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Supplementary appendix

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APPENDIX

Table S1. SONIC Study sites

Country	Individual sites
Belgium	• University Hospitals Leuven
Bulgaria	• University Specialized Hospital for Active Treatment in Endocrinology
Canada	• St. Paul's Hospital/Vancouver General Hospital
Czechia	• Vseobecna fakultni nemocnice v Praze – III. Interni klinika VFN a 1. LF UK
Denmark	• Aarhus University Hospital • Herlev Hospital, Research Unit • Odense Universitets Hospital • Rigshospitalet, Copenhagen University Hospital
France	• Hopital de la CONCEPTION Service d'Endocrinologie, Diabete et Maladies Metaboliques
Georgia	• David Metreveli Medical Centre
Germany	• Max-Planck-Institute of Psychiatry • Med Clinic I - University of Lubeck
Hungary	• MH - Egeszsegugyi Kozpont • Semmelweis University
Israel	• Bnai Zion Medical Center Institute of Endocrinology & Metabolism • Institute of Endocrinology & Metabolism Rabin Medical Center, Beilinson Campus • Sourasky Medical Center • Ziv Medical Center
Italy	• Azienda Ospedaliera - Universitaria Ancona • Azienda Ospedaliero - Universitaria Careggi • Azienda Ospedaliero - Universitaria Città della Salute e della Scienza di Torino • Istituto Auxologico Italiano • Policlinico GB Rossi • Policlinico Universitario Agostino Gemelli • SCDU Medicina Interna I, Universita di Torino • University of Genova, IRCCS AOU San Martino-IST • University of Naples Federico II • University of Padua • UOC di Endocrinologia, Dipartimento di Medicina, AOU Policlinico G. Martino • UOC Endocrinologia, Azienda Ospedaliera Sant'Andrea
Netherlands	• Leiden University Medical Center • Polikliniek Endocrinologie, Erasmus MC
Poland	• Instytut Centrum Zdrowia Matki Polki • Outpatient Clinic: Reuma Centrum • Samodzielny Publiczny Szpital Kliniczny Nr 1 • Szpital Kliniczny im. Heliodora Swiecickiego • Terpa Sp.z.o.o

Serbia	• Clinical Center of Serbia • Clinical Center of Vojvodina Clinic for Endocrinology
Spain	• Hospital de la Santa Creu i Sant Pau • Hospital Universidad de la Ribera • Hospital Universitario Reina Sofia
Sweden	• Sahlgrenska University Hospital
Turkey	• Bezmi Alem Vakif Universitesi Endokrinoloji Bolumu Adnan • Dokuz Eylul University Medical Faculty • Istanbul University Medical Faculty
United Kingdom	• Manchester Royal Infirmary • Salford Royal NHS Foundation Trust
United States	• Allegheny Neuroendocrinology Center • Cleveland Clinic • Icahn School of Medicine at Mount Sinai • Johns Hopkins University • Massachusetts General Hospital • Memorial Sloan Kettering Cancer Center • Oregon Health & Science University • Swedish Hospital • University of California, Los Angeles, School of Medicine • University of Florida • University of Michigan Medical Center • University of New Mexico HSC

Table S2. Inclusion and exclusion criteria

Inclusion criteria:

1. Male or female ≥18 years of age
2. Able to provide written informed consent prior to any study procedures being performed; eligible patients must be able to understand the informed consent form prior to inclusion into the study.
3. Confirmed diagnosis of newly diagnosed, persistent or recurrent Cushing's disease (CD) or endogenous CS of other etiology if patients are not candidates for surgery or radiotherapy within the 18 months after enrollment.

   Previous medical records will be collected and used to support the diagnosis of CD or endogenous CS of other etiology, including the following etiologies:
   - Ectopic adrenocorticotropic hormone (ACTH) secretion, i.e. ACTH not of pituitary origin
   - Ectopic corticotropin-releasing hormone (CRH) secretion
   - Adrenal-dependent CS (i.e. adrenal adenoma (NOT carcinoma), adrenal hyperplasia, etc.)
   - Etiology unknown.

   In the absence of pathological or post-surgical confirmation of the diagnosis of CD (i.e. documented adrenal insufficiency post-adenomectomy or hypophysectomy, which will be considered diagnostic). The following historical evidence will be considered satisfactory to establish the diagnosis of CD: **Plasma corticotropin** (ACTH) level >20 pg/mL (4.5 pmol/L) or greater (Note: ACTH ≥5 pg/mL (1.1 pmol/L) and ≤20 pg/mL will generally suffice only if accompanied by either a positive CRH stimulation test or Dexamethasone Suppression Test (DST) or combined CRH-DST) **PLUS** one of the diagnostic strategies described below based on pituitary magnetic resonance imaging (MRI)/computed tomography (CT) findings (Note: pituitary imaging preceding the original diagnosis is a requirement for eligibility):

   For tumors ≥6 mm by imaging:
   - Inferior petrosal sinus sampled (IPSS) ACTH central:plasma gradient ≥2 before CRH or ≥3 after CRH, OR if IPSS was not done then:
   - Positive ACTH and/or cortisol response to CRH/desmopressin or combined CRH-desmopressin stimulation **plus** high-dose (8 mg) dexamethasone suppression of plasma cortisol, ideally on more than one occasion, performed and interpreted according to internationally recognized standards of diagnosis
   - In the absence of IPSS and the combination of tests described, an individual might be eligible if CD was otherwise confirmed via adequate testing. Such cases must be discussed with and explicitly approved by the Medical Monitor, and the specific diagnostic criteria used to establish the diagnosis of CD must be documented.

   For tumors <6 mm or not visible by MRI:
   - IPSS with ACTH central:plasma gradient ≥2 before CRH or ≥3 after CRH
   - In the absence of IPSS, an individual might be eligible if CD was otherwise confirmed via adequate testing. Such cases must be discussed with and explicitly approved by the Medical Monitor, and the specific diagnostic criteria used to establish the diagnosis of CD must be documented.
4. Regardless of the etiology of endogenous CS, patients MUST have elevated mean 24-hour UFC levels ≥1.5X ULN based on the normative range of the central lab assay and on a minimum of four measurements from adequately collected urine. Urine will ideally be collected on sequential days.
5. In addition to elevated mean UFC, presence of abnormal values from **one** of the following tests:
   - Abnormal DST: Elevated 8 AM serum cortisol ≥1.8 μg/dL (50 nmol/L) after 1 mg dexamethasone orally at 11 PM the evening prior (if not conducted already in the diagnostic workup of the patient within the previous 2 months before start of Screening Phase; in that case previous test results and details of conduct will need to be available by the Baseline Visit)
   - Elevated late night salivary cortisol concentrations (at least two measurements) >ULN

NOTE: For patients with estimated glomerular filtration rate (eGFR as determined by Modified Diet in Renal Disease MDRD equation) >40 and <60 mL/min/1.73 m^2 in addition to meeting the UFC criteria, late night salivary cortisol test results (≥2 measurements) **MUST** also demonstrate evidence of CS.

6. Previously irradiated patients with CD or endogenous CS of other etiology will be allowed as long as the radiation treatment occurred > 4 years ago and patients have not exhibited evidence for improvement in their underlying CD for 6 months prior to the Screening visit. The total number of previously irradiated patients enrolled in this study will not exceed 10.
7. Patients with CD or CS of other etiology who are not candidates for surgery, refuse surgery, or in whom surgery will be delayed for at least 18 months following enrollment. Patients may be allowed to participate in the trial while awaiting surgery, but must agree to complete this study prior to surgery. For patients who have already undergone surgery, a minimum of 6 weeks should have elapsed before the patient can be deemed a surgical failure. Patients who have undergone surgery should be stable post-surgery (i.e., no significant post-operative sequelae remain and the risk of such sequelae is considered negligible).
8. Patients on treatment for CD or endogenous CS of other etiology for whom treatment has been inadequate or not well tolerated must agree to the following minimum washout periods prior to the Baseline Visit:
    - Ketoconazole or metyrapone: 2 weeks
    - Dopamine agonists: bromocriptine (2 weeks), cabergoline (8 weeks)
    - Octreotide acetate LAR, lanreotide Autogel®, pasireotide LAR: 12 weeks
    - Lanreotide SR: 8 weeks
    - Octreotide acetate (immediate release) or short-acting pasireotide: 1 week
    - Mifepristone (RU 486, KORLYM®): 4 weeks
9. Patients on megestrol acetate or medroxyprogesterone acetate (and selected other synthetic progestins) must agree to a washout period of at least 6 weeks prior to the Baseline Visit
10. A female is eligible to enter and participate in the study if she is of:
    Non-child bearing potential (i.e. physiologically incapable of becoming pregnant, including any female who is post-menopausal or surgically sterile). Surgically sterile females are defined as those with a documented hysterectomy and/or bilateral oophorectomy or tubal ligation. Post-menopausal females are defined as being amenorrheic for greater than 1 year with an appropriate clinical profile, e.g. age > 45 years, in the absence of hormone replacement therapy. However, in questionable cases, a blood sample with follicle stimulating hormone (FSH) > 40MIU/ml and estradiol < 40pg/ml (<140 pmol/L) is confirmatory.
    OR
    Child-bearing potential and agrees to use highly effective methods of birth control while participating in the study and for 2 weeks after the study is completed.
11. Fertile men must also agree to use a medically acceptable form of birth control while on study drug and up to 2 weeks after the study is completed.
12. Able to comprehend and comply with procedures.

Exclusion criteria:

1. Patients with Pseudo-Cushing's syndrome based on assessment of the Investigator.
2. Patients with cyclic CS based on assessment of the Investigator
3. Patients with a non-endogenous source of hypercortisolism such as exogenous source of glucocorticoids or therapeutic use of ACTH.
4. Known inherited syndrome as the cause of hypercortisolism, including but not limited to multiple endocrine neoplasia Type 1, McCune Albright Syndrome and Carney Complex
5. Patients with adrenal carcinoma

6. History of malignancy, other than thyroid, early-stage prostate, squamous cell and basal cell carcinoma, within 3 years prior to the Screening Phase. Patients with history of such allowed carcinoma must have a life expectancy of >18 months and must be considered medically stable. Patients with early stage prostate cancer undergoing no treatment due to low grade potential may be enrolled.
7. Clinical or radiological signs of compression of the optic chiasm.
8. Major surgery within 1 month prior to enrollment (informed consent form signing)
9. Patients with clinically significant abnormality in 12-lead ECGs during the Screening Phase needing medical intervention.
10. Patients with QTc interval of >470 msec during the Screening Phase.
11. Patients with a history of Torsades des Pointes, or ventricular tachycardia, or ventricular fibrillation, or history of prolonged QT syndrome (including family history), or use of medications resulting in QT/QTc prolongation, or hypokalemia <3.0 mEq/L.
12. Pre-existing hepatic disease; patients with mild to moderate hepatic steatosis consistent with fatty infiltration (non-alcoholic fatty liver disease [NAFLD] are allowed).
13. Positive for hepatitis B surface antigen (HbsAg) or positive hepatitis C test.
14. History or symptoms of recurrent symptomatic cholelithiasis or pancreatitis.
15. Liver function tests (LFT) must not be above the following cut-offs during the Screening Phase:
    - Alanine transaminase (ALT) and/or aspartate transaminase (AST) >3 X ULN
    - Total bilirubin (TBN) >2 X ULN

    If all LFTs are within normal limits (WNL) and TBN is elevated, examination of direct and indirect bilirubin may be conducted. Patients with isolated indirect TBN up to 3X ULN are presumed to have Gilbert's syndrome and may be enrolled if all other LFTs are within normal levels.
16. History of documented or suspected drug-induced liver injury requiring drug discontinuation of ketoconazole or any azole antifungals.
17. Pregnant or lactating women
18. Human immunodeficiency virus (HIV)-positive.
19. History of persistent, uncontrolled hypertension (>180/120 mmHg) despite medical intervention.
20. Patients with hypercholesterolemia who are currently treated with atorvastatin, lovastatin or simvastatin and not willing or unable to change to alternative therapies, i.e. pravastatin, fluvastatin, or rosuvastatin within 2 weeks of start of the Screening Phase.
21. Body habitus preventing repeated venipuncture as required by protocol.
22. Patient is currently in another study or has received any investigational treatment (drug, biological agent or device) within 30 days or five half-lives of treatment, whichever is longer.
23. Repeated hospitalization for hyperglycemia or for any complication of hyperglycemia and diabetes during the last 12 months
24. Patients with decreased renal function as defined by eGFR <40 mL/min/1.73 m_2, using MDRD equation.
25. Any other clinically significant medical condition, as determined by the Investigator that precludes enrollment and participation in the study through completion, including conditions that would preclude the patient from being able to follow instructions or to perform the necessary procedures (for example, psychiatric instability or severe disability).
26. Abnormal free thyroxine (T4). Patients with thyroid stimulating hormone (TSH) < lower limit of normal (LLN) and normal free T4 are permitted to participate in the study.
27. Patients who have a history of alcohol or drug abuse in the 6-month period prior to enrollment.
28. Patients who have been treated with mitotane within 6 months of the Screening Phase.

Table S3. Prohibited medications

Class	**Selected Medications**
Steroidogenesis inhibitors	Etomidate, ketoconazole, metyrapone, mitotane, trilostane
Systemic corticosteroids	Hydrocortisone, prednisolone, prednisone
Dopamine agonists	Cabergoline, dihydroergotamine/ergotamine, levodopa
Synthetic progestins	Medroxyprogesterone acetate, megestrol acetate, micronized progesterone
Somatostatin analogues	Lanreotide, octreotide, pasireotide
Weight loss medications	Bupropion/naltrexone, phentermine, topiramate
Strong CYP3A4 inducers	Phenytoin, pioglitazone, St. John's wort
Strong CYP3A4 inhibitors	Clarithromycin, conivaptan, itraconazole
Drugs predicted to interfere with the absorption of levoketoconazole	Histamine H2 receptor antagonists, proton-pump inhibitors, sucralfate
Drugs whose systemic exposure is predicted to be significantly increased by levoketoconazole	HMG-COA reductase inhibitors (NOT fluvastatin, pravastatin, rosuvastatin)
Drugs that can cause QTc prolongation*	Azithromycin, citalopram, venlafaxine
Other	Acetaminophen (paracetamol) >3g total daily dose

*Unless no acceptable alternative is available.

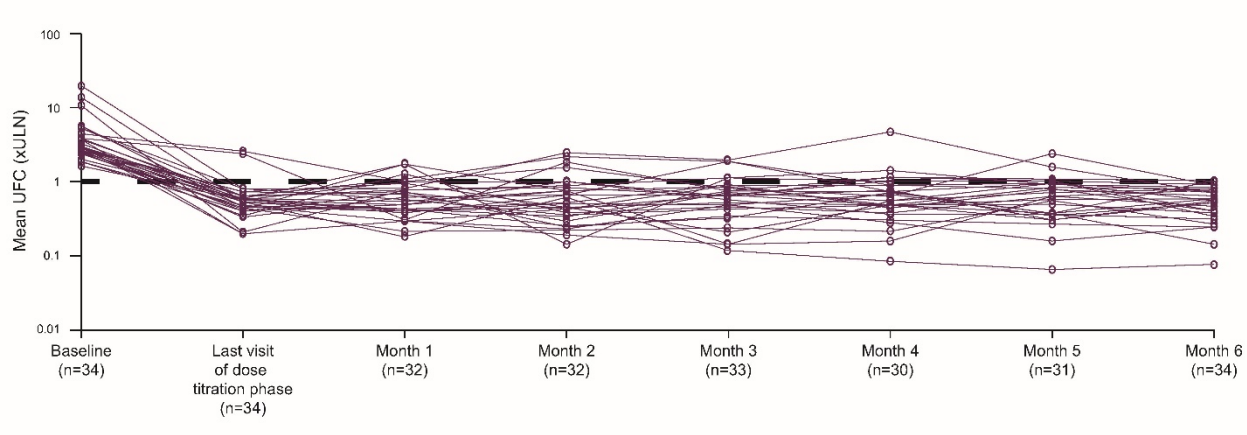
Table S4. Clinical responses for patients entering the maintenance phase

Response, n (%)	Levoketoconazole dose/day at the end of dose titration phase*							
	300 mg (n=24)	**450 mg (n=12)**	**600 mg (n=11)**	**750 mg (n=10)**	**900 mg (n=8)**	**1050 mg (n=3)**	**1200 mg (n=9)**	**All (n=77)**
Complete response (mUFC ≤ ULN)	24 (100)	12 (100)	8 (72·7)	6 (60)	6 (75)	2 (66·7)	4 (44·4)	62 (80·5)
mUFC decrease of ≥50% from baseline	0	0	1 (9·1)	3 (30)	1 (12·5)	0	1 (11·1)	6 (7·8)
Clinically meaningful partial response†	0	0	1 (9·1)	0	0	0	2 (22·2)	3 (3·9)
Not determined‡	0	0	1 (9·1)	1 (10)	1 (12·5)	1 (33·3)	2 (22·2)	6 (7·8)

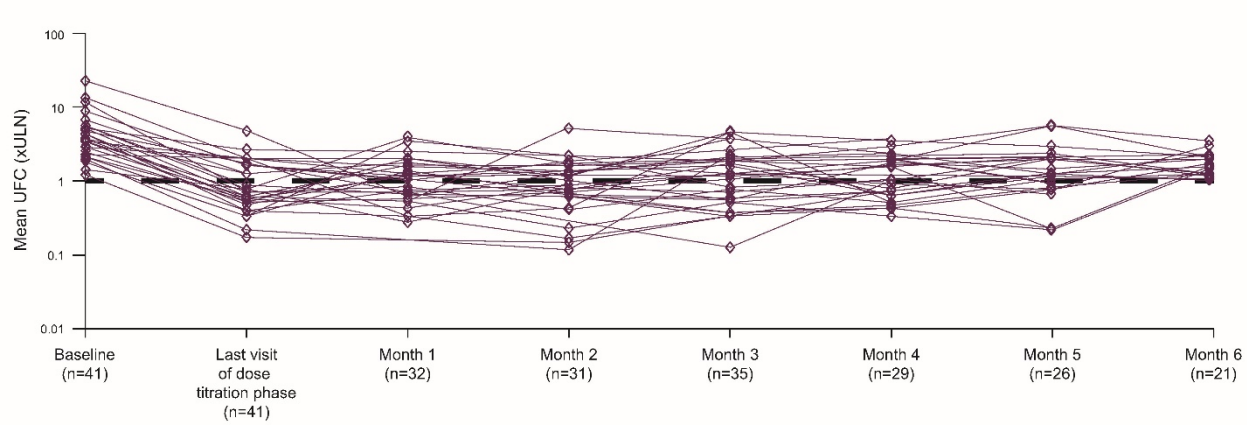
*Three patients were switched to another dose at the start of the maintenance phase. one patient was switched from 300 to 450 mg/day; one patient from 450 to 300 mg/day; and one from 600 to 450 mg/day. Therefore, the number of patients in the maintenance phase are 24, 11, 12, 10, 8, 3, and 9 for 300, 450, 600, 750, 900, 1050, and 1200 mg/day dose levels, respectively. †As judged by the investigator after titration to 1200 mg/day or maximum tolerated dose; mUFC was reduced by 46%, 38%, and 35% in these three patients. ‡Three patients had samples taken at their last dose, but they were all inadequate samples. Three patients did not have any urine samples for UFC after the start of the last dose level during the dose titration phase. mUFC=mean urinary free cortisol. ULN=upper limit of normal.

Figure S1. Individual line plots of mUFC (×ULN) for (A) responders (mUFC ≤ULN at EoM; n=34) and (B) nonresponders (mUFC >ULN at EoM; n=41) regardless of dose increase

(A)



(B)



Two patients had no mUFC values in the maintenance phase. They were considered as nonresponders but are not represented in this figure. The dashed line represents the ULN for urinary free cortisol (138 nmol/24 hours [50 µg/24 hours]). EoM=end of maintenance. mUFC=mean urinary free cortisol. ULN=upper limit of normal.

Table S5. Shift from baseline to month 6 (or last assessed visit in the maintenance phase) in category of markers of comorbid conditions (maintenance population)

Variable	Baseline category	End of maintenance phase category	
Total cholesterol (n=73)	<5·17 mmol/L (200 mg/dL) (n=29)	<5·17 mmol/L	28 (97%)
		5·17–6·18 mmol/L	1 (3%)
		≥6·21 mmol/L	0
	5·17–6·18 mmol/L (200–239 mg/dL) (n=22)	<5·17 mmol/L	17 (77%)
		5·17–6·18 mmol/L	5 (23%)
		≥6·21 mmol/L	0
	≥6·21 mmol/L (240 mg/dL) (n=22)	<5·17 mmol/L	9 (41%)
		5·17–6·18 mmol/L	7 (32%)
		≥6·21 mmol/L	6 (27%)
Low-density lipoprotein (n=74)	<1·81 mmol/L (70 mg/dL) (n=5)	<1·81 mmol/L	4 (80%)
		1·81–<2·59 mmol/L	1 (20%)
		2·59–<3·36 mmol/L	0
		≥3·36 mmol/L	0
	1·81–<2·59 mmol/L (70–<100 mg/dL) (n=21)	<1·81 mmol/L	9 (43%)
		1·81–<2·59 mmol/L	10 (48%)
		2·59–<3·36 mmol/L	2 (10%)
		≥3·36 mmol/L	0
	2·59–<3·36 mmol/L (100–<130 mg/dL) (n=13)	<1·81 mmol/L	1 (8%)
		1·81–<2·59 mmol/L	10 (77%)
		2·59–<3·36 mmol/L	2 (15%)
		≥3·36 mmol/L	0
	≥3·36 mmol/L (130 mg/dL) (n=35)	<1·81 mmol/L	1 (3%)
		1·81–<2·59 mmol/L	10 (29%)
		2·59–<3·36 mmol/L	17 (49%)
		≥3·36 mmol/L	7 (20%)
High-density lipoprotein (n=74)	<1·03 mmol/L (40 mg/dL) (n=3)	<1·03 mmol/L	2 (67%)
		1·03–<1·55 mmol/L	1 (33%)
		≥1·55 mmol/L	0
	1·03–<1·55 mmol/L (40–<60 mg/dL) (n=31)	<1·03 mmol/L	4 (13%)
		1·03–<1·55 mmol/L	22 (71%)
		≥1·55 mmol/L	5 (16%)
		<1·03 mmol/L	3 (8%)

	≥1·55 mmol/L (60 mg/dL) (n=40)	1·03–<1·55 mmol/L ≥1·55 mmol/L	18 (45%) 19 (48%)
Haemoglobin A1c (n=76)	<5·7% (<38·8 mmol/mol) (n=39)	<5·7% 5·7–<6·5% 6·5–<8% ≥8%	38 (97%) 1 (3%) 0 0
	5·7–<6·5% (38·8–<47·5 mmol/mol) (n=21)	<5·7% 5·7–<6·5% 6·5–<8% ≥8%	10 (48%) 11 (52%) 0 0
	6·5–<8% (47·5–<63·9 mmol/mol) (n=9)	<5·7% 5·7–<6·5% 6·5–<8% ≥8%	2 (22%) 2 (22%) 4 (44%) 1 (11%)
	≥8% (≥63·9 mmol/mol) (n=7)	<5·7% 5·7–<6·5% 6·5–<8% ≥8%	1 (14%) 2 (29%) 1 (14%) 3 (43%)
Fasting blood glucose (n=75)	<6·1 mmol/L (110 mg/dL) (n=55)	<6·1 mmol/L 6·1–6·9 mmol/L >6·9 mmol	54 (98%) 1 (2%) 0
	6·1–6·9 mmol/L (110–125 mg/dL) (n=9)	<6·1 mmol/L 6·1–6·9 mmol/L >6·9 mmol	6 (67%) 2 (22%) 1 (11%)
	>6·9 mmol (125 mg/dL) (n=11)	<6·1 mmol/L 6·1–6·9 mmol/L >6·9 mmol	6 (55%) 3 (27%) 2 (18%)
BMI (n=75)	<18·5 kg/m^2 (n=0)		
	18·5–<25·0 kg/m^2 (n=17)	<18·5 kg/m^2 18·5–<25·0 kg/m^2 25·0–<30·0 kg/m^2 30·0–<40·0 kg/m^2 ≥40 kg/m^2	2 (12%) 15 (88%) 0 0 0
	25·0–<30·0 kg/m^2 (n=26)	<18·5 kg/m^2 18·5–<25·0 kg/m^2	0 14 (54%) 11 (42%)

		25·0–<30·0 kg/m^2	1 (4%)
		30·0–<40·0 kg/m^2	0
		≥40 kg/m^2	0
	30·0–<40·0 kg/m^2 (n=25)	<18·5 kg/m^2	2 (8%)
		18·5–<25·0 kg/m^2	7 (28%)
		25·0–<30·0 kg/m^2	15 (60%)
		30·0–<40·0 kg/m^2	1 (4%)
		≥40 kg/m^2	0
	≥40 kg/m^2 (n=7)	<18·5 kg/m^2	0
		18·5–<25·0 kg/m^2	0
		25·0–<30·0 kg/m^2	2 (29%)
		30·0–<40·0 kg/m^2	5 (71%)
		≥40 kg/m^2	
Data are n (%). BMI=body mass index.			

Table S6**.** Change in concomitant medication use during the maintenance phase (N=77)

Type of medication, n (%)	Patients taking medication before the start of levoketoconazole						Medication started after baseline*
	Total	Started new and significant medication	Dose increased or restarted after gap	Dose decreased	No change from baseline	Stopped taking medication	
Antidiabetic	24 (31·2)†	2 (2·6)	1 (1·3)	3 (3·9)	15 (19·5)	2 (2·6)	3 (3·9)
Cholesterol lowering	12 (15·6)†	0	1 (1·3)	1 (1·3)	9 (11·7)	0	6 (7·8)
Antihypertensive	49 (63·6)	9 (11·7)	9 (11·7)	4 (5·2)	26 (33·8)	1 (1·3)	3 (3·9)

*Patients who were not taking the medication at baseline but started it during the maintenance phase.
†One patient had a clinically insignificant change (same dose of a different formulation or pharmacological equivalent dose of a different drug).

Table S7. Patient-reported outcomes: Cushing's QoL questionnaire and Beck Depression Inventory II[*] (maintenance population)

Patient-reported outcome	**Baseline**	**Mean (SE) (95% CI) change from baseline to EoM**	**p value[†]**
Cushing's QoL questionnaire total score[‡]	44·3 (21·3) n=74	10·6 (2·0)(6·7 to 14·6) n=51	<0·0001
Beck Depression Inventory II total score[§,¶]	17·1 (12·9) n=59	–4·3 (1·4) –(7·2 to –1·4) n=40	0·0043

Data are mean (SD), number of participants, unless otherwise indicated.
[*]Analysis based on the 77 patients who entered the maintenance phase. [†]Two-sided p value from the paired t test performed on the change from baseline to EoM. [‡]Cushing's QoL questionnaire score: range from 0 [worst] to 100 [best]. An increase from baseline corresponds to improvement. [§] Beck Depression Inventory II score: range from 0 (best) to 63 (worst). A decrease from baseline corresponds to improvement. [¶]Only patients who consented to study participation under protocol amendments 5 and 6 had this assessment. CI = confidence interval. EoM=end of maintenance (month 6). QoL=quality of life. SD=standard deviation. SE=standard error.