Supporting Information

Population PK and Semi-Mechanistic PK/PD Modeling and Simulation of Relugolix Effects on Testosterone Suppression in Men with Prostate Cancer

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Supplementary Material

Overview of the five clinical studies included in the PopPK and PopPK/PD analyses				
Study (region, year completed)	**Design**	**Population**	**Drug, dose, and duration**	**Number of subjects/ patients enrolled**
Phase I				
C27001 (EudraCT Number 2011-002868-24) (UK, 2013)	Four-part, randomized, double-blind, placebo-controlled study	Healthy adult men	Part 1 (single dose): relugolix 80, 120, 180, 360 mg, or placebo* Part 2 (QD for 14 days): relugolix 80 mg QD; 180 mg QD; 360 mg on Day 1 then 40 mg QD; 320 mg on Day 1, 240 mg on Day 2, 160 mg on Day 3, then 20 mg QD; 320 mg on Day 1 and 160 mg on Day 2 then 20 mg QD; placebo* Part 3 (QD for 28 days): relugolix 320 mg on Day 1 and 160 mg on Day 2 then 40 mg QD; relugolix 160 mg QD with no loading regimen; placebo Part 4 (QD for 28 days): relugolix 60 mg QD, 80 mg QD, or placebo	Relugolix: 128 Placebo: 48*
TB-AK160108 (NCT02141659) (Japan, 2017)	Two-part, open-label, dose-range finding study	Hormone treatment-naïve adult men with nonmetastatic prostate cancer	Part A Cohort 1: relugolix 320 mg on Day 1 then 80 mg QD on Day 2 to 28 Cohort 2: relugolix 320 mg on Day 1 then 120 mg QD on Day 2 to 28 Cohort 3: relugolix 320 mg on Day 1 then 160 mg QD on Day 2 to 28 Cohort 4: relugolix 360 mg on Day 1 then 120 mg QD on Day 2 to 28 Part B 80 mg: relugolix 320 mg on Day 1 then 80 mg QD from Day 2 for 48 to 96 weeks 120 mg: relugolix 320 mg on Day 1 then 120 mg QD from Day 2 for 48 to 96 weeks	Relugolix: 43

Phase II				
C27002 (NCT02083185) (North America, 2017)	Three-arm, randomized, open-label, parallel-group, dose-finding study	Adult men with advanced prostate cancer requiring first-line ADT	Arm 1: relugolix 320 mg on Day 1 then 80 mg QD from Day 2 for 48 to 96 weeks Arm 2: relugolix 320 mg on Day 1 then 120 mg QD from Day 2 for 48 to 96 weeks Arm 3: leuprolide 22.5 mg subcutaneous depot injection every 12 weeks for 48 weeks*	Relugolix: 110 Leuprolide: 24*
C27003 (NCT02135445) (United States and UK, 2016)	Two-arm, randomized, open-label, parallel-group study	Adult men with localized prostate cancer requiring neoadjuvant/ adjuvant ADT with EBRT	Arm 1: relugolix 320 mg on Day 1 then 120 mg QD from Day 2 for 24 weeks Arm 2: two degarelix 120 mg subcutaneous depot injections on Day 1 then degarelix 80 mg subcutaneous injection every 4 weeks from Week 5 for 24 weeks*	Relugolix: 65 Degarelix: 38*
Phase III				
HERO (NCT03085095) (Multinational, primary analysis completed in 2020)	Randomized, open-label, parallel-group study	Adult men with advanced prostate cancer	Relugolix: 360 mg on Day 1 then 120 mg QD from Day 2 for 48 weeks Leuprolide: 22.5 mg (or 11.25 mg in Japan, Taiwan, and China) every 3 months by subcutaneous depot injection for 48 weeks*	Primary analysis: Relugolix: 624 Leuprolide: 310*

ADT, androgen deprivation therapy; EBRT, external beam radiation therapy; PD, pharmacodynamic; PopPK, population pharmacokinetic; QD, once daily.

*, Excluded from the PopPK or PopPK/PD analyses because subjects/patients were not treated in a representative food regimen (C27001, Part 1), treated in an inpatient setting (C27001, Part 2), or not treated with relugolix.

Additional information on the five clinical studies included in the PopPK and PopPK/PD analyses *(continued)*

Bioanalytical assays for determination of relugolix in human plasma

All bioanalytical methods used to determine relugolix concentrations in human plasma from the five clinical studies included in the PopPK and PopPK/PD analyses (C27001, TB-AK160108, C27002, C27003, and HERO) were based on high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Four bioanalytical laboratories, Bioanalytical Systems, Inc. (BASi; West Lafayette, IN, USA), Sumika Chemical Analysis Services, Ltd. (Osaka, Japan), QPS (Newark, DE, USA), and Labcorp Pharmaceutical R&D (Shanghai) Co., Ltd (Shanghai, China) validated the bioanalytical methods and assayed samples from the five clinical studies. Bioanalytical methods were also cross validated between Bioanalytical Systems, Inc. and Sumika Chemical Analysis Services, Ltd., and between QPS and Labcorp Pharmaceutical R&D (Shanghai) Co, Ltd. A summary of performance of the validated bioanalytical methods are provided in the table below.

Clinical Study	Bioanalytical Laboratory	LLOQ	Range	Accuracy (%RE)[a]	Precision (%CV)[a]
C27001	BASi	0.01 ng/mL	0.01 – 50 ng/mL	2.0%	6.0%
C27002		0.05 ng/mL	0.05 – 50 ng/mL	2.0%	6.0%
C27003					
TB-AK160108	Sumika	0.01 ng/mL	0.01 – 50 ng/mL	5.3%	9.6%
HERO	QPS	0.05 ng/mL	0.05 – 50 ng/mL	3.2%	8.3%
	Labcorp Shanghai	0.05 ng/mL	0.05 – 50 ng/mL	0.8%	8.8%

[a] Inter-run accuracy (presented as % relative error [RE]) and precision (presented as % coefficient of variation [CV]) at LLOQ.

C27001

C27001 is a randomized, double-blind, parallel-group, placebo-controlled, four-part study to assess the safety and tolerability, pharmacokinetics, and pharmacodynamics of relugolix. Part 1 evaluated escalating single doses (fasted); in each of the four cohorts, participants were randomized 6:2 to relugolix:placebo. Part 1 included a second (fed) period for Cohort 3; participants received the same treatment (relugolix or placebo) as they did in Period 1. Part 2 evaluated escalating repeat doses for 14 days; in each of five cohorts, participants were randomized 6:2 to relugolix:placebo. Part 3 evaluated two doses of relugolix for 28 days; participants were randomized 1:1:1 to each dose of relugolix or placebo. Part 4 evaluated two doses of relugolix for 28 days; in each cohort, participants were randomized 14:4 to relugolix:placebo. Part 1 enrolled healthy adult male subjects, 18–50 years of age inclusive. Part 2 enrolled healthy adult male subjects, 40–70 years of age inclusive. Parts 3 and 4 enrolled healthy adult male subjects, 45–75 years of age inclusive.

In Part 1 (single dose), blood and urine samples for the determination of relugolix plasma and urine concentrations, and blood samples for determination of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and dihydrotestosterone (DHT) serum

concentrations were collected predose and at prespecified time points up to 48 hours postdose.

In Part 2 (14-day dosing), blood and urine samples for the determination of relugolix plasma and urine concentrations and blood samples for determination of testosterone, LH, FSH, and DHT serum concentrations were collected predose and at prespecified time points up to 24 hours postdose (Day 1) and predose, and at prespecified time points up to 48 hours postdose (urine collected up to 24 hours postdose) (Day 14). Blood samples for determination of relugolix plasma concentrations and testosterone, LH, FSH, and DHT serum concentrations were collected predose on certain days between Day 3 and Day 13.

In Parts 3 and 4 (28-day dosing), Day 1 and Day 28, blood samples for the determination of relugolix plasma concentrations and determination of testosterone and LH serum concentrations were collected predose and at prespecified time points up to 24 hours postdose; additional samples were collected 672 hours after the last dose on Day 28 and predose on Days 7, 14, and 21. Blood samples for determination of FSH, DHT, and insulin-like growth factor 1 serum concentrations were collected predose on Days 1, 14, and 28.

TB-AK160108 (NCT02141659)

TB-AK160108 is a completed, multicenter, phase I, open-label, dose-range-finding study that evaluated the tolerability, safety, pharmacokinetics, and pharmacodynamics of relugolix in 43 hormone treatment-naïve men with nonmetastatic prostate cancer. Criteria for inclusion and exclusion are listed below.

Inclusion criteria:

1. Participants judged by the investigator to have the capacity to understand the study and follow the study rules.
2. Participants whose written consent (signature or printed name and personal seal on informed consent form) can be obtained before any study procedures are performed.
3. Japanese male participants ≥20 years of age at the time of informed consent.
4. Participants who, if they have a female partner who could become pregnant, agree to practice appropriate means of contraception from the time of informed consent throughout the entire study treatment period and for 4 months after the last dose of study drug.
5. Participants in stable medical condition, including the absence of acute exacerbations of chronic illnesses, serious infections, or major surgery within 4 weeks (28 days) prior to study treatment initiation.
6. Participants with histologically or cytologically confirmed prostate cancer.
7. Participants whose clinical diagnosis is T1–4 N0 M0, or Tx N0 M0 for participants who have undergone radical prostatectomy.
8. Participants who are considered eligible for hormone therapy for prostate cancer.

9. Participants who have not received hormone therapy (e.g., gonadotropin-releasing hormone [GnRH] receptor agonist, GnRH receptor antagonist, steroidal antiandrogen, nonsteroidal androgen) for prostate cancer.
10. Participants who have not undergone surgical castration.
11. Participants with serum testosterone at screening >150 ng/dL.
12. Participants meeting either of the following criteria for prostate-specific antigen (PSA) at screening. Untreated prostate cancer: PSA at screening >4.0 ng/mL. Treated prostate cancer: PSA at screening >0.2 ng/mL.
    - Treated participants were defined as who have undergone prostatectomy or either or both of high intensity focused ultrasound therapy or radiotherapy (excluding 125I-brachytherapy) prior to the start of this study.
13. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1.
14. Body mass index (BMI) at screening ≥18.0 kg/m^2.

Exclusion criteria:

1. Participants exhibiting symptoms judged related to prostate cancer by the investigator (e.g., bone pain, pelvic pain, ureteral obstruction) who urgently require hormone therapy such as GnRH receptor agonist, GnRH receptor antagonist, or complete androgen blockade (CAB)/maximal androgen blockade (MAB) therapy, chemotherapy, or radiotherapy.
2. Participants who have received 5-alpha reductase inhibitors (except for participants who have been treated for male-pattern alopecia).
3. Participants who have received chemotherapy for prostate cancer (including estramustine).
4. Participants who have received 125I-brachytherapy.
5. Participants who received radiotherapy (except for 125I-brachytherapy) within 16 weeks (112 days) before study treatment initiation.
6. Participants who underwent prostatectomy within 16 weeks (112 days) before study treatment initiation.
7. Treatment with any investigational compound within the 4 weeks (28 days) prior to the first dose of study drug or ongoing participation in another experimental trial related to the treatment of prostate cancer.
8. Participants with diagnosis of or treatment for another systemic malignancy within 2 years before study treatment initiation, or who had received a diagnosis of another malignancy before that and have evidence of residual disease. Participants with nonmelanoma skin cancer or carcinoma in situ who have undergone complete resection will not be excluded from the study.
9. Participants taking drugs with moderate to strong cytochrome P450 3A4 (CYP3A4) inhibitory or inducing effects, or any medications, supplements, or food products with

P-glycoprotein (P-gp) inhibitory effects, in the 2 weeks (14 days) prior to study treatment initiation.

10. Participants who have received TAK-385 in a past clinical study.
11. Participants for whom it would be difficult to collect blood from a peripheral vein.
12. Participants with uncontrolled and clinically significant nervous, circulatory, pulmonary, hepatic, renal, metabolic, gastrointestinal, urogenital, or endocrine disorders, or other abnormalities (except for the targeted disease) that could affect study participation or the study results. Also, participants meeting any of the criteria (A through C) below.

    A. Participants with uncontrolled diabetes (hemoglobin A1c [HbA1C] >8% at screening). However, participants whose HbA1c is brought under control with diabetes medications may be rescreened.

    B. Participants with uncontrolled hypertension (systolic blood pressure >150 mmHg and diastolic blood pressure >90 mmHg at two separate measurements taken no more than 60 minutes apart at screening). Participants whose blood pressure is brought under control by antihypertensive medication may be rescreened.

    C. Participants with myocardial infarction, unstable symptomatic ischemic heart disease, arrhythmias of common terminology criteria for adverse events (CTCAE) Grade >2, thromboembolism (deep vein thrombosis, pulmonary embolism, or symptomatic cerebrovascular events), or other heart diseases (e.g., pericardial effusion, restrictive cardiomyopathy). However, chronic stable atrial fibrillation controlled by stable anticoagulant therapy will be allowed.
13. Participants with bilateral hydronephrosis or bladder neck outlet obstruction.
14. Known hypersensitivity to TAK-385, TAK-385 excipients, or GnRH receptor antagonists.
15. Participants with a past history of gastrointestinal tract treatments (including gastrectomy) or gastrointestinal disease that could affect the drug absorption or tolerability (malabsorption, esophageal reflux, peptic ulcer, erosive esophagitis).
16. Participants positive for hepatitis B surface antigens (HBsAg), hepatitis C antibodies (HCV), human immunodeficiency virus (HIV) antibodies, or serologic test for syphilis, or with life-threatening disease other than cancer, at screening.
17. Clinically relevant electrocardiogram (ECG) abnormalities, or the following ECG abnormalities, at screening.
    - Q-wave infarction, unless identified ≥6 months prior to TAK-385 treatment initiation.
    - QTcF interval >450 msec (when calculating the QTc interval, Fridericia's equation [QT/RR0.33] will be used).
18. Participants with congenital QT prolongation.
19. Current use of Class 1A or Class 3 antiarrhythmic medications.
20. New York Heart Association Class III or IV heart failure.
21. Participants with clinical laboratory abnormalities suggesting clinically relevant underlying disease, or with any of the following abnormal results, at screening.

- Serum creatinine ≥2.0 mg/dL.
- Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥1.5*upper limit of normal (ULN) for the study site.
- Total bilirubin ≥2*ULN for the study site.
- Neutrophil count <1500/mm^3, platelet count <100,000 per microliter, hemoglobin <10.0 g/dL.
- Results of heart-related tests (creatine kinase MB [CK-MB] and cardiac troponin T) exceeding the study sites reference value.

22. Participants found to have clinical problems on the basis of examination findings, ECG findings, or chest X-ray findings at screening.
23. Participants considered unlikely by investigators to be able to follow the study protocol or considered ineligible for the study by investigators for other reasons.

The study consisted of a dose-rising phase (Part A) and a 96-week expansion phase (Part B). In Part A (*N* = 13), a loading dose of relugolix (320 or 360 mg) was administered on Day 1 followed by once daily oral dosing on Days 2 through 28, with the dosage dependent on tolerability in each individual Cohort of three to four patients. In Part B, 30 patients received a maximum of 96 weeks of treatment at doses of 80 or 120 mg once daily (*N* = 15 each group), with a loading dose of 320 mg on Day 1.

The primary objectives of the study were to evaluate the safety and tolerability of relugolix in hormone treatment-naïve patients with nonmetastatic prostate cancer. The secondary objectives were to evaluate relugolix pharmacokinetics, its effects on serum testosterone, and the change over time in PSA levels in hormone treatment-naïve patients with nonmetastatic prostate cancer.

Blood samples for determination of plasma relugolix trough plasma concentrations were collected predose and at various time points up to 24 hours postdose on Day 1 and Day 14 (8 time points) and Day 28 (5 time points) and predose on various days from Day 2 to Day 35 in Part A of the study, and predose for specified weeks from Week 1 to Week 49 and at 2 hours postdose on Day 1 of Week 1 and Week 5 in Part B of the study. In Part A, the pharmacokinetics of relugolix, such as maximum observed concentration [C_{max}] and area under the plasma concentration-time curve [AUC]), were calculated by noncompartment analysis of the plasma concentration profiles of relugolix in the pharmacokinetic analysis set. In Part B, summary statistics of relugolix concentrations by time point were calculated.

Blood samples for determination of LH, FSH, testosterone, DHT, and sex hormone binding globulin (SHBG) serum concentrations were measured at baseline and predose on specified days (Part A) and specified weeks (Part B) throughout the study. Hormone and SHBG concentrations were measured by an external laboratory in accordance with specific procedures.

C27002 (NCT02083185)

C27002 was a completed phase II dose-finding study in men with androgen-sensitive advanced prostate cancer. This open-label, parallel-group study enrolled men with androgen-sensitive advanced prostate cancer, including patients with PSA biochemical relapse following primary surgical treatment or radiotherapy, newly diagnosed metastatic prostate cancer, or advanced localized disease for which immediate primary surgical treatment or radiotherapy was unlikely to be curative. Inclusion and exclusion criteria are listed below.

Inclusion criteria:

1. Male participant 18 years or older.
2. Histologically or cytologically confirmed diagnosis of prostate adenocarcinoma.
3. Candidate for androgen deprivation therapy (ADT) for the management of hormone-sensitive prostate cancer with 1 of the following clinical disease states: 1) advanced localized disease not suitable for primary therapy, 2) evidence of prostate-specific antigen (PSA) biochemical or clinical relapse following primary surgery or radiation therapy of curative intent, or 3) newly diagnosed metastatic disease that is asymptomatic or not threatening to vital organs.
4. Appropriate serum testosterone and serum PSA concentration at screening as specified in the protocol.
5. A body mass index (BMI) ≥18.0 at screening and/or baseline.
6. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1 at screening and/or baseline.
7. Male participants, even if surgically sterilized, who agree to practice effective barrier contraception or agree to practice true abstinence.
8. Voluntary written consent must be given before performance of any study-related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the participant at any time without prejudice to future medical care.
9. Suitable venous access for the study-required blood sampling, including pharmacokinetic (PK) and pharmacodynamic (PD) sampling.

Exclusion criteria:

1. In participants with advanced, localized M0N1 or M1 disease, the presence of clinically significant symptoms or threat to vital organs requiring immediate gonadotropin-releasing hormone (GnRH)/combined or complete androgen blockade (CAB) therapy, chemotherapy, or radiotherapy.
2. Previously received androgen deprivation therapy (ADT) for >8 months total duration (if ADT was received for ≤8 months, then that ADT must have been completed ≥2 years prior to screening).
3. Visceral metastases (liver or lung).
4. Features of the participant's medical condition that may make ADT unnecessary or not indicated.

5. Scheduled for additional surgical or (salvage) radiation therapy within 6 months after baseline evaluations.
6. History of surgical castration.
7. Diagnosis of or treatment for another malignancy within the 2 years before the first dose of study drug, or previously diagnosed with another malignancy and have any evidence of residual disease. Participants with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection.
8. Abnormal screening and/or baseline laboratory values as specified in the protocol.
9. History of any significant cardiac condition within 6 months before receiving the first dose of study drug.
10. Electrocardiogram (ECG) abnormalities as specified in the protocol.
11. Congenital long QT syndrome.
12. Current use of Class IA (e.g., quinidine, procainamide) or Class III (e.g., amiodarone, sotalol) antiarrhythmic medications.
13. Uncontrolled hypertension despite appropriate medical therapy. Participants may be re-screened after referral and further management of hypertension.
14. Known, previously diagnosed human immunodeficiency virus (HIV) infection, active chronic hepatitis B or C, life-threatening illness unrelated to prostate cancer, or any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with participation in the study. Specific screening for chronic viral illness is at the discretion of the site and/or local institutional review board (IRB).
15. Treatment with any investigational products within 3 months before the first dose of study drug.
16. A primary family member (spouse, parent, child, or sibling of the participant) is involved in the conduct of the study or is a study-site employee.
17. Known gastrointestinal (GI) disease or GI procedure that could interfere with the oral absorption or tolerance of TAK-385, including difficulty swallowing tablets.
18. Use of any medication or food products listed in the excluded medications and dietary products table within 2 weeks before the first dose of study drug. Participant must have no history of amiodarone use in the 6 months before the first dose of TAK-385.
19. Admission or evidence of alcohol or drug abuse or use of illicit drugs.

A total of 134 patients were enrolled into one of three groups to receive oral relugolix for up to 48 weeks at doses of 80 mg (*N* = 56) or 120 mg (*N* = 54) orally once daily (after a single oral loading dose of 320 mg), or into a reference control group to receive GnRH receptor agonist therapy (leuprorelin [i.e., leuprolide], 22.5 mg subcutaneous every 12 weeks, *N* = 24). An extension to the core 48-week study was completed by 35 patients, which provided an additional 48 weeks of treatment (total of 96 weeks) for patients in the relugolix groups, continuing their previously assigned doses (20 patients from the 80-mg group and 15 patients from the 120-mg group).

The study's primary endpoint was the sustained castration (<50 ng/dL) rate through Week 25 Day 1 (Day 169). No formal statistical differences were sought or hypothesized either between the two relugolix dosing arms or between relugolix and leuprorelin. Secondary endpoints were testosterone and PSA kinetics, quality of life, safety, pharmacokinetics, and pharmacodynamics.

Blood samples for determination of relugolix trough plasma concentrations were collected at various time points throughout the study (Week 1 at predose on Day 1 and Day 4, Week 5 and Week 13 at predose and 2 [± 30 min] hours postdose on Day 1, during visits from Week 2 to Week 49 at predose on Day 1) and analyzed using validated bioanalytical methods.

Blood samples for determination of LH, FSH, testosterone, and SHBG serum concentrations were collected at baseline and at various predose time points throughout the study (LH and testosterone: Week 1 Day 4, during subsequent visits from Week 2 to Week 49, at the end of treatment, follow-up, and end of study visits; FSH and SHBG: during visits from Weeks 1 (if screening visit was >3 days from the Week 1 visit), 2, 5, 13, 25, 37, and 49, and at the end-of-study visit. For screening, testosterone concentrations were assayed using a conventional immunoassay. A validated and sensitive liquid chromatography mass spectrometry method (lower limit of quantification of 5 ng/dL) performed at a central facility (LabCorp) was used for all subsequent scheduled measurements. Testosterone concentrations determined at local laboratories could be used for clinical decision-making.

C27003 (NCT02135445)

C27003 was a completed phase II, two-arm, randomized, active-control, open-label, parallel-group study that enrolled patients with a histologically confirmed diagnosis of localized prostate cancer of intermediate prognostic risk for whom 6 months of ADT was indicated as neoadjuvant or adjuvant ADT, with EBRT administered after 12–14 weeks of ADT. Inclusion and exclusion criteria are listed below.

Inclusion criteria:

1. Is male, 18 years of age or older.
2. Has histologically confirmed diagnosis of localized prostate adenocarcinoma of intermediate risk for which 6-month neoadjuvant and adjuvant androgen deprivation therapy (ADT) to EBRT is indicated. Intermediate risk per National Comprehensive Cancer Network (NCCN) guidelines includes one of the following:
    - T2b–T2c disease, or
    - Gleason score 7, or
    - Prostate-specific antigen (PSA) 10–20 ng/mL.
3. Is scheduled for EBRT to begin ≥12 weeks after the baseline visit.
4. Has serum testosterone at screening >150 ng/dL (5.2 nmol/L).
5. Has screening serum PSA concentration >2 ng/mL.
6. Has body mass index (BMI) ≥18.0 at screening or baseline.

7. Has Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1 at screening or baseline.
8. Is a male participant, even if surgically sterilized (that is, status postvasectomy), who agrees to practice effective barrier contraception during the entire study treatment period and through 4 months after the last dose of study drug, or agrees to practice true abstinence, when this is in line with the preferred and usual lifestyle of the participant. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal are not acceptable methods of contraception.)
9. Has given voluntary written consent before performance of any study-related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the participant at any time without prejudice to future medical care.
10. Has suitable venous access for the study-required blood sampling, including pharmacokinetic (PK) and pharmacodynamic sampling.

Exclusion criteria:

1. Has metastatic disease (based on investigator evaluation and assuming no likely metastatic pelvic lymph nodes >1.0 cm in long axis diameter).
2. Had prior or current use of a gonadotropin-releasing hormone (GnRH) analog or androgen receptor antagonist as first-line hormone therapy, unless total use was <6 months and not more recently than 1 year before the planned baseline visit.
3. Had diagnosis of or treatment for another malignancy within 2 years before the first dose of study drug, or previous diagnosis of another malignancy with evidence of residual disease. Participants with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection.
4. Has abnormal screening and/or baseline laboratory values that suggest a clinically significant underlying disease, or the following laboratory values:
    a. Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) >1.5 * institutional upper limit of the normal range (ULN);
    b. Serum creatinine >2.0 mg/dL;
    c. Total bilirubin >2.0 * institutional ULN (unless documented Gilbert's disease);
    d. Uncontrolled diabetes (hemoglobin A1c [HbA1c] >10%) or previously undiagnosed diabetes mellitus with HbA1c >8%.
5. Has history of myocardial infarction, unstable symptomatic ischemic heart disease, any ongoing cardiac arrhythmias of Grade >2 (chronic stable atrial fibrillation on stable anticoagulant therapy is allowed), thromboembolic events (e.g., deep vein thrombosis, pulmonary embolism, or symptomatic cerebrovascular events), or any other significant cardiac condition (e.g., pericardial effusion, restrictive cardiomyopathy) within 6 months before receiving the first dose of study drug.
6. Has electrocardiogram (ECG) abnormalities of:
    a. Q-wave infarction, unless identified ≥6 months before screening;

b. Heart rate-corrected QT interval millisecond (msec) (QTcF interval) >480 msec. If QTcF is prolonged in a participant with a pacemaker, the participant may be enrolled in the study upon discussion with the project clinician;
c. If the QTcF interval is 450–480 msec inclusive, in a participant with current use of medications with known effects on QT interval, the participant may be enrolled in the study following discussion with the project clinician.

7. Has congenital long QT syndrome.
8. Is currently using Class IA (e.g., quinidine, procainamide) or Class III (e.g., amiodarone, sotalol) antiarrhythmic medications.
9. Has uncontrolled hypertension despite appropriate medical therapy (sitting blood pressure [BP] of >160 mmHg systolic and 90 mmHg diastolic at two separate measurements no more than 60 minutes apart during the screening visit). Participants with systolic BP measurements >160 mmHg may be rescreened. Participants with systolic BP measurements 141–160 mmHg, although eligible, should be referred for further management of hypertension if indicated.
10. Has known, previously diagnosed human immunodeficiency virus (HIV) infection, active chronic hepatitis B or C, life-threatening illness unrelated to prostate cancer, or any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with participation in the study. Specific screening for chronic viral illness is at the discretion of the site and/or local institutional review board (IRB).
11. Has received treatment with any investigational products within 3 months before the first dose of study drug.
12. Is a primary family member (spouse, parent, child, or sibling) of anyone involved in the conduct of the study or is a study-site employee.
13. Has known gastrointestinal (GI) disease, condition or procedure that could interfere with the oral absorption or tolerance of TAK-385, including difficulty swallowing tablets.
14. Is using any medication or food products listed in the excluded medications and dietary products table within 2 weeks before the first dose of study drug. This list includes moderate and strong inhibitors or inducers of cytochrome P450 (CYP3A4/5) and P-glycoprotein (P-gp). Participants must have no history of amiodarone use in the 6 months before the first dose of TAK-385.
15. Has admission or evidence of alcohol or drug abuse or use of illicit drugs.

Patients were randomized to relugolix 120 mg once daily (after a single oral loading dose of 320 mg) (*N* = 65) or to degarelix 80 mg subcutaneously every 4 weeks for 24 weeks (after a single loading dose of 240 mg) (*N* = 38). The degarelix group was included to provide a reference control of a depot GnRH peptide receptor antagonist with a mechanism of action similar to that of relugolix.

The primary endpoint was the rate of effective castration between Week 5 Day 1 (Day 29) to Week 25 Day 1 (Day 169) as determined by the estimated proportion of patients who had testosterone concentrations <50 ng/dL at all scheduled visits. Secondary endpoints were

testosterone and PSA kinetics, changes in prostate gland size, quality of life, safety, pharmacokinetics, and pharmacodynamics.

Blood samples for determination of plasma relugolix concentrations were collected at various time points throughout the study (Week 1, Day 1 and Day 4 at predose, Week 5 and Week 13 at predose and 2 [± 30 min] hours postdose on Day 1, during visits from Week 2 to Week 37 at predose on Day 1) and analyzed using validated bioanalytical methods.

Blood samples for determination of LH, FSH, testosterone, and SHBG serum concentrations were collected at baseline and at various predose time points throughout the study (LH and testosterone: Week 1, Day 1, Day 2 and Day 4, during subsequent visits from Week 2 to Week 25, at the end of treatment, follow-up and end of study visits; FSH and SHBG: Day 1 of Weeks 1, 2, 5, 13, and 25 and at the end-of-treatment visit).

HERO (NCT03085095)
HERO was a phase III, multinational, randomized, open-label, parallel-group study designed to evaluate the safety and efficacy of relugolix in men with androgen-sensitive advanced prostate cancer who required ≥1 year of continuous ADT. Key inclusion and exclusion criteria are listed below.

Key inclusion criteria:

1. Has histologically or cytologically confirmed diagnosis of adenocarcinoma of the prostate.
2. Is a candidate for, in the opinion of the investigator, ≥1 year of continuous androgen deprivation therapy for the management of androgen-sensitive advanced prostate cancer with 1 of the following clinical disease state presentations:
    a. Evidence of biochemical (PSA) or clinical relapse following local primary intervention with curative intent, such as surgery, radiation therapy, cryotherapy, or high-frequency ultrasound and not a candidate for salvage treatment by surgery; or
    b. Newly diagnosed androgen-sensitive metastatic disease; or
    c. Advanced localized disease unlikely to be cured by local primary intervention with either surgery or radiation with curative intent.
3. Has a serum testosterone at the screening visit of ≥150 ng/dL (5.2 nmol/L).
4. Has a serum PSA concentration at the screening visit of >2.0 ng/mL (2.0 µg/L), or, when applicable, post radical prostatectomy of >0.2 ng/mL (0.2 µg/L) or post radiotherapy, cryotherapy, or high frequency ultrasound >2.0 ng/mL (2.0 µg/L) above the post interventional nadir.
5. Has an Eastern Cooperative Oncology Group Performance Status of 0 or 1 at initial screening and at baseline.

Key exclusion criteria:

1. In the investigator's opinion, is likely to require chemotherapy or surgical therapy for symptomatic disease management within 2 months of initiating androgen deprivation therapy.
2. Previously received gonadotropin-releasing hormone analog or other form of androgen deprivation therapy (estrogen or antiandrogen) for >18 months' total duration. If androgen deprivation therapy was received for ≤18 months' total duration, then that therapy must have been completed ≥3 months prior to baseline. If the dosing interval of the depot is >3 months, then the prior androgen deprivation therapy must have been completed at least as long as the dosing interval of the depot.
3. Previous systemic cytotoxic treatment for prostate cancer (e.g., taxane-based regimen).
4. Metastases to brain per prior clinical evaluation.
5. Participants with myocardial infarction, unstable symptomatic ischemic heart disease, cerebrovascular events, or any significant cardiac condition within the prior 6 months.
6. Active conduction system abnormalities.
7. Uncontrolled hypertension.

A total of 1078 patients were randomized at 160 centers globally, including Europe, North and South America, Asia, and Rest of World. The study included patients with PSA biochemical relapse following primary surgical treatment or radiotherapy with curative intent, newly diagnosed metastatic prostate cancer, or advanced localized disease for which immediate primary surgical treatment or radiotherapy was unlikely to be curative. Patients with metastatic disease could have been categorized as either 'newly diagnosed metastatic prostate cancer' or 'PSA biochemical relapse following primary surgical treatment or radiotherapy with curative intent'.

To support the primary analysis, a total of 934 patients were randomized (2:1) to relugolix 120 mg (*N* = 624) orally once daily following a single oral loading dose of relugolix 360 mg, or to leuprolide 3-monthly depot injections (*N* = 310). To support the final analysis, an additional 144 patients (139 of whom had metastatic disease) were randomized to the study, resulting in a total of 719 patients in the relugolix group and 359 patients in the leuprolide group. The primary endpoint was the sustained castration rate (<50 ng/dL) from Week 5 Day 1 (Day 29) through Week 49 Day 1 (Day 337). Key secondary efficacy endpoints included assessments of testosterone, PSA concentrations, FSH concentrations at Week 25 Day 1 (Day 169), clinical recurrence-free survival (CRFS), and testosterone recovery. Further endpoints included quality of life, pharmacokinetics, pharmacodynamics, and safety. Safety assessments included adverse events and physical examinations including visual acuity, vital signs, clinical laboratory tests, and 12-lead electrocardiogram (ECG).

Blood samples for determination of plasma relugolix trough concentrations were collected at various time points in the relugolix group and analyzed using validated bioanalytical methods. Blood samples for determination of FSH, LH, DHT, and sex hormone binding globulin (SHBG) serum concentrations in the relugolix and leuprolide groups were collected at baseline and at

various time points throughout the study. Serum samples were analyzed using standard clinical assays.

Table S1 Parameter estimates of the final PopPK model and the sensitivity analysis

	Final model				**Sensitivity Analysis***	
Parameter (unit)	**Estimate**	**RSE (%)****	**95% CI****	**Shrinkage (%)**	**Estimate**	**RSE (%)****
F1 (-)	1.000	-	-	-	1.000	-
Ka (1/h)	3.326	-	-	-	3.326	-
CL/F (L/h)	468	1.87	451, 486	-	464	1.53
Vc/F (L)	7053	6.58	6240, 8120	-	6900	2.25
Vp1/F (L)	11730	-	-	-	11730	-
Vp2/F (L)	14550	-	-	-	14550	-
Q1/F (L/h)	16.49	-	-	-	16.49	-
Q2/F (L/h)	584.9	-	-	-	584.9	-
Lag time (h)	0.273	-	-	-	0.273	-
Dose ~ F1 (-)***	0.389	3.6	0.361, 0.416	-	0.358	1.73
Age at baseline on CL/F (-)***	-0.419	22.9	-0.628, -0.234	-	-0.381	25.7
Black/African American on CL/F (-)***	-0.284	17.3	-0.379, -0.186	-	-0.269	18.1
Weight at baseline on CL/F (-)***	0.322	19.5	0.197, 0.453	-	0.75	-
Black/African American on Vc/F (-)***	-0.681	12.8	-0.821, -0.476	-	-0.669	12.3
Inter-individual variability						
IIV Ka (CV%)	159.9	15.1	121, 211	70.4	159.7	20.3
IIV CL/F (CV%)	46.93	2.85	44.5, 49.7	7.52	47.33	5.3
Correlation CL/F ~ Vc/F	70.19	-	-	-	70.19	-
IIV Vc/F (CV%)	122.9	4.64	112, 134	32.9	132.2	8.4
Residual unexplained variability						

	Final model				Sensitivity Analysis*	
Parameter (unit)	**Estimate**	**RSE (%)****	**95% CI****	**Shrinkage (%)**	**Estimate**	**RSE (%)****
Additive error on log scale (-)	0.404	0.681	0.399, 0.409	5.57	0.404	0.267

AGE, age in years; CI, confidence interval; CL/F, apparent systemic clearance after oral administration in litre per hour (L/h); CV, coefficient of variation; η, individual random effect; F1, relative bioavailability; IIV, inter-individual variability; Ka, absorption rate constant; PopPK, population pharmacokinetic; Q1/F, apparent intercompartmental clearance with first peripheral compartment; Q2/F, apparent intercompartmental clearance with second peripheral compartment; RSE, relative standard error; Vc/F, apparent volume of distribution in the central compartment in litre (L); Vp1/F, apparent volume of distribution in the first peripheral compartment; Vp2/F, apparent volume of distribution in the second peripheral compartment; WT, body weight in kg.
*, Sensitivity analysis reflects a fit of the model with fixed allometric exponents of 0.75 and 1 on clearance and volume parameters, respectively.
**, Relative standard errors and 95% confidence intervals were computed from the sampling importance resampling process. The relative standard errors for omega and sigma are reported on the approximate standard deviation scale (SE/variance estimate)/2.
***, Covariate effects were incorporated as shown in equations below. The reference individual across the five clinical studies for development of the PopPK model is a non-Black/African American male with median body weight of 80.6 kg and median age of 71 years.

$$\frac{CL}{F}\ (\mathrm{L/h}) = 467.6 \cdot \left({WT}/{80.6}\right)^{0.322} \cdot \left({AGE}/{71}\right)^{-0.419} \cdot (1 - 0.284 \cdot Black) \cdot e^{\eta CL/F}$$

$$\frac{Vc}{F}\ (\mathrm{L}) = 7053 \cdot (1 - 0.681 \cdot Black) \cdot e^{\eta Vc}$$

$$F1{=}1 \cdot \left({Dose}/{80}\right)^{0.389}$$

Table S2 Parameter estimates of the final PopPK/PD model

Parameter	Estimate	RSE (%)*	95% CI*	Shrinkage (%)
Population parameter				
KinT (pg/mL/h)	640	4.03	592, 691	-
KD (pg/mL)	0.209	4.66	0.192, 0.23	-
KouT (1/h)	0.0915	3.37	0.0858, 0.0979	-
DR_{50} (unitless)	0.313	0.462	0.31, 0.316	-
KouR (1/h)	0.0118	1.68	0.0114, 0.0122	-
BLA (unitless)	1	-	-	-
nH (unitless)	7.96	1.26	7.76, 8.16	-
HILFD (unitless)	1	-	-	-
Age~KinT**	-0.662	17.6	-0.89, -0.442	-
Inter-individual variability				
ETA KinT (CV%)	45.7	2.63	43.5, 48.3	2.8
Residual variability				
Additive error on log scale	0.42	0.601	0.415, 0.425	3.1

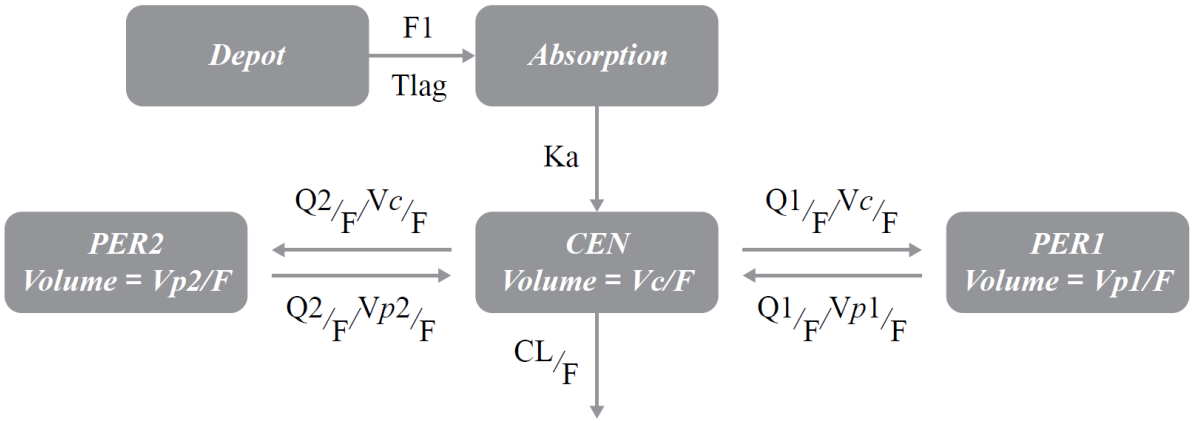
AGE, age in years; BLA, baseline value for the endogenous agonist (GnRH); CI, confidence interval; CV, coefficient of variation; DR, receptor downregulation; DR_{50}, level of receptor activation at which receptor downregulation is at 50% of maximum; ETA, individual random effect; GnRH, gonadotropin-releasing hormone (endogenous agonist); HILFD, Hill coefficient of the testosterone-feedback on GnRH production; KD, receptor equilibrium dissociation constant; KinT, zero order testosterone production rate constant; KouR, GnRH receptor degradation rate constant; KouT, testosterone degradation rate constant; nH, Hill coefficient of the down regulation of GnRH receptor production; PD, pharmacodynamic; PopPK, population pharmacokinetic; RSE, relative standard error; TVKinT, value of KinT for a typical individual with an age of 71 years (median in the observed data from the five clinical studies for development of the PopPK/PD model).

*, Relative standard errors and 95% confidence intervals were computed from the sampling importance resampling process. The relative standard errors for omega and sigma are reported on the approximate standard deviation scale (SE/variance estimate)/2.

**, The statistically significant effect of age on KinT denoted a decrease in testosterone production with increasing age (exponent estimated at -0.662) as shown in the equation below:

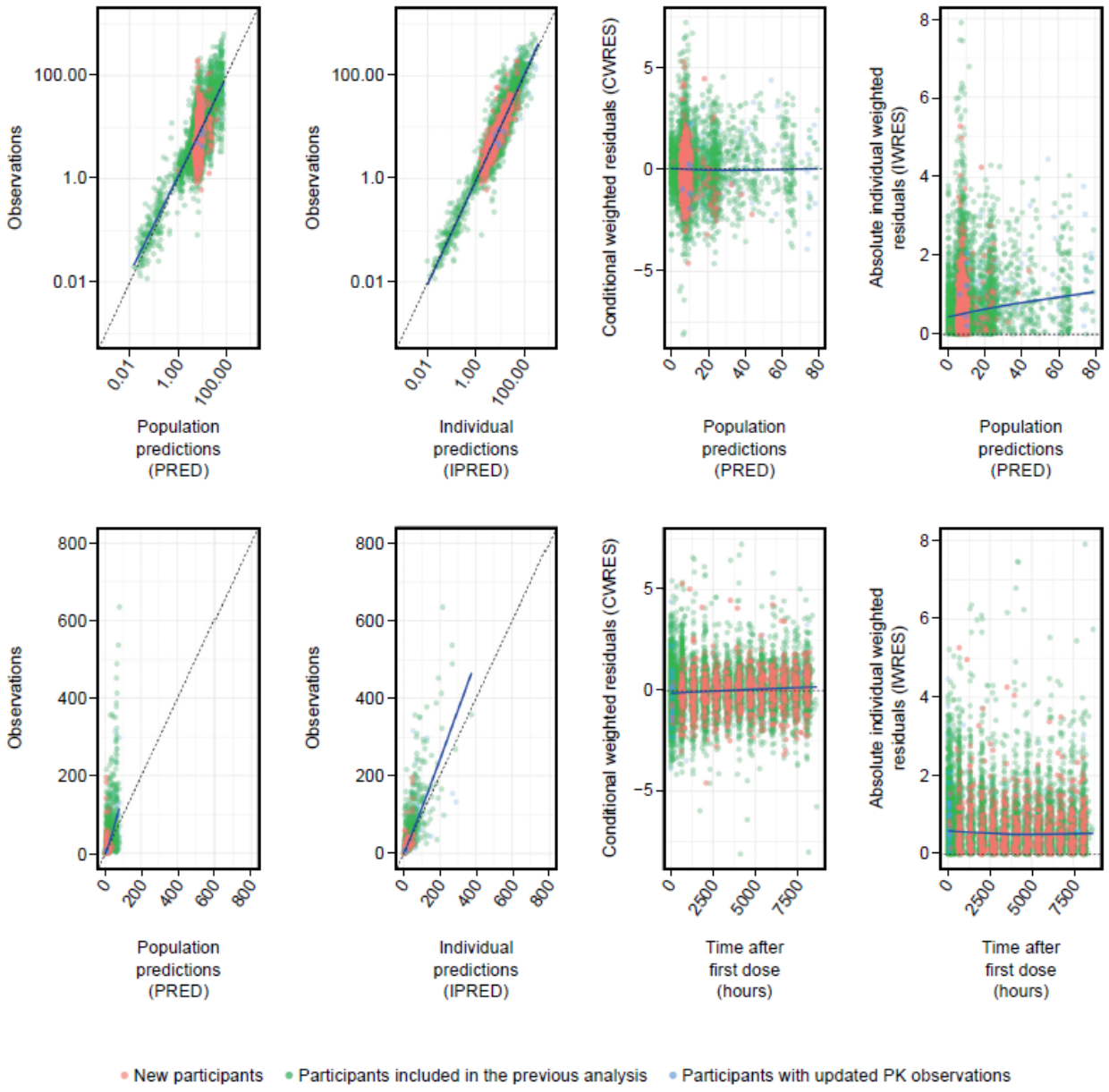
$$KinT\ (\mathrm{pg/mL/h}) = TVKinT \cdot \left({}^{AGE}/_{71}\right)^{-0.662} \cdot e^{\eta_{KinT}}$$

Figure S1 Schematic representation of the base PopPK model



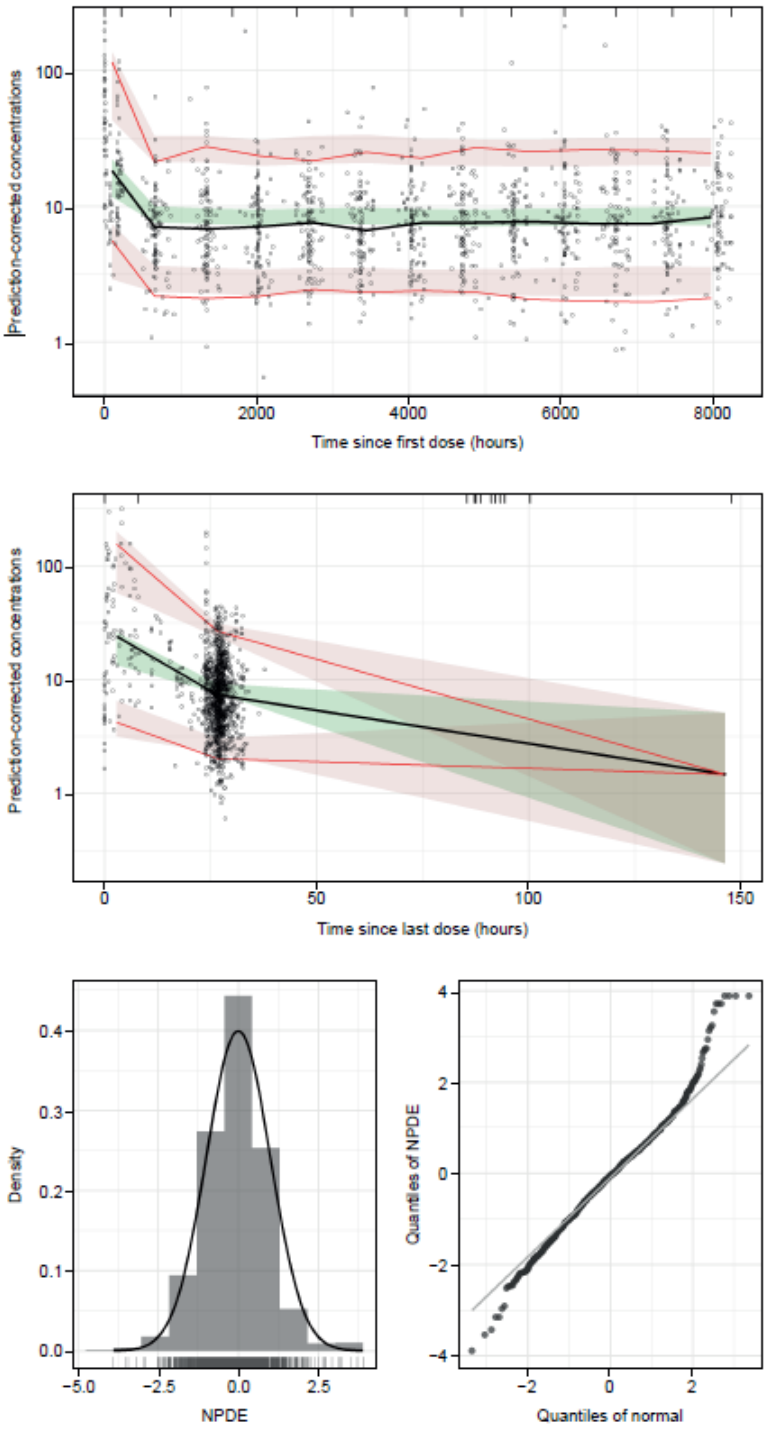
CEN, central compartment; CL/F, apparent systemic clearance after oral administration; F1, relative bioavailability; Ka, absorption rate constant; PER1, first peripheral compartment; PER2, second peripheral compartment; PopPK, population pharmacokinetic; Q1/F, apparent intercompartmental clearance with first peripheral compartment; Q2/F, apparent intercompartmental clearance with second peripheral compartment; Tlag, lag time; Vc/F, apparent volume of distribution in the central compartment; Vp1/F, apparent volume of distribution in the first peripheral compartment; Vp2/F, apparent volume of distribution in the second peripheral compartment.

Figure S2 Goodness-of-fit plots for the final PopPK model



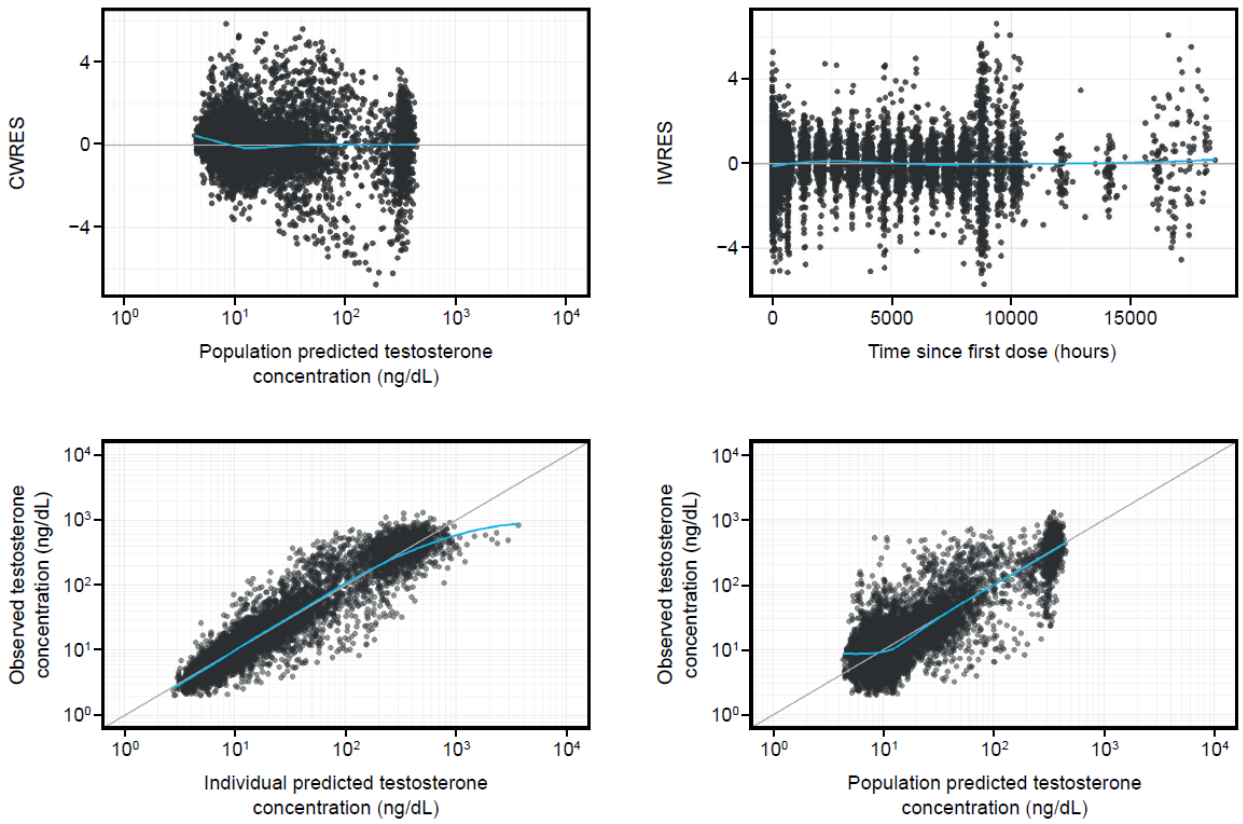
CWRES, conditional weighted residuals; IPRED, individual predictions; IWRES, individual weighted residuals; PRED, population predictions.
Notes: The colored circles represent individual observations; the solid blue lines represent the loess line of the presented data, and the dashed lines represent the identity line for plots of observations versus PRED and observations versus IPRED, and the zero line for CWRES and IWRES plots.

Figure S3 pcVPC and NPDE for the external validation of the PopPK model



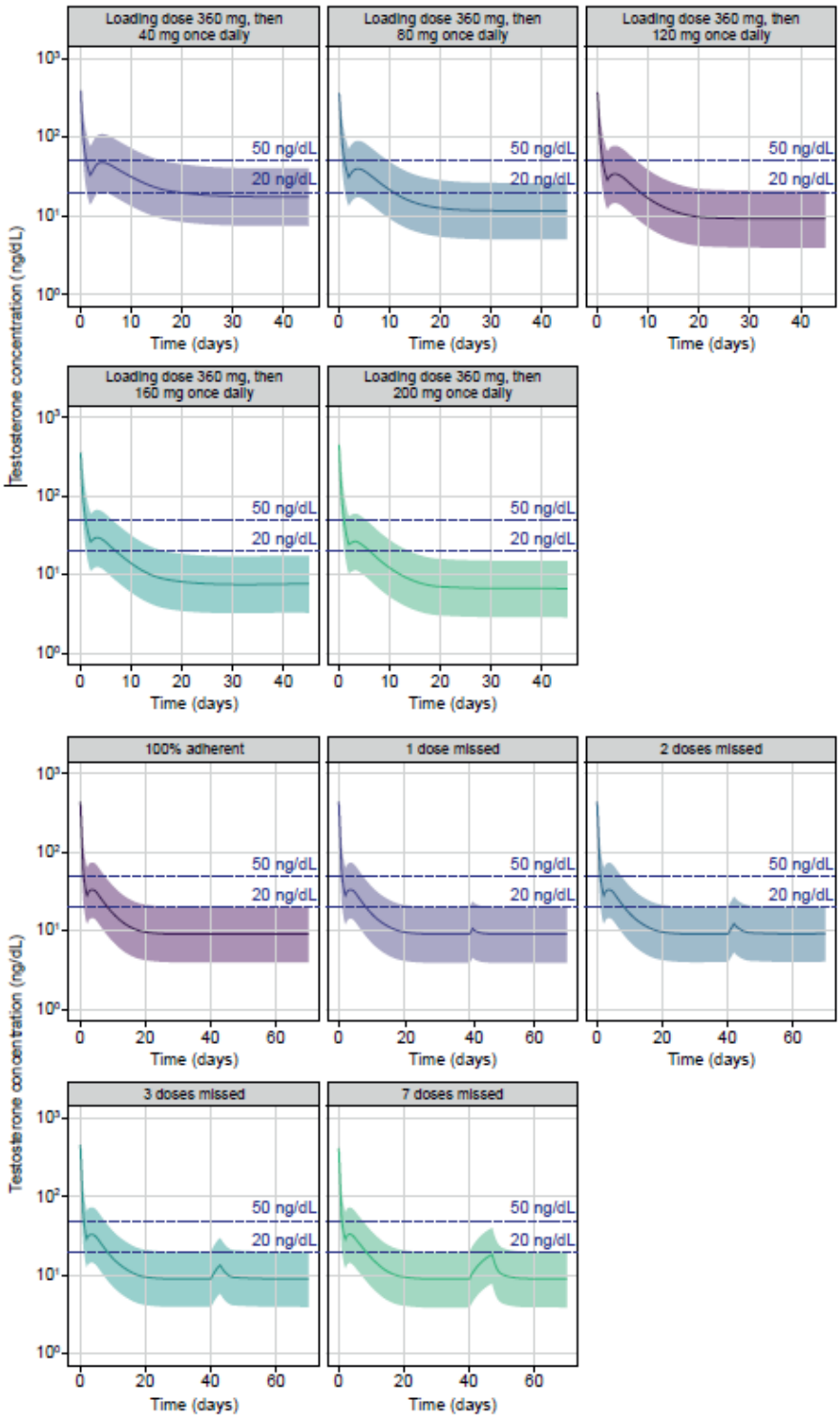
NPDE, normalized prediction distribution error; pcVPC, prediction-corrected visual predictive checks; PopPK, population pharmacokinetic.
Note for the pcVPC plots (top two graphs): The markers represent the observed data. The solid black and red lines represent the median and the 5th and 95th percentiles of the observed data, respectively. The green- and red-shaded areas represent the 90% confidence interval of the median and the 5th and 95th percentiles of the simulated data, respectively.

Figure S4 Goodness-of-fit plots for the final PopPK/PD model



CWRES, conditional weighted residuals; IWRES, individual weighted residuals.
Note: The grey circles represent individual observations; the light blue solid lines represent the loess smooth curve of the presented data and the grey solid lines represent the identity line for DV versus IPRED and DV versus PRED plots, and the zero line for CWRES versus PRED and IWRES versus TIME plots.

Figure S5 Simulations of testosterone profiles with different dosing regimens and durations of treatment interruption



Note: The shaded areas represent the 90% prediction interval (5th to 95th percentile) of the simulations. The solid lines represent the median of the simulations. Relugolix was simulated to be administered for 45 days with 100% adherence to different dosing regimens (the top graphs), or was simulated to be administered for 40 days with 100% adherence to the recommended dosing regimen (a 360-mg loading dose, followed by a 120-mg daily dose) before interruptions of treatment (the bottom graphs).