

Inhibition of ovulation and pharmacologic mechanism of action of relugolix combination therapy

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Objective: To assess the effects of relugolix combination therapy (CT) on ovarian function in healthy, ovulatory, premenopausal women.

Design: This was an open-label, single-cohort, pharmacodynamic, safety, and tolerability study consisting of five study periods: a pretreatment period to confirm ovulatory status, three 28-day treatment periods, and a posttreatment period to assess the duration of time required to return to ovulation after treatment discontinuation. Ovarian function was assessed by transvaginal ultrasonography and serum hormone concentrations.

Subjects: Healthy, premenopausal female participants, 18–35 years of age.

Exposure: Relugolix CT (relugolix 40 mg, with estradiol 1 mg and norethindrone acetate 0.5 mg) was taken orally once daily for 84 days.

Main Outcome Measures: The primary endpoint was the proportion of women in whom ovulation was inhibited during the 84-day treatment period. Secondary endpoints included proportion of women in whom ovulation was inhibited within each treatment period, number of women who fulfilled the Landgren criterion; characterization of follicular diameter, hormone concentrations, and endometrial thickness; time to return to ovulation after treatment discontinuation; proportion of women who returned to ovulation within 36 days after treatment discontinuation; safety and tolerability.

Results: Seventy women were enrolled in the study, 67 of whom completed treatment. Treatment with relugolix CT inhibited ovulation in 100% of women who completed treatment (95% confidence interval, 94.6–100.0). Systemic concentrations of luteinizing hormone and follicle-stimulating hormone were suppressed and maintained at low concentrations during treatment, with an absence of a preovulatory luteinizing hormone surge. Median estradiol concentrations across all women were consistently maintained between 36.8 and 39.1 pg/mL (range, 12.1–121.1 pg/mL) during treatment. All individual progesterone concentrations during treatment remained below 1.57 ng/mL (5 nmol/L).

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Attestation statements:

- The subjects in this trial have not concomitantly been involved in other randomized trials (if applicable). Yes
- Data regarding any of the subjects in the study has not been previously published unless specified.
- Two abstracts have previously been published:
- ASRM 2020: Ingrid Duijkers, Elizabeth M. Migoya, Juan Camilo Arjona Ferreira, Christine Klipping. Characterization of pituitary and ovarian hormone concentrations during treatment with relugolix combination therapy. *Fertility and Sterility*, Volume 114, Issue 3, Supplement, 2020, Page e81, ISSN 0015-0282, <https://doi.org/10.1016/j.fertnstert.2020.08.248>. (<https://www.sciencedirect.com/science/article/pii/S0015028220310025>)
- ESHRE 2020: I. Duijkers, C. Klipping, C. Draeger, B. Schug, R.S. Wedemeyer, J. Li, J.C. Arjona Ferreira, E. Migoya. Suppression of ovarian activity during co-administration of the oral gonadotropin-releasing hormone receptor antagonist relugolix, estradiol, and norethindrone acetate in healthy female volunteers. *Human Reproduction*. P-287. Volume 35, Supplement 1, 2020, Page i268. doi.org/10.1093/humrep/35.Supplement_1.1
- Data will be made available to the editors of the journal for review or query on request. Yes

Authors data sharing statements: The data underlying this article are available in the article. Data sets from Myovant Sciences, GmbH (now Sumitomo Pharma Switzerland, GmbH) and Pfizer-sponsored clinical research will not be shared and no other documents will be available. Access criteria for data sharing is not applicable. Individual participant data (including data dictionaries) will not be available. General study information is available under the EudraCT number: EudraCT 2018-004130-15.

- Will individual participant data be available (including data dictionaries)? No.
- What data will be shared? Not available.
- What other documents will be available? Not available.
- When will data be available (start and end dates)? Not applicable.
- By what access criteria will data be shared (including with whom, for what types of analyses, and by what mechanism)? Not applicable.
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After treatment discontinuation, all women ovulated or initiated menses. The mean time to return to ovulation was 23.5 days. Treatment was generally well tolerated with no safety or tolerability issues identified.

Conclusion: Relugolix CT inhibits ovulation, which, in the context of this study, was achieved within the first cycle after treatment initiation. The rapid and predictable return of ovarian activity and ovulation after treatment discontinuation is advantageous for patients who wish to conceive thereafter. (Fertil Steril® 2025; ■:■-■. ©2025 by American Society for Reproductive Medicine.)

Key Words: Relugolix combination therapy, GnRH receptor antagonist, ovulation inhibition, estradiol, luteinizing hormone

Relugolix is an orally active, nonpeptide, gonadotropin-releasing hormone (GnRH) receptor antagonist that competitively binds to GnRH receptors, blocking the binding of native GnRH and thereby preventing the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) into the systemic circulation. Consequently, downstream production of estradiol (E2) and progesterone (P) by the ovaries is reduced (1, 2). Relugolix 40 mg, in combination with E2 1 mg and norethindrone acetate (NETA) 0.5 mg (relugolix combination therapy, taken orally), is approved in the United States for the management of heavy menstrual bleeding associated with uterine fibroids (UFs) and moderate-to-severe pain associated with endometriosis (3), and in the European Union for the treatment of moderate-to-severe symptoms of UFs in adult women of reproductive age (4). Because UFs and endometriosis affect women of reproductive age (5), understanding whether relugolix CT inhibits ovulation was important to inform contraception requirements during treatment. Currently, product information differs in terms of contraception recommendations for women with UFs and endometriosis treated with relugolix CT, depending on the region and country (3, 4, 6–8). A study (SERENE, NCT04756037) into the safety and contraceptive efficacy (assessed using the Pearl Index) of relugolix CT in women with UFs or endometriosis who are at risk for pregnancy is currently ongoing (9).

In a previous pharmacokinetic and pharmacodynamic study, healthy premenopausal women were randomized to receive either relugolix 40 mg alone ($n = 25$) or in combination with E2 1 mg and NETA 0.5 mg once daily for 6 weeks ($n = 23$) (10). Pituitary (FSH and LH) and ovarian (E2 and P) hormone concentrations were maintained at low physiologic concentrations during treatment in both treatment groups, reflecting the effects of relugolix on the hypothalamic-pituitary-gonadal (HPG) axis. The low physiologic E2 concentrations were thought to be the result of the suppression of FSH concentrations, although E2 concentrations in the relugolix combination therapy group were maintained above that associated with bone mineral density (BMD) loss, which was considered imperative to long-term treatment. The low systemic P concentrations and the lack of an observed postovulatory rise in P typically observed during an untreated menstrual cycle suggest that ovulation could have been inhibited (10). However, a postovulatory rise in P could have been missed because hormone concentrations were only collected once weekly and ovulation was not evaluated using a standardized methodology (10). To overcome these limitations, the current study was conducted to assess if relugolix CT

suppresses ovarian function and inhibits ovulation in women of reproductive age and to determine the time for ovulation to return after treatment discontinuation.

MATERIALS AND METHODS

This study was conducted at a single research center (dinox GmbH, Berlin, Germany) from April to November 2019. Study-related documents were submitted for ethical review and approval by the independent ethics committee of the Landesamt für Gesundheit und Soziales (Berlin, Germany) and written approval by the independent ethics committee and the Competent Authority (Bundesinstitut für Arzneimittel und Medizinprodukte) was obtained before study initiation. Participants provided written informed consent before study-related procedures were performed. The study was conducted in accordance with the International Council on Harmonisation E6 Good Clinical Practice (11), applicable participant privacy requirements, and the ethical principles outlined in the Declaration of Helsinki 2013 (12).

Study participants

Premenopausal female participants, 18 to 35 years of age with a body mass index 18.5 to 30.0 kg/m², who were considered to be medically healthy on the basis of medical history, physical, breast and gynecologic examinations, electrocardiogram, clinical laboratory tests, prespecified criteria for heart rate, blood pressure, QTc interval, and lack of serum creatinine and transaminase elevations (13), were eligible. Participants were either nonsmokers or, if ≤ 30 years of age, smoked no more than 10 cigarettes per day, or if >30 years of age, were ex-smokers within 3 months before the screening visit. Short-acting hormonal contraceptives were discontinued at least one menstrual cycle before the pretreatment period, and GnRH analogues and depot hormonal injectables were restricted from use for various durations corresponding to their respective biological effects. Participants agreed to use non-hormonal contraception or practice abstinence throughout the study.

Exclusion criteria included significant endocrine, hepatic, renal, hematologic, pulmonary, cardiovascular, gastrointestinal, urologic, immunologic, or neurologic disorders; pregnancy; lactation; abnormal cervical smear; contraindication to treatment with E2 or NETA; concomitant use of hormonal preparations, anticoagulants, glucocorticoids, or oral P-glycoprotein and/or cytochrome P450 3A inducers or inhibitors.

Analysis populations

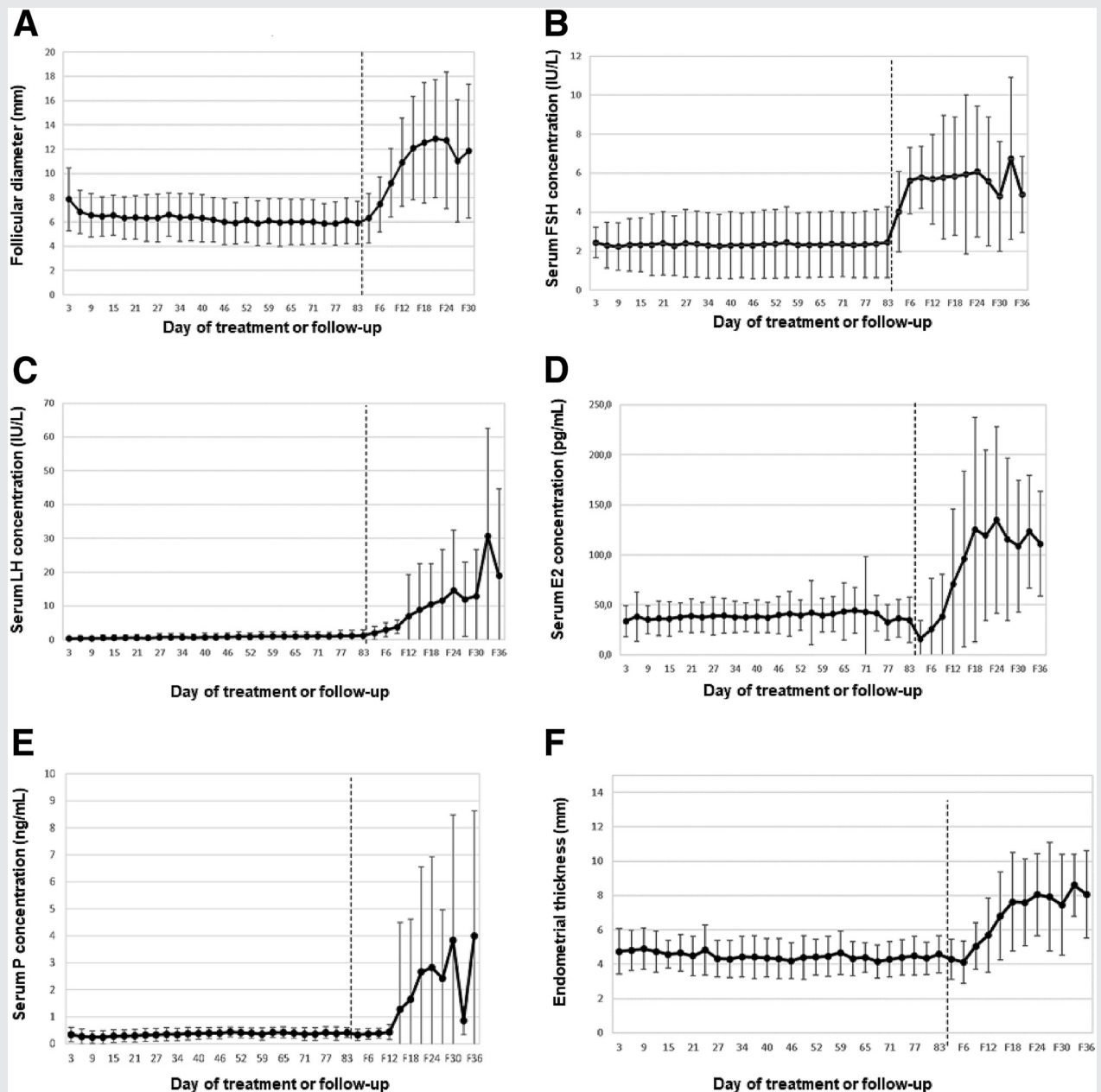
The evaluable population was defined as all study participants who received at least one dose of study drug and provided at least one Hoogland–Skouby score (HSS). The completers population was defined as all study participants in the evaluable population who completed the 84-day treatment period and did not miss more than two consecutive doses of study medication. The safety population was defined as all study participants who received at least one dose of study medication. The

included population was defined as study participants who attended at least one study visit during the pretreatment period (Fig. 1). All analysis populations were defined before the study start.

Study treatment

Relugolix 40-mg tablets were supplied as blister strips (Myovant Sciences, GmbH, now Sumitomo Pharma Switzerland,

FIGURE 1



Assessment of follicular diameter (A), FSH (B), LH (C), E2 (D), P (E), concentrations, and endometrial thickness (F) in women during and after treatment with relugolix combination therapy (evaluable population). E2 = estradiol; FSH = follicle-stimulating hormone; LH = luteinizing hormone; P = progesterone.

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GmbH, Basel, Switzerland), and E2/NETA ([1 mg/0.5 mg] Acti-velle; Novo Nordisk, Bagsværd, Denmark) was supplied as commercially available dial packs. Participants were instructed to self-administer the study drug once daily, at the same time every day, and to take relugolix and E2/NETA tablets simultaneously with water after an overnight fast.

Study design

This was an open-label, single-cohort study. The study consisted of five consecutive study periods: a pretreatment period (Pre-TP) of approximately 28 days, three 28-day treatment periods (TP-1, -2, and -3), and a Post-TP of up to 36 days (Supplemental Fig. 1, available online).

In each study period, participants attended study visits every 3 (± 1) days beginning on day 9 (± 1) after the first day of menstrual bleeding in the Pre-TP, day 3 (± 1) of TP-1 through day 27 (± 1) of TP-3 and throughout the Post-TP until ovulation was observed. During each visit, transvaginal ultrasound scans (TVUS) were performed to measure the size of the dominant follicle and endometrial thickness, and to monitor for follicular rupture (ovulation). Pituitary (FSH and LH) and ovarian (E2 and P) hormone concentrations were determined to assess pituitary and ovarian function, and to confirm ovulation. In the Pre-TP, if follicular rupture was observed, serum P concentrations were determined 2 (± 1) days and, if necessary, 4 (± 1) days postovulation; ovulation was confirmed if P concentrations were ≥ 5 ng/mL (16 nmol/L) at either visit. If follicular rupture was not observed on or before day 27 (± 1) or if both postovulation serum P concentrations were < 5 ng/mL, the study participant was considered not to be eligible. Ovulatory participants returned to the clinic 6 (± 1) days postovulation to receive the study medication. On the first day of menstrual bleeding (day 1 of TP-1), participants initiated self-administration of relugolix CT.

In each treatment period, if ovulation was observed or suspected by TVUS, P concentrations were determined 2-, 4-, and 6-days postovulation to confirm ovulation and to assess the adequacy of the luteal phase. In the Post-TP, after ovulation was observed, P concentrations were determined 2 (± 1) days thereafter and, if P concentrations were < 5 ng/mL (16 nmol/L), also 4 (± 1) days thereafter, to confirm ovulation. A final examination was performed 6 (± 1) days after ovulation. If ovulation was not observed by day 36 (± 1), a final examination was performed and participants attended weekly visits for up to 6 additional weeks, during which TVUS scans were performed and P concentrations determined until ovulation was observed or menstruation commenced.

Throughout the study, all participants maintained a daily paper diary to record vaginal bleeding data; the date, time, and result of the urine pregnancy test (TP-1); the date and time of daily study drug self-administration, and fasting status (TP-1, -2, and -3). Safety was assessed by repeated clinical and laboratory evaluations, including vital sign measurements, reporting of adverse events, and clinical laboratory tests. At each study visit, the study staff checked the blister packs of study medications to monitor intake of study medication, which were reconciled with daily diary entries, to evaluate treatment compliance.

Study endpoints and measurements

The primary study endpoint was the proportion of women in whom ovulation was inhibited (HSS (14) of < 5 [$P \leq 1.57$ ng/mL; see Supplemental Table 1, available online]) during the 84-day treatment period. Secondary endpoints included the proportion of women in whom ovulation was inhibited by treatment period, the number of women who fulfilled the Landgren criterion (15), characterization of follicular diameter, hormone concentrations, and endometrial thickness by study period, the time to return to ovulation after treatment discontinuation, the proportion of women who returned to ovulation within 36 days after treatment discontinuation, and safety and tolerability.

Inhibition of ovulation, or absence of luteal activity, was evaluated using the HSS (Supplemental Table 1) (14, 16, 17), a 6-point scale on the basis of the presence, size, and persistence of follicle-like structures (FLSs), as measured by TVUS, combined with E2 and P concentrations. An HSS of < 5 indicates the absence of luteal activity (14). The diameter of the dominant follicle in both ovaries (mean of measurements in two directions [height, width]) and double-layer endometrial thickness (on longitudinal images of the uterus) were measured by TVUS using a Voluson E8 Expert device (GE Medical Systems). Follicular rupture (ovulation) was determined by TVUS visualization and confirmed with up to three sequential (every 2 days) P measurements. During treatment, the postovulatory luteal phase was considered adequate if three P concentrations were > 5 ng/mL (16 nmol/L), in accordance with the Landgren criterion (P concentrations > 5 ng/mL [16 nmol/L] for at least 5 consecutive days) (15). Follicle-stimulating hormone, LH, and P serum concentrations were quantified using a validated chemiluminescent microparticle immunoassay (Synlab Pharma Analytics & Services, Berlin, Germany). Serum E2 concentrations were analyzed using a validated high-performance liquid chromatography coupled to tandem mass spectrometry assay (QPS, Newark, DE, USA). Assays were conducted in accordance with the Food and Drug Administration's Guidance for Industry, Bioanalytical Method Validation (18).

Statistical analysis

The proportion of women in whom ovulation inhibition was demonstrated during the 84-day treatment period (and within each treatment period) and the associated exact 95% confidence intervals (CIs; Clopper–Pearson) were calculated (completers population). If the HSS in any of the three 28-day TPs could not be determined for any reason, the participant was excluded from evaluation of the primary endpoint.

Follicular diameter, endometrial thickness, and pituitary (FSH and LH) and ovarian (E2 and P) hormone concentrations were listed and summarized descriptively by TP and the Post-TP (evaluatable population). All hormone concentrations were reported as mean, with the exception of E2, which was reported as median for consistency and comparability across published studies. Participant maximum and mean follicular diameter, endometrial thickness, and hormone concentrations were evaluated by treatment period and the overall 84-day treatment period. Return of ovulation was confirmed

by TVUS within 36 days after discontinuation of treatment (completers population). The day of ovulation was calculated from the day after the last dose of study drug in TP-3. The Kaplan–Meier method was used to characterize the time to event (confirmed ovulation) and estimate the proportion of women in whom a return of ovulation was confirmed within 36 days after treatment.

Adverse event verbatim text was coded and classified by body system and preferred (coded) term using Medical Dictionary for Regulatory Activities (Version 22.0). The number and proportion of study participants reporting at least one adverse event, overall and by study period, were generated (included population). Descriptive summary statistics for serum chemistry and hematology parameters by treatment period and study visit day were generated (safety population).

Sample size estimation

Assuming ovulation would occur in 5% of women during treatment, a sample size of 40 women completing the 84-day treatment period would allow the lower bound of the 95% CI for the proportion of women in whom ovulation was inhibited to be 86.3%. Based on a dropout rate of 30% and 25%–30% of women anticipated not to demonstrate ovulation in the Pre-TP, approximately 80 study women would be required to initiate the Pre-TP with 110 women screened.

RESULTS

Of the 110 healthy premenopausal women screened, 71 were ovulatory, 70 were enrolled and received study treatment, and 67 completed the study treatment period (Fig. 1). Of the 70 women enrolled, 61 (87%) were white. The mean (range) age was 29 years (18–35 years) and body weight was 66.1 kg (49.5–91.0 kg) (Table 1). Three women were prematurely discontinued from the study: one for an adverse event (depression) on day 46, another for treatment noncompliance on day 47, and the third for withdrawal of consent on day 62. Treatment compliance in the completers population was 100%.

Assessment of ovarian activity and HSS

Ovulation was inhibited in 100% (95% CI, 94.6%–100%) of women who completed study treatment (Table 2). Additionally, ovulation was inhibited in 100% (95% CI, 94.6%–100%) of women in each 28-day TP (Table 2). In most women, ovarian activity was completely suppressed (HSS 1, largest FLS of ≤ 10 mm; Supplemental Table 2). Six women had FLSs of > 13 mm at several or all study visits during treatment (HSS 3 or 4). Two women had FLSs assessed as ovarian cyst, three women had FLSs > 13 mm that disappeared with study treatment, and one woman had a growing FLS > 13 mm observed on day 25 of TP-1, reaching a maximum of 13.5 mm on day 34, with an associated E2 concentration of 107 pg/mL (393 pmol/L), both of which decreased thereafter. In all six women, P concentrations were below 1.57 ng/mL (5 nmol/L), hence no luteinized unruptured follicles (HSS = 5) or ovulations (HSS = 6) were observed. Inhibition of ovulation

TABLE 1

Demographics and baseline characteristics (safety population).

Variable	Safety population (n = 70)
Age (y)	
Mean (SD)	29.2 (3.8)
Median (min–max)	30.0 (18.0–35.0)
Height (m)	
Mean (SD)	1.67 (0.07)
Median (min–max)	1.67 (1.54–1.81)
Weight (kg)	
Mean (SD)	66.09 (9.53)
Median (min–max)	64.45 (49.5–91.0)
BMI (kg/m ²)	
Mean (SD)	23.57 (3.06)
Median (min–max)	22.80 (18.6–30.0)
Ethnicity, n (%)	
Asian	1 (1.4)
Black or African American	1 (1.4)
Black or African American and White	1 (1.4)
White	61 (87.1)
White and American Indian or Alaska Native	1 (1.4)
Other	5 (7.1)

Note: BMI = body mass index; max. = maximum; min. = minimum.

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was also demonstrated in the evaluable population (Supplemental Fig. 2) in which no women had an HSS of 5 or 6.

Follicular diameter

Follicular growth was considerably and consistently suppressed during treatment (Fig. 1A). The mean dominant follicle size (diameter) within each TP was maintained at approximately 6 mm (Table 3). In contrast, mean [\pm SD] dominant follicle diameter for the Pre- and Post-TPs was considerably higher (13.0 [± 2.5] and 10.4 [± 1.8] mm, respectively), reflecting normal follicular growth before and after treatment (Table 3).

Pituitary and ovarian hormones

Pituitary secretion of FSH and LH was markedly suppressed during treatment (Fig. 1B and 1C). Mean FSH and LH concentrations within each treatment period were consistently maintained at approximately 2.3 U/L and at or below 1.0 U/L, respectively (Table 3). After discontinuation of treatment, mean [\pm SD] FSH and LH concentrations were 5.6 [± 1.1] and 7.8 [± 4.6] U/L, respectively (Table 3). Importantly, the preovulatory LH increase was absent in each treatment period, whereas, after discontinuation of treatment, mean LH concentrations began to increase by day 12, consistent with the return of an LH surge.

Systemic E2 concentrations were reduced during treatment with an absence of cyclical fluctuations typically observed during a natural menstrual cycle (Fig. 1D). Median E2 concentrations were consistently maintained between 36.8 and 39.1 pg/mL (range, 12.1–121.1 pg/mL) (Table 3).

TABLE 2

Proportion of participants demonstrating inhibition of ovulation (HSS < 5) (completers population).

Treatment period	HSS < 5			Proportion Yes	Type	95% CI	
	Yes	No	N			Lower limit	Upper limit
Treatment period 1	67	0	67	1.00	Clopper–Pearson (exact)	0.9464	1.0000
Treatment period 2	67	0	67	1.00	Clopper–Pearson (exact)	0.9464	1.0000
Treatment period 3	67	0	67	1.00	Clopper–Pearson (exact)	0.9464	1.0000
Overall 84-day treatment period	67	0	67	1.00	Clopper–Pearson (exact)	0.9464	1.0000

Note: CI = confidence interval; HSS = Hoogland–Skouby Score.

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After discontinuation of treatment, E2 concentrations began to increase on day 6 with a median concentration of 71.9 pg/mL (range, 27.1–215.1 pg/mL) (Table 3).

Progesterone concentrations were reduced during treatment without the postovulatory rise typically observed during a natural menstrual cycle (Fig. 1E). Mean P concentrations were consistently maintained between 0.3 and 0.4 ng/mL (Table 3). Importantly, the maximum P concentration among all participants during treatment remained below 1.57 ng/mL (5 nmol/L). Thus, the Landgren criterion was not met for any participant during treatment with relugolix CT. After discontinuation of study treatment, P concentrations began to increase with a mean [\pm SD] concentration of 2.8 [\pm 1.7] ng/mL (Table 3 and Fig. 1E).

Endometrial thickness

Endometrial proliferation was suppressed during treatment with relugolix CT. Mean endometrial thickness in each treatment period (Fig. 1F) was consistently maintained between 4.4 and 4.7 mm (Table 3). In contrast, mean [\pm SD] endometrial thickness in the Pre- and Post-TPs was 7.8 [\pm 1.5] and 6.2 [\pm 1.3] mm, respectively (Table 3).

Return of ovulation

After discontinuation of study treatment, all women either ovulated (follicle rupture observed by TVUS) or menstruated (a single woman on day 39 in whom ovulation was not observed because of missed visits). By day 36, 97% (65 of 67) of women ovulated; ovulation could not be confirmed on day 36 in two women, one who missed her visits and another who did not ovulate until day 43. The overall mean time to return to ovulation was 23.5 days (range, 15–43 days) (Supplemental Fig. 3).

Safety

Relugolix CT was generally well tolerated with no major safety issues identified in healthy adult premenopausal women. A total of 69 of 71 (97.2%) women reported at least one adverse event during the study (Supplemental Table 3). Most adverse events were mild or moderate in severity and, with the exception of 15 adverse events, were resolved by the end of the study.

The most frequently reported adverse events during treatment (in > 10% of participants) were nasopharyngitis (40.9%), headache (38.0%), nausea (22.5%), mood altered (16.9%), pelvic pain (12.7%), acne (12.7%), and dizziness (11.3%) (Supplemental Table 3).

One woman with a medical history of depression not requiring medication was prematurely discontinued from the study on day 46 because of a moderate adverse event of depression that was considered by the investigator as probably related to the study drug. Another participant reported a serious adverse event of severe back pain on day 83 requiring hospitalization that was not considered related to the study drug. No pregnancies were reported during the study.

DISCUSSION

In this study, relugolix CT inhibited ovulation in 100% of women who completed study treatment. Secretion of pituitary and ovarian hormones was rapidly suppressed, with low systemic concentrations that were consistently maintained over time. Follicular growth was also suppressed, with little or no ovarian activity (as assessed by the HSS) in nearly all women. Postovulation increases in P concentrations were not observed, indicating the absence of luteal activity and, consequently, no risk for pregnancy.

The results from this study characterize the mechanism of action of relugolix CT. As a GnRH receptor antagonist, relugolix inhibits secretion of FSH and LH (1). The resultant low FSH concentrations diminish natural follicular growth and development (as evidenced by a mean dominant follicle size of < 13 mm during treatment), suppressing ovarian production of E2. A 40-mg dose of relugolix suppresses ovarian E2 production and when combined with exogenous administration of a 1-mg dose of E2, median E2 concentrations are consistently maintained within a narrow range (36.8 and 39.1 pg/mL [range, 12.1–121.1 pg/mL]), similar to values observed in the early follicular phase of a natural menstrual cycle (40.8 pg/mL [range, 13.6–97.8 pg/mL]) (19).

Furthermore, the reduction in systemic LH concentrations prevents corpus luteum formation, thereby suppressing the production of P, as evidenced by the low systemic P concentrations during treatment. The progestin NETA, included as a component of relugolix CT, minimizes risk for endometrial

TABLE 3

Descriptive statistics of participant-wise mean values per period of pituitary hormones, ovarian function parameters, and endometrial thickness (evaluative population).

Variable	Measure	Pretreatment period (N = 70)	Treatment period 1 (N = 70)	Treatment period 2 (N = 70)	Treatment period 3 (N = 68)	Posttreatment period (N = 67)
Dominant follicle size (diameter, mm)	Mean ± SD	13.0 ± 2.5	6.6 ± 1.5	6.2 ± 1.6	6.0 ± 1.7	10.4 ± 1.8
	Median	13.0	6.3	5.8	5.6	10.4
	Min.–max.	5.7–19.6	4.8–15.3	4.4–15.4	4.3–15.4	5.2–15.6
FSH concentration (IU/L)	Mean ± SD	—	2.3 ± 1.3	2.3 ± 1.7	2.3 ± 1.7	5.6 ± 1.1
	Median	—	2.2	2.1	2.2	5.3
	Min.–max.	—	0.3–5.4	0.1–6.3	0.1–6.5	2.7–8.1
LH concentration (IU/L)	Mean ± SD	—	0.5 ± 0.7	0.8 ± 1.1	1.0 ± 1.4	7.8 ± 4.6
	Median	—	0.2	0.3	0.5	6.8
	Min.–max.	—	0.0–3.4	0.0–4.5	0.0–6.6	1.9–25.6
E2 concentration (pg/mL)	Mean ± SD	—	36.9 ± 12.2	39.1 ± 13.7	39.7 ± 16.7	78.9 ± 36.0
	Median	—	36.8	39.1	38.4	71.9
	Min.–max.	—	14.2–77.3	14.6–90.6	12.1–121.1	27.1–215.1
Progesterone concentration (ng/mL)	Mean ± SD	—	0.29 ± 0.17	0.39 ± 0.15	0.39 ± 0.16	2.80 ± 1.66
	Median	—	0.31	0.39	0.42	2.49
	Min.–max.	—	0.02–0.74	0.00–0.82	0.05–0.76	0.44–12.06
Endometrial thickness (mm)	Mean ± SD	7.8 ± 1.5	4.7 ± 0.6	4.4 ± 0.7	4.4 ± 0.6	6.2 ± 1.3
	Median	7.7	4.7	4.4	4.44	6.1
	Min.–max.	5.1–12.4	3.3–6.5	3.1–6.2	3.2–5.8	4.0–12.8

Note: E2 = estradiol; FSH = follicle-stimulating hormone; LH = luteinizing hormone; max. = maximum; min. = minimum.

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hyperplasia resulting from unopposed estrogen. Endometrial proliferation was markedly suppressed during treatment (mean endometrial thickness, 4.4–4.7 mm).

Pituitary function and ovarian activity rapidly resumed after treatment discontinuation, resulting in ovulation. Follicle-stimulating hormone concentrations began to rise by 3 days and increased to near normal by 6 days after treatment discontinuation, promoting follicular growth and subsequent increases in E2 concentrations and endometrial thickness shortly thereafter.

Ovulation (observed by TVUS and confirmed with P concentrations) occurred in almost all women ($n = 66$ of 67; 98.5%). It was not observed in one woman because of missed study visits. However, this individual did start menstruating on day 39, which may indicate that ovulation had occurred. The earliest ovulation was observed 15 days after treatment discontinuation, with 97% of women returning to ovulation by day 36, indicating that even after robust suppression of ovarian activity, follicular development and the requisite LH surge for ovulation resume on treatment discontinuation. The rapid and predictable recovery of ovarian function is advantageous for women who wish to conceive after stopping treatment with relugolix CT.

Interestingly, literature reports indicate that elagolix, another GnRH receptor antagonist, is associated with dose-dependent suppression of ovarian function, with an ovulation rate of 27% with the highest dose of 300 mg twice daily (20). Although the ovulation rate was lower (10%) in women who received elagolix 300 mg twice daily with 1 mg E2 and 0.5 mg NETA once daily (20), ovulation still occurred, suggesting incomplete or inconsistent suppression of the HPG axis. Although both relugolix and elagolix are GnRH receptor antagonists, inherent differences in the respective compound profiles, such as potency, half-life, or potential inductive effects of E2 metabolism, may impact the ability to achieve complete suppression of the HPG axis with elagolix (21). Study-related issues, such as compliance with study treatment, may also have contributed to the discrepant results (20).

Relugolix CT effectively reduces heavy menstrual bleeding associated with UFs (leiomyomas) and moderate-to-severe pain associated with endometriosis, and balances the improvement in clinical symptoms while minimizing the frequency and severity of vasomotor symptoms and risk for BMD loss (22, 23). The current investigation demonstrated that systemic E2 concentrations during treatment with relugolix CT, reflecting both the suppression of endogenous production of E2 and the exogenous administration of a 1 mg dose of E2, are maintained within a range that is efficacious for the treatment of estrogen-driven conditions while minimizing the risks associated with hypoestrogenism (24–27). The resulting E2 concentrations are consistent with the physiological concentrations observed during the early follicular phase of a natural menstrual cycle.

A limitation of this study is that treatment compliance in clinical practice may be lower than the observed 100% treatment compliance rate, which could potentially result in

incomplete ovarian suppression. The SERENE study (NCT04756037) is currently ongoing to assess the contraceptive efficacy of relugolix CT in women with UF or endometriosis who are at risk for pregnancy.

CONCLUSION

The results of this study showed that ovulation was inhibited in 100% of women during treatment with relugolix CT, an effect that was manifested within the first treatment period. After treatment discontinuation, ovarian activity rapidly returned, and all women either ovulated or initiated menses. Ovulation occurred after a mean of 23.5 days, demonstrating the rapid return to physiological functioning of the HPG axis. The rapid onset and offset of action demonstrated in this study are consistent with the mechanism of action associated with relugolix as a competitive, but reversible, GnRH receptor antagonist. The robust characterization of E2 concentrations in this study was within a range associated with therapeutic efficacy while minimizing the risk for BMD loss and vasomotor symptoms associated with hypoestrogenism (27), confirming the rationale for inclusion of a 1-mg dose of E2 as a component of relugolix CT. Additionally, the lack of endometrial proliferation during treatment, as evidenced by measurement of endometrial thickness with TVUS, highlights the rationale for inclusion of a 0.5-mg dose of the progestin NETA as a component of relugolix CT.

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CRedit Authorship Contribution Statement

Ingrid J.M. Duijkers: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Christine Klipping:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation,

Conceptualization. **Corinna Draeger**: Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Barbara S. Schug**: Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Ralph-Steven Wedemeyer**: Writing – review & editing, Validation, Software, Formal analysis, Data curation, Conceptualization. **Yulan Li**: Writing – review & editing, Methodology, Conceptualization. **Juan Camilo Arjona Ferreira**: Writing – review & editing, Methodology, Conceptualization. **Elizabeth M. Migoya**: Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of Interests

I.J.M.D. is the director of dinox consultancy, a company that received funding from Myovant Sciences GmbH for consultancy services. C.K. is the director of dinox consultancy and director of dinox GmbH. C.D. is an employee of dinox GmbH, a contract research organization that received funding from Myovant Sciences, GmbH (now Sumitomo Pharma Switzerland, GmbH) and Pfizer for consultancy services and conduct of the study, respectively. B.S.S. was Managing Director of SocraTec R&D and SocraMetrics GmbH (at the time of the study), contract research organizations responsible for monitoring, data management, and evaluation; is now a Scientific Consultant at SocraMetrics GmbH, reports funding from Myovant, sponsorship of trial and support for medical writing for the submitted work. R.–S.W. is Head of Pharmacokinetics and Statistics at SocraMetrics GmbH and an employee of SocraTec R&D GmbH; reports funding from Myovant, sponsorship of trial and support for medical writing for the submitted work; and stock options from various companies as part of ETFs outside the submitted work. Y.L. was an employee of Myovant Sciences, Inc. (now Sumitomo Pharma America, Inc.) at the time the study was conducted. J.C.A.F. was an employee of Myovant Sciences, Inc. (now Sumitomo Pharma America, Inc.) at the time the study was conducted. E.M.M. was an employee of Myovant Sciences, Inc. (now Sumitomo Pharma America, Inc.) at the time the study was conducted.

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