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ORIGINAL RESEARCH ARTICLE

A Randomized Open-Label Study of Relugolix Alone or Relugolix Combination Therapy in Premenopausal Women

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Abstract

Background and Objective Relugolix is a gonadotropin-releasing hormone receptor antagonist. Relugolix 40-mg monotherapy is associated with vasomotor symptoms and long-term bone mineral density loss due to hypoestrogenism. This study assessed whether the addition of estradiol (E2) 1 mg and norethindrone acetate (NETA) 0.5 mg to relugolix 40 mg (relugolix combination therapy) provides systemic E2 concentrations in the 20–50 pg/mL range to minimize these undesirable effects.

Methods This was a randomized, open-label, parallel-group study to assess the pharmacokinetics, pharmacodynamics, safety, and tolerability of relugolix 40 mg alone or in combination with E2 1 mg and NETA 0.5 mg in healthy premenopausal women. Eligible women were randomized 1:1 to receive relugolix alone or relugolix plus E2/NETA for 6 weeks. Study assessments included pharmacokinetic parameters of E2, estrone, and relugolix in both treatment groups, and norethindrone in the relugolix plus E2/NETA treatment group at weeks 3 and 6.

Results Median E2 24 h average concentrations with the relugolix plus E2/NETA group ($N = 23$) were 31.5 pg/mL, 26 pg/mL higher compared with the relugolix-alone group (6.2 pg/mL) ($N = 25$). There were 86.4% of participants in the relugolix plus E2/NETA group who had E2 average concentrations exceeding 20 pg/mL, the threshold expected to minimize bone mineral density loss, compared with 21.1% in the relugolix-alone group. Both treatments were generally safe and well tolerated.

Conclusions Relugolix 40 mg in combination with E2 1 mg and NETA 0.5 mg provided systemic E2 concentrations within a range expected to minimize the risk of undesirable effects of hypoestrogenism associated with the administration of relugolix alone.

Clinical Trial Registration Clinicaltrials.gov identifier no. NCT04978688. Trial registration date: 27 July, 2021; retrospectively registered.

1 Introduction

Relugolix is an orally active, non-peptide, gonadotropin-releasing hormone (GnRH) receptor antagonist that competitively binds to GnRH receptors, preventing the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) into the systemic circulation [1, 2]. Follicular growth and development are consequently suppressed, reducing ovarian production of estradiol (E2) [3]. Together with reductions in LH concentrations and the absence of a pre-ovulatory LH surge, ovulation and formation of the corpus luteum are prevented, thereby suppressing the production of progesterone (P) [1, 3]. Upon oral administration of relugolix, rapid (within hours) dose-dependent decreases in systemic FSH, LH, E2, and P concentrations are observed, with near-maximum decreases in E2 concentrations associated with a 40-mg dose (data on file).

In phase II dose-finding studies in women with uterine fibroids or endometriosis, 10-, 20-, or 40-mg doses of relugolix once daily for 12 weeks were associated with dose-related, statistically significant reductions in menstrual blood loss volume or pelvic pain compared with placebo ($p < 0.001$ and $p < 0.05$ [40 mg: $p = 0.001$], respectively) [4, 5]. In the relugolix 40-mg dose groups of the phase II studies, hot flush was reported more frequently compared

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Key Points

The current study characterizes the range of systemic estradiol concentrations in premenopausal women associated with relugolix combination therapy.

The data highlight the importance of a 1-mg dose of estradiol as a component of relugolix combination therapy in order to achieve systemic exposures to estradiol within a range that provides therapeutic effects while minimizing the frequency and severity of vasomotor symptoms and bone mineral density loss.

with placebo (38.9% vs 3.5% and 52.4% vs 8.2%, respectively) and a greater mean (± standard deviation) percent change from baseline in bone mineral density (BMD; measured by a dual-energy X-ray absorptiometry scan) was observed at week 12 (− 2.27 ± 2.21% vs − 0.2 ± 2.22% and − 2.1 ± 2.20% vs − 0.1 ± 1.7%, respectively) [4, 5]. Treatment with relugolix monotherapy for longer than 6 months was therefore not considered appropriate because of the risk for BMD loss [4–6] associated with the mechanistic reduction in systemic E2 concentrations. However, uterine fibroids and endometriosis are chronic debilitating conditions [7, 8] that require long-term treatment. Therefore, the goal of developing relugolix for the treatment of symptoms associated with uterine fibroids or endometriosis was to provide a long-term therapeutic option for patients [9, 10].

Barbieri hypothesized that achieving systemic E2 concentrations between 20 and 50 pg/mL would lead to the improvement of symptoms associated with these conditions while minimizing the undesirable consequences of hypoestrogenism [11]. Additionally, an assessment of the relationship between systemic E2 concentrations and the change from baseline in BMD loss associated with various GnRH receptor agonists or antagonists demonstrated that E2 concentrations > 20 pg/mL minimized the risk for BMD loss [12]. Moreover, in dose-finding studies for commercially available E2/norethindrone acetate (NETA) combination products (e.g., Activelle®) indicated for the treatment of moderate-to-severe vasomotor symptoms associated with menopause and the prevention of postmenopausal osteoporosis, a 1-mg dose of E2 and a 0.5-mg dose of NETA effectively mitigated the signs and symptoms associated with hypoestrogenism and prevented BMD loss [13]. Higher doses of E2 (2 mg) and NETA (1 mg) did not provide additional benefit [14].

Relugolix 40 mg with E2 1 mg and NETA 0.5 mg (relugolix combination therapy) was therefore developed to achieve near-maximum suppression of E2 by relugolix, prevent hypoestrogenism by exogenous E2, and protect the estrogen-related proliferative effects on the endometrium from unopposed estrogen by NETA. Relugolix combination therapy is approved in the USA for the management of heavy menstrual bleeding associated with uterine fibroids and moderate-to-severe pain associated with endometriosis and in the European Union for the treatment of moderate-to-severe symptoms of uterine fibroids in adult women of reproductive age [17–19].

The current study was conducted prior to initiation of the pivotal phase III studies with relugolix combination therapy in women with uterine fibroids or endometriosis. The goal was to assess whether relugolix 40 mg in combination with E2 1 mg and NETA 0.5 mg achieves systemic E2 concentrations within the range of 20–50 pg/mL previously hypothesized to treat symptoms associated with uterine fibroids or endometriosis, while minimizing the undesirable effects of hypoestrogenism [11, 12, 20].

2 Methods

This study was conducted in accordance with the International Conference on Harmonisation E6 Good Clinical Practice guidelines [21], applicable participant privacy guidelines, and ethical principles outlined in the Declaration of Helsinki 2013 [22]. The study protocol was reviewed and approved by an independent institutional review board and all participants provided written informed consent prior to study-related procedures being performed.

2.1 Study Design and Interventions

This was a randomized, open-label, parallel-group study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of relugolix 40 mg as monotherapy or combination therapy with E2/NETA in healthy premenopausal women. Following a screening period of up to 45 days, eligible participants ($N = 48$) were randomized 1:1 to receive either relugolix 40 mg alone (Myovant Sciences, Inc.) or with E2 1 mg and NETA 0.5 mg (relugolix + E2/NETA [Activelle®; NovoNordisk A/S, Bagsværd, Denmark]) once daily for 6 weeks (Fig. 1). Study drug administration was initiated on day 1, coinciding with the first to sixth day of the menstrual cycle. Participants returned to the study site on a weekly basis for outpatient visits throughout the study treatment period and remained domiciled for 48 h at the week 3 and week 6 visits for pharmacokinetic and pharmacodynamic assessments. Blood samples for the determination of relugolix (4 mL/sample), norethindrone (NET; active form of NETA; relugolix + E2/NETA group only), and ethinylestradiol (EE; a metabolite of NETA;

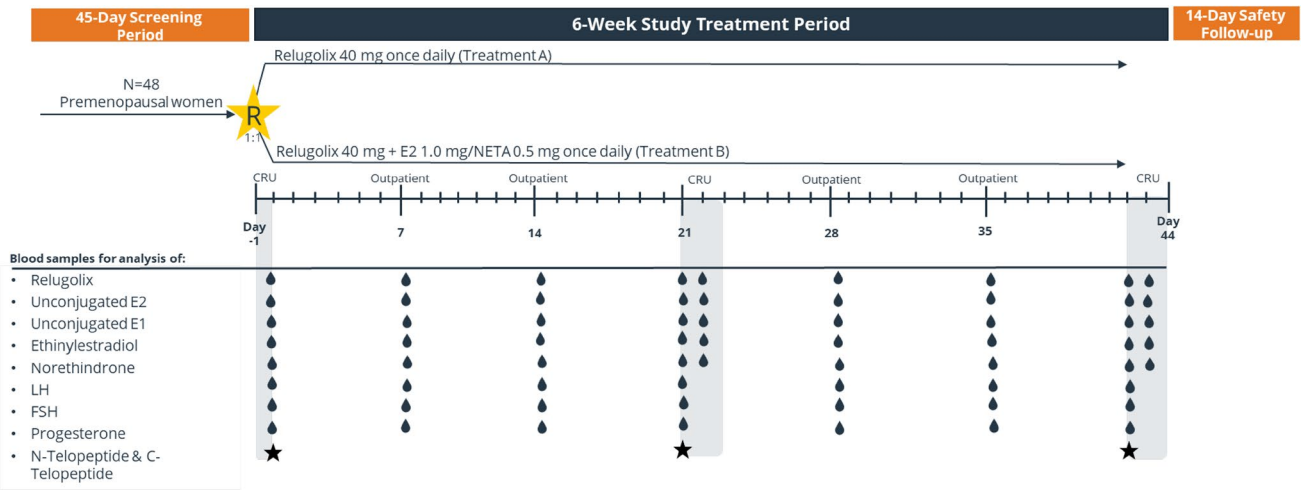

Fig. 1 Study schema. *AE* adverse event, *CRU* clinical research unit, *E1* estrone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *NETA* norethindrone acetate, *PD* pharmacodynamics, *PK* pharmacokinetic, *R* randomization

relugolix + E2/NETA group only) (8 mL/sample) plasma concentrations and unconjugated E2 and unconjugated estrone (E1) (6 mL/sample) serum concentrations were collected pre-dose on day 1 (baseline) and at weeks 1, 2, 4, and 5, and at pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h post-dose at week 3 (day 21) and week 6 (day 42). Blood samples for the determination of LH, FSH, and P serum concentrations were collected pre-dose once weekly from week 1 to week 6. Blood samples for determination of the bone resorption biomarkers N-telopeptide (NTx) and C-telopeptide (CTx) (2.5 mL/sample) were collected pre-dose on day 1 and at week 3 and week 6. Participants used daily diaries throughout the treatment period to record their adherence to study treatment, menstruation details, incidence and severity of hot flush, adverse events, and concomitant medications. For at-home self-administration of the study drug, site staff confirmed treatment compliance with a documented mid-weekly phone call. At the end of the 6-week treatment period, participants attended a safety follow-up visit 14 days (±2 days) after administration of the last dose of the study drug.

2.2 Study Participants

Eligible participants were those considered to be healthy based on clinical evaluations and laboratory tests, premenopausal women between 18 and 48 years old with a body weight ≥ 45 kg and a body mass index of 20–36 kg/m^2. Participants were required to have a history of regular menstrual periods (≥ 3 consecutive days of bleeding requiring protection) with cycle lengths of 21–35 days for 3 months prior to enrollment. Other inclusion criteria were E2, P, LH, and FSH concentrations within 0.5 times the lower limit and two times the upper limit of the normal ranges.

Participants were excluded if they had various conditions inconsistent with being a healthy subject (e.g., diabetes mellitus; hypertension; gynecologic; pulmonary; hepatic; or renal disease: clinically significant cardiovascular disease; or electrocardiogram abnormalities) or that would be exacerbated by study treatments (e.g., hyperlipidemia, cholelithiasis, osteoporosis, or osteopenia). Medications that could confound interpretation of study endpoints (e.g., hormone-containing products [including oral, injectable, or intrauterine device contraceptives], danazol or GnRH receptor agonists) were not permitted within specific timeframes prior to the study start nor were medications (e.g., investigational products, prescription or non-prescription drugs or supplements) or conditions (renal or hepatic impairment, gastrointestinal dysfunction, or anatomical abnormalities) that might interfere with the absorption, metabolism, and/or excretion of study treatments. Pregnant or lactating women were excluded from participation.

2.3 Study Objectives

The primary study objective was to assess the pharmacokinetic parameters for relugolix, unconjugated E2 and unconjugated E1 in both treatment groups, and EE and NET in the relugolix plus E2/NETA group only, at week 3 and week

6. Of note, systemic E2 concentrations reflect both endogenously produced E2 and exogenously administered E2 as a component of relugolix combination therapy; therefore, unconjugated E2 concentrations serve as a pharmacokinetic and pharmacodynamic endpoint, characterizing the exposure to E2 achieved with relugolix combination therapy and the mechanism of action associated with relugolix, namely the suppression of ovarian production of E2. Secondary study objectives included safety and tolerability parameters (see Sect. 2.6) and characterization of FSH, LH, P, NTx, and CTx serum concentrations.

2.4 Bioanalytical Methods and Pharmacokinetic Analyses

2.4.1 Bioanalytical Methods

Plasma or serum concentrations for relugolix, NET, EE, unconjugated E2, and unconjugated E1 were quantified using validated liquid chromatography/tandem mass spectrometry following standard procedures of the bioanalytical laboratory (QPS, Newark, DE, USA). Concentrations below the lower limit of quantitation (LLOQ) were reported as 0. The LLOQ for relugolix, NET, and EE in plasma was 0.05 ng/mL, 0.05 ng/mL, and 2.5 pg/mL, respectively. The LLOQ for unconjugated E2 and unconjugated E1 in serum were 2.5 pg/mL and 5 pg/mL, respectively. Luteinizing hormone, FSH, and P serum concentrations were quantified by standard clinical laboratory assays, with the LLOQ of 0.3 IU/L, 0.1 IU/L, and 0.67 nmol/L, respectively. N-telopeptide and CTx serum concentrations were quantified by enzyme-linked immunosorbent assays, with the detection range of 5–40 nM and 0.146–3.041 ng/mL, respectively.

2.4.2 Pharmacokinetic Analyses

Pharmacokinetic parameters for relugolix, NET, unconjugated E2, and unconjugated E1, including the area under the concentration–time curve (AUC) from time zero to 24 h post-dose ($AUC_{0–24}$), maximum concentration (C_{max}), time to C_{max}, trough concentration (C_{trough}), average concentration (C_{avg}; calculated as $AUC_{0–24}$ divided by 24 h), and terminal elimination half-life ($t_{1/2}$), were calculated using Phoenix® WinNonlin® version 6.3 or later (Certara USA, Inc., Princeton, NJ, USA).

A pharmacokinetic analysis was performed for all participants who underwent blood sampling for determination of plasma or serum drug concentrations and who had evaluable pharmacokinetic parameters. Pharmacokinetic parameters were calculated from the actual dosing, and sampling times and missing data were not imputed. For participants whose last quantifiable concentrations were collected prior to 24 h post-dose, the AUC from time zero to the last quantifiable concentration was represented as $AUC_{0–24}$.

2.5 Statistical Analyses

No formal hypothesis testing was planned. The sample size estimate for the study was determined using the variability associated with C_{avg} of E2 at steady state in order to achieve a desired precision to characterize C_{avg}. Assuming a coefficient of variation of 50% for the C_{avg} at steady state, a sample size of 20 evaluable participants per treatment group was estimated to yield lower and upper bounds of the 95% confidence interval (CI) within 19% of the point estimate of the calculated C_{avg} value.

Concentration–time profiles and descriptive statistics of the pharmacokinetic parameters of relugolix, unconjugated E2, and unconjugated E1 were provided by timepoint (week 3 and week 6) and treatment group (relugolix alone; relugolix + E2/NETA). The potential effects of exogenously administered E2 and NETA on the $AUC_{0–24}$ and C_{max} of relugolix at week 3 and week 6 were analyzed using a linear mixed-effects model with treatment, time, and treatment-by-time interaction as fixed effects and participant as a random effect. The $AUC_{0–24}$ and C_{max} of relugolix at weeks 3 and 6 were natural log-transformed prior to analysis. The geometric least-squares mean ratios (relugolix alone/relugolix + E2/NETA) and 90% CIs were estimated for $AUC_{0–24}$ and C_{max} of relugolix by taking the exponent of mean differences and the corresponding 90% CIs. The time to achieve steady-state plasma concentration of relugolix was evaluated using Helmert contrast testing of C_{trough} from week 1 to week 6 of treatment [23]. A contrast was not statistically significant if the 90% CI of the contrast coefficient fell within prespecified comparability bounds (80%, 125%).

Descriptive statistics for pharmacodynamic endpoints were provided by timepoint and treatment group. Endpoints were analyzed with a change from baseline (day 1) as the response, using a linear mixed-effects model incorporating treatment, time, and treatment-by-time interaction as factors and the baseline value as a covariate, assuming a compound symmetric covariance structure among times within each participant. Summary statistics were calculated using R version 3.02 or later [24]. Tables including descriptive statistics of pharmacokinetic and pharmacodynamic data and summaries of safety parameters were generated using SAS version 9.2 or higher [25]. Figures of concentration–time data for each analyte were generated using GraphPad Prism version 9.4 (GraphPad Software, San Diego, CA, USA).

2.6 Safety Monitoring

Safety was assessed throughout the study by repeated clinical and laboratory evaluations including physical examinations, clinical laboratory tests, vital sign measurements, 12-lead electrocardiograms, and reporting of adverse events.

3 Results

3.1 Participants

The study was conducted between 16 June and 21 September, 2016. A total of 48 premenopausal women were enrolled, with 25 women randomized to receive relugolix alone (all of whom completed the study) and 23 women to receive relugolix plus E2/NETA (21 of whom completed the study) (Fig. 2). Demographics for participants enrolled in this study are summarized in Table 1.

3.2 Adherence to Study Treatment

Three participants (all in the relugolix alone group) reported protocol deviations of missing a dose of study drug on a single day, and three participants (two in the relugolix alone group; one in the relugolix + E2/NETA group) returned fewer tablets than expected upon study drug reconciliation. These and other protocol deviations that resulted in missing data were judged by the investigators not to have an impact on study integrity or participant safety.

3.3 Pharmacokinetics

The pharmacokinetic parameters of relugolix, E2, E1, and NET at steady state after administration of relugolix plus E2/NETA once daily for 6 weeks in healthy premenopausal women are summarized in Table 2. Additional pharmacokinetic results specific to relugolix, E2, E1, NET, and EE are described below.

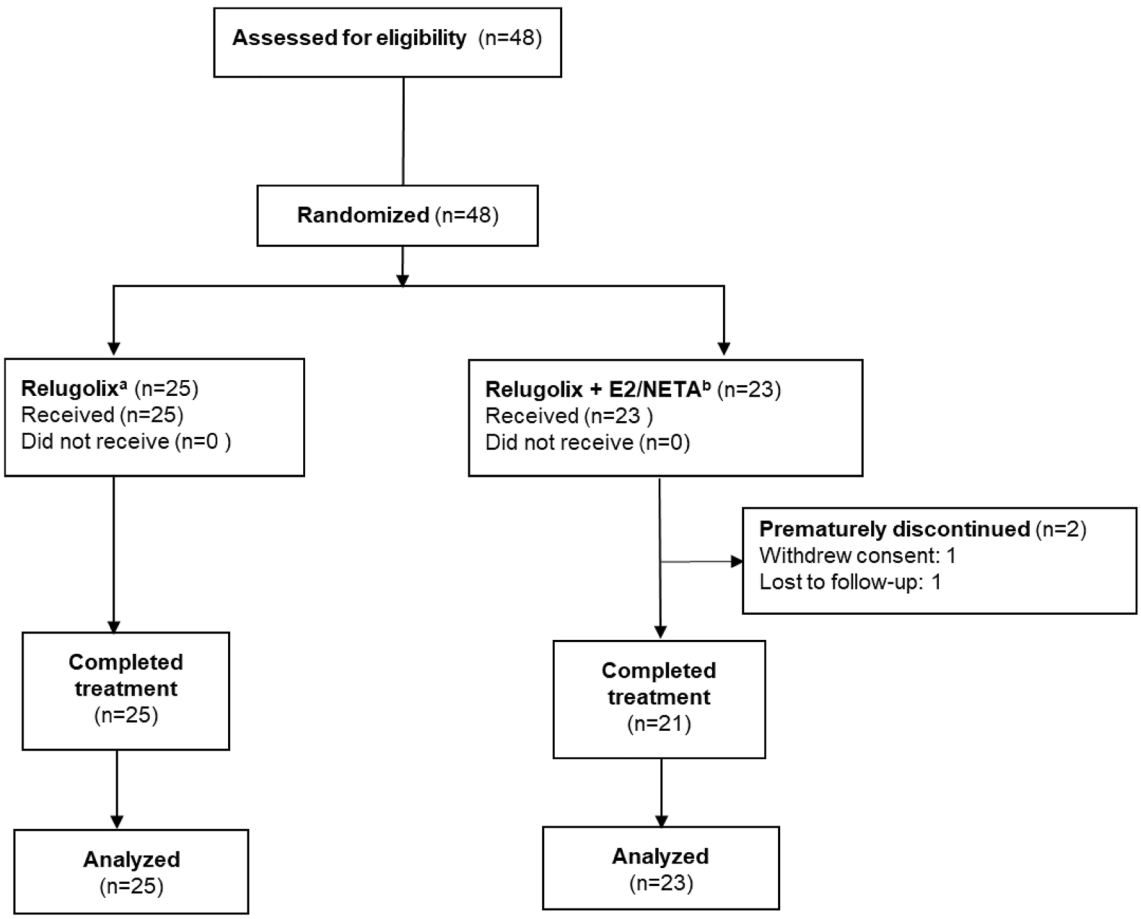

Fig. 2 Participant disposition. [a]Relugolix 40 mg alone for 6 weeks. [b]Relugolix 40 mg with estradiol (E2) 1 mg and norethindrone acetate (NETA) 0.5 mg for 6 weeks

Table 1 Demographics

	Relugolix[a] ($n = 25$)	Relugolix + E2/NETA[b] ($n = 23$)	Overall ($N = 48$)
Race, *n* (%)			
White	20 (80.0)	15 (65.2)	35 (72.9)
Black	4 (16.0)	4 (17.4)	8 (16.7)
Asian	1 (4.0)	0	1 (2.1)
Native American or Alaska Native	0	1 (4.3)	1 (2.1)
Other	0	3 (13.0)	3 (6.3)
Age (years), mean (SD)	33.9 (7.9)	34.1 (6.5)	34.0 (7.2)
Body mass index (kg/m^2), mean (SD)	26.0 (3.9)	24.2 (3.0)	25.2 (3.6)

E2 estradiol, *NETA* norethindrone acetate, *SD* standard deviation

[a]Relugolix 40 mg alone for 6 weeks

[b]Relugolix 40 mg with E2 1 mg and NETA 0.5 mg for 6 weeks

Table 2 Pharmacokinetic parameters of relugolix, unconjugated E2, unconjugated E1, and NET in pre-menopausal women after once-daily administration of relugolix plus E2/norethindrone acetate for 6 weeks

	Relugolix	E2	E1	NET
C_{max} (ng/mL or pg/mL)	26 (21.4)	46.8 (17.3)	303 (137)	5.21 (1.53)
t_{max} (h)	3 (0.5, 6)	3 (0.50, 12.00)	4 (1, 8.08)	1 (1, 2)
C_{avg} (ng/mL or pg/mL)	6.53 (3.94)	32.6 (10.9)	186 (82.4)	1.06 (0.474)
C_{trough} (ng/mL or pg/mL)	2.96 (1.74)	20.8 (7.81)	96.4 (45)	0.302 (0.229)
AUC_{0-24} (ng*h/mL or pg*h/mL)	157 (94.7)	784 (262)	4450 (1980)	25.5 (11.4)
$t_{1/2}$ (hr)	ND	17.1 (4.03)	13.9 (4.14)	8.28 (1.87)

Arithmetic means and standard deviations are shown except for t_{max}, where median and range (minimum, maximum) are shown. AUC_{0-24} is presented in ng*h/mL for relugolix and NET and in pg*h/mL for unconjugated E2 and unconjugated E1. C_{max} is presented in ng/mL for relugolix and NET and in pg/mL for unconjugated E2 and unconjugated E1. The terminal elimination half-life for relugolix was not included, as the 24 h duration of sample collection in this study was deemed inadequate for capturing the elimination phase with a $t_{1/2}$ of 61.5 h reported previously

AUC_{0-24} area under the concentration–time curve during a dosing interval (24), *C_{avg}* average concentration, *C_{max}* maximum observed concentration, *C_{trough}* trough concentration, *E1* estrone, *E2* estradiol, *h* hour, *ND* not determined, *NET* norethisterone, *$t_{1/2}$* terminal elimination half-life, *t_{max}* time to the maximum observed concentration

3.3.1 Relugolix

Mean concentration–time profiles and pharmacokinetic parameters of relugolix after administration of relugolix alone or relugolix plus E2/NETA at week 3 and week 6 were generally comparable (Fig. 3). The geometric mean ratios (relugolix + E2/NETA/relugolix alone) for the AUC_{0-24} and C_{max} of relugolix at week 6 approximated 1.1 and the 90% CI interval for the ratios included 1.00 (Table 3),with the upper bound slightly outside of the comparability bounds (80%, 125%), indicating that exposure to relugolix after administration of relugolix alone or relugolix plus E2/NETA once daily for 6 weeks was similar.

3.3.2 E2 and Estrone

In both treatment groups, median E2 C_{trough} values measured once weekly were relatively consistent from day 8 to day 43 (Fig. 4), with ranges from 4.66 to 6.34 pg/mL and from 24.5 to 27.4 pg/mL for the relugolix alone and the relugolix plus E2/NETA treatment groups, respectively, demonstrating sustained suppression of endogenous E2 production by relugolix. Median E2 concentrations over the 24 h dosing interval for the relugolix-alone group at week 6 (day 43) were low (5.46–6.35 pg/mL) and corresponded to the median E2 C_{trough} values. Conversely, for the relugolix plus E2/NETA group, median E2 concentrations initially increased to the maximum of 49.2 pg/mL at 3 h post-dose and declined thereafter in a monophasic manner to a concentration of 21.4 pg/mL at 24 h post-dose (Fig. 5, Table 4). Median E2 C_{trough} values were considerably higher compared with corresponding values for the relugolix-alone group (21.4 vs 5.7 pg/mL, respectively) and were associated with a narrower range (3.6–39.0 pg/mL vs 1.3–255.0 pg/mL, respectively) [Table 4]. Median E2 C_{avg} values were 26 pg/mL higher for the relugolix plus

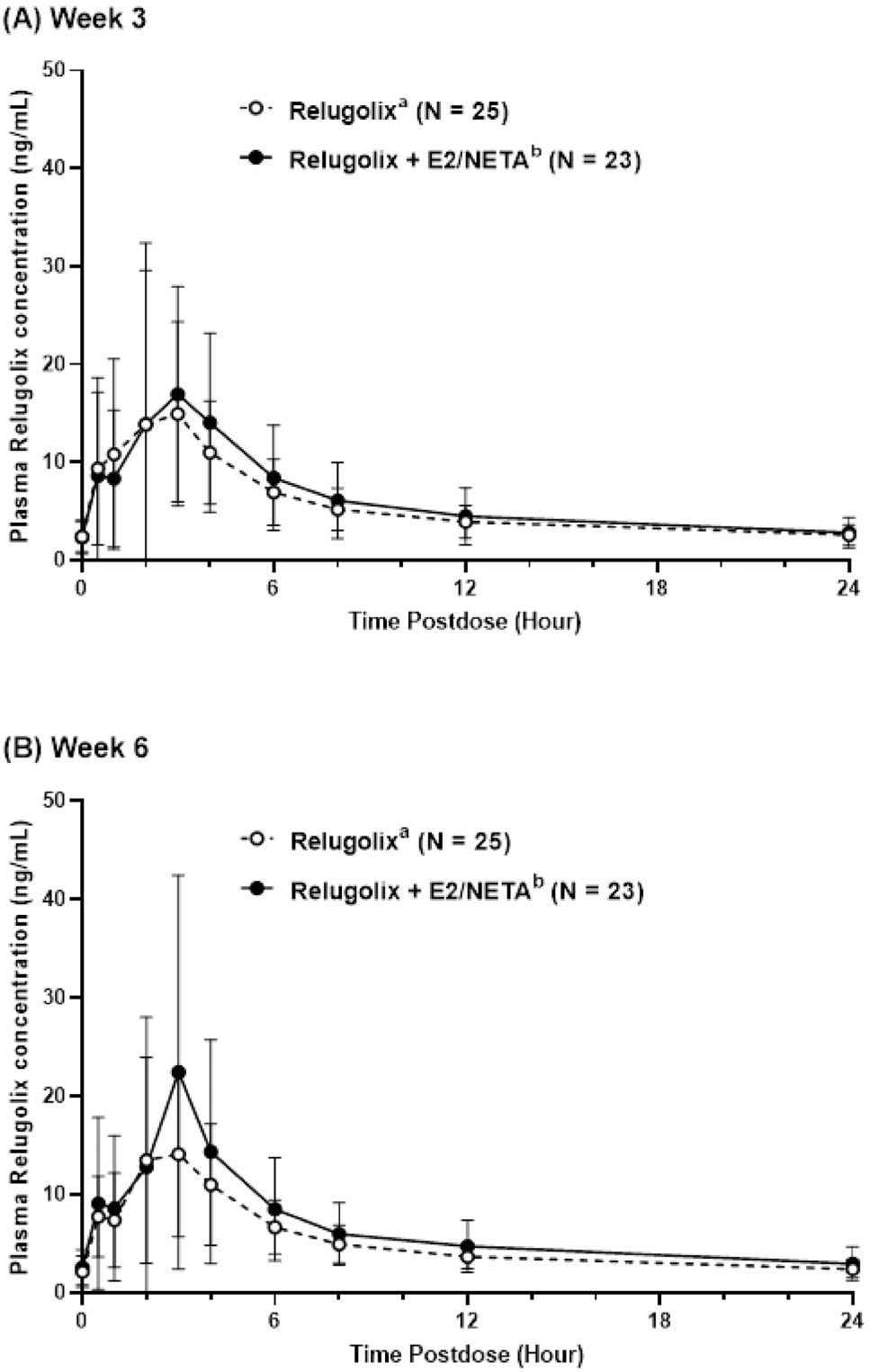

Fig. 3 Mean (standard deviation) relugolix concentration–time profiles at **a** week 3 and **b** week 6. [a]Relugolix 40 mg alone. [b]Relugolix 40 mg with estradiol (E2) 1 mg and norethindrone acetate (NETA) 0.5 mg

E2/NETA group compared with the relugolix-alone group (31.5 pg/mL and 6.2 pg/mL, respectively) [Table 4]. The proportion of participants with E2 C_{avg} values ≥ 20 pg/mL was 86.4% for the relugolix + E2/NETA group, compared with 21.1% for the relugolix-alone group.

Median E1 concentrations over the 24 h post-dose period after administration of relugolix alone at week 6 were consistently low (14.2–22.8 pg/mL), whereas median E1 concentrations upon coadministration of relugolix plus E2/NETA initially increased to a maximum of 270 pg/mL at 4 h post-dose and

Table 3 Statistical analysis for pharmacokinetic parameters of relugolix after administration of relugolix alone or relugolix plus E2/NETA in healthy adult premenopausal women for 6 weeks

Pharmacokinetic parameter	*N*	Geometric mean	CV_W (%)	Geometric mean ratio[a]	90% CI
Week 6					
AUC_{0-24} (ng*h/mL)					
Relugolix alone	25	116			
Coadministration (relugolix + E2/NETA)	22	128	24.6	1.10	(0.84, 1.44)
C_{max} (ng/mL)					
Relugolix alone	25	17.6			
Coadministration (relugolix + E2/NETA)	22	18.9	40.6	1.07	(0.76, 1.51)

AUC_{0-24} and C_{max} parameters were analyzed on a natural log scale; only participants with evaluable parameter values in both Test and Reference treatments were included in the statistical analysis for AUC_{0-24} and C_{max}

AUC area under the concentration–time curve, AUC_{0-24} AUC from time 0 to 24 h, *CI* confidence interval, C_{max} maximum observed concentration, CV_W within-subject coefficient of variation, *E2* estradiol, *h* hour, *n* number of participants included in summary statistics, *NETA* norethindrone acetate

[a]Ratio of geometric mean between Test (relugolix plus E2/NETA) and Reference (relugolix alone). From a mixed-effects model for the log-transformed parameter results with treatment as fixed effect and participant as a random effect

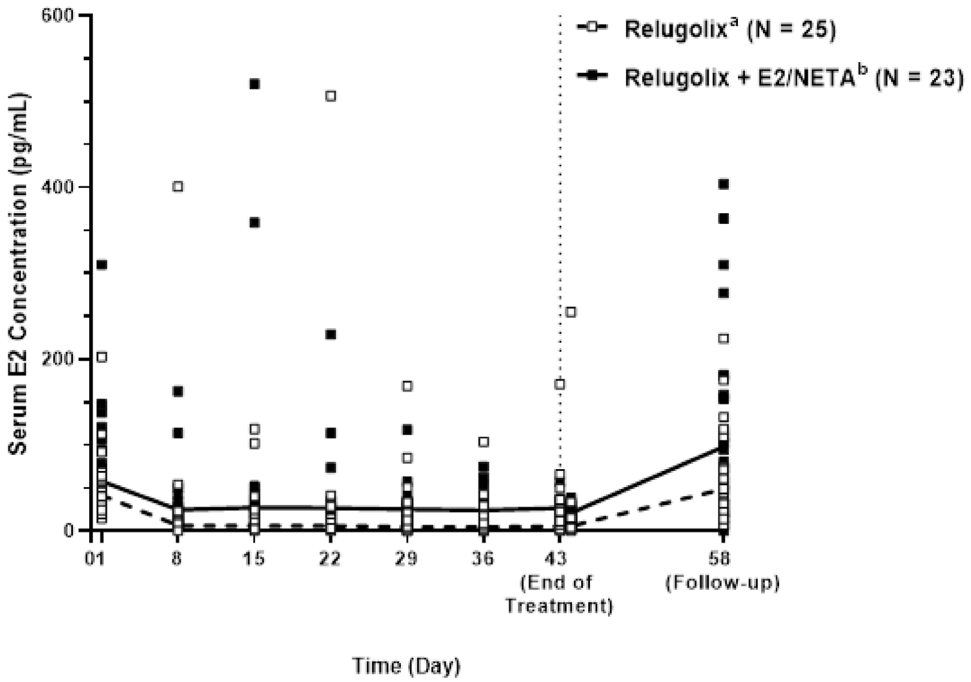

Fig. 4 Weekly median (and individual) predose (trough) E2 (estradiol) serum concentration–time profiles during the 6-week treatment period and at the follow-up visit. [a]Relugolix 40 mg alone. [b]Relugolix 40 mg with E2 1 mg and norethindrone acetate (NETA) 0.5 mg

declined thereafter in a monophasic manner with a concentration of 98.6 pg/mL at 24 h post-dose (Fig. 5, Table 4) [26]. Overall, E1 concentrations were higher than E2 concentrations over the entire 24 h dosing interval in both treatment groups.

3.3.3 NET and EE

The mean plasma concentration–time profiles and pharmacokinetic parameters for NET were generally similar for week 3 and week 6 for the relugolix plus E2/NETA treatment group. Overall, NET concentrations initially increased, reaching mean peak concentrations of 5.2 ng/mL at approximately 1 h post-dose, declining thereafter in a biphasic manner (Fig. 1 of the Electronic Supplementary Material). Concentrations of EE, an active estrogenic metabolite of NET, were below the limit of quantitation (2.5 ng/mL) for most timepoints (> 98%); therefore, pharmacokinetic parameters were not determined for this analyte.

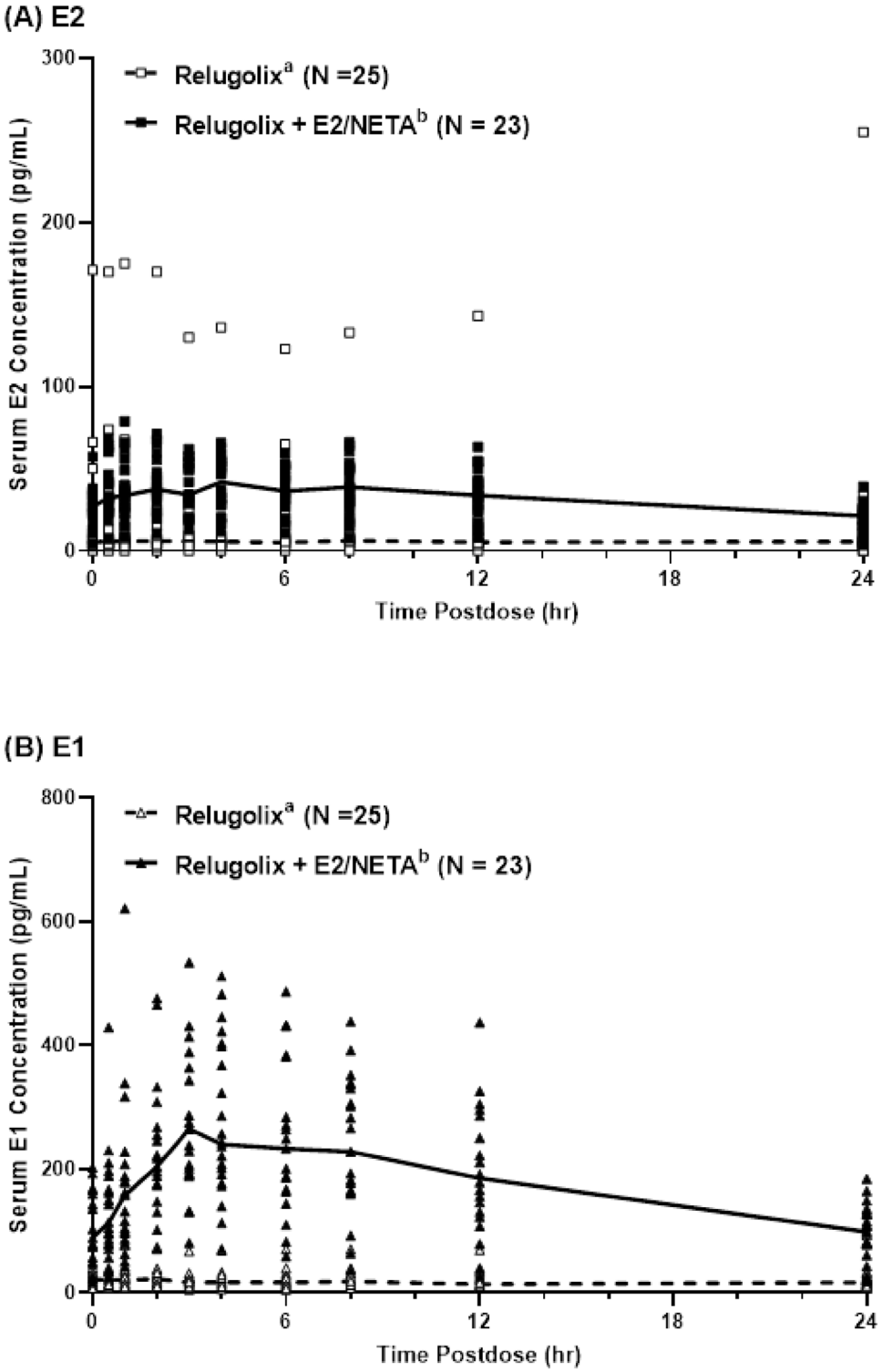

Fig. 5 Individual and median (range) **a** estradiol (E2) and **b** estrone (E1) concentration–time profiles at week 6. [a]Relugolix 40 mg alone. [b]Relugolix 40 mg with estradiol 1 mg and norethindrone acetate (NETA) 0.5 mg. *hr* hour

3.4 Pharmacodynamics

3.4.1 FSH, LH, and P Concentrations

Serum concentrations of LH, FSH, and P showed a comparable degree of suppression in both treatment groups during the 6-week treatment period (Fig. 6). Follicle-stimulating hormone, LH, or P concentrations between the relugolix-alone group and the relugolix plus E2/NETA group from week 1 (day 8) to week 6 (day 43) were not statistically different ($p > 0.05$). The consistently low P concentrations with no obvious post-ovulatory rise in P is suggestive of

Table 4 Pharmacokinetic parameters of E2 and E1 concentrations at week 6

	E2		E1	
	Relugolix[a]	Relugolix + E2/NETA[b]	Relugolix	Relugolix + E2/NETA
C_{max} (pg/mL)				
n	21	22	25	22
Mean (SD)	28.5 (55.3)	46.8 (17.3)	25.3 (16.6)	303 (137)
Median (range)	7.22 (2.74, 255)	49.2 (13.0, 78.9)	23.1 (7.98, 93.6)	270 (80.8, 621)
C_{avg} (pg/mL)				
n	19	22	25	22
Mean (SD)	20.0 (38.2)	32.6 (10.9)	19.7 (14.1)	186 (82.4)
Median (range)	6.17 (2.89, 170)	31.5 (7.73, 50.2)	17.2 (6.95, 76.3)	170 (36.7, 329)
C_{trough} (pg/mL)				
n	22	22	25	22
Mean (SD)	18.8 (53.2)	20.8 (7.8)	20.9 (16.7)	96.4 (45.0)
Median (range)	5.7 (1.3, 255)	21.4 (3.6, 39.0)	16.9 (7.04, 93.6)	98.6 (20.1, 184)

C_{avg} average concentration, C_{max} maximum observed plasma concentration, C_{trough} trough concentration, *E1* estrone, *E2* estradiol, *NETA* norethindrone acetate, *SD* standard deviation

[a]Relugolix 40 mg alone

[b]Relugolix 40 mg with E2 1 mg and NETA 0.5 mg

inhibition of ovulation; however, because sampling was performed only once weekly, a definitive conclusion could not be made.

3.4.2 NTx and CTx

The between-group differences of the change from baseline to day 43 in NTx and CTx of 2.24 nmol/L (20.97%) and 0.13 µg/L (33.31%), respectively, were statistically significant ($p = 0.019$ and $p = 0.030$, respectively), indicating that the 1-mg dose of E2 as a component of relugolix combination therapy minimizes the effect on these bone resorption biomarkers (Fig. 7). In the relugolix plus E2/NETA group, the percent change from baseline to week 6 for both NTx and CTx were small (5.54% and 10.36%, respectively) with both 95% CIs including 0 (Fig. 7), suggesting there was no significant change in bone resorption after 6 weeks of treatment.

3.5 Safety

Overall, administration of 40-mg doses of relugolix alone or relugolix plus E2/NETA once daily for 6 weeks was generally safe and well tolerated (Table 5). Adverse events were reported for 23 of 25 (92.0%) participants who received relugolix alone and 20 of 23 (87%) participants who received relugolix plus E2/NETA. A total of 80 and 92 adverse events were reported, respectively, and of these, 58 adverse events in each group (reported by 21 and 18 participants, respectively) were considered by the investigator to be drug related. Most adverse events were mild or moderate in intensity, with the exception of seven adverse events of hot flush (four participants in the relugolix-alone group and three participants with relugolix plus E2/NETA group) rated as severe. The most commonly reported drug-related adverse events included nausea, headache, delay in menstruation, and hot flush. Overall, the frequency of adverse events reported in participants in the relugolix-alone or relugolix plus E2/NETA groups was similar, except for hot flush, which was more frequent in the relugolix-alone group (21 events in 19 participants [76.0%]) vs ten events in ten participants [43.5%], respectively). Adverse events of uterine bleeding were more frequent in the first 28 days of study treatment (prior to week 6) attributed to shedding of the existing uterine lining as a result of a rapid decline in P concentrations, a pharmacologic effect of relugolix. As expected, the number of participants who reported no menstrual bleeding (except spotting) over the last 28 days of treatment was greater after administration of relugolix alone (88.0%) compared with relugolix plus E2/NETA (47.8%). No clinically significant changes in clinical laboratory test values, vital sign measurements, or electrocardiogram parameters were reported.

4 Discussion

The current study was conducted to assess whether relugolix combination therapy achieves systemic E2 concentrations within a range expected to provide therapeutic effects while minimizing the frequency and severity of vasomotor symptoms and the risk for BMD loss. In phase II studies, relugolix

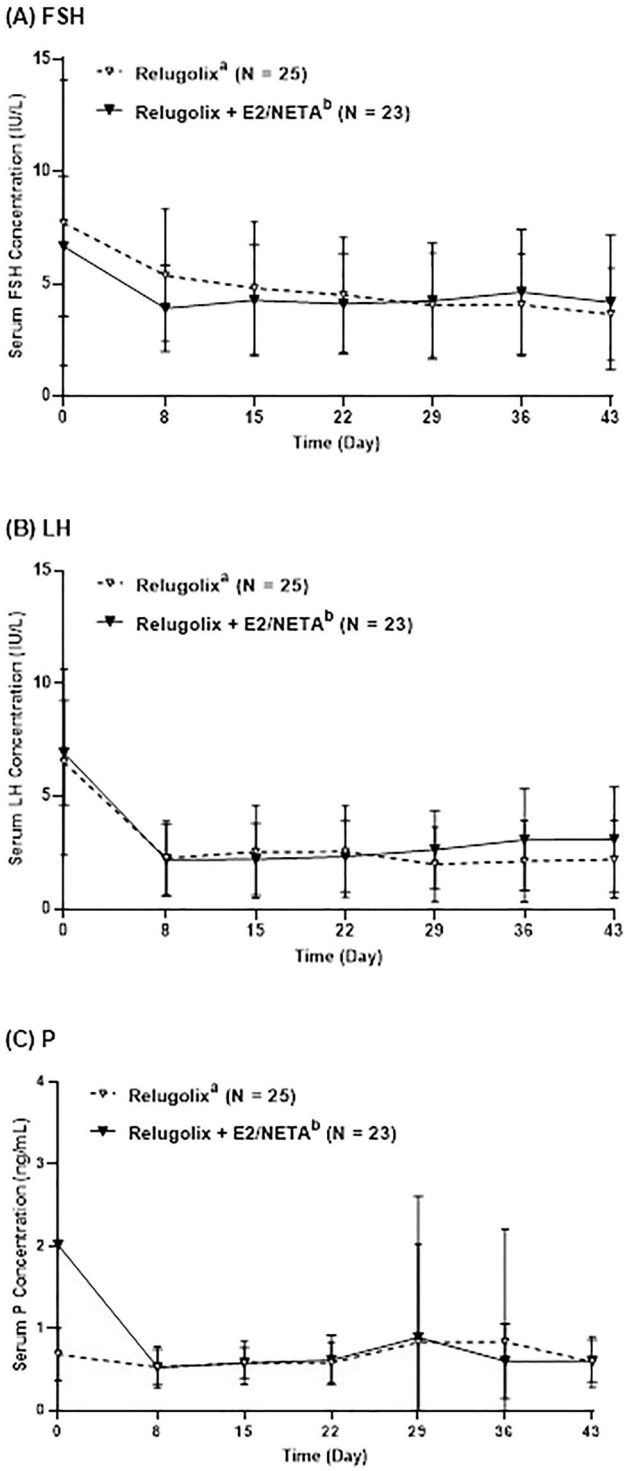

Fig. 6 Mean (± standard deviation) pre-dose **a** follicle-stimulating hormone (FSH), **b** luteinizing hormone (LH), and **c** progesterone (P) serum concentrations over time with relugolix alone and relugolix + estradiol/norethindrone acetate (E2/NETA). [a]Relugolix 40 mg alone. [b]Relugolix 40 mg with E2 1 mg and NETA 0.5 mg

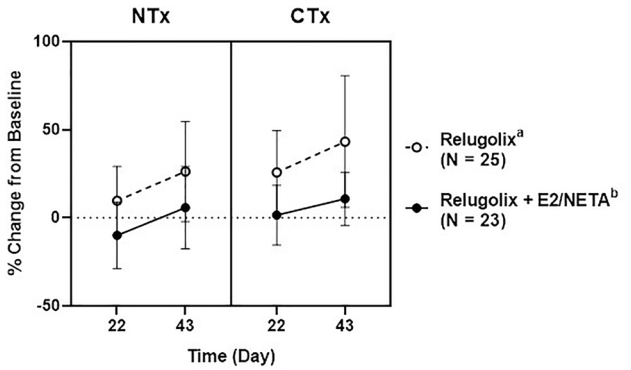

Fig. 7 Mean (± standard deviation) percent change from baseline in N-telopeptide (NTx) and C-telopeptide (CTx). [a]Relugolix 40 mg alone. [b]Relugolix 40 mg with estradiol (E2) 1 mg and norethindrone acetate (NETA) 0.5 mg

40 mg as monotherapy showed dose-dependent and statistically significantly reductions in menstrual blood loss volume in women with heavy menstrual bleeding associated with uterine fibroids or pelvic pain in women with endometriosis compared with placebo [4, 5]; however, the degree of BMD loss with relugolix monotherapy would limit the duration of treatment [4–6].

As shown in this study, the median E2 C_{avg} value in the relugolix plus E2/NETA group, reflecting both endogenously produced E2 (which is suppressed by relugolix) and exogenous administration of E2 (as a component of relugolix combination therapy), was 31.5 pg/mL, 26 pg/mL higher than the corresponding value after administration of relugolix alone and above the 20-pg/mL threshold associated with the risk for BMD loss [12]. In the relugolix plus E2/NETA group, 86.4% of participants had E2 C_{avg} exceeding 20 pg/mL, compared with 21.1% in the relugolix-alone group. The observed small percent of change from baseline to week 6 of the bone resorption biomarkers NTx and CTx with relugolix combination therapy are also consistent with the maintenance of E2 concentrations > 20 pg/mL. Estradiol inhibits osteoclastic activity responsible for bone resorption, hence, when E2 concentrations are too low, bone resorption biomarkers would reflectively increase as a result of increased osteoclastic activity. The frequency of vasomotor symptoms such as hot flush was reduced from 76.0% of participants with relugolix alone to 43.5% of participants in the relugolix plus E2/NETA group, also highlighting the importance of the 1-mg dose of E2 as a component of relugolix combination therapy.

In the replicate, double-blind, randomized, placebo-controlled, 24-week, phase III studies, relugolix combination

Table 5 Summary of TEAEs

	Number of TEAEs/number of participants (% of participants)		
	Relugolix[a]	Relugolix + E2/NETA[b]	Overall
TEAE	80/23 (92.0)	92/20 (87.0)	172/43 (89.6)
TEAE causally related to study drug	58/21 (84.0)	58/18 (78.3)	116/39 (81.3)
Treatment-emergent SAE	0	2/1 (4.3)	2/1 (2.1)
Treatment-emergent SAE causally related to study drug	0	0	0
Death	0	0	0
Withdrawal from study because of TEAE	0	0	0

E2 estradiol, *NETA* norethindrone acetate, *SAE* serious adverse event, *TEAE* treatment-emergent adverse event

[a]Relugolix 40 mg alone

[b]Relugolix 40 mg with E2 1 mg and NETA 0.5 mg

therapy (relugolix + E2/NETA) provided effective reductions in menstrual blood loss volume in women with heavy menstrual bleeding associated with uterine fibroids [9] and dysmenorrhea and non-menstrual pelvic pain in women with endometriosis [10]. Additionally, no meaningful differences in mean percent change from baseline to week 24 in BMD at the lumbar spine (L1–L4) were observed between the relugolix plus E2/NETA and placebo groups, and no deleterious effects on the endometrium were observed in the active treatment group in any of the studies [9, 10]. These effects were generally maintained for up to 2 years in extension studies for both programs [27–29], indicating the potential for long-term use of relugolix combination therapy.

The limitations of this study include the lack of blinding and the relatively small sample size. Although no blinding to study treatment was implemented, participants were randomly assigned to study treatment and, other than reporting of adverse events, the study endpoints were objective rather than subjective assessments. A systematic bias with respect to grading or assigning causality of adverse events was minimized by multiple (four) study sites participating in the study. The study was sufficiently powered to estimate E2 concentrations within each group, although there was considerable variability in E2 concentrations, likely owing to the initiation of study treatment on day 1–6 of the menstrual cycle. This resulted in incomplete suppression of endogenous E2 production in some women, in whom high E2 concentrations consistent with the ovulatory phase of the menstrual cycle were observed. Nevertheless, E2 concentrations over a 24 h dosing interval (including C_{max}, C_{avg}, and C_{trough}) at week 3 and week 6 were considerably higher and less variable in the relugolix plus E2/NETA group compared with the relugolix-alone group.

5 Conclusions

Results from the study demonstrate that suppression of endogenous E2 production by relugolix 40 mg in combination with exogenous administration of E2 1 mg provides systemic E2 concentrations within a range expected to deliver clinical efficacy while minimizing the frequency and severity of vasomotor symptoms and the risk for BMD loss observed with relugolix 40 mg as monotherapy.

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Declarations

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Conflicts of interest/competing interests EM, TYL, YL, and JCAF are employees of Myovant Sciences and own stock/options in the company. AL has received grants, consulting fees, or honoraria from Myovant Sciences and payment for lectures from Myovant Sciences and AbbVie. AL is a principal investigator for several companies including: AbbVie, Bayer, Merck, Myovant, Organon, NIH, Estetra SRL and Lundbeck LLC. BJ is an employee and stockholder of Roivant Sciences, the parent company of Myovant Sciences. BJ also holds stock options in Myovant Sciences and is a co-inventor on some Myovant Sciences relugolix patents.

Ethics approval Independent ethics committee review and approval of the study protocol, informed consent forms, and other information requiring pre-approval were provided by an institutional review board (IntegReview, Austin, TX, USA). This study was performed in compliance with International Council for Harmonisation Good Clinical Practices, with US 21 Code of Federal Regulations 312.3(b), and with the ethical principles of the Declaration of Helsinki 2008.

Consent to participate All participants provided written informed consent before the initiation of study procedures.

Consent for publication Not applicable (no data are reported that could identify individuals).

Availability of data and material Data from this article will not be deposited in a data repository.

Code availability Not applicable.

Authors' contributions Study conception: BJ. Study design: BJ. Data interpretation: AL, EM, BJ, T-YL, YL, JCAF. Manuscript drafting/revision: AL, EM, BJ, T-YL, YL, JCAF. Approval of final manuscript: AL, EM, BJ, T-YL, YL, JCAF. Agreement to take public responsibility for appropriate portions of the content: AL, EM, BJ, T-YL, YL, JCAF.

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