Supplementary information

The Effect of Modafinil on the Safety and Pharmacokinetics of Lorlatinib: A Phase 1 Study in Healthy Participants

Journal: *Clinical Pharmacokinetics*

Jerry Li, Yazdi K. Pithavala, Jason Gong, Robert R. LaBadie, Josué Kunjom Mfopou, Joseph Chen

Corresponding author: Jerry Li (jerry.li@pfizer.com), Global Product Development, Pharmacometrics, Oncology, Pfizer Inc, San Diego, CA, USA

S2 Methods

S2.3 PK Assessments

Briefly, lorlatinib and its stable isotopes labeled internal standard PF-06687235 were isolated from 50.0 μL of human plasma using a liquid-liquid extraction procedure with 100 μL of 0.1% ammonium hydroxoide in water and 1.00 mL of methyl-tert-butyl ether: ethyl acetate (50:50, v: v). After vortexing and centrifugation, the organic phase was transferred to a 96-well plate and evaporated to dryness under a stream of nitrogen. The dry residue was reconstituted with 250 μL of water: methanol (70:30, v:v), vortex-mixed and analyzed by electrospray ionization liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using positive ionization mode. The chromatographic separation was achieved using Chromolith®Speed ROD RP-18e/Merck/50 × 4.6 mm and gradient elution. Assay precision (% CV) and accuracy (% RE) were ≤7.1 and -3.8 to 0.9, respectively.

PF-06895751 (M8) and its stable isotopes labeled internal standard PF-06927100 were isolated from 100 μL of human plasma using a liquid-liquid extraction (LLE) procedure with 100 μL of Water: Formic Acid (100:0.3) and 1.00 mL of Ethyl Acetate. After vortexing and centrifugation, the organic phase was transferred to a 96-well plate and evaporated to dryness under a stream of nitrogen. The dry residue was reconstituted with 200 μL of Acetonitrile:Water (20:80, v:v), vortex-mixed and analyzed by atmospheric pressure chemical ionization (APCI) liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using negative ionization mode. The chromatographic separation was achieved using Waters, Atlantis ® dC 18 5μm 2.1*150 mm and gradient elution. Assay precision (% CV) and accuracy (% RE) were ≤7.3 and -2.0 to 1.6, respectively.

S2.4 Other Assessments

Cholesterol and 4β-hydroxycholesterol are endogenous in human plasma, therefore the calibration standards were prepared in a surrogate matrix consisting of 4% bovine serum albumin in 10 mM phosphate buffered saline and subsequently treated with 50 μg/mL butylated

hydroxytoluene (BHT). Quality Control (QC) samples (QC-L, QC-M, and QC-H) were prepared in human plasma and their concentrations were corrected for the endogenous concentrations in the control plasma used to prepare the samples. The endogenous concentration for the control plasma lot used in this study was determined in four analytical runs for cholesterol and three analytical runs for 4β-hydroxycholesterol. For cholesterol, assay precision (% CV) and accuracy (% RE) were ≤10.2 and 3.1 to 7.4, respectively. For 4β-hydroxycholesterol, assay precision and accuracy were ≤2.2 and -17.2 to -9.9, respectively.

Total cholesterol was extracted from 10 µL of human plasma treated with 50 µg/mL BHT or surrogate matrix sample by saponification, liquid/liquid extraction and subsequent derivatization with picolinic acid. Plasma samples were quantified by the surrogate matrix standard curve. Stable-isotope labeled (SIL) cholesterol (cholesterol-d7) was added prior to extraction for use as the internal standard.

Total 4β-hydroxycholesterol was extracted from 80 µL of human plasma treated with 50 µg/mL BHT or surrogate matrix sample by saponification and liquid/liquid extraction derivatization. Plasma samples were quantified by the surrogate matrix standard curve. SIL 4β-hydroxycholesterol (4β- hydroxycholesterol-d7) was added prior to extraction for use as the internal standard.

Plasma sample extracts were analyzed by high performance LC-MS/MS in the positive ion mode.

To analyze cortisol and 6β-hydroxycortisol, a 100 µL matrix aliquot was fortified with 25 µL of internal standard working solution. Analytes were isolated through solid phase extraction using Waters Oasis MAX 30 mg, 96-well SPE plates and eluted with 700 µL of methanol. The eluate was evaporated under a nitrogen stream at room temperature, and the remaining residue is reconstituted with 1000 µL of 10:90:0.1 acetonitrile/water/acetic acid, v/v/v. For cortisol, assay precision (% CV) and accuracy (% RE) were ≤10.0 and -3.59 to 3.99, respectively. For 6β-hydroxycortisol, assay precision and accuracy were ≤8.09 and 2.22 to 4.66, respectively.

Urine sample extracts were analyzed via HPLC with MS/MS detection using negative ion electrospray.

Table S1 PK and biomarker sampling (Period 1)

Study day	**1**								**2**		**3**		**4**	**5**	**6**
Hours before/after dose	**0**	**0.5**	**1**	**1.5**	**2**	**4**	**6**	**12**	**24**	**36**	**48**	**60**	**72**	**96**	**120**
Lorlatinib administration[a]	X														
PK blood sample for lorlatinib and potential metabolite(s)[b]	X[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Endogenous biomarker blood sample[d]	X	X	X	X	X	X	X	X	X						

[a]An oral dose of lorlatinib was administered as oral tablets on the morning of Day 1 after an overnight fast of at least 10 hours. Participants remained fasted until at least 4 hours post lorlatinib dosing.

[b]At each specified time point, one 6 mL blood sample should be drawn for determination of plasma lorlatinib concentration and concentration(s) of potential lorlatinib metabolite(s).

[c]Pre-lorlatinib dose.

[d]One 3 mL blood sample for endogenous biomarkers was drawn once prior to lorlatinib dose on Day 1 of Period 1 and at each timepoint through 24 hours post-lorlatinib dose.

PK pharmacokinetic

Table S2 PK sampling (Period 2)

Study day	**1**	**2-13**	**14**	**15**									**16**		**17**		**18**	**19**	**20**
Hours before/after dose				**0**	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**6**	**12**	**24**	**36**	**48**	**60**	**72**	**96**	**120**
Modafinil administration[a]	X	X[h]	X	X									X		X		X	X	
Lorlatinib administration[b]				X															
PK blood sample for lorlatinib and potential metabolite(s)[d]				X[c]	X	X	X	X		X	X	X	X	X	X	X	X	X	X
PK blood sample for modafinil[f]				X[c]					X										
Endogenous biomarker blood sample[e]				X[c]	X	X	X	X		X	X	X	X						
Morning spot urine sample for 6β-OHC/C ratio[g]	X	X[h]		X[c]															
Plasma sample for 4β-OHC/C ratio[g]	X	X[h]		X[c]															

[a]Oral doses of 400 mg modafinil at least one hour before or at least 2 hours after a meal once a day were administered at approximately the same time each day from Day 1 to Day 19 of Period 2, except on Day 15 where the administration was in fasted state. On Day 15 of Period 2, modafinil was dosed concurrently with lorlatinib within 5 minutes of each other.

[b]The dose of lorlatinib was administered as oral tablets on the morning of Day 15 after an overnight fast of at least 10 hours. Participants remained fasted until at least 4 hours post lorlatinib dose.

[c]Pre-lorlatinib and modafinil doses.

[d]At each specified time point, one 6 mL blood sample was drawn for determination of plasma lorlatinib concentration and concentration(s) of potential lorlatinib metabolite(s).

[e]One 3 mL blood sample for endogenous biomarkers was drawn once prior to lorlatinib dose on Day 15 of Period 2 and at each timepoint through 24 hours post-lorlatinib dose.

[f]At each specified time point, one 3 mL blood sample was drawn for determination of plasma modafinil concentration. These modafinil PK samples were only assayed in the event of an unexpected SAE or if the investigator had reason to believe the patient had not achieved moderate CYP3A induction due to compliance issues.

[g]One 10 mL morning spot urine sample for 6β-OHC/C and one 3 mL blood sample for 4β-OHC/C ratios were collected prior to modafinil dosing on Days 1, 8, and 15 of Period 2. The morning spot urine samples were collected at time 0-0 (pre-modafinil dose) on Day 1 and between times 0-10 on Day 8 and Day 15.

[h]6β-OHC/C and 3 mL blood sample for 4β-OHC/C ratios were collected at the CRU only on Day 8 in the Days 2-13 time period. Modafinil 400 mg was self-administered outside of the CRU on Day 2 to Day 7 and Day 9 to Day 13. Modafinil 400 mg was administered at the CRU on Day 8 of Period 2, and participants were instructed to not self-administer the 400 mg dose of modafinil on Day 8.

4β-OHC/C 4β-hydroxycholesterol/cholesterol; *6β-OHC/C* 6β-hydroxycortisol/cortisol; *CRU* clinical research unit; *PK* pharmacokinetic; *SAE* serious adverse event

Table S3 Inclusion and exclusion criteria

Inclusion criteria
1. Female participants of non-childbearing potential and/or male participants must be 18–55 years of age, inclusive, at the time of signing the informed consent document (ICD). 2. Male and female participants who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests, and electrocardiogram (ECG). 3. Adequate renal function, including: a. Estimated creatinine clearance ≥90 mL/min as calculated using chronic kidney disease epidemiology collaboration equation. In equivocal cases, a 24-h urine collection test can be used to estimate the creatinine clearance more accurately. 4. Adequate liver function, including: a. Total serum bilirubin within normal limits (WNL). b. Aspartate and alanine aminotransferase (AST and ALT) WNL. c. Alkaline phosphatase WNL. 5. Participants who are willing and able to comply with all scheduled visits, the treatment plan, laboratory tests, lifestyle considerations, and other study procedures. 6. Male participants of childbearing potential must agree to use a highly effective method of contraception from the first screen throughout the study and for 97 days after the last dose of assigned treatment. A participant is of childbearing potential if, in the opinion of the investigator, he/she is biologically capable of having children and is sexually active. 7. Body mass index of 17.5–30.5 kg/m^2; and a total body weight >50 kg (110 lb). 8. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the ICD and in the protocol.

Exclusion criteria

1. Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurological, or allergic disease (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at the time of dosing).
2. Any condition possibly affecting drug absorption (e.g., gastrectomy, cholecystectomy).
3. History of human immunodeficiency virus (HIV) infection, hepatitis B, or hepatitis C; positive testing for HIV, hepatitis B surface antigen, or hepatitis C antibody. A positive HepBsAb serology indicating hepatitis B vaccination is allowed.
4. Other acute or chronic medical or psychiatric condition including recent (within the past year) or active suicidal ideation or behavior or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the participant inappropriate for entry into this study.
    - Psychiatric adverse experiences have been reported in patients treated with modafinil with many, but not all, with prior psychiatric history. Participants with a history of psychosis, depression, or mania are excluded from this study.
5. Use of prescription or non-prescription drugs and dietary and herbal supplements within 7 days or five half-lives (whichever is longer) prior to the first dose of investigational product. Note, however, that the use of strong cytochrome P450 (CYP) 3A (CYP3A4) inhibitors or strong CYP3A inducers is prohibited at least 14 days prior to the first dose of the investigational product. As an exception, acetaminophen (paracetamol) may be used at doses of ≤1 g/day. Limited use of non-prescription medications that are not believed to affect participant safety or the overall results of the study may be permitted on a case-by-case basis following approval by the sponsor.

6. Previous administration with lorlatinib within 30 days (or as determined by the local requirement) or five half-lives preceding the first dose of lorlatinib used in this study (whichever is longer).
7. Previous treatment with lorlatinib.
8. A positive urine drug test, as confirmed by a single repeat.
9. Screening supine blood pressure (BP) ≥130 mm Hg (systolic) or ≥80 mm Hg (diastolic), following at least 5 min of supine rest. If BP is ≥130 mm Hg (systolic) or ≥80 mm Hg (diastolic), the BP should be repeated two more times and the average of the three BP values should be used to determine the participant's eligibility.
10. History of pancreatitis (elevated amylase or lipase) or hyperlipidemia.
11. Screening supine 12-lead ECG demonstrating PR interval >200 msec, corrected QT (QTc) >450 msec, or a QRS interval >120 msec.
12. History of cardiac arrhythmia (excluding asymptomatic sinus arrhythmia with heart rate >50, and occasional asymptomatic premature atrial contraction and premature ventricular contractions), atrioventricular block, symptomatic bradycardia, or QTc prolongation.
13. Participants with ANY of the following abnormalities in clinical laboratory tests at screening:
    - AST or ALT level > upper limit of normal (ULN);
    - Total bilirubin level > ULN; participants with a history of Gilbert's syndrome may have direct bilirubin measured and would be eligible for this study provided the direct bilirubin level is ≤ ULN.
14. History of alcohol abuse or binge drinking and/or any other illicit drug use or dependence within 6 months of screening. Binge drinking is defined as a pattern of five (male) and four (female) or more alcoholic drinks in about 2 h. As a general rule, alcohol intake should not exceed 14 units per week for males or seven units per week for females (1 unit = 8 ounces [240 mL] beer, 1 ounce [30 mL] of 40% spirit, or 3 ounces [90 mL] of wine).
15. As modafinil is a controlled substance, participants with a history of drug abuse will be excluded.

16. Blood donation (excluding plasma donations) of approximately 1 pint (500 mL) or more within 60 days prior to dosing.
17. History of sensitivity to lorlatinib or modafinil.
18. Unwilling or unable to comply with the criteria in the Lifestyle Considerations described in the protocol.
19. Male participants with partners currently pregnant; male participants able to father children who are unwilling or unable to use a highly effective method of contraception as outlined in the protocol for the duration of the study and for at least 97 days after the last dose of investigational product or longer based upon the compound's half-life characteristics, and refrain from sperm donation for the duration of the study and for at least 97 days after the last dose of investigational product.
20. Investigator site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or Pfizer employees, including their family members, directly involved in the conduct of the study.

Table S4 Treatment-related TEAEs

Number of participants by SYSTEM ORGAN CLASS and preferred term	Lorlatinib 50 mg single dose (Cohort 1) (*N*=2)	Modafinil 400 mg QD (Cohort 1) (*N*=2)	Lorlatinib 50 mg single dose + modafinil 400 mg QD (Cohort 1) (*N*=2)	Lorlatinib 75 mg single dose (Cohort 2) (*N*=2)	Modafinil 400 mg QD (Cohort 2) (*N*=2)	Lorlatinib 75 mg single dose + modafinil 400 mg QD (Cohort 2) (*N*=2)	Lorlatinib 100 mg single dose (Cohorts 3 & 4 combined) (*N*=12)	Modafinil 400 mg QD (Cohorts 3 & 4 combined) (*N*=12)	Lorlatinib 100 mg single dose + modafinil 400 mg QD (Cohorts 3 & 4 combined) (*N*=6)
Any AE	0	1	1	0	1	0	6	12	4
CARDIAC DISORDERS									
Palpitations	0	0	0	0	1	0	1	7	0
EYE DISORDERS									
Mydriasis	0	0	0	0	0	0	0	1	0
Vision blurred	0	0	0	0	0	0	1	1	0
GASTROINTESTINAL DISORDERS									
Abdominal pain	0	0	0	0	0	0	2	0	0
Diarrhea	0	1	0	0	0	0	1	1	1
Dry mouth	0	0	0	0	0	0	0	4	0
Flatulence	0	0	0	0	0	0	1	0	0
Hematochezia	0	0	0	0	0	0	1	0	0

Mouth ulceration	0	0	1	0	0	0	0	1	0
Nausea	0	0	0	0	0	0	0	5	1
Vomiting	0	1	0	0	0	0	0	2	0
GENERAL DISORDERS AND ADMINISTRATION-SITE CONDITIONS									
Asthenia	0	0	0	0	0	0	0	1	0
Chest pain	0	0	0	0	0	0	0	2	0
Energy increased	0	0	0	0	0	0	1	2	0
Fatigue	0	0	0	0	0	0	0	5	0
Feeling hot	0	0	0	0	0	0	1	3	0
Feeling of relaxation	0	0	0	0	0	0	0	0	1
Malaise	0	0	0	0	0	0	0	1	0
INJURY, POISONING, AND PROCEDURAL COMPLICATIONS									
Lip injury	0	0	0	0	0	0	0	1	0
INVESTIGATIONS									
Blood pressure increased	0	0	0	0	0	0	0	1	0
Weight decreased	0	0	0	0	0	0	0	1	0
METABOLISM AND NUTRITION DISORDERS									
Decreased appetite	0	0	0	0	1	0	0	7	1

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS									
Back pain	0	0	0	0	0	0	0	1	0
Myalgia	0	0	0	0	0	0	0	1	0
NERVOUS SYTEM DISORDERS									
Disturbance in attention	0	0	0	0	0	0	0	3	0
Dizziness	0	0	0	0	0	0	0	2	0
Dyskinesia	0	0	0	0	0	0	0	1	0
Head discomfort	0	0	0	0	0	0	1	0	0
Headache	0	1	0	0	0	0	1	5	0
Hypoesthesia	0	0	0	0	1	0	0	0	0
Paresthesia	0	0	0	0	0	0	0	1	0
Tremor	0	0	0	0	0	0	0	3	0
PSYCHIATRIC DISORDERS									
Affect lability	0	0	0	0	0	0	0	1	0
Aggression	0	0	0	0	0	0	0	1	0
Anxiety	0	0	0	0	0	0	0	5	0
Euphoric mood	0	0	0	0	0	0	1	0	2
Insomnia	0	0	0	0	1	0	0	6	1
Irritability	0	0	0	0	0	0	0	1	0
Libido decreased	0	0	0	0	0	0	1	0	0

REPRODUCTIVE SYSTEM AND BREAST DISORDERS									
Erectile dysfunction	0	0	0	0	0	0	0	1	0
RESPIRATORY, THORACIC, AND MEDIASTINAL DISORDERS									
Dry throat	0	0	0	0	0	0	0	1	0
Dyspnea	0	0	0	0	0	0	0	1	0
SKIN AND SUBCUTANEOUS TISSUE DISORDERS									
Acne	0	0	0	0	0	0	0	1	0
Alopecia	0	0	0	0	0	0	0	1	0
Hyperhidrosis	0	0	0	0	0	0	1	2	0

Participants were counted only once per treatment per event

AE adverse event; *TEAE* treatment-emergent adverse event; *QD* once daily

Table S5 Summary of lorlatinib and metabolite (M8) PK parameters with and without modafinil

Parameter, units	Lorlatinib 50 mg single dose (Cohort 1) (*N*=2)	Lorlatinib 50 mg single dose + modafinil 400 mg QD (Cohort 1) (*N*=2)	Lorlatinib 75 mg single dose (Cohort 2) (*N*=2)	Lorlatinib 75 mg single dose + modafinil 400 mg QD (Cohort 2) (*N*=2)	Lorlatinib 100 mg single dose (Cohorts 3 & 4 combined) (*N*=12)	Lorlatinib 100 mg single dose + modafinil 400 mg QD (Cohorts 3 & 4 combined) (*N*=6)
Lorlatinib						
AUC_{inf}, ng.h/mL	3306 (27)	2212 (32)	6552 (77)	5021 (28)	7791 (26)	5932 (23)
AUC_{last}, ng.h/mL	3182 (27)	2104 (32)	6309 (73)	4857 (26)	7615 (26)	5773 (23)
C_{max}, ng/mL	230.9 (19)	155.4 (23)	411.6 (41)	327.2 (23)	569.3 (34)	433.0 (21)
T_{max}, h	1.76 (1.50–2.02)	1.26 (1.00–1.52)	1.25 (1.00–1.50)	1.25 (1.00–1.50)	1.50 (1.00–2.02)	1.50 (1.00–1.52)
$t_{½}$, h	27.4 ± 2.2	23.8 ± 2.3	23.7 ±9.5	23.7 ± 8.2	22.5 ± 4.6	23.1 ± 7.9
CL/F, L/h	15.12 (27)	22.61 (31)	11.44 (77)	14.92 (28)	12.85 (26)	16.84 (23)
V_Z/F, L	595.5 (36)	774.9 (42)	375.6 (28)	495.2 (8)	408.0 (33)	529.6 (32)
M8						
AUC_{inf}, ng.h/mL	3475 (2)	2190 (9)	3877 (32)	2387 (15)	6043 (24)	3326 (21)
AUC_{last}, ng.h/mL	3143 (5)	2055 (10)	3551 (24)	2240 (10)	5547 (24)	3108 (17)

C_{max}, ng/mL	47.92 (14)	36.24 (11)	55.19 (3)	37.74 (2)	83.46 (27)	51.76 (20)
T_{max}, h	18.0 (12.0–24.0)	17.9 (12.0–23.8)	30.0 (12.0–48.0)	30.0 (23.9–36.0)	36.0 (24.0–48.0)	12.0 (12.0–23.8)
$t_{½}$, h	30.1 ± 5.6	26.9 ± 0.8	26.7 ± 9.1	25.6 ± 8.5	26.7 ± 6.7	25.9 ± 8.8

Geometric mean (geometric % coefficient of variation) for all except median (range) for T_{max} and arithmetic mean ± standard deviation for $t_{½}$

AUC_{inf}, area under the plasma concentration-time profile from time 0 extrapolated to infinite time; *AUC_{last}* area under the plasma concentration-time profile from time 0 to C_{last}; *CL/F* apparent oral clearance; *C_{last}* last measured plasma concentration; *C_{max}* maximum observed plasma concentration; *PK* pharmacokinetic; *QD* once daily; *$t_{½}$* terminal plasma half-life; *T_{max}* time of peak concentration; *V_Z/F* apparent volume of distribution.

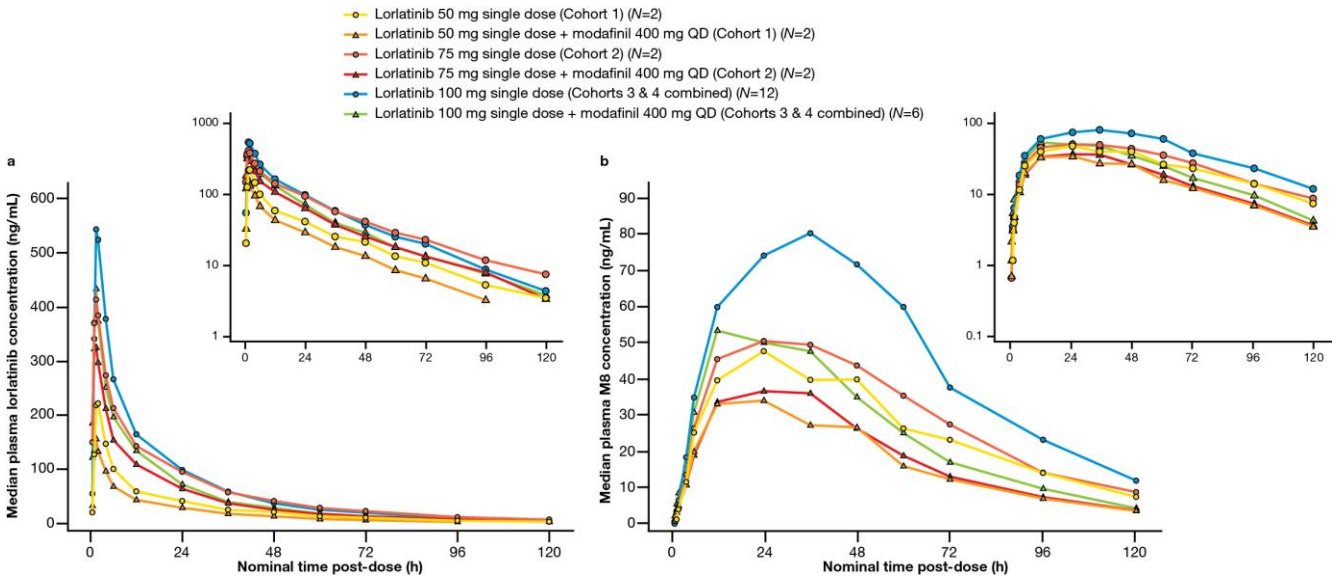
Table S6 Summary of plasma 4β-hydroxycholesterol/cholesterol ratios (x 10^{-5})

Analysis visit		Lorlatinib 50 mg single dose → lorlatinib 50 mg single dose + modafinil 400 mg QD (Cohort 1) (*N*=2)	Lorlatinib 75 mg single dose → lorlatinib 75 mg single dose + modafinil 400 mg QD (Cohort 2) (*N*=2)	Lorlatinib 100 mg single dose → lorlatinib 100 mg single dose + modafinil 400 mg QD (Cohorts 3 & 4 combined) (*N*=12)
Period 2 Day 1	n	2	2	12
	Mean	1.22	0.93	1.04
	SD	0.33	0.47	0.29
	CV (%)	27	50	28
	Median	1.23	0.93	0.99
	Range	0.99–1.46	0.60–1.26	0.62–1.72
Period 2 Day 8	n	2	2	9
	Mean	1.91	0.86	1.79
	SD	0.43	0.29	0.96
	CV (%)	22	34	53
	Median	1.91	0.86	1.70
	Range	1.60–2.21	0.65–1.06	0.81–4.07
Period 2 Day 15	n	2	2	6
	Mean	2.49	1.19	1.99
	SD	0.41	0.20	0.83
	CV (%)	17	17	42
	Median	2.49	1.19	2.04
	Range	2.20–2.79	1.05–1.33	0.84–3.22

N=number of participants in each treatment group; n=number of participants contributing to the summary statistics

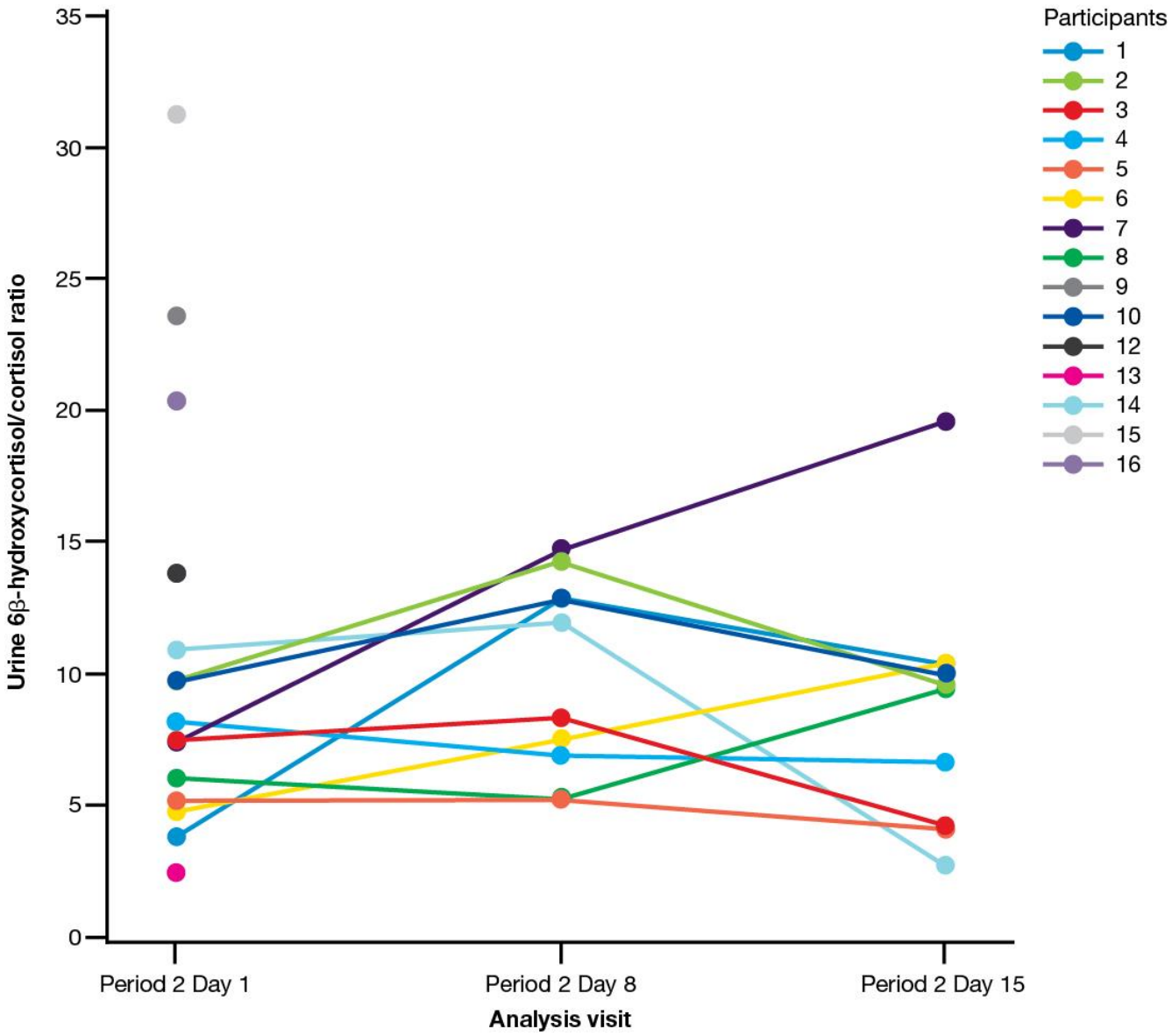
CV coefficient of variation; *QD* once daily; *SD* standard deviation

Fig. S1 a) Plasma lorlatinib and b) metabolite (M8) concentration-time profiles following administration of a single oral dose of lorlatinib alone and in the presence of steady-state modafinil (linear scale; semi-logarithmic scale inset)



QD once daily

Fig. S2 Matchstick plots for urine 6β-hydroxycortisol/cortisol concentration ratios pre- and post-modafinil QD treatments



Only participants who had at least one urine 6β-hydroxycortisol/cortisol ratio are represented

Data represent individual participant values (participants 1 and 2 were in Cohort 1 [lorlatinib 50 mg], participants 3 and 4 were in Cohort 2 [lorlatinib 75 mg], and participants 5–16 were in Cohorts 3 & 4 combined (lorlatinib 100 mg])

QD once daily