 mg lynestrenol once daily for 14 days	10	5 women with >50 HFs/week and 5 women with <10 HFs/week
Group 2	E2V 2 mg	Once daily for 28 days		10	5 women with >50 HFs/week and 5 women with <10 HFs/week
Group 3	E4 10 mg	Once daily for 28 days		10	10 women with >50 HFs/week
Group 4	E4 20 mg	Once daily for 28 days		10	hysterectomized postmenopausal women
Group 5	E4 40 mg	Once daily for 28 days		9	hysterectomized postmenopausal women

PD objectives are presented below:

- Endocrinology (FSH, LH, E2, prolactine [Groups 3-5], testosterone [Groups 4, 5])
- Carrier protein (SHBG)
- Haemostatic factors (plasminogen [Groups 1, 2], prothrombin fragment 1+2 [Groups 3-5], normalized activated protein C ratio (nAPCr) [Groups 1, 2], and tissue plasminogen activator (tPA) [Groups 1, 2])
- Lipids and lipoproteins (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol)

- Carbohydrates (fasting glucose)
- Bone turnover markers (osteocalcin, CTX-1)
- Immunology [Group 3] (lymphocyte differentiation, C-reactive protein, complement C3 and C4 and immunoglobulins [Ig] IgG, IgA and IgM)
- Vaginal cytology (maturation index)
- Endometrium [Groups 1-3] (endometrial thickness and biopsy)

Due to the proliferative endometrial effect of E4 10 mg observed during study PR3050, E4 20 mg and E4 40 mg treatments were administered to H women while NH women participating in the E4 2 mg, E4 10 mg, or E2V study arms received 5 mg lynestrenol for 14 days starting on Day 31 (i.e. 3 days after cessation of estrogen intake).

Pharmacodynamic results

E2

The E2 levels in the 2 mg, and 10 mg E4 groups were in general not affected by the E4 administration. However, mean E2 levels had increased by 41% (from a mean of 50.7 to 70.0 pmol/L) on Day 7 in the 20 mg E4 group and by over 75% in the 40 mg E4 group on Days 7, 14, and 28 (from 33.0 to 52.6, 52.3, and 50.3 pmol/L, respectively). These results might be explained by a possible cross-reaction between E4 and E2 in the E2 immunoassay.

FSH

A dose-dependent decrease was seen in FSH levels up to 87% in the E4 40 mg dose group. The decrease of FSH levels of the 2 mg E2-valerate group was in between the decrease seen in the 2 mg and 10 mg E4 groups.

LH

LH levels decreased in a dose-dependent manner after a small increase during the first days of E4 administration. LH levels in the E2V group showed only a small decrease that was comparable to the E4 10 mg dose group on Day 28.

Prolactin

Prolactin was measured in the E4 10 mg, 20 mg, and 40 mg groups at baseline and on Day 28. Baseline values were 151.6, 138.2 and 140.9 mU/L in the E4 10 mg, 20 mg and 40 mg groups, respectively. Mean prolactin levels were higher on Day 28 compared to Day 1 in the E4 10 mg and E4 40 mg groups (relative change from baseline 32% and 46%, respectively) but had only slightly changed in the E4 20 mg group (relative change from baseline 8%).

Total and free testosterone

Testosterone was measured only in the E4 20 mg and E4 40 mg groups and baseline levels were 0.6 and 0.5 nmol/L, respectively. There were no relevant changes in the total testosterone levels in both groups. Free testosterone was only calculated in the 40 mg E4 group and had a baseline value of 7.4 pmol/L. During treatment with 40 mg E4, the mean free testosterone level gradually decreased from 7.4 pmol/L on Day 1 to 2.4 pmol/L on Day 28, being a decrease of 58.1% from baseline. No relevant changes were observed for total testosterone (20 and 40 mg group only). This can be explained by the increase in SHBG levels after E4 treatment, resulting in more bound testosterone and less free testosterone.

SHBG

SHBG levels showed a dose-dependent increase from Day 7 onward after administration of E4. In the E2-valerate group the increase in SHBG levels was comparable to the increase observed for the 10 mg E4 dose group.

Haemostasis
Prothrombin fragment 1+2 levels seemed to increase with increasing doses of E4 in the 2 mg to 20 mg dose range: actual mean change from baseline was -6% for the 2 mg E4 group, 23% for the 10 mg E4 group, 51% for the 20 mg E4 group, and 37% for the 40 mg E4 group. The mean actual change from baseline for the 2 mg E2-valerate was between the increase seen in the 2 mg and 10 mg E4 groups (11%). Decreases in tissue plasminogen activator (tPA) levels were observed in the 10 mg, 20 mg and 40 mg E4 groups and these decreases suggested a relation between tPA changes and estetrol levels. No changes were seen in plasminogen (2 mg dose groups) and normalized activated protein C ratio (nAPCr) (10, 20 and 40 mg E4 dose groups).

Immunology parameters, and glucose
No clinically relevant changes were observed in immunology parameters (10 mg only) and glucose levels (fasting state) were also not affected.

Lipids
The results indicated that dose-dependent decreases of LDL-cholesterol levels and the total cholesterol to HDL-cholesterol ratio might be present after administration of E4, and a possible dose-dependent increase in HDL-cholesterol levels in the 20 mg and 40 mg E4 groups. LDL-cholesterol also decreased in the 2 mg E2-valerate group comparable to the decrease observed in the 10 mg E4 group. HDL-cholesterol levels did not change in the 2 mg E2-valerate group, while total cholesterol showed a small decrease comparable to the E4 groups (except that no effect was seen in the 2 mg E4 group). The total cholesterol to HDL-cholesterol ratio also decreased in the 2 mg E2-valerate group and this decrease was in between the decreases observed for the 10 mg and 20 mg E4 groups. Triglyceride levels increased in the 2 mg E2-valerate group, and this increase was much higher than the increases observed in the 20 mg and 40 mg E4 groups.

Bone turnover markers
CTX-1 levels decreased in the 3 highest E4 dose groups and the E2-valerate group. In addition, in the two highest E4 dose groups a small decrease was seen in osteocalcin levels while a small increase was observed in the 2 mg E4 group.

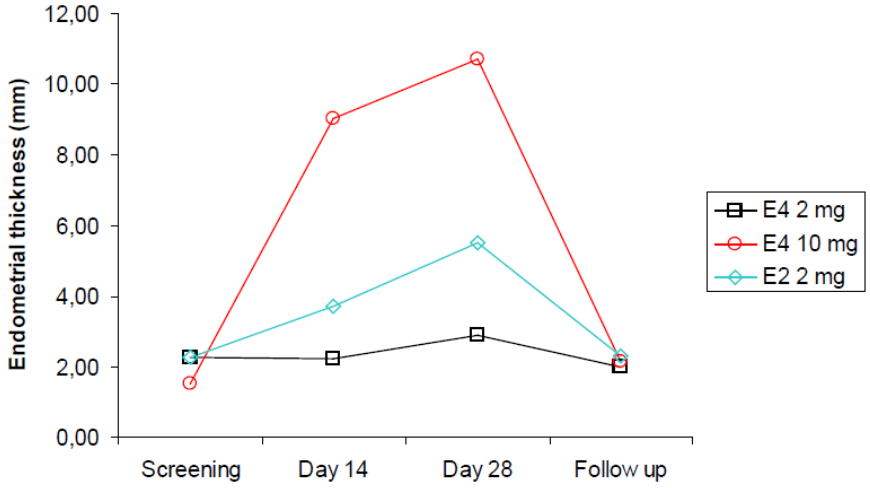
Hot flushes/sweating
The effect on the number of hot flushes and sweating per day was studied in the 2 mg and 10 mg E4 groups and the 2 mg E2-valerate group until Day 56. Only subjects who reported to have 35 or more hot flushes per week at screening were evaluated. In all three dose groups after start of dosing, a modest decrease in the mean number of hot flushes was seen. The mean number of hot flushes per day on Day 1 was 11.5 in the 2 mg E2-valerate, 9.0 in the 2 mg E4, and 8.4 in the 10 mg E4 group. By Day 28, the mean number of daily hot flushes had decreased to 6.8, 5.6, and 5.0 for the 2 mg E2-valerate, the 2 mg E4, and the 10 mg E4 groups, respectively. The number of sweating per day had decreased on Day 28 in all three treatment groups. No difference between the treatment groups was seen in the change of the number of sweating.

Vaginal cytology (maturation index)
In the 10 mg, 20 mg and 40 mg E4 groups there was a clear shift in the relative contributions of the three cell types from parabasal cells at baseline to superficial cells at Day 28. In the 2 mg E4 group the shift was mainly from parabasal cells at baseline to intermediate cells at Day 28. The percentage of superficial cells was approximately the same at baseline and at Day 28. The shift in cell types seen in the 2 mg E2-valerate group was in between those observed in the 2 mg and the 10 mg E4 groups. In some subjects a shift in relatively more intermediate cells was seen and in other subjects a shift in relatively more superficial cells.

Endometrial thickness

The endometrial thickness did not change during treatment in the 2 mg E4 group. The endometrium of subjects in the 10 mg E4 group had increased sevenfold on Day 28 (up to 10.71 mm) and returned to baseline values at Day 56. The endometrial thickness of the 2 mg E2-valerate group increased to more than double on Day 28 and also returned to baseline values on Day 56.

Figure 4: Mean endometrial thickness actual values for the 2 mg and 10 mg E4 groups and the 2 mg E2-valerate group until Day 56



Endometrial biopsy outcome

An endometrial biopsy using a Pipelle device was performed in Groups 1-3 only at screening and on Day 28 (in Groups 1 and 2 only in subjects in whom the endometrium had increased in thickness from Screening by more than 50% (double layer) as measured by transvaginal ultrasound. All of the 18 reported biopsies obtained on Day 28 were classified as proliferative (3 biopsies in the 2 mg E4 group, 8 biopsies in the 2 mg E2-valerate group and 7 biopsies in the 10 mg E4 group). Endometrial biopsy samples obtained at Screening were classified as atrophic/inactive, while those obtained on Day 28 were classified as proliferative.

All but two subjects in Groups 1-3 reported withdrawal bleedings at the end of the study after intake of lynestrenol.

Pharmacodynamic (+ biological) evaluations in Phase 2 study

MIT-Do0001-C201 (E4 RELIEF study) phase 2 dose-finding, randomised, double-blind, placebo-controlled study to select the minimal daily oral dose of estetrol (E4) for the treatment of vasomotor symptoms in hysterectomized and non-hysterectomized post-menopausal women. In this study, 2.5, 5, 10, or 15 mg E4 were compared to placebo for 12 weeks. For details on the study design, see 5.3.1.

<u>Pharmacodynamic parameters (part of the other secondary endpoints):</u>

- Change from baseline to week 13 in Vaginal pH
- Change from baseline to week 13 in Vaginal Maturation Index
- Change from baseline to week 13 in lipid and glucose metabolism markers, haemostatic variables and bone variables

Effect on Vaginal pH

The mean change in vaginal pH from baseline at Week 13 was not statistically significant in any E4 group compared to placebo (ANCOVA, ITT population).

Effect on Vaginal Maturation Index

Intermediate cells were more abundant in all groups at baseline. This remained unchanged in the placebo group, but in all E4 groups, especially the E4 10 mg and the E4 15 mg groups, the percentage of intermediate cells was considerably lower at Week 13. The percentage of parabasal cells decreased as well in the E4 groups, while the percentage of superficial cells increased. The maturation value (MV), which showed only minor changes from baseline to end of treatment (EOT) in the placebo group, markedly increased in all E4 groups. Pairwise comparisons revealed a statistically significant increase in the vaginal MV between each E4 treatment group and placebo at Week 13.

Markers of Lipid and Glucose Metabolism

For only 2 markers of lipid and glucose metabolism, the ANCOVA indicated statistically significant differences among the 5 treatment groups regarding changes from baseline to EOT: HDL-cholesterol, and glycated haemoglobin.

The HDL-cholesterol concentrations increased from baseline to EOT in all E4 groups, with the increase being most pronounced in the 15 mg group. There were no statistically significant differences among the 5 treatment groups in changes from baseline to EOT for triglycerides, total cholesterol and LDL-cholesterol.

Glycated haemoglobin (HbA1C) concentrations decreased from baseline to EOT in the 10 mg and 15 mg groups (point estimations: -0.82 and -1.48 mmol/mol haemoglobin, respectively) and slightly increased in the other groups. Pairwise comparisons showed a statistically significant difference from placebo for the 10 and 15 mg groups. Although fasting glucose levels did not change from baseline to EOT in any of the treatment groups, there was a dose-related trend to a decrease in fasting insulin. This was also reflected in a trend of the homeostasis model assessment-estimated insulin resistance to decrease (ie, to improve) with increasing dose of E4. This is in good agreement with the statistically significant decrease in glycated haemoglobin in the 10 and 15 mg groups.

Hemostasis Variables

For only 2 haemostasis variables, the ANCOVA indicated statistically significant differences among the 5 treatment groups regarding changes from baseline to EOT: SHBG and free protein-S.

The SHBG concentrations increased from baseline to EOT in the 5, 10, and 15 mg groups, with the increase being most pronounced in the 15 mg group (point estimation: 39.93 nmol/L). Pairwise comparisons showed a statistically significant difference from placebo for the 10 and 15 mg groups.

Concentrations of free protein-S decreased from baseline to EOT in all treatment groups, with the decrease being most pronounced in the 15 mg group (point estimation: 5.91%). Pairwise comparisons showed a statistically significant difference from placebo for 15 mg group. All other haemostasis variables assessed from baseline to EOT – prothrombin fragment 1 + 2, D-dimers, antithrombin, Factor XIV/protein C, Factor VIII, and free tissue pathway inhibitor – did not show any change over time or with dose of E4 vs placebo.

Effect on biomarkers for bone turnover

Decreases in CTX-1 and osteocalcin were observed. Only the change in the E4 10 mg arm differed statistically significantly ($p<0.005$) from that in the placebo arm.

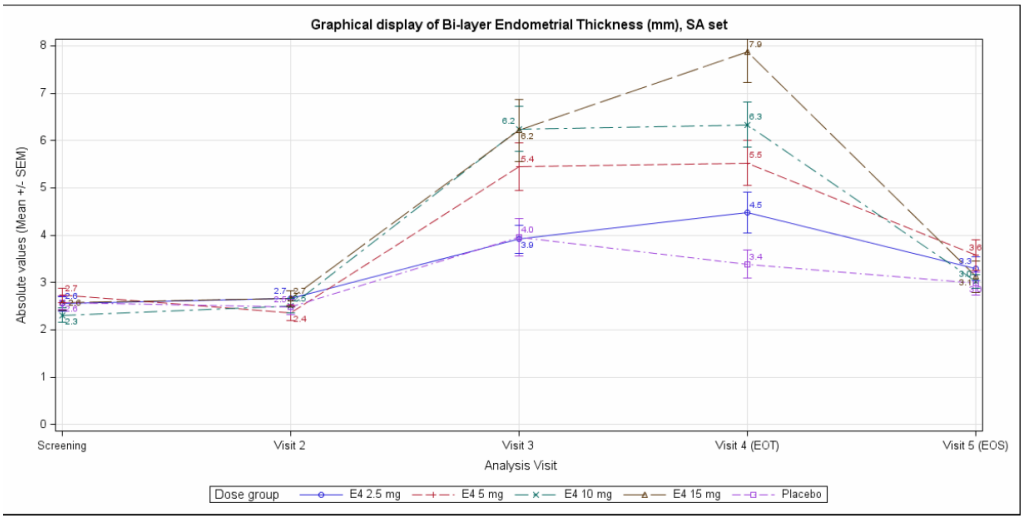
Osteocalcin concentrations decreased from baseline to end of treatment in the E4 5 mg, E4 10 mg, and E4 15 mg arms, with the decrease being most pronounced in the E4 15 mg arm, only the change in the E4 15 mg arm differed statistically significantly ($p<0.05$) from that in the placebo arm.

Endometrial thickness

Measurements of endometrial thickness by TVUS were performed in all 225 non-hysterectomized subjects at screening and baseline and in most subjects also at Visit 3 and at EOT and EOS.

The maximum thickness measured at screening and baseline was 5 mm as stipulated in the admission criteria. The mean thickness versus time curves show that the endometrial thickness increased with increasing dose during treatment with E4 and returned towards pretreatment level at EOS, ie, after 2-week intake of progestin. There were no major differences in endometrial thickness at EOS among the 5 treatment groups. Mean endometrial thickness in subjects from the placebo group did not exceed 5 mm during the entire study. In single subjects, however, values up to 19 mm were measured occasionally.

Figure 5: Endometrial thickness before, during, and after 12-week treatment with E4 or placebo (SA set) (Protocol MIT-Do0001-C201)



Pharmacodynamic (+ biological) evaluations in Phase 3 studies

MIT-Do001-C302: A randomized double-blind placebo-controlled Phase 3 trial to evaluate the efficacy and safety of estetrol for the treatment of moderate to severe vasomotor symptoms in postmenopausal women (E4Comfort Study II). See section 6.3.2 for details on the study design.

Haemostasis (C302-ESP)

For the majority of haemostasis variables assessed from baseline to EOT (prothrombin fragment 1 + 2, D-dimers, antithrombin, Factor XIV/protein C, Factor VIII, and free tissue pathway inhibitor), changes observed over time were minimal with E4 vs placebo.

Concentrations of free protein-S decreased from baseline to EOT in all study arms, with the decrease being most pronounced in the E4 15 mg arm (point estimation: 5.91%); using an ANCOVA model, pairwise comparisons of this data showed a statistically significant difference from placebo for the E4 15 mg arm ($p<0.05$). Values remained within reference ranges demonstrating a neutral profile on this parameter.

Glucose metabolism (C302-ESP)

The effect of E4 treatment on glucose metabolism parameters was variable and inconsistent between study arms and at the different time points analyzed. A statistically significant ($p<0.05$) reduction in the difference of LS means from baseline for HbA1c was observed in the E4 20 mg arm at Week 12, compared with the placebo arm.

No clinically significant abnormalities in glucose metabolism were reported as TEAEs that were considered related to study treatment.

Study C301

Glucose metabolism (EPS)

Statistically significant (p<0.05) decreases in the difference of LS means vs placebo for fasting plasma glucose and HbA1c were observed after E4 15 mg and E4 20 mg treatment at Week 12.
Decreases in the difference of LS means for insulin levels and HOMA-IR in the E4 study arms compared to the placebo arm did not reach statistical significance. No clinically relevant abnormalities in glucose metabolism were reported as TEAEs considered related to study treatment.

Glucose metabolism (SSP)

Compared with baseline, treatment with E4 20 mg + P4 100 mg daily resulted in a decrease in the mean HbA1c, fasting glycaemia, insulin resistance (HOMA-IR), and insulin levels, at Week 52.
The only abnormality in glucose metabolism that was reported as a TEAE and considered related to study treatment was diabetes mellitus of moderate intensity in 1 subject. The sponsor assessed this case and concluded no causal relation. It considers only one subject, with obesity and aged 53. The difference in fasting glucose from baseline to week 53 was 2.17 mmol/L. The threshold fasting glucose is 7.0 mmol/L, indicating that this women at baseline already borderline (6.93 mmol/L).

Bone turn-over

Treatment with E4 15 mg or E4 20 mg resulted in statistically significant (p<0.05) reductions from baseline compared with placebo at Weeks 12 and 52 for the bone turnover parameters CTX 1 and PINP.

Secondary pharmacology

Cardiac safety

Study MIT-Do001-C104 - A randomized, placebo- and active-controlled 4-way crossover study in healthy subjects to evaluate the effect of E4 on the QTc interval of therapeutic (20 mg) and supratherapeutic (100 mg dose) doses. The primary objective of this study was to evaluate the effects of therapeutic (E4 20 mg dose) and supratherapeutic (E4 100 mg dose) plasma concentrations of estetrol on heart rate-corrected QT interval (QTc).

Results

In the concentration-QTc analysis, a linear model with a treatment effect-specific intercept was fitted for E4 plasma concentrations, which represented the data in an acceptable way. The estimated slope of E4 plasma concentration in the concentration-QTc relationship was negative and not statistically significant (-0.0016 ms per ng/mL [90% CI: -0.02422 to 0.02109]) with a very small and not statistically significant treatment effect-specific intercept of 0.45 ms. The effect on placebo-corrected change-from-baseline (ΔΔ) QTcF at the GM peak E4 concentration can be predicted to 0.42 ms (90% CI: -0.20 to 1.05) and 0.32 ms (90% CI: -1.50 to 2.14) for the 20 mg and 100 mg dose groups, respectively.
In the by-time point analysis of the QT interval, least squares (LS) mean change from baseline (Δ) QTcF on E4 closely followed the placebo pattern across postdose time points. LS mean ΔΔQTcF varied from -2.5 ms at 6 h postdose in the 20 mg group to 3.7 ms at 24 h postdose in the 100 mg dose group, without an indication of dose-dependency. The upper limit of the CI remained below 10 ms at all time points.

E4 at the studied doses did not have a clinically relevant effect on HR or cardiac conduction, i.e. the PR and QRS intervals.

The same linear model with a treatment effect-specific intercept as in the concentration-QTc analysis was used in the assay sensitivity analysis for moxifloxacin. The slope of the relationship was positive

and statistically significant: 0.0038 ms per ng/mL (90% CI: 0.00305 to 0.00450), and the lower bound of the 2-sided CI of the predicted QT effect (16.49 ms [90% CI: 14.06 to 18.91]) at the GM peak moxifloxacin concentration (3881.1 ng/mL) was above 5 ms, thereby demonstrating assay sensitivity.

Cardiodynamics conclusion: E4 at the studied doses had no clinically relevant effects on studied ECG parameters. Based on the concentration-QTc analysis, an effect on ΔΔQTcF exceeding 10 ms can be excluded within the full observed range of plasma concentrations of E4 up to approximately 177 ng/mL. These results constitute a negative thorough QT (TQT) study, as described in the International Council for Harmonisation (ICH) E14 clinical guidance document.

5.2.3.3. Pharmacodynamic interactions with other medicinal products or substances

During clinical trials with the hepatitis C virus combination drug regimen ombitasvir/paritaprevir/ritonavir with and without dasabuvir, ALT elevations greater than 5 times the ULN were significantly more frequent in women using ethinylestradiol-containing medicinal products such as combined hormonal contraceptives. Women using medicinal products containing estrogens other than ethinylestradiol, such as estradiol, had a rate of ALT elevation similar to those not receiving any estrogens; however, due to the limited number of women taking these other estrogens, caution is warranted for co-administration with the combination drug regimen ombitasvir/paritaprevir/ritonavir with or without dasabuvir and also the regimen with glecaprevir/pibrentasvir.

No data is available on the interaction of E4 with combination drug regimens used for hepatitis C virus. This was reflected accordingly in the SmPC.

5.2.4. Dose selection and therapeutic window

The key PK parameters showed dose-proportionality. Therefore, the dose selection process was not directly driven by PK, but by the efficacy and safety outcomes consecutive to the multiple daily administration of the E4 treatment. Those aspects are extensively described in the Efficacy and Safety sections.

5.2.5. Overall discussion and conclusions on clinical pharmacology

5.2.5.1. Discussion

Pharmacokinetics

Bioanalytical method

The applicant performed no cross-validation between different analytical methods and analytical sites. However, the analytical methods of the main pharmacokinetic studies used comparable methods with comparable calibration ranges and adequate precision and sensitivity. Additionally, pharmacokinetic results over the different studies were sufficiently comparable, therefore a cross-validation is not considered necessary.

Absorption

The results of the *in vitro* study indicate that estetrol is a medium to highly permeable compound. However, this should be interpreted carefully, as only one high permeability control was included in this study, and no medium and low permeability controls were included.
It is worth noting that in all the conducted experiments (even at the highest estetrol concentrations of 200 µM), the ratio of B to A/A to B had values well above 2, implying the active efflux of estetrol. Thus, the currently reported apparent permeability value for estetrol cannot be regarded as the

parameter describing solely a passive permeability, but rather a net value of both active and passive transport processes. Since the transporters in Caco-2 cells were not saturated/inhibited in the present experiments, no precise conclusions about the passive permeability of estetrol can be made based on these results.
Enterohepatic recirculation as the mechanism of the secondary peaks in plasma of estetrol is supported by the observation that metabolites of estetrol also exhibit secondary peaks, which can only be formed if the parent compound is absorbed. Secondary peaks of the metabolites were also observed after IV administration of estetrol. Other possible mechanisms for secondary peaks like delayed or biphasic absorption cannot explain these observations. Furthermore, data of the PK profiles of the parent and metabolites from intravenous infusion of estetrol support this.
The absolute bioavailability of estetrol has not been determined. In a mass balance study (MIT-Es0001-C105), at least 69% of the administered radioactivity was recovered in urine. The additional 21.9% of the dose was recovered in faeces, as unchanged estetrol. No clinical data was generated during the drug development to quantify the relative contribution of faecal excretion of unabsorbed estetrol versus estetrol recycled through biliary excretion. However, the (*in vitro*) high permeability of estetrol, the low early faecal recovery (2.5%) of estetrol within the first 24 hours, the delayed recovery of estetrol of 8–9% in faeces after 72 hours in the mass-balance study, and the PK-profile of estetrol with secondary peaks, make it more likely that the majority of the estetrol found in faeces is due to biliary excretion of absorbed estetrol. However, unabsorbed estetrol cannot be ruled out with the available data. No glucuronide conjugates were identified in faeces.

Food effect
In the food interaction study a tablet formulation of 30 mg was used, instead of the to-be-marketed tablet formulations of 15 mg and 20 mg. However, the pharmacokinetics of estetrol tablets is linear across a dose range from 5 mg to 100 mg. The formulations of the different tablets used during clinical development are very similar. No deviation from dose-proportionality was observed from the clinical data across the different tablet formulations. Therefore, the results of the food interaction study can be extrapolated to the commercial formulations.

Inter conversion
The potential of estetrol for interconversion was not discussed.
Still, considering the *in vitro* and *in vivo* metabolic profile of estetrol in humans, the lack of detection of epimers and/or keto-derivatives of estetrol, together with considerations about feasibility of the chemical process, it seems unlikely that inter-conversion at specific chiral centers of estetrol in the D-ring would be of any clinical significance. Estetrol is a terminal product of estrogen metabolism, and it is not interconverted into other estrogens *in vivo*. The dataset supports that estetrol is metabolised mainly by conjugation, without back-conversion or pharmacologically active metabolites.
Furthermore, the probability of local deconjugation of estetrol-glucuronides to regenerate clinically meaningful free estetrol at target tissues is low, as bacterial β-glucuronidases hydrolyse the glucuronide conjugates.

Consequences of possible genetic polymorphisms
Estetrol is metabolised by UGT2B7. This enzyme is considered to be highly polymorphic. Several UGT2B7 genotypes have been identified, amongst others UGT2B7*1 and UGT2B7*2 genotypes. UGT2B7*1 polymorphism has been associated with an increase in oral clearance of zidovudine of 196% (*Kwara, Lartey et al. 2009*) while UGT2B7*2 was not associated with altered *in vivo* enzyme activity (*Court 2010*). In view of the impact of UGT2B7 polymorphism, a similar impact may be expected of such polymorphism on estetrol.
Furthermore, the frequencies of the UGT2B7*1 and UGT2B7*2 alleles in Caucasian and Japanese populations has been identified. In the Caucasian population there was an approximately equal

distribution of subjects homozygous for each allele (homozygote UGT2B7*1 and UGT2B7*2 frequencies were 23.75% and 21.75% respectively), while in the Japanese UGT2B7*1 homozygotes were over 10-fold more prevalent than UGT2B7*2 homozygotes (homozygote UGT2B7*1 and UGT2B7*2 were 45.0% and 6.0% respectively) (*Bhasker, McKinnon et al. 2000*).
No *in vivo* or *in vitro* studies were performed to determine the effect of UGT polymorphism on the metabolism and clearance of estetrol. However, a study was conducted to compare the estetrol PK between the Caucasian and Japanese population (study MIT-Es0001-C109). The results of this study showed that 15 mg estetrol displayed similar exposure (in terms of AUC) in Japanese and Caucasian subjects, suggesting that the existing differences in UGT2B7*1 and UGT2B7*2 genotypes between the Caucasian and Japanese population do not result in a difference in estetrol pharmacokinetics between both groups.
In conclusion, the contribution of UGT polymorphism can be considered of no clinical relevance.

Transporters
For OAT3 the results of the *in vitro* studies are inconclusive. The OAT3 transporter could potentially play a role as an uptake transporter in the kidneys for estetrol. However, from the mass balance study, it was observed that estetrol is not excreted as parent compound in urine. Additionally, OAT3 could play a role with glucuronidation at the site of the kidneys by UGT2B7. However, the uptake ratio of 3.09 observed only at the concentration of 1 µM estetrol, the minimal effect of the positive inhibitor on the uptake of estetrol, and the large difference compared to the results for the probe substrate all indicate that if OAT3 plays a minor role in the uptake of estetrol, and its clinical importance would be low. Therefore, no additional studies are warranted for further investigation of the role of OAT3.

Renal impairment and hepatic impairment
The increase in $AUC_{0\text{-}inf}$ for estetrol in the groups with moderate and severe impairment could be of clinical relevance, and use of estetrol in moderate and severe renal impairment is not recommended in section 4.2 of the proposed SmPC. Since there is an absence of long-term safety data on a 2-fold estetrol exposure in terms of AUC during long term multiple dosing, it is agreed that estetrol is not recommended in the moderate and severe renal impairment population.
Overall, it appears reasonable to not exclude patients with mild and moderate hepatic impairment due to a 1.7- and 1.9-fold increase in C_{max}, respectively, while AUC is comparable to that observed in healthy individuals after a single dose of estetrol. In severe hepatic impairment patients, the Cmax increased 5.3-fold to 111.2 ng/mL, which falls outside the therapeutic window for C_{max}. Furthermore, the 90% CI for $AUC_{0\text{-}inf}$ completely shifted above the usual bioequivalence limits. Therefore, estetrol is contraindicated in patients with severe hepatic impairment.
Furthermore, the increased exposure to the metabolites is not of clinical relevance, as it has been found that both glucuronide metabolites have weak estrogenic activity compared to the parent compound. Additional simulations of 14 days of repeated dosing for the estetrol-3-glucuronide and estetrol-16-glucuronide metabolites in participants with varying degrees of renal and hepatic impairment by superposition of the data generated in the hepatic impairment and renal impairment studies show an increase of <7.2-fold for both glucuronide metabolites which is not considered relevant due to the several hundred fold less potency of both metabolites compared to the parent compound estetrol.

Pharmacodynamics

Two phase 1 studies have been performed, a placebo-controlled single dose study evaluating 0.1, 1, 10, 100 mg E4 versus placebo (PR3050), and a multiple dose study (PR3054) evaluating 2, 10, 20, and 40 mg E4 of which 2, and 10 mg were compared versus 2 mg estradiol valerate (E2-valerate) in

healthy postmenopausal women. Next to endocrinologic PD endpoints, biological endpoints (lipids, haemostasis, glucose) have been evaluated, in line with the recommendations of the CHMP guideline on HRT. Additionally, endometrial thickness and endometrial biopsies have been evaluated. Further preliminary efficacy has been evaluated in VMS and vaginal atrophy.

LH, FSH, testosterone (free and total), prolactin, E2 level

- In study PR3050, a clear dose-dependent inhibition of plasma LH levels by E4 was noted, from a slight decrease in 10 mg, and immediate decrease after 100 mg as compared to placebo. A profound decrease in FSH levels was noted in the E4 100 mg group (only measured in 100 mg).
- In study PR3054, LH levels decreased in a dose-dependent manner after a small increase during the first days of E4 administration. In the 2 mg E2-valerate group only a small decrease in LH was noted that was comparable to the E4 10 mg dose group. A dose-dependent decrease was seen in FSH levels up to 87% in the E4 40 mg dose group. The decrease in FSH levels of the 2 mg E2-valerate group was in between the decrease seen in the 2 mg and 10 mg E4 groups. For total testosterone (in E4 20 and E4 40 mg), no relevant changes were noted in both groups. Free testosterone gradually decreased from 7.4 pmol/L on Day 1 to 2.4 pmol/L on Day 28 (58.1%). This can be explained by the increase in SHBG levels after E4 treatment, resulting in more bound testosterone and less free testosterone.

Mean Prolactin levels were increased on Day 28 in the E4 10 mg and E4 40 mg groups (32% and 46%, respectively) but had only slightly in the E4 20 mg group (8%).

No change in E2 levels was observed after administration of E4 with 2 and 10 mg, a single rise in the 20 mg E4 group, and consistent increases in the 40 mg E4 dose group. According to the Applicant, these results in the 20 and 40 mg E4 group might be explained by a possible cross-reaction between E4 and E2 in the E2 immunoassay. Furthermore, a rebound increase was seen after withdrawal in the 10 mg group, not at higher doses. Similar endocrine effects were confirmed in ovulatory women in Study PR3081. It was explained that the different E2 patterns in PR3054 were the consequence of inter-subject variability, RIA cross-reactivity with circulating E4, and a positive bias for E2 in a concentration proportionally presence of E4. In addition, the spiking data indicated interference in the assay at low E2 levels. This finding supports the conclusion that the E2 increases in PR3054 were analytical artefacts rather than a true pharmacodynamic effect. Since the Phase 3 data (E4 15–20 mg) showed no change in E2 versus placebo for up to 13 weeks, this reduces the concerns about a true pharmacologic effect on endogenous E2. Thus, the divergent E2 patterns in PR3054 do not appear to significantly impact the overall benefit–risk ratio.

Biological endpoints lipids, bone markers, SHBG, CBG, hemostasis

Lipids

- Study PR3050: with E4 100 mg, a slight increase in triglycerides and a slight decrease in LDL-cholesterol and apolipoprotein-B100 was noted.
- Study PR3054: a dose-dependent decrease of LDL-cholesterol levels and the total cholesterol to HDL-cholesterol ratio was noted. The total cholesterol to HDL-cholesterol ratio also decreased in the 2 mg E2-valerate group, which decreased in between the decreases observed for the 10 mg and 20 mg E4 groups. LDL-cholesterol also decreased with 2 mg E2-valerate which effect was comparable to the decrease with 10 mg E4. A possible dose-dependent increase in HDL-cholesterol levels with 20 and 40 mg E4 was noted, and no change with 2 mg E2-valerate. Total cholesterol showed a small decrease in the E4 groups but not in the 2 mg E4 group. Triglyceride levels increased in the 2 mg E2-valerate group, and this increase was higher than the increases observed in the 20 mg and 40 mg E4 groups.

Glucose levels

Study PR3054: glucose levels (fasting state) were not affected.

SHBG

- Study PR3050: A slight increase in SHBG and CBG was observed.
- Study 3054: SHBG levels showed a dose-dependent increase from Day 7 onward after administration of E4. In the E2-valerate group, the increase in SHBG levels was comparable to the increase observed for the 10 mg E4 dose group.

Hemostasis

- Study PR3050: No clear changes were observed in clotting parameters Factor XII and plasminogen.
- Study PR3054, prothrombin fragment 1+2 levels increased with increasing doses of E4 in the 2 mg to 20 mg dose range: actual mean change from baseline was -0.28 nmol/L (-6%) for the 2 mg E4 group, 0.19 nmol/L (23%) for the 10 mg E4 group, 0.42 nmol/L (51%) for the 20 mg E4 group, and 0.37 nmol/L (37%) for the 40 mg E4 group. The mean actual change from baseline for the 2 mg E2-valerate was 0.10 nmol/L (11%): the increase in prothrombin fragment 1+2 in the 2 mg E2-valerate dose group was in between the increase seen in the 2 mg and 10 mg E4 groups. Decreases in tissue plasminogen activator (tPA) levels were observed in the 10 mg, 20 mg and 40 mg E4 groups and these decreases suggested a relation between tPA changes and estetrol levels. No changes were seen in plasminogen (2 mg dose groups) and normalized activated protein C ratio (nAPCr) (10, 20 and 40 mg E4 dose groups).

Immunology parameters

No clinically relevant changes were observed in immunology parameters (10 mg only) and glucose levels (fasting state) were also not affected.

Bone turnover markers

Study PR3050: Slight changes were observed in osteocalcin and C-telopeptide.
Study PR3054: CTX-1 levels decreased in the 3 highest E4 dose groups and in the E2-valerate group. In the 20 and 40 mg E4 dose groups a small decrease was seen in osteocalcin levels.

Hot flushes/sweating

Study PR3054: The mean number of daily hot flushes on Day 1 was 11.5 in the 2 mg E2-valerate, 9.0 in the 2 mg E4, and 8.4 in the 10 mg E4 group. By Day 28, the mean number of daily hot flushes had decreased to 6.8, 5.6, and 5.0 for the 2 mg E2-valerate, the 2 mg E4, and the 10 mg E4 groups, respectively. The number of sweating per day had decreased on Day 28 in all treatment groups. No difference between the treatment groups was seen in the change of the number of sweating.

Vaginal cytology (maturation index)

Study PR3054: In the 10 mg, 20 mg and 40 mg E4 groups there was a clear shift in the relative contributions of the three cell types from parabasal cells at baseline to superficial cells at Day 28. The shift in cell types seen in the 2 mg E2-valerate group was in between those observed in the 2 mg and the 10 mg E4 groups.

Endometrial thickness

Study PR3054: The endometrial thickness did not change during treatment in the 2 mg E4 group. The endometrium of subjects in the 10 mg E4 group had increased sevenfold on Day 28 (up to 10.71 mm) and returned to baseline values at Day 56. The endometrial thickness of the 2 mg E2-valerate group increased to more than double on Day 28 and also returned to baseline values on Day 56. This difference in increase of endometrial thickness in between postmenopausal women who were treated for 28 days with 10 mg E4 versus women treated with the marketed 2 mg E2-valerate (7-fold versus 2-fold) is considerable and considered relevant in the discussion of endometrial safety and tolerability of E4 in the doses selected for marketing (15 and 20 mg).

Endometrial biopsy outcome
An endometrial biopsy was performed in the 2 and 10 mg E4 and in the 2 mg E2-valerate group at screening and on Day 28 (in 2mg E4 and E2-valerate groups only in subjects in whom endometrial thickness was increased from Screening by more than 50% (double layer) as measured by transvaginal ultrasound. All 18 biopsies obtained on Day 28 were classified as proliferative (3 biopsies in the 2 mg E4 group, 8 biopsies in the 2 mg E2-valerate group and 7 biopsies in the 10 mg E4 group). Endometrial biopsy samples obtained at screening were classified as atrophic/inactive.

Effect of progestogen administration after end of study
Study PR3050: In the single dose of E4 100 mg group, 10 mg of dydrogesterone daily for 10 days starting 14 days after E4 dosing was given to counteract any E4-induced stimulatory effect on the endometrium. None of the subjects experienced a withdrawal bleeding after discontinuation of the progestin, suggesting no endometrial stimulation after single dose of E4 in the tested doses.
Study PR3054: All but two subjects reported withdrawal bleedings at the end of the study after intake of lynestrenol, indicating significant endometrial stimulation occurred with all doses tested.

Haemostasis parameters evaluated in the Phase 2 and Phase 3 studies included prothrombin fragment 1 + 2, antithrombin, endogenous thrombin potential based activated protein C sensitivity ratio (APCsr ETP), Protein-C, free Protein-S, and Factor VIII. Haemostasis data after 13 weeks of treatment are limited, as these were not assessed in C301 ESP.

Overall, there was no clinically relevant impact of E4 treatment on the haemostasis parameters, although some statistically significant changes from baseline between both E4 15 mg and E4 20 mg arms were observed: antithrombin III activity (decrease), APCsr ETP (increase), and free protein S (decrease). These hemostasis parameters were still within normal ranges and APCsr ETP, the global marker of coagulation, was weakly impacted by E4 treatment.

PD interactions

Dedicated pharmacodynamic interaction studies with commonly used cardiovascular or metabolic medicines were not conducted. Limited Phase 3 data did not indicate clinically relevant effects on blood pressure, glucose or lipids versus placebo.

E-R analysis

No formal E-R analysis was performed. The Applicant provided a justification for not conducting a formal E–R analysis, highlighting that LH, FSH, and E2 were not measured in parallel with E4 PK in the clinical pharmacology studies with the E4 product. The phase 3 descriptive summaries showed E2 medians broadly similar across arms over time. E2 medians remained stable and similar through Week 13. In addition, performing an E–R analysis for efficacy, in which VMS change and responder status are related to E4 exposure with adjustment for baseline VMS, age, BMI, and site may not be fully reliable due to several problems: timing mismatch (VMS at Weeks 4/12 vs single E4 concentrations at Weeks 5/13), simple linear regressions without covariates, and reliance on single-point concentrations from compliance samples. This was accepted, since it did not raise any dose-selection concerns.

5.2.5.2. Conclusions

The clinical pharmacokinetics was assessed in healthy female premenopausal and postmenopausal volunteers and in female patients. Overall, the pharmacokinetics of estetrol is substantiated adequately.

Overall, the estrogenic effects of E4 in PD and biological parameters are comparable with those

observed for E2-valerate when administered to postmenopausal women. However, in comparison to 2 mg E2-valerate, the effects of 10 mg E4 and higher are more pronounced for almost all endpoints tested. Considering all endocrinologic PD endpoints, the effect of E2-valarate was in between the effect of the 2 mg and 10 mg E4 groups. The largest differences in comparison to E2-valerate were noted for increase in endometrial thickness, which was considerable and relevant in the discussion of endometrial safety and tolerability of E4 in the doses selected for marketing (15 and 20 mg).

Slight dose-related changes were noted in SHMBG, lipids, bone markers, and haemostatic parameters of which the latter suggested slight effects on the anticoagulant parameters as is known to occur with estrogens.

5.3. Clinical efficacy

5.3.1. Dose response study

MIT-Do0001-C201 (E4 RELIEF study) - A phase 2 multicentre dose-finding, randomised, double-blind, placebo-controlled study to select the daily oral dose of estetrol (E4) for the treatment of vasomotor symptoms in post-menopausal women.

Dose selection

Initial clinical safety phase 1 dose-finding studies were performed using E4 0.1 mg to E4 100 mg (single dose) (PR3050, FIH) or E4 2 mg to E4 40 mg (MD, 28 days) (PR3054). The PR3054 study concluded that E4 up to a dose of E4 40 mg/day given for 28 days was well tolerated.

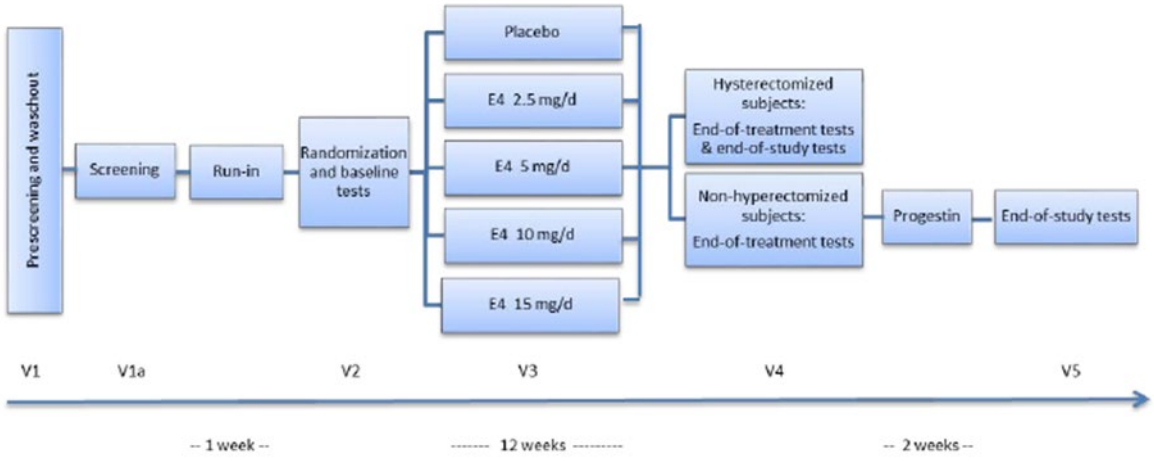
With regard to efficacy and safety, in the multiple dosing study (PR3054), four doses of E4 (2, 10, 20 and 40 mg) were administered once daily (QD) for 28 days in groups of 10 subjects each and compared to 2 mg estradiol valerate (E2V)(*Pantarhei Bioscience B.V., Visser and Coelingh Bennink, 2009*) . The number of hot flushes by day for the 2 mg and 10 mg E4 groups decreased over time, i.e. showed positive effects on VMS symptoms. The endometrial thickness increased both in the 2 mg and 10 mg dose group; however, at follow-up 1 month after EOT, the endometrial thickness had returned to baseline values in all subjects after progestin administration. Based on this data, the sponsor has defined four dosages to be evaluated from 2.5 mg up to 15 mg.

Study design C201

This is a phase 2, multi-centre, randomised, double-blind, placebo-controlled dose-finding study to select the minimum daily dose of E4 for the treatment of VMS in post-menopausal hysterectomised (H) or non-hysterectomised (NH) women. The study was performed in Belgium, Czech Republic, Great Britain, Ireland, Poland, and the Netherlands.

Eligible women were randomized 1:1:1:1:1 to receive once daily treatment with E4 2.5, 5, 10, or 15 mg or placebo for 12 weeks. After completion of the E4/placebo treatment, all NH women received treatment for endometrial protection with dydrogesterone 10 mg once daily for 14 days. In addition, if abnormal uterine bleeding occurred or a bilayer endometrial thickness ≥15 mm was detected during the E4/placebo treatment, an endometrial biopsy was performed and the subject received dydrogesterone 10 mg once daily (14 days on, 14 days off regimen) in addition to the E4/placebo treatment until the end of Week 11. The study design is presented in the figure below.

Figure 6: Study design (Protocol MIT-Do0001-C201)



V1, V2, V3 …; Visit 1, Visit 2, Visit 3 ….
Prescreening and washout (if required): within 6 weeks before screening visit (V1a); screening visit (V1a) within 4 weeks before randomization; run-in period: 7 days before randomization (V2); treatment period: starting on the day after V2 and ending with the end-of-treatment visit (V4); progestin treatment (nonhysterectomized subjects only): 14 days; end-of-study visit: 3 to 4 weeks after end of treatment.

Primary efficacy objective:

To define the minimum effective dose of orally taken Estetrol (E4) by evaluating changes in frequency and in severity of moderate to severe vasomotor symptoms (VMS).

Secondary efficacy objectives:

To evaluate the effects of different doses of E4 on genitourinary syndrome of menopause (GSM) (also referred to as vulvovaginal atrophy), vaginal maturation index, vaginal pH, Menopause Rating Scale scores, lipid and glucose metabolism, and haemostatic and bone laboratory variables.

The four co-primary efficacy endpoints were:

- Change in weekly frequency of moderate to severe VMS from baseline to Week 4.
- Change in weekly frequency of moderate to severe VMS from baseline to Week 12.
- Change in severity of moderate to severe VMS from baseline to Week 4.
- Change in severity of moderate to severe VMS from baseline to Week 12.

The secondary efficacy endpoints were:

- Weekly weighted score
- Change from baseline to week 13 in the VVA symptoms (VVA subject self-assessment
- Change from baseline to week 5 and week 13 in Menopause Rating Scale (MRS)
- Change from baseline to week 13 in Vaginal pH
- Change from baseline to week 13 in Vaginal Maturation Index
- Change from baseline to week 13 in lipid and glucose metabolism markers, haemostatic variables and bone variables

Study population

The main inclusion criteria were healthy non-hysterectomized (NH) and hysterectomized postmenopausal women aged 40-65 years inclusive, with a BMI of 18-35 kg/m^2 inclusive, presenting ≥7 moderate to severe VMS per day or ≥50 moderate to severe VMS per week. In- and exclusion criteria were consistent with other HRT studies.

Results:

Recruitment

The study was conducted between 12 May 2016 (first subject enrolled) and 22 January 2018 (last subject, last visit) at 31 sites in the EU and 4 sites in the UK.

Study participants

In total, 257 eligible postmenopausal women were randomized and received at least one dose of E4 2.5, 5, 10, or 15 mg or placebo for 12 weeks. The majority of subjects (225 [87.5%]) were NH. In the ITT set, 10.9% of subjects required a washout period before screening due to prior use of estrogen- or progestin-containing drugs or nonhormonal treatment to reduce VMS.

In total, 200 of 260 (77%) randomized subjects completed the study. Discontinuation rate was the lowest in subjects treated with E4 15 mg (16.3%) compared with the other treatment groups (range: 17.0%-28.3%), including placebo (25.5%). The most common reasons for premature discontinuation were withdrawal by subject (9.3%) and protocol deviation (5.8%).

In addition to E4 or placebo, 14 subjects in total who experienced vaginal bleeding or had bilayer endometrial thickness ≥15 mm received progestin during the E4/placebo treatment period. These were 0, 3, 5, 2, and 4 subjects in the 2.5 mg, 5 mg, 10 mg, 15 mg, and placebo group, respectively. Hysterectomized subjects also received progestin after the end of E4/placebo treatment. Treatment compliance as assessed based on capsule counts was between 94.9% for placebo and 96.8% for E4 2.5 mg (mean percentages) with a median of 100% for all treatment groups.

Primary efficacy endpoints

Weekly frequency of moderate to severe VMS (week 4 and 12)

Primary efficacy analysis

At Week 4 of treatment with E4 15 mg, the reduction from baseline in the weekly frequency of moderate to severe VMS was statistically significant ($p<0.05$) compared to placebo. Using an ANCOVA model, the difference in LS means was: -12.24 (95% CI: -23.77, -0.71; p=0.0335). The reduction was not statistically significant at Week 12 (-8.91 [95% CI: -20.44, 2.63], p=0.1800). There were no statistically significant differences in the pairwise comparisons for the E4 2.5, 5 and 10 mg doses compared to placebo.

Table 15: Mean Change in the Weekly Frequency of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set; ANCOVA) – MIT-Do0001-C201

Visit	Statistics	E4 2.5 mg (N = 53)	E4 5 mg (N = 47)	E4 10 mg (N = 53)	E4 15 mg (N = 49)	Placebo (N = 55)
Week 4						
	n	50	45	51	46	50
Model-Adjusted Change from Baseline	LS mean	-32.50	-27.89	-36.68	-45.24	-33.00
	(95% CI)	(-39.19, -25.82)	(-34.66, -21.12)	(-43.06, -30.31)	(-52.01, -38.47)	(-39.45, -26.55)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	0.49	5.11	-3.68	-12.24	
	(95% CI)	(-11.00, 11.99)	(-6.46, 16.68)	(-14.91, 7.54)	(-23.77, -0.71)	
	p-value between vs. Placebo	0.9999	0.6540	0.8385	**0.0335**	
Week 12						
	n	43	38	37	39	40
Model-Adjusted Change from Baseline	LS mean	-43.21	-40.90	-50.25	-57.97	-49.06
	95% CI	(-49.82, -36.60)	(-47.65, -34.14)	(-57.20, -43.30)	(-64.65, -51.29)	(-55.65, -42.47)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	5.85	8.17	-1.19	-8.91	
	95% CI	(-5.72, 17.42)	(-3.48, 19.81)	(-12.92, 10.54)	(-20.44, 2.63)	
	p-value between vs. Placebo	0.5386	0.2518	0.9974	0.1800	

CI = Confidence Interval, E4 2.5 mg = estetrol monohydrate 2.5 mg (equivalent to estetrol 2.36 mg), E4 5 mg = estetrol monohydrate 5 mg (equivalent to estetrol 4.72 mg), E4 10 mg = estetrol monohydrate 10 mg (equivalent to estetrol 9.44 mg), E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), ITT = intent-to-treat, LS = least squares, ANCOVA = Analysis of Covariance, N = number of subjects in the statistical analysis set, n = number of observations per study group, VMS = vasomotor symptoms, vs = versus.

Statistically significant values ($p<0.05$) are written in bold.

ANCOVA with fixed factors treatment and pooled site and covariate baseline value, including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).

Sensitivity analyses on the 2 coprimary efficacy endpoints of VMS frequency led to similar results.

Mean severity of moderate to severe VMS (week 4 and 12)

At Weeks 4 and Week 12, E4 2.5, 5, 10, and 15 mg once daily did not lead to statistically significant reduction of the severity of moderate to severe VMS from baseline compared to placebo.

Table 16: Mean Change in the Mean Severity Score of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set; ANCOVA) – MIT-Do0001-C201

Visit	Statistics	E4 2.5 mg (N = 53)	E4 5 mg (N = 47)	E4 10 mg (N = 53)	E4 15 mg (N = 49)	Placebo (N = 55)
Week 4						
	n	47	43	45	40	49
Model-Adjusted Change from Baseline	LS mean (95% CI)	-0.11 (-0.17, -0.04)	-0.11 (-0.18, -0.04)	-0.18 (-0.25, -0.12)	-0.21 (-0.28, -0.14)	-0.11 (-0.17, -0.04)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means (95% CI)	0.00 (-0.12, 0.12)	-0.01 (-0.13, 0.11)	-0.08 (-0.20, 0.04)	-0.10 (-0.23, 0.02)	
	p-value between vs. Placebo	1.0000	0.9998	0.3008	0.1156	
Week 12						
	n	37	34	29	24	31
Model-Adjusted Change from Baseline	LS mean	-0.16	-0.16	-0.22	-0.28	-0.26
	95% CI	(-0.24, -0.08)	(-0.24, -0.07)	(-0.31, -0.12)	(-0.39, -0.18)	(-0.35, -0.17)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	0.10	0.10	0.04	-0.03	
	95% CI	(-0.05, 0.25)	(-0.05, 0.26)	(-0.11, 0.20)	(-0.19, 0.14)	
	p-value between vs. Placebo	0.2840	0.2774	0.9021	0.9861	

CI = Confidence Interval, E4 2.5 mg = estetrol monohydrate 2.5 mg (equivalent to estetrol 2.36 mg), E4 5 mg = estetrol monohydrate 5 mg (equivalent to estetrol 4.72 mg), E4 10 mg = estetrol monohydrate 10 mg (equivalent to estetrol 9.44 mg), E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), ITT = intent-to-treat, LS = least squares, ANCOVA = Analysis of Covariance, N = number of subjects in the statistical analysis set, n = number of observations per study group, VMS = vasomotor symptoms, vs = versus.

Statistically significant values ($p<0.05$) are written in bold. ANCOVA with fixed factors treatment and pooled site and covariate baseline value, including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).

Sensitivity analyses on the 2 coprimary efficacy endpoints of VMS severity led to similar results.

Secondary efficacy endpoints

The secondary efficacy endpoints on weekly weighted score of VMS, Menopause Rating Scale (MRS), and VVA symptoms are considered explorative (non-alpha controlled). The weekly weighted score of moderate to severe VMS markedly decreased in all treatment groups – including the placebo group – as of Week 1. E4 10 mg and E4 15 mg achieved a larger decrease from baseline than all other treatment groups from Week 3 and Week 2 onwards, respectively. With regards to vulvovaginal atrophy (VVA) symptoms, the results are suggestive of a positive effect of E4. The favourable effects were most pronounced at the E4 15 mg dosage.

Results on PD endpoints of vaginal pH, and vaginal maturation index, lipids, HbA1C, SHBG, haemostatic- and bone turnover parameters are discussed in section 5.2.3.2.

In conclusion, the highest E4 dose tested in this study, E4 15 mg, showed discernible effects on the frequency of moderate to severe VMS and on other efficacy variables. Based upon the results obtained in this study, E4 15 mg was considered as the potential minimum effective dose (MED), to be confirmed in phase 3 studies. Since statistical significance was not obtained for all coprimary endpoints with this dose, a higher dose of E4 20 mg would also be tested in the phase 3 studies.

5.3.2. Main studies

5.3.2.1. MIT-Do001-C301 and MIT-Do001-C302

5.3.2.1.1. Study titles

- **MIT-Do001-C301**: A randomized double-blind placebo-controlled phase 3 trial to evaluate the efficacy and safety of estetrol for the treatment of moderate to severe vasomotor symptoms in postmenopausal women (E4Comfort Study I)
- **MIT-Do001-C302**: A randomized double-blind placebo-controlled phase 3 trial to evaluate the efficacy and safety of estetrol for the treatment of moderate to severe vasomotor symptoms in postmenopausal women (E4Comfort Study II)

5.3.2.1.2. Study designs

Clinical efficacy of E4 15 and 20 mg in the proposed indication is based on the 12 Week placebo-controlled two pivotal phase 3 studies, i.e. C301 ESP and C302 ESP. Both C301 and C302 consist of an efficacy study part (ESP) and a Safety Study Part (SSP):

- An **ESP**, which had a randomized, double-blind, placebo-controlled design. The ESP was conducted in H and NH postmenopausal subjects aged 40 to 65 years, inclusive, not using estrogen- or progestogen-containing drug(s) at Screening, and presenting ≥7 moderate to severe bothersome VMS per day or ≥50 moderate to severe bothersome VMS per week in the last 7 consecutive days of the screening period.
    - In MIT-Do001-C301 ESP, the efficacy of daily oral administration of E4 15 mg or E4 20 mg administered for 12 weeks was evaluated in comparison to placebo.
    - In MIT-Do001-C302 ESP, the efficacy of daily oral administration of E4 15 mg or E4 20 mg for 12 weeks was evaluated versus placebo, and treatment was continued up to 53 weeks to assess secondary efficacy endpoints and the safety of long-term exposure to E4.
- An Endometrial and General Safety Study Part (MIT-Do001-C301 SSP) or Safety Study Part (MIT-Do001-C302 SSP) (abbreviated '**SSP**' in both instances), which had an open label design. The SSPs were conducted in subjects experiencing ≥1 moderate to severe VMS per week.
    - MIT-Do001-C301 SSP evaluated the endometrial and general safety of E4 20 mg plus continuous daily dose of P4 100 mg for up to 53 weeks.
    - MIT-Do001-C302 SSP evaluated the general safety and secondary efficacy of E4 during treatment with E4 20 mg for up to 53 weeks.

The SSPs were not controlled and not designed for efficacy assessment and therefore, are not considered for the analysis of clinical efficacy. The safety results of these studies are discussed in Section 5.4.

12-week placebo-controlled Efficacy study parts (ESPs) of C301 and 302

The ESP of both C301 and 302 has a randomized, double-blinded placebo-controlled design and evaluates the efficacy (and safety) of estetrol (E4) in hysterectomized and non-hysterectomized postmenopausal women. Subjects will be randomly allocated (1:1:1) to one of the 3 arms and will receive E4 15 mg (arm 1), E4 20 mg (arm 2), or Placebo (arm 3). During the first 12 weeks of the ESPs, the efficacy of E4 on VMS versus placebo will be evaluated. However, in the placebo-controlled part of study C301 subjects were treated for a total duration of up 13 weeks, while in study C302 placebo-controlled treatment proceeded for a total duration of up to 53 weeks.

For endometrial protection, after completion of the E4/placebo treatment, all non-hysterectomized subjects received treatment with 200 mg progesterone (P4) once daily for 14 consecutive days.

Safety study parts (SSPs) of C301 and C302– see Safety section 6.4

SSP in C301 (C301 SSP):

SSP in C301 was the Endometrial and General Safety Study part of this study, which has an uncontrolled design and evaluates the general safety, and endometrial safety (see section 4.5) of E4 in non-hysterectomized postmenopausal women. All subjects received E4 20 mg in combination with 100 mg P4 continuously for up to 53 weeks (**arm 4**).

SSP in C302 (C302 SSP):

SSP in C302 was conducted in hysterectomized and non-hysterectomized postmenopausal women and has an uncontrolled design. This SSP evaluated the general safety, and endometrial safety. Secondary efficacy endpoints were evaluated for up to 53 weeks treatment with E4 20 mg (**arm 4**).

Figure 7: Study schema MIT-Do001-C301

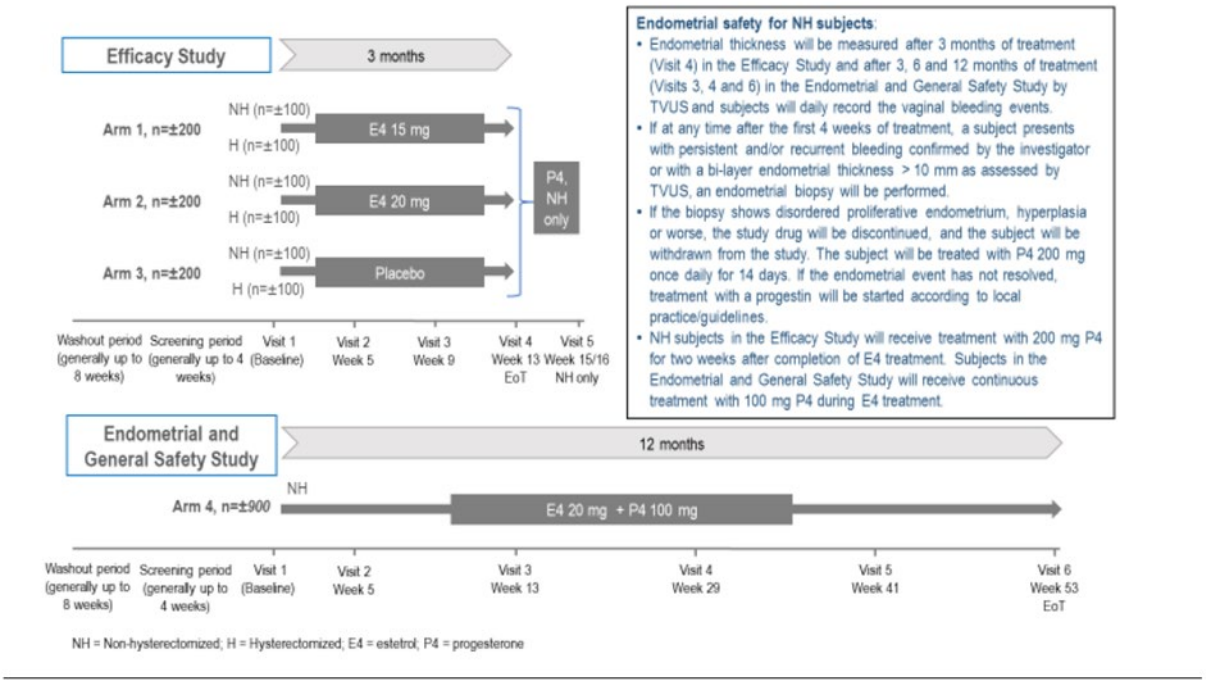
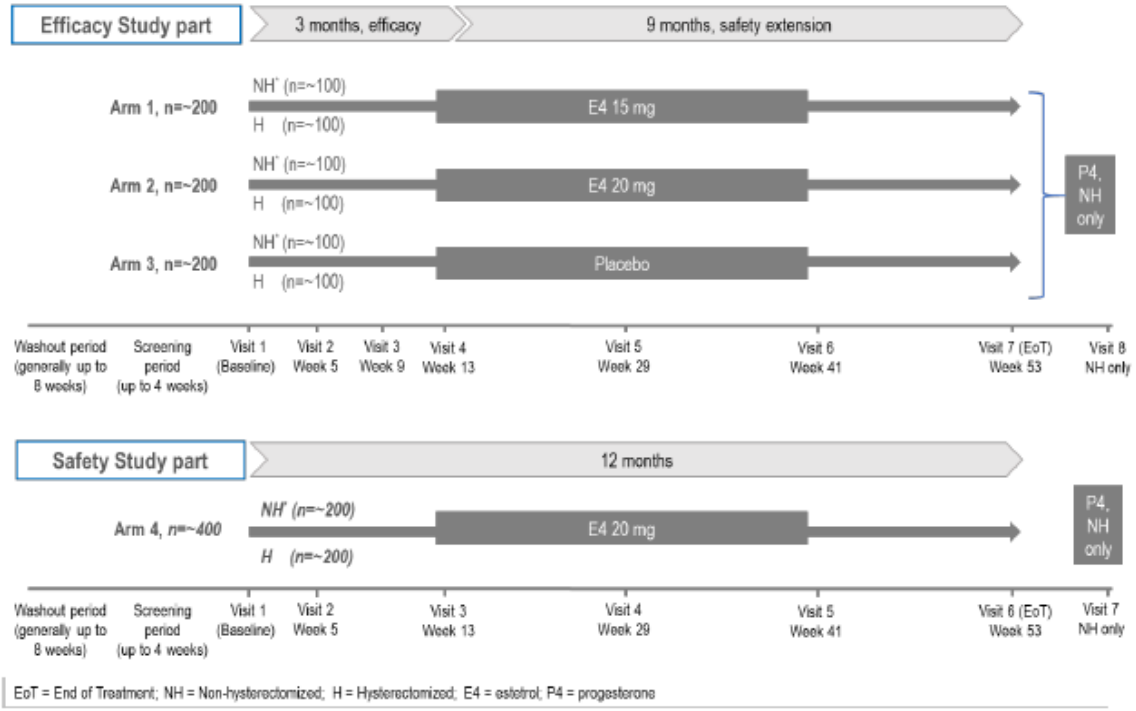


Figure 8: Study schema MIT-Do001-C302



EoT = End of Treatment; NH = Non-hysterectomized; H = Hysterectomized; E4 = estetrol; P4 = progesterone

*For non-hysterectomized (NH) subjects endometrial thickness will be measured after 3, 6, 9 and 12 months (Visits 4, 5, 6 and 7 of the Efficacy Study part and Visits 3, 4, 5 and 6 of the Safety Study part respectively) of treatment by TVUS and subjects will record daily the vaginal bleeding events. If after the first 4 weeks of treatment a subject presents with a bi-layer endometrial thickness >10 mm as assessed by TVUS or presents with persistent and/or recurrent bleeding, confirmed by the investigator, an endometrial biopsy will be performed. If the biopsy shows disordered proliferative endometrium, hyperplasia or worse, the study drug will be discontinued, and the subject will be withdrawn from the study. The subject will be treated with P4 200 mg once daily for 14 days. If the endometrial event has not resolved, treatment with a progestin will be started according to local practice/guidelines. All NH subjects will receive treatment with 200 mg P4 for two weeks after completion of E4 treatment.

Treatment

In both the ESPs (C301 and C302), eligible women were randomized 1:1:1 to receive once daily oral treatment with E4 15 mg, E4 20 mg or placebo **for 12−13 weeks** (C301) or **for 12 months** (C302).

After completion of the E4/placebo treatment, all NH women received treatment for endometrial protection with P4 200 mg once daily for 14 days. In addition, if at any time after the first 4 weeks of treatment a subject presented with persistent and/or recurrent bleeding, or with a bilayer endometrial thickness >10 mm as assessed by TVUS, an endometrial biopsy was performed. If the biopsy showed disordered proliferative endometrium, hyperplasia or worse, the study drug was discontinued, and the subject was withdrawn from the study and treated with P4 200 mg once daily for 14 days.

Subjects were instructed to take the trial medication at approximately the same time each day. If P4 was co-administered, this was preferably taken at bedtime, within 2 hours of food intake. For compliance reason, it was preferable to take E4 together with P4. The E4 and placebo trial medication were supplied as tablets. The progesterone treatment (P4) was supplied as capsules.

Concomitant and rescue therapies

Treatment for VMS symptoms, such as other estrogen and/or progestin therapies and the use of non-hormonal medication were not allowed. A washout period was necessary as described. Regarding lipid lowering drugs the subject should have been on a stable dose for at least 1 month before the start of screening. Thyroid treatments were allowed as long as the subject has normal TSH levels or low or high TSH levels with normal free T4 at screening. Subjects with mild to moderate hypertension who

were controlled on a stable antihypertension regimen were enrolled if they met the blood pressure criteria at screening, excluding subjects using methyldopa or clonidine.

Randomisation

Eligible women were randomized 1:1:1 to receive once daily treatment with E4 15 mg, E4 20 mg or placebo. Randomisation was computer-generated.

Blinding

Study centre personnel, study subjects, contract research organization (CRO) and Sponsor were blinded to group assignment. Active study drug was supplied as tablets indistinguishable from placebo.

Patient population

Study *C301 ESP* was conducted in the EU, UK, Russia, Argentina, Brazil, the US and Canada, whereas Study *C302 ESP* was conducted in the US and Canada.

The study population of C301 ESP and C302 ESP consisted of postmenopausal women aged 40−65 years (inclusive), hysterectomized (H) or non-hysterectomized (NH), with ≥7 moderate to severe bothersome VMS per day or ≥50 moderate to severe bothersome VMS per week. The eligibility criteria were similar between both phase 3 studies. Those listed below are from MIT-Do001-C301 ESP CSR and any relevant differences compared to the eligibility criteria of study MIT-Do001-C302 were clearly indicated.

Main **inclusion criteria**:

1. Females, ≥40 up to ≤65 years of age at randomization/treatment allocation.

2. For hysterectomized subjects: documented hysterectomy must have occurred ≥ 6 weeks prior to screening. Hysterectomy can be total or subtotal (i.e., cervix was not removed).

3. For NH subjects: uterus with bi-layer endometrial thickness ≤ 4 mm on TVUS.

4. For NH subjects: an evaluable endometrial biopsy taken at screening with no abnormal results, i.e., hyperplasia, carcinoma, or disordered proliferative endometrium findings. The screening biopsy should have sufficient endometrial tissue for diagnosis.

5. Seeking treatment for relief of VMS associated with menopause:
   - For ESP: ≥7 moderate to severe bothersome VMS per day or at least 50 moderate to severe bothersome VMS per week in the last 7 consecutive days during the Screening period
     To note, eligible women for the SSP had to had at least 1 moderate to severe VMS per week

6. BMI ≥18.0 kg/m² to ≤38.0 kg/m².

7. A mammogram with no sign of significant disease performed at screening or <9 months prior to screening.

8. Postmenopausal status defined as any of the following:

   - For NH subjects:
     - ≥12 months of spontaneous amenorrhea with serum follicle stimulating hormone (FSH) >40 mIU/mL (value obtained after washout of estrogen/progestin containing drugs);

- or ≥6 months of spontaneous amenorrhea with serum FSH >40 mIU/mL and E2 <20 pg/mL (<73.4 pmol/L, value obtained after washout of estrogen/progestin containing drugs)
- or at least 6 weeks postsurgical bilateral oophorectomy.

- For hysterectomized subjects:
  - serum FSH >40 mIU/mL and E2 <20 pg/mL (<73.4 pmol/L, values obtained after washout of estrogen/progestin containing drug);
  - or at least 6 weeks postsurgical bilateral oophorectomy.

Main **exclusion criteria**:

1. History of malignancy, with exception of basal cell or squamous cell carcinoma of the skin if diagnosed more than 1 year prior to the screening visit.

2. Any clinically significant findings at the breast examination and/or on mammography suspicious of breast malignancy requiring additional clinical testing to rule out breast cancer (however, simple cysts confirmed by ultrasound are allowed).

3. PAP test with atypical squamous cells undetermined significance (ASC-US) or higher (low-grade squamous intraepithelial lesion [LSIL], atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion [HSIL] [ASC-H], HSIL, dysplastic or malignant cells) in sub-totally hysterectomized and NH subjects. Note: ASC-US is allowed if a reflex human papilloma virus (HPV) testing is performed and is negative for high-risk oncogene HPV subtypes 16 and 18.

4. For NH subjects:
   - History or presence of uterine cancer, endometrial hyperplasia, or disordered proliferative endometrium;
   - Presence of endometrial polyps;
   - Undiagnosed vaginal bleeding or undiagnosed abnormal uterine bleeding;
   - Endometrial ablation;
   - Any uterine/endometrial abnormality that in the judgment of the investigator contraindicates the use of estrogen and/or progestin therapy. This includes presence or history of adenomyosis or significant myoma.

5. Systolic BP higher than 130 mmHg, diastolic blood pressure higher than 80 mmHg during screening.

6. History of venous or arterial thromboembolic disease, or first-degree family history of venous thromboembolism event.

7. History of known acquired or congenital coagulopathy or abnormal coagulation factors, including known thrombophilia's.

8. Laboratory values of fasting glucose above 125 mg/dL (>6.94 mmol/L) and/or glycated haemoglobin above 7%.

9. Dyslipoproteinaemia (LDL >190 mg/dL [>4.91 mmol/L] and/or triglycerides >300 mg/dL [>3.39 mmol/L]).

10. Subjects smoking >15 cigarettes per day.

11. Presence or history of gallbladder disease, unless cholecystectomy has been performed.

12. Systemic lupus erythematosus.

13. Any malabsorption disorders including gastric bypass surgery.

14. History of acute liver disease in the preceding 12 months before the start of screening or presence or history of chronic or severe liver disease [alanine transaminase (ALT) or aspartate transaminase (AST) >2x upper limit of normal (ULN), bilirubin >1.5 ULN], or liver tumours.

15. Chronic or current acute renal impairment (estimated glomerular filtration rate <60 ml/min).

16. Porphyria.

17. Diagnosis or treatment of major psychiatric disorder (e.g., schizophrenia, bipolar disorder, etc.) in the judgement of the Investigator.

18. Use of estrogen/progestin containing drug(s).

19. Use of androgen/dehydroepiandrosterone containing drugs.

20. Use of phytoestrogens or black cohosh for the treatment of VMS.

21. For the women participating in the Efficacy Study part: use of prescription or over-the-counter products used for the treatment of VMS, e.g., anti-depressants: paroxetine, escitalopram, methyldopa, opioid and clonidine up to 4 weeks before the start of screening, and venlafaxine and desvenlafaxine up to 3 months before the start of screening, and not willing to stop these during their participation in the trial.

22. Inadequately treated hyperthyroidism with abnormal TSH and free T4 at screening. Subjects with low or high TSH are allowed if free T4 at screening is within normal range.

23. History of alcohol or substance abuse (including marijuana, even if legally allowed) or dependence in the previous 12 months before the start of screening as determined by the Investigator, based on reported observations.

24. For NH subjects (to be included in the USA and Canada in C301): history or presence of allergy to peanuts.

5.3.2.1.3. Objectives and estimands

Primary objective

The primary objective of C301 ESP and C302 ESP was to measure the effect of treatment with E4 15 mg or E4 20 mg compared to placebo on the frequency and severity of moderate to severe VMS in postmenopausal women at 4 and 12 weeks.

The primary endpoint consisted of 4 co-primary efficacy endpoints:

- Mean change in weekly frequency of moderate to severe VMS from Baseline to Week 4
- Mean change in weekly frequency of moderate to severe VMS from Baseline to Week 12
- Mean change in severity of moderate to severe VMS from Baseline to Week 4
- Mean change in severity of moderate to severe VMS from Baseline to Week 12

The hypotheses of the coprimary endpoints were defined as:

All statistical tests were to be two-sided with a significance level of $\alpha = 0.05$, unless specified otherwise and were performed using SAS Grid version 9.4 or higher. Confidence intervals (CIs) were presented as 2-sided 95% CIs unless specified differently in specific analysis.

The two dose groups of E4 were compared to placebo regarding the four co-primary endpoints. The null hypothesis was rejected if there was statistical evidence of significantly lower average change from baseline in the primary efficacy endpoint in dose groups of E4 compared to placebo. This was checked based on estimates of difference between treatment effects (difference to placebo) and corresponding 95 % CIs based on Dunnett's test.

Table 17: Definitions used in the studies

Term	Definition
Clinical severity of VMS	Subject rating of VMS clinical severity as recorded in the diary. The following scores were used: • None (0) = No VMS symptoms. • Mild (1) = Sensation of heat without sweating. • Moderate (2) = Sensation of heat with sweating; able to continue activity. • Severe (3) = Sensation of heat with sweating; causing cessation of activity.
Daily Severity Score of VMS at Baseline for the phase 3 studies	[((2 x number of moderate VMS) + (3 x number of severe VMS))/(total number of moderate + severe VMS)], if at least one moderate to severe VMS was recorded during the day. In case of documented absence of moderate to severe VMS during the day, the daily severity was set to zero.
Daily Severity Score of VMS at Week X by the EMA scoring method for the phase 3 studies	Same as the daily severity score of VMS at Baseline (for the phase 3 studies).
Mean (weekly) severity score of VMS at Baseline	Arithmetic mean of the daily severity score values of VMS (moderate or severe) observed from Day -7 to Day -1.
Mean (weekly) severity score of VMS at Week X by the EMA scoring method	Arithmetic mean of the daily severity score values of moderate to severe VMS observed during the Week X.
Weekly frequency of moderate to severe VMS at Baseline	Sum of all recorded moderate to severe VMS experienced between Day -7 and Day -1.
Weekly frequency of moderate to severe VMS at Week X	Sum of all recorded moderate to severe VMS experienced during the Week X.

Estimand for the primary objective

Table 18: Estimand for primary objective

Population	Post-menopausal subjects with at least 7 moderate to severe bothersome menopausal related VMS per day or at least 50 moderate to severe bothersome VMS per week in the last 7 consecutive days during the screening period, under any treatment assignment.
Treatment conditions	Assignment to E4 arms (E4 15 mg or E4 20 mg) compared to assignment to Placebo arm, regardless of study discontinuation, of lack or compliance to E4 study medication intake, or of concomitant use of E2-containing medication.
Endpoint (variable)	4 co-primary endpoints: • Mean change in weekly frequency of moderate to severe VMS from Baseline to Week 4 • Mean change in weekly frequency of moderate to severe VMS from

	Baseline to Week 12 • Mean change in severity of moderate to severe VMS from Baseline to Week 4 • Mean change in severity of moderate to severe VMS from Baseline to Week 12
Population-level summary	Least squares mean difference between E4 arms (E4 15 mg and E4 20 mg) and placebo at Week 4 and Week 12
ICEs and strategy to handle them	
Study discontinuation	Hypothetical strategy, considering the MMRM missing-at-random (MAR) handling of missing data without any formal imputation
Lack of compliance with E4 study medications	Treatment policy, where data is analyzed regardless of whether or not the intercurrent event occurs
Concomitant use of E2-containing medications	Treatment policy, where data is analyzed regardless of whether or not the intercurrent event occurs

Statistical methods for estimation and sensitivity analysis on primary estimand

Additional sensitivity analyses were implemented to assess the robustness of the results.

- A comparison between treatment arms of the change from baseline at week 1 to week 4/week 12 in weekly frequency of moderate to severe VMS was made by using MMRM. The MMRM included treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status, status*treatment and pooled trial centers as fixed effects and baseline value of weekly frequency of moderate to severe VMS as a covariate. If the pooled center effect included in the model was significant, treatment by center interaction was included in the model. Treatment effects were assessed at week 4 and 12. LS Mean estimates and 95% CIs for the respective treatment effects (difference to placebo) were presented at week 4 and 12 along with the corresponding p-value. The analysis was done on the mITT and PP populations.
- A comparison between treatment arms of the change from baseline at week 1 to week 4/week 12 in weekly frequency of moderate to severe VMS was made by using an ANCOVA model with treatment (E4 15 mg, E4 20 mg, Placebo), status (hysterectomized/non hysterectomized), status*treatment and pooled trial centers as fixed effects and the baseline measurement as a covariate. This analysis was done based on ITT, mITT and PP Population. Missing data was imputed using LOCF method. LS Mean estimates and 95% CIs for the respective treatment effects (difference to placebo) were presented at week 4 and 12 along with the corresponding p-value. Line plot for the change from baseline of the weekly frequency of moderate to severe VMS on week 4 and week 12 with the result of the ANCOVA was provided.

Secondary objectives

- To measure effect of treatment with E4 15 mg or E4 20 mg compared to placebo on frequency and severity of mild, moderate and severe VMS in postmenopausal women weekly up to 12 weeks
- To measure clinical meaningfulness of E4 15 mg or E4 20 mg compared to placebo on the reduction of VMS at weeks 4 and 12
- To evaluate effect of treatment with E4 15 mg or E4 20 mg compared to placebo on symptoms of VVA
- To evaluate effect of treatment with E4 15 mg or E4 20 mg compared to placebo on lipid and glucose metabolism (and hemostasis and bone turnover, in C302 ESP)
- To evaluate effect of treatment with E4 15 mg or E4 20 mg compared to placebo on HRQoL and treatment satisfaction (TS)

Secondary efficacy endpoints (non-alpha controlled)

- change in weekly frequency and severity of moderate to severe VMS from baseline to Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
- change in weekly frequency and severity of mild, moderate and severe VMS from baseline to Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
- % subjects with ≥50% and ≥75% reduction from Baseline in the weekly frequency of moderate to severe VMS at weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12
- % subjects with ≥50% and ≥75% reduction from Baseline in the weekly frequency of mild, moderate and severe VMS at weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12
- % subjects with a CID compared to Baseline in the weekly frequency of moderate to severe VMS after weeks 4 and 12 using the CGI questionnaire
- change from baseline to week 12 in VVA symptoms (subject self-assessment) using VVA questionnaire (only in C301 ESP),
- change from baseline to week 12 in VVA symptom (subject self-assessment) that is initially identified by the subject as being the most bothersome using VVA questionnaire (only in C301 ESP)
- change from baseline to Week 12 in HRQoL using the MENQOL questionnaire (to week 52 in C302 ESP)
- total score in Treatment Satisfaction (TS) after 4 and 12 weeks of treatment using the clinical global impression (CGI) questionnaire (to week 52 in C302 ESP)
- change from baseline to Week 12 in: lipid and glucose metabolism markers (to week 52 in C302 ESP)
- change from baseline to Week 12 and Week 52 in: haemostasis lab variables, SHBG, and bone lab variables (only in C302 ESP)

Table 19: Definitions used in the studies

Term	Definition
Clinical severity of VMS	Subject rating of VMS clinical severity as recorded in the diary. The following scores were used: • None (0) = No VMS symptoms. • Mild (1) = Sensation of heat without sweating. • Moderate (2) = Sensation of heat with sweating; able to continue activity. • Severe (3) = Sensation of heat with sweating; causing cessation of activity.
Daily Severity Score of VMS at Baseline for the phase 3 studies	[((2 x number of moderate VMS) + (3 x number of severe VMS))/(total number of moderate + severe VMS)], if at least one moderate to severe VMS was recorded during the day. In case of documented absence of moderate to severe VMS during the day, the daily severity was set to zero.
Daily Severity Score of VMS at Week X by the EMA scoring method for the phase 3 studies	Same as the daily severity score of VMS at Baseline (for the phase 3 studies).
Mean (weekly) severity score of VMS at Baseline	Arithmetic mean of the daily severity score values of VMS (moderate or severe) observed from Day -7 to Day -1.
Mean (weekly) severity score of VMS at Week X by the EMA scoring method	Arithmetic mean of the daily severity score values of moderate to severe VMS observed during the Week X.
Responders (moderate to severe)	Subjects with ≥50% and, separately, ≥75% reduction in the weekly frequency of moderate to severe VMS from Baseline to week 1 to 12 (for assessment of clinical meaningfulness).
Weekly frequency of moderate to severe VMS at Baseline	Sum of all recorded moderate to severe VMS experienced between Day -7 and Day -1.
Weekly frequency of moderate to severe VMS at Week X	Sum of all recorded moderate to severe VMS experienced during the Week X.
Weekly Weighted Score of VMS (phase 3 studies) at Baseline and at Week X by the EMA scoring method	[(2 x number of moderate VMS) + (3 x number of severe VMS)] if at least one moderate to severe VMS was recorded and zero otherwise.
Clinically Important Difference (CID)	It includes the ratings of much improved and very much improved based on CGI questionnaire.
Clinical Meaningfulness using the CGI questionnaire	- For the assessment of clinical meaningfulness, the CGI responses were grouped into 3 subgroups: clinically meaningful (much or very much improved) corresponding to CIDs, minimally improved corresponding to MCIDs and no change or worse (no change to very much worse).
Minimally Clinically Important Difference (MCID)	- It includes the rating of minimally improved based on CGI questionnaire.
Treatment satisfaction (TS)	Percentage of subjects in each category of the CGI questionnaire (very much improved, much improved, minimally improved, no change, minimally worse, much worse, and very much worse) at week X for a treatment group.

Estimands for the secondary objectives

Not applicable.

Statistical methods for estimation and sensitivity analysis on the secondary estimands

Variables were summarized separately using descriptive statistics based on the ITT Set for each treatment arm by visit. The same MMRM model was used as in primary analysis. The proportion of subjects with 50% and 75% reduction from baseline in the weekly frequency of moderate to severe VMS at weeks 1 to 12 and the proportion of subjects with 50% and 75% reduction from baseline in the weekly frequency of mild to severe VMS at weeks 1 to 12, were provided by number and percentage for each treatment group in ITT Set. Missing data were not imputed. Treatment differences were analysed with the Chi-square test. The two-sided 95% CI between (15 mg E4 versus Placebo, 20 mg E4 versus Placebo) was calculated. Line plot for the proportion of subjects with 50% and 75% reduction from baseline in the weekly frequency of moderate to severe VMS at weeks 1 to 12 was provided.

5.3.2.1.4. Results

Participant flow and numbers analysed

Subject Disposition

Study C301 ESP: The study was conducted between 30 December 2019 (study initiation date) and 10 August 2021 (last subject, last visit) at 151 sites in 14 countries (EU, UK, Russia, Argentina, Brazil, the US and Canada). A total of 3496 subjects were screened of whom 2204 (63.0%) subjects were screen failures.

Study C302 ESP: The study was conducted between 27 September 2019 (study initiation date) and 16 August 2022 (study completion date) at 110 sites in the US and Canada. A total of 3974 subjects were screened of whom 2959 (74.5%) subjects were screen failures.

Subject disposition is summarized in the tables below for the two phase 3 ESPs.

Of note, the discontinuations for both studies correspond to study discontinuations and not treatment discontinuations.

Table 20: Summary of Subject Disposition – MIT-Do001-C301 ESP

	Total			Hysterectomized			Non-Hysterectomized		
Subject Disposition	**E4 15 mg n (%)**	**E4 20 mg n (%)**	**Placebo n (%)**	**E4 15 mg n (%)**	**E4 20 mg n (%)**	**Placebo n (%)**	**E4 15 mg n (%)**	**E4 20 mg n (%)**	**Placebo n (%)**
Randomized	213	214	214	110	110	110	103	104	104
Treated (ITT Set)	213 (100)	213 (99.5)	214 (100)	110 (100)	110 (100)	110 (100)	103 (100)	103 (99.0)	104 (100)
Completed study	166 (77.9)	164 (77.0)	174 (81.3)	94 (85.5)	96 (87.3)	91 (82.7)	72 (69.9)	68 (66.0)	83 (79.8)
Discontinued treatment at week 12	40 (18.8)	43 (20.2)	35 (16.4)	14 (12.7)	11 (10.0)	18 (16.4)	26 (25.2)	32 (31.1)	17 (16.3)
Discontinued study	47 (22.1)	49 (23.0)	40 (18.7)	16 (14.5)	14 (12.7)	19 (17.3)	31 (30.1)	35 (34.0)	21 (20.2)

Treated subjects (ITT Set) are expressed as a percentage of randomized subjects. Subjects who completed the study and subjects who discontinued are expressed as a percentage of treated subjects (ITT Set).

Table 21: Summary of Subject Disposition – MIT-Do001-C302 ESP

	Total			Hysterectomized			Non-Hysterectomized		
Subject Disposition	**E4 15 mg n (%)**	**E4 20 mg n (%)**	**Placebo n (%)**	**E4 15 mg n (%)**	**E4 20 mg n (%)**	**Placebo n (%)**	**E4 15 mg n (%)**	**E4 20 mg n (%)**	**Placebo n (%)**
Randomized	194	194	196	100	100	100	94	94	96
Treated (ITT Set)	192 (99.0)	193 (99.5)	194 (99.0)	99 (99.0)	100 (100)	99 (99.0)	93 (98.9)	93 (98.9)	95 (99.0)
Completed study	96 (50.0)	85 (44.0)	108 (55.7)	62 (62.6)	59 (59.0)	60 (60.6)	34 (36.6)	26 (28.0)	48 (50.5)
Discontinued treatment at week 12	27 (14.1)	30 (15.5)	30 (15.5)	12 (12.1)	12 (12.0)	16 (16.2)	15 (16.1)	18 (19.4)	14 (14.7)
Discontinued study	96 (50.0)	108 (56.0)	86 (44.3)	37 (37.4)	41 (41.0)	39 (39.4)	59 (63.4)	67 (72.0)	47 (49.5)

Treated subjects (ITT Set) are expressed as a percentage of randomized subjects. Subjects who completed the study and subjects who discontinued are expressed as a percentage of treated subjects (ITT Set).

The reasons for discontinuation are summarized in the table below.

Table 22: Reasons for discontinuation (ITT* Set) – MIT-Do001-C301 ESP and MIT-Do001-

C302 ESP

	MIT-Do001-C301 ESP				MIT-Do001-C302 ESP			
	E4 15 mg (N=213)	**E4 20 mg (N=213)**	**Placebo (N=214)**	**Total (N=640)**	**E4 15 mg (N=192)**	**E4 20 mg (N=193)**	**Placebo (N=194)**	**Total (N=579)**
Total Discontinued Subjects, n (%)	47 (22.1)	49 (23.0)	40 (18.7)	136 (21.3)	96 (50.0)	108 (56.0)	86 (44.3)	290 (50.1)
Primary reason for discontinuation, n (%)								
Withdrawal of Consent for Another Reason	11 (5.2)	11 (5.2)	6 (2.8)	28 (4.4)	15 (7.8)	17 (8.8)	21 (10.8)	53 (9.2)
Adverse Event	11 (5.2)	9 (4.2)	4 (1.9)	24 (3.8)	19 (9.9)	25 (13.0)	8 (4.1)	52 (9.0)
Lost to Follow-Up	5 (2.3)	4 (1.9)	4 (1.9)	13 (2.0)	22 (11.5)	25 (13.0)	25 (12.9)	72 (12.4)
Lack of Efficacy	4 (1.9)	3 (1.4)	6 (2.8)	13 (2.0)	1 (0.5)	1 (0.5)	9 (4.6)	11 (1.9)
Other	3 (1.4)	4 (1.9)	5 (2.3)	12 (1.9)	10 (5.2)	6 (3.1)	5 (2.6)	21 (3.6)
Protocol Violation	1 (0.5)	7 (3.3)	3 (1.4)	11 (1.7)	3 (1.6)	5 (2.6)	2 (1.0)	10 (1.7)
Withdrawal of Consent Due to Lack of Efficacy	4 (1.9)	2 (0.9)	3 (1.4)	9 (1.4)	2 (1.0)	2 (1.0)	6 (3.1)	10 (1.7)
Endometrial Biopsy Showing Proliferative Disordered Pattern or worse Requiring Treatment with a Progestin	5 (2.3)	2 (0.9)	0	7 (1.1)	7 (3.6)	9 (4.7)	1 (0.5)	17 (2.9)
Non-Compliance to Trial Protocol: Others	1 (0.5)	2 (0.9)	2 (0.9)	5 (0.8)	2 (1.0)	2 (1.0)	1 (0.5)	5 (0.9)
COVID-19	1 (0.5)	0	3 (1.4)	4 (0.6)	2 (1.0)	2 (1.0)	3 (1.5)	7 (1.2)
Serious Adverse Event	0	3 (1.4)	1 (0.5)	4 (0.6)	7 (3.6)	11 (5.7)	1 (0.5)	19 (3.3)
Non-Compliance to Trial Protocol: VMS Count Compliance	1 (0.5)	1 (0.5)	0	2 (0.3)	0	0	1 (0.5)	1 (0.2)
Non-Compliance to Trial Protocol: Study Drug Intake Compliance	0	1 (0.5)	1 (0.5)	2 (0.3)	1 (0.5)	0	1 (0.5)	2 (0.3)
Study Terminated by Sponsor	0	0	2 (0.9)	2 (0.3)	1 (0.5)	1 (0.5)	1 (0.5)	3 (0.5)
Death	0	0	0	0	1 (0.5)	0	0	1 (0.2)
Endometrial Findings that in the judgement of the investigator Precludes Further Treatment	0	0	0	0	1 (0.5)	1 (0.5)	0	2 (0.3)
Physician Decision	0	0	0	0	2 (1.0)	1 (0.5)	1 (0.5)	4 (0.7)

COVID-19 = coronavirus disease 2019, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, n = number of subjects in category of interest, N = number of subjects, VMS = vasomotor symptoms.

* The percentages were calculated using the number of subjects in the Safety Set as the denominator.

Data analysis sets

Randomized Set: included all subjects randomly allocated to one of the 3 treatment arms in Efficacy Study Part (through Interactive Web Response System).

Safety Analysis Set: included all randomized subjects who received at least one dose of randomized study medication. The Safety Analysis Set was used for all analyses of safety, tolerability, and background characteristics and all analyses on this set were based on treatment received.

Intent-to-treat (ITT) Set: consisted of all randomized subjects who received at least one dose of randomized study medication. The ITT Set was the primary analysis population for the efficacy analyses and all analyses on this set were based on randomized treatment. Based on handling of missing VMS diary information, only subjects who had at least 4 days of VMS diary data for one on-treatment week following initiation of treatment were included in primary analysis.

Modified Intent-to-treat (mITT) Set: consisted of all randomized subjects in the ITT Set excluding subjects presenting at least one of the following features

- At least one post-baseline E4 concentration level below the lower limit of quantification (not applicable to placebo arm).
- At least one post screening E2 concentration level superior to 40 pg/mL.

Per-Protocol (PP) Set: included all subjects in the ITT Set who did not have major protocol deviations. The major protocol deviations were defined at the time of the evaluability assessment between the database soft lock and hard lock before unblinding. The PP Set was used for efficacy sensitivity analyses and all analyses on this set were based on randomized treatment.

Table 23: Summary of analysis sets – MIT-Do001-C301 ESP

	E4 15 mg n (%)	E4 20 mg n (%)	Placebo n (%)	Total n (%)
Randomized	213	214	214	641
Treated (Safety set)	213 (100)	213 (99.5)	214 (100)	640 (99.8)
Treated (ITT set)	213 (100)	213 (99.5)	214 (100)	640 (99.8)
mITT set	155 (72.8)	154 (72.0)	199 (93.0)	508 (79.3)
PP set	111 (52.1)	114 (53.3)	120 (56.1)	345 (53.8)

E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, m = modified, n = number of subjects, PP = per-protocol.
The ITT set is the same as the Safety set.
Note: Percentages (%) were based on the number of subjects in the Randomized Set in each treatment arm or total column.

Table 24: Summary of analysis sets – MIT-Do001-C302 ESP

	E4 15 mg n (%)	E4 20 mg n (%)	Placebo n (%)	Total n (%)
Randomized	194	194	196	584
Treated (Safety set)	192 (99.0)	193 (99.5)	194 (99.0)	579 (99.1)
Treated (ITT set)	192 (99.0)	193 (99.5)	194 (99.0)	579 (99.1)
mITT set	143 (73.7)	134 (69.1)	170 (86.7)	447 (76.5)
PP set	107 (55.2)	128 (66.0)	120 (61.2)	355 (60.8)

E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, m = modified, n = number of subjects, PP = per-protocol
The ITT set is the same as the Safety set.
Note: Percentages (%) were based on the number of subjects in the Randomized Set in each treatment arm or total column.

Treatment compliance

C301 ESP - Overall, a 100.0% (median) treatment compliance was achieved in all 3 treatment arms. The mean (SD) compliance achieved was 97.4% (21.84%) in total and greater than 96% in all 3 treatment arms. Treatment compliance between 80 to 120% was achieved by 177 (83.1%) subjects in the E4 15 mg arm, 182 (85.4%) subjects in the E4 20 mg arm, and 186 (86.9%) subjects in the placebo arm.

C302 ESP - The mean (SD) compliance achieved was 87.1 (27.28%) (median 98.7%) in total and greater than 85% in each of the 3 treatment arms. Overall, 414 (71.5%) subjects achieved a treatment compliance of 80 to 120%; 137 (71.4%) subjects in the E4 15 mg arm, 136 (70.5%) subjects in the E4 20 mg arm, and 141 (72.7%) subjects in the placebo arm.

Deviations from study plan

Protocol amendments

For study C301 5 amendments were made to the original protocol and for study C302 7 amendments were made.

Of importance, the secondary objective and endpoints for frequency and severity of VMS have been updated to further define the evaluation of these endpoints (Amendment 4.1, 5 August 2021 for C301, and Amendment 6.1, 30 July 2021 for C302). Further, several amendments were made after start of the recruitment for both studies, including changes in in- and exclusion criteria.

Major protocol deviations

For Study C301 ESP- Overall, 295 of 640 (46.1%) subjects had at least one major protocol deviation during the study. The percentage of subjects with major protocol deviations were similar between the 3 treatment arms. The most commonly (>10% incidence) reported protocol deviations were related to inclusion/exclusion criteria (96 [15.0%] subjects), issues with ICF (73 [11.4%] subjects), study drug non-compliance (71 [11.1%] subjects), and procedures/tests non-compliance (68 [10.6%] subjects).

Study C302 ESP**-** Overall, 224 of 579 (38.7%) subjects had at least one major protocol deviation during the study. The most commonly reported (> 5% incidence) protocol deviations were related to procedures/tests (69 [11.9%] subjects), inclusion/exclusion criteria (63 [10.9%] subjects), visit schedules (51 [8.8%] subjects), and issues with ICF (50 [8.6%] subjects).

Baseline data

Of note, only results from C301 and C302 **ESPs** will be described in this section. Results from C301 and C302 SSP will be described in the safety part of this assessment report.

Demographic characteristics

In study *C301 ESP*, 89.2% of women were White and 6.3% were Black or African American. 27.0% of women were of Hispanic/Latino ethnicity (20.6%-31.9% across treatment arms). Mean (SD) age was 53.9 (4.87) years and mean (SD) BMI was 27.49 (4.389) kg/m^2.

In study *C302 ESP*, which took place in North America, 70.5% of women were White and 27.5% were Black or African American. 34.4% of women were of Hispanic/Latino ethnicity (30.6%-38.5% across treatment arms). Mean (SD) age was 54.6 (4.88) years and mean (SD) BMI was 28.50 (4.547) kg/m^2.

Baseline characteristics

In study C301 ESP, of the total 640 subjects in the ITT set, 330 (51.6%) were hysterectomized (H) and 310 (48.4%) were non-hysterectomized (NH). Baseline characteristics were generally similar between H and NH subjects except for VMS frequency in the E4 20 mg arm where it was higher in NH subjects (93.21 VMS/week) than in hysterectomized subjects (74.93 VMS/week).

In study C302 ESP, of the total 579 subjects in the ITT set, 298 (51.5%) were hysterectomized and 281 (48.5%) were NH. Baseline characteristics were generally similar between hysterectomized and NH subjects except for VMS frequency.

Intake of drugs containing sex hormones and modulators of genital system, including derivatives of E2 and P4, were reported in 5.8% (37 of 640, C301 ESP) and 6.4% (37 of 579, C302 ESP) subjects before first intake of study drug. The use of non-hormonal medication (prescription medication or over the counter) for the treatment of VMS and estrogen and/or progestin therapies were not allowed, and a washout period was applied.

Table 25: Summary of Key Baseline Characteristics for Efficacy, Overall and by Hysterectomy Status – MIT-Do001-C301 ESP and MIT-Do001-C302 ESP (ITT Set)

	MIT-Do001-C301 ESP									MIT-Do001-C302 ESP								
	E4 15 mg			E4 20 mg			Placebo			E4 15 mg			E4 20 mg			Placebo		
	All	H	NH	All	H	NH	All	H	NH	All	H	NH	All	H	NH	All	H	NH
N (%)	213 (100)	110 (51.6)	103 (48.4)	213 (100)	110 (51.6)	103 (48.4)	214 (100)	110 (51.4)	104 (48.6)	192 (100)	99 (51.6)	93 (48.4)	193 (100)	100 (51.8)	93 (48.2)	194 (100)	99 (51.0)	95 (49.0)
VMS Frequency																		
n	209	106	103	212	109	103	212	108	104	190	98	92	193	100	93	192	98	94
mean	78.94	77.19	80.75	83.81	74.93	93.21	75.49	73.77	77.27	79.93	86.51	72.92	79.04	86.21	71.33	78.86	75.92	81.93
SD	37.580	37.849	37.399	51.183	44.204	56.363	34.666	30.940	38.222	51.289	66.757	24.837	50.139	63.029	29.249	40.337	35.811	44.559
Missing	4	4	0	1	1	0	2	2	0	2	1	1	0	0	0	2	1	1
VMS Severity																		
n	209	106	103	212	109	103	212	108	104	190	98	92	193	100	93	192	98	94
mean	2.44	2.44	2.43	2.41	2.40	2.41	2.38	2.37	2.39	2.46	2.46	2.47	2.46	2.48	2.44	2.46	2.43	2.50
SD	0.284	0.292	0.275	0.273	0.266	0.280	0.265	0.258	0.273	0.284	0.231	0.332	0.230	0.224	0.236	0.241	0.246	0.231
Missing	4	4	0	1	1	0	2	2	0	2	1	1	0	0	0	2	1	1
VVA – Vaginal Dryness score																		
n	211			209			202											
mean	1.5			1.3			1.4											
SD	1.08			1.04			1.05											
Missing	2			4			12											
VVA – Vaginal and/or Vulvar Irritation/Itching score																		

	MIT-Do001-C301 ESP									MIT-Do001-C302 ESP								
	E4 15 mg			E4 20 mg			Placebo			E4 15 mg			E4 20 mg			Placebo		
	All	H	NH	All	H	NH	All	H	NH	All	H	NH	All	H	NH	All	H	NH
n	210			211			204											
mean	0.8			0.8			0.8											
SD	0.97			0.95			0.98											
Missing	3			2			10											
VVA – Dysuria score																		
n	211			211			204											
mean	0.4			0.3			0.4											
SD	0.74			0.67			0.74											
Missing	2			2			10											
VVA – Vaginal Pain Associated with Sexual Activity score																		
n	211			211			203											
mean	1.2			0.9			1.0											
SD	1.18			1.06			1.11											
Missing	2			2			11											
VVA – Vaginal Bleeding Associated with Sexual Activity																		
Presence, n (%)	6 (2.8)			6 (2.8)			7 (3.3)											
Absence, n (%)	205 (96.2)			205 (96.2)			196 (91.6)											
Missing, n (%)	2 (0.94)			2 (0.94)			11 (5.14)											

	MIT-Do001-C301 ESP									MIT-Do001-C302 ESP								
	E4 15 mg			E4 20 mg			Placebo			E4 15 mg			E4 20 mg			Placebo		
	All	H	NH	All	H	NH	All	H	NH	All	H	NH	All	H	NH	All	H	NH
Modified MENQOL Total Score																		
n	213	110	103	213	110	103	214	110	104	192	99	93	193	100	93	194	99	95
mean	5.40	5.41	5.38	5.33	5.20	5.47	5.31	5.34	5.27	4.90	4.98	4.81	4.85	4.82	4.89	4.93	4.90	4.97
SD	1.378	1.445	1.309	1.394	1.412	1.367	1.240	1.261	1.223	1.383	1.404	1.362	1.325	1.306	1.352	1.448	1.363	1.537

E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, H = hysterectomized, ITT = intent-to-treat, NH = non-hysterectomized, MENQOL = menopause specific quality of life, n = number of subjects in category of interest, N = total number of subjects in the treatment group (overall or by hysterectomy status), SD = standard deviation, VMS = vasomotor symptoms, VVA = vulvovaginal atrophy.
Percentages are calculated with N as the denominator.
Grey cells indicate non available data.
Note: 'VMS Frequency' designates here the weekly frequency of moderate to severe VMS at baseline, defined as the sum of all recorded moderate to severe VMS experienced between Day -7 and Day -1. 'VMS Severity' designates here the mean severity score of VMS at baseline, defined as the arithmetic mean of the daily severity score values of VMS (moderate or severe) observed from Day -7 to Day -1. VVA-related items are part of the VVA questionnaire.

Outcomes and estimation

Primary efficacy endpoint

Of note, C301/C302 ESPs assessed the change from Baseline to Week 1, 2, 3, 5, 6, 7, 8, 9, 10, and 11 in the weekly frequency and severity of moderate to severe VMS as a secondary efficacy endpoint. Because the assessment of this secondary efficacy endpoint is complementary to the primary efficacy analysis of change from baseline to week 4 and week 12, both are presented in this section.

Weekly frequency of moderate to severe VMS (at week 4 and 12)

Study C301 ESP

Primary efficacy (MMRM model): at Week 4 and Week 12 of treatment with E4 15 mg and E4 20 mg once daily, the reduction from baseline in weekly frequency of moderate to severe VMS was statistically significant compared to placebo.

Table 26: Change in the weekly frequency of moderate to severe VMS from baseline to week 4 and week 12 (ITT Set) – MIT-Do001-C301 ESP

	E4 15 mg (N=213)	E4 20 mg (N=213)	Placebo (N=214)
Baseline, n	209	212	212
Mean	78.94	83.81	75.49
(95% CI for Mean)	(73.82, 84.07)	(76.88, 90.74)	(70.79, 80.18)
Week 4, n	197	196	202
Mean	38.48	32.91	45.59
(95% CI for Mean)	(32.69, 44.28)	(27.55, 38.27)	(39.65, 51.53)
Week 12, n	169	162	169
Mean	20.97	15.26	32.76
(95% CI for Mean)	(16.06, 25.88)	(10.75, 19.77)	(27.16, 38.35)
Change from Baseline to Week 4, n	193	195	200
Mean	-40.65	-51.75	-30.42
(95% CI for Mean)	(-46.44, -34.86)	(-58.46, -45.04)	(-35.23, -25.60)
Change from Baseline to Week 12, n	166	161	167
Mean	-55.96	-70.71	-42.17
(95% CI for Mean)	(-62.08, -49.84)	(-79.24, -62.18)	(-48.11, -36.23)

CI = confidence interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, n = number of subjects in the analysis, N = number of subjects in the analysis sets, VMS = vasomotor symptoms.

Table 27: Mean Change in the Weekly Frequency of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set; MMRM) – MIT-Do001-C301 ESP

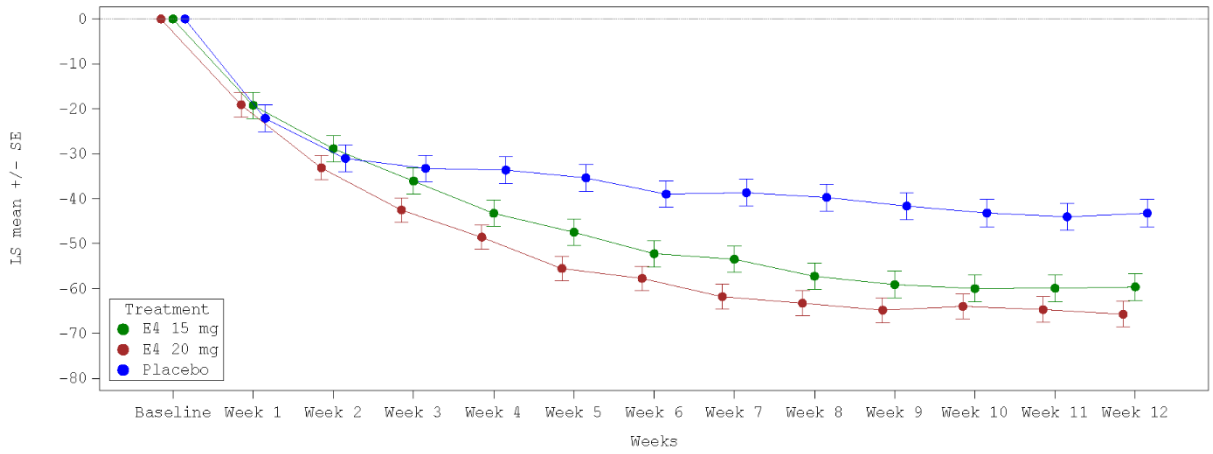
Visit	Statistics	E4 15 mg (N = 213)	E4 20 mg (N = 213)	Placebo (N = 214)
Week 4				
	n	193	195	200
Model-Adjusted Change from Baseline	LS mean	-43.25	-48.56	-33.62
	(95% CI)	(-48.99, -37.50)	(-53.91, -43.21)	(-39.41, -27.83)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	-9.63	-14.94	
	(95% CI)	(-18.81, -0.45)	(-23.85, -6.04)	
	p-value between vs. Placebo	**0.0380**	**0.0004**	
Week 12				
	n	166	161	167
Model-Adjusted Change from Baseline	LS mean	-59.63	-65.71	-43.22
	(95% CI)	(-65.57, -53.68)	(-71.30, -60.12)	(-49.26, -37.17)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	-16.41	-22.49	
	(95% CI)	(-25.95, -6.87)	(-31.79, -13.19)	
	p-value between vs. Placebo	**0.0003**	**<0.0001**	

CI = Confidence Interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = Mixed-effect Models for Repeated-Measures model, N = number of subjects in the statistical analysis set, n = number of observations per study group, VMS = vasomotor symptoms, vs = versus. Statistically significant values ($p<0.05$) are written in bold.
MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).

<u>Weekly frequency</u> of moderate to severe VMS (at week 1 to week 12, <u>Secondary endpoint</u>)

Secondary efficacy (MMRM model): compared to placebo, treatment with E4 15 mg and E4 20 mg once daily achieved a nominally significant reduction from baseline in the weekly frequency of moderate to severe VMS as early as Week 3 and Week 4 with E4 20 mg and E4 15 mg, respectively, with a continuous further reduction up to Week 12 in both groups.

Figure 9: Mean change from Baseline to Week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 in the Weekly Frequency of Moderate to Severe VMS (ITT Set; MMRM) – MIT-Do001-C301 ESP



E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, SE = standard error, MMRM = Mixed-effect Models for Repeated-Measures model, VMS = vasomotor symptoms. MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment and pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett). The autoregressive covariance is used in the modeling of the within-patient errors in the analysis.

Sensitivity analyses on co-primary efficacy endpoints for VMS frequency

Sensitivity analyses were performed with respect to the ITT, mITT and PP set with different analysis methods (MMRM (without status and center effect), ANCOVA-LOCF (without status and center effect)).

Most sensitivity analyses on the 2 co-primary efficacy endpoints of VMS frequency were consistent with the primary analysis.

Study C302 ESP

Primary endpoint- Frequency of moderate to severe VMS (at week 4 and 12)

The mean weekly frequency of moderate to severe VMS at Baseline, Week 4 and Week 12, and the mean change from Baseline to Week 4 and Week 12 are presented in the table below.

Table 28: Change in the weekly Frequency of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set) – MIT-Do001-C302 ESP

	E4 15 mg (N=192)	E4 20 mg (N=193)	Placebo (N=194)
Baseline, n	190	193	192
Mean	79.93	79.04	78.86
(95% CI for Mean)	(72.59,87.27)	(71.92,86.15)	(73.12,84.60)
Week 4, n	173	179	181
Mean	39.83	39.21	46.85
(95% CI for Mean)	(34.61,45.04)	(33.03,45.38)	(40.65,53.05)
Week 12, n	157	153	151
Mean	24.51	22.10	33.76
(95% CI for Mean)	(20.55,28.46)	(16.77,27.43)	(27.88,39.65)
Change from Baseline to Week 4, n	172	179	179
Mean	-39.77	-40.11	-31.82
(95% CI for Mean)	(-45.75, -33.79)	(-45.51, -34.71)	(-36.96, -26.67)
Change from Baseline to Week 12, n	156	153	150
Mean	-55.84	-59.47	-43.27
(95% CI for Mean)	(-63.58, -48.09)	(-66.98, -51.95)	(-48.82, -37.71)

CI = confidence interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, n = number of subjects in the analysis, N = number of subjects in the analysis sets, VMS = vasomotor symptoms.

Primary efficacy (MMRM model): at Week 4 and Week 12 of treatment with E4 15 mg and E4 20 mg once daily, the reduction from baseline in weekly frequency of moderate to severe VMS was statistically significant compared to placebo.

Table 29: Mean Change in the Weekly Frequency of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set; MMRM) – MIT-Do001-C302 ESP

Visit	Statistics	E4 15 mg (N = 192)	E4 20 mg (N = 193)	Placebo (N = 194)
Week 4				
	n	172	179	179
Model-Adjusted Change from Baseline	LS mean (95% CI)	-41.38 (-46.44, -36.32)	-43.17 (-48.24, -38.09)	-32.96 (-38.20, -27.71)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means (95% CI)	-8.42 (-16.64, -0.20)	-10.21 (-18.44, -1.98)	
	p-value between vs. Placebo	**0.0436**	**0.0117**	
Week 12				
	n	156	153	150
Model-Adjusted Change from Baseline	LS mean (95% CI)	-57.54 (-62.73, -52.34)	-60.82 (-66.09, -55.56)	-45.33 (-50.79, -39.88)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means (95% CI)	-12.20 (-20.69, -3.71)	-15.49 (-24.04, -6.94)	
	p-value between vs. Placebo	**0.0029**	**0.0001**	

CI = confidence interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least square, MMRM = Mixed-effect Models for Repeated Measures, n = number of subjects in the analysis, N = number of subjects in the analysis sets, VMS = vasomotor symptoms, vs = versus.
Statistically significant values ($p<0.05$) are written in bold.
MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).

Sensitivity analyses on coprimary efficacy endpoints for VMS frequency

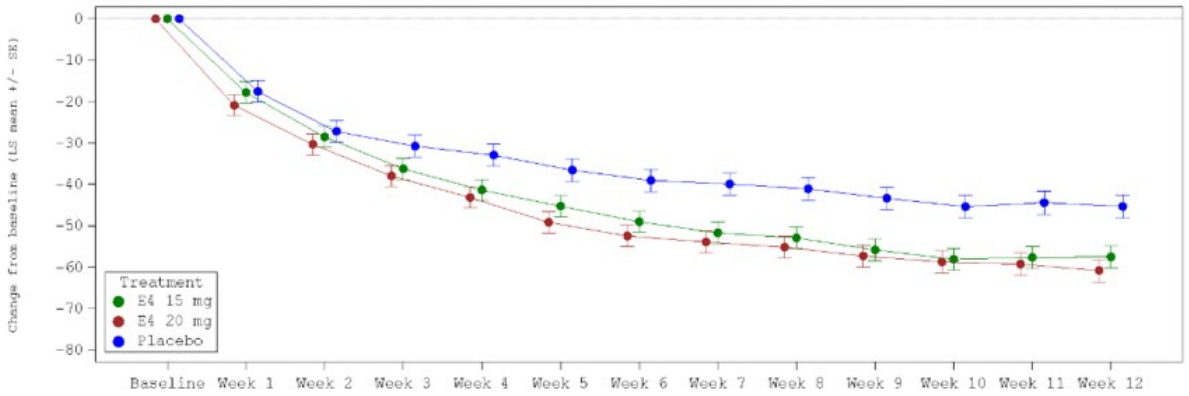
Additional sensitivity analyses were performed, similar to primary analysis, to assess the robustness of the results. These included sensitivity with respect to 1) populations studied (mITT and PP); 2) an imputation of missing data (last observation carried forward [LOCF] or other); and 3) the modelling strategy (analysis of covariance [ANCOVA]).

The sensitivity analyses on the 2 co-primary efficacy endpoints of VMS frequency conducted on the ITT and mITT Sets were consistent with the primary analysis. Those conducted on the PP Set showed statistically significant reduction in VMS frequency only for E4 20 mg treatment arm while the results of the sensitivity analyses on the PP set with E4 15 mg were inconsistent.

Weekly frequency of moderate to severe VMS (at week 1 to week 12, Secondary endpoint)

Secondary efficacy (MMRM model): compared to placebo, treatment with E4 15 mg and E4 20 mg once daily achieved a nominally significant reduction from baseline in the weekly frequency of moderate to severe VMS from Week 4, with a continuous further reduction up to Week 12 in both arms.

Figure 10: Mean Change from Baseline to Week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 in the Weekly Frequency of Moderate to Severe VMS (ITT Set; MMRM) – MIT-Do001-C302 ESP



E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = Mixed-effect Models for Repeated-Measures model, SE = standard error, VMS = vasomotor symptoms.
The MMRM model includes treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett). The autoregressive covariance is used in the modeling of the within-subject errors in the analysis.

Primary endpoints Mean <u>severity</u> of moderate to severe VMS (at week 4 and 12)

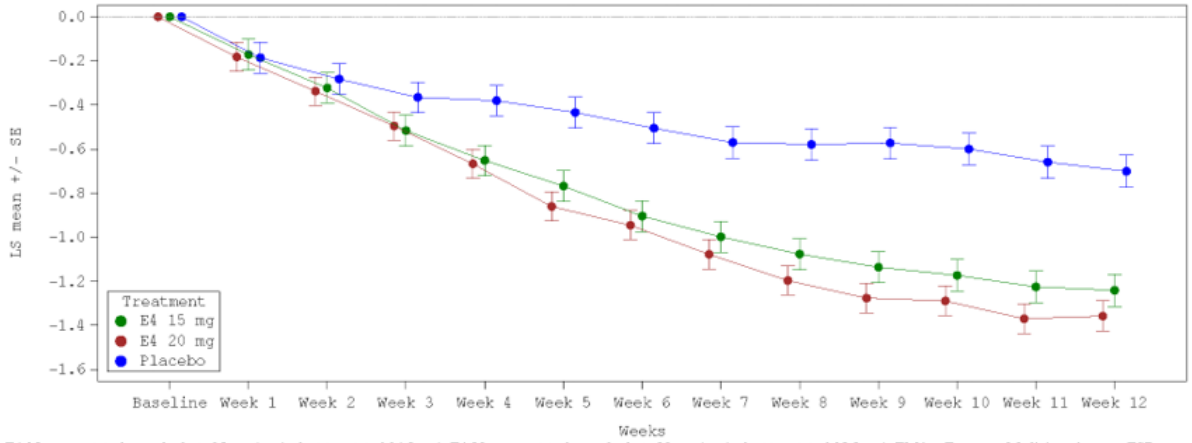
<u>Study C301 ESP</u>

The mean severity of moderate to severe VMS decreased in all treatment groups – including the placebo group – as of Week 1 with a continuous further reduction up to Week 12. From Week 3 onwards, the decrease was more pronounced in the E4 treatment groups compared with the placebo group.

Primary efficacy (MMRM model): at Week 4 and Week 12 of treatment with E4 15 mg and E4 20 mg once daily, the reduction from baseline in mean severity score of moderate to severe VMS was statistically significant compared to placebo (see table below).

Secondary efficacy (MMRM model): compared to placebo, treatment with E4 15 mg and E4 20 mg once daily achieved a nominally significant reduction from baseline in the weekly severity of moderate to severe VMS from Week 4 for both E4 doses with a continuous further reduction up to Week 12 (see figure below).

Figure 11: Mean Change from Baseline to Week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 in the Mean Severity of Moderate to Severe VMS (ITT Set; MMRM; EMA Scoring Method) – MIT-Do001-C301 ESP



E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), EMA = European Medicines Agency, ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = Mixed-effect Models for Repeated-Measures model, SE = standard error, VMS = vasomotor symptoms. MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment and pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett). The autoregressive covariance is used in the modeling of the within-patient errors in the analysis.

Table 30: Mean Change in the Mean Severity Score of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set; MMRM; EMA Scoring Method) – MIT-Do001-C301 ESP

Visit	Statistics	E4 15 mg (N = 213)	E4 20 mg (N = 213)	Placebo (N = 214)
Week 4				
	n	193	195	200
Model-Adjusted Change from Baseline	LS mean	-0.65	-0.67	-0.38
	(95% CI)	(-0.79, -0.52)	(-0.79, -0.54)	(-0.52, -0.24)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	-0.27	-0.29	
	(95% CI)	(-0.49, -0.05)	(-0.50, -0.08)	
	p-value between vs. Placebo	**0.0109**	**0.0051**	
Week 12				
	n	166	161	167
Model-Adjusted Change from Baseline	LS mean	-1.24	-1.36	-0.70
	95% CI	(-1.38, -1.10)	(-1.49, -1.22)	(-0.85, -0.56)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	-0.54	-0.66	
	95% CI	(-0.77, -0.31)	(-0.88, -0.43)	
	p-value between vs. Placebo	**<0.0001**	**<0.0001**	

CI = Confidence Interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), EMA = European Medicines Agency, ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = Mixed-effect Models for Repeated-Measures, N = number of subjects in the statistical analysis set, n = number of observations per study group, VMS = vasomotor symptoms, vs = versus.

Statistically significant values ($p<0.05$) are written in bold.

MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).

Sensitivity analyses on co-primary efficacy endpoints for VMS severity

Sensitivity analyses were performed with respect to the ITT, mITT and PP set with different analysis methods (MMRM (without status and center effect), ANCOVA-LOCF (without status and center effect)). Most sensitivity analyses on the 4 coprimary efficacy endpoints of VMS severity were consistent with the primary analysis.

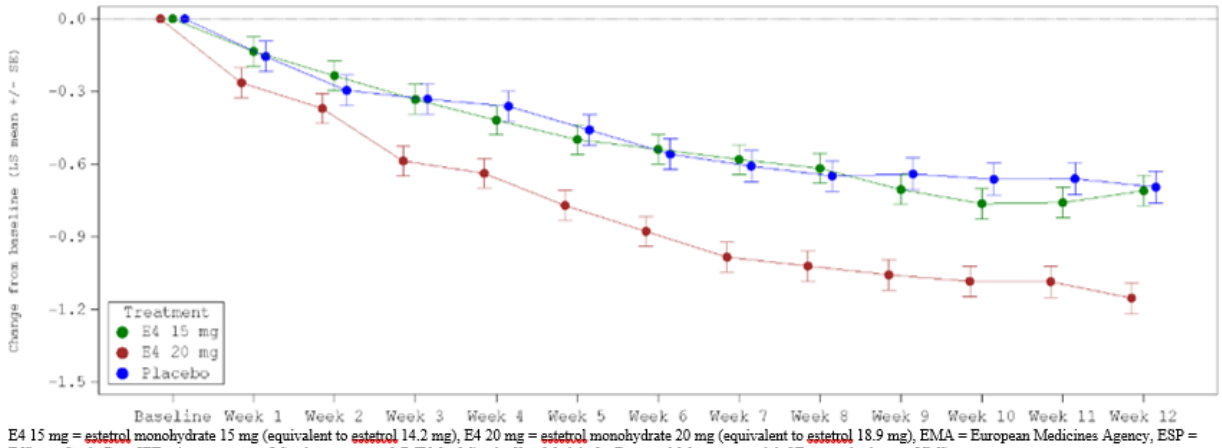
Study C302 ESP

The mean severity of moderate to severe VMS decreased in all treatment groups – including the placebo group – as of Week 1 with a continuous further reduction up to Week 12. The decrease was similar in the E4 15 mg and placebo groups but steeper in the E4 20 group (see figure below).

Primary efficacy (MMRM model): at Week 4 and Week 12 of treatment with E4 20 mg once daily, the reduction from baseline in mean severity score of moderate to severe VMS was statistically significant compared to placebo. Single daily oral doses of E4 15 mg treatment did not result in a statistically significant reduction in the severity of moderate to severe VMS from baseline at Weeks 4 and 12 compared to placebo (see table below).

Secondary efficacy (MMRM model): compared to placebo, treatment with E4 20 mg once daily achieved a nominally significant reduction from baseline in the weekly severity of moderate to severe VMS as early as Week 3 with a continuous further reduction up to Week 12. Treatment with E4 15 mg once daily did not result in a statistically significant reduction in the severity of moderate to severe VMS from baseline at any of the timepoints of assessment compared to placebo (see figure below).

Figure 12: Mean Change from Baseline to Week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 in the Mean Severity of Moderate to Severe VMS (ITT Set; MMRM; EMA Scoring Method) – MIT-Do001-C302 ESP



E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), EMA = European Medicines Agency, ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = Mixed-effect Models for Repeated-Measures model, SE = standard error, VMS = vasomotor symptoms.
The MMRM model includes treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett). The autoregressive covariance is used in the modeling of the within-subject errors in the analysis.

Table 31: Mean Change in the Mean Severity Score of Moderate to Severe VMS from Baseline to Week 4 and Week 12 (ITT Set; MMRM; EMA Scoring Method) – MIT-Do001-C302 ESP

Visit	Statistics	E4 15 mg (N = 192)	E4 20 mg (N = 193)	Placebo (N = 194)
Week 4				
	n	172	179	179
Model-Adjusted Change from Baseline	LS mean	-0.42	-0.64	-0.36
	(95% CI)	(-0.54, -0.30)	(-0.76, -0.52)	(-0.48, -0.24)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	-0.06	-0.28	
	(95% CI)	(-0.25, 0.14)	(-0.47, -0.08)	
	p-value between vs. Placebo	0.7368	**0.0031**	
Week 12				
	n	156	153	150
Model-Adjusted Change from Baseline	LS mean	-0.71	-1.15	-0.70
	(95% CI)	(-0.83, -0.59)	(-1.28, -1.03)	(-0.83, -0.56)
Model-Adjusted Change from Baseline vs. Placebo	Difference of LS Means	-0.01	-0.46	
	(95% CI)	(-0.22, 0.19)	(-0.66, -0.25)	
	p-value between vs. Placebo	0.9809	**<0.0001**	

CI = Confidence Interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), EMA = European Medicines Agency, ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = Mixed-effect Models for Repeated-Measures, N = number of subjects in the statistical analysis set, n = number of observations per study group, VMS = vasomotor symptoms, vs = versus.

Statistically significant values ($p<0.05$) are written in bold.

MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).

Sensitivity analyses on co-primary efficacy endpoints for VMS severity

The sensitivity analyses on the ITT Set using the MMRM model without status and center effect showed statistically significant reduction in VMS severity with E4 20 mg treatment only at Week 12. The sensitivity analyses on the ITT Set using ANCOVA (LOCF), with and without status and center effect, were consistent with the primary analysis. The sensitivity analyses on the mITT Set using the MMRM model were consistent with the primary analysis, while those conducted on the PP Set showed statistical significance only in the E4 20 mg treatment arm at Week 12.

Secondary efficacy endpoints

Changes from baseline in weekly frequency and severity of moderate to severe VMS over time

Please also refer to the primary efficacy endpoint section, which includes secondary efficacy data from all weeks (1-12), of which week 4 and 12 are used for the primary efficacy analysis.

Study C301 ESP

Frequency - The difference in the adjusted LS mean of the weekly frequency of moderate to severe VMS compared with placebo was nominally significant ($p<0.05$) in the E4 15 mg arm from Weeks 4 to

12 and in the E4 20 mg arm from Weeks 3 to 12. The LS mean difference varied from -9.63 to -17.53 in the E4 15 mg arm and -9.26 to -23.54 in the E4 20 mg arm.

Severity - Based on EMA method (excludes mild VMS at baseline and post-baseline), using MMRM model, the difference in the adjusted LS mean of the *severity* of moderate to severe VMS compared with placebo was nominally significant ($p<0.05$) in both the E4 15 mg and E4 20 mg arms from Weeks 4 to 12. The LS mean difference varied from -0.27 to -0.57 in the E4 15 mg arm and from -0.29 to -0.71 in the E4 20 mg arm.

Study C302 ESP

Frequency - The difference in the adjusted LS mean change from Baseline of the frequency of moderate to severe VMS was nominally significant ($p < 0.05$) in both the E4 15 mg and E4 20 mg arms compared to placebo from Weeks 4 through 12. No statistically significant differences were observed in the adjusted LS mean change from Baseline at Weeks 1 through 3 for either E4 treatment arm compared to placebo. The LS mean difference in the frequency of moderate to severe VMS from Baseline compared to placebo varied from -0.22 (Week 1) to -13.24 (Week 11) in the E4 15 mg arm and from -3.10 (Week 2) to -15.49 (Week 12) in the E4 20 mg arm.

Severity - Based on EMA method the LS mean difference in the severity score of moderate to severe VMS from Baseline compared to placebo varied from -0.07 (Week 2) to -0.46 (Week 12) in the E4 20 mg arm and was close to 0 in the E4 15 mg arm with a maximum of -0.10 at Week 11. The difference in the adjusted LS mean of the severity score of moderate to severe VMS was nominally significant ($p < 0.05$) in the E4 20 mg arm compared to placebo from Weeks 3 through 12. No statistically significant differences were observed in the adjusted LS mean change from Baseline at any time point analyzed for the E4 15 mg arm compared to placebo.

50% and 75% Responder analyses for the weekly frequency of *moderate to severe* VMS over time

Study C301 ESP

A nominally significantly higher proportion of subjects experienced a ≥50% reduction from baseline in the weekly frequency of moderate to severe VMS from Week 3 until Week 12 in both E4 15 mg and E4 20 mg arms compared with the placebo arm. At Week 12, this proportion was 82.5% and 87.0% with E4 15 mg and E4 20 mg treatment, respectively, compared to 60.5% with placebo.

A nominally significantly higher proportion of subjects experienced a ≥75% reduction from baseline in the weekly frequency of moderate to severe VMS from Week 3 until Week 12 in both E4 15 mg and E4 20 mg arms compared with the placebo arm. At Week 12, this proportion was 63.3% and 74.5% of subjects in the E4 15 mg and E4 20 mg treatment arms, respectively, compared to 39.5% with placebo.

Study C302 ESP

A nominally significantly higher proportion of subjects experienced a ≥50% reduction from baseline in the weekly frequency of moderate to severe VMS from Week 5 until Week 12 with E4 20 mg compared to placebo and at weeks 5, 6, and 9 until 12 with E4 15 mg. At Week 12, this proportion was 81.3% and 81.7% with E4 15 mg and E4 20 mg treatment, respectively, compared to 61.3% with placebo.

A nominally significantly higher proportion of subjects experienced a ≥75% reduction from baseline in the weekly frequency of moderate to severe VMS from Week 4 until Week 12 with E4 20 mg compared to placebo and at weeks 11 and 12 with E4 15 mg. At Week 12, this proportion was 49.0% and 56.9% of subjects in the E4 15 mg and E4 20 mg treatment arms, respectively, compared to 37.3% with placebo.

Table 32: Summary Table for the Responder Analysis for the Weekly Frequency of Moderate to Severe VMS at Week 12 (ITT Set)

	VMS Reduction from Baseline	Proportion of Responders at Week 12				
		E4 15 mg		E4 20 mg		Placebo
		%	p-value*	%	p-value*	%
MIT-Do001-C301 ESP	**≥50%**	82.5	**<0.0001**	87.0	**<0.0001**	60.5
	≥75%	63.3	**<0.0001**	74.5	**<0.0001**	39.5
MIT-Do001-C302 ESP	**≥50%**	81.3	**0.0001**	81.7	**<0.0001**	61.3
	≥75%	49.0	**0.0392**	56.9	**0.0007**	37.3

E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, VMS = vasomotor symptoms.
*p-value vs placebo. Statistically significant values ($p<0.05$) are written in bold.
Note: Percentages (%) are based on the number of subjects with a non-missing percent change from Baseline result in each treatment arm by visit in the ITT Set.

Weekly Weighted Score of VMS

Study C301 ESP

The weekly weighted score (EMA scoring method) of moderate to severe VMS markedly decreased in all treatment groups – including the placebo group – as of Week 1. From Week 2 and Week 3 onwards with E4 20 mg and E4 15 mg, respectively, E4 achieved a larger decrease from baseline than placebo, with E4 20 mg achieving the largest decrease at all timepoints. The weekly weighted score of moderate to severe VMS has a profile comparable to that of the weekly frequency of moderate to severe VMS for all 3 treatment groups.

Compared to placebo, single daily oral doses of E4 15 mg and E4 20 mg treatment achieved a nominally significant reduction from baseline of the weekly weighted VMS scores at week 4 and as early as week 3 in the E4 15 mg and E4 20 mg arm, respectively, with a continuous further reduction up to week 12 in both arms. The LS mean difference (95% CI) from baseline compared to placebo was:

- E4 15 mg: -23.95 (-43.10, -4.80) at Week 4 and -40.66 (-60.73, -20.58) at Week 12
- E4 20 mg: -25.48 (-44.47, -6.49) at Week 3 to -59.83 (-79.92, -39.74) at Week 12

Study C302 ESP

The weekly weighted score (EMA scoring method) of moderate to severe VMS markedly decreased in all treatment groups – including the placebo group – as of Week 1. At all timepoints, E4 achieved a larger decrease from baseline, with E4 20 mg achieving the largest decrease. The weekly weighted score of moderate to severe VMS has a profile comparable to that of the weekly frequency of moderate to severe VMS for all 3 treatment groups. Compared to placebo, oral doses of E4 15 mg and E4 20 mg treatment once daily achieved a nominally significant reduction from baseline of the weekly weighted VMS scores at week 4 with a continuous further reduction up to week 12. The LS mean difference (95% CI) from baseline compared to placebo was:

- E4 15 mg: -21.39 (-42.41, -0.36) at Week 4 and -29.03 (-50.74, -7.32) at Week 12
- E4 20 mg: -26.37 (-47.42, -5.32) at Week 4 and -38.25 (-60.11, -16.39) at Week 12

Clinical important difference (CID) using the Clinical Global Impression (CGI) questionnaire

Study C301 ESP

The percentage of subjects with a CID was nominally significantly higher in the E4 arms compared with placebo at both Week 4 and Week 12. The percentage of subjects with Minimally Clinically Important Difference (MCID, it includes the rating of minimally improved based on clinical global impression (CGI) questionnaire) or no change or worsening of their condition was consistently lower in the E4 arms compared with placebo at both Week 4 and Week 12. The proportion of subjects with a CID compared to baseline in the weekly frequency of moderate to severe VMS was higher in the E4 15 mg (52.9% at Week 4 and 73.3% at Week 12) and E4 20 mg (59.8% at Week 4 and 77.8% at Week 12) compared to placebo (27.9% at Week 4 and 47.0% at Week 12).

Study C302 ESP

The percentage of subjects with a CID was nominally significantly higher in the E4 20 mg arm compared with placebo at both Week 4 and Week 12. The percentage of subjects with MCID was lower in the E4 20 mg arm compared to placebo at Week 12. With E4 15 mg, the increase in the percentage of subjects with a CID compared to placebo at Week 12 was not statistically significant. The percentage of subjects with no change or worsening in their condition was nominally significantly lower in both E4 arms compared to placebo at Week 4 but nominal significance was not maintained at Week 12. In subjects with a CID, both E4 doses achieved a nominally significant reduction from baseline in the weekly frequency of moderate to severe VMS compared to placebo at Week 12. In subjects with a MCID or reporting no change or worsening of their condition, no nominally significant change in moderate to severe VMS frequency from baseline at Week 4 or Week 12 was observed compared to placebo.

HRQoL using the MENQOL questionnaire

Study C301 ESP

Significant improvement in quality of life was observed at Week 12 with nominally significant decrease versus placebo in the total MENQOL score and in all domain scores (i.e. vasomotor, psychosocial, physical, and sexual functioning) after E4 20 mg treatment, and in the total MENQOL score and vasomotor, psychosocial, and sexual functioning domain scores after E4 15 mg treatment. For the E4 15 mg arm, the LS mean changes compared with placebo from Baseline to Week 12 were nominally significant for vasomotor, psychosocial, and sexual functioning domain scores while the physical function domain scores failed to meet statistical significance (p=0.0728). For the E4 20 mg arm, the LS mean changes compared with placebo from baseline to Week 12 were nominally significant for all 4 domain scores.

Study C302 ESP

Improvement in quality of life was observed at Week 52 with nominally significant decrease versus placebo in the total MENQOL score and in the vasomotor domain score after E4 15 mg treatment; nominally significant improvement versus placebo in the vasomotor domain score was observed at both Weeks 12 and 52 after E4 20 mg treatment.

Treatment Satisfaction (TS) using CGI questionnaire

Study C301 ESP

Compared to baseline, the condition of the subjects receiving E4 treatment nominally significantly improved at Week 4 and Week 12 compared with placebo with most subjects rating their overall condition as "very much improved" or "much improved". The proportion of subjects who rated "no change" from baseline in their overall condition (no improvement) was nominally significantly higher in the placebo arm compared to the E4 15 mg and E4 20 mg arms at Weeks 4 and 12. The number of

subjects experiencing worsening of the overall condition was too low in the E4 treatment arms to conclude on the treatment effect compared with placebo.

Study C302 ESP

At Week 4, nominally significantly fewer subjects rated their overall condition as "no change" from Baseline in the E4 15 mg arm compared to the placebo arm (treatment difference [95% CI]: -10.1 [-18.4, -1.9]). At this time point, no nominally significant differences between the E4 20 mg and placebo arms were evidenced for any of the improvement score categories.

At Week 12, nominally significantly more subjects in the E4 20 mg treatment arm rated their overall condition as "very much improved" from Baseline compared to the placebo arm (10.5 [1.2, 19.8]) while nominally significantly fewer subjects rated it as "minimally improved" (-13.7 [-23.5, -4.0] or as "no change" (-9.0 [-15.8, -2.3]) and nominally significantly more subjects rated it as "minimally worse" (3.5 [-0.0, 7.0]). The proportion of subjects in the "no change" category was also nominally significantly lower compared to the placebo arm in the E4 15 mg arm (-8.6 [-15.4, -1.8]).

At Week 52, nominally significantly fewer subjects in the E4 15 mg (-8.1 [-15.6, -0.6]) and E4 20 mg (-9.9 [-17.1, -2.8]) treatment arms reported "no change" from Baseline compared to the placebo arm.

Vulvovaginal atrophy (VVA)

Study C301 ESP (only)

Severity of VVA symptoms (including vaginal dryness, vaginal and/or vulvar irritation/itching, dysuria, and dyspareunia) decreased in all treatment groups after 12 weeks of treatment. The proportion of subjects with vaginal bleeding associated with sexual activity was low in the E4 treatment arms and placebo at baseline so that no reliable conclusions could be drawn.

A nominally significant reduction in vaginal dryness and vaginal pain associated with sexual activity was observed with E4 15 mg compared to placebo at Week 12. There was no nominally significant decrease in severity in any VVA symptoms in subjects who received E4 20 mg, compared to placebo.

Another VVA-related secondary efficacy endpoint was the change from Baseline to Week 12 in the VVA symptom that was initially identified by the subject as being the most bothersome using the VVA questionnaire at baseline (subject self-assessment).

Nominally significant treatment effect was observed with E4 15 mg compared to placebo at Week 12 for the reduction in vaginal dryness (-0.47 [95% CI: -0.84, -0.09]) when it was initially identified by the subject as the "most bothersome" VVA symptom at baseline. There was no nominally significant decrease in severity in any other VVA symptoms when initially identified as the "most bothersome" with E4 15 mg. In subjects who received E4 20 mg, no nominally significant reduction in severity was observed compared to placebo for any VVA symptom initially identified as the "most bothersome".

Pre-defined and post-hoc subgroup analyses

Pre-defined analyses

Frequency and Severity of Moderate to Severe VMS by Subgroup (Prespecified Analysis)

C301 ESP

Weekly Frequency of Moderate to Severe VMS by Subgroup

Subgroup analyses outcomes suggested that:

- In all subgroups, the efficacy of E4 20 mg in terms of reduction of VMS frequency was confirmed at both timepoints, except for the subgroup BMI ≥30 kg/m^2, in which the reduction was not statistically significant.
- In all subgroups, the efficacy of E4 15 mg in terms of reduction of VMS frequency at Week 12 was confirmed, except for the subgroups Hispanic or Latino and BMI ≥30 kg/m^2, in which the reduction was not statistically significant.
- The efficacy of E4 15 mg in terms of reduction of VMS frequency at Week 4 was confirmed in Not Hispanic or Latino, White, Non-smoker, and BMI: 25 to <30 kg/m^2. In subgroups ≥55 years and BMI <25 kg/m^2, although the reduction was not statistically significant, a clear trend was visible (-11.67 [-24.10, 0.75], p=0.0693 and -12.97 [-27.42, 1.49] p=0.0855, respectively).

Mean Severity of Moderate to Severe VMS by Subgroup

Subgroup analyses outcomes suggested that:

- At Week 12, the efficacy of E4 15 mg and E4 20 mg in terms of reduction of VMS severity was confirmed in all subgroups with a sufficient sample size.
- At Week 4, the efficacy of E4 15 mg and E4 20 mg in terms of reduction of VMS severity was confirmed for Not Hispanic or Latino, < 55 years (E4 15 mg), ≥ 55 years (E4 20 mg), White, BMI ≥ 25 to <30 kg/m^2, and Non-smokers. With E4 20 mg, in Hispanic or Latino, although the decrease was not statistically significant, a clear trend was visible (-0.35 [-0.73, 0.02] p=0.0628). With E4 15 mg, in 'BMI ≥30 kg/m^2, although the decrease was not statistically significant, a clear trend was visible (-0.34 [-0.72, 0.05], p=0.0975).

C302 ESP

Weekly Frequency of Moderate to Severe VMS by Subgroup

Subgroup analyses outcomes suggested that:

- In most subgroups the efficacy of E4 20 mg in terms of reduction of VMS frequency was confirmed at Week 12, except for 'Hispanic or Latino', in which the reduction was not statistically significant. In '≥55 years and 'BMI <25 kg/m^2', although the reduction was not statistically significant, a clear trend was visible (-9.73 [-19.52, 0.05], p=0.0513 and -18.25 [-37.07, 0.57] p=0.0589, respectively).
- In a few subgroups, the efficacy of E4 20 mg in terms of reduction of VMS frequency at Week 4 was not confirmed: in 'Hispanic or Latino', no decrease was observed; in '<55 years' and 'BMI 25 to <30 kg/m^2', the reduction was not statistically significant. In 'BMI ≥30 kg/m^2', although the reduction was not statistically significant, a clear trend was visible (-12.05 [-25.61, 1.52], p=0.0905).
- In most subgroups, the efficacy of E4 15 mg in terms of reduction of VMS frequency was confirmed at Week 12, except for: 'Hispanic or Latino' and 'BMI ≥30 kg/m^2', in which the reduction was not statistically significant. In 'White' and 'BMI 25 to <30 kg/m^2', although the reduction was not statistically significant, a clear trend was visible (-7.05 [-14.99, 0.89], p=0.0897 and -10.11 [-21.56, 1.35], p=0.0922, respectively).
- The efficacy of E4 15 mg in terms of reduction of VMS frequency at Week 4 was confirmed in 'Not Hispanic or Latino, ≥55 years, and BMI: <25 kg/m^2. Efficacy of E4 15 mg was not confirmed: in Hispanic or Latino, <55 years, White, BMI 25 to <30 kg/m^2 and BMI ≥30 kg/m^2, the reduction was not statistically significant. In Non-Smokers, although the reduction was not statistically significant, a clear trend was visible (-6.96 [-14.25, 0.32], p=0.0635).

Mean Severity of Moderate to Severe VMS by Subgroup

Subgroup analyses outcomes suggested that:

- In all subgroups the efficacy of E4 20 mg in terms of reduction of VMS severity was confirmed at Week 12, except for: 'Hispanic or Latino' and 'BMI <25 kg/m^2', in which the reduction was not statistically significant.
- In a few subgroups, the efficacy of E4 20 mg in terms of reduction of VMS severity was not confirmed at Week 4: in 'Hispanic or Latino', '<55 years' and the 3 BMI categories, the reduction was not statistically significant. In '≥55 years', although the reduction was not statistically significant, a clear trend was visible -0.21 (-0.44, 0.03), p=0.0933).

Of note, although E4 15 mg did not achieve a statistically significant reduction in VMS severity at either timepoints compared to placebo in the overall population, the subgroup analyses showed a statistically significant reduction in subgroups 'Not Hispanic or Latino' and 'Black or African American' at Week 12.

***Post hoc* analyses**

Frequency and Severity of Moderate to Severe VMS by Hysterectomy Status (*post hoc* analyses)

To note, the analyses by subgroup were not powered to show differences by hysterectomy status.

C301 ESP

The results of the *post hoc* subgroup analysis by hysterectomy status were overall comparable to the overall population. The only exceptions were noted for the statistical significance of reduction of VMS frequency or severity versus placebo at Week 4. In the hysterectomized population treated with E4 15 mg, the reduction from baseline in the weekly frequency became significantly more pronounced compared to placebo as from Week 8 instead of Week 4. In the NH population treated with E4 20 mg, the reduction of the severity score became significantly more pronounced compared to placebo as from Week 5 instead of Week 4.

C302 ESP

The *post hoc* subgroup analysis by hysterectomy status showed that, in hysterectomized subjects, a statistically significant decrease in the frequency of VMS was obtained with both doses at Week 12, and a statistically significant decrease in severity of VMS was obtained with the 20 mg dose at Week 12. Statistically significant reductions in frequency and severity were not obtained at Week 4. In NH subjects, statistical significance was obtained for the decrease in the severity of moderate to severe VMS with the 20 mg dose at both Week 4 and Week 12. The observed decrease in VMS frequency in NH subjects was not statistically significant with either dose at either timepoint.

Frequency and Severity of Moderate to Severe VMS by Race (post hoc analyses)

Post hoc analyses of the co-primary efficacy endpoints 'frequency and severity of VMS at Week 4 and at Week 12' and the secondary efficacy endpoint 'frequency and severity of VMS from Week 1 to Week 12' by race (Black or African American, White, and Other) are presented. The conclusions of the *post hoc* analyses are consistent with the conclusions of the prespecified subgroup analyses for subgroups 'White' and 'Black or African American'. Subgroups 'Other' were small such that no reliable conclusions could be drawn.

Pre-defined and *post hoc* sensitivity analyses

Please be referred to the outcomes section above for the corresponding sensitivity analyses.

Ancillary analyses

Post-hoc analyses (exclusion NH women 6-12 months since last menses)

The indication can only be approved for non-hysterectomized women with at least 12 months since last menses and hysterectomized women. Therefore, post-hoc analyses excluding non-hysterectomized women with at least 6 months till 12 months since last menses were performed.

Table 33:Primary Efficacy: Effect of E4 15 mg and E4 20 mg on the Weekly Frequency and Mean Severity (EMA Scoring Method) of Moderate to Severe VMS at Week 4 and Week 12 – ITT Population, NH Women ⩾12 Months since Last Menses and Hysterectomized Women (Post-Hoc Analysis)

				Frequency of VMS			Severity of VMS		
C301 ESP				**E4 15 mg**	**E4 20 mg**	**Placebo**	**E4 15 mg**	**E4 20 mg**	**Placebo**
				(N=200)	**(N=197)**	**(N=200)**	**(N=200)**	**(N=197)**	**(N=200)**
	Week 4		**n**	181	182	187	181	182	187
		Model-adjusted change from baseline	**LS mean**	-43.31	-48.45	-32.17	-0.65	-0.69	-0.37
			Std Error	2.984	2.852	3.103	0.071	0.068	0.073
			95% CI	(-49.16, -37.46)	(-54.04, -42.86)	(-38.25, -26.08)	(-0.79, -0.51)	(-0.82, -0.56)	(-0.51, -0.22)
			p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		Model-adjusted change from baseline vs. Placebo	**Difference of LS Means**	-11.14	-16.28[a]		-0.29	-0.33	
			Std Error	4.299	4.219		0.102	0.100	
			95% CI	(-20.64, -1.65)	(-25.60, -6.96)		(-0.51, -0.06)	(-0.55, -0.10)	
			p-value vs. Placebo	**0.0181**	**0.0002**		**0.0096**	**0.0022**	
	Week 12		**n**	157	149	156	157	149	156
		Model-adjusted change from baseline	**LS mean**	-59.33	-64.46	-41.81	-1.25	-1.36	-0.71
			Std Error	3.098	2.984	3.238	0.074	0.072	0.077
			95% CI	(-65.41, -53.26)	(-70.31, -58.61)	(-48.16, -35.46)	(-1.39, -1.10)	(-1.50, -1.22)	(-0.86, -0.56)
			p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		Model-adjusted change from baseline vs. Placebo	**Difference of LS Means**	-17.52	-22.65		-0.54	-0.65	
			Std Error	4.475	4.408		0.107	0.106	
			95% CI	(-27.41, -7.64)	(-32.39, -12.92)		(-0.78, -0.30)	(-0.89, -0.42)	
			p-value vs. Placebo	**0.0002**	**<.0001**		**<.0001**	**<.0001**	

				Frequency of VMS			Severity of VMS		
C302 ESP				**E4 15 mg**	**E4 20 mg**	**Placebo**	**E4 15 mg**	**E4 20 mg**	**Placebo**
				(N=185)	**(N=186)**	**(N=185)**	**(N=185)**	**(N=186)**	**(N=185)**
	Week 4		**n**	166	172	171	166	172	171
		Model-adjusted change from baseline	**LS mean**	-42.09	-42.83	-32.38	-0.42	-0.61	-0.35
			Std Error	2.736	2.699	2.801	0.063	0.063	0.065
			95% CI	(-47.45, -36.72)	(-48.12, -37.53)	(-37.87, -26.89)	(-0.55, -0.30)	(-0.73, -0.48)	(-0.47, -0.22)
			p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		Model-adjusted change from baseline vs. Placebo	**Difference of LS Means**	-9.71	-10.44		-0.08	-0.26[b]	
			Std Error	3.916	3.889		0.091	0.090	
			95% CI	(-18.36, -1.05)	(-19.04, -1.85)		(-0.28, 0.12)	(-0.46, -0.06)	
			p-value vs. Placebo	**0.0249**	**0.0138**		0.5901	**0.0075**	
	Week 12		**n**	150	149	144	150	149	144
		Model-adjusted change from baseline	**LS mean**	-58.34	-60.61	-45.01	-0.73	-1.12	-0.69
			Std Error	2.806	2.789	2.916	0.066	0.066	0.068
			95% CI	(-63.84, -52.84)	(-66.08, -55.14)	(-50.73, -39.30)	(-0.86, -0.60)	(-1.25, -0.99)	(-0.82, -0.55)
			p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		Model-adjusted change from baseline vs. Placebo	**Difference of LS Means**	-13.32	-15.59		-0.04	-0.43	
			Std Error	4.047	4.035		0.095	0.095	
			95% CI	(-22.26, -4.38)	(-24.51, -6.67)		(-0.25, 0.17)	(-0.64, -0.22)	
			p-value vs. Placebo	**0.0020**	**0.0002**		0.8533	**<.0001**	

CI = confidence interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = mixed-effect models for repeated-measures, p = p-value, VMS = vasomotor symptoms

[a] Statistically significant as early as Week 3: -10.81 (-20.13, -1.50), p=0.0195.

[b] Statistically significant as early as Week 3: -0.23 (-0.43, -0.04), p=0.0171.

Statistically significant results (p<0.05) are shown in bold.

The mean change in weekly frequency and mean severity score of moderate to severe VMS were calculated using an MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/non-hysterectomized), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate. The autoregressive covariance was used in the modeling of the within-patient errors in the analysis.

5.3.3 Clinical studies in special populations

Please be referred to section 4.3.2 pharmacokinetics.

5.3.4 Supportive studies

As described before, both pivotal phase 3 studies (C301 and C302) contained a SSP. The SSP parts of studies C301 and C302 were not controlled and not designed for efficacy assessment and, therefore, were not considered supportive for the analysis of primary clinical efficacy.

Secondary efficacy endpoints were assessed in both SSP parts. However, due to the difference in included population in ESP and SSP (specifically the low number of VMS at baseline in SSP) it is not possible to compare the data regarding reduction in VMS and responder analysis, and these studies are not considered supportive.

5.3.5 Analysis performed across trials (pooled analyses and meta-analysis)

Cross-study comparison

The results of the primary efficacy analyses of E4 15 mg and E4 20 mg across the phase 2 and phase 3 studies are summarized in the table below.

Table 34: Primary Efficacy Summary: Effect of E4 15 mg and E4 20 mg on the Weekly Frequency and Mean Severity of Moderate to Severe VMS at Week 4 and Week 12 (ITT Set)

		VMS Frequency LS Mean Difference (95% CI) vs Placebo		VMS Severity LS Mean Difference (95% CI) vs Placebo	
		Week 4	Week 12	Week 4	Week 12
MIT-Do0001-C201	**E4 15 mg**	**-12.24 (-23.77, -0.71) p=0.0335**	-8.91 (-20.44, 2.63) p=0.1800	-0.10 (-0.23, 0.02) p=0.1156	-0.03 (-0.19, 0.14) p=0.9861
MIT-Do001-C301 ESP	**E4 15 mg**	**-9.63 (-18.81, -0.45) p=0.0380**	**-16.41 (-25.95, -6.87) p=0.0003**	**-0.27 (-0.49, -0.05) p=0.0109**	**-0.54 (-0.77, -0.31) p<0.0001**
	E4 20 mg	**-14.94[a] (-23.85, -6.04) p=0.0004**	**-22.49 (-31.79, -13.19) p<0.0001**	**-0.29 (-0.50, -0.08) p=0.0051**	**-0.66 (-0.88, -0.43) p<0.0001**
MIT-Do001-C302 ESP	**E4 15 mg**	**-8.42 (-16.64, -0.20) p=0.0436**	**-12.20 (-20.69, -3.71) p=0.0029**	-0.06 (-0.25, 0.14) p=0.7368	-0.01 (-0.22, 0.19) p=0.9809
	E4 20 mg	**-10.21 (-18.44, -1.98) p=0.0117**	**-15.49 (-24.04, -6.94) p=0.0001**	**-0.28[b] (-0.47, -0.08) p=0.0031**	**-0.46 (-0.66, -0.25) p<0.0001**

ANCOVA = Analysis of Covariance, CI = confidence interval, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), ESP = Efficacy Study Part, ITT = intent-to-treat, LS = least squares, MMRM = mixed-effect models for repeated-measures, p = p-value, VMS = vasomotor symptoms, vs = versus.
[a] Statistically significant as early as Week 3: -9.26 (-18.16, -0.37), p=0.0397 (MIT-Do001-C301 ESP CSR).
[b] Statistically significant as early as Week 3: -0.26 (-0.45, -0.06), p=0.0069 (MIT-Do001-C302 ESP CSR).
For study MIT-Do0001-C201, mean change in weekly frequency and mean severity score of moderate to severe VMS were calculated using an ANCOVA with fixed factors treatment and pooled site and covariate baseline value, including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).
In addition, the severity scores of moderate to severe VMS used a different method of calculation in study MIT-Do0001-C201 compared to the Phase 3 studies, which precludes any comparison (details in **Section 2.1.1.1**).
For the Phase 3 ESPs, the mean change in weekly frequency and mean severity score of moderate to severe VMS were calculated based using an MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment, status (hysterectomized/NH), status*treatment, pooled trial centers and treatment*pooled trial centers as fixed effects and baseline as a covariate including pairwise comparisons vs placebo (with adjustment for multiple comparisons according to Dunnett).
Statistically significant results (p<0.05) are shown in bold.

Post-hoc analyses (pooled ITT set)

Post-hoc analyses excluding non-hysterectomized women with at least 6 months till 12 months since last menses were performed in pooled ITT sets by hysterectomy status.

Table 35: Model-adjusted change from baseline to Week 4 and Week 12 for the weekly frequency of moderate to severe VMS, by hysterectomy status – Pooled ITT Sets of MIT-Do001-C301 ESP and -C302 ESP, Non-hysterectomized ⩾12 months since last menses & Hysterectomized (MMRM; post-hoc analysis*)*

HYSTERECTOMIZED

Visit		Statistics	E4 15 mg (N= 209)	E4 20 mg (N=210)	Placebo (N= 209)
Week 4		n	187	195	195
	Model-adjusted change from baseline	LS Mean	-42.47	-49.26	-37.94
		Std Error	2.636	2.505	2.555
		95% CI	(-47.64, -37.30)	(-54.18, -44.35)	(-42.95, -32.93)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-4.52	-11.32	
		Std Error	3.384	3.327	
		95% CI	(-12.01, 2.97)	(-18.69, -3.96)	
		p-value between vs. Placebo	0.3056	0.0013	
Week 12		n	171	175	164
	Model-adjusted change from baseline	LS Mean	-60.08	-65.02	-46.46
		Std Error	2.704	2.590	2.667
		95% CI	(-65.38, -54.78)	(-70.10, -59.94)	(-51.68, -41.23)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-13.62	-18.56	
		Std Error	3.514	3.468	
		95% CI	(-21.40, -5.85)	(-26.24, -10.89)	
		p-value between vs. Placebo	0.0002	<.0001	

NON-HYSTERECTOMIZED ≥12 MONTHS SINCE LAST MENSES

Visit		Statistics	E4 15 mg (N=176)	E4 20 mg (N=173)	Placebo (N=176)
Week 4		n	160	159	163
	Model-adjusted change from baseline	LS Mean	-42.15	-43.03	-27.38
		Std Error	2.720	2.738	2.774
		95% CI	(-47.49, -36.82)	(-48.40, -37.66)	(-32.82, -21.94)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-14.77	-15.65	
		Std Error	3.695	3.648	
		95% CI	(-22.95, -6.60)	(-23.72, -7.58)	
		p-value between vs. Placebo	0.0001	<.0001	
Week 12		n	136	123	136
	Model-adjusted change from baseline	LS Mean	-56.53	-61.40	-41.62
		Std Error	2.842	2.918	2.908
		95% CI	(-62.10, -50.96)	(-67.12, -55.68)	(-47.33, -35.92)
		p-value	<.0001 <.0001 <.0001		
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-14.91	-19.78	
		Std Error	3.880	3.888	
		95% CI	(-23.49, -6.32)	(-28.38, -11.17)	
		p-value between vs. Placebo	0.0002	<.0001	

CI = Confidence Interval; E4 = Estetrol; LS = least square; MMRM = Mixed-effects Model for Repeated Measures; VMS = vasomotor symptoms.

Weekly frequency of moderate to severe VMS at Week x = Total number (sum) of all recorded moderate to severe VMS experienced during the last 7 consecutive days during the week x.

MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment and pooled trial centres as fixed effects and baseline as a covariate.

The autoregressive covariance was used in the modelling of the within-patient errors in the analysis.

Table 36: Model-adjusted change from baseline to Week 4 and Week 12 for the mean severity of moderate to severe VMS (EMA scoring method), by hysterectomy status – Pooled ITT Sets of MIT-Do001-C301 ESP and -C302 ESP, Non-hysterectomized ⩾12 months since last menses & Hysterectomized (MMRM; post-hoc analysis)

HYSTERECTOMIZED

Visit		Statistics	E4 15 mg (N=209)	E4 20 mg (N=210)	Placebo (N=209)
Week 4		n	187	195	195
	Model-adjusted change from baseline	LS Mean	-0.60	-0.68	-0.40
		Std Error	0.064	0.061	0.062
		95% CI	(-0.73, -0.48)	(-0.80, -0.56)	(-0.52, -0.28)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-0.20	-0.28	
		Std Error	0.083	0.082	
		95% CI	(-0.39, -0.02)	(-0.46, -0.09)	
		p-value between vs. Placebo	0.0284	0.0016	
Week 12		n	171	175	164
	Model-adjusted change from baseline	LS Mean	-1.01	-1.26	-0.75
		Std Error	0.066	0.064	0.066
		95% CI	(-1.14, -0.88)	(-1.39, -1.14)	(-0.88, -0.62)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-0.26	-0.51	
		Std Error	0.088	0.087	
		95% CI	(-0.45, -0.07)	(-0.71, -0.32)	
		p-value between vs. Placebo	0.0061	<.0001	

NON-HYSTERECTOMIZED ≥12 MONTHS SINCE LAST MENSES

Visit		Statistics	E4 15 mg (N=176)	E4 20 mg (N=173)	Placebo (N=176)
Week 4		n	160	159	163
	Model-adjusted change from baseline	LS Mean	-0.49	-0.51	-0.27
		Std Error	0.063	0.064	0.064
		95% CI	(-0.62, -0.37)	(-0.64, -0.39)	(-0.40, -0.15)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-0.22	-0.24	
		Std Error	0.086	0.085	
		95% CI	(-0.41, -0.03)	(-0.43, -0.05)	
		p-value between vs. Placebo	0.0196	0.0097	
Week 12		n	136	123	136
	Model-adjusted change from baseline	LS Mean	-1.02	-1.11	-0.60
		Std Error	0.067	0.069	0.068
		95% CI	(-1.15, -0.89)	(-1.25, -0.98)	(-0.74, -0.47)
		p-value	<.0001	<.0001	<.0001
	Model-adjusted change from baseline vs. Placebo	Difference of LS Means	-0.42	-0.51	
		Std Error	0.092	0.093	
		95% CI	(-0.62, -0.21)	(-0.72, -0.31)	
		p-value between vs. Placebo	<.0001	<.0001	

CI = Confidence Interval; E4 = Estetrol; LS = least square; MMRM = Mixed-effects Model for Repeated Measures; VMS = vasomotor symptoms.

Mean severity score of VMS at Week x = Arithmetic mean of the daily severity score values of VMS (moderate or severe) observed during the Week x.

MMRM model with treatment (E4 15 mg, E4 20 mg, Placebo), week, week*treatment and pooled trial centres as fixed effects and baseline as a covariate.

The autoregressive covariance was used in the modelling of the within-patient errors in the analysis.

5.3.6 Overall discussion and conclusions on clinical efficacy

5.3.6.1 Discussion

Overview of the main clinical studies

One clinical phase 2 dose-response study (C201) of 12 weeks has been performed to define the minimum effective dose (MED) of E4 in the requested indication for evaluation in the phase 3 studies.

Two pivotal phase 3 studies (C301) and (C302) were performed to support the proposed indication. Both studies consisted of a placebo-controlled Efficacy Study Part (ESP) of 12 weeks in hysterectomized (H) and non-hysterectomized (NH) postmenopausal women.

Rationale for dose selection in the phase 3 clinical studies

Study C201 (E4 RELIEF study) was a phase 2, randomised, double-blind, placebo-controlled dose-finding study to select the minimum effective dose (MED) of E4 for the treatment of moderate to severe vasomotor symptoms (VMS) in hysterectomized (H) or non-hysterectomized (NH) women. In total, 257 eligible postmenopausal women, presenting at least 7 moderate to severe hot flushes/day or at least 50 moderate to severe hot flushes/week, were randomized 1:1:1:1:1 and received E4 2.5, 5, 10, or 15 mg or placebo for 12 weeks. The inclusion criteria are supported.

Primary efficacy analysis was based on 4 co-primary endpoints of change in weekly frequency and severity of moderate to severe VMS from baseline to Week 4 and Week 12. The reduction from baseline in the weekly *frequency* of VMS was statistically significant versus placebo at Week 4 ($p < 0.05$), but not at Week 12. There were no statistically significant differences in the pairwise comparisons for the E4 2.5, 5 and 10 mg doses compared to placebo. At both weeks 4 and 12, none of the doses of E4 (2.5, 5, 10, and 15 mg) resulted in a statistically significant reduction of the *severity* of moderate to severe VMS from baseline compared to placebo. Sensitivity analyses for all 4 co-primary endpoints led to similar results.

The **secondary efficacy** endpoints (non-alpha controlled) included weekly weighted score of VMS, Menopause Rating Scale (MRS) and Vulvovaginal atrophy (VVA) symptoms are considered supportive. The weekly weighted score of moderate to severe VMS markedly decreased in all treatment groups – including the placebo group – as of Week 1. E4 10 mg and E4 15 mg achieved a larger decrease from baseline than all other treatment groups from Week 3 and Week 2 onwards, respectively. With regards to vulvovaginal atrophy (VVA) symptoms, the results are suggestive of a positive effect of E4. The favourable effects were most pronounced at the E4 15 mg dosage. Results on PD endpoints of vaginal pH, and vaginal maturation index, lipids, HbA1C, SHBG, haemostatic- and bone turnover parameters are discussed in PD section 5.2.3.2.

In conclusion, the 15 mg dose met only one of the four co-primary endpoints, which is minimal supportive for defining a MED. Furthermore, the minimal support for a MED of 15 mg is acknowledged by the applicant as also a higher dose of E4 (i.e. 20 mg) in the pivotal phase 3 studies has been evaluated. The 20 mg dose deviates from the previous scientific advice (EMA/171260/2019) in which a dosing regimen of 30 mg was mentioned by the applicant. The rationale to select the 20 mg as second dose in the pivotal phase 3 studies is mainly based on the rationale from a safety perspective as it appears likely that the highest dose is limited to 20 mg, by minimizing the risk of additional adverse events and maintaining the overall safety profile of E4, as stated by the applicant. This was accepted. Further, the placebo effect was high, but it is in line in most trials in this therapeutic area.

Design and conduct of pivotal phase 3 studies C301 ESP and C302 ESP

The Efficacy Study Parts (ESPs) of C301 and C302 are similar with regard to the primary efficacy analysis at 12 weeks, although the overall study designs in the Safety Study Parts (SSP) are different (see safety section).
In the ESP of both studies, for the primary efficacy analysis both NH and H patients are treated with either E4 15 mg, E4 20 mg or placebo for 13 weeks (i.e. 3 months). However, in study C302, randomized controlled treatment with E4 or placebo was continued up to 53 weeks to assess secondary efficacy endpoints and the safety of long-term exposure to E4 only.
The study design including the 12-week duration and the placebo-controlled design (no active comparator arm) is consistent with the CHMP guideline on clinical investigation of HRT

(EMEA/CHMP/021/97 Rev. 1, 2005). However, an evaluation of efficacy and safety of E4 as monotherapy in non-hysterectomized postmenopausal women, i.e. without concomitant progestogen therapy is not mandatory according to this CHMP guideline due to the increased risk of endometrial malignancy. The introduction of this endometrial safety risk is considered undesirable in the EU. As also acknowledged by the applicant, in standard clinical practice, women with an intact uterus, who are prescribed systemic estrogen therapy, should always receive a progestogen for endometrial protection, as also stated in section 4.2 of the proposed SmPC and in line with the EU Core SmPC for HRT. However, the applicant stated they investigated the effect of E4 only for up to 1 year, as requested by the FDA guideline (*Estrogen and Estrogen/Progestin Drug Products to Treat Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms — Recommendations for Clinical Evaluation, 2003*). Actually, the use of P4 was an exclusion criterion for the phase 3 studies and if P4 administration was deemed necessary for medical reasons (endometrial safety), this subject was discontinued from the trial. On the other hand, it is not clear why all NH-subjects, including the NH-subjects in placebo group received P4 for 14 days at the end of the study. As already stated in the discussion on the phase 2 study C201, the placebo-treated NH-subjects do not need to receive treatment for endometrial protection with P4 200 mg. Still, considering that did not affect the primary outcome of the study, this issue was not further pursued.
The selected **patient population** consisted of women, aged 40–65 years, with ≥7 moderate to severe VMS per day or ≥50 moderate to severe VMS per week and seeking treatment for relief of VMS associated with menopause, which is in line with the CHMP guideline on HRT. Main **inclusion criteria**: for hysterectomized (H) subjects a documented hysterectomy must have occurred ≥6 weeks prior to screening, and for non-hysterectomized (NH) subjects a uterus with bi-layer endometrial thickness ≤ 4 mm on TVUS, and an evaluable endometrial biopsy at screening with no abnormal result. Postmenopausal status was defined for NH subjects as ≥12 months of spontaneous amenorrhea with FSH >40 mIU/mL or ≥6 months of spontaneous amenorrhea with serum FSH >40 mIU/mL and E2 <20 pg/mL or ≥ 6 weeks postsurgical bilateral oophorectomy) and H subjects as serum FSH >40 mIU/mL and E2 <20 pg/mL or at least 6 weeks postsurgical bilateral oophorectomy. Main **exclusion** criteria were the use of estrogen/progestin and androgen/dehydroepiandrosterone containing drug(s) from 1 week to 6 months before screening, depending on the product. Also, other hormonal and non-hormonal products used for the treatment of VMS were prohibited. Furthermore, a history or current malignancy (with a few exceptions), history of acute liver disease, chronic or current acute renal impairment, diagnosis or treatment of major psychiatric disorder, history of alcohol or substance abuse excluded subjects. In general, the inclusion and exclusion criteria are in line with other studies evaluating HRT and reflect the proposed target population (i.e. postmenopausal women with oestrogen deficiency symptoms) and are considered acceptable.

Eligible women were **randomized** 1:1:1 to once daily treatment with E4 15 mg, E4 20 mg or placebo. The procedures for blinding and randomization were appropriate. The trial medication should be taken once daily, at approximately the same time each day, which is consistent with section 4.2 of the proposed SmPC.

The applicant provided an overview with the allowed and prohibited **concomitant treatment** during the trial. In general, this was acceptable. The methods used for the assessment of efficacy are considered appropriate and consistent in all treatment arms.

The **primary efficacy analysis** in both pivotal phase 3 studies C301 ESP and C301 ESP consisted of 4 co-primary endpoints, i.e. the mean change in weekly *frequency* and *severity* of moderate to severe VMS from Baseline to Week 4 and Week 12. The 2 co-primary endpoints regarding the change in *frequency of moderate to severe VMS* are in line with the recommendations in the CHMP 'guideline on HRT' and considered acceptable. The 2 co-primary endpoints regarding the change in *severity of moderate to severe VMS* are in line with the recommendations on efficacy endpoints required by the

FDA guideline on HRT but is not required as primary endpoint in an EU-application. Nevertheless, the 4 co-primary endpoints are considered acceptable. MMRMs were used for the primary analysis, and ANCOVA for the sensitivity analysis. All primary analysis were done on the ITT set, which was considered acceptable. *Post hoc*, an estimand definition was provided. The retrospective definitions are in agreement with what has been described in the protocol and are overall appropriate.

Multiple **secondary endpoints** were defined, which were mainly similar for both pivotal phase 3 studies. No hierarchy or key secondary endpoints were defined. Therefore, all secondary endpoints were treated explorative (non-alpha controlled), and all p-values are considered nominal. Of the secondary endpoints selected, the 50% and 75% responder rate is considered the most relevant as it presents efficacy in VMS on a patient level. Other secondary endpoints included the effect of treatment on frequency and severity of VMS for every week (until week 12), the clinical meaningfulness based on clinical global impression (CGI), health-related quality of life (HRQoL) and Treatment satisfaction (TS) are considered supportive for the primary analysis. The secondary endpoint on the effect on symptoms of VVA (*C301 ESP*) is acknowledged, but systemic acting HRT for solely symptoms of VVA is not recommended in clinical guidelines. The selection of secondary PD and biological endpoints on the effect on lipid and glucose metabolism, hemostasis and bone turnover (*C302 ESP*) are PD biomarkers are in line with the CHMP guideline on HRT, but results are discussed in PD section 4.3.1.

Five **protocol amendments** to the original study protocol of C301 and 7 amendments to the original study protocol of C302 have been made, which generally have been sufficiently described by the applicant.

Efficacy results of pivotal phase 3 studies C301 ESP and C302 ESP

Regarding the **subject disposition** in *C301 ESP,* 3496 subjects were screened, 641 subjects were randomized, and 640 received treatment of which 213 subjects in each E4 15 mg and 20 mg group and 214 subjects in the placebo-group. In total 78.7% (n=504) of subjects completed the study, with 77.9%, 77.0%, and 81.3% for the 15 mg E4, 20 mg E4 and placebo groups, respectively. This appears to be in line with previous HRT studies. The most common reasons for discontinuation are withdrawal of consent for another reason, adverse event, lost to follow-up and lack of efficacy (1.9%, 1.4%, 2.8%, respectively), but see safety section for details.

In *C302 ESP,* 3974 subjects were screened and 584 subjects were randomized, of which 579 received treatment of which 192 in E4 15 mg group, 193 in 20 mg group and 194 in placebo-group. The discontinuation numbers at 12 weeks are considered most important for the (primary) efficacy analysis. The percentage of subjects who completed 12 weeks of treatment in C302 (85% (n=492) was higher than in C301 ESP (81.6%, n=522), with 85.9%, 84.5%, and 84.5% for the 15 mg E4, 20 mg E4 and placebo groups, respectively.

The percentage of women that completed treatment up to 12 weeks is higher in the hysterectomized subgroup compared to non-hysterectomized women. The main reasons were adverse events, which is in agreement with the safety dataset assessment.

Regarding the data analysis sets, the analysis is based on the ITT set, which is appropriate. Sensitivity analyses are planned analogous to the primary analysis, but are based on the mITT and PP set, representing patient groups which are stronger adhering to the treatment regimen, which is acceptable.

The mean **treatment compliance** was relatively high in both studies, with C302 ESP with > 96% in all treatment arms in C301 EPS and with > 85% in study C302 ESP), but C302 has a longer treatment duration of 53 weeks in the C302 study compared to 12 weeks duration in C301. The treatment compliance percentages that are listed by the applicant are based on the number of tablets that are dispensed and returned but also blood samples for analysis of E4 concentrations were taken during

Visits 2, 3 and 4 to confirm treatment compliance. A clear link between E4 levels in blood and treatment compliance could not be found. Considering the very high standard deviations and the above-mentioned warnings with regards to interpreting the data, this was accepted. The number of subjects with at least one major **protocol deviation** during the study was 46.1% in study *C301 ESP* and 38.7% in study *C302 ESP*, which is considered high. In general, the numbers and type of PDs are similarly divided over the study arms.

In general, the **demographic characteristics** were well balanced between the treatment groups in both studies. The subjects had a median age of 54 and 55 years and a median BMI of 27.30 kg/m^2 and 28.40 kg/m^2 for C301 ESP and C302 ESP, respectively.

In both studies ca. 52% of the subjects were hysterectomized and most **baseline characteristics** were well balanced between the treatment groups for both studies, except for VMS frequency. In *C301 ESP*, VMS frequency in the E4 20 mg arm was higher in NH subjects (93.21 VMS/week) than in H-subjects (74.93 VMS/week), a less pronounced but similar trend was also found in the E4 15 mg (80.75 and 77.19 VMS/week) and placebo group (77.27 and 73.77 VMS/week). Overall, the VMS frequency was higher in the E4 treated groups (NH+H combined) compared to placebo (78.94 VMS/week, 83.81 VMS/week and 75.49 VMS/week for 15 mg E4, 20 mg E4 and placebo, respectively). The higher VMS frequency at baseline in the E4-treated groups in C301 compared to the placebo groups could have an advantage in favour of the E4 groups, because these subjects start with a higher number of VMS. However, the applied statistical analysis would take into account baseline differences, and the differences in VMS percentage at baseline in E4 groups compared to placebo are not expected to introduce significant differences. Based on the data, it is justified that these imbalances have not affected the final results.

In *C302 ESP*, VMS frequency in both the E4 15 mg and E4 20 mg arms was lower in NH subjects than in H-subjects (72.92 VMS/week vs 86.51 VMS/week and 71.33 VMS/week vs 86.21 VMS/week). Overall, the VMS frequency was similar in the E4 treated groups combined compared to placebo (79.93 VMS/week, 79.04 VMS/week and 78.86 VMS/week for 15 mg E4, 20 mg E4 and placebo, respectively).

Primary efficacy analysis

Frequency of moderate to severe VMS from baseline to week 4 and week 12:

In study C301, treatment with estetrol 15 mg and 20 mg resulted in significant reductions in the frequency of moderate to severe VMS from baseline to week 4 and 12 compared to placebo. For 15 mg E4, the difference of LS mean was -9.63 ((95% CI: -18.81, -0.45), p=0.0380) and -16.41 ((-25.95, -6.87), p=0.0003) at week 4 and week 12, respectively and for 20 mg E4 this was -14.94 ((-23.85, -6.04), p=0.0004) and -22.49 ((-31.79, -13.19) p<0.0001)), at week 4 and week 12, respectively.
Also, in study C302, treatment with estetrol 15 mg and 20 mg resulted in significant reductions in the frequency of moderate to severe VMS from baseline to week 4 and 12 compared to placebo. For 15 mg E4 the difference of LS mean was -8.42 ((95% CI: -16.64, -0.20), p=0.0436) and -12.20 ((-20.69, -3.71), p=0.0029)) at week 4 and week 12, respectively) and for 20 mg E4 the difference of LS mean was -10.21 ((-18.44, -1.98), p=0.0117) and -15.49 ((-24.04, -6.94), p=0.0001) at week 4 and week 12, respectively).
For C301, most sensitivity analyses on the co-primary efficacy endpoints of VMS frequency were consistent with the primary analysis. For C302 the sensitivity analyses on the ITT and mITT Sets were consistent, but in the PP set a consistent effect was only shown with the 20 mg E4 dose, but not for E4 15 mg.

Severity of moderate to severe VMS from baseline to week 4 and week 12:

In study C301, treatment with estetrol 15 and 20 mg resulted in significant reductions in the severity of moderate to severe VMS from baseline to week 4 and 12 compared to placebo. For 15 mg E4 the difference of LS mean was -0.27 ((95% CI: -0.49, -0.05), p=0.0109) and -0.54 ((-0.77, -0.31),

p=<0.0001), at week 4 and week 12, respectively) and for 20 mg E4 this was -0.29 ((-0.50, -0.08), p=0.0051) and -0.66 ((-0.88, -0.43), p<0.0001), at week 4 and week 12, respectively).
However, in study C302, treatment with estetrol resulted in significant reductions in the severity of moderate to severe VMS from baseline to week 4 and 12 compared with placebo for 20 mg E4 (difference of LS mean (95% CI) of -0.28 ((-0.47, -0.08), p=0.0031) and -0.46 ((-0.66, -0.25), p<0.0001), at week 4 and week 12, respectively) but not for the 15 mg E4 dose (-0.06 ((-0.25, 0.14), p=0.74) and -0.01 ((-0.22, 0.19), p=0.9809), at week 4 and week 12, respectively. These findings were supported by sensitivity analyses in which a significant effect to the primary analyses on severity was only shown with the 20 mg E4 dose.
Taken together, the justification of the place of the 15 mg dose can be accepted for non-hysterectomized women, but not for hysterectomized women. For the latter group, only the 20 mg is considered approvable. This has been addressed by the different dose recommendations for the two patient groups in section 4.2 of the SmPC.

Regarding the **secondary endpoints** (non-alpha controlled), of those which are related to the primary efficacy endpoint of VMS reduction, the 50% and 75% responder analysis for the weekly frequency of moderate to severe VMS (not mild) is considered most relevant and most supportive for the primary efficacy objective as this analysis presents efficacy regarding reduction of VMS in the individual patient. Other secondary endpoints related to the effect on frequency and severity of VMS at other time points, weekly weighted score in VMS, subjects with a clinically important difference (CID) in VMS, health-related quality of life (HRQoL), Treatment satisfaction (TS) and effect on VVA.
Regarding the **responder analysis,** in C301 ESP at Week 12, the proportion of subjects that experienced a ≥50% reduction from baseline in the weekly frequency of moderate to severe VMS was 82.5% and 87.0% with E4 15 mg and E4 20 mg treatment, respectively, compared to 60.5% with placebo (nominal p<0.0001). At Week 12, the proportion of subjects that experienced a ≥75% reduction from baseline was 63.3% and 74.5% of subjects in the E4 15 mg and E4 20 mg treatment arms, respectively, compared to 39.5% with placebo (nominal p<0.0001). In C302 ESP at Week 12, the proportion of subjects that experienced a ≥50% reduction from baseline in the weekly frequency of moderate to severe VMS was 81.3% and 81.7% with E4 15 mg and E4 20 mg treatment, respectively, compared to 61.3% with placebo (nominal p-value of 0.0001 and p<0.0001). At Week 12, the proportion of subjects that experienced a ≥75% reduction from baseline was 49.0% and 56.9% of subjects in the E4 15 mg and E4 20 mg treatment arms, respectively, compared to 37.3% with placebo (nominal p-value of 0.0392 and 0.0007). In conclusion, the responder analyses for both dosages of estetrol in observed in both studies are considered supportive for the primary efficacy analysis.
The **weekly weighted score** of moderate to severe VMS has a profile comparable to that of the weekly frequency of moderate to severe VMS for all 3 treatment groups in both studies. The percentage of subjects with a **clinically important difference (CID)**, assessed using the Clinical Global Impression (CGI) questionnaire was nominally significant for E4 20 mg in both pivotal studies at week 4 and week 12. For the 15 mg dosage, a nominally significant result was found in study C301 (week 4 and 12), but not in study C302. This result is consistent with the primary efficacy endpoint of severity for which no significant reduction was observed for the E4 15 mg dose in study C302. HRQoL was assessed using the **MENQOL questionnaire**, for which a significant improvement at week 12 for both doses (nominal p<0.0001) in study C301 was observed, but not in study C302 ESP. The results obtained with regards to **treatment satisfaction**, using the CGI questionnaire, showed an improvement in the condition at week 4 and 12 (and 52 in C302). Nominally significant differences were mainly seen with the E4 20 mg dose at week 12. The effect on **vulvovaginal atrophy (VVA)** symptoms was only assessed in C301 ESP. The 15 mg E4 dose showed a nominally significant reduction in vaginal dryness (-0.31, nominal p-value =0.0030) and vaginal pain associated with sexual

activity (-0.23, nominal p-value=0.0142) compared to placebo at week 12. Somewhat surprisingly, the 20 mg E4 dose did not show a significant effect for any of the VVA symptoms tested.

Subgroup analyses for the primary endpoint were performed for race, ethnicity, age, BMI, and smoking status. The results of the analyses in general suggested similar effects as observed for the 4 co-primary efficacy endpoints in study C301 and C302. The subgroup analyses however revealed some differences in outcome regarding ethnicity and BMI >30 kg/m^2:

- The results were mostly negative for Hispanic and Latino women. In trial C302 neither frequency nor severity was significant for either dose at either timepoint. In trial C301 only the 20 mg dose was significant for frequency, but not severity.
- In both trials approximately the same amount of women with a BMI 25-30 kg/m^2 and >30 kg/m^2 were included. In trial C301, both doses were significant at both timepoints for BMI 25-30 kg/m^2, but not for BMI >30 kg/m^2. In C302, frequency was significant for BMI >30 kg/m^2 at week 12 for 20 mg and for BMI 25-30 kg/m^2 , but for BMI <25 it was significant for 15 mg at week 4 and 12, and for 20 mg at week 4, with week 12 just being outside the boundary (despite that this group was smaller, having less power). Overall, it seems that there is a potential effect based on weight, and that a significant effect cannot always be ensured for the highest weight groups.

The reasons why statistical significance was not reached in all subgroups in either both or one of the trials was most likely caused by insufficient statistical power. Post-hoc analysis with pooled data from both trials and subgroup analyses of the pooled analysis are in line with the main results, and no reason to doubt the efficacy in any subgroup exists.

Since the group of non-hysterectomized women with at least 6 months till 12 months since last menses are excluded from the indication, additional post-hoc analyses from both trials were performed. In general, these results were consistent with those obtained in the prespecified analysis on the whole ITT set.

The pooled efficacy analyses by hysterectomy status on the pooled datasets of C301 ESP and C302 ESP showed statistical significance for all coprimary endpoints in NH women. For the H women, only the reduction in VMS frequency vs. placebo at week 4 for the 15 mg dose was not met.

5.3.6.2 Conclusions on the clinical efficacy

Based on the primary efficacy analyses obtained in the placebo-controlled 12 weeks of two pivotal ESP studies (C301 ESP and C302 ESP), a statistical significant treatment effect of estetrol compared to placebo has been demonstrated in terms of reduction in frequency of VMS at week 4 and 12 for both the 15 and the 20 mg dose. Furthermore, in terms of a reduction in severity of VMS at week 4 and 12, a statistical significant treatment effect has been demonstrated for the 20 mg dose in both trials, but for the 15 mg dose only in C301 and not C302. However, the 15 mg dose complies with the efficacy requirement of the EMA guideline on HRT, which only states that demonstration of reduction in frequency of VMS is a mandatory efficacy endpoint and not severity, which is an FDA requirement. With regards to the selected primary endpoint of reduction in frequency of VMS, as recommended by the EMA guideline on HRT, both studies demonstrated a significant reduction in VMS frequency versus placebo at week 4 and week 12 for the 15 and 20 mg selected for marketing. It should, however, be noted that the 15 mg dose suggested a lower effect, as compared to the 20 mg dose, specifically in study C302 ESP. Further, the pooled safety data of the 15 mg of E4 were suggestive for less AEs, in particular endometrial AEs, as compared to the 20 mg dose.

Furthermore, of secondary endpoints (non-alpha controlled), the 50% and 75% responder analysis for the weekly frequency of moderate to severe VMS at week 4 and week 12 was considered the most relevant endpoint and most supportive for the primary efficacy objective for both dosages.

Altogether, the currently provided efficacy and safety data might be suggestive for a less effective (although still within acceptable ranges), but safer 15 mg dose, as compared to the 20 mg dose of E4. Therefore, in agreement with the statement in the core SmPC for HRT, it is preferred to have a second (lower) dose available in clinical practice to achieve shared decision-making.

However, the safety advantage for the lower dose is suggested to be mainly endometrium-related and will not play a role for hysterectomized women. For non-hysterectomized women it is considered important to have a lower dose available, which should be the initial starting dose. For hysterectomized women it is considered undesirable to start with a less effective dose, because no safety advantage is required, but it is preferred to start immediately with the highest, most effective dose. Taken together, the justification of the place of the 15 mg dose can be accepted for non-hysterectomized women, but not for hysterectomized women. For the latter group, only the 20 mg is considered approvable. The SmPC reflects this accordingly.

5.4. Clinical safety

Please refer to the table of studies in section 5.3.2.

For the purpose of this document, the following definitions apply:

'Adverse event – AE' means any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

'Serious adverse event – SAE' means any untoward medical occurrence that at any dose requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death. The definition (in line with ICH E2A) includes important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

'Adverse Drug Reaction – ADR' means any untoward and unintended response to a medicinal product related to any dose administered, for which, after a thorough assessment, a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, based for example, on their comparative incidence in clinical trials, or findings from epidemiological studies and/or on an evaluation of causality from individual case reports.

5.4.1. Safety data collection

The clinical safety database for estetrol (E4) is based on the following data:

- Three individual key clinical studies conducted in postmenopausal women for the treatment of vasomotor symptom (VMS), i.e. one phase 2 study (C201) and two phase 3 studies (C301 and C302).
  - The placebo-controlled phase 2 study C201 had a treatment duration of 12 weeks
  - The phase 3 study C301 consisted of a placebo-controlled Efficacy Study Part (ESP) with a treatment duration of 12 weeks and a single-arm Endometrial and General Safety Study Part (SSP) of 52 weeks
  - The phase 3 study C302 consisted also of a 12-weeks placebo-controlled ESP, extended to 9 months for safety (placebo-controlled) and a single-arm SSP with a treatment duration of 52 weeks.
- an Integrated Summary of Safety (ISS) pool, which consisted of placebo-controlled 12-weeks data from the two Phase 3 studies C301 (ESP) and C302 (ESP), and the Phase 2 study C201.
- Twelve supportive Phase 1 and Phase 2 clinical studies conducted in pre- and postmenopausal women.

Table 37: Overview of Key Phase 2 and Phase 3 Clinical Studies with E4 in the Treatment of Vasomotor Symptoms in Postmenopausal Women (Safety Populations)

Study ID	No of study centers Location Study start	Study Phase Objective (brief)	Study design Diagnosis	Number of subjects in the Safety Population	Mean age (range)	Study arms	Duration of Treatment
MIT-Do0001-C201[a]	35 BE (6 sites), CZ (8 sites), UK (4 sites), IE (2 sites), and PL (15 sites) May 2016	2 Efficacy/safety (dose-finding)	R, MC, DB, PC Postmenopausal women (40 to ≤65 years) presenting with VMS	257	54.2 years (41-64)	Placebo: n=55[a] E4 2.5 mg: n=52 E4 5 mg: n=47 E4 10 mg: n=54 E4 15 mg: n=49[a]	12 weeks After completed E4 or placebo treatment, all NH subjects received dydrogesterone 10 mg once daily for 14 days[b]
MIT-Do001-C301	151 PL (32 sites), US (27 sites), BR (24 sites), CZ (19 sites), RO (12 sites), RU (11 sites), UK (9 sites), AR (9 sites), CA (8 sites), LT (8 sites), IT (5 sites), ES (4 sites), HU (2 sites), and SK (1 site) December 2019	3 Efficacy/safety	*Efficacy Study Part, Arms 1-3* R, MC, DB, PC Postmenopausal women (40 to ≤65 years) seeking treatment for moderate to severe VMS associated with menopause	640	53.9 years (41-65)	Placebo: n=214 E4 15 mg: n=213 E4 20 mg: n=213	12 weeks After completed E4 or placebo treatment, all NH subjects received P4 200 mg once daily for 14 days[c]
	211 PL (32 sites), US (63 sites), BR (24 sites), CZ (20 sites), RO (12 sites), RU (11 sites), UK (9 sites), AR (9 sites), CA (11 sites), LT (8 sites), IT (5 sites), ES (4 sites), HU (2 sites), and SK (1 site) December 2019	3 Safety	*Endometrial and General Safety Study Part, Arm 4* MC, OL Postmenopausal women (40 to ≤65 years) seeking treatment for moderate to severe VMS	922 (346 in the Endometrial Safety Analysis Set)	53.9 years (40-65)	E4 20 mg + P4 100 mg: n=922	12 months
				853 with ≥12 months since last menses (316 in the Endometrial Safety Analysis Set)	54.2 years (40-65)	E4 20 mg + P4 100 mg: n=853	

Study ID	No of study centers Location Study start	Study Phase Objective (brief)	Study design Diagnosis	Number of subjects in the Safety Population	Mean age (range)	Study arms	Duration of Treatment
MIT-Do001-C302	110 US (105 sites) and CA (5 sites) September 2019	3 Efficacy/safety	*Efficacy Study Part, Arms 1-3* R, MC, DB, PC Postmenopausal women (40 to ≤65 years) seeking treatment for moderate to severe VMS	579	54.6 years (40-65)	Placebo: n=194 E4 15 mg: n=192 E4 20 mg: n=193	12 months (3 months efficacy evaluation followed by 9 months safety extension) After completed E4 or placebo treatment, all NH subjects received P4 200 mg once daily for 14 days[c]
			Safety Study Part, Arm 4 MC, OL Postmenopausal women (40 to ≤65 years) seeking treatment for moderate to severe VMS	430	54.4 years (41-65)	E4 20 mg: n=430	12 months After completed E4 treatment, all NH subjects received P4 200 mg once daily for 14 days[c]

AR = Argentina, BE = Belgium, BR = Brazil, CA = Canada, CZ = Czech Republic, DB = double-blind, DPE = disordered proliferative endometrium, E4 = estetrol monohydrate ES = Spain, HU = Hungary, IE = Ireland; ISS = Integrated Summary of Safety, IT = Italy, LT = Lithuania, MC = multicenter, n = number per treatment group, NH = Non-hysterectomized, OL = open-label, P4 = progesterone, PC = placebo-controlled, PL = Poland, R = randomized, RO = Romania, RU = Russia, SK = Slovakia, TVUS = transvaginal ultrasonography, UK = United Kingdom, US = United States, VMS = vasomotor symptoms.

a. Only data from the placebo arm and the E4 15 mg study arm were pooled in the ISS analysis.
b. If a bi-layer endometrial thickness ≥15 mm was detected or abnormal uterine bleeding occurred in a NH woman during E4/placebo treatment, the subject received dydrogesterone 10 mg once daily until the end of Week 11 (14 days on, 14 days off).
c. If at any time after the first 4 weeks of treatment a subject presented with persistent and/or recurrent bleeding, or with a bi-layer endometrial thickness >10 mm as assessed by TVUS, an endometrial biopsy was performed. If the biopsy showed DPE, hyperplasia or worse, the study drug was discontinued, and the subject was withdrawn from the study and treated with P4 200 mg once daily for 14 days.

5.4.2. Patient exposure

Table 38: Patient exposure

Study identifier	Study period (study initiation, database lock) No. of subjects	Dose arms Treatment duration	Number of women treated* / completed treatment	Duration of treatment (mean (SD))
Blinded studies (placebo-controlled)				
C201	12-05-2016 to 22-01-2018 Total screened: 609 Total treated: 260 Total completed: 200	E4 15 mg Placebo 13 weeks	49/41 55/41	80.2 (19.21) 76.2 (22.98)

Study identifier	Study period (study initiation, database lock) No. of subjects	Dose arms Treatment duration	Number of women treated* / completed treatment	Duration of treatment (mean (SD))
C301 ESP	30-12-2019 to 07-12-2021 Total screened: 3496 Total treated: 640 Total completed: 504	E4 15 mg E4 20 mg Placebo 13 weeks	213/166 213/164 214/174	80.6 (21.1) 78.9 (25.88) 80.2 (23.23)
C302 ESP	27-09-2019 to 23-11-2022 Total screened (ESP and SSP combined): 3974 Total treated: 579 Total completed: 289	E4 15 mg E4 20 mg Placebo 3/12 months	192/96 193/85 194/108	254.2 (133.85) 239.4 (137.13) 276.8 (132.98)
Open studies (uncontrolled)				
C301 SSP (Endometrial safety study)	30-12-2019 to 13-05-2024 Total screened: unknown Total treated: 922 Total completed: 402	E4 + P4 12 months	922/402 853/372** *Endometrial safety set n=346 n=316**	E4: 222.8 (144.9) P4: 234.6 (140.91) E4: 221.8 (145.44)** P4: 233.8 (140.98)**
C302 SSP	27-09-2019 to 23-11-2022 Total screened (ESP and SSP combined): 3974 Total treated: 430 Total completed: 149	E4 20 mg 12 months	430/149	203.5 (141.22)

**(⩾12 months since last menses)

53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

In total, 579 subjects were exposed to at least 1 dose of E4 or placebo. In the overall safety population, the mean duration of exposure was similar among all 3 study arms: 254.2 days in the E4 15 mg arm, 239.4 days in the E4 20 mg arm, and 276.8 days in the placebo arm.

In the subgroup analysis by hysterectomy status, mean duration of exposure to E4 was longer in the E4 study arms for H subjects (292.9 days in the E4 15 mg arm, 281.6 days in the E4 20 mg arm) than for NH subjects (213.0 days in the E4 15 mg arm, 193.9 days in the E4 20 mg arm), whereas the durations were similar for subjects in the placebo arm, irrespective of hysterectomy status.

13-week placebo-controlled H + NH safety population (E4 monotherapy) - ISS pooled data

The majority (99.5%) of all allocated subjects in the pooled arms were included in the Safety Population. A total of 1323 were treated, 454 with E4 15 mg, 406 with E4 20 mg and 463 with placebo.

The mean duration of exposure was similar between the pooled E4 15 mg (83.7 days) and pooled E4 20 mg (82.4 days) arms and their respective placebo arms (83.6 days and 84.4 days, respectively).

The mean duration of exposure was similar between H (85.4 days in the pooled E4 15 mg arm vs 85.2 days in its placebo arm, 85.3 days in the pooled E4 20 mg arm vs 85.8 days in its placebo arm) and NH subjects (82.1 days in the pooled E4 15 mg arm vs 82.2 days in its placebo arm, 79.2 days in the pooled E4 20 mg arm vs 82.8 days in its placebo arm) within each of the pooled arms.

53-week uncontrolled NH safety population (with E4 + P4 combination therapy) - study C301 SSP

In total, 922 subjects were exposed to at least 1 dose of study drug. The mean (SD) duration of exposure to E4 20 mg was 222.8 (144.90) days and to P4 was 234.6 (140.91).

53-week uncontrolled H + NH safety population (E4 monotherapy) - study C302 SSP

In total, 430 subjects were exposed to at least 1 dose of E4 20 mg. The mean (SD) duration of exposure to E4 20 mg was 203.5 (141.22) days.

In the subgroup analysis by hysterectomy status, duration of exposure to E4 20 mg was longer in H subjects (274.7 (133.87) days for N=201) than in NH subjects (141.0 [115.80] days for N=229). One hundred and seventy-one (74.7%) NH subjects received P4 200 mg once daily for 2 weeks after completion of E4 treatment or early discontinuation due to the results of endometrial biopsy.

Patient disposition, demographics and baseline characteristics

For study C301 ESP and C302 ESP, please refer to section 5.3.2.1.4.

For study C301 SSP (endometrial safety study) please refer to the section endometrial and General Safety Study Part of this safety section.

Patient disposition, demographics and baseline characteristics of study C302 SSP are short presented below.

A total of 431 subjects were included in the SSP (Included Set). One subject did not receive the assigned study drug. A total of 149 of 430 (34.7%) treated subjects completed treatment with the study drug and 281 (65.3%) subjects discontinued the treatment.

The most common reasons (>5% subjects overall) for treatment discontinuation included AEs (20 H subjects [10.0%) and 84 [36.7%] NH subjects), withdrawal of consent for another reason (9.5% and 8.7% for H and NH), loss to follow-up (8.5% and 8.3% for H and NH subjects), and SAEs (3 [1.5%] H subjects and 29 [12.7%] NH subjects). As mandated by the protocol, 26 (11.4%) subjects with disordered proliferative endometrium (DPE) or worse on biopsy were discontinued from the study drug. COVID-19 was directly responsible for 7 (1.6%) subjects discontinuing the study drug.

The summary of demographics is presented below.

Table 39: Summary of Demographics – SSP – SAF MIT-Do001-C302-SSP

Characteristic	E4 20 mg (N = 430)
Age (years)	
Mean (SD)	54.4 (4.88)
Median (Min, Max)	54.0 (41, 65)
Race, n (%)	
American Indian or Alaska Native	0
Asian	5 (1.2)
Black or African American	95 (22.1)
Native Hawaiian or Other Pacific Islander	1 (0.2)
White	325 (75.6)
Other	4 (0.9)
Ethnic Origin, n (%)	
Hispanic/Latino	83 (19.3)
Not Hispanic/Latino	347 (80.7)
Education Level, n (%)	
Less than Upper Secondary School	6 (1.4)
Completed Upper Secondary School	126 (29.3)
Completed Vocational School	42 (9.8)
Completed College	204 (47.4)
Completed Graduate School	46 (10.7)
Unknown	6 (1.4)
Weight (kg)	
Mean (SD)	76.25 (13.350)
Median (Min, Max)	75.70 (45.6, 119.7)
Height (cm)	
Mean (SD)	163.88 (6.321)
Median (Min, Max)	162.70 (148.0, 188.0)
BMI (kg/m^2)	
Mean (SD)	28.33 (4.542)
Median (Min, Max)	28.15 (18.3, 37.9)

Abbreviations: BMI = body mass index; E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); Max = maximum; Min = minimum; n = number of subjects in category of interest; N = number of subjects; SAF = safety analysis set; SD = standard deviation; SSP Safety Study Part

Note: Percentages (%) were based on the number of subjects in the SAF

Baseline characteristics

Of the 430 subjects, 201 (46.7%) subjects were hysterectomized, and 229 (53.3%) subjects were NH. Among hysterectomized subjects, 138 (68.7%) subjects underwent a "total" hysterectomy, and 63

(31.3%) subjects underwent a "subtotal" hysterectomy. A total of 65 (15.1%) subjects underwent a bilateral oophorectomy of whom 46 (70.8%) subjects had documentation available for the procedure.

At Baseline, the mean (SD) weekly frequency of moderate to severe VMS was 26.80 (19.276).

5.4.3. Adverse events

Adverse Events

53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

A total of 354 (61.1%) subjects experienced TEAEs. The majority of TEAEs were mild or moderate.

The overall number of subjects with TEAEs in either subgroup (H or NH) was similar between the E4 15 mg and the E4 20 mg arms and lowest in the placebo arms. Study drug-related TEAEs were reported by a greater proportion of NH subjects than H subjects in the E4 15 mg arm (47.3% vs 25.3%, respectively) and in the E4 20 mg arm (61.3% vs 29.0%, respectively), and by a similar proportion of NH and H subjects in the placebo arms (16.8% vs 22.2%, respectively). In the E4 arms there was a greater proportion of NH subjects with study drug-related TEAEs in the Reproductive System and Breast Disorders SOC, particularly vaginal haemorrhage and endometrial disorder.

Serious TEAEs were reported by a greater proportion of NH subjects than H subjects in the E4 15 mg arm (5.4% vs 4%, respectively) and in the E4 20 mg arm (9.7% vs 0%, respectively), and by a similar proportion of NH and H subjects in the placebo arms (3.2% vs 1.0%, respectively). All study drug-related serious TEAEs were reported in the E4 arms of NH subjects (3 [3.2%] in the E4 15 mg arm, 8 [8.6%] in the E4 20 mg arm) and were findings on endometrial biopsy.

As expected, given the definition of AESIs in this study, all AESIs were reported in NH subjects.

Table 40: Overall Summary of TEAEs in Each Overall Study arm and by Hysterectomy Status Within Each Study arm – MITDo001- C302 ESP (Safety Population)

	E4 15 mg						E4 20 mg						Placebo					
	H (N=99)		NH (N=93)		All (N=192)		H (N=100)		NH (N=93)		All (N=193)		H (N=99)		NH (N=95)		All (N=194)	
	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E
Subjects With At Least One TEAE	61 (61.6)	153	65 (69.9)	202	126 (65.6)	355	60 (60.0)	132	68 (73.1)	209	128 (66.3)	341	52 (52.5)	119	48 (50.5)	123	100 (51.5)	242
Subjects With At Least One Severe TEAE	6 (6.1)	11	5 (5.4)	6	11 (5.7)	17	2 (2.0)	2	2 (2.2)	2	4 (2.1)	4	2 (2.0)	3	1 (1.1)	1	3 (1.5)	4
Subjects With At Least One Study Drug-related TEAE	25 (25.3)	36	44 (47.3)	132	69 (35.9)	168	29 (29.0)	49	57 (61.3)	161	86 (44.6)	210	22 (22.2)	43	16 (16.8)	29	38 (19.6)	72
Subjects With At Least One Serious TEAE [a]	4 (4.0)	7	17 (18.3)	17	21 (10.9)	24	0	0	21 (22.6)	21	21 (10.9)	21	1 (1.0)	1	2 (2.1)	2	3 (1.5)	3
Subjects With At Least One Serious TEAE, with Endometrial Disorders Removed [a]	4 (4.0)	7	5 (5.4)	5	9 (4.7)	12	0	0	9 (9.7)	9	9 (4.7)	9	1 (1.0)	1	2 (2.1)	2	3 (1.5)	3
Subjects With At Least One Study Drug-related Serious TEAE [a]	0	0	15 (16.1)	15	15 (7.8)	15	0	0	20 (21.5)	20	20 (10.4)	20	0	0	0	0	0	0
Subjects With At Least One Study Drug-related Serious TEAE, with	0	0	3 (3.2)	3	3 (1.6)	3	0	0	8 (8.6)	8	8 (4.1)	8	0	0	0	0	0	0

Endometrial Disorders Removed [a]																		
Subjects With At Least One TEAE Leading to Study Drug Discontinuation	4 (4.0)	5	29 (31.2)	47	33 (17.2)	52	7 (7.0)	9	35 (37.6)	45	42 (21.8)	54	4 (4.0)	6	6 (6.3)	11	10 (5.2)	17
Subjects With At Least One TEAE Leading to Death	1 (1.0)	1	0	0	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Subjects With At Least One AESI [b]	0	0	11 (11.8)	12	11 (5.7)	12	0	0	13 (14.0)	14	13 (6.7)	14	0	0	1 (1.1)	1	1 (0.5)	1

AE = adverse event, AESI = adverse event of special interest, CSR = Clinical Study Report, DPE = disordered proliferative endometrium, E = number of events, E4 = estetrol monohydrate, ESP = Efficacy Study Part, H = hysterectomized, n = number of subjects in category of interest, N = number of subjects, NH = non-hysterectomized, SAE = serious adverse event, SAF = Safety Analysis Set, TEAE = treatment emergent adverse event.
Percentages (%) were based on the number of subjects in the SAF in each study arm or its subgroup by hysterectomy status. TEAEs were defined as AEs with an onset date on or after the date of the first dose of study drug and before the date of the last Visit. If the start date of the AE was unknown, an AE was assumed to be after the date of the first dose of study drug unless the stop date was before the first dose.
a. Serious TEAEs include endometrial biopsy findings in NH subjects that were to be reported by means of an SAE report form (up to Protocol Amendment 4 [Protocol Version 5.0]) and serious AESIs.

13-week placebo controlled H + NH safety population (E4 monotherapy) - ISS pooled data

15 mg

The table below provides a summary of TEAE reported up to 13 weeks, pooled for C201, C302 ESP and C302 ESP, combined for H and NH women treated with E4 monotherapy 15 mg.

There was a lower incidence of drug-related TEAEs in H subjects compared with the NH subjects (21.2% and 37.4%, respectively). This was driven by the incidence of drug-related TEAEs in the E4 15 mg arm (25.2% and 58.1% in the H and NH groups, respectively). There was a lower incidence of serious TEAEs in H subjects (0.9%) compared with NH subjects (3.0%). There were no drug-related serious TEAEs reported in H subjects whereas 2.5% of subjects in the NH group experienced a serious TEAE in the E4 15 mg arm and 0.4% in the placebo arm. The incidence of TEAEs leading to study drug discontinuation was lower in H subjects (1.4% [E4 15 mg arm 1.4%; placebo 1.4%]) compared with NH subjects (9.9% [E4 15 mg arm 16.5%; placebo 3.6%]). The majority of these TEAEs were considered related to study drug.

No TEAESIs were reported in H subjects. In NH subjects, a higher proportion of TEAESIs were reported for subjects in the E4 15 mg arm compared with the placebo arm (19.5% and 2.8%, respectively).

20 mg

The table below provides a summary of TEAE reported up to 13 weeks, pooled for C201, C302 ESP and C302 ESP, combined for H and NH women treated with E4 monotherapy 20 mg.

Table 41: Overall summary of AEs reported up to 13 weeks in each arm of each pooled data set and by hysterectomy status within each arm of the pooled data set – Pooled E4 15 mg and Placebo, Pooled E4 20 mg and Placebo (ISS safety populations)

	E4 15 mg			Placebo			E4 20 mg			Placebo		
	H (N=218)	NH (N=236)	All (N=454)	H (N=215)	NH (N=248)	All (N=463)	H (N=210)	NH (N=196)	All (N=406)	H (N=209)	NH (N=199)	All (N=408)
	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E	n (%) E

Any TEAE	95 (43.6) 218	155 (65.7) 465	250 (55.1) 683	80 (37.2) 192	107 (43.1) 250	187 (40.4) 442	94 (44.8) 210	150 (76.5) 479	244 (60.1) 689	79 (37.8) 189	82 (41.2) 185	161 (39.5) 374
Any Study Drug-related TEAE	55 (25.2) 97	137 (58.1) 351	192 (42.3) 448	37 (17.2) 77	44 (17.7) 77	81 (17.5 154	59 (28.1) 110	132 (67.3) 363	191 (47.0) 473	36 (17.2) 76	32 (16.1) 54	68 (16.7) 130
Any Serious TEAE	2 (0.9) 3	19 (8.1) 19	21 (4.6) 22	0	2 (0.8) 2	2 (0.4) 2	2 (1.0) 2	24 (12.2) 28	26 (6.4) 30	0	1 (0.5) 1	1 (0.2) 1
Any Serious TEAE, with Endometrial Disorders Removed	2 (0.9) 3	7 (3.0) 7	9 (2.0) 10	0	2 (0.8) 2	2 (0.4) 2	2 (1.0) 2	14 (7.1) 18	16 (3.9) 20	0	1 (0.5) 1	1 (0.2) 1
Any Study Drug-related Serious TEAE	0	18 (7.6) 18	18 (4.0) 18	0	1 (0.4) 1	1 (0.2) 1	0	20 (10.2) 22	20 (4.9) 22	0	1 (0.5) 1	1 (0.2) 1
Any Study Drug-related Serious TEAE, with Endometrial Disorders Removed	0	6 (2.5) 6	6 (1.3) 6	0	1 (0.4) 1	1 (0.2) 1	0	10 (5.1) 12	10 (2.5) 12	0	1 (0.5) 1	1 (0.2) 1
Any TEAE Leading to Study Drug Discontinuation	3 (1.4) 4	39 (16.5) 55	42 (9.3) 59	3 (1.4) 3	9 (3.6) 18	12 (2.6) 21	6 (2.9) 7	34 (17.3) 44	40 (9.9) 51	3 (1.4) 3	7 (3.5) 14	10 (2.5) 17
Any Study Drug-related TEAE Leading to Study Drug Discontinuation	3 (1.4) 3	38 (16.1) 50	41 (9.0) 53	3 (1.4) 3	9 (3.6) 13	12 (2.6) 16	6 (2.9) 7	32 (16.3) 42	38 (9.4) 49	3 (1.4) 3	7 (3.5) 9	10 (2.5) 12
Any TEAE Leading to Death	2 (0.9) 3	0	2 (0.4) 3	0	0	0	0	0	0	0	0	0
Any Study Drug-related TEAE Leading to Death	0	0	0	0	0	0	0	0	0	0	0	0
Any AESI	0	46 (19.5) 50	46 (10.1) 50	0	7 (2.8) 7	7 (1.5) 7	0	49 (25.0) 54	49 (12.1) 54	0	7 (3.5) 7	7 (1.7) 7

AESI = adverse event of special interest, E = number of events, E4 = estetrol monohydrate, ESP = Efficacy Study Part, H = hysterectomized, ISS = Integrated Summary of Safety, n = number of observations per treatment group, N = number of subjects in the statistical analysis set, NH = non-hysterectomized, TEAE = treatment emergent adverse event.

Percentages (%) based on the number of subjects in the Safety Population in each pooled treatment arm or its subgroup by hysterectomy status. TEAEs were defined as those AEs occurring from time point of first ingestion of investigational product until last Visit or any event already present that worsened in either intensity or frequency following exposure to the treatment. If the start date of the AE was unknown, it was assumed to be after the start of study drug unless the stop date was before the first dose.

Pooled E4 15 mg and Placebo: PHASE 3 STUDIES MIT-Do001-C301 ESP and MIT-Do001-C302 ESP, PHASE 2 STUDY MIT-Do0001-C201

Pooled E4 20 mg and Placebo: PHASE 3 STUDIES MIT-Do001-C301 ESP and MIT-Do001-C302 ESP

The pooled placebo arm for the E4 15 mg dataset contained the same subjects from studies MIT-Do001-C301 ESP and MIT-Do001-C302 ESP as the pooled placebo arm for the E4 20 mg dataset; however, the pooled placebo arm for the E4 15 mg dataset also contained subjects from the placebo arm of study MIT-Do001-C201.

When analyzed by hysterectomy status, the overall incidence of TEAEs was lower in H subjects (41.3% [E4 20 mg 44.8%; placebo 37.8%]) compared with NH subjects (58.7% [E4 20 mg 76.5%; placebo 41.2%]). This was also seen for drug-related TEAEs with a lower incidence in H subjects (22.7% [E4 20 mg 28.1%; placebo 17.2%]) compared with NH subjects (41.5% [E4 20 mg 67.3%; placebo 16.1%]). There was a lower incidence in serious TEAEs in H subjects (E4 20 mg 1.0%; placebo 0%]) compared with NH subjects (E4 20 mg 7.1%; placebo 1.0%]). None of the serious TEAEs in H subjects was considered drug-related, whereas the majority of serious TEAEs in NH subjects were considered drug-related (5.1%). There was a lower incidence of TEAEs leading to study drug discontinuation in H subjects (2.1% [E4 20 mg 2.9%; placebo 1.4%]) compared with NH subjects (10.4% [E4 20 mg 17.3%; placebo 3.5%]). In H subjects all these TEAEs were considered study drug-related. In NH subjects the majority were considered study drug-related (9.9%).

No TEAESIs were reported in H subjects. In NH subjects there was a higher incidence of TEAESIs reported for the E4 20 mg arm (25.0%) compared with the placebo arm (3.5%).

53-week uncontrolled NH safety population (with E4 + P4 combination therapy) - study C301 SSP

A total of 783 (84.9%) NH subjects experienced 4026 TEAEs. The majority of TEAEs were mild or moderate. A total of 64 severe TEAEs were reported in 45 (4.9%) NH subjects.

Study drug-related TEAEs were reported in 737 (79.9%) subjects. Serious TEAEs were reported in 32 (3.5%) subjects, and in 20 (2.2%) subjects these were study drug-related. TEAEs led to study drug discontinuation in 269 (29.2%) subjects. A total of 801 AESIs were reported by 537 (58.2%) subjects.

In the sub-population, 717 (84.1%) NH subjects with ≥12 months since last menses experienced 3665 TEAEs. The majority of TEAEs were mild or moderate. A total of 59 severe TEAEs were reported in 40 (4.7%) NH subjects with ≥12 months since last menses.

Study drug-related TEAEs were reported in 675 (79.1%) subjects with ≥12 months since last menses. Serious TEAEs were reported in 30 (3.5%) subjects, and in 20 (2.3%) subjects these were study drug-related. TEAEs led to study drug discontinuation in 251 (29.4%) subjects. A total of 726 AESIs were reported by 489 (57.3%) subjects.

Table42: Overall Summary of TEAEs in NH Subjects – MIT-Do001-C301 SSP (Safety Population)

	E4 20 mg – 100 mg P4 Full population (N=922)		E4 20 mg – 100 mg P4 Sub-population: ≥12 months since last menses (N=853)	
	n (%)	E	n (%)	E
Number Of Subjects With At Least One TEAE	783 (84.9)	4026	717 (84.1)	3665
Subjects With At Least One Severe TEAE	45 (4.9)	64	40 (4.7)	59
Subjects With At Least One Study Drug-related TEAE	737 (79.9)	3312	675 (79.1)	3019
Subjects With At Least One Serious TEAE [a]	32 (3.5)	36	30 (3.5)	34
Subjects With At Least One Serious TEAE, with Endometrial Disorders Removed [a]	25 (2.7)	29	23 (2.7)	27
Subjects With At Least One Study Drug-related Serious TEAE [a]	20 (2.2)	20	20 (2.3)	20
Subjects With At Least One Study Drug-related Serious TEAE, with Endometrial Disorders Removed [a]	13 (1.4)	13	13 (1.5)	13
Subjects With At Least One TEAE Leading to Dose Interruption	69 (7.5)	114	60 (7.0)	101
Subjects With At Least One TEAE Leading to Study Drug Discontinuation	269 (29.2)	349	251 (29.4)	325
Subjects With At Least One TEAE Leading to Death	0	0	0	0
Subjects With At Least One Study Drug-related TEAE Leading to Death	0	0	0	0

	E4 20 mg – 100 mg P4 Full population (N=922)		E4 20 mg – 100 mg P4 Sub-population: ≥12 months since last menses (N=853)	
	n (%)	E	n (%)	E
Subjects With At Least One AESI [b]	537 (58.2)	801	489 (57.3)	726

AESI = adverse events of special interest, CSR = Clinical Study Report, E = number of events, E4 = estetrol monohydrate (, n = number of subjects in category of interest, N = number of subjects, NH = non-hysterectomized, P4 = progesterone, SSP = Safety Study Part, TEAE = treatment emergent adverse event.
Note: Percentages (%) are based on the number of subjects in the Safety Analysis Set.
a. Serious TEAEs include serious AESIs.
b. AESIs include both serious and non-serious AESIs.

53-week uncontrolled H + NH safety population (E4 monotherapy) - study C302 SSP

The overall number of subjects with TEAEs was higher in the NH subgroup (188 [82.1%]) than in H subgroup (126 [62.7%]). Likewise, the number of subjects with severe TEAEs, study drug-related TEAEs, and serious TEAEs were higher in the NH subgroup (23 [10.0%], 172 [75.1%], 14 [6.1%], respectively) than in the H subgroup (11 [5.5%], 76 [37.8%], 4 [2.0%]). Study drug-related serious TEAEs were reported in 2 (1%) H subjects and 10 (4.4%) NH subjects. Discontinuations due to TEAEs were also higher in NH subjects (145 [63.3%]) than in H subjects (24 [11.9%]). The Reproductive System and Breast Disorders SOC accounted for the main difference between the number of NH and H subjects with the different types of TEAEs. As might be expected given the definition of AESIs in this study, all AESIs were reported in NH subjects.

Table 43: Overview of TEAEs in Overall Safety Population and by Hysterectomy Status – MIT-Do001-C302 SSP (Safety Population)

	E4 20 mg					
	H (N=201)		NH (N=229)		All (N=430)	
	n (%)	E	n (%)	E	n (%)	E
Subjects With At Least One TEAE	126 (62.7)	326	188 (82.1)	677	314 (73.0)	1003
Subjects With At Least One Severe TEAE	11 (5.5)	15	23 (10.0)	34	34 (7.9)	49
Subjects With At Least One Study Drug-related TEAE	76 (37.8)	135	172 (75.1)	542	248 (57.7)	677
Subjects With At Least One Serious TEAE [a]	4 (2.0)	7	56 (24.5)	59	60 (14.0)	66
Subjects With At Least One Serious TEAE, with Endometrial Disorders Removed [a]	4 (2.0)	7	14 (6.1)	15	18 (4.2)	22
Subjects With At Least One Study Drug-related Serious TEAE [a]	2 (1.0)	2	54 (23.6)	57	56 (13.0)	59
Subjects With At Least One Study Drug-related Serious TEAE, with Endometrial Disorders Removed [a]	2 (1.0)	2	10 (4.4)	11	12 (2.8)	13
Subjects With At Least One TEAE Leading to Study Drug Discontinuation	24 (11.9)	39	145 (63.3)	215	169 (39.3)	254
Subjects With At Least One TEAE Leading to Death	0	0	0	0	0	0

	E4 20 mg					
	H (N=201)		NH (N=229)		All (N=430)	
	n (%)	E	n (%)	E	n (%)	E
Subjects With At Least One AESI [b]	0	0	51 (22.3)	57	51 (11.9)	57

AE = adverse event, AESI = adverse event of special interest, CSR = Clinical Study Report, E = number of events, E4 = estetrol monohydrate H = hysterectomized; n = number of subjects in category of interest, N = number of subjects, NH = non-hysterectomized, SAE = serious adverse event, SAF = safety analysis set, SSP = Safety Study Part, TEAE = treatment emergent adverse event.
Percentages (%) were based on the number of subjects in the SAF. TEAEs were defined as AEs with an onset date on or after the date of the first dose of study drug and before the date of the last Visit. If the start date of the AE was unknown, an AE was assumed to be after the date of the first dose of study drug unless the stop date was before the first dose.
a Serious TEAEs reported in NH subjects included endometrial biopsy findings that were to be reported by means of an SAE report form (up to Protocol Amendment 4; Protocol Version 5.0) and serious AESIs.
b AESIs included both serious and non-serious AESIs.

Common Adverse events

53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

The overall incidence of TEAEs of the reproductive system and breast disorders SOC, and abdominal pain, was higher in the non-hysterectomized (NH) group (284 TEAEs in 116 [41.3%] subjects) than in the hysterectomized group (84 TEAEs in 61 [20.5%] subjects).

Among hysterectomized subjects, 25 (25.3%) subjects in the E4 15 mg arm reported 29 TEAEs within the reproductive system and breast disorders SOC; 22 (22.0%) subjects reported 28 TEAEs in the E4 20 mg arm, and 9 (9.1%) subjects reported 16 TEAEs in the placebo arm. Breast tenderness was reported by 15 (15.2%) subjects (16 TEAEs) in the E4 15 mg arm; 3 (3.0%) subjects reported 3 events of nipple pain; 2 (2.0%) subjects reported 2 events of breast pain; and 2 (2.0%) subjects reported 2 events of vaginal discharge. Within the E4 20 mg arm, 14 (14.0%) subjects reported 14 events of breast tenderness; 4 (4.0%) subjects reported 4 events of nipple pain; 2 (2.0%) subjects reported 2 events of vaginal discharge; and 2 (2.0%) subjects reported 2 events of breast pain. In the placebo arm, 6 (6.1%) subjects reported 6 events of breast tenderness; 2 (2.0%) subjects reported 3 events of nipple pain; 1 (1.0%) subject reported 1 event of breast pain, and no subjects reported vaginal discharge.

In the E4 15 mg arm, 2 (2.2%) subjects reported 2 events of abdominal pain, and 1 (1.0%) subject reported a single event of abdominal pain lower. In the E4 20 mg arm; 3 (3.0%) subjects experienced 3 events of abdominal pain lower while 2 (2.0%) subjects reported 2 events of abdominal pain. In the placebo arm, 2 (2.0%) subjects reported 2 events of abdominal pain upper, and 1 (1.0) subject reported a single event of abdominal pain lower.

Among NH subjects, 46 (49.5%) subjects in the E4 15 mg arm reported 120 TEAEs within the reproductive system and breast disorders SOC; 55 (59.1%) subjects reported 140 such TEAEs in the E4 20 mg arm, and 12 (12.6%) subjects reported 16 such TEAEs in the placebo arm. A summary of the 10 most frequent TEAEs reported by NH subjects in this SOC is provided here.

Within the E4 15 mg treatment arm, vaginal haemorrhage was reported by 28 (30.1%) subjects (47 TEAEs, of which 30 were vaginal bleeding and 17 vaginal spotting); 21 (22.6%) subjects reported 21 events of endometrial disorder; 15 (16.1%) subjects reported 16 events of endometrial thickening; 7 (7.5%) subjects reported 7 events of breast tenderness; 7 (7.5%) subjects reported 7 events of vaginal discharge; 4 (4.3%) subjects reported 4 events of endometrial hyperplasia; 4 (4.3%) subjects reported 4 events of uterine haemorrhage (all were uterine bleeding); 2 (2.2%) subjects reported 2

events of uterine spasm; 2 (2.2%) subjects reported 2 events of nipple pain; and no subjects reported ovarian cyst. Within the E4 20 mg arm, 35 (37.6%) subjects reported 56 events of vaginal haemorrhage (of these 17 events were vaginal spotting); 23 (24.7%) subjects reported 23 events of endometrial disorder; 18 (19.4%) subjects reported 19 events of endometrial thickening; 10 (10.8%) subjects reported 10 events of breast tenderness; 11 (11.8%) subjects reported 11 events of endometrial hyperplasia; 4 (4.3%) subjects reported 4 events of uterine haemorrhage; 4 (4.3%) subjects reported 4 events of uterine spasm; 2 (2.2%) subjects reported 2 events of nipple pain; 2 (2.2%) subjects reported 2 events of ovarian cyst; and 1 (1.1%) subject reported 1 event of vaginal discharge. Within the placebo arm, 5 (5.3%) subjects reported 7 events of vaginal haemorrhage; 3 (3.2%) subjects reported 3 events of ovarian cyst; 1 (1.1%) subject reported 1 event of endometrial disorder; 1 (1.1%) subject reported 1 event of endometrial thickening; 1 (1.1%) subject reported 1 event of vaginal discharge; and no subjects reported breast tenderness, endometrial hyperplasia, uterine haemorrhage, uterine spasm, or nipple pain.

In the E4 15 mg arm, 2 (2.2%) subjects reported 2 events of abdominal pain, and 1 (1.1%) subject reported 1 event of abdominal pain upper. In the E4 20 mg treatment arm, 3 (3.2%) subjects reported 3 events of abdominal pain, while in the placebo arm 2 (2.1%) subjects reported 2 events of abdominal pain.

13-week placebo-controlled H + NH safety population (E4 monotherapy) - ISS pooled data

15 mg

The most reported TEAEs (in ≥5% of subjects) were vaginal haemorrhage (17.0%), endometrial disorder (12.6%), breast tenderness (6.8%), breast pain (5.3%) in the Reproductive System and Breast Disorders SOC, and headache (7.5%) in the Nervous System Disorders SOC. In the placebo arm, headache (9.7%) was the only event reported in ≥5% of subjects.

Markedly fewer TEAEs in the Reproductive System and Breast Disorders SOC were reported for H subjects compared with NH subjects, (12.0% and 33.3%, respectively). This difference was mainly due to absence of endometrium related AEs and a lower incidence of vaginal haemorrhage. In NH subjects, the most reported TEAEs (≥5%) were vaginal haemorrhage (18.8%), endometrial disorder (12.8%), headache (8.9%). In H subjects, headache was the only TEAE reported in ≥5% of total subjects (8.3%).

In hysterectomized subjects, there were 6 severe TEAEs in 5 (2.3%) subjects in the E4 15 mg arm and 3 in 3 (1.4%) subjects in the placebo arm. In NH subjects, there were 8 severe TEAEs in 6 (2.5%) subjects in the E4 15 mg arm and 10 in 7 (1.4%) subjects in the placebo arm. Severe Headache in both H and NH subjects and arthralgia in H subjects were the only severe TEAEs reported in more than one subject.

Table44: TEAEs Reported up to 13 Weeks in ⩾1.0% of Total Subjects of either Hysterectomized or Non-hysterectomized group (Safety Population) - Pooled E4 15 mg and Placebo

	Hysterectomized			Non-hysterectomized		
System Organ Class Preferred Term	E4 15 mg (N=218)	Placebo (N=215)	Total (N=433)	E4 15 mg (N=236)	Placebo (N=248)	Total (N=484)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Any TEAEs	95 (43.6%) 218	80 (37.2%) 192	175 (40.4%) 410	155 (65.7%) 465	107 (43.1%) 250	262 (54.1%) 715
Reproductive System and	43 (19.7%) 55	9 (4.2%) 14	52 (12.0%) 69	128 (54.2%) 292	33 (13.3%) 47	161 (33.3%) 339

	Hysterectomized			Non-hysterectomized		
System Organ Class Preferred Term	**E4 15 mg (N=218)**	**Placebo (N=215)**	**Total (N=433)**	**E4 15 mg (N=236)**	**Placebo (N=248)**	**Total (N=484)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Breast Disorders						
Vaginal haemorrhage	1 (0.5%) 1	0	1 (0.2%) 1	76 (32.2%) 123	15 (6.0%) 24	91 (18.8%) 147
Endometrial disorder	0	0	0	57 (24.2%) 57	5 (2.0%) 5	62 (12.8%) 62
Breast tenderness	18 (8.3%) 18	3 (1.4%) 3	21 (4.8%) 21	13 (5.5%) 13	1 (0.4%) 1	14 (2.9%) 14
Endometrial thickening	0	0	0	21 (8.9%) 22	1 (0.4%) 1	22 (4.5%) 23
Breast pain	14 (6.4%) 16	0	14 (3.2%) 16	10 (4.2%) 10	3 (1.2%) 3	13 (2.7%) 13
Vaginal discharge	4 (1.8%) 4	0	4 (0.9%) 4	11 (4.7%) 11	2 (0.8%) 2	13 (2.7%) 13
Uterine haemorrhage	0	0	0	9 (3.8%) 11	2 (0.8%) 2	11 (2.3%) 13
Nipple pain	6 (2.8%) 6	3 (1.4%) 4	9 (2.1%) 10	6 (2.5%) 6	0	6 (1.2%) 6
Endometrial hyperplasia	0	0	0	6 (2.5%) 6	0	6 (1.2%) 6
Menopausal symptoms	0	1 (0.5%) 1	1 (0.2%) 1	4 (1.7%) 4	1 (0.4%) 1	5 (1.0%) 5
Infections and Infestations	**21 (9.6%) 24**	**18 (8.4%) 22**	**39 (9.0%) 46**	**23 (9.7%) 28**	**32 (12.9%) 37**	**55 (11.4%) 65**
COVID-19	7 (3.2%) 7	5 (2.3%) 5	12 (2.8%) 12	4 (1.7%) 4	2 (0.8%) 2	6 (1.2%) 6
Urinary tract infection	5 (2.3%) 5	2 (0.9%) 2	7 (1.6%) 7	1 (0.4%) 1	7 (2.8%) 7	8 (1.7%) 8
Nasopharyngitis	2 (0.9%) 2	2 (0.9%) 2	4 (0.9%) 4	2 (0.8%) 3	3 (1.2%) 3	5 (1.0%) 6
Upper respiratory tract infection	0	0	0	1 (0.4%) 2	4 (1.6%) 4	5 (1.0%) 6
Gastrointestinal Disorders	**17 (7.8%) 26**	**18 (8.4%) 19**	**35 (8.1%) 45**	**30 (12.7%) 36**	**23 (9.3%) 31**	**53 (11.0%) 67**
Nausea	7 (3.2%) 11	3 (1.4%) 3	10 (2.3%) 14	5 (2.1%) 5	3 (1.2%) 3	8 (1.7%) 8
Abdominal pain	3 (1.4%) 3	4 (1.9%) 4	7 (1.6%) 7	6 (2.5%) 6	4 (1.6%) 4	10 (2.1%) 10
Abdominal distension	1 (0.5%) 1	0	1 (0.2%) 1	5 (2.1%) 6	3 (1.2%) 3	8 (1.7%) 9
Abdominal pain upper	0	2 (0.9%) 2	2 (0.5%) 2	3 (1.3%) 3	3 (1.2%) 3	6 (1.2%) 6
Diarrhoea	3 (1.4%) 3	2 (0.9%) 2	5 (1.2%) 5	2 (0.8%) 2	4 (1.6%) 4	6 (1.2%) 6
Abdominal pain lower	3 (1.4%) 3	1 (0.5%) 1	4 (0.9%) 4	4 (1.7%) 4	1 (0.4%) 1	5 (1.0%) 5
Nervous System Disorders	**23 (10.6%) 33**	**21 (9.8%) 51**	**44 (10.2%) 84**	**21 (8.9%) 27**	**30 (12.1%) 45**	**51 (10.5%) 72**
Headache	18 (8.3%) 28	18 (8.4%) 37	36 (8.3%) 65	16 (6.8%) 21	27 (10.9%) 37	43 (8.9%) 58
Dizziness	3 (1.4%) 3	2 (0.9%) 3	5 (1.2%) 6	4 (1.7%) 4	2 (0.8%) 2	6 (1.2%) 6
Musculoskeletal and Connective Tissue Disorders	**13 (6.0%) 13**	**15 (7.0%) 21**	**28 (6.5%) 34**	**9 (3.8%) 12**	**17 (6.9%) 21**	**26 (5.4%) 33**
Back pain	4 (1.8%) 4	1 (0.5%) 1	5 (1.2%) 5	2 (0.8%) 2	7 (2.8%) 9	9 (1.9%) 11
Arthralgia	2 (0.9%) 2	4 (1.9%) 4	6 (1.4%) 6	2 (0.8%) 4	4 (1.6%) 4	6 (1.2%) 8
Pain in extremity	2 (0.9%) 2	3 (1.4%) 3	5 (1.2%) 5	1 (0.4%) 1	2 (0.8%) 2	3 (0.6%) 3
Investigations	**10 (4.6%) 13**	**10 (4.7%) 10**	**20 (4.6%) 23**	**5 (2.1%) 6**	**7 (2.8%) 8**	**12 (2.5%) 14**

	Hysterectomized			Non-hysterectomized		
System Organ Class Preferred Term	**E4 15 mg (N=218)**	**Placebo (N=215)**	**Total (N=433)**	**E4 15 mg (N=236)**	**Placebo (N=248)**	**Total (N=484)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Weight increased	8 (3.7%) 8	4 (1.9%) 4	12 (2.8%) 12	3 (1.3%) 3	2 (0.8%) 2	5 (1.0%) 5
Skin and subcutaneous tissue disorders	**11 (5.0%) 12**	**9 (4.2%) 9**	**20 (4.6%) 21**	**8 (3.4%) 10**	**2 (0.8%) 2**	**10 (2.1%) 12**
Rash	3 (1.4%) 3	3 (1.4%) 3	6 (1.4%) 6	0	0	0
General disorders and administration site conditions	**8 (3.7%) 8**	**8 (3.7%) 9**	**16 (3.7%) 17**	**8 (3.4%) 10**	**8 (3.2%) 10**	**16 (3.3%) 20**
Fatigue	3 (1.4%) 3	2 (0.9%) 2	5 (1.2%) 5	2 (0.8%) 2	3 (1.2%) 3	5 (1.0%) 5
Psychiatric Disorders	**6 (2.8%) 6**	**7 (3.3%) 9**	**13 (3.0%) 15**	**4 (1.7%) 4**	**8 (3.2%) 11**	**12 (2.5%) 15**
Anxiety	3 (1.4%) 3	2 (0.9%) 2	5 (1.2%) 5	2 (0.8%) 2	2 (0.8%) 2	4 (0.8%) 4
Insomnia	0	1 (0.5%) 3	1 (0.2%) 3	1 (0.4%) 1	4 (1.6%) 4	5 (1.0%) 5
Respiratory, Thoracic and Mediastinal Disorders	**4 (1.8%) 4**	**3 (1.4%) 3**	**7 (1.6%) 7**	**5 (2.1%) 6**	**9 (3.6%) 11**	**14 (2.9%) 17**
Metabolism and Nutrition Disorders	**3 (1.4%) 3**	**7 (3.3%) 7**	**10 (2.3%) 10**	**7 (3.0%) 7**	**6 (2.4%) 6**	**13 (2.7%) 13**
Vitamin D deficiency	2 (0.9%) 2	3 (1.4%) 3	5 (1.2%) 5	3 (1.3%) 3	2 (0.8%) 2	5 (1.0%) 5
Injury, poisoning and procedural complications	**9 (4.1%) 11**	**2 (0.9%) 2**	**11 (2.5%) 13**	**2 (0.8%) 3**	**5 (2.0%) 5**	**7 (1.4%) 8**
Renal and urinary disorders	**4 (1.8%) 4**	**5 (2.3%) 5**	**9 (2.1%) 9**	**7 (3.0%) 7**	**4 (1.6%) 4**	**11 (2.3%) 11**
Vascular Disorders	**3 (1.4%) 3**	**5 (2.3%) 5**	**8 (1.8%) 8**	**4 (1.7%) 4**	**5 (2.0%) 5**	**9 (1.9%) 9**
Hypertension	3 (1.4%) 3	2 (0.9%) 2	5 (1.2%) 5	3 (1.3%) 3	1 (0.4%) 1	4 (0.8%) 4

Abbreviations: COVID-19 = coronavirus disease 2019, e = number of events, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), N = number of subjects in the statistical analysis set, n = number of observations per treatment group, PT = preferred term, SOC = system organ class, TEAE = treatment emergent adverse event.
Note: Percentage (%) based on the number of subjects in Safety population in each treatment arm or total column.
Classifications of adverse events based on the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0).
TEAEs defined as those AEs occurring from time point of first ingestion of investigational product until last visit or any event already present that worsens in either intensity or frequency following exposure to the treatment. If the start date of the AE is unknown, it will be assumed to be after the start of study drug unless the stop date is before the first dose.
Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.
PHASE 3 STUDIES: MIT-Do001-C301 ESP and MIT-Do001-C302 ESP, PHASE 2 STUDY: MIT-Do0001-C201

20 mg

For the E4 20 mg arm, the most reported TEAEs (in ≥5% of subjects) were vaginal haemorrhage (20.9%), endometrial disorder (12.3%), breast tenderness (8.6%), breast pain (6.4%), endometrial thickening (5.9%) in the Reproductive System and Breast Disorders SOC and headache (6.2%) in the Nervous System Disorders SOC. In the placebo arm, headache (9.6%) was the only event reported in ≥5% of subjects.

Markedly fewer Reproductive System and Breast Disorders SOC TEAEs were reported for H subjects compared with NH subjects (13.8% and 38.5%, respectively). This difference was mainly due to absence of endometrium related TEAEs and a lower incidence of vaginal haemorrhage. The number of breast related TEAEs (breast tenderness, breast pain, nipple pain and breast discomfort) in subjects treated with E4 20 mg was higher in H (a total of 53 TEAEs) compared with NH subjects (35 TEAEs).

In H subjects, headache and breast tenderness were the only TEAEs reported in ≥5% of total subjects (7.2% and 5.7%, respectively). In NH subjects, the most commonly reported TEAEs (≥5%) were vaginal haemorrhage (24.3%), endometrial disorder (13.9%), headache (8.6%), and endometrial thickening (6.1%).

There was no marked difference in the incidence of severe TEAEs when analyzed by hysterectomy status for the pooled E4 20 mg and placebo group (<3% in both subgroups). Headache was the only severe TEAE reported in more than one subject in H subjects (0.5%), and severe vaginal haemorrhage was the only severe TEAE reported in more than one subject in NH subjects (1.0%).

Table 45: TEAEs Reported up to 13 Weeks in ⩾1.0% of Total Subjects of Either Hysterectomized or Non-hysterectomized Group by SOC and PT, (Safety Population) - Pooled E4 20 mg and Placebo

	Hysterectomized			Non-hysterectomized		
SOC PT	**E4 20 mg (N=210)**	**Placebo (N=209)**	**Total (N=419)**	**E4 20 mg (N=196)**	**Placebo (N=199)**	**Total (N=395)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Any TEAE	**94 (44.8%) 210**	**79 (37.8%) 189**	**173 (41.3%) 399**	**150 (76.5%) 479**	**82 (41.2%) 185**	**232 (58.7%) 664**
Reproductive System and Breast Disorders	**49 (23.3%) 68**	**9 (4.3%) 14**	**58 (13.8%) 82**	**127 (64.8%) 315**	**25 (12.6%) 35**	**152 (38.5%) 350**
Vaginal haemorrhage	1 (0.5%) 1	0	1 (0.2%) 1	84 (42.9%) 155	12 (6.0%) 18	96 (24.3%) 173
Endometrial disorder	0	0	0	50 (25.5%) 50	5 (2.5%) 5	55 (13.9%) 55
Endometrial thickening	0	0	0	24 (12.2%) 24	0	24 (6.1%) 24
Breast tenderness	21 (10.0%) 21	3 (1.4%) 3	24 (5.7%) 24	14 (7.1%) 17	0	14 (3.5%) 17
Breast pain	16 (7.6%) 16	0	16 (3.8%) 16	10 (5.1%) 10	2 (1.0%) 2	12 (3.0%) 12
Nipple pain	10 (4.8%) 12	3 (1.4%) 4	13 (3.1%) 16	5 (2.6%) 5	0	5 (1.3%) 5
Endometrial hyperplasia	0	0	0	12 (6.1%) 12	0	12 (3.0%) 12
Vaginal discharge	3 (1.4%) 3	0	3 (0.7%) 3	6 (3.1%) 7	2 (1.0%) 2	8 (2.0%) 9
Pelvic pain	0	2 (1.0%) 2	2 (0.5%) 2	6 (3.1%) 8	0	6 (1.5%) 8
Breast discomfort	4 (1.9%) 4	1 (0.5%) 1	5 (1.2%) 5	3 (1.5%) 3	2 (1.0%) 2	5 (1.3%) 5
Uterine haemorrhage	0	0	0	4 (2.0%) 4	0	4 (1.0%) 4
Uterine spasm	0	0	0	4 (2.0%) 4	0	4 (1.0%) 4
Vulvovaginal pruritus	2 (1.0%) 2	0	2 (0.5%) 2	2 (1.0%) 2	2 (1.0%) 2	4 (1.0%) 4
Infections and Infestations	**22 (10.5%) 25**	**18 (8.6%) 22**	**40 (9.5%) 47**	**24 (12.2%) 29**	**22 (11.1%) 24**	**46 (11.6%) 53**
COVID-19	5 (2.4%) 5	5 (2.4%) 5	10 (2.4%) 10	6 (3.1%) 6	2 (1.0%) 2	8 (2.0%) 8
Upper respiratory tract infection	0	0	0	1 (0.5%) 1	4 (2.0%) 4	5 (1.3%) 5

	Hysterectomized			Non-hysterectomized		
SOC PT	**E4 20 mg (N=210)**	**Placebo (N=209)**	**Total (N=419)**	**E4 20 mg (N=196)**	**Placebo (N=199)**	**Total (N=395)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Urinary tract infection	3 (1.4%) 3	2 (1.0%) 2	5 (1.2%) 5	1 (0.5%) 1	3 (1.5%) 3	4 (1.0%) 4
Pneumonia	1 (0.5%) 1	3 (1.4%) 3	4 (1.0%) 4	1 (0.5%) 1	0	1 (0.3%) 1
Nervous System Disorders	**16 (7.6%) 19**	**21 (10.0%) 51**	**37 (8.8%) 70**	**18 (9.2%) 22**	**22 (11.1%) 31**	**40 (10.1%) 53**
Headache	12 (5.7%) 14	18 (8.6%) 37	30 (7.2%) 51	13 (6.6%) 15	21 (10.6%) 28	34 (8.6%) 43
Dizziness	2 (1.0%) 2	2 (1.0%) 3	4 (1.0%) 5	3 (1.5%) 3	0	3 (0.8%) 3
Gastrointestinal Disorders	**20 (9.5%) 21**	**18 (8.6%) 19**	**38 (9.1%) 40**	**19 (9.7%) 27**	**19 (9.5%) 25**	**38 (9.6%) 52**
Abdominal pain	2 (1.0%) 2	4 (1.9%) 4	6 (1.4%) 6	6 (3.1%) 6	4 (2.0%) 4	10 (2.5%) 10
Nausea	7 (3.3%) 7	3 (1.4%) 3	10 (2.4%) 10	6 (3.1%) 6	2 (1.0%) 2	8 (2.0%) 8
Abdominal pain lower	3 (1.4%) 3	1 (0.5%) 1	4 (1.0%) 4	5 (2.6%) 8	1 (0.5%) 1	6 (1.5%) 9
Diarrhoea	0	2 (1.0%) 2	2 (0.5%) 2	1 (0.5%) 1	4 (2.0%) 4	5 (1.3%) 5
Abdominal distension	0	0	0	1 (0.5%) 1	3 (1.5%) 3	4 (1.0%) 4
Musculoskeletal and Connective Tissue Disorders	**11 (5.2%) 14**	**14 (6.7%) 20**	**25 (6.0%) 34**	**12 (6.1%) 13**	**12 (6.0%) 15**	**24 (6.1%) 28**
Back pain	2 (1.0%) 3	1 (0.5%) 1	3 (0.7%) 4	2 (1.0%) 2	4 (2.0%) 5	6 (1.5%) 7
Pain in extremity	3 (1.4%) 4	3 (1.4%) 3	6 (1.4%) 7	4 (2.0%) 4	2 (1.0%) 2	6 (1.5%) 6
Arthralgia	3 (1.4%) 3	3 (1.4%) 3	6 (1.4%) 6	2 (1.0%) 2	3 (1.5%) 3	5 (1.3%) 5
Psychiatric Disorders	**6 (2.9%) 7**	**6 (2.9%) 8**	**12 (2.9%) 15**	**15 (7.7%) 18**	**5 (2.5%) 8**	**20 (5.1%) 26**
Insomnia	1 (0.5%) 1	1 (0.5%) 3	2 (0.5%) 4	7 (3.6%) 7	3 (1.5%) 3	10 (2.5%) 10
Irritability	1 (0.5%) 1	0	1 (0.2%) 1	1 (0.5%) 1	3 (1.5%) 3	4 (1.0%) 4
General disorders and administration site conditions	**9 (4.3%) 11**	**8 (3.8%) 9**	**17 (4.1%) 20**	**11 (5.6%) 11**	**8 (4.0%) 10**	**19 (4.8%) 21**
Fatigue	5 (2.4%) 5	2 (1.0%) 2	7 (1.7%) 7	3 (1.5%) 3	3 (1.5%) 3	6 (1.5%) 6
Oedema peripheral	1 (0.5%) 1	2 (1.0%) 2	3 (0.7%) 3	2 (1.0%) 2	2 (1.0%) 2	4 (1.0%) 4
Investigations	**6 (2.9%) 9**	**10 (4.8%) 10**	**16 (3.8%) 19**	**5 (2.6%) 6**	**5 (2.5%) 6**	**10 (2.5%) 12**
Weight increased	2 (1.0%) 2	4 (1.9%) 4	6 (1.4%) 6	0	2 (1.0%) 2	2 (0.5%) 2
Skin and subcutaneous tissue disorders	**8 (3.8%) 8**	**8 (3.8%) 8**	**16 (3.8%) 16**	**6 (3.1%) 7**	**1 (0.5%) 1**	**7 (1.8%) 8**
Pruritus	2 (1.0%) 2	2 (1.0%) 2	4 (1.0%) 4	0	0	0
Rash	2 (1.0%) 2	2 (1.0%) 2	4 (1.0%) 4	1 (0.5%) 1	0	1 (0.3%) 1
Metabolism and Nutrition Disorders	**5 (2.4%) 5**	**7 (3.3%) 7**	**12 (2.9%) 12**	**8 (4.1%) 9**	**5 (2.5%) 5**	**13 (3.3%) 14**
Vitamin D deficiency	2 (1.0%) 2	3 (1.4%) 3	5 (1.2%) 5	3 (1.5%) 3	2 (1.0%) 2	5 (1.3%) 5
Respiratory, Thoracic and	**6 (2.9%) 7**	**3 (1.4%) 3**	**9 (2.1%) 10**	**3 (1.5%) 4**	**8 (4.0%) 9**	**11 (2.8%) 13**

	Hysterectomized			Non-hysterectomized		
SOC PT	E4 20 mg (N=210)	Placebo (N=209)	Total (N=419)	E4 20 mg (N=196)	Placebo (N=199)	Total (N=395)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Mediastinal Disorders						
Injury, poisoning and procedural complications	**1 (0.5%) 1**	**2 (1.0%) 2**	**3 (0.7%) 3**	**2 (1.0%) 2**	**5 (2.5%) 5**	**7 (1.8%) 7**
Renal and urinary disorders	**2 (1.0%) 2**	**5 (2.4%) 5**	**7 (1.7%) 7**	**3 (1.5%) 3**	**4 (2.0%) 4**	**7 (1.8%) 7**
Vascular Disorders	**2 (1.0%) 2**	**5 (2.4%) 5**	**7 (1.7%) 7**	**3 (1.5%) 3**	**3 (1.5%) 3**	**6 (1.5%) 6**
Immune System Disorders	**4 (1.9%) 4**	**0**	**4 (1.0%) 4**	**0**	**1 (0.5%) 1**	**1 (0.3%) 1**

Abbreviations: COVID-19 = coronavirus disease 2019, e = number of events, E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), N = number of subjects in the statistical analysis set, n = number of observations per treatment group, PT = preferred term, SOC = system organ class, TEAE = treatment emergent adverse event.
Note: Percentage (%) based on the number of subjects in Safety population in each treatment arm or total column.
Classifications of adverse events based on the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0).
Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.
TEAEs defined as those AEs occurring from time point of first ingestion of investigational product until last visit or any event already present that worsens in either intensity or frequency following exposure to the treatment. If the start date of the AE is unknown, it will be assumed to be after the start of study drug unless the stop date is before the first dose. Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.
PHASE 3 STUDIES: MIT-Do001-C301 ESP and MIT-Do001-C302 ESP

53-week uncontrolled NH safety population (with E4 + P4 100 mg combination therapy) - study C301 SSP

A summary of TEAEs by SOC and PT reported in at least 1.0% of subjects in the Safety Analysis Set is provided in the table below.

TEAEs were most commonly reported in the SOC of reproductive system and breast disorders (76.5%), infections and infestations (17.4%), gastrointestinal disorders (16.3%), and nervous system disorders (13.3%).

Regarding reproductive system and breast disorders, TEAEs by PT with the highest incidence were vaginal haemorrhage (66.9%), endometrial disorder (16.5%), breast pain (9.7%), breast tenderness (6.9%), and endometrial thickening (6.0%).

For gastrointestinal disorders, TEAEs by PT with the highest incidence were lower abdominal pain (5.2%) and abdominal pain (4.0%).

COVID-19 (6.6%) was the most frequently reported TEAE in the infections and infestations SOC.

Headache (9.5%) was the most frequently reported TEAE in the nervous system disorders SOC.

In the sub-population of subjects with ≥12 months since last menses, the most commonly reported TEAEs in ≥5% of subjects were: vaginal haemorrhage (562 [65.9%] subjects); endometrial disorder (138 [16.2%]); breast pain (83 [9.7%), breast tenderness (61 [7.2%]); endometrial thickening (53

[6.2%]); abdominal pain lower (43 [5.0%]); COVID-19 (56 [6.6%]); and headache (81 [9.5%]). Headache, COVID-19, and abdominal pain lower were the only commonly reported TEAEs outside of the Reproductive System and Breast Disorders SOC.

Table 46: Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term Reported in ⩾1.0% of Subjects - Safety Study Part - Safety Analysis Set

SOC/PT	E4 20 mg + P4 100 mg			
	Full population (N=922)		Sub-population: ≥12 months since last menses (N=853)	
	n[a] (%)	E[b]	n[a] (%)	E[b]
Subjects with any TEAE	**783 (84.9)**	**4026**	**717 (84.1)**	**3665**
Reproductive System and Breast Disorders	**705 (76.5)**	**3008**	**643 (75.4)**	**2735**
Vaginal haemorrhage	617 (66.9)	2385	562 (65.9)	2151
Vaginal bleeding[c]	566 (61.4)	1216	516 (60.5)	1106
Spotting vaginal[c]	207 (22.5)	1110	189 (22.2)	995
Bleeding vaginal[c]	12 (1.3)	59	9 (1.1)	50
Endometrial disorder[d]	152 (16.5)	154	138 (16.2)	139
Breast pain	89 (9.7)	95	83 (9.7)	89
Breast tenderness	64 (6.9)	67	61 (7.2)	64
Endometrial thickening	55 (6.0)	55	53 (6.2)	53
Uterine spasm	10 (1.1)	33	10 (1.2)	33
Nipple pain	24 (2.6)	25	24 (2.8)	25
Breast discomfort	23 (2.5)	24	22 (2.6)	23
Uterine haemorrhage	14 (1.5)	23	14 (1.6)	23
Uterine bleeding[c]	13 (1.4)	22	13 (1.5)	22
Vaginal discharge	17 (1.8)	21	17 (2.0)	21
Menopausal symptoms	11 (1.2)	18	11 (1.3)	18
Ovarian cyst	13 (1.4)	14	9 (1.1)	10
Breast swelling	12 (1.3)	12	11 (1.3)	11
Pelvic pain	10 (1.1)	12	8 (0.9)	10
Gastrointestinal Disorders	**150 (16.3)**	**228**	**136 (15.9)**	**204**
Abdominal pain lower	48 (5.2)	58	43 (5.0)	53
Abdominal pain	37 (4.0)	48	34 (4.0)	43
Abdominal pain upper	20 (2.2)	20	18 (2.1)	18
Nausea	18 (2.0)	19	16 (1.9)	17
Abdominal discomfort	10 (1.1)	10	9 (1.1)	9
Constipation	9 (1.0)	9	8 (0.9)	8
Diarrhoea	9 (1.0)	9	7 (0.8)	7
Infections and Infestations	**160 (17.4)**	**197**	**146 (17.1)**	**179**
COVID-19	61 (6.6)	62	56 (6.6)	57
Upper respiratory tract infection	19 (2.1)	20	19 (2.2)	20
Nasopharyngitis	18 (2.0)	18	18 (2.1)	18
Urinary tract infection	14 (1.5)	15	13 (1.5)	14
Nervous system Disorders	**123 (13.3)**	**189**	**114 (13.4)**	**178**

SOC/PT	E4 20 mg + P4 100 mg			
	Full population (N=922)		Sub-population: ≥12 months since last menses (N=853)	
	n[a] (%)	E[b]	n[a] (%)	E[b]
Headache	88 (9.5)	133	81 (9.5)	124
Dizziness	14 (1.5)	14	14 (1.6)	14
Musculoskeletal and Connective Tissue Disorders	**63 (6.8)**	**85**	**61 (7.2)**	**83**
Back pain	23 (2.5)	27	23 (2.7)	27
Pain in extremity	14 (1.5)	16	14 (1.6)	16
Arthralgia	10 (1.1)	10	10 (1.2)	10
General disorders and Administration Site Conditions	**43 (4.7)**	**53**	**39 (4.6)**	**48**
Asthenia	9 (1.0)	10	8 (0.9)	9
Skin and Subcutaneous Tissue Disorders	**35 (3.8)**	**40**	**29 (3.4)**	**34**
Alopecia	10 (1.1)	10	8 (0.9)	8
Pruritus	9 (1.0)	9	6 (0.7)	6
Psychiatric Disorders	**35 (3.8)**	**39**	**32 (3.8)**	**36**
Insomnia	11 (1.2)	11	10 (1.2)	10
Neoplasms benign, Malignant and Unspecified (incl Cysts and Polyps)	**31 (3.4)**	**33**	**27 (3.2)**	**29**
Uterine leiomyoma	29 (3.1)	30	25 (2.9)	26
Investigations	**26 (2.8)**	**29**	**23 (2.7)**	**25**
Weight increased	11 (1.2)	11	11 (1.3)	11

AE = adverse event, COVID-19 = Coronavirus disease of 2019, CSR = Clinical Study Report, DPE = disordered proliferative endometrium, E = number of events, E4 = estetrol monohydrate , LLT = lower level term, MedDRA = Medical Dictionary for Regulatory Activities, n = number of subjects in category of interest, N = number of subjects, NH = non-hysterectomized, PT = preferred term, P4 = progesterone, SOC = system organ class, SSP = Safety Study Part, TEAE = treatment emergent adverse event.
Note: Percentages (%) are based on the number of subjects in the Safety Analysis Set. Classification of TEAEs is based on MedDRA Version 25.0. TEAEs are defined as AEs with an onset date on or after the date of the first dose of study drug and before the date of the last Visit. If the start date of the AE was unknown, an AE was assumed to be after the date of the first dose of study drug unless the stop date was before the first dose.
a. Number of subjects reporting at least one TEAE within SOC/ PT/ LLT. Totals for the number of subjects at a higher level are not necessarily the sum of those at the lower levels since a subject may report two or more different TEAEs within the higher level category.
b. The number of individual occurrence of the TEAE within SOC/PT/LLT. Subjects can be represented more than once.
c. LLTs are included for clarification only within the PTs vaginal haemorrhage and uterine haemorrhage.
d. As there is no MedDRA PT for DPE, these events were coded as endometrial disorder which is the PT that is most appropriate, but not exclusive, for coding the biopsy result of DPE. Other biopsy results such as weakly proliferative endometrium and active proliferative endometrium were also coded to this PT if the biopsy result was considered reportable by the Investigator. Events of simple/complex hyperplasia with/without atypia were coded to the PT endometrial hyperplasia. For the Phase 3 studies, TEAEs of endometrial disorder and endometrial hyperplasia were reported based upon the Initial or Safety diagnosis, whereas the Final/Consensus diagnosis was used to evaluate overall endometrial safety

53-week uncontrolled H + NH safety population (E4 monotherapy) - study C302 SSP

A total of 314 (73.0%) subjects experienced 1003 TEAEs. The most commonly reported TEAEs were in the reproductive system and breast disorders SOC (534 TEAEs in 231 [53.7%] subjects).

Overall, a greater proportion of NH subjects (188 [82.1%]) reported TEAEs than H subjects (126 [62.7%]).

Of the most commonly reported TEAEs in ≥5% of the total safety population, endometrial disorder, and endometrial thickening were reported only in NH subjects. The majority of TEAEs with vaginal haemorrhage were reported in NH subjects (only 1 H subject had vaginal haemorrhage). Those reported in a similar proportion of H and NH subjects were: breast tenderness (28 [13.9%] and 34

[14.8%], respectively); nipple pain (14 [7.0%] and 14 [6.1%], respectively); and headache (9 [4.5%] and 13 [5.7%], respectively). A total of 10 events of endometrial hyperplasia were reported in 10 (2.3%) subjects.

Among the 201 hysterectomized subjects, 59 (29.4%) subjects reported 78 TEAEs of the reproductive system and breast disorders SOC, and abdominal pain. The PT of breast tenderness was the most common TEAE among the hysterectomized subjects, reported by 28 (13.9%) subjects (28 TEAEs); 14 (7.0%) subjects reported 15 TEAEs of nipple pain; 7 (3.5%) subjects reported 8 TEAEs of breast pain; 5 (2.5%) subjects reported 5 TEAEs of breast discomfort and vaginal discharge; 3 (1.5%) subjects reported 3 TEAEs of menopausal symptoms; 2 (1.0%) subjects reported 2 TEAEs of breast enlargement and ovarian cyst, and a single (0.5%) subject each reported single TEAEs of breast mass, vaginal spotting, breast swelling, fibrocystic breast disease, ovarian vein thrombosis, pruritus genital, and vulvovaginal dryness. A total of 3 (1.5%) subjects reported 3 TEAEs of abdominal pain.

Among the 229 NH subjects, 175 (76.4%) subjects reported 480 TEAEs of the reproductive system and breast disorders SOC, and abdominal pain. The most common PT was vaginal haemorrhage, with 104 (45.4%) subjects reporting 177 events (65 [36.7%] of these events were spotting vaginal). Endometrial disorder was reported by 85 (37.1%) subjects (86 TEAEs) and endometrial thickening was reported by 59 (25.8%) subjects (59 TEAEs).

The TEAEs reported by 5−15% of NH subjects were: breast tenderness (34 [14.8%] subjects, 34 events); uterine spasm (15 [6.6%] subjects, 17 events); nipple pain (14 [6.1%] subjects, 14 events) and vaginal discharge (14 [6.1%] subjects, 14 events). Menopausal symptoms, pelvic pain, uterine polyp and ovarian cyst were reported by 1−2% of subjects. Abnormal uterine bleeding, breast discomfort, breast pain, dyspareunia and postmenopausal haemorrhage were reported by 2 (0.9%) subjects each (2 events for each PT except postmenopausal haemorrhage which had 3 events). A single event of breast swelling, cervical cyst, cervical polyp, dysmenorrhea, endometrial metaplasia, pelvic discomfort, uterine enlargement, uterine pain, vulva cyst and vulvovaginal pruritus were reported by 1 (0.4%) subject each.

The PTs of endometrial disorder, endometrial thickening, uterine spasm, uterine haemorrhage (all events were uterine bleeding), endometrial hyperplasia, pelvic pain, uterine polyp, abnormal uterine bleeding, dyspareunia, postmenopausal haemorrhage, cervical cyst, cervical polyp, dysmenorrhea, endometrial metaplasia, pelvic discomfort, uterine enlargement, uterine pain, vulva cyst, and vulvovaginal pruritus were observed only among NH subjects.

Drug-related AE evaluated by the investigator

AEs following treatment with E4 alone for up to 13 weeks

C201: Approximately 50% of the AEs were considered related to study drug. The majority of study drug-related AEs were reported in the Reproductive System and Breast Disorders SOC. The most frequent drug-related AE was vaginal haemorrhage, mainly reported in the E4 10 mg arm (18.5%) and in the E4 15 mg arm (20.4%). Other drug-related AEs reported in >5% of the total safety population were uterine haemorrhage and breast pain with a higher proportion of subjects in the E4 10 mg (7.4% subjects each) and E4 15 mg (8.2% subjects each) arms than in the other arms.

C301 ESP: The number of subjects with study drug-related AEs was higher in the E4 15 mg (51.6%) and E4 20 mg (57.3%) arms than in the placebo arm (20.6%). The majority of study drug-related AEs were reported in the Reproductive System and Breast Disorders SOC.

Study drug-related AEs reported in ≥5% of subjects in any of the study arms were (in the E4 15 mg, E4 20 mg and placebo arms, respectively): vaginal haemorrhage (23.5%, 29.1%, 5.1%), endometrial

disorder (21.6%), 19.7%, 1.9%), breast pain (8.0%, 10.8%, 0.9%), endometrial thickening (4.2%, 6.1%, 0%), breast tenderness (4.2%, 5.6%, 0%), headache (6.6%, 4.7%, 5.6%).

Study drug-related AEs were reported by a greater proportion of NH subjects than H subjects in the E4 15 mg arm (77.7% vs 27.3%, respectively), E4 20 mg arm (84.5% vs 31.8%, respectively), and in the placebo arm (25.0% vs 16.4%, respectively) Of the study drug-related AEs reported in ≥2% of subjects in any study arm, the following were reported only in NH subjects (in the E4 15 mg, E4 20 mg, and placebo arms, respectively): endometrial disorder (44.7%, 40.8%, 3.8% of NH subjects), endometrial thickening (8.7%, 12.6%, 0% of NH subjects), endometrial hyperplasia (2.9%, 4.9%, 0% of NH subjects), uterine haemorrhage (2.9%, 1.9%, 0% of NH subjects). The majority of the following AEs were reported in NH subjects (in the E4 15 mg, E4 20 mg, and placebo arms, respectively): vaginal haemorrhage (47.6%, 59.2%,10.6% of NH subjects), vaginal discharge (3.9%, 4.9%, 1% of NH subjects), · pelvic pain (1%, 3.9%, 0% of NH subjects). In total, only 2 H subjects had vaginal spotting (lower-level term), 3 H subjects had vaginal discharge, and 1 H subject had pelvic pain.

E4 alone in studies up to 53 weeks duration

C302 ESP: Following E4 treatment for up to 53 weeks, the number of subjects with study drug-related AEs was higher in the E4 15 mg (35.9%) and E4 20 mg (44.6%) arms than in the placebo arm (19.6%), and the majority of study drug-related AEs were reported in the Reproductive System and Breast Disorders SOC. For the E4 15 mg, E4 20 mg and placebo, respectively, in terms of PT study-drug-related AEs reported in ≥5% of subjects in any study arm were: vaginal haemorrhage (12.0%, 17.6%, 2.1%), breast tenderness (10.9%, 12.4%, 3.1%), endometrial disorder (9.9%, 11.9%, 0.5%), endometrial thickening (7.3%, 8.8%, 0.5%), endometrial hyperplasia (2.1%, 5.7%, 0%). There was a trend towards a higher proportion of study-drug related AEs in subjects on E4 20 mg compared to 15 mg. This was most obvious for vaginal haemorrhage and endometrial hyperplasia. Study drug-related AEs were reported by a greater proportion of NH subjects than H subjects in the E4 15 mg arm (47.3% vs 25.3%, respectively) and in the E4 20 mg arm (61.3% vs 29.0%, respectively), and by a similar proportion of NH and H subjects in the placebo arms (16.8% vs 22.2%, respectively). Of the study drug-related AEs reported in ≥5% of subjects in any study arm, the following events were reported only in NH subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively: vaginal haemorrhage (24.7%, 36.6%, 4.2%), endometrial disorder (20.4%, 24.7%, 1.1%), endometrial thickening (15.1%, 8.3%, 1.1%), endometrial hyperplasia (4.3%, 11.8%, 0%). Study drug-related breast tenderness was reported in a greater number of H subjects than NH subjects (14.1%, 14.0%, 6.1% in the E4 15 mg, E4 20 mg, and placebo arms, respectively in H subjects vs 7.5%, 10.8%, 0% in NH subjects). Study drug-related vaginal discharge was reported in a greater number of NH subjects than H subjects (5.4%, 1.1%, 1.1% in NH subjects vs 0%, 2.0%, 0% in H subjects).

C302 SSP: Overall, study drug-related AEs were reported in 57.7% subjects. The majority of study drug-related AEs were reported in the Reproductive System and Breast Disorders SOC. Study drug-related AEs reported in >5% of the total safety population were: vaginal haemorrhage (23.3%), endometrial disorder (19.5%), breast tenderness (14.2%), endometrial thickening (13.5%), nipple pain (6.3%). Overall, a greater proportion of NH subjects (82.1%) reported AEs than H subjects (62.7%). Of the study drug-related AEs reported in ≥5% of the total safety population, the following were reported only in NH subjects: endometrial disorder (36.7%), endometrial thickening (25.3%). The following were reported in a greater number of NH subjects vs H subjects: vaginal haemorrhage (43.2% in NH subjects vs 0.5% in H subjects), vaginal discharge (6.1% vs 2.0%), abdominal pain (5.2% vs 1.0%). The following study drug-related AEs were reported in a similar number of H and NH subjects: breast tenderness (13.4% in H subjects vs 14.8% in NH subjects), nipple pain (7.0% vs 5.7%).

E4 plus P4 in a study of up to 53 weeks duration

C301 SSP: Study drug-related AEs were reported in 79.9% of subjects. The majority of study drug-related AEs were reported in the Reproductive System and Breast Disorders SOC. Study drug-related AEs reported in ≥5% of all subjects were: vaginal haemorrhage (66.4%), endometrial disorder (16.4%), breast pain (9.2%), breast tenderness (6.6%), endometrial thickening (5.9%), headache (5.7%).

In summary, overall, a similar pattern of drug-related AEs was noted in the different studies. The most common study drug-related AEs were from the Reproductive System and Breast Disorders SOC. The most common reported event in all studies was vaginal haemorrhage. Other commonly reported drug-related events were endometrial disorder, endometrial thickening, and breast tenderness. Outside the Reproductive System and breast disorders SOC, headache was the most frequently reported event. A greater proportion of NH subjects reported AEs than H subjects in the E4 15 mg and E 20 mg study arms whereas in the placebo arms the proportion of subjects with study drug related AEs was similar between the NH and H subsets.

5.5.1.1. Adverse drug reactions

Table 47: Summary of ADRs proposed for inclusion by the applicant in the SmPC

Reproductive system and breast disorders	
Very Common	Vaginal haemorrhage, Endometrial thickening
Common	Disordered proliferative endometrium, Breast pain, Breast tenderness, Nipple pain, Uterine spasm, Vaginal discharge, Vulvovaginal pruritus
Uncommon	Endometrial hyperplasia, Endometrial polyp, Adenomyosis, Breast mass, Breast swelling, Ovarian cyst
Infections and infestations	
Very Common	
Common	Vulvovaginal candidiasis
Uncommon	
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	
Very Common	
Common	Uterine leiomyoma
Uncommon	
Vascular disorders	
Very Common	
Common	
Uncommon	Venous thromboembolism
Nervous system disorders	
Very Common	
Common	Dizziness
Uncommon	
Gastrointestinal disorders	
Very Common	
Common	Abdominal pain lower, Abdominal pain, Abdominal distension, Nausea Constipation
Uncommon	
Musculoskeletal and connective tissue disorders	
Very Common	
Common	Pain in extremity
Uncommon	

Skin and subcutaneous tissue disorders	
Very Common	
Common	
Uncommon	Urticaria
General disorders and administration site conditions	
Very Common	
Common	Asthenia
Uncommon	Peripheral swelling
Investigations	
Very Common	
Common	Weight increased
Uncommon	

The basic methodology of how all AEs were analysed in order to select the ADRs:

- During the initial review of AEs reported in studies C301 ESP, C302 ESP (up to 53 weeks) and C201 for 15 mg (i.e., all placebo-controlled clinical trials), it became apparent that there was only a slight difference in the overall incidence of AEs between subjects receiving E4 15 mg and E4 20 mg, and between subjects that were hysterectomised and non-hysterectomised, except in the SOC Reproductive system and breast disorders. Therefore, it was decided to pool data from the E4 15 mg and E4 20 mg treatment arms, irrespective of hysterectomy status for the analysis and selection of ADRs.
- Comparison of occurrence of AEs in the pooled E4 with the placebo arms, in the three abovementioned studies, was done to initially make a preliminary decision on inclusion of the AEs for further analysis. All AEs in the SOC Reproductive system and breast disorders that were uterus-related events (e.g. endometrial events) were analysed separately based on the open-label study C301 SSP with E4 20 mg + P4 100 mg treatment as per clinical practice in non-hysterectomised women, see below.
- AEs with a higher frequency in the pooled E4 arm compared to placebo were considered for further analysis, including comparison of the frequency of AEs reported as related by Investigators. In case of AEs for which the medical assessment of relatedness required complete knowledge of the patient's overall condition and clinical picture or presence of confounding factors (e.g. infections, abdominal distension or vaginal discharge), the Applicant relied on the Investigator's medical judgement and causality assessment.
- In cases where the frequency of an AE was higher in the E4 arm compared to placebo, but it was reported as related by the Investigators with lower frequency in the pooled E4 arm than in the placebo arm, an individual decision was made by the Applicant to judge if the Investigators' assessment should prevail for final ADR selection (e.g. constipation).
- If a particular pooled AE frequency was greater in the pooled E4 arm compared to placebo, but it comprised terms relating to natural disease progression, e.g. menopausal symptoms, or medical judgment indicated that a relationship to E4 was implausible (e.g. COVID-19), it was excluded from the further analysis.
- Pooling of AEs reported as related by Investigators was also performed in the placebo-controlled trials to allow the further analysis. ADRs with a higher frequency in the pooled E4 arm vs placebo were then analysed, taking into account primarily the causality relationship as reported by the Investigator, but in some cases also as assessed by the Applicant based upon

individual cases' medical history, vital signs or laboratory results, e.g. anaemia or chest discomfort.

- An additional step was implemented during selection of ADRs to avoid missing any ADR for selection. If an ADR was reported with a higher frequency in the pooled E4 arm vs placebo but the frequency of the AE term (related and non-related) in the pooled E4 arm was similar/lower vs placebo, then such event was scrutinised by the Applicant, checking medical history, vital signs or laboratory results allowing to exclude or include the event as an ADR (e.g. tinnitus, chest pain or oedema peripheral).
- Data from open label studies, C301 SSP (E4 20 mg + P4 100 mg) and C302 SSP (E4 20 mg) was used as supportive information for making the final decision on ADR inclusion, (e.g. pain in extremity or bacterial vaginosis).
- Some events were considered for inclusion as ADRs based on pre-defined data collected in the relevant studies, such as endometrial thickening (> 4 mm in sub-population of subjects with ≥ 12 months since last menses), endometrial hyperplasia or DPE using Final/Consensus diagnosis from independent expert pathologists' panel, in case of the relevant sub-population of subjects with at least 12 months since last menses.
- AEs related to the uterus were considered for inclusion as ADRs primarily based upon data from the open-label study C301 SSP which is relevant for the non-hysterectomised target population. Data from non-hysterectomised subjects in the pooled E4 and placebo arms and data from non-hysterectomised subjects in C302 SSP was supportive for decision making. As these uterine-related events were medically independent from Investigator's causality assessment, the frequency of such ADRs was calculated from number of all AEs in this target population.
- Following the final decision to include an AE term as an ADR, the frequency of such ADR was calculated. The frequency calculation of ADR was in general based on the frequency of pooled data of AEs considered related by Investigator. For uterus-related events the frequency of the ADRs was based on the frequency of AEs in the C301 SSP, with the exception of some events described above (endometrial thickening, endometrial hyperplasia and DPE) for which objective measures such as ultrasound examination data or biopsy results were taken into account. The denominator used in such calculation was from the relevant population from pooled placebo-controlled studies and, for uterine-related ADRs, the sub-population of subjects with at least 12 months since last menses.

5.4.4 AEs of special interest, serious adverse events and deaths, other significant events

Serious Adverse Events

Of note, the pathologic diagnoses of DPE, hyperplasia, and endometrial carcinoma were reported as serious TEAEs for MIT-Do001-C301 Protocol Versions 1.0 to 3.0 and for MIT-Do001-C302 Protocol Versions 1.0 to 5.0, and the subject was followed up until the resolution of the event. However, in Protocol Amendment 3 for MIT-Do001-C301 and Protocol Amendment 5 for MIT-Do001-C302, this requirement was removed, and the definition of SAEs was in line with the regulatory definition of SAEs (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use [ICH] E6 [R2]) (Guideline for Good Clinical Practice E6[R2], Committee for Human Medicinal Products, ICH [EMA/CHMP/ICH/135/1995]). Adverse events of special interest (AESIs) were defined and,

therefore, following the implementation of Protocol Version 4.0 (MIT-Do001-C301) and Protocol Version 6.0 (MIT-Do001-C302), these events were reported as AESIs.
When reporting the AESI, the Investigator specified whether it was serious (i.e. fulfilled the ICH criteria of an SAE) or non-serious.

53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

The overall number of subjects with serious TEAEs was similar between the E4 15 mg and E4 20 mg arms (21 [10.9% for each arm]), but lower in the placebo arm (3 [1.5%]). All serious TEAEs recovered/resolved except for 1 event of gastric adenocarcinoma in the E4 15 mg arm, 2 events of endometrial disorder in the E4 20 mg arm, and 1 event of stage 4 pancreatic cancer in the placebo arm which did not recover/resolve. In addition, the outcome of 1 serious TEAE of endometrial disorder in the E4 20 mg arm was unknown.

The number of subjects with serious TEAEs was lower in the H subgroup than in the NH subgroup in the E4 15 mg arm (4 [4%] vs 17 [18.3%] respectively) and in the E4 20 mg arm (0% vs 21 [22.6%]. The number of subjects with serious TEAEs was similar for H and NH subjects in the placebo arm (1 [1.0%] vs 2 [2.1%], respectively). In total, 8 serious TEAEs were reported in 5 (1.7%) of the 298 hysterectomized subjects; 4 (4.0%) subjects in the E4 15 mg arm, none in the E4 20 mg arm, and 1 (1.0%) subject from the placebo arm. In NH subjects, 40 (14.2%) subjects reported 40 serious TEAEs; 17 (18.3%) subjects in the E4 15 mg arm, 21 (22.6%) subjects in the E4 20 mg arm, and 2 (2.1%) subjects from the placebo arm.

Reclassification of SAEs

The number of NH subjects with serious TEAEs decreased from 40 (14.2%) before the SAE reclassification (17 [18.3%], 21 [22.6%], 2 [2.1%] in the E4 15 mg, E4 20 mg, and placebo arms, respectively) to 6 (2.1%) after the SAE reclassification (3 [3.2%], 1 [1.1%], 2 [2.1%] in the E4 15 mg, E4 20 mg, and placebo arms, respectively). After the SAE reclassification, all serious TEAEs occurred in a single subject each and the incidence of serious TEAEs was similar between treatment arms (3 [3.2%], 1 [1.1%], 2 [2.1%] in the E4 15 mg, E4 20 mg, and placebo arms, respectively).

The only SOC affected by the SAE reclassification was the Reproductive System and Breast Disorder SOC which had 35 (12.5%) NH subjects with 35 serious TEAEs before the SAE reclassification (15 [16.1%] and 20 [21.5%] subjects in the E4 15 mg and E4 20 mg arm, respectively) and only 1 (0.4%) NH subject with 1 serious TEAE after the SAE reclassification. The latter serious TEAE was endometrial hyperplasia of moderate intensity in the E4 15 mg arm that was considered related to study drug.

No thrombotic events or study drug-related by the investigator cardiovascular events were reported.

13-week placebo-controlled H + NH safety population (E4 monotherapy) - ISS pooled data

15 mg

Overall, a higher incidence of serious TEAEs was reported in the E4 15 mg arm (4.6%) compared with the placebo arm (0.4%). The majority of serious TEAEs were reported in the Reproductive System and Breast Disorders SOC. Only endometrial disorder and endometrial hyperplasia were reported in more than 1 subject, both reported in the E4 15 mg arm (in 12 [2.6%] and 5 [1.1%] subjects, respectively).

No serious TEAEs in the Reproductive System and Breast Disorders SOC were reported for H subjects. In NH subjects, the incidence of serious TEAEs in the E4 15 mg arm and placebo arm was 8.1% and 0.8%, respectively. Only endometrial disorder and endometrial hyperplasia were reported in more than 1 subject, both reported in the E4 15 mg arm (in 12 [5.1%] and 5 [2.1%] subjects, respectively).

Table48: Serious TEAEs Reported up to 13 Weeks by SOC and PT by Hysterectomy Status (Safety Population) – Pooled E4 15 mg and Placebo

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 15 mg (N=218)	Placebo (N=215)	Total (N=433)	E4 15 mg (N=236)	Placebo (N=248)	Total (N=484)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Any Serious TEAEs	**2 (0.9%) 3**	**0**	**2 (0.5%) 3**	**19 (8.1%) 19**	**2 (0.8%) 2**	**21 (4.3%) 21**
Reproductive System and Breast Disorders	**0**	**0**	**0**	**18 (7.6%) 18**	**1 (0.4%) 1**	**19 (3.9%) 19**
Endometrial disorder	0	0	0	12 (5.1%) 12	0	12 (2.5%) 12
Endometrial hyperplasia	0	0	0	5 (2.1%) 5	0	5 (1.0%) 5
Abnormal uterine bleeding	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Hydrosalpinx	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Musculoskeletal and Connective Tissue Disorders	**0**	**0**	**0**	**1 (0.4%) 1**	**1 (0.4%) 1**	**2 (0.4%) 2**
Back pain	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Intervertebral disc protrusion	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Infections and Infestations	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**0**	**0**	**0**
COVID-19	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0
Injury, poisoning and procedural complications	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**0**	**0**	**0**
Road traffic accident	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0
Respiratory, Thoracic and Mediastinal Disorders	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**0**	**0**	**0**
Acute respiratory failure	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0

Abbreviations: COVID-19 = coronavirus disease 2019, e = Number of events, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg),; N = number of subjects in the statistical analysis set, n = number of observations per treatment group, PT = preferred term, SOC = system organ class, TEAE = treatment emergent adverse event.
Note: Percentage (%) based on the number of subjects in Safety population in each treatment arm or total column.
Classifications of adverse events based on the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0).
TEAEs defined as those AEs occurring from time point of first ingestion of investigational product until last visit or any event already present that worsens in either intensity or frequency following exposure to the treatment. If the start date of the AE is unknown, it will be assumed to be after the start of study drug unless the stop date is before the first dose.
Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.
PHASE 3 STUDIES: MIT-Do001-C301 ESP and MIT-Do001-C302 ESP, PHASE 2 STUDY: MIT-Do0001-C201

20 mg

Overall, a higher incidence of serious TEAEs was reported in the E4 20 mg arm (6.4%) compared with the placebo arm (0.2%). As seen in the pooled E4 20 mg and placebo arm, the majority of serious TEAEs were reported in the Reproductive System and Breast Disorders SOC and only endometrial disorder and endometrial hyperplasia were reported in more than 1 subject, both in the E4 20 mg arm (in 10 [2.5%] and 9 [2.2%] subjects), respectively.

No serious TEAEs in the Reproductive System and Breast Disorders SOC were reported for H subjects. In NH subjects, the incidence of serious TEAEs in the E4 20 mg arm and placebo arm was 12.2% and 0.5%, respectively. Only endometrial disorder and endometrial hyperplasia were reported in more than 1 subject, both in the E4 20 mg arm (in 10 [5.1%] and 9 [4.6%] subjects, respectively).

Table49: Serious TEAEs Reported up to 13 Weeks by SOC and PT by Hysterectomy Status (Safety Population) – Pooled E4 20 mg and Placebo

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 20 mg (N=210)	Placebo (N=209)	Total (N=419)	E4 20 mg (N=196)	Placebo (N=199)	Total (N=395)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Any Serious TEAEs	**2 (1.0%) 2**	**0**	**2 (0.5%) 2**	**24 (12.2%) 28**	**1 (0.5%) 1**	**25 (6.3%) 29**
Reproductive System and Breast Disorders	**0**	**0**	**0**	**20 (10.2%) 22**	**1 (0.5%) 1**	**21 (5.3%) 23**
Endometrial disorder	0	0	0	10 (5.1%) 10	0	10 (2.5%) 10
Endometrial hyperplasia	0	0	0	9 (4.6%) 9	0	9 (2.3%) 9
Endometrial metaplasia	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Hydrosalpinx	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Uterine polyp	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Vaginal haemorrhage	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Infections and Infestations	**0**	**0**	**0**	**2 (1.0%) 3**	**0**	**2 (0.5%) 3**
COVID-19	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Cholecystitis infective	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Peritonitis	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Hepatobiliary disorders	**0**	**0**	**0**	**1 (0.5%) 1**	**0**	**1 (0.3%) 1**
Cholecystitis acute	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Neoplasms Benign, Malignant and Unspecified (incl cysts and polyps)	**0**	**0**	**0**	**1 (0.5%) 1**	**0**	**1 (0.3%) 1**
Intraductal proliferative breast lesion	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Nervous System Disorders	**0**	**0**	**0**	**1 (0.5%) 1**	**0**	**1 (0.3%) 1**
Essential tremor	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 20 mg (N=210)	**Placebo (N=209)**	**Total (N=419)**	**E4 20 mg (N=196)**	**Placebo (N=199)**	**Total (N=395)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Eye disorders	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**0**	**0**	**0**
Retinal detachment	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0
Metabolism and Nutrition Disorders	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**0**	**0**	**0**
Hypocalcaemia	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0

Abbreviations: COVID-19 = coronavirus disease 2019, e = Number of events, E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), N = number of subjects in the statistical analysis set, n = number of observations per treatment group, PT = preferred term, SOC = system organ class, TEAE = treatment emergent adverse event.
Note: Percentage (%) based on the number of subjects in Safety population in each treatment arm or total column.
Classifications of adverse events based on the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0).
TEAEs defined as those AEs occurring from time point of first ingestion of investigational product until last visit or any event already present that worsens in either intensity or frequency following exposure to the treatment. If the start date of the AE is unknown, it will be assumed to be after the start of study drug unless the stop date is before the first dose.
Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.
PHASE 3 STUDIES: MIT-Do001-C301 ESP and MIT-Do001-C302 ESP

53-week uncontrolled NH safety population (with E4 + P4 combination therapy) - study C301 SSP

A total of 32 (3.5%) subjects experienced 36 serious TEAEs. In the sub-population 30 (3.5%) subjects with ≥12 months since last menses experienced 34 serious TEAEs. All serious TEAEs recovered/resolved except for 1 event of road traffic accident which recovered or resolved with sequelae, 1 event of cerebrovascular accident whose outcome was unknown, and 1 event of endometrial disorder whose outcome was reported as recovering/resolving. These three events were reported by subjects in the sub-population of subjects with ≥12 months since last menses.

The Reproductive System and Breast Disorders SOC accounted for serious TEAEs in 13 (1.4%) NH subjects. In the sub-population, the Reproductive System and Breast Disorders SOC accounted for serious TEAEs in 13 (1.5%) subjects with ≥12 months since last menses. Serious TEAEs reported in ≥0.2% of all subjects were endometrial disorder in 7 (0.8%) subjects, vaginal haemorrhage in 5 (0.5%), lower limb fracture in 2 (0.2%), and COVID-19 in 4 (0.4%), all in the sub-population of subjects with ≥12 months since last menses except for lower limb factures. All other serious TEAEs were reported by 1 NH subject each and no trends were observed.

As no serious TEAEs of endometrial disorder were reported prior to implementation of protocol version 4, no reclassification was applied.

53-week H + NH uncontrolled safety population (E4 monotherapy) - study C302 SSP

A total of 60 (14.0%) subjects experienced 66 serious TEAEs. All serious TEAEs recovered/resolved except for 3 events of endometrial disorder which did not recover/resolve, 1 event of DVT which was recovering/resolving, and 3 events of endometrial disorder and 1 event of endometrial hyperplasia whose outcome was unknown.

A total of 56 (13.0%) subjects experienced 58 study drug-related serious TEAEs, and the majority were due to endometrial disorder (45 [10.5%]), endometrial hyperplasia (6 [1.4%]), or vaginal haemorrhage (3 [0.7%]). All other study drug-related serious TEAEs were reported by 1 subject each (ovarian vein thrombosis, DVT, superficial vein thrombosis, and anaemia). Study drug-related serious TEAEs of severe intensity were endometrial disorder in 3 (0.7%) subjects, and those reported in a single subject each (endometrial hyperplasia, vaginal haemorrhage, DVT, and anaemia). These recovered/resolved except for 1 event of endometrial disorder which did not recover/resolve and 1 event of DVT which was recovering/resolving.

Serious TEAEs were reported in a lower number of H subjects (4 [2.0%]) than NH subjects (56 [24.5%]). As expected, endometrial disorder, endometrial hyperplasia, and vaginal haemorrhage were only reported in NH subjects.

Reclassification of SAEs

The number of NH subjects with serious TEAEs decreased from 56 (24.5%) before the SAE reclassification to 6 (2.6%) after the SAE reclassification. As expected, the only SOC affected by the SAE reclassification was the Reproductive System and Breast Disorder SOC which had 53 (23.1%) NH subjects with 55 serious TEAEs before the SAE reclassification and 3 (1.3%) NH subjects with 3 serious TEAEs after the SAE reclassification. These 3 serious TEAEs were all study drug-related (moderate endometrial disorder, moderate vaginal haemorrhage, and severe vaginal haemorrhage).

Deaths

One H subject in the E4 15 mg arm in C301 ESP died during the study of severe acute respiratory failure due to COVID-19 infection. One H subject in the E4 15 mg arm in C302 ESP experienced a fatal TEAE of road traffic accident. None were considered related to study drug.

In C301 SSP and C302 SSP, no deaths were reported.

Thromboembolic Events

In C301 ESP, no thromboembolic events were reported in the E4 study arms. There was 1 event of superficial thrombophlebitis (left leg) of moderate intensity reported in a NH subject in the placebo arm that was considered not related to the study drug.

In C302 ESP, no thromboembolic events were reported; 1 cardiovascular (CV) event was reported, a serious event of angina pectoris of severe intensity reported in a H subject in the E4 15 mg arm, which was considered not related to study drug.

In C302 SSP (E4 20 mg), in H subjects, no CV events were reported; 2 serious thromboembolic events considered related to the study drug were reported: 1 event of ovarian vein thrombosis of moderate intensity and 1 event of superficial vein thrombosis of mild intensity. In NH subjects, 2 thromboembolic events considered related to the study drug were reported: 1 serious event of DVT of severe intensity and 1 non-serious event of retinal vein occlusion of mild intensity; 1 subject was reported with serious acute myocardial infarction of severe intensity considered not related to study drug.

In C301 SSP (E4 20 mg + P4 100 mg, all NH subjects), 7 thromboembolic events were reported: 1 serious event of transverse sinus thrombosis of severe intensity, 1 serious event of superficial thrombophlebitis of moderate intensity, and 1 non-serious event of venous thrombosis limb of mild intensity that were considered related to study drug and 1 non-serious event of thrombophlebitis of mild intensity considered not related to the study drug, 1 serious event of cerebrovascular accident of severe intensity and 1 serious event of TIA of severe intensity considered related to study drug, and 1 non-serious event of cerebrovascular accident of mild intensity considered not related to study drug.

Adverse Events of Special Interest

The following categories of TEAESIs were defined in the SAP:

1. Vaginal bleeding events grade 2 (evidence of blood loss requiring more than one pad, tampon, or panty liner per day) as assessed using the vaginal bleeding events scale;
2. Events resulting from endometrial biopsy reading limited to:
   a) Disordered proliferative disorder (DPE);
   b) Simple hyperplasia without atypia;
   c) Complex hyperplasia without atypia;
   d) Simple hyperplasia with atypia;
   e) Complex hyperplasia with atypia;
   f) Carcinoma.

53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

There were no AESIs in hysterectomized subjects.

In total, 27 AESIs occurred in 25 (8.9%) NH subjects; 12 AESIs in 11 (11.8%) subjects in the E4 15 mg arm, 14 AESIs in 13 (14.0%) subjects in the E4 20 mg arm, and 1 AESI in 1 (1.1%) subject in the placebo arm.

The most common PTs included 19 AESIs of endometrial disorder in 19 (6.8%) subjects; 4 AESIs of endometrial hyperplasia in 4 (1.4%) subjects and 4 events of vaginal haemorrhage (vaginal bleeding) in 4 (1.4%) subjects.

Endometrial disorder was more frequent in the E4 treatment arms (8 events in 8 [8.6%] subjects in the E4 15 mg arm and 10 events in 10 [10.8%] subjects in the E4 20 mg arm) than in the placebo arm (1 event in 1 [1.1%] subject). The PTs of endometrial hyperplasia and vaginal haemorrhage were reported only in the E4 treatment arms.

10 (3.6%) NH subjects reported 12 AESIs which were mild in intensity, and 15 (5.3%) NH subjects reported 15 events which were moderate in intensity. No AESIs were severe in intensity.

13-week placebo-controlled H + NH safety population (E4 monotherapy) - ISS pooled data

15 mg

No TEAESIs were reported in H subjects. In NH subjects, a higher incidence of TEAESIs were reported for subjects in the E4 15 mg arm compared with the placebo arm (19.5% and 2.8%, respectively). The most commonly reported TEAESIs (≥5% in either arm) were endometrial disorder (13.1% and 2.0%, respectively) and vaginal haemorrhage (6.8% and 0.8%, respectively). There was one case of hyperplasia (0.4%).

20 mg

No TEAESIs were reported for H subjects. In NH subjects, a higher incidence of TEAESIs were reported for subjects in the E4 20 mg arm compared with the placebo arm (25.0% and 3.5%, respectively). As seen in the pooled the E4 20 mg and placebo arm, the most commonly reported TEAESIs (≥5% in either arm) were endometrial disorder (16.8% and 2.5%, respectively) and vaginal haemorrhage (9.2% and 1.0%, respectively). There were 2 cases of hyperplasia (1.0%).

53-week uncontrolled NH safety population (with E4 + P combination therapy) - study C301 SSP

In the sub-population of subjects ≥12 months since last menses the following AESI were reported: vaginal haemorrhage in 372 (43.6%) subjects, endometrial disorder in 117 (13.7%), and endometrial hyperplasia in 1 (0.1%). Four (0.5%) subjects with ≥12 months since last menses reported severe AESIs (vaginal haemorrhage in 4 subjects). Four AESIs were reported as study drug-related serious events (3 events of vaginal haemorrhage and 1 event of endometrial disorder).

53-week uncontrolled H + NH safety population (E4 monotherapy) - study C302 SSP

Naturally, there were no AESIs in H subjects in this study.

In NH subjects, AESIs occurred in 51 (22.3%) subjects. AESIs were reported as: endometrial disorder in 40 (17.5%) subjects, vaginal haemorrhage in 12 (5.2%) subjects, and endometrial hyperplasia in 4 (1.7%) subjects. Four (1.7%) subjects reported severe AESIs (endometrial hyperplasia in 2 subjects and endometrial disorder and vaginal haemorrhage in a single subject each). Three AESIs were reported as study drug-related serious events (1 event of endometrial disorder and 2 events of vaginal haemorrhage).

Endometrial and General Safety Study Part – C301 SSP – E4+P4 in NH subjects

As standard clinical practice, women with an intact uterus who are prescribed systemic estrogen therapy, should receive progestogen for endometrial protection. However, during the clinical development, based on a request by the FDA, in study MIT-Do001-C302, all women (including NH women) who received active treatment took unopposed E4 alone, up to 1 year, in order to evaluate the effect of E4 alone on the endometrium. A total of 621 of 1543 (40.2%) NH women in the phase 3 studies received unopposed E4 treatment where the progestogen was taken after completing E4-alone treatment.

In study MIT-Do001-C301 SSP, E4 20 mg was given concurrently with once daily P4 100 mg to control the proliferative effect of E4 on the endometrium. The endometrial safety results from this study are the most clinically relevant as per clinical practice.

Methods

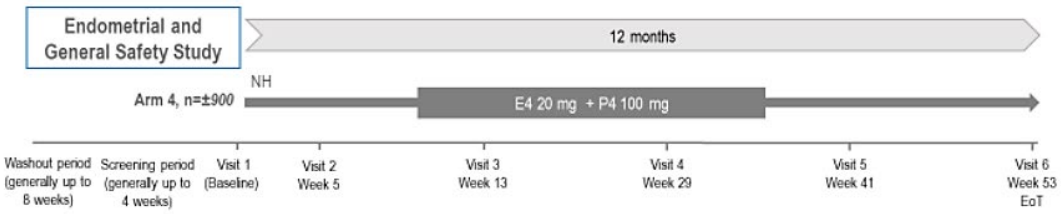
Study design

This was a phase 3, multicenter study in postmenopausal women, aged 40 to 65 years, with moderate to severe VMS. All subjects received daily E4 20 mg + P4 100 mg continuously for up to 53 weeks. It had an open-label, uncontrolled design and evaluated the primary endometrial safety, secondary general safety (TEAEs, haematology and biochemistry, physical, gynaecological and breast examinations, and electrocardiogram [ECG]), and secondary and exploratory efficacy (HRQoL and TS, lipid and glucose metabolism, and VMS) of E4 20 mg in combination with P4 100 mg in non-hysterectomized postmenopausal women.

A total of ~900 non-hysterectomized subjects were planned to be enrolled. The study population consisted of non-hysterectomized women seeking treatment for the relief of VMS associated with menopause, with at least 1 moderate to severe menopause-related VMS per week.

At least 8 visits were planned in this study part: a Washout visit, Screening visit followed by a Baseline and Treatment Allocation visit (Visit 1 at Day 1) and 5 on-treatment visits (Visit 2 at Week 5, Visit 3 at Week 13, Visit 4 at Week 29, Visit 5 at Week 41, and Visit 6 at Week 53). The last treatment visit (Visit 6) was the end of treatment (EoT) visit, which also occurred in case of early discontinuation (ED). The Screening period required at least 1 visit, to occur as per-protocol, at the beginning of the period, but some assessments (i.e., mammography, biopsy, transvaginal ultrasound [TVUS], ECG, and Papanicolaou [PAP] test) could require additional visits in case these assessments could not be performed during the same visit. During the treatment period, additional visits could also be required for ad hoc safety assessments (i.e., TVUS).

Figure 13: Study Design – Flow Chart



Abbreviations: E4 20 mg=estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); EoT=end of treatment; n=number of subjects; NH=non-hysterectomized; P4=progesterone

Study participants

In- and exclusion criteria

Please refer to the efficacy section, C301 ESP.

Treatments

A single treatment arm was included in the Endometrial and General Safety Study Part: E4 20 mg + progesterone (P4) 100 mg.

Safety objectives

Primary safety objective and safety endpoint

To evaluate the effect of treatment with E4 20 mg/P4 100 mg on the endometrium

- Incidence of endometrial hyperplasia with up to 12 months of treatment based on endometrial biopsies

Secondary safety objectives and safety endpoints

1. To evaluate the general safety of treatment with E4 20 mg/P4 100 mg.
   - Frequency of treatment-emergent adverse events (TEAEs) (including serious TEAEs).
   - Frequency of changes in results in physical and gynaecological examination, vital signs, electrocardiogram (ECG), mammography, and breast examination at each measured time point.
   - Frequency of changes in routine clinical laboratory tests results (haematology and chemistry) at each measured time point.
2. To evaluate the effect of treatment with E4 20 mg/P4 100 mg on vaginal bleeding.
   - Frequency of women with vaginal bleeding and/or spotting during each 28-day cycle of treatment based on recording in their subject diary.
   - Number of days with bleeding and/or spotting during each 28-day cycle of treatment based on recording in the subject diary.
   - Frequency of women with amenorrhea (absence of any bleeding or spotting) during each 28-day cycle of treatment, based on recording in the subject diary.
   - Cumulative rates of amenorrhea defined as the percentage of women who reported consecutive cycles of amenorrhea for a given cycle of time.
3. To further evaluate the effect of treatment with E4 20 mg/P4 100 mg on the endometrium.
   - Change from baseline to each measured time point in endometrial thickness measured by ultrasound.
   - Frequency of subjects in the different endometrial categories according to Blaustein's Pathology in subjects for whom an endometrial biopsy has been taken.

Statistical methods

Primary and Secondary Endometrial Safety Endpoints

The frequency of endometrial events was computed on the evaluable biopsies of the endometrium (Final diagnosis) or adequate specimens (Initial and Safety), as well as non-evaluable and inadequate (respectively) biopsies. In addition, counts (n) and percentages were provided for the subcategories of the evaluable/adequate biopsies. The rate of endometrial events was generated on the evaluable endometrium. The 95% two-sided CI was computed on the observed frequency of each type of endometrial events.

Evaluation of Endometrial Tissue

Standardized criteria provided in Blaustein's Pathology text (Pathology of the Female Genital Tract 8) were used to characterize the endometrial tissue. Endometrial polyps were fully characterized as to the glandular proliferation and atypia.

The endometrial tissue obtained by endometrial biopsy at screening, during the conduct of the study, and at the end of the study was processed in the same manner by a central laboratory.

Screening Biopsies/Initial Diagnosis

Biopsies obtained at screening were initially read by one safety pathologist (initial pathology report). The investigator decided upon inclusion of the subject into the study or screen failure based on the initial pathology report at screening.

All inadequate tissue records, defined as no tissue or tissue insufficient for diagnosis, were disregarded in the derivation of the Initial Diagnosis; the diagnosis was based on adequate tissue samples only, read by the safety pathologist.

Screening biopsies were also read by 2 other expert pathologists after enrolment. If a subject was enrolled incorrectly, she was discontinued.

On study Biopsies

Biopsy samples collected during the study and at the EoT/ED (Visit 6) were evaluated by 3 expert pathologists for the Safety and Final/Consensus Diagnosis. These 3 expert pathologists were independent and belonged to different institutions. They were blinded to each other's readings. The Safety Diagnosis was based on readings from pathologists 1, 2, and 4, whereas the Final/Consensus Diagnosis relied on readings from pathologists 2, 4, and 5. During the study, the investigator was informed if subsequent readings impacted the initial decision.

Safety Diagnosis

The investigator received the final pathology report (or Safety Diagnosis), defined as "*the worst reading of adequate specimens, from the 3 pathologists involved in safety reading, where disordered proliferative was the best and carcinoma the worst*. *If none of the adequate specimen readings were disordered proliferative or worse, the first reading with an adequate specimen was kept as Safety Diagnosis. If the 3 readers concurred on "no tissue or tissue insufficient", then no Safety Diagnosis was derived"*.

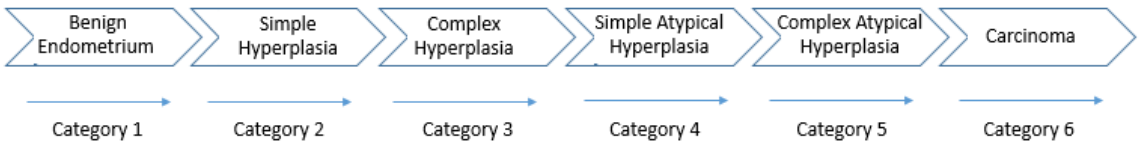
Final/Consensus Diagnosis

The Final/Consensus Diagnosis signified "*the concurrence of diagnosis of at least 2 of the 3 pathologists. If there was no agreement among at least 2 pathologists, the most severe pathologic diagnosis was used as the Final/Consensus Diagnosis. If the 2 or 3 readers concurred on "no tissue or tissue insufficient", then no Final/Consensus Diagnosis was derived*".

The Final/Consensus Diagnosis was only derived for evaluable biopsies. All inadequate tissue records were disregarded; evaluable biopsies were defined as at least 2 readers assessing adequate tissue samples.

The Final/Consensus Diagnosis was used for the analysis of the endometrial safety endpoint. The categorization for Final/Consensus Diagnosis is defined in Figure below.

Figure 14: Category Indicators for Final Diagnosis (from Least to Most Severe)



Discontinuation

If a biopsy showed DPE, hyperplasia, or carcinoma, the study drug was stopped, treatment with P4 200 mg for 14 days was given, and the subject returned to the site for the last visit. If the event was not resolved, the subject was followed up until resolution according to local practice/guidelines.

In case of bleeding events Grade 2, DPE, or worse, the event had to be reported as an AESI.

Analysis of Endometrial Tissue

The primary safety endpoint was the incidence of endometrial hyperplasia with up to 12 months of treatment based on endometrial biopsies. The primary endpoint for endometrial safety is based on the worst-case analysis across post-baseline visits (Final/Consensus Diagnosis) in the Endometrial Safety Analysis Set.

The secondary safety endpoint was the frequency of subjects in the different endometrial categories according to Blaustein's Pathology in subjects for whom an endometrial biopsy has been taken. The secondary endpoint for endometrial safety is based on the worst-case analysis across post-baseline visits (Final/Consensus Diagnosis) in the Endometrial Safety Analysis Set.

- Initial Diagnosis (from first safety reader, based on adequate samples only)
  - By visit analysis: The Initial Diagnosis was summarized at each visit.
- Safety Diagnosis
  - By visit analysis: The Safety Diagnosis was summarized at each visit.
- Final/Consensus Diagnosis
  - By visit analysis: The Final/Consensus Diagnosis was summarized at each visit that occurred on-treatment.
  - Worst-case analysis across post-baseline visits: The individual worst-case derived from post-baseline visits, including unscheduled visits, was summarized for Final/Consensus Diagnosis.

To analyse inter-reader variability, Kappa statistics (coefficient of agreement between the reviewing pathologists) was calculated. The simple (Cohen) kappa coefficient measure of interrater agreement was used to estimate agreement among all the pair-wise comparisons of readers. For the comparison of Consensus diagnosis (between readers 2, 4, and 5), Safety Diagnosis (between readers 1, 2, and 4), and overall comparison (between all readers), the subject level comparison/repeated nature of the biopsies was not of interest, and therefore, the SAS MAGREE macro was used to calculate Fleiss's kappa statistics for nominal responses.

Determination of Sample Size

The number of subjects in the Endometrial and General Safety Study part was selected to meet the requirement stated in EMA guideline (EMEA/CHMP/021/97 Rev. 1, 2005) with regards to the number of paired endometrial biopsies required to evaluate endometrial safety. It was planned to enrol approximately 900 subjects to ensure a total of 300 completers. However, if during the study the drop-out rate appeared to be lower than 67%, less subjects were to be enrolled to reach the target of 300 completers.

Dataset included

- **Included Set:** included a total of 929 (100%) subjects. This was the main analysis set for the disposition table and all the listings of the Endometrial and General Safety Study part.
- **Safety Analysis Set:** included a total of 922 (99.2%) subjects who received at least 1 dose of study drug. Seven subjects (0.8%) did not receive any dose of the study drug and were excluded from the Safety Analysis Set. The Safety Analysis Set was used for all analyses of safety, tolerability, and background characteristics
- **Endometrial Safety Analysis Set:** included a total of 346 (37.2%) subjects. Three (0.3%) subjects were excluded from the Endometrial Safety Analysis Set due to unavailability of an evaluable biopsy at baseline and 573 (61.7%) subjects were excluded because they did not have an evaluable biopsy at Month 12 (defined by a visit window as on or after Day 326) and did not have a diagnosis of endometrial hyperplasia prior to Month 12. Seven subjects (0.8%) were excluded because they did not receive any dose of the study drug.
- **PP Set:** included a total of 424 (45.6%) subjects. Seven subjects (0.8%) were excluded from the PP Set because they did not receive any dose of the study drug. A total of 498 (53.6%)

subjects were excluded from the PP Set due to major protocol deviations. The PP Set was used for efficacy sensitivity analyses.

Results

Participant flow

A total of 929 subjects were included in the study. A total of 922 (99.2%) subjects were treated with at least 1 dose of the study drug and were included in the Safety Analysis Set.

A total of 402 (43.6%) subjects completed the treatment, and 520 (56.4%) subjects discontinued the treatment. The most common reasons for treatment discontinuation can be found in the table below.

Of note, in the disposition table no subjects were reported as having discontinued due to endometrial biopsy showing proliferative disordered pattern or worse or due to endometrial safety findings, because the reason for discontinuation (for disposition evaluation purposes) was assessed as a different category (e.g., AE, physician decision).

Table 50: Subject Disposition - Safety Study Part - Included Set

Subject Disposition	E4 20 mg + P4 100 mg	
	Full population n (%)	Sub-population ≥12 months since last menses n (%)
Subjects in Safety Study Part (Included Set)	929	859
Subjects Treated (Safety Analysis Set)	922 (99.2)	853 (99.3)
Subjects Completed Treatment	402 (43.6)	372 (43.6)
Subjects Discontinued Treatment	520 (56.4)	481 (56.4)
Adverse Event	273 (29.6)	249 (29.2)
Serious Adverse Event	18 (2.0)	17 (2.0)
Death	0	0
Endometrial Biopsy Showing Proliferative Disordered Pattern or Worse Requiring Treatment with a Progestin	0	0
Endometrial Safety Findings that in the Judgement of the Investigator Precludes Further Treatment	0	0
Lack of Efficacy	6 (0.7)	6 (0.7)
Withdrawal of Consent Due to Lack of Efficacy	19 (2.1)	17 (2.0)
Withdrawal of Consent for Another Reason	80 (8.7)	76 (8.9)
Protocol Violation	3 (0.3)	3 (0.4)
Non-Compliance to Trial Protocol: VMS Count Compliance	0	0
Non-Compliance with Study Drug	13 (1.4)	12 (1.4)
Non-Compliance to Trial Protocol: Others	6 (0.7)	5 (0.6)
Withdrawal by Subject	2 (0.2)	2 (0.2)
Study Terminated by Sponsor	2 (0.2)	1 (0.1)
Physician Decision	37 (4.0)	36 (4.2)
Lost to Follow-Up	33 (3.6)	31 (3.6)
COVID-19	7 (0.8)	6 (0.7)
Other	21 (2.3)	20 (2.3)

Note: For Subjects Treated, percentages (%) are based on the number of subjects in the Included Set.
For Subjects Completed Treatment and Subjects Discontinued Treatment, percentages (%) are based on the number of subjects in the Safety Analysis Set.

Protocol deviations

Overall, 498 (54.0%) subjects had at least 1 major protocol deviation or violation during the study. The most commonly (>10% incidence) reported protocol deviations or violations were related to other reasons (188 [20.4%] subjects), study drug non-compliance (149 [16.2%] subjects), procedures/tests non-compliance (135 [14.6%] subjects), and issues with ICF (113 [12.3%] subjects).

Conduct of the study

Number of participants (planned and analysed)

- Included Set: included a total of 929 (100%) subjects.
- Safety Analysis Set: included a total of 922 (99.2%) subjects who received at least 1 dose of study drug. Seven subjects (0.8%) did not receive any dose of the study drug and were excluded from the Safety Analysis Set.
- Endometrial Safety Analysis Set: included a total of 346 (37.2%) subjects. Three (0.3%) subjects were excluded from the Endometrial Safety Analysis Set due to unavailability of an evaluable biopsy at baseline and 573 (61.7%) subjects were excluded because they did not have an evaluable biopsy at Month 12 (defined by a visit window as on or after Day 326) and did not have a diagnosis of endometrial hyperplasia prior to Month 12. Seven subjects (0.8%) were excluded because they did not receive any dose of the study drug.
- PP Set: included a total of 424 (45.6%) subjects. Seven subjects (0.8%) were excluded from the PP Set because they did not receive any dose of the study drug. A total of 498 (53.6%) subjects were excluded from the PP Set due to major protocol deviations.

Baseline data

Demographics and Baseline Characteristics

Mean (SD) age of the study population was 53.9 (4.76) years, ranging from 40 to 65 years. Mean (SD) BMI was 26.77 (4.183) kg/m2. The majority (91.4%) of subjects were White and <5% were Black or African American (4.7%) or another race. The majority (78.5%) of subjects were not Hispanic/Latino.

Overall, 85.2% of subjects were non-smokers and 14.6% of subjects were smokers (with the majority having ≤15 cigarettes per day). Of the 922 subjects, only 8 (0.9%) subjects had a history of bilateral oophorectomy, confirmed with available documentation. The majority (89.3%) of subjects had normal PAP results at baseline and 10.4% of subjects had an abnormal but non-clinically significant PAP result. One (0.1%) subject had an abnormal clinically significant PAP result at baseline. Overall, 75.9% of subjects had a BI-RAD score of 1 or 2, a requirement for study enrolment. Breast Imaging-Reporting and Data System (BI-RAD) score was 0 for 7 (0.8%) subjects; per-protocol, further assessment was done confirming non-clinically significant (NCS) observations. BI-RAD score was not assessed at baseline for 215 subjects as a historical score was available. It is important to note that the protocol did not require the BI-RAD score to be documented in the EDC system if a historical score was accessible and met inclusion criterion.

Outcomes and estimation

Endometrial Biopsy

Of note, reporting of AESIs was implemented from Protocol Version 4.0, (Amendment 3) onwards. In previous protocol versions, an endometrial biopsy reading of DPE, hyperplasia or worse, was to be reported as an SAE.

Analysis was performed on the Endometrial Safety Analysis Set (this included 346 subjects who received at least one dose of study medication and had an evaluable biopsy at Baseline and at Month

12 or had a diagnosis of endometrial hyperplasia prior to Month 12). Of this Set, 325 subjects had an available Final/Consensus diagnosis.

The primary endometrial safety endpoint showed an incidence rate of hyperplasia of 0.3% (2-sided 95% confidence interval [CI]: 0.0–1.7) with E4 20 mg + P4 100 mg daily oral treatment for up to 1 year. This was 1 (0.3%) subject with complex hyperplasia without atypia. There were no cases of endometrial carcinoma.

Of the subjects with an evaluable biopsy, benign endometrium was diagnosed in 324 (99.7%); of these, 15 (4.6%) subjects were diagnosed with DPE.

In sub-population of subjects with at least 12 months since last menses, the incidence rate of hyperplasia was 0.3% (2-sided 95% CI: 0.0–1.9) in 298 subjects and with evaluable biopsies: 1 subject had a Final/Consensus diagnosis of complex hyperplasia without atypia. Benign endometrium was diagnosed in 99.7% of subjects, and of these, 5.0% were diagnosed with DPE. There were no cases of endometrial carcinoma or hyperplasia with atypia.

The incidence rate of DPE was also calculated based on the Final/Consensus diagnosis in the Safety Analysis Set, which includes all subjects who received at least one dose of study drug.

For the full study population, in the Safety Analysis Set (N=922), endometrial biopsy was performed in 629 subjects, 548 of whom had an evaluable biopsy. Among these subjects, 27 (4.9%) were diagnosed with DPE based on the Final/Consensus diagnosis.

For the sub-population of NH women with ≥12 months since last menses, in the Safety Analysis Set (N=853), endometrial biopsy was performed in 577 subjects, 503 of whom had an evaluable biopsy. Among these subjects, 27 (5.4%) were diagnosed with DPE based on the Final/Consensus diagnosis.

Table 51: Summary of Endometrial Biopsy Results by Visit: Safety Diagnosis - Safety Study Part - Safety Analysis Set

	E4 20 mg + P4 100 mg (N=922)						
Frequency of diagnosis	**Baseline N=919[a] n (%)**	**Week 5 N=52[a] n (%)**	**Week 13 N=94[a] n (%)**	**Week 29 N=45[a] n (%)**	**Week 41 N=17[a] n (%)**	**Week 53 N=325[a] n (%)**	**ED N=149[a] n (%)**
Atrophic	748 (81.4)	1 (1.9)	9 (9.6)	0	2 (11.8)	70 (21.5)	37 (24.8)
Inactive	114 (12.4)	5 (9.6)	16 (17.0)	11 (24.4)	3 (17.6)	87 (26.8)	44 (29.5)
Proliferative	39 (4.2)	35 (67.3)	63 (67.0)	29 (64.4)	9 (52.9)	129 (39.7)	50 (33.6)
Weakly proliferative	23 (2.5)	8 (15.4)	19 (20.2)	7 (15.6)	1 (5.9)	37 (11.4)	21 (14.1)
Active proliferative	13 (1.4)	5 (9.6)	11 (11.7)	8 (17.8)	2 (11.8)	39 (12.0)	5 (3.4)
Disordered proliferative	3 (0.3)	22 (42.3)	33 (35.1)	14 (31.1)	6 (35.3)	53 (16.3)	24 (16.1)
Secretory	11 (1.2)	0	4 (4.3)	4 (8.9)	1 (5.9)	24 (7.4)	5 (3.4)
Cyclic type	5 (0.5)	0	4 (4.3)	3 (6.7)	1 (5.9)	15 (4.6)	2 (1.3)
Progestational type (including stromal decidualization)	6 (0.7)	0	0	1 (2.2)	0	9 (2.8)	3 (2.0)
Menstrual type	7 (0.8)	11 (21.2)	2 (2.1)	1 (2.2)	2 (11.8)	15 (4.6)	12 (8.1)
Simple hyperplasia without atypia	0	0	0	0	0	0	0
Simple hyperplasia with atypia	0	0	0	0	0	0	0

	E4 20 mg + P4 100 mg (N=922)						
Frequency of diagnosis	**Baseline N=919[a] n (%)**	**Week 5 N=52[a] n (%)**	**Week 13 N=94[a] n (%)**	**Week 29 N=45[a] n (%)**	**Week 41 N=17[a] n (%)**	**Week 53 N=325[a] n (%)**	**ED N=149[a] n (%)**
Complex hyperplasia without atypia	0	0	0	0	0	0	1 (0.7)
Complex hyperplasia with atypia	0	0	0	0	0	0	0
Carcinoma	0	0	0	0	0	0	0

Abbreviations: E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); ED=early discontinuation; P4 = progesterone.

a. Number of subjects with at least one adequate biopsy in the Safety Analysis Set. Adequate biopsies included all biopsies, except for biopsies with no tissue or insufficient tissue.

Safety Diagnosis: The worst reading of adequate specimens from the 3 pathologists.

Unscheduled visits are included and have been clustered into regular analysis visits. Subjects can have more than one endometrial biopsy result within an analysis visit. In those cases, the earlier adequate biopsy result is selected.

Table52: Summary of Endometrial Biopsy by Visit: Final/Consensus Diagnosis - Safety Study Part - Endometrial Safety Analysis Set)

	E4 20 mg + P4 100 mg (N=346)					
Frequency of diagnosis	**Week 5 N=9[a] n (%)**	**Week 13 N=30[a] n (%)**	**Week 29 N=21[a] n (%)**	**Week 41 N=12[a] n (%)**	**Week 53 N=303[a] n (%)**	**ED N=13[a] n (%)**
Benign endometrium	9 (100)	30 (100)	21 (100)	12 (100)	303 (100)	12 (92.3)
Disordered proliferative endometrium	0	5 (16.7)	1 (4.8)	1 (8.3)	6 (2.0)	2 (15.4)
Simple hyperplasia without atypia	0	0	0	0	0	0
Simple hyperplasia with atypia	0	0	0	0	0	0
Complex hyperplasia without atypia	0	0	0	0	0	1 (7.7)
Complex hyperplasia with atypia	0	0	0	0	0	0
Carcinoma	0	0	0	0	0	0

Abbreviations: E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); ED=early discontinuation; P4 = Progesterone.

a. Number of subjects with an evaluable biopsy in the Endometrial Safety Analysis Set. Evaluable biopsies included all biopsies, except for biopsies with no tissue or insufficient tissue.

Final/Consensus Diagnosis: The concurrence of at least 2 adequate specimens from 2 of the 3 pathologists. If there is no agreement among at least 2 of the 3 pathologists, the most severe pathologic diagnosis is used as the final diagnosis. Unscheduled visits are included and have been clustered into regular analysis visits. Subjects can have more than one endometrial biopsy result within an analysis visit. In those cases, the earlier evaluable biopsy result is selected.

Table 53: Summary of the Worst-Case Analysis Across Post-Baseline Visits (Final/Consensus Diagnosis) - Safety Study Part - Endometrial Safety Analysis Set

	E4 20 mg + P4 100 mg (N=346)	
Parameter	**n (%)**	**95% CI**
Subjects with performed biopsy [a]	346 (100)	(98.9, 100.0)
Number of subjects with evaluable biopsies [b]	325 (93.9)	(90.9, 96.2)
Benign Endometrium [c]	324 (99.7)	(98.3, 100.0)
Disordered proliferative [c]	15 (4.6)	(2.6, 7.5)
Simple Hyperplasia Without Atypia [c]	0	(0.0, 1.1)
Simple Hyperplasia With Atypia [c]	0	(0.0, 1.1)
Complex Hyperplasia Without Atypia [c]	1 (0.3)	(0.0, 1.7)
Complex Hyperplasia With Atypia [c]	0	(0.0, 1.1)
Carcinoma [c]	0	(0.0, 1.1)

Abbreviations: CI = confidence interval; E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); P4 = Progesterone.

Note: Denominator for the computation of percentage (%) and 95% CIs:

[a] Number of subjects in the Endometrial Safety Analysis Set.

[b] Number of subjects with performed biopsy in the Endometrial Safety Analysis Set.

[c] Number of subjects with an evaluable biopsy in the Endometrial Safety Analysis Set.

Final/Consensus Diagnosis: The concurrence of at least 2 adequate specimens from 2 of the 3 pathologists. If there is no agreement among at least 2 of the 3 pathologists, the most severe pathologic diagnosis is used as the final diagnosis.

Table 54: Summary of the Worst-Case Analysis Across Post-Baseline Visits (Final/Consensus Diagnosis)
Safety Study Part - Endometrial Safety Analysis Set Sub-population: ⩾ 12 months amenorrhea (N=316)

	E4 20 mg+ P4 100 mg	
Parameter	**n (%)**	**95% CI**
Subjects with performed biopsy [a]	316 (100.0)	[98.8, 100.0]
Subjects with an evaluable biopsy [b]	298 (94.3)	[91.1, 96.6]
Benign Endometrium [c]	297 (99.7)	[98.1, 100.0]
Disordered proliferative [c]	15 (5.0)	[2.8, 8.2]
Simple hyperplasia without atypia [c]	0	[0.0, 1.2]
Simple hyperplasia with atypia [c]	0	[0.0, 1.2]
Complex hyperplasia without atypia [c]	1 (0.3)	[0.0, 1.9]
Complex hyperplasia with atypia [c]	0	[0.0, 1.2]
Carcinoma [c]	0	[0.0, 1.2]

CI = confidence interval; E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); P4 = Progesterone.

Note: Denominator for the computation of percentage (%) and 95% CIs:

[a] Number of subjects in the Endometrial Safety Analysis Set.

[b] Number of subjects with performed biopsy in the Endometrial Safety Analysis Set.

[c] Number of subjects with an evaluable biopsy in the Endometrial Safety Analysis Set.

Final/Consensus Diagnosis: The concurrence of at least two adequate specimens from two of the three pathologists. If there is no agreement amon g the
three pathologists, the most severe pathologic diagnosis is used as the final diagnosis.

To analyze inter-reader variability, Kappa statistics (coefficient of agreement) were calculated for the endometrial biopsy reading agreement between the reviewing pathologists. The level of agreement between the readers was poor for overall (kappa=0.1498), Safety Diagnosis (kappa=0.1083), and Consensus Diagnosis (kappa=0.1348), respectively. For pair-wise readers comparisons, the level of agreement was poor between readers #1 and #2 (kappa=0.0584), readers #2 and #4 (kappa=0.1658), and readers #2 and #5 (kappa=0.0521). The level of agreement was fair between readers #1 and #4 (kappa=0.2247), readers #1 and #5 (kappa=0.2206), and readers #4 and #5 (kappa=0.2324).

Transvaginal ultrasound

Abnormal clinical significant (CS) TVUS findings were noted in 3 (0.3%) subjects at baseline, 33 (3.6%) subjects at Week 13, 23 (2.5%) subjects at Week 29, 24 (2.6%) subjects at ED, and 8 (0.9%) subjects at Week 53 visits.

Shift tables for TVUS findings indicated the following shifts from baseline:

- From normal to abnormal CS reported in 19 (3.2%) subjects at Week 13, 13 (2.9%) subjects at Week 29, 13 (3.5%) subjects at ED visit, and 3 (0.8%) subjects at Week 53.
- From abnormal NCS to abnormal CS reported in 13 (2.2%) subjects at Week 13, 9 (2.0%) subjects at Week 29, 11 (3.0%) subjects at ED visit, and 4 (1.0%) subjects at Week 53.

Endometrial Thickness

The mean (SD) endometrial thickness increased from 2.61 (0.969) mm at baseline, to 5.51 (2.781) mm at Week 13, 5.19 (2.539) mm at Week 29, 4.85 (2.626) mm at Week 53, and 4.66 (3.175) mm at the ED visit. In the sub-population of subjects with at least 12 months since last menses, the mean endometrial thickness at Baseline was 2.58 mm and increased to 5.48 mm, 5.16 mm, and 4.82 mm at Week 13, Week 29, and Week 53, respectively. In subjects who prematurely discontinued the study, the mean endometrial thickness at the early discontinuation visit was 4.68 mm.

Table 55: Summary of Endometrial Thickness (mm) and Change from Baseline by Visit - Safety Study Part - Safety Analysis Set

		E4 20 mg + P4 100 mg (N=922)	
Visit	**Statistics**	**Observed Value**	**Change from Baseline**
Baseline	n	918	
	Mean (SD)	2.61 (0.969)	
Week 13	n	596	593
	Mean (SD)	5.51 (2.781)	2.92 (2.832)
Week 29	n	456	455
	Mean (SD)	5.19 (2.539)	2.61 (2.600)
Week 53	n	385	384
	Mean (SD)	4.85 (2.626)	2.29 (2.678)
ED	n	370	369
	Mean (SD)	4.66 (3.175)	2.04 (3.176)

Abbreviations: E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg); ED=early discontinuation; P4 = Progesterone; SD=standard deviation.
Note: Baseline is defined as the last non-missing value recorded prior to the first dose of study drug.
Missing category is displayed only when there are missing values.

Vaginal bleeding (over time)

A summary of vaginal bleeding and/or spotting (overall and after exclusion of bleeding/spotting within 6 days after a biopsy or after discontinuation of E4 treatment) assessed from available bleeding information in diaries for each 28-day cycle during study treatment (Cycles 1 to 13). Percentages are calculated based on the number of subjects with available bleeding/spotting diary information during each cycle in the Safety Analysis Set.

Overall, the proportion of subjects with any bleeding and/or spotting during the cycle increased up to 67.6% in Cycle 3 and then decreased gradually to a range of 51.9% to 54% across Cycle 9 to Cycle 13. In the majority of cycles, 20%-25% of the subjects reported only spotting. The proportion of subjects with bleeding only throughout the 13 cycles ranged from 0.8%-5.6%.

Similar results were observed when excluding vaginal bleeding and/or spotting values within 6 days after a biopsy or after discontinuation of E4 treatment.

In the sub-population, the proportion of subjects with ≥12 months since last menses (treated with E4 20 mg + P4 100 mg) with any bleeding and/or spotting increased from Cycle 1 (361 [44.3%]) to Cycle 2 (501 [66.4%]). By Cycle 3, 447 (66.2%) subjects reported any bleeding and/or spotting. The proportion then gradually decreased, with the proportion of subjects with any bleeding and/or spotting being 53.1% during Cycle 11, 51.1% during Cycle 12, and 53.3% during Cycle 13. In the majority of cycles, 20−25% of the subjects reported spotting only. The proportion of subjects with bleeding only during a cycle ranged from 0.8−5.6% over the 13 cycles period. A similar trend was seen when values of spotting and/or bleeding within a 6-day window after a biopsy, or all values after discontinuation of E4, were excluded.

Amenorrhea

The cumulative rate of amenorrhea is summarized below.

Overall, 660 subjects had at least one cycle with amenorrhea during the study. Amenorrhea was observed in 365 (55.3%) of these subjects at Cycle 13. Cumulative amenorrhea was observed in 140 (36.7%) subjects over Cycles 11-13, in the sub-group of women at least 12 months since last menses this was 130 (36.9%).

Table 56: Summary of Cumulative Rate of Amenorrhea by Cycle - Safety Study Part - Safety Analysis Set

Cycle/ month	Full study population (N = 922)		Post-menopausal women with at least 12 months since last menses (N = 853)	
	Number of subjects who completed all corresponding cycles	Number of subjects with amenorrhea in all the corresponding cycles (%)	Number of subjects who completed all corresponding cycles	Number of subjects with amenorrhea in all the corresponding cycles (%)
Cycles 1-3/ months 1-3	689	142 (20.6)	632	136 (21.5)
Cycles 4-6/ months 4-6	515	154 (29.9)	473	145 (30.7)
Cycles 7-10/ months 7-9	409	125 (30.6)	379	117 (30.9)
Cycles 11-13/ months 10-12	381	140 (36.7)	352	130 (36.9)

Venous Thrombotic Events and Cardiovascular Events

Please refer to 5.4.4. - Thromboembolic Events

Mammography

At EoT/ED, 384 mammograms were performed and assessed by the site-investigator. Four TEAEs related to mammograms at EoT/ED were reported: 2 cases of breast cyst, 1 case of increased breast density and 1 case of higher breast density. Increased and higher breast density cases were both coded as 'mammogram abnormal' PT. Each of these TEAEs was assessed as non-serious, of mild intensity and related to study drug.

Endometrial safety from phase 3 studies in NH subjects treated with E4 monotherapy

53-week placebo-controlled safety population (E4 monotherapy) - study C302 ESP

Of the subjects with an evaluable biopsy, benign endometrium was diagnosed in 41 (93.2%), 40 (88.9%), and 20 (100%) subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively; of these, DPE was diagnosed in 14 (31.8%), 23 (51.1%), and 0 (0%) subjects, respectively.

Endometrial hyperplasia was diagnosed in 3 (6.8%), 5 (11.1%), and 0 (0%) subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively. Of these, 1 (2.3%) subject in the E4 15 mg arm and 3 (6.7%) subjects in the E4 20 mg arm had simple hyperplasia without atypia; 1 (2.2%) subject in the E4 20 mg arm had simple hyperplasia with atypia; 1 subject in each of the E4 15 mg and E4 20 mg arms had complex hyperplasia without atypia; and 1 (2.3%) subject in the E4 15 mg arm reported complex hyperplasia with atypia.

No subjects had carcinoma of the endometrium on biopsy.

All evaluable biopsies taken in subjects at the Follow-Up Visit, after treatment with P4 200 mg, showed benign type of endometrium with no cases of DPE or hyperplasia.

13-week placebo-controlled safety population (E4 monotherapy) – study C301 ESP

Of the subjects with an evaluable biopsy, benign endometrium was diagnosed in 65 (97.0%), 59 (95.2%), and 56 (100%) subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively; of these, DPE was diagnosed in 33 (49.3%), 29 (46.8%), and 1 (1.8%) subjects, respectively.

Endometrial hyperplasia was diagnosed in 2 (3.0%), 3 (4.8%), and 0 (0%) subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively; of these, 1 (1.5%) subject in the E4 15 mg arm and 3 (4.8%) in the E4 20 mg arm had simple hyperplasia without atypia, and 1 (1.5%) subject in the E4 15 mg had complex hyperplasia without atypia.

No subjects had carcinoma of the endometrium or hyperplasia with atypia.

Biopsies taken in subjects at the Week 15/16 Follow-up Visit, after treatment with P4 200 mg, showed benign type of endometrium with no cases of DPE.

53-week uncontrolled safety population (E4 monotherapy) - study C302 SSP

Of the subjects with an evaluable biopsy, benign endometrium was diagnosed in 125 (94.7%); of these, DPE was diagnosed in 70 (50.3%) subjects.

Endometrial hyperplasia was diagnosed in 7 (5.3%) subjects, and all of these were simple hyperplasia without atypia.

There were no cases of endometrial carcinoma.

Biopsies taken in subjects at the Follow-up Visit, after treatment with P4 200 mg, showed benign type of endometrium with no cases of DPE or hyperplasia.

Table 57: Summary of Final/Consensus Diagnosis of Endometrial Biopsies Across all Post-baseline Visits Based on Endometrial Biopsy Readings Per Protocol – MIT-Do001-C301 ESP

(Safety Population), MIT-Do001-C301 SSP (Endometrial Safety Analysis Set), MIT-Do001-C302 (Safety Population)

Study number	MIT-Do001-C301			MIT-Do001-C302			MIT-Do001-C302	MIT-Do001-C301	
Study part (duration)	ESP (12 weeks)			ESP (52 weeks)			SSP (52 weeks)	SSP (52 weeks)	
Study drug/s	E4 15 mg n (%)	E4 20 mg n (%)	Placebo n (%)	E4 15 mg n (%)	E4 20 mg n (%)	Placebo n (%)	E4 20 mg n (%)	E4 20 mg + P4 100 mg Full population n (%) (95% CI)	E4 20 mg + P4 100 mg Sub-population: ≥12 months since last menses n (%) (95% CI)
Number of subjects with an evaluable biopsy	67	62	56	44	45	20	132	325[a]	298[a]
Benign endometrium	65 (97.0)	59 (95.2)	56 (100)	41 (93.2)	40 (88.9)	20 (100)	125 (94.7)	324 (99.7) (98.3, 100)	297 (99.7) (98.1, 100)
DPE	33 (49.3)	29 (46.8)	1 (1.8)	14 (31.8)	23 (51.1)	0	70 (53.0)	15 (4.6) (2.6, 7.5)	15 (5.0) (2.8, 8.2)
Endometrial hyperplasia	2 (3.0)	3 (4.8)	0	3 (6.8)	5 (11.1)	0	7 (5.3)	1 (0.3) (0, 1.7)	1 (0.3) (0, 1.9)
Simple hyperplasia without atypia	1 (1.5)	3 (4.8)	0	1 (2.3)	3 (6.7)	0	7 (5.3)	0 (0) (0, 1.1)	0 (0) (0, 1.2)
Complex hyperplasia without atypia	1 (1.5)	0	0	1 (2.3)	1 (2.2)	0	0	1 (0.3) (0, 1.7)	1 (0.3) (0, 1.9)
Simple hyperplasia with atypia	0	0	0	0	1 (2.2)	0	0	0 (0) (0, 1.1)	0 (0) (0, 1.2)
Complex hyperplasia with atypia	0	0	0	1 (2.3)	0	0	0	0 (0) (0, 1.1)	0 (0) (0, 1.2)
Endometrial carcinoma	0	0	0	0	0	0	0	0	0
Hyperplasia and carcinoma	-	-	-	-	-	-	-	1 (0.3) (0, 1.7)	1 (0.3) (0, 1.9)

CI = confidence interval, CSR = Clinical Study Report, DPE = disordered proliferative endometrium, E4 = estetrol monohydrate, ESP = Efficacy Study Part, n = number of subjects in category of interest, P4 = progesterone, SSP = Safety Study Part, US = United States

The concurrence of at least two evaluable diagnoses from two of the three pathologists is summarized. If there was no agreement among at least two of the three pathologists, the most severe pathologic diagnosis was used as the Final/Consensus diagnosis.
a. Number of subjects of the Endometrial Safety Analysis Set with an available Final/Consensus diagnosis.

Information on re-reading of the endometrial biopsies

Statistical analysis of inter- and intra-reader variability in the individual diagnoses of endometrial biopsies revealed significant diagnostic divergences between the 3 Pathologists in the first panel that conducted the per protocol readings. Notably, these divergences were in the diagnosis of hyperplasia, and significant inconsistencies between readings of the same biopsies for one of the Pathologists. The high inter- and intra-reader variability triggered uncertainty about the conclusion to be drawn for the endometrial safety of treatment with estetrol (E4), and E4 combined with progesterone (P4) in non-hysterectomized (NH) women in the Phase 3 studies. Therefore, a re-read of the biopsy slides from the Phase 3 studies (obtained during unscheduled, end of treatment [EoT] or early discontinuation [ED] visit) with a new panel of 3 independent expert pathologists has been performed. The re-reading approach has been discussed with the FDA and with the EMA.

Although a re-reading was not encouraged by the CHMP because it is not per protocol and not per SAP, the CHMP acknowledged that it may be acceptable, if deemed necessary, to properly evaluate the endometrial effect of E4 and the endometrial protection by a progestogen.

The applicant reported that it was unable to demonstrate that the outlier reader from the first panel was diagnosing incorrectly. It was therefore accepted to only present and assess the endometrial biopsies from the first (per protocol) read.

5.4.5 Discontinuation due to adverse events

53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

Overall, 123 TEAEs leading to study drug discontinuation occurred in 85 (14.7%) subjects: 33 (17.2%) subjects (52 events) in the E4 15 mg arm; 42 (21.8%) subjects (54 events) in the E4 20 mg arm; and 10 (5.2%) subjects (17 events) in the placebo arm.

Most of these TEAEs were either mild (47 TEAEs in 31 [5.4%] subjects) or moderate (72 TEAEs in 63 [10.9%] subjects) in intensity. Severe intensity TEAEs (n=4) leading to study drug discontinuation occurred in 1 (0.5%) subject in the E4 15 mg arm (1 event of adenocarcinoma gastric), 2 (1.0%) subjects in the E4 20 mg arm (a single event of breast tenderness and intraductal proliferative breast lesion), and in 1 (0.5%) subject in the placebo arm (1 event of cholecystitis acute).

A total of 3 participants discontinued due to a SAE, two NH women in the 15 mg arm (endometrial hyperplasia, adenocarcinoma) and one NH women in the placebo arm (cholecystitis acute).
An overview of the AEs leading to study drug discontinuation per SOC and split for H and NH subjects is presented below.

Table 58: Summary of TEAEs Leading to Study Drug Discontinuation by SOC and PT – MIT-Do001-C302 ESP (Safety Population)

SOC/ PT	E4 15 mg						E4 20 mg						Placebo					
	H (N=99)		NH (N=93)		All (N=192)		H (N=100)		NH (N=93)		All (N=193)		H (N=99)		NH (N=95)		All (N=194)	
	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]
Subjects with Any TEAE Leading to Study Drug Discontinuation	**4 (4.0)**	**5**	**29 (31.2)**	**47**	**33 (17.2)**	**52**	**7 (7.0)**	**9**	**35 (37.6)**	**45**	**42 (21.8)**	**54**	**4 (4.0)**	**6**	**6 (6.3)**	**11**	**10 (5.2)**	**17**
Reproductive System and Breast Disorders	**1 (1.0)**	**1**	**26 (28.0)**	**33**	**27 (14.1)**	**34**	**4 (4.0)**	**4**	**31 (33.3)**	**39**	**35 (18.1)**	**43**	**2 (2.0)**	**3**	**2 (2.1)**	**2**	**4 (2.1**	**5**
Endometrial disorder[c]	0	0	14 (15.1)	14	14 (7.3)	14	0	0	15 (16.1)	15	15 (7.8)	15	0	0	1 (1.1)	1	1 (0.5)	1
Vaginal haemorrhage	0	0	7 (7.5)	7	7 (3.6)	7	0	0	8 (8.6)	8	8 (4.1)	8	0	0	0	0	0	0
Endometrial hyperplasia[c]	0	0	3 (3.2)	3	3 (1.6)	3	0	0	9 (9.7)	9	9 (4.7)	9	0	0	0	0	0	0
Endometrial thickening	0	0	6 (6.5)	6	6 (3.1)	6	0	0	3 (3.2)	3	3 (1.6)	3	0	0	0	0	0	0
Uterine haemorrhage	0	0	2 (2.2)	2	2 (1.0)	2	0	0	3 (3.2)	3	3 (1.6)	3	0	0	0	0	0	0

SOC/ PT	E4 15 mg						E4 20 mg						Placebo					
	H (N=99)		NH (N=93)		All (N=192)		H (N=100)		NH (N=93)		All (N=193)		H (N=99)		NH (N=95)		All (N=194)	
	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]
Breast tenderness	1 (1.0)	1	0	0	1 (0.5)	1	2 (2.0)	2	0	0	2 (1.0)	2	1 (1.0)	1	0	0	1 (0.5)	1
Nipple pain	0	0	0	0	0	0	1 (1.0)	1	0	0	1 (0.5)	1	1 (1.0)	1	0	0	1 (0.5)	1
Breast pain	0	0	0	0	0	0	1 (1.0)	1	0	0	1 (0.5)	1	0	0	0	0	0	0
Heavy menstrual bleeding	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Ovarian cyst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1
Vaginal discharge	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Vulvovaginal dryness	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)	1	0	0	1 (0.5)	1
Investigations	**0**	**0**	**2 (2.2)**	**3**	**2 (1.0)**	**3**	**1 (1.0)**	**1**	**1 (1.1)**	**1**	**2 (1.0)**	**2**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**
Weight increased	0	0	1 (1.1)	1	1 (0.5)	1	1 (1.0)	1	0	0	1 (0.5)	1	0	0	1 (1.1)	1	1 (0.5)	1
Lymphocyte count abnormal	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Neutrophil count abnormal	0	0	1 (1.1)	1	1 (0.5	1	0	0	0	0	0	0	0	0	0	0	0	0
Platelet count increased	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Nervous System Disorders	**1 (1.0)**	**1**	**1 (1.1)**	**1**	**2 (1.0)**	**2**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**	**1 (1.0)**	**1**	**2 (2.1)**	**2**	**3 (1.5)**	**3**
Headache	1 (1.0)	1	1 (1.1)	1	2 (1.0)	2	0	0	0	0	0	0	1 (1.0)	1	2 (2.1)	2	3 (1.5)	3
Migraine	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Gastrointestinal Disorders	**1 (1.0)**	**1**	**2 (2.2)**	**2**	**3 (1.6)**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.1)**	**2**	**1 (0.5)**	**2**
Nausea	1 (1.0)	1	1 (1.1)	1	2 (1.0)	2	0	0	0	0	0	0	0	0	0	0	0	0
Abdominal discomfort	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Abdominal distension	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1
Flatulence	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1
Skin and Subcutaneous Tissue Disorders	**2 (2.0)**	**2**	**1 (1.1)**	**1**	**3 (1.6)**	**3**	**1 (1.0)**	**1**	**0**	**0**	**1 (0.5)**	**1**	**1 (1.0)**	**1**	**0**	**0**	**1 (0.5)**	**1**
Rash	1 (1.0)	1	0	0	1 (0.5)	1	1 (1.0)	1	0	0	1 (0.5)	1	1 (1.0)	1	0	0	1 (0.5)	1
Acne	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Alopecia	1 (1.0)	1	0	0	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
General Disorders and Administration Site Conditions	**0**	**0**	**0**	**0**	**0**	**0**	**3 (3.0)**	**3**	**0**	**0**	**3 (1.6)**	**3**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**
Fatigue	0	0	0	0	0	0	2 (2.0)	2	0	0	2 (1.0)	2	0	0	1 (1.1)	1	1 (0.5)	1
Chest pain	0	0	0	0	0	0	1 (1.0)	1	0	0	1 (0.5)	1	0	0	0	0	0	0

SOC/ PT	E4 15 mg						E4 20 mg						Placebo					
	H (N=99)		NH (N=93)		All (N=192)		H (N=100)		NH (N=93)		All (N=193)		H (N=99)		NH (N=95)		All (N=194)	
	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]	n[a] (%)	E[b]
Neoplasms Benign, Malignant and Unspecified (incl Cysts and Polyps)	**0**	**0**	**3 (3.2)**	**3**	**3 (1.6)**	**3**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Uterine leiomyoma	0	0	2 (2.2)	2	2 (1.0)	2	0	0	0	0	0	0	0	0	0	0	0	0
Adenocarcinoma gastric	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Intraductal proliferative breast lesion	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Musculoskeletal and connective tissue disorders	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**	**1 (1.0)**	**1**	**1 (1.1)**	**1**	**2 (1.0)**	**2**
Arthralgia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1
Back pain	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Muscle spasms	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)	1	0	0	1 (0.5)	1
Blood and Lymphatic System Disorders	**0**	**0**	**2 (2.2)**	**2**	**2 (1.0)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Anaemia	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
White blood cell disorder	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Infections and Infestations	**0**	**0**	**2 (2.2)**	**2**	**2 (1.0)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Helicobacter gastritis	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Subcutaneous abscess	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0	0	0	0	0	0	0
Psychiatric Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.1)**	**2**	**1 (0.5)**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
Anger	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Depression	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1	0	0	0	0	0	0
Cardiac Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**
Sinus tachycardia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1
Hepatobiliary Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.1)**	**1**	**1 (0.5)**	**1**
Cholecystitis acute	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.1)	1	1 (0.5)	1

13-week placebo-controlled H + NH safety population (E4 monotherapy) - ISS pooled data

15 mg

Overall, TEAEs leading to study drug discontinuation were reported in 5.9% of subjects. For the E4 15 mg arm, TEAEs leading to study drug discontinuation were reported in 9.3% of subjects. The

commonly reported TEAEs (in ≥1% of subjects) leading to study drug discontinuation were endometrial disorder (3.7%), vaginal haemorrhage (2.0%), endometrial hyperplasia (1.1%) and endometrial thickening (1.1%). In the placebo arm, TEAEs leading to study drug discontinuation were reported in 2.6% of subjects and no TEAEs leading to study drug discontinuation were reported in ≥1% of subjects. The majority of TEAEs leading to study drug discontinuation were mild or moderate, with no severe TEAE reported in more than 1 subject.

When analyzed by hysterectomy status, the majority of TEAEs leading to study drug discontinuation were reported in the Reproductive System and Breast Disorders SOC in NH subjects. In H subjects no TEAE leading to study drug discontinuation for more than 1 subject was reported in either the E4 15 mg or placebo arms. In NH subjects, the most commonly reported (in ≥5% of subjects) TEAE leading to study drug discontinuation was endometrial disorder (7.2% in the E4 15 mg arm). TEAEs leading to study drug discontinuation for more than 1 subject (≥0.8% of subjects) in the E4 15 mg arm were all reported in NH subjects and included endometrial disorder (7.2%), vaginal haemorrhage (3.8%), endometrial hyperplasia (2.1%), endometrial thickening (2.1%), uterine haemorrhage (0.8%) and uterine leiomyoma (0.8%). TEAEs leading to study drug discontinuation for more than 1 subject in the placebo arm included headache (0.8%) and insomnia (0.8%). The majority of TEAEs leading to study drug discontinuation by hysterectomy status, were mild or moderate, with no severe TEAE reported in more than 1 subject.

A total of 13 SAE leading to study discontinuation were reported in the E4 arm (endometrial disorder (DPE), n=9, endometrial hyperplasia, n=4), and 1 in the placebo arm (hydrosalpinx).

Table 59: TEAEs Leading to Study Drug Discontinuation Reported up to 13 Weeks by SOC and PT, by Hysterectomy Status (Safety Population) - Pooled E4 15 mg and Placebo

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 15 mg (N=218)	Placebo (N=215)	Total (N=433)	E4 15 mg (N=236)	Placebo (N=248)	Total (N=484)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Any TEAEs Leading to Study Drug Discontinuation	**3 (1.4%) 4**	**3 (1.4%) 3**	**6 (1.4%) 7**	**39 (16.5%) 55**	**9 (3.6%) 18**	**48 (9.9%) 73**
Reproductive System and Breast Disorders	**1 (0.5%) 1**	**1 (0.5%) 1**	**2 (0.5%) 2**	**36 (15.3%) 42**	**4 (1.6%) 4**	**40 (8.3%) 46**
Endometrial disorder	0	0	0	17 (7.2%) 17	1 (0.4%) 1	18 (3.7%) 18
Vaginal haemorrhage	0	0	0	9 (3.8%) 9	0	9 (1.9%) 9
Endometrial hyperplasia	0	0	0	5 (2.1%) 5	0	5 (1.0%) 5
Endometrial thickening	0	0	0	5 (2.1%) 5	0	5 (1.0%) 5
Breast tenderness	1 (0.5%) 1	1 (0.5%) 1	2 (0.5%) 2	0	0	0
Breast pain	0	0	0	1 (0.4%) 1	1 (0.4%) 1	2 (0.4%) 2
Uterine haemorrhage	0	0	0	2 (0.8%) 2	0	2 (0.4%) 2
Abnormal uterine bleeding	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Breast swelling	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Hydrosalpinx	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Menopausal symptoms	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Vaginal discharge	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Gastrointestinal Disorders	**0**	**1 (0.5%) 1**	**1 (0.2%) 1**	**3 (1.3%) 3**	**3 (1.2%) 4**	**6 (1.2%) 7**
Abdominal discomfort	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Abdominal distension	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Abdominal pain	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Constipation	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Diarrhoea	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Flatulence	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Nausea	0	1 (0.5%) 1	1 (0.2%) 1	1 (0.4%) 1	0	1 (0.2%) 1

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 15 mg (N=218)	Placebo (N=215)	Total (N=433)	E4 15 mg (N=236)	Placebo (N=248)	Total (N=484)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Investigations	**0**	**0**	**0**	**2 (0.8%) 3**	**1 (0.4%) 1**	**3 (0.6%) 4**
Weight increased	0	0	0	1 (0.4%) 1	1 (0.4%) 1	2 (0.4%) 2
Lymphocyte count abnormal	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Neutrophil count abnormal	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Nervous System Disorders	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**1 (0.4%) 1**	**2 (0.8%) 2**	**3 (0.6%) 3**
Headache	1 (0.5%) 1	0	1 (0.2%) 1	1 (0.4%) 1	2 (0.8%) 2	3 (0.6%) 3
Neoplasms Benign, Malignant and Unspecified (incl cysts and polyps)	**0**	**0**	**0**	**2 (0.8%) 2**	**0**	**2 (0.4%) 2**
Uterine leiomyoma	0	0	0	2 (0.8%) 2	0	2 (0.4%) 2
Psychiatric Disorders	**0**	**0**	**0**	**0**	**2 (0.8%) 3**	**2 (0.4%) 3**
Insomnia	0	0	0	0	2 (0.8%) 2	2 (0.4%) 2
Irritability	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Vascular Disorders	**0**	**0**	**0**	**0**	**2 (0.8%) 2**	**2 (0.4%) 2**
Hot flush	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Superficial vein thrombosis	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Blood and lymphatic system disorders	**0**	**0**	**0**	**1 (0.4%) 1**	**0**	**1 (0.2%) 1**
White blood cell disorder	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
General disorders and administration site conditions	**0**	**0**	**0**	**0**	**1 (0.4%) 1**	**1 (0.2%) 1**
Fatigue	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Infections and Infestations	**0**	**0**	**0**	**1 (0.4%) 1**	**0**	**1 (0.2%) 1**
Subcutaneous abscess	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Injury, poisoning and procedural complications	**0**	**0**	**0**	**1 (0.4%) 1**	**0**	**1 (0.2%) 1**
Contusion	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 15 mg (N=218)	**Placebo (N=215)**	**Total (N=433)**	**E4 15 mg (N=236)**	**Placebo (N=248)**	**Total (N=484)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Musculoskeletal and Connective Tissue Disorders	**0**	**1 (0.5%) 1**	**1 (0.2%) 1**	**0**	**1 (0.4%) 1**	**1 (0.2%) 1**
Arthralgia	0	0	0	0	1 (0.4%) 1	1 (0.2%) 1
Muscle spasms	0	1 (0.5%) 1	1 (0.2%) 1	0	0	0
Renal and urinary disorders	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**0**	**0**	**0**
Urinary incontinence	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0
Skin and subcutaneous tissue disorders	**1 (0.5%) 1**	**0**	**1 (0.2%) 1**	**1 (0.4%) 1**	**0**	**1 (0.2%) 1**
Acne	0	0	0	1 (0.4%) 1	0	1 (0.2%) 1
Rash	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0

Abbreviations: e = Number of events, E4 15 mg = estetrol monohydrate 15 mg (equivalent to estetrol 14.2 mg), N = number of subjects in the statistical analysis set, n = number of observations per treatment group, PT = preferred term, SOC = system organ class, TEAE = treatment emergent adverse event.
Note: Percentage (%) based on the number of subjects in Safety population in each treatment arm or total column.
Classifications of adverse events based on the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0).
TEAEs defined as those AEs occurring from time point of first ingestion of investigational product until last visit or any event already present that worsens in either intensity or frequency following exposure to the treatment. If the start date of the AE is unknown, it will be assumed to be after the start of study drug unless the stop date is before the first dose.
Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.

20 mg

Overall, TEAEs leading to study drug discontinuation were reported in 6.1% of subjects. For the E4 20 mg arm, TEAEs leading to study drug discontinuation were reported in 9.9% of subjects; the commonly reported TEAEs (in ≥1% of subjects) leading to study drug discontinuation were endometrial disorder and vaginal haemorrhage (both reported in 3.0%), endometrial hyperplasia (1.7%), and endometrial thickening (1.0%). In the placebo arm, TEAEs leading to study drug discontinuation were reported in 2.5% of subjects, no TEAEs leading to study drug discontinuation were reported in ≥1% of placebo subjects, and headache was the only TEAE leading to study drug discontinuation reported in more than 1 subject (2 subjects [0.5%]). The majority of TEAEs leading to study drug discontinuation were mild or moderate, with no severe TEAE leading to study drug discontinuation reported in more than 1 subject.

When analyzed by hysterectomy status, the majority of TEAEs leading to study drug discontinuation were reported in NH subjects in the Reproductive System and Breast Disorders SOC. In NH subjects, the most commonly reported (in ≥5% of subjects) TEAE leading to study drug discontinuation was endometrial disorder (6.1% in the E4 20 mg arm) and vaginal haemorrhage (6.1% in the E4 20 mg arm). TEAEs leading to study drug discontinuation for more than 1 subject in the E4 20 mg arm of NH subjects included endometrial disorder (6.1%), vaginal haemorrhage (6.1%), endometrial hyperplasia (3.6%), endometrial thickening (2%) and uterine haemorrhage (1%), and in the E4 20 mg arm of H women breast pain (1%) and breast tenderness (1%). No TEAEs leading to study drug discontinuation for more than 1 subject were reported in the placebo arm. The majority of TEAEs leading to study drug discontinuation by hysterectomy status, were mild or moderate, with no severe TEAE reported in more than 1 subject.

For the 20 mg dose, no SAE leading to study discontinuation were reported for H women. For NH women a total of 15 SAE were reported in the E4 arm (endometrial disorder (DPE), n=7, endometrial hyperplasia, n=6, vaginal haemorrhage, n=1 and intraductal proliferative breast lesion, n=1), and 1 in the placebo arm (hydrosalpinx).

Table 60: TEAEs Leading to Study Drug Discontinuation Reported up to 13 Weeks by SOC and PT, by Hysterectomy Status (Safety Population) - Pooled E4 20 mg and Placebo

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 20 mg (N=210)	Placebo (N=209)	Total (N=419)	E4 20 mg (N=196)	Placebo (N=199)	Total (N=395)
	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e	n (%) e
Any TEAEs Leading to Study Drug Discontinuation	**6 (2.9%) 7**	**3 (1.4%) 3**	**9 (2.1%) 10**	**34 (17.3%) 44**	**7 (3.5%) 14**	**41 (10.4%) 58**
Reproductive System and Breast Disorders	**5 (2.4%) 5**	**1 (0.5%) 1**	**6 (1.4%) 6**	**31 (15.8%) 40**	**3 (1.5%) 3**	**34 (8.6%) 43**
Endometrial disorder	0	0	0	12 (6.1%) 12	1 (0.5%) 1	13 (3.3%) 13
Vaginal haemorrhage	0	0	0	12 (6.1%) 12	0	12 (3.0%) 12
Endometrial hyperplasia	0	0	0	7 (3.6%) 7	0	7 (1.8%) 7
Endometrial thickening	0	0	0	4 (2.0%) 4	0	4 (1.0%) 4
Breast tenderness	2 (1.0%) 2	1 (0.5%) 1	3 (0.7%) 3	0	0	0
Breast pain	2 (1.0%) 2	0	2 (0.5%) 2	0	0	0
Uterine haemorrhage	0	0	0	2 (1.0%) 2	0	2 (0.5%) 2
Adenomyosis	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Heavy menstrual bleeding	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Hydrosalpinx	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Menopausal symptoms	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Uterine polyp	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Nipple pain	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0
Gastrointestinal Disorders	**1 (0.5%) 2**	**1 (0.5%) 1**	**2 (0.5%) 3**	**0**	**2 (1.0%) 3**	**2 (0.5%) 3**
Nausea	1 (0.5%) 1	1 (0.5%) 1	2 (0.5%) 2	0	0	0
Abdominal distension	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Diarrhoea	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Flatulence	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Vomiting	1 (0.5%) 1	0	1 (0.2%) 1	0	0	0
Investigations	**0**	**0**	**0**	**1 (0.5%) 1**	**1 (0.5%) 1**	**2 (0.5%) 2**
Platelet count increased	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Weight increased	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1

SOC PT	Hysterectomized			Non-hysterectomized		
	E4 20 mg (N=210)	**Placebo (N=209)**	**Total (N=419)**	**E4 20 mg (N=196)**	**Placebo (N=199)**	**Total (N=395)**
	n (%) e	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**	**n (%) e**
Nervous System Disorders	**0**	**0**	**0**	**0**	**2 (1.0%) 2**	**2 (0.5%) 2**
Headache	0	0	0	0	2 (1.0%) 2	2 (0.5%) 2
Psychiatric Disorders	**0**	**0**	**0**	**1 (0.5%) 2**	**1 (0.5%) 2**	**2 (0.5%) 4**
Anger	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Depression	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Insomnia	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Irritability	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
General disorders and administration site conditions	**0**	**0**	**0**	**0**	**1 (0.5%) 1**	**1 (0.3%) 1**
Fatigue	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Musculoskeletal and Connective Tissue Disorders	**0**	**1 (0.5%) 1**	**1 (0.2%) 1**	**0**	**1 (0.5%) 1**	**1 (0.3%) 1**
Arthralgia	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1
Muscle spasms	0	1 (0.5%) 1	1 (0.2%) 1	0	0	0
Neoplasms Benign, Malignant and Unspecified (incl cysts and polyps)	**0**	**0**	**0**	**1 (0.5%) 1**	**0**	**1 (0.3%) 1**
Intraductal proliferative breast lesion	0	0	0	1 (0.5%) 1	0	1 (0.3%) 1
Vascular Disorders	**0**	**0**	**0**	**0**	**1 (0.5%) 1**	**1 (0.3%) 1**
Superficial vein thrombosis	0	0	0	0	1 (0.5%) 1	1 (0.3%) 1

Abbreviations: e = Number of events, E4 20 mg = estetrol monohydrate 20 mg (equivalent to estetrol 18.9 mg), N = number of subjects in the statistical analysis set, n = number of observations per treatment group, PT = preferred term, SOC = system organ class, TEAE = treatment emergent adverse event.
Note: Percentage (%) based on the number of subjects in Safety population in each treatment arm or total column.
Classifications of adverse events based on the Medical Dictionary for Regulatory Activities (MedDRA) (version 25.0).
TEAEs defined as those AEs occurring from time point of first ingestion of investigational product until last visit or any event already present that worsens in either intensity or frequency following exposure to the treatment. If the start date of the AE is unknown, it will be assumed to be after the start of study drug unless the stop date is before the first dose.
Endometrial disorder is the MedDRA PT that is most appropriate but not exclusive for coding the biopsy result of disordered proliferative endometrium.
Other biopsy results such as weakly proliferative endometrium have also been coded to this PT if the biopsy result was considered reportable by the investigator.
PHASE 3 STUDIES: MIT-Do001-C301 ESP and MIT-Do001-C302 ESP

53-week uncontrolled safety population (with E4 + P combination therapy) - study C301 SSP

TEAEs led to study drug discontinuation in 269 (29.2%) NH subjects. Eighteen (2%) subjects reported serious TEAEs leading to study drug discontinuation, and these were study drug-related in 15 subjects.

The most commonly reported TEAEs leading to study drug discontinuation were in the Reproductive System and Breast Disorders SOC, and those reported in ≥1% of subjects were: vaginal haemorrhage (138 [15.0%]); endometrial disorder (77 [8.4%]); breast pain (9 [1.0%]); and headache (9 [1.0%]). Within the gastrointestinal disorders SOC, 24 TEAEs leading to study drug discontinuation were reported in 20 (2.2%) subjects, with any TEAE by PT being reported in less than 1% of subjects.

In most subjects, TEAEs leading to study drug discontinuation were mild or moderate. There were 26 severe TEAEs leading to study drug discontinuation in 20 (2.2%) subjects. Severe TEAEs that led to study drug discontinuation in ≥2 subjects were: vaginal haemorrhage (4 [0.4%] subjects); breast pain (2 [0.2%]); and migraine (2 [0.2%]). All other severe TEAEs led to study drug discontinuation in 1 subject each.

Table 61: Summary of TEAEs Leading to Study Drug Discontinuation by SOC and PT – MIT-Do001-C301 SSP (Safety Population)

SOC/PT	E4 20 mg + P4 100 mg			
	Full population (N=922)		Sub-population: ≥12 months since last menses (N=853)	
	n[a] (%)	E[b]	n[a] (%)	E[b]
Subjects with Any TEAE Leading to Study Drug Discontinuation	**269 (29.2)**	**343**	**251 (29.4)**	**319**
Reproductive System and Breast Disorders	**232 (25.2)**	**270**	**216 (25.3)**	**253**
Vaginal haemorrhage	138 (15.0)	146	127 (14.9)	134
Endometrial disorder[c]	77 (8.4)	77	73 (8.6)	73
Breast pain	9 (1.0)	9	9 (1.1)	9
Endometrial thickening	8 (0.9)	8	8 (0.9)	8
Uterine haemorrhage	5 (0.5)	5	5 (0.6)	5
Breast tenderness	4 (0.4)	4	4 (0.5)	4
Nipple pain	4 (0.4)	4	4 (0.5)	4
Menopausal symptoms	3 (0.3)	3	3 (0.4)	3
Breast discomfort	2 (0.2)	2	2 (0.2)	2
Pelvic pain	2 (0.2)	2	2 (0.2)	2
Vaginal discharge	2 (0.2)	2	2 (0.2)	2
Breast cyst	1 (0.1)	1	1 (0.1)	1
Breast swelling	1 (0.1)	1	1 (0.1)	1
Breast ulceration	1 (0.1)	1	1 (0.1)	1
Endometrial hyperplasia[c]	1 (0.1)	1	1 (0.1)	1
Hydrosalpinx	1 (0.1)	1	0	0
Intermenstrual bleeding	1 (0.1)	1	1 (0.1)	1
Uterine spasm	1 (0.1)	1	1 (0.1)	1
Vulvovaginal pruritus	1 (0.1)	1	1 (0.1)	1
Gastrointestinal Disorders	**20 (2.2)**	**24**	**18 (2.1)**	**22**
Abdominal pain lower	8 (0.9)	8	7 (0.8)	7

SOC/PT	E4 20 mg + P4 100 mg			
	Full population (N=922)		Sub-population: ≥12 months since last menses (N=853)	
	n[a] (%)	E[b]	n[a] (%)	E[b]
Abdominal discomfort	4 (0.4)	4	4 (0.5)	4
Abdominal pain	3 (0.3)	3	2 (0.2)	2
Abdominal distension	2 (0.2)	2	2 (0.2)	2
Abdominal pain upper	2 (0.2)	2	2 (0.2)	2
Constipation	1 (0.1)	1	1 (0.1)	1
Diarrhoea	1 (0.1)	1	1 (0.1)	1
Gastritis	1 (0.1)	1	1 (0.1)	1
Nausea	1 (0.1)	1	1 (0.1)	1
Vomiting	1 (0.1)	1	1 (0.1)	1
Nervous System Disorders	**17 (1.8)**	**17**	**17 (2.0)**	**17**
Headache	9 (1.0)	9	9 (1.1)	9
Cerebrovascular accident	2 (0.2)	2	2 (0.2)	2
Migraine	2 (0.2)	2	2 (0.2)	2
Dizziness	1 (0.1)	1	1 (0.1)	1
Transient ischaemic attack	1 (0.1)	1	1 (0.1)	1
Transverse sinus thrombosis	1 (0.1)	1	1 (0.1)	1
Vascular dementia	1 (0.1)	1	1 (0.1)	1
Skin and Subcutaneous Tissue Disorders	**5 (0.5)**	**5**	**5 (0.6)**	**5**
Alopecia	2 (0.2)	2	2 (0.2)	2
Acne	1 (0.1)	1	1 (0.1)	1
Hirsutism	1 (0.1)	1	1 (0.1)	1
Rash macular	1 (0.1)	1	1 (0.1)	1
General Disorders and Administration Site Conditions	**4 (0.4)**	**4**	**2 (0.2)**	**2**
Chest discomfort	1 (0.1)	1	1 (0.1)	1
Chest pain	1 (0.1)	1	0	0
Fatigue	1 (0.1)	1	1 (0.1)	1
Oedema peripheral	1 (0.1)	1	0	0
Investigations	**4 (0.4)**	**4**	**2 (0.2)**	**2**
Weight increased	2 (0.2)	2	2 (0.2)	2
Aspartate aminotransferase increased	1 (0.1)	1	0	0
Blood pressure increased	1 (0.1)	1	0	0
Neoplasms Benign, Malignant and Unspecified (incl Cysts and Polyps)	**4 (0.4)**	**4**	**3 (0.4)**	**3**
Uterine leiomyoma	4 (0.4)	4	3 (0.4)	3
Psychiatric Disorders	**3 (0.3)**	**4**	**3 (0.4)**	**4**
Insomnia	2 (0.2)	2	2 (0.2)	2
Mood swings	1 (0.1)	1	1 (0.1)	1
Sleep disorder	1 (0.1)	1	1 (0.1)	1
Infections and Infestations	**3 (0.3)**	**3**	**3 (0.4)**	**3**
COVID-19	2 (0.2)	2	2 (0.2)	2
Cystitis	1 (0.1)	1	1 (0.1)	1

SOC/PT	E4 20 mg + P4 100 mg			
	Full population (N=922)		Sub-population: ≥12 months since last menses (N=853)	
	n[a] (%)	E[b]	n[a] (%)	E[b]
Vascular Disorders	**3 (0.3)**	**3**	**3 (0.4)**	**3**
Cyanosis	1 (0.1)	1	1 (0.1)	1
Superficial vein thrombosis	1 (0.1)	1	1 (0.1)	1
Venous thrombosis limb	1 (0.1)	1	1 (0.1)	1
Injury, Poisoning and Procedural Complications	**1 (0.1)**	**2**	**1 (0.1)**	**2**
Road traffic accident	1 (0.1)	1	1 (0.1)	1
Tibia fracture	1 (0.1)	1	1 (0.1)	1
Musculoskeletal and Connective Tissue disorders	**2 (0.2)**	**2**	**2 (0.2)**	**2**
Back pain	2 (0.2)	2	2 (0.2)	2
Ear and Labyrinth Disorders	**1 (0.1)**	**1**	**1 (0.1)**	**1**
Tinnitus	1 (0.1)	1	1 (0.1)	1

COVID-19 = Coronavirus disease of 2019, CSR = Clinical Study Report, DPE = disordered proliferative endometrium, E = number of events, E4 = estetrol monohydrate, MedDRA = Medical Dictionary for Regulatory Activities, n = number of subjects in category of interest, N = number of subjects, NH = non-hysterectomized, P4= progesterone, PT = preferred term, SOC = system organ class, SSP = Safety Study Part, TEAE = Treatment Emergent Adverse Event
Note: Percentages (%) are based on the number of subjects in the Safety Analysis Set.
a. Number of subjects reporting at least one TEAE within SOC/ PT. Totals for the number of subjects at a higher level are not necessarily the sum of those at the lower levels since a subject may report two or more different TEAEs within the higher level category.
b. The number of individual occurrences of the TEAE within SOC/ PT. Subjects can be represented more than once in the any event and SOC categories (only once in the PT category).
c. As there is no MedDRA PT for DPE, these events were coded as endometrial disorder which is the PT that is most appropriate, but not exclusive, for coding the biopsy result of DPE. Other biopsy results such as weakly proliferative endometrium and active proliferative endometrium were also coded to this PT if the biopsy result was considered reportable by the Investigator. Events of simple/complex hyperplasia with/without atypia were coded to the PT endometrial hyperplasia. For the Phase 3 studies, TEAEs of endometrial disorder and endometrial hyperplasia were reported based upon the Initial or Safety diagnosis, whereas the Final/Consensus diagnosis was used to evaluate overall endometrial safety.

A total of 18 subjects discontinued due to a SAE in C301 SSP. A total of 7 SAE were endometrial disorder (all DPE), of which 1 was a delayed diagnosis from screening. The other SAE DPE cases were reported between D47 and D99. There were two cases of vaginal haemorrhage, for which hospitalization was required, one case of uterine haemorrhage, one case of uterine leiomyoma. The other included cerebrovascular accident, transverse sinus thrombosis, COVID-19, superficial vein thrombosis, toad traffic accident, tibia fracture, migraine and transient ischaemic attack.
The majority of AEs/SAEs of endometrial disorder or vaginal haemorrhage, including AEs leading to study drug discontinuation, occurred during the first quarter of study, Cycle 1-3. There is a gradual reduction in the number of these events over time with the exception of AEs of endometrial disorder in Cycle 13+ which increased. The reason for this increase is due to the number of endometrial biopsies required to be performed at the End of Treatment visit (Visit 6), as per protocol.

Table 62: Number of subjects with AEs/SAEs of endometrial disorder or vaginal haemorrhage and among these AEs leading to study drug discontinuation, over time, C301 SSP full population, safety analysis set (N = 922)

Period	Endometrial disorder			Vaginal haemorrhage[a]		
	AEs	SAEs	AEs leading to study drug discontinuation	AEs	SAEs	AEs leading to study drug discontinuation

	n [b]	n [b]	n [b]	n [b]	n [b]	n [b]
Cycle 1-3	48	3	40	557	5	121
Cycle 4-6	28	3	22	200	0	18
Cycle 7-9	18	0	12	138	1	5
Cycle 10-13[c]	5	0	3	110	0	2
Cycle 13+[d]	55	1	0	11	0	0

Cycle = 28 days
[a] Vaginal haemorrhage includes PTs of vaginal haemorrhage and uterine haemorrhage.
[b] Number of subjects reporting at least one AE/SAE.
[c] The period 'Cycle 10-13' includes 4 cycles while other periods include 3 cycles.
[d] AEs reported during the follow-up period, i.e. after cycle 13 (Week 52).

53-week uncontrolled H + NH safety population (E4 monotherapy) - study C302 SSP

TEAEs leading to study drug discontinuation occurred in 169 (39.3%) subjects. 57 (13.3%) subjects reported serious TEAEs leading to study drug discontinuation, and these were study drug-related in 55 subjects.

In most subjects TEAEs leading to study drug discontinuation were either mild or moderate. There were 26 severe TEAEs leading to study drug discontinuation in 20 (4.7%) subjects. The most commonly reported TEAEs leading to study drug discontinuation by SOC were in the Reproductive System and Breast Disorders SOC, and those reported in ≥1% of subjects were: endometrial disorder (71 [16.5%]); vaginal haemorrhage (41 [9.5%]); endometrial thickening (24 [5.6%]); endometrial hyperplasia (9 [2.1%]); breast tenderness (8 [1.9%]); menopausal symptoms (5 [1.2%], uterine haemorrhage (5 [1.2%]); abdominal pain (7 [1.6%]); weight increased (6 [1.4%]); and headache (5 [1.2%]).

Of the most commonly reported TEAEs leading to study drug discontinuation in ≥1% of subjects, endometrial disorder, endometrial hyperplasia, endometrial thickening, uterine haemorrhage, and abdominal pain were reported only in NH subjects. The majority of events of vaginal haemorrhage, menopausal symptoms, or headache were reported in NH subjects (each event was reported only in 1 H subject). Breast tenderness led to study drug discontinuation in a similar proportion of H (3 [1.5%]) and NH subjects (5 [2.2%]). Breast pain, nipple pain, breast discomfort, and breast swelling only led to study drug discontinuation in H subjects (3 [1.5%], 3 [1.5%], 1 [0.5%], 1 [0.5%], respectively). Weight increased led to study drug discontinuation in a similar proportion of H and NH subjects (3 [1.5%], 3 [1.3%], respectively).

Seven participants discontinued due to a SAE. In H women this concerned ovarian vein thrombosis, superficial vein thrombosis, papillary thyroid cancer. In NH women this concerned endometrial disorder (DPE), vaginal haemorrhage, deep vein thrombosis and anemia. All PTs were reported ones, except for vaginal haemorrhage, this was twice reported.

5.4.6 Safety in special populations

Analyses of safety by BMI, race, ethnicity, age and smoking status were performed for the pooled Safety Populations of the key clinical studies up to 13 weeks in the ISS. No analysis was performed in the supportive studies due to the limited number of subjects in these studies and the even smaller number of subjects in specific categories.

TEAE by BMI

In the E4 15 mg arm, a lower incidence of TEAEs was observed for subjects with a with a BMI ≥30 kg/m^2 (47.3%) compared with subjects with a BMI <25 kg/m^2 or ≥25 kg/m^2 to <30 kg/m^2 (60.9% and

57.1%, respectively). In the placebo arm, a lower incidence of TEAEs was observed for subjects with a BMI <25 kg/m^2 (35.4%) than seen for subjects with a BMI ≥25 kg/m^2 to <30 kg/m^2 or ≥30 kg/m^2 (44.4% and 40.0%, respectively).

In the pooled clinical studies of the ISS, a lower proportion of subjects with drug-related TEAEs was observed in the subgroup with a BMI ≥30 kg/m^2 (32.2 % and 42.5% in the pooled E4 15 mg and E4 20 mg arms, respectively) compared with the subgroup with a BMI <25 kg/m^2 (48.9 % and 49.1% in the pooled E4 15 mg and E4 20 mg arms, respectively) or BMI ≥25 kg/m^2 to <30 kg/m^2 (45.7 % and 48.6%, in the pooled E4 15 mg and E4 20 mg arms, respectively). This was mainly due to a lower proportion of subjects with drug-related TEAEs in the Reproductive System and Breast Disorders SOC (including vaginal haemorrhage, endometrial disorder, and endometrial hyperplasia), probably because of the lower proportion of NH subjects at Baseline in the BMI ≥30 kg/m^2 subgroup.

TEAE by Race

In the pooled clinical studies of the ISS, the subgroup analyses for race should be considered with caution because the sample size was very limited for non-White subjects especially the Other race subgroup.

In the pooled clinical studies of the ISS, a lower proportion of subjects with drug-related TEAEs was observed in the Black or African American subgroup (31.0% and 34.8% in the pooled E4 15 mg and E4 20 mg arms, respectively) compared with the White subgroup (44.1% and 48.9% in the pooled E4 15 mg and E4 20 mg arms, respectively). This was mainly due to a lower proportion of subjects with drug-related TEAEs in the Reproductive System and Breast Disorders SOC including vaginal haemorrhage and endometrial disorder. The lower sample size of the Black or African America subgroup and the lower proportion of NH subjects at Baseline in this subgroup may have partially accounted for this difference.

TEAE by ethnicity

In the pooled clinical studies of the ISS, a lower proportion of subjects with drug-related TEAEs was observed for Hispanic or Latino subjects (32.4% and 39.2% in the pooled E4 15 mg and E4 20 mg arms, respectively) compared with subjects of Not Hispanic or Latino ethnicity (46.4% and 50.3% in the pooled E4 15 mg and E4 20 mg arms, respectively). This was mainly due to a lower proportion of Hispanic or Latino subjects with drug-related TEAEs in the Reproductive System and Breast Disorders SOC. In each of the pooled E4 treatment arms, the proportion of subjects reporting breast tenderness was clearly lower in the Hispanic or Latino subgroup.

TEAE by Smoking status

In the pooled clinical studies of the ISS, the subgroup analyses for smoking should be considered with caution because the sample size was very limited for Smokers.

In each pooled E4 treatment arm, drug-related TEAEs were reported by a smaller proportion of Non-Smokers (41.9% and 46.6% in the pooled E4 15 mg and E4 20 mg arms, respectively) compared with Smokers (48.8% and 52.4% in the pooled E4 15 mg and E4 20 mg arms, respectively). This was mainly due to a lower proportion of subjects with drug-related TEAEs in the Reproductive System and Breast Disorders SOC including vaginal haemorrhage and endometrial disorder.

TEAE by Age

In the pooled clinical studies of the ISS, there were no marked differences between the age categories in the proportion of subjects reporting drug related TEAEs (for subjects of age <55 years, 41.4% and 47.6% in the pooled E4 15 mg and E4 20 mg arms, respectively; for subjects of age ≥55 years, 43.4% and 46.4% in the pooled E4 15 mg and E4 20 mg arms, respectively). No marked differences between

age groups were seen for the total TEAEs in any SOC. In the pooled E4 20 mg arm, the proportion of subjects reporting TEAEs of vaginal haemorrhage and endometrial thickening was higher in subjects <55 years of age, probably because of the higher proportion of NH subjects at Baseline in this age subgroup.

Hepatic Impairment

In study MIT-Do001-C102, 32 female subjects with varying degrees of hepatic impairment received a single dose of E4 20 mg. The study included 8 subjects with normal hepatic function, 8 subjects with mild hepatic impairment, 8 subjects with moderate hepatic impairment, and 8 subjects with severe hepatic impairment as defined using the Child-Pugh classification at Screening. Overall, the treatment was well tolerated in all study groups. No safety concerns or signals were identified during the study. No deaths, SAEs, or other significant AEs occurred during this study. The most common TEAE was diarrhoea (gastrointestinal disorders) with 3 subjects (9.4%). Diarrhoea was reported by 1 subject in the group with normal hepatic function and by 2 subjects in the group with moderate hepatic impairment (one was considered related to the drug).

Renal Impairment

A single oral dose of E4 20 mg in women with normal renal function (n=10) or mild (n=8), moderate (n=8), or severe renal impairment (n=8) was well tolerated. No serious TEAEs or other significant TEAEs occurred during this study. The most frequently reported TEAEs were hypoglycaemia and headache in 2 subjects each. Hypoglycaemia was reported by 1 subject with moderate renal impairment and by 1 subject with severe renal impairment. Headache was reported by 1 subject with mild renal impairment and 1 subject with normal renal function. Most of the TEAEs reported during this study were considered not related to the study drug except for dysfunctional uterine bleeding reported by 1 subject with normal renal function and breast discharge and uterine haemorrhage reported by 1 subject each with severe renal impairment.

No safety concerns or signals were identified during the study.

5.4.7 Safety related to drug-drug interactions and other interactions

Please refer to section 4.2.3.3.

5.4.8 Vital signs and laboratory findings

Laboratory parameters

General Haematology and Biochemistry

During administration of E4 15 mg or E4 20 mg (with and without P4) in studies up to 13 weeks and up to 53 weeks, no clinically relevant effect of the treatment on haematology or biochemistry parameters was observed. No significant differences in shift patterns were observed between the E4 arms and the placebo arm. In the E4 arms of the different studies, a very limited number of haematology abnormalities were reported as drug-related AEs and none occurred in more than 1 subject each.

Lipid metabolism

Overall, a very limited number of lipid abnormalities were reported as drug-related AEs, none of which occurred in more than 1 subject by study arm.

Glucose metabolism

With the exception of 1 AE (diabetes mellitus of moderate intensity) that was considered related to study treatment reported in 1 NH subject in study MIT-Do001-C301 SSP, no clinically relevant abnormalities in glucose metabolism were reported as AEs that were considered related to study treatment in any studies. The sponsor assessed this case and concluded no causal relation. Based on the subject's ethnic origin, BMI, baseline HbA1c and fasting glucose levels above the normal range at screening and baseline (with no knowledge from the Applicant of the subject's glucose level and HbA1c value prior to study entry), the Applicant considers that the AE of diabetes mellitus reported in this single subject was not related to study drugs.

Haemostasis parameters

Haemostasis parameters evaluated in the phase 2 and phase 3 studies included prothrombin fragment 1 + 2, antithrombin, endogenous thrombin potential based activated protein C sensitivity ratio (APCsr ETP), Protein-C, free Protein-S, and Factor VIII.

Overall, there was no clinically relevant impact of E4 treatment on the haemostasis parameters, although some statistically significant changes from baseline between the E4 arms and the placebo arms were observed, these changes were small in amplitude and remained within established normal ranges. The majority of individual haemostasis parameters were still within normal ranges and APCsr ETP, the global marker of coagulation, was weakly impacted by E4 treatment. See PD section 5.2.3 for details.

Vital signs and ECG

Vital signs

The most commonly assessed vital signs throughout the clinical studies included body weight, BMI, sitting systolic and diastolic blood pressures, and heart rate.

During a 13-week or 53-week administration of E4 15 mg or E4 20 mg, no clinically relevant effect of E4 on vital signs was observed. A very limited number of abnormalities in vital signs was reported as drug-related AE in the Phase 3 studies. The supportive studies were consistent with this. Overall, no relevant effect of treatment with E4 15 mg or E4 20 mg on any vital sign was observed, including blood pressure and heart rate.

ECG

ECGs were collected at baseline and at the end of studies (Week 13 or Week 53) in the Phase 3 studies and in Study MIT-Do0001-C201. Overall, the vast majority of ECGs were normal at baseline, and no shift to clinically significant abnormal ECG was observed at the end of the studies in any of the study arms.

5.4.9 Post marketing experience

To date, E4 alone has not been used in any marketed product for postmenopausal women.

5.4.10 Overall discussion and conclusions on clinical safety

5.4.10.1 Discussion

5.4.10.1.1. Overall assessment of available safety data

The standard HRT registration dossiers submitted for MAA in Europe through decentralized or mutual recognition procedures (none of the HRTs has been approved by centralized procedure) do not consist of studies in which women with a uterus (non-hysterectomized, NH) receive estrogen without a progestogen for protection of the increased risk of endometrial hyperplasia or endometrial cancer. However, the current dossier is mainly based of FDA guideline requirements, which request assessment of the safety of estrogen monotherapy in hysterectomized (H) and non-hysterectomized women up to 52 weeks. Therefore, all NH women in all studies were also treated with E4 monotherapy except for one study (C301 SSP), the endometrial safety of E4 20 mg + progesterone 100 mg has been evaluated over 52 weeks as recommended by the CHMP guideline on HRT.

The **safety database** consists of one phase 2 study, C201, and two phase 3 studies, C301 and C302. For a complete overview of the designs, please refer to the efficacy section.

- C201 phase 2 study:
  - placebo-controlled phase 2 study in H + NH women of 13 weeks who received 15 or 20 mg E4 monotherapy or placebo.
- C301 phase 3 study:
  - placebo-controlled Efficacy Study Part (ESP) of 13 weeks in H + NH women who received 15 or 20 mg E4 monotherapy or placebo
  - uncontrolled Endometrial and General Safety Study Part (SSP) of 53 weeks in NH women who received 20 mg E4 + 100 mg progesterone (P4).
- C302 phase 3 study:
  - placebo-controlled ESP of 12 weeks with a 9 months safety extension (total 53 weeks), in women who received 15 or 20 mg E4 monotherapy or placebo
  - uncontrolled Safety Study Part (SSP) of 53 weeks in women who received 20 mg E4 monotherapy

Further, the applicant conducted an Integrated Summary of Safety (ISS) analysis, which consisted of placebo-controlled 12-weeks data from the two phase 3 studies C301 (ESP) and C302 (ESP), and the phase 2 study C201.

The **safety** is presented in 4 safety populations:

• 53-week placebo-controlled H + NH safety population (E4 monotherapy) - study C302 ESP

• 13-week placebo-controlled safety H + NH population (E4 monotherapy) - ISS pooled data

• 53-week uncontrolled NH safety population (with E4 + P combination therapy) - study C301 SSP

• 53-week uncontrolled H + NH safety population (E4 monotherapy) - study C302 SSP

Study C302 SSP was a uncontrolled long-term safety study of 20 mg E4 without progesterone in H and NH women. This study was not earlier discussed in the efficacy section. The primary objective was the general safety of E4 20 mg monotherapy, secondary objectives were effect on endometrium and vaginal bleeding in NH women. 46.7% were H women and 53.3% NH subjects. A low percentage completed the study,60.2% of the H women and only 12.2% of the NH women. Adverse events were the major reason (10.0% in H women and 36.7% in NH women), followed by withdrawal of consent for

another reason (9.5% and 8.7% for H and NH women) and lost to follow up (8.5% and 8.3%) and serious adverse event (1.5% and 12.7%). Adverse endometrial biopsy outcome resulted in discontinuation in 11.4%. Overall, the demographic characteristics of the subjects were as expected and similar to the ESP of study C302, the mean (SD) age was 54.4 (4.88) years and mean (SD) BMI was 28.33 (4.542) kg/m2. Approximately 76% of the study population was white and about 22% were black or African American.

Based on above study designs, study C301 SSP was the only study in which NH women received E4 combined with progesterone (P4). In studies 301 ESP, 302 ESP and 302 SSP, both NH and H women received E4 only, not combined with P4, for up to 13 weeks and even up to 53 weeks. These studies are not suitable for evaluation of the safety profile in NH women. Furthermore, it is expected that study discontinuation and adverse events in these studies are driven by NH women.

Given this information, the assessment of the **general safety** profile was primarily based on 1) the 53-week placebo-controlled safety population (study C302 ESP)**,** supported by the 12-week placebo-controlled safety population (ISS pooled data of studies C201, C301 ESP and C302 ESP), and 2) the 53-week uncontrolled safety population with E4 + P4 combination therapy (study C301 SSP) and supported by the 53-week uncontrolled safety population (study C302 SSP).
Further, the 53-week uncontrolled safety population with E4 + P4 combination therapy (study C301 SSP) will be primarily used for assessment of **endometrial safety**.

Regarding **exposure,** in the 53-week placebo-controlled safety population (study C302 ESP), the mean treatment duration in H women was 293 (n=99) and 282 days (n=100) in respectively the 15 mg and 20 mg arms, respectively, and 275 days in the placebo arm (n=99). In NH women, the exposure was shorter, i.e. 213 days (n=93), 194 (n=93) and 278 (n=95) for 15 mg, 20 mg and placebo, respectively. For the pooled 13 weeks safety data set (ISS), a total of 1323 women were treated, of who 454 with E4 15 mg, 406 with E4 20 mg and 463 with placebo. The median duration of exposure to E4 was 84 days in each treatment group. There were no differences between the H and NH women in treatment duration. For the 53-week uncontrolled safety population (E4+P4, C301 SSP), 922 NH women started treatment with E4 and P4. The mean duration of exposure was 223 days, a total of 402 women completed treatment, of who 346 women were included in the endometrial safety set. For the supportive 53-week uncontrolled safety population (C302 SSP), the mean duration was 275 days in H women (n=201) and 141 days in NH women (n=229). According to the guideline ICH-E1 'Population Exposure', more than 100 patients were exposed for a minimum of one year at dosage levels intended for clinical use. Therefore, the exposure to 15 mg and 20 mg E4 and exposure to 20 mg E4 + 100 mg P4 in the safety data base is acceptable.

Of note, in all studies except for the endometrial safety study 301 SSP, H and NH women were treated with E4 alone. As a result, the common AE pattern for NH women will not be in line with the pattern expected in clinical practice.

Overall, **adverse events (AE)** were commonly reported in all safety studies. In the 53-week placebo-controlled study C302 ESP, in which both H and NH women were treated with unopposed E4, the overall number of any AE was slightly higher in the NH women (69.9% and 73.1% for 15 and 20 mg), as compared to H women (61.6% and 60.0% for 15 and 20 mg), in the placebo arms this was ~50%. The difference between NH and H was larger in the ISS, as compared to C302 ESP. The overall AE incidence rates in NH subjects was 65.7% and 76.5%, in H subjects this was 43.6% and 44.8% for 15 and 20 mg, respectively, while the incidence in the placebo arm was ~40%.
In the 53-week uncontrolled NH safety population C301 SSP, treated with E4+P4, the percentage of women with at least one AE was considered very high, 84.9%. In the 53-week safety analysis of the open-label uncontrolled data of C302 SSP, the number of participants who reported an AE was 62.7% for H women and 82.1% for NH women.

Most frequently reported AEs. In the 53-week placebo-controlled safety population C302 ESP, in H women, the most frequently reported AE was breast tenderness, reported in 15.2% and 14.0% in 15 and 20 mg vs 6.1% in placebo, followed by nipple pain (3.0% and 2.0% vs 2.0% in placebo) and breast pain (2.0% for both arms and 1.0% in placebo). Abdominal pain was reported in 2.2% in the 15 mg arm and 2.0% in the 20 mg arm. Lower abdominal pain in respectively 1.0% and 2.0%. In the placebo arm, 2.0% reported abdominal pain upper and 1.0% abdominal pain lower. For NH women, the incidence of endometrial AE and vaginal haemorrhage was high, as expected, with an incidence for endometrial hyperplasia of 4.3% and 11.8% in the 15 and 20 mg respectively, compared to none in the placebo arm. Incidences of breast tenderness, nipple pain and abdominal pain were in general comparable to the incidence in H women. Further, the AEs with ≥2 AE higher incidence in the active treatment arms vs placebo did not reveal worrisome events. A similar AE pattern was observed in the supportive 13-weeks ISS data. The most frequent AEs reported were breast tenderness, breast pain and nipple pain, with frequencies in line with C302 ESP for both NH and H women. Concerning other SOCs, headache was reported in 8.3%, 7.6% and 8.6%, for respectively 15 mg, 20 mg and placebo. Apart from endometrial safety, the AE pattern was generally similar in NH and H subjects with no major difference between the 15 and 20 mg doses.
In the 53-week single arm endometrial safety study (C301 SSP) in NH women who received E4+P4, the most frequently reported AE were vaginal haemorrhage (66.9%), endometrial disorder (16.5%), breast pain (9.7%), headache (9.5%), breast tenderness (6.9%), COVID-19 infection (6.6%) and endometrial thickening (6.0%) and lower abdominal pain (5.2%). A total of 29 subjects reported with 30 AEs of uterine leiomyoma and 1 (0.1%) subject with one AE of leiomyoma (reported term: myoma growth) in C301 SSP. In the 52-week study C302 ESP, also 5 subjects were reported with uterine leiomyoma while, no AE of uterine leiomyoma was reported in the placebo arm. Also, in C302 SSP, 5 (1.2%) subjects were reported with an AE of uterine leiomyoma. The difference in incidence between C301 SSP (E4 + P4) and C302 ESP and C302 SSP (only E4), might be explained by the addition of P4 of E4, in line with the report of *Templeman et al. (2009)* which showed a higher RR for the combination estrogen-progestin (RR = 2.38; 95% CI: 1.66-3.41) compared to estrogen only HRT (RR = 2.03; 95% CI: 1.17-3.52). As expected, uterine leiomyoma has been included as ADR in section 4.8 of the SmPC. The AE pattern and incidence in the supportive 53-week single arm study C302 SSP was in line with the other unopposed studies.

Adverse events considered **related to the study drug by the investigator**. Concerning C302 ESP, study drug-related breast tenderness was reported in 14.1%, 14.0%, 6.1% in the E4 15 mg, E4 20 mg, and placebo arms in H subjects. Based on the endometrial safety study C301 ESP, in NH women, study drug-related AEs were reported in 79.9% of subjects, as expected mainly in the Reproductive System and Breast Disorders SOC. Study drug-related AEs reported in ≥5% of all subjects were: vaginal haemorrhage (66.4%), endometrial disorder (16.4%), breast pain (9.2%), breast tenderness (6.6%), endometrial thickening (5.9%) and headache (5.7%).

Adverse drug reactions (ADR). Please refer to the adverse drug reactions in the SmPC. Regarding **serious adverse events** (SAEs), concerning H women in C302 ESP, SAE were reported in 4%, 0% and 1% for 15 mg, 20 mg and placebo, respectively. In the ISS, this was 0.9% and 1.0% vs. 0% in placebo, respectively. Concerning C302 SSP, this was 2.0% for H women. For the endometrial safety study (C301 SSP), a total of 3.5% subjects reported an SAE. Endometrial disorder was reported in 0.8%, vaginal haemorrhage in 0.5%, lower limb fracture in 0.2%, and COVID-19 in 0.4% of patients. Seven events of endometrial disorder in seven participants were classified as serious adverse event. In 3 out of 7 subjects disordered proliferative endometrium was diagnosed despite a TVUS of <10 mm. Further, the number of bleedings days resulting in TVUS and biopsy significantly differ between the participants, ranging from a couple of days up to almost a year in one participant. Four DPE SAE diagnoses occurred in the first 3 cycles (up to and including D86), two in cycles 4-6 and 1 in cycles 11-13. In all safety populations, PTs are reported once, except for the events of COVID-19, road traffic

accident and PTs withing the SOC Reproductive System and Breast Disorders. Two **deaths** were reported during the studies, one case of severe acute respiratory failure due to COVID-19 infection and one case of a fatal TEAE of road traffic accident, both are not considered related with the use of E4.

A total of 8 **thromboembolic events** were reported. A total of 5 **cardiovascular events** were reported, of which 3 were considered not related, the causal relation of the 5 cases can currently be considered weak, and it is accepted to not include ATE in section 4.8. VTE is a known risk of HRT and reflected in section 4.4 of the proposed SmPC. VTE is, in line with the Core SmPC for HRT included in the table in section 4.8 of the SmPC.

Regarding AEs related to mammography, for assessment on breast-cancer, a low number (4 out of 384) of AEs was identified, of which 2 cases of breast cysts, 1 case of increased breast density and 1 case of higher breast density.

The following **Adverse Events of Special Interest** (AESIs) were defined: vaginal bleeding events grade 2 and events resulting from endometrial biopsy reading. Since vaginal bleeding and endometrial disorder only affect NH women, and monotherapy is not in line with clinical practice, only data concerning C301 SSP, the endometrial safety study will be discussed. The incidence of AESI was vaginal haemorrhage of 52.5%, endometrial disorder 14.1%, and endometrial hyperplasia of 0.1% (n=1). All AESIs were collected in the safety database, and the narratives of vaginal bleeding events were re-classified. This re-assessment by the Applicant showed less than the initially reported 52.5% (484 subjects) were confirmed as grade 2. The Applicant confirmed that 40.3% (372 subjects) of vaginal bleeding events were classified as AESI grade 2. Based on the sub-analysis on women ≥ 12 months since last menses, it appears that all grade 2 bleedings were reported in this sub-population, resulting in a percentage of grade 2 bleeding in this sub-population of 43.6%. Events resulting from endometrial biopsy reading limited to DPE or worse were included as an AESI. In the initial documentation, a total of 14.1% (130 events) endometrial disorder was reported as AESI, in the currently provided re-classification a total of 129 cases were determined as DPE (14.0%). Almost all endometrial disorder (117 cases, 13.7%) and the one case of hyperplasia was reported in the sub-population of women ≥ 12 months since last menses.

Endometrial safety evaluation – C**301 SSP**

Study design C301 SSP. Study C301 SSP was a 53-week uncontrolled multicentre phase 3 study investigate the long-term safety of E4 with 100 mg progesterone (P4) in women suffering from at least 1 HF per week. The study design is considered appropriate. A duration of 53 weeks of treatment is adequate, as it is in line with the 1-year duration recommended for assessment of endometrial safety in the CHMP guideline on HRT. This guideline also recommends including at least 300 patients in whom endometrial biopsies are taken at study entry and at the end of week 52. The **inclusion and exclusion criteria** are in general in line with those for 301 ESP, except for the number of HF and acceptable for adequate assessment of endometrial safety during use of E4. The required number of HF is low, but this is not of relevance for investigating the safety. Women included should have ≥12 months of spontaneous amenorrhea with FSH >40 mIU/mL or ≥6 months of spontaneous amenorrhea with serum FSH >40 mIU/mL and E2 <20 pg/mL. However, both populations were treated with 100 mg P4 in a continuous dosing regimen, however a continuous dose regimen is only approved for women with ≥12 months of spontaneous amenorrhea. Following the Core SmPC for HRT, women ≥6 months since last menses should be treated with a cyclic or a continuous sequential dosing regimen, i.e. the oestrogen is dosed continuously or cyclic with a treatment-free interval, usually 21 days on and 7 days off in each 28-cycle and a progestogen should be added for 12-14 days (or more) of every 28-day cycle. Furthermore, the approved dosing regimen for adjuvant progesterone in a sequential or cyclic treatment regimen is 200 mg. As a result, the subjects of ≥6 months since last menses included in the study have not been treated according to the approved dosing schedule and approved P4 dose.

Therefore, endometrial safety cannot be assessed adequately. As a result, the endometrial safety data are not applicable for the currently proposed indication and dose regimen in the current SmPC. The endometrial data provided can only be used in support of a continuous dose regimen. As a results, the indication for non-hysterectomized women was restricted to "Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women at least 12 months since last menses" and split for H and NH women, since the 12 months restriction is not applicable for H women. Consequently, the posology only includes continuous combined dose regimen, since for this population a continuous combined regimen is regular, and this regimen is also in line with the design of the pivotal study C301 SSP.

The **primary safety endpoint** was the incidence of endometrial hyperplasia up to 12 months of treatment. **The secondary endpoints** include vaginal bleeding per 28-day cycle or amenorrhea and change in endometrial thickness measured by ultrasound (TVUS). The primary and secondary safety objectives and endpoints are considered acceptable for assessment of the long-term safety and endometrial health of E4. Concerning the **diagnostic work-up,** frequency (percentages) of endometrial AEs was computed on evaluable biopsies. The pre-specified upper limit of the two-sided 95% CI of the incidence of hyperplasia/carcinoma was ≤ 2%, which is in line with the CHMP *'Guideline on clinical investigation of medicinal products for hormone replacement therapy of oestrogen deficiency symptoms in postmenopausal women' (EMEA/CHMP/021/97 Rev. 1)*. Biopsies obtained at screening were initially read by one safety pathologist (initial pathology report). The investigator decided upon inclusion of the subject into the study or screen failure based on the initial pathology report at screening. Following FDA requirements, this pathologist could not be involved in the final/consensus diagnosis. The endometrial safety analysis (safety diagnosis) was based on the diagnosis evaluated from an additional 2 pathologists (i.e. in total 3 pathologists), defined as the worst reading from the 3 pathologists involved in safety reading. The final/consensus diagnosis was the concordance of 3 pathologists' diagnoses: if at least 2 pathologists agreed, the result was included, if none of them agreed, then the worst result was used. This assessment protocol applied for the final diagnosis of endometrial hyperplasia, endometrial cancer and disordered proliferative endometrium is considered in line with the recommendations of the CHMP guideline for HRT regarding the diagnostic approach of adverse effects on the endometrium.

Results C301 SSP. A total of 402 subjects (43.6%) completed the 53-week treatment period, for the post-hoc analysis on subjects ≥12 months since last menses, this was 372 subjects (43.6%), which can be considered relatively low. It was noted in Amendment 4 of C301 that the enrolment has been increased, as the drop-out rate turned out to be higher than expected. As a result of the higher drop-out of 66% (instead of 50%), the sample size was increased by 300 participants. The majority of discontinuations was due to an AE (29.6%), please refer the section on discontinuations due to AE for more information. The **baseline data and demographics** are in general in line with those for the efficacy studies, including the 53-week placebo-controlled safety population (C301 ESP), however it is requested to provide the number of subjects ≥6 and ≥12 months since last menses at baseline (part of safety MO on indication). The mean age was 53.9 (4.76) years. The majority (91.4%) of subjects were White, and the majority (78.5%) of subjects were not Hispanic/Latino.

Endometrial safety results

The Endometrial Safety Analysis Set included 346 subjects (of which 316 subjects were ≥12 months since last menses), i.e. subjects who had an evaluable biopsy at baseline and an evaluable biopsy at Month 12 (defined by a visit window as on or after Day 326) or did have a diagnosis of endometrial hyperplasia prior to Month 12. The number of patients in the Endometrial Safety Analysis Set is considered sufficient and acceptable, as this is in line with the *CHMP's 'guideline on clinical investigation of medicinal products for hormone replacement therapy of oestrogen deficiency symptoms in postmenopausal women'* with regard to precision of the point estimate of incidence of

endometrial hyperplasia/cancer, which is set at approximately 300 patients with biopsy treated for one year. Of this set, 325 subjects (of which 298 subjects were ≥12 months since last menses) had an available Final/Consensus diagnosis, defined as 'the number of subjects with performed biopsy in the Endometrial Safety Analysis Set'.

Endometrial hyperplasia/carcinoma incidence. One (1) patient was diagnosed with complex hyperplasia without atypia, for which the incidence rate (IR) was calculated to be 0.3% (two-sided 95% CI 0.0-1.7%), in subjects with ≥12 months since last menses, the primary endometrial safety endpoint also showed an incidence rate of hyperplasia of 0.3% (2-sided 95% confidence interval [CI]: 0.0–1.9). There were no cases of endometrial carcinoma observed.
It can be concluded that the findings of E4 in study C301 SSP fulfilled the FDA's and CHMP's specific requirements on endometrial safety.

Disordered proliferative endometrium. Based on the final/consensus diagnosis of the Safety Analysis Set (i.e. all patients who received study drug), a total of 27 (4.9%) subjects received benign disordered proliferative endometrium (DPE) as final diagnosis, these were all diagnosed in the subgroup of women at least 12 months since last menses (5.4%). Of note, the safety diagnosis atrophic endometrium was diagnosed in 81.4% at baseline, and decreased to 21.5% at week 53, inactive endometrium increased from 12.4% at baseline to 26.8% and proliferative (including weakly, active and disordered proliferative) increased from 4.2% to 39.7%.

Endometrial thickness. Regarding results of the transvaginal ultrasound (TVUS), the mean endometrial thickness at baseline was 2.61 mm, which increased to 5.51 mm at week 13, and remained generally consistent over time with 5.19 mm at week 29 and 4.85 mm at week 53 and was equal for the sub-population of women ≥12 months since last menses. This is above normal limits (<4 mm in menopausal women) defined by the Applicant. Further, abnormal clinical significant (CS) TVUS findings were observed in 3 (0.3%) subjects at baseline, while the incidence increased to 33 (3.6%) subjects at Week 13, 23 (2.5%) subjects at Week 29, 24 (2.6%) subjects at ED, and 8 (0.9%) subjects at Week 53 visits for the full study population. Further, endometrial thickening was a common TEAE reported in 6.0% of subjects. In conclusion, the TVUS data points to a slight increase on endometrial thickness and a higher incidence of abnormal clinically significant TVUS findings after 1 year of treatment with E4 and progesterone, compared to baseline.

Vaginal bleeding. Subjects with any bleeding and/or spotting during at least one cycle in cycles 1-3 were 77.2% and decreased to 62.2% in cycles 11-13 for the sub-group of ≥12 months since last menses, the majority reported only spotting (i.e. not bleeding). Further, vaginal haemorrhage was the most commonly reported TEAE, occurring in 66.9% of subjects (617 subjects). Vaginal haemorrhage grade 2 was also identified as an AESI and reported in 372 (43.6%) of the subjects ≥12 months since last menses. The frequency of vaginal haemorrhage was high and implies a tolerability issue, leading to discontinuation of treatment in 15% of NH subjects (see further below).

Cumulative amenorrhea. Cumulative amenorrhea per trimester for the sub-group of participants at least 12 months since last menses was 21.5% for cycles 1-3, 30.7% for cycles 4-6, 30.9% for cycles 7-10 and 36.9% for cycles 11-13.

Concluding, the incidence rate of hyperplasia is in line with the acceptable regulatory threshold for hyperplasia incidence rate set in the EMA guideline on HRT, which is reassuring. Nevertheless, there were a high number of vaginal haemorrhage and endometrial disorder events reported, a low percentage of cumulative amenorrhea at cycles 11-13 observed, and scientific literature data pointed to insufficiency of a dose of 100 mg P4 for endometrium protection, while no predicting factors for higher risk at bleeding/ discontinuation were found. Given this, in general, vaginal bleeding and DPE are sufficiently described in the SmPC (in line with the HRT core SmPC) and these events are considered manageable for clinical practice. An additional warning was included in section 4.4 of the

SmPC, that a higher dose of progesterone or another approved progestogen might be used, since 100 mg P4 might not be optimal for every patient.

Further, considering the higher incidence of disordered proliferative endometrium in non-hysterectomized women, it might be expected that these women have an increased risk of endometrium carcinoma. Therefore, endometrium neoplasm malignancy has been included as an important potential risk in the summary of safety specifications of the RMP.

Supplemental data on endometrial health in NH women treated with E4 monotherapy. Data on endometrial safety is also collected by endometrial biopsies in NH women participating in the other phase 3 studies, who were all treated with unopposed E4. In the 53-week placebo-controlled study C302 ESP, endometrial hyperplasia was diagnosed in 3 (6.8%), 5 (11.1%), and 0 (0%) subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively. In the 13-week placebo-controlled study C301 ESP, endometrial hyperplasia was diagnosed in 2 (3.0%), 3 (4.8%), and 0 (0%) subjects in the E4 15 mg, E4 20 mg, and placebo arms, respectively. In the 53-week single arm safety population (C302 SSP), endometrial hyperplasia was diagnosed in 7 (5.3%) subjects. Of note, in all unopposed studies, the incidence of DPE in NH women was between 31.8% to 53%. No cases of endometrial carcinoma were observed in any of the NH women in these studies. These data clearly show the need for opposing E4 with P in women with a uterus to counteract the adverse effects of estrogen only treatment on the endometrium.

Concerning **discontinuations due to AE**. In the 53-week placebo-controlled study (C302 ESP), the incidence of study-drug discontinuations in H women was overall low, 4.0% in the 15 mg group, 7.0% in the 20 mg arm and comparable to the placebo group (4.0%). The incidence in NH women was, as expected due to the unopposed E4 treatment, much higher. In H subjects, in SOC reproductive system and breast disorders, most PTs were reported once, except for breast tenderness. Of note, uterine leiomyoma was reported as reason for discontinuation in 2 subjects. In the ISS safety population, the incidence of study drug discontinuation in H women was 1.4% for 15 mg and 2.9% in 20 mg, compared to 1.4% in the placebo arms. In the 15 mg arm in H, all AE leading to discontinuation were isolated cases, in the 20 arm only breast tenderness and breast pain were both in 2 subjects (1.0%). The incidence of discontinuation in NH women was, as expected, driven by endometrial disorders, endometrial hyperplasia and endometrial thickening and vaginal haemorrhage. A total of 13 SAE leading to study discontinuation were reported in the E4 arm, and 1 in the placebo arm. For the 20 mg dose, no SAE leading to study discontinuation were reported for H women. For NH women a total of 15 SAE were reported in the E4 arm, and 1 in the placebo arm. In C301 SSP endometrial safety study, the incidence of AE leading to study drug discontinuation in was relatively high, with 29.2%, of which 18 were SAEs; 7 SAE were endometrial disorder (all DPE), two SAE of vaginal haemorrhage, the other cases were all isolated cases. The most frequent AEs leading discontinuations were vaginal haemorrhage (15.0%). Endometrial disorder was reported in 8.4% and breast pain in 1.0%. PTs in the SOC gastrointestinal disorder and nervous system were reported less than 1%, except for headache (1%). Concerning discontinuations over time due to vaginal haemorrhage, 121 events occurred within the cycles 1-3, accounting for 82.8% of the discontinuations, followed by 18 (12.3%), 5 (3.4%) and 2 (1.4%) events in cycles 4-6, 7-9 and 10-13 respectively. A total of 77 participants (8.4%) discontinued due to an AE of endometrial disorder (DPE), 40 subjects, accounting for 52% of all discontinuations due to DPE within cycle 1-3. A total of 22 (28.5%), 12 (15.6%) and 3 (3.9%) discontinued in cycles 4-6, 7-9 and 10-13, respectively. Discontinuation from the 53-week open-label uncontrolled study (C302 SSP), was 11.9% for H subjects and much higher, 63.3% in NH subjects. PTs occurring in >1 subject in H women were in general comparable to C302 ESP, except for pain (1.0%), increased weight (1.5%), mood swings (1.0%). Of note, none of the NH women discontinued due to breast pain or nipple pain. Discontinuation due to gastrointestinal disorders was reported in 2 subjects (1.0%) in H

women, compared to 10 (4.4%) in NH women, mainly abdominal pain. Seven participants discontinued due to a SAE.

Special population analysis (subgroup analysis) is provided for the ISS in H and NH women who received E4 monotherapy (i.e. 13-week placebo-controlled data). Subgroup analyses were performed for BMI, race, ethnicity, smoking status, age, hepatic and renal impairment. No clear trends were observed in most subgroups, however especially for race and smoking status, the subgroups were small. Furthermore, the analysis was hampered by imbalances in the proportion of NH and H women in several subgroups.

Laboratory findings. In the phase 3 studies, overall, no clinically relevant changes were observed in the mean change from baseline at various time points of measurement in haematology parameters. The following clinically significant abnormalities reported as TEAEs (and considered related by the investigator) in the E4 arms reported more than in 1 subject were anaemia (n=3) and iron deficiency (n=2). No safety signals in the abnormalities of biochemistry were identified. Concerning vital signs, during a 13-week or 53-week administration of E4 15 mg or E4 20 mg, no clinically relevant effect of E4 on blood pressure or heart rate was observed. Based on the non-clinical and phase 1 study findings, no QT prolongation was detected (please refer to secondary PD section). Therefore, no QT prolongation potential was anticipated to be expected during the confirmatory clinical phase in a target population generally not at increased risk for arrhythmias. Accordingly, only standard ECG monitoring has been performed in the confirmatory study phase, which is considered appropriate. No clinically relevant changes in the ECG parameters or clinically meaningful differences between study arms have been found in the safety populations data sets, which is reassuring. No further information is needed. Overall, there was no clinically relevant impact of E4 treatment on the haemostasis parameters. Concerning lipid metabolism and glucose metabolism, none clinically relevant abnormalities were reported in more than 1 subject by study arm as AE that was considered related to de study drug by the investigator. Effect of E4 on lipid and glucose levels is further discussed in the PD section. Of note, physical (i.e. weight), gynaecological and breast examinations are included and discussed in the adverse events sections. The effect on laboratory parameters is limited.

5.4.10.1.2 Adverse drug reactions in the SmPC

AEs of C301 ESP, C302 ESP (up to week 53) and C201 were pooled for 15 mg and 20 mg, irrespective of the hysterectomy status, except for the SOC Reproductive system and breast disorders. Comparison of occurrence of AEs in the pooled E4 with the placebo arms, was done to initially make a preliminary decision on inclusion of the AEs for further analysis. This is acknowledged and accepted.

In general, all AEs (i.e. not only drug-related TEAE, as defined by the investigator) with increased frequency compared to placebo (or in case of an uncontrolled study with a predefined cut-off frequency) were assessed by default as an ADR in section 4.8 of the SmPC, unless this is thoroughly justified.

Further, an ADR analysis was provided on all AEs with a frequency of pooled data of treatment-related AEs. Both frequency calculations (all AE frequency and after ADR analysis, i.e. those considered related by the investigator) are presented in the table below. The highest frequency has been used for categorization in section 4.8 of the SmPC.

Table 63: ADRs proposed for inclusion in the SmPC by the Rapporteur

	AE frequency	ADR frequency
Gastrointestinal disorders		
Abdominal distension	1.05%	0.7%
Abdominal pain grouped with abdominal pain upper to abdominal pain term	2.44%+0.35%=2.79%	1.51%+0.35%=1.86%
Abdominal pain lower; grouped with pelvic pain to abdominal pain lower term	2.09%+1.4% = 3.49%	1.4%+0.93%=2.33%
Constipation	1.28%	-
Nausea	3.02%	2.44%
General disorders and administration site conditions		
Asthenia; grouped with fatigue as asthenia term	0.58%+1.74%=2.32%	0.35%+1.28%=1.63%
Oedema peripheral; grouped with peripheral swelling to peripheral swelling term	0.35%+0.47%=0.82%	0.35%+0.12%=0.47%
Infections and infestations		
Candida infection; grouped with vulvovaginal candidiasis and vulvovaginal mycotic infection to Vulvovaginal candidiasis term	0.47%+0.58%+0.35% =1.4%	0.12%+0.35%+0%= 0.47%
Investigations		
Weight increased; grouped with obesity to weight increased term	1.74%+0.12%= 1.86%	1.51%+0.12%=1.63%
Musculoskeletal and connective tissue disorders		
Pain in extremity	1.16%	0.7%
Neoplasms benign, malignant and unspecified (incl cysts and polyps)		
Uterine leiomyoma; grouped with leiomyoma as uterine leiomyoma term	3.05%	3.05%
Nervous system disorders		
Dizziness	1.86%	0.81%
Reproductive system and breast disorders		
Adenomyosis	0.59%	0.35%
Breast cyst; grouped with breast mass, Phyllodes tumour and breast scan abnormal to breast mass term.	0.23%+0.12%+0.12% +0.12%=0.58%	0.23%+0.12%+0.12% +0.12%=0.58%
Breast discomfort; grouped with breast tenderness to breast tenderness term	1.05%+7.91%=8.96%	0.93%+7.79%=8.72%
Breast engorgement; grouped with breast swelling and breast enlargement to breast swelling term	0.12%+0.58%+0.23% =0.93%	0.12%+0.58%+0.23% =0.93%
Breast pain	5.81%	5.58%
Disordered proliferative endometrium	5.4%	5.4%
Endometrial hyperplasia	0.3%	0.3%
Endometrial hypertrophy; grouped with endometrial thickening to endometrial thickening term	71.3%	71.3%

Intermenstrual bleeding; grouped with vaginal haemorrhage and uterine haemorrhage to vaginal haemorrhage term	66.8%	66.8%
Nipple pain	3.14%	2.91%
Ovarian cyst	0.93%	0.58%
Uterine polyp; grouped with cervical polyp to endometrial polyp term	0.59%+0.23%=0.82%	0.59%+0.23%=0.82%
Uterine spasm	1.17%	1.17%
Vaginal discharge	3.14%	2.33%
Vulvovaginal pruritus	1.16%	0.47%
Skin and subcutaneous tissue disorders		
Urticaria	0.12%	0.12%
Vascular disorder		
Venous thromboembolism	8 cases	0.14%

5.4.10.2 Conclusions on clinical safety

Based on the currently available data, E4 appears to be generally well tolerated in H subjects. Based on the 53-week placebo-controlled study in H women, a total of 4.0% and 7.0% in the 15 mg and 20 mg study arm, as compared to 4.0% in the placebo arm, discontinued due to adverse events. Most common AEs were breast tenderness, nipple pain, breast pain and (lower) abdominal pain.

Concerning endometrial safety in NH subjects, the incidence rate of endometrial hyperplasia fulfils CHMP's specific requirements on endometrial safety. Nevertheless, there was a high number of vaginal bleedings, and disordered proliferative endometrium, although no predicting factors for higher risk at bleeding or discontinuation were found. Given this, in general, vaginal bleeding and DPE are sufficiently described in the SmPC (in line with the HRT core SmPC) and these events are considered manageable for clinical practice, with an additional warning, that a higher dose of progesterone or another approved progestogen might be used, since 100 mg P4 might be not optimal for every patient. Further, considering the higher incidence of disordered proliferative endometrium in non-hysterectomized women, it might be expected that these women have an increased risk of endometrium carcinoma. This risk is therefore included as an important potential risk in the summary of safety specifications of the RMP.

In conclusion, based on the current provided data, the safety profile of E4 does not suggest major safety concerns, without relevant differences between the 15 mg and 20 mg dose for H women. Concerning NH women, the incidence rate of hyperplasia fulfils CHMP's specific requirements on endometrial safety, although issues regarding vaginal bleeding and DPE remain, but these are appropriately addressed in the SmPC.

6. Risk management plan

6.1. Safety specification

6.1.1. Proposed safety specification

The applicant proposed the following summary of safety concerns in the RMP:

Table 64: Summary of safety concerns in the proposed RMP

Summary of safety concerns	
Important identified risks	None
Important potential risks	Endometrium neoplasm malignancy
Missing information	None

6.1.2. Discussion on proposed safety specification

The incidence rate of endometrial hyperplasia fulfils CHMP's specific requirements on endometrial safety. Nevertheless, there was a high number of vaginal bleedings, and disordered proliferative endometrium, although no predicting factors for higher risk at bleeding or discontinuation were found. Given this, in general, vaginal bleeding and DPE are sufficiently described in the SmPC (in line with the HRT core SmPC) and these events are considered manageable for clinical practice. In section 4.4 of the SmPC, an additional warning was included, that a higher dose of progesterone or another approved progestogen might be used, since 100 mg P4 might not be optimal for every patient. Further, considering the higher incidence of disordered proliferative endometrium in non-hysterectomized women, it might be expected that these women have an increased risk of endometrium carcinoma. Therefore, this risk is included as important potential risk to the list of safety concerns in the RMP. Although Thromboembolic Events, both VTE and ATE, are risks associated with HRT, these are extensively discussed in the SmPC and well known in clinical practice. It is considered sufficient to review these in PSURs.

6.2. Pharmacovigilance plan

6.2.1. Proposed pharmacovigilance plan

Routine pharmacovigilance activities

Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection:

Specific adverse reaction follow-up questionnaires

In order to further characterise the important potential risk of 'Endometrium neoplasm malignancy' and to ensure high quality reports and facilitate better causality assessment, a targeted follow-up questionnaire is utilised for safety concern of 'Endometrium neoplasm malignancy'.

Considering that most of the initial post-marketing cases are poorly documented, targeted follow-up questionnaires provide help in better assessment of the cases and thus are considered necessary outstanding routine pharmacovigilance activities by the applicant.

Other forms of routine pharmacovigilance activities

None proposed.

Additional pharmacovigilance activities

Study and status	Summary of objectives	Safety concerns addressed	Milestones	Due Dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
Feasibility report on potential real-world data study Planned	To investigate whether real-world data study is suitable to characterise a rare potential risk of 'endometrium neoplasm malignancy' in patients exposed to FYLREVY, considering the required sample size, data sources and the expected market uptake of estetrol in non-hysterectomised women for such study	Important potential risk of 'endometrium neoplasm malignancy'	Feasibility report	Within 3 months after European Commission Decision (June 2026)

6.2.2. Discussion on the Pharmacovigilance Plan

The routine pharmacovigilance activities, as well as the specific adverse reaction follow-up questionnaires and the feasibility report to investigate whether a real-world data study is suitable to characterise the important potential risk of 'endometrium neoplasm malignancy', are considered acceptable.

6.3. Plans for post-authorisation efficacy studies

The Applicant did not propose a post authorisation efficacy study (PAES). Based on the efficacy results (please refer to the efficacy section), this is considered acceptable.

6.4. Risk minimisation measures

The applicant only proposed routine risk minimisation measures, as no safety concerns were identified.

It is agreed that no additional risk minimisation measures are necessary.

6.5. RMP Summary and RMP Annexes overall conclusion

The RMP Summary and RMP Annexes are considered acceptable.

6.6. Overall conclusion on the Risk Management Plan

The PRAC considers that the risk management plan version 0.4 is acceptable.

7. Pharmacovigilance

7.1. Pharmacovigilance system

The CHMP considers that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

7.2. Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list, and a new entry will be required. The new list of Union reference dates (EURD list) entry uses the European birth date (EBD) to determine the forthcoming Data Lock Points.

8. Product information

8.1. Summary of Product Characteristics (SmPC)

8.1.1. SmPC section 4.1 justification

Please refer also to the efficacy discussion section (6.3.10) and safety discussion section (6.4.12) for more information.

Summarized, non-hysterectomized women <12 months since last menses were excluded from the indication since those were treated in a continuous combined regimen of estetrol with 100 mg progesterone, instead of the required cyclic or a continuous sequential dosing regimen with 200 mg progesterone. Therefore, these subjects have not been treated according to the approved dosing schedule. As a result, the endometrial data provided can only be used in support of a continuous dose regimen and the indication for non-hysterectomized women was restricted to "Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women at least 12 months since last menses" and split for H and NH women, since this 12 months restriction is not applicable for H women.

8.1.2. SmPC section 5.1 justification

According to the guideline (*Draft Assessment of SmPC section 5.1: A Guide for Assessors of Centralised Applications*) Section 5.1 should be limited to the indication, target population and posology that are authorised.

Since the indication can only be approved for non-hysterectomized (NH) women with at least 12 months since last menses and hysterectomized (H) women, the results of the *post-hoc* efficacy analyses, in which NH women with at least 6 months till 12 months since last menses are excluded, were included in section 5.1.

8.2. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use*.

8.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, FYLREVY is included in the additional monitoring list since is included in the additional monitoring list since it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet include a statement that this medicinal product is subject to additional monitoring and will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

9. Benefit-risk assessment

9.1. Therapeutic context

9.1.1. Disease or condition, proposed therapeutic indication

The Applicant initially proposed the following indication:

"*Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women"*

Menopause is characterized by decreased estradiol levels due to decreased ovarian function, and a resultant increase in gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus leading to high luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations (*Freeman et al. 2005*). Decreased estrogen levels are thought to be a main cause for vasomotor symptoms (VMS).

VMS are transient (lasting between 1-5 minutes) episodes of flushing and intense heat sensation (*Bansal and Aggarwal 2019*). Up to 80% of menopausal women suffer from VMS, which last a mean duration of 7 to 9 years. In one-third of women, VMS can last more than 10 years (*NAMS 2023*).

VMS can contribute towards physical and psycho-social impairment, with a consequent reduction in health-related quality of life (HRQoL) and are one of the main reasons why women may seek medical care for the menopause (*Santoro 2008*).

The medicinal product consists of estetrol monohydrate 15 mg and 20 mg (E4 15 mg and E4 20 mg) equivalent to estetrol (E4) 14.2 mg and 18.9 mg, respectively. In the documents, except if explicitly mentioned, doses refer to the dose of estetrol monohydrate. E4 belongs to the pharmacotherapeutic group sex hormones and modulators of the genital system, with anatomical therapeutic chemical (ATC) code G03CA10.

E4 is a naturally occurring estrogen produced only by the human foetal liver. Pregnant women are exposed to E4 as it reaches the maternal circulation through the placenta. The physiological function of E4 during pregnancy remains unclear. It is developed as an estrogen in a dose of 14.2 mg E4 in combination with the known progestogen drospirenone in a dose of 3 mg for the indication of oral contraception (approved by centralized procedure in 2021), and currently as estrogen for hormone replacement therapy. All current HRTs consist of the estrogen estradiol.

9.1.2. Available therapies and unmet medical need

Currently available therapies

Hormonal treatment

Hormone replacement therapy (HRT) is the broad term used to describe the use of estrogen only (unopposed estrogen) in hysterectomized women or combined estrogen-progestogen therapy in women with a uterus to relieve vasomotor symptoms associated with estrogen deficiency during menopause. Currently the estrogen component consists of estradiol (E2) in a dose of 0.5, 1 mg, and 2 mg, and estradiol valerate (E2V) 1 and 2 mg. E2 can be increased or decreased based on clinical response, E2V can be increased to 2 mg in case of insufficient treatment response. The addition of a progestogen to the estrogen regimen in women with a uterus is aimed at preventing the increase in endometrial cancer risk associated with estrogen therapy. In Europe, the following progestogens are approved for this indication: dydrogesterone, norethisterone, progesterone and medroxyprogesterone.

Recent international menopause guidelines such as the European Menopause and Andropause Society (EMAS) 2022 and North American Menopause Society (NAMS) position statement 2022 (Hormone therapy) and 2023 (non-hormone therapy), identify HRT as the first-line treatment for VMS in menopausal women. They recommend an individualized approach, considering each patient's symptoms, health status, and preferences. A systemic estrogen or combined estrogen-progestogen therapy (depending on the hysterectomized status) is advised and is the preferred first line-treatment, in menopausal women aged younger than 60 years, and particularly within 10 years of menopause onset for optimal benefits (NAMS 2022, 2023).

Non-hormonal treatment

A variety of non-hormonal therapies can be offered to women who cannot use HRT because of contraindications or personal preference. As presented in the EMAS position statement, several non-hormonal agents can be used for hot flushes, including clonidine, selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), gabapentin, although these are less effective than systemic estrogens (*Rees et al. 2022*). SSRIs and SNRIs are not approved for the treatment of VMS and would therefore be used off-label.

Fezolinetant, a non-hormonal selective neurokinin 3 (NK3) receptor antagonist, has recently been approved in the US and in Europe for the treatment of moderate-severe VMS.

Over-the-counter-remedies

Natural over-the-counter remedies, including herbal/non-prescription products, have not been approved for VMS treatment, and their safety and efficacy are not well established. Furthermore, these products do not address the burden of disease considering the very limited evidence to support their use and limited data to support their mechanisms of action.

Unmet medical need

Not applicable.

9.2. Main clinical studies

For a detailed description of the main clinical studies supporting this application, please refer to section 6.3.2 of this document.

Two pivotal phase 3 studies, C301 and C302, have been submitted, both consisting of an Efficacy Study Part (ESP) and a Safety Study Part (SSP).

Efficacy Study Parts

The pivotal **C301 ESP** and **C302 ESP** have large similarities in the study design. Both studies are placebo-controlled, randomized (1:1:1 to E4 15 mg, E4 20 mg and placebo), 12-week double-blind studies in hysterectomized (H) and non-hysterectomized (NH) postmenopausal women. Of note, the placebo-controlled C302 ESP has a duration of 12 months due to a safety extension of 9 months. A total of 1219 women (n=213 on E4 15 mg, n=213 on E4 20 mg and n= 214 on placebo in C301 ESP and n=192 on E4 15 mg, n=193 on 20 mg and n=194 on placebo in C302 ESP) aged 40–65 years, with ≥7 moderate to severe VMS per day or ≥50 moderate to severe VMS per week and seeking treatment for relief of VMS associated with menopause were included. The co-primary endpoints were the mean change in weekly frequency and severity of moderate to severe VMS from Baseline to Week 4 and Week 12 for E4 15 mg and E4 20 mg vs placebo.

Safety Study Parts

Both SSPs are uncontrolled, single-arm design, 53-week studies. C301 SSP is the Endometrial and General Safety in NH women, treated with E4 20 mg and 100 mg progesterone (P4), and considered the pivotal study regarding endometrial safety. C302 SSP is a general safety study, conducted in H and NH women, treated with only E4 20 mg. The study populations are largely in line with the ESPs, with the exception of the number of required hot flushes per week (i.e. at least 1 instead of ≥50). Of note, in both placebo-controlled studies C301 ESP and C302 ESP, safety data was collected, as well as in the dose-finding study C201. Therefore, 4 safety populations were presented.

- **C302 ESP** - 53-week placebo-controlled study in N + NH women on E4 monotherapy. A total of 579 were included. For H women, n=99 on 15 mg E4, n=100 on 20 mg E4 and n = 99 on placebo. For NH women n=93 on E4 15 mg, n=93 for E4 20 mg and n=95 on placebo.
- **ISS** - 13-week placebo-controlled studies in H + NH on E4 monotherapy. It consists of pooled data from the phase 2 study C201, and the phase 3 studies C301 ESP and C302 ESP (i.e. the first 13 weeks of this study), a total of 1323 women were treated, of who 454 on E4 15 mg, 406 with E4 20 mg and 463 with placebo.
- **C301 SSP** - 53-week uncontrolled NH safety population treated with E4 + P4 combination therapy. A total of 922 NH women started treatment, 402 women completed treatment, of who 346 women were included in the endometrial safety set.
- **C302 SSP** - 53-week uncontrolled in H + NH women on E4 monotherapy. A total of 201 H and 229 NH women were treated.

9.3. Favourable effects

Regarding the **primary analysis** of C301 and C302, the **co-primary endpoints** on **frequency** of moderate to severe VMS have been met for both dosages. Treatment with either 15 or 20 mg estetrol resulted in a higher reduction in frequency of moderate to severe VMS from baseline to week 4 and week 12, compared to placebo:

In *C301*, the difference in LS means (95% CI) were statistically significant for 15 and 20 mg estetrol compared to placebo at week 12; respectively -16.41 (-25.95, -6.87) and -22.49 (-31.79, -13.19).

In *C302*, the difference in LS means (95% CI) were statistically significant for 15 and 20 mg estetrol compared to placebo; respectively -12.20 (-20.69, -3.71) and -15.49 (-24.04, -6.94).

Similar findings were seen in the reduction of VMS frequency at week 4.

Regarding the **primary analysis** of C301 and C302, the **co-primary endpoints** on **severity** of moderate to severe VMS have been met for both dosages in C301. Treatment with either 15 mg or 20 mg estetrol resulted in a higher reduction in severity of moderate to severe VMS from baseline to week 4 and week 12, compared to placebo, in study C301 and for 20 mg in study C302 as well.

In *C301*, the difference in LS means (95% CI) were statistically significant for 15 and 20 mg estetrol compared to placebo; respectively -0.54 (-0.77, -0.31) and -0.66 (-0.88, -0.43).

In *C302*, the difference in LS means (95% CI) was statistically significant for 20 mg Estetrol compared to placebo; -0.46 (-0.66, -0.25), though the observed reduction in severity was not statistically significant for the 15 mg.

Similar findings were seen in the reduction of VMS severity at week 4.

Post-hoc analyses excluding non-hysterectomized women with at least 6 months till 12 months since last menses have shown generally similar data.

Overall, the **sensitivity analyses** confirmed the robustness of the results and that the results are not relevantly impacted by the exact chosen methodology, including imputation and assumptions about the underlying data.

Of the **secondary endpoints** (non-alpha controlled), the responder analysis for the weekly frequency of moderate to severe VMS is considered most relevant and most supportive for the primary efficacy objective as this endpoint presents efficacy in reduction of VMS at a patient level.

Regarding the **responder analysis,** in *C301 ESP* at Week 12, the proportion of subjects that experienced a ≥75% reduction from baseline in the weekly frequency of moderate to severe VMS was 63.3% and 74.5% of subjects in the E4 15 mg and E4 20 mg treatment arms, respectively, compared to 39.5% with placebo (nominal p<0.0001). In *C302 ESP* at week 12, the proportion of subjects that experienced a ≥75% reduction from baseline was 49.0% and 56.9% of subjects in the E4 15 mg and E4 20 mg treatment arms, respectively, compared to 37.3% with placebo (nominal p-value of 0.0392 and 0.0007).

Similar findings were seen in the **≥50% reduction** of VMS at week 12. With regards to other secondary endpoints selected those related to the primary endpoint (frequency and severity of VMS at other time points, subjects with a clinically important difference (CID) in VMS, health-related quality of life (HRQoL) and Treatment satisfaction (TS), the results were in general supportive.

9.3.1. Uncertainties and limitations about favourable effects

Regarding the 15 mg dose, although the primary endpoint for frequency has been reached, in line with the EMA HRT guideline, in the primary analysis of study C302, the co-primary endpoints on moderate to severe VMS, severity have not been met for the E4 15 mg dose, as the difference in LS means (95% CI) was not statistically significant for 15 mg Estetrol compared to placebo; -0.01 (-0.22, 0.19). Further, the pooled safety data of the 15 mg of E4 were suggestive for less AEs, in particular endometrial AEs, as compared to the 20 mg dose. The safety advantage for this lower dose of 15 mg is suggested to be mainly endometrium-related and will, thus, not play a role for hysterectomized women.

9.4. Unfavourable effects

In general, the **safety database** was sufficient. Regarding exposure in the endometrial safety study (C301 SSP), a total of 402 (43.6%) completed treatment, of which 346 were included in the

endometrial safety dataset. In the 53-week placebo-controlled safety population (C302 ESP), a total of 199 H women were exposed to E4 with a mean duration of ~290 days. Therefore, the exposure to 15 mg and 20 mg E4 and exposure to 20 mg E4 + 100 mg progesterone (P4) in the safety data base is considered acceptable.

In all placebo-controlled study parts combined, in which hysterectomised (H) and non-hysterectomized (NH) were treated with E4 monotherapy, **adverse events** (AE) were slightly more common in the E4 15 and 20 mg treatment groups as compared to placebo, concerning the 53 weeks placebo-controlled study C302 ESP in hysterectomized (H) subjects, this was 61.6% and 60.0% for 15 and 20 mg, in the placebo arms this was ~50%. The most frequently reported AE in H women were breast tenderness (15.2% and 14.0% in 15 and 20 mg vs 6.1% in placebo), nipple pain (3.0% and 4.0% vs 2.0% in placebo) and breast pain (2.0% for both active treatment arms vs 1.0% in placebo).

In the endometrial safety study C301 SSP, the overall percentage of AEs in NH women, treated with E4 20 mg continuously combined with P4 100 mg, was 84.9%. The most frequently reported AE were vaginal haemorrhage (66.9%), endometrial disorder (16.5%), breast pain (9.7%), headache (9.5%), breast tenderness (6.9%), COVID-19 infection (6.6%) and endometrial thickening (6.0%) and lower abdominal pain (5.2%).

In all placebo-controlled study parts on E4 monotherapy combined, the incidence of **serious adverse events** (SAE) in H subjects was relatively low (4% for 15 mg, 0% for 20 mg vs 1.0% for placebo). For NH women in C301 SSP, this was also relatively low 3.5%, the most frequent SAE were endometrial disorder (0.8%), vaginal haemorrhage (0.5%), lower limb fracture (0.2%), and COVID-19 (0.4%).

Two **deaths** were reported in the studies, one case of severe acute respiratory failure due to COVID-19 infection and one case of a fatal TEAE of road traffic accident, both are not considered related with the use of E4.

Two **adverse events of special interest** (AESI) were: pathological results of endometrial biopsies (disordered proliferative endometrium (DPE), hyperplasia, and endometrial carcinoma) and vaginal bleeding grade 2. The AESI of vaginal haemorrhage occurred in 372 (43.6%) subjects, endometrial disorder in 117 (13.7%) of the population at least 12 months since last menses. One (1) patient was diagnosed with complex hyperplasia without atypia, for which the incidence rate (IR) was calculated to be 0.3% (two-sided 95% CI 0.0-1.7%) and 0.3% (two-sided 95% CI 0.0-1.9%) in the sub-group of subjects ≥12 months since last menses. There were no cases of endometrial carcinoma observed.

A total of eight **venous thrombotic events** and five **cardiovascular events** were reported in all studies combined, of these, 4 venous thrombotic events and 3 cardiovascular events were reported in the endometrial safety study C301 SSP.

The incidence of **AEs leading to discontinuation** in H subjects was relatively low and generally similar in the estetrol group (4.0% in the 15 mg group and 7.0% in the 20 mg group), as compared with the placebo group (4.0%) in the placebo-controlled 53-week study. In study C301-SSP, the percentage discontinuation due to AE in the NH subjects in the combined E4 + 100 mg P4 group was high, with 29.4% in the sub-group of women ≥12 months since last menses. The most frequent AEs leading discontinuations were **vaginal haemorrhage** (14.9%), endometrial disorder (8.6%); breast pain (1.1%).

No safety signals with respect to **hematology**, **biochemistry**, **vital signs** (including QT prolongation), **lipid** and **glucose metabolism**, **hemostasis**, have been observed in phase 3 studies.

9.4.1. Uncertainties and limitations about unfavourable effects

Hysterectomized subjects

There are no specific uncertainties about the unfavourable effects in hysterectomized women.

Non-hysterectomized subjects

The incidence rate of hyperplasia is in line with the acceptable regulatory threshold for hyperplasia incidence rate set in the EMA guideline on HRT, which is reassuring. Nevertheless, there were a high number of vaginal haemorrhage and endometrial disorder events reported, a low percentage of cumulative amenorrhea at cycles 11-13 observed, and scientific literature data pointed to insufficiency of a dose of 100 mg P4 for endometrium protection, while no predicting factors for higher risk at bleeding/ discontinuation were found. Given this, in general, vaginal bleeding and DPE are sufficiently described in the SmPC (in line with the HRT core SmPC) and these events are considered manageable for clinical practice. However, at the start of section 4.4 of the SmPC, an additional warning was included, that a higher dose of progesterone or another approved progestogen might be used, since 100 mg P4 might not be optimal for every patient.

Further, considering the higher incidence of disordered proliferative endometrium in non-hysterectomized women, it might be expected that these women have an increased risk of endometrium neoplasm malignancy. This risk is, therefore, included as an important potential risk in the summary of safety specifications of the RMP.

9.5. Effects Table

Table 65: Effects Table for FYLREVY as HRT for oestrogen deficiency symptoms in postmenopausal women (data cut-off: 13 May 2024)*

Effect (short description)	*Estetrol 15 mg*	*Estetrol 20 mg*	*Placebo*	*Uncertainties/ Strength of evidence*
Frequency (model-adjusted change in weekly frequency of moderate to severe VMS from Baseline to week 12) LS mean (95% CI)	-59.63 (-65.57, -53.68) [1] -57.54 (-62.73, -52.34) [2]	-65.71 (-71.30, -60.12) [1] -60.82 (-66.09, -55.56) [2]	-43.22 (-49.26, -37.17) [1] -45.33 (-50.79, -39.88) [2]	**Co-primary endpoint** **SoE:** • Diff. in LS means vs. placebo (95% CI): **15 mg** -16.41 (-25.95, -6.87) [1] -12.20 (-20.69, -3.71) [2] **20 mg** -22.49 (-31.79, -13.19) [1] -15.49 (-24.04, -6.94) [2] • Consistent in sensitivity analyses Consistent with results at week 4
Severity (model-adjusted change in severity of moderate to severe VMS from Baseline to Week 12) LS mean (95% CI)	-1.24 (-1.38, -1.10) [1] -0.71 (-0.83, -0.59) [2]	-1.36 (-1.49, -1.22) [1] -1.15 (-1.28, -1.03) [2]	-0.70 (-0.85, -0.56) [1] -0.70 (-0.83, -0.56) [2]	**Co-primary endpoint** **SoE:** • Diff. in LS means vs. placebo (95% CI): **15 mg** -0.54 (-0.77, -0.31) [1] **20 mg** -0.66 (-0.88, -0.43) [1] -0.46 (-0.66, -0.25) [2] • Consistent in sensitivity analysis • Consistent with results at week 4 **Unc:** • Diff. in LS means vs. placebo (95% CI): **15 mg** -0.01 (-0.22, 0.19) [2]

Effect (short description)	Estetrol 15 mg	Estetrol 20 mg	Placebo	Uncertainties/ Strength of evidence
Responders (Proportion of Responders with moderate and severe VMS reduction ≥75% at Week 12) percentage (%)	63.3 [1] 49.0 [2]	74.5 [1] 56.9 [2]	39.5 [1] 37.3 [2]	**Secondary endpoint** **SoE:** • Nominal p-values <0.05 compared to placebo • Consistent with results of VMS reduction ≥50%: 82.5%, 87.0% and 60.5% [1] 81.3%, 81.7% and 61.3% [2] **Unc: -**
Discontinuations due to AE (%) *H subjects*	4.0[2]	7.0 [2]	4.0[2]	**SoE:** Consistent results in all H safety populations
Discontinuations due to AE (%) *NH subjects*		29.4 [3]		
Hyperplasia (n; %, 95% CI)		n=1; 0.3 (0.0-1.9) [3]		**SoE:** fulfils CHMP's specific requirements on endometrial safety
Vaginal haemorrhage grade 2		43.6% [3]		

Abbreviations: Unc: uncertainties; SoE: strength of evidence; H: hysterectomized; NH: non-hysterectomized; DPE: disordered proliferative endometrium
[1]= C301 ESP, [2]= C302 ESP, [3] = C301 SSP (endometrial safety study), post-hoc sub-group analysis of women ⩾ 12 months since last menses
* information comprised in the effects table refers to the full dataset, whilst the information included in SmPC section 5.1 refers to the post-hoc analysis

9.6. Benefit-risk assessment and discussion

9.6.1. Importance of favourable and unfavourable effects

For the initially proposed indication "*Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in postmenopausal women*" pivotal evidence for efficacy is based on the two 12-week placebo-controlled phase 3 studies C301 ESP and C302 ESP, of which the design and duration is in line with the recommendations of the CHMP guideline on HRT. With regards to the selected primary endpoint of reduction in **frequency of VMS**, as recommended by the CHMP guideline, both studies demonstrated a significant reduction in VMS frequency versus placebo at week 4 and week 12 for the 15 and 20 mg selected for marketing. With regards to the degree of reduction in VMS, the reduction in frequency of VMS observed for the highest dose of 20 mg can also be considered clinically relevant as the daily reduction in number of VMS versus placebo was reduced with ≥ 2 per day. Notably, although these are indirect comparisons, the reduction in frequency of VMS seems to be in the same range as noted for other oestrogen replacement therapy (*Cochrane Data Base Syst. Rev. 2001*). The primary endpoint of reduction in **severity of VMS**, demonstrated also a significant reduction versus placebo for the 20 mg dose, but not for the 15 mg dose in one of the 2 studies. Efficacy of both dosages is further supported by a nominally significantly higher **responder rate** (i.e. percentage of subjects with ≥50% or ≥75% reduction in the weekly frequency of VMS at Week 12) compared to placebo in both studies. In line with the reductions in VMS, results of other secondary endpoints selected were suggested supportive of the primary endpoint. For non-hysterectomized women it is considered important to have a lower dose available, which should be the initial starting dose. For hysterectomized women it is considered undesirable to start with a less effective dose, because no safety advantage is required, but it is preferred to start immediately with the highest, most effective dose. The justification of the place of the 15 mg dose can be accepted for non-hysterectomized women, but not for hysterectomized women. For the latter group, only the 20 mg is considered approvable.

Concerning safety in hysterectomized women, the safety dataset did not raise major concerns, with breast tenderness being the most important adverse event. Estetrol was well tolerated since discontinuations due to AE were low in all hysterectomized safety populations.

Concerning safety in non-hysterectomized women, treated with 20 mg Estetrol and 100 mg progesterone (P4), the CHMP's specific requirements on evaluation of endometrial safety are fulfilled, i.e. in a population of at least 300 women, the upper limit of the two-sided 95% confidence interval of the observed frequency of endometrial events did not exceed 2%.

Nevertheless, there were a high number of vaginal haemorrhage and endometrial disorder events reported, a low percentage of cumulative amenorrhea at cycles 11-13 observed, and scientific literature data pointed to insufficiency of a dose of 100 mg P4 for endometrium protection, while no predicting factors for higher risk at bleeding/ discontinuation were found. Given this, in general, vaginal bleeding and DPE are sufficiently described in the SmPC (in line with the HRT core SmPC) and these events are considered manageable for clinical practice.

9.6.2. Balance of benefits and risks

In terms of benefit, 15 mg and 20 mg estetrol have shown to provide a statistically significant greater reduction in the frequency of moderate to severe VMS in post-menopausal women compared to placebo, which is the most important and EMA guideline-recommended endpoint. The reduction in frequency was accompanied by a reduction in the severity of VMS. This was shown for both dosages in study C301 and confirmed for the 20 mg dosage in the second pivotal study C302, but not for the 15 mg dosage. Efficacy of both dosages is further supported by a higher responder rate (i.e. percentage of subjects with ≥50% or ≥75% reduction in the weekly frequency of VMS at Week 12) compared to placebo. The justification of the place of the 15 mg dose can be accepted for non-hysterectomized women, but not for hysterectomized women. For the latter group, only the 20 mg is considered approvable.

The use of estetrol in *hysterectomized* women appeared to be well tolerated with an acceptable safety profile. Concerning *non-hysterectomized* women, the incidence of endometrial hyperplasia fulfils CHMP's specific requirements on endometrial safety of E4 20 mg combined with progesterone 100 mg. Nevertheless, there were a high number of vaginal haemorrhage and endometrial disorder events reported, a low percentage of cumulative amenorrhea at cycles 11-13 observed, while no predicting factors for higher risk at bleeding/ discontinuation were found. Given this, in general, vaginal bleeding and DPE are sufficiently described in the SmPC (in line with the HRT core SmPC) and these events are considered manageable for clinical practice. However, at the start of section 4.4 of the SmPC, an additional warning is included, that a higher dose of progesterone or another approved progestogen might be used, since 100 mg P4 might not be optimal for every patient. Since the subgroup of women ≥6 months since last menses in the endometrial safety study has not been treated according to the approved dosing schedule and P4 dose, the indication for non-hysterectomized women was restricted to ≥12 months since last menses and a separate indication for hysterectomized women is recommended, since this restriction is not applicable for hysterectomized women.

Based on the results, from a clinical point of view, the benefit/risk balance is positive for the subgroup of *hysterectomized women* and the subgroup of *non-hysterectomized* women ≥12 months since last menses. However, for the *hysterectomized women* only the 20 mg is considered approvable. That is why the final indication reads:

"Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in hysterectomised postmenopausal women.

Hormone replacement therapy (HRT) for oestrogen deficiency symptoms in non-hysterectomised postmenopausal women with at least 12 months since last menses."

9.6.3. Additional considerations on the benefit-risk balance

Not applicable.

9.7. Benefit-risk conclusions

9.7.1. Final CHMP conclusions

The benefit/risk balance is positive for both strengths in the subgroup of *hysterectomized women* and for the subgroup of *non-hysterectomized women ≥12 months since last menses*. However, for the *hysterectomized women,* only the 20 mg dose of estetrol is considered approvable.