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Efficacy and safety of levoketoconazole in the treatment of endogenous Cushing's syndrome (SONICS): a phase 3, multicentre, open-label, single-arm trial

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Summary

Background Levoketoconazole is a ketoconazole stereoisomer in development for treatment of Cushing's syndrome and has not been assessed previously in a clinical trial in patients with Cushing's syndrome. We aimed to investigate the efficacy and safety of levoketoconazole in patients with endogenous Cushing's syndrome.

Methods SONICS is a phase 3, multicentre, open-label, non-randomised, single-arm study in which we recruited adults (≥18 years) with confirmed Cushing's syndrome and a mean 24-h urinary free cortisol (mUFC) of at least 1·5 times the upper limit of normal from 60 hospital and community sites in 19 countries (15 countries in Europe, and Canada, Israel, Turkey, and the USA). Patients were treated with oral levoketoconazole in a 2–21 week incremental dose-titration phase starting at 150 mg twice daily (150 mg increments until mUFC normalisation, maximum 600 mg twice daily) and a 6-month maintenance phase. The primary outcome was the proportion of patients with mUFC normalisation at end of maintenance, without dose increase during the maintenance phase (in the intention-to-treat population). Prespecified adverse events of special interest were potential liver toxicity, corrected QT prolongation, and adrenal insufficiency. This trial is registered with ClinicalTrials.gov, NCT01838551.

Findings Between July 30, 2014, and June 30, 2017, 201 individuals were screened and 94 patients were enrolled and received at least one dose of study medication. Of the 94 patients, 80 (85%) had pituitary Cushing's syndrome. Mean mUFC at baseline was 671·4 nmol/24 h (243·3 µg/24 h), which is 4·9 times the upper limit of normal. Of the 77 patients who advanced to the maintenance phase, 62 (81%) had mUFC normalisation by end-of-dose titration. At the end of the 6-month maintenance phase, 29 (31%) of 94 patients were responders; the least-squares mean estimate of the proportion of responders was 0·30 (95% CI 0·21–0·40; p=0·0154 *vs* null hypothesis of ≤0·20). The most common adverse events in the 94 patients were nausea (30 [32%]) and headache (26 [28%]). Adverse events led to study discontinuation in 12 (13%) of 94 patients. Two patients had a QT interval (Fridericia corrected) of more than 500 ms, and three patients had suspected adrenal insufficiency. Alanine aminotransferase reversibly increased to more than three times the upper limit of normal in ten (11%) patients. Four patients had serious adverse events that were considered probably or definitely related to the study drug: abnormal liver function test results (n=1), prolonged QT interval (n=2), and adrenal insufficiency (n=1). One person died from colon carcinoma unrelated to study medication.

Interpretation Twice-daily oral levoketoconazole treatment led to sustained improvements in urinary free cortisol, with an acceptable safety and tolerability profile. Levoketoconazole might represent a useful therapeutic option for the medical treatment of Cushing's syndrome.

Funding Strongbridge Biopharma.

Introduction

Endogenous Cushing's syndrome is a rare, serious endocrine condition characterised by chronic over-production of cortisol, most often caused by a pituitary adenoma (ie, Cushing's disease).[1] Other causes include ectopic adrenocorticotropic hormone production or primary adrenal neoplasia.[2] Patients with Cushing's syndrome have increased mortality, mainly as a result of cardiovascular complications.[3–5]

Surgical removal of the underlying lesion is first-line therapy, sometimes preceded by preoperative medical treatment.[6] The choice of second-line therapy (medications, further surgery, or radiotherapy) depends on individual patient characteristics and treatment efficacy and risks.[6,7] Medical treatments suppress excessive adrenocorticotropic hormone or cortisol production or decrease cortisol activity.[5,8]

Ketoconazole, a racemic mixture of two enantiomers (2S,4R-ketoconazole and 2R,4S-ketoconazole), is an azole antifungal drug that is approved for treatment of endogenous Cushing's syndrome by the European Medicines Agency[9] and is used off-label for this purpose in the USA (where the recognised use by the US Food and Drug Administration [FDA] is for endemic mycoses

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Research in context

Evidence before this study

Medical treatments for endogenous Cushing's syndrome are used to reduce cortisol production or activity when surgery to resect the underlying lesion is delayed, contraindicated, or unsuccessful. We searched PubMed using the terms "Cushing's syndrome," "Cushing's disease," "Cushing syndrome," and "Cushing disease" for clinical trial reports published from database inception to Feb 13, 2019. Few medications have been evaluated in well-designed prospective studies. Ketoconazole, an azole antifungal drug that inhibits steroidogenesis, is approved for the treatment of endogenous Cushing's syndrome in Europe and used off-label in the USA. Retrospective chart reviews suggest that ketoconazole can reduce urinary free cortisol concentrations in patients with endogenous Cushing's syndrome, but its use is limited by side-effects (such as hepatotoxicity and QT interval prolongation) and the potential for drug interactions. Levoketoconazole, the 2S,4R enantiomer of ketoconazole, decreases cortisol synthesis via potent inhibition of several enzymes in the steroidogenic pathway and might have lower risk of hepatotoxicity and an improved side-effect profile relative to ketoconazole.

Added value of this study

To our knowledge, this phase 3 study is the first clinical trial to investigate levoketoconazole in patients with endogenous Cushing's syndrome. Efficacy was assessed with a robust, conservative analytical approach that counted all patients who discontinued from the study as treatment failures, irrespective of the reason, as well as all patients with missing data at the 6 month assessment. Levoketoconazole normalised urinary free cortisol levels after 6 months of maintenance therapy (without a dose increase after establishing a therapeutic dose) in about 30% of patients. Additionally, levoketoconazole was associated with improvements in biomarkers of cardiovascular risk (such as fasting blood glucose concentration, HbA_{1c}, LDL cholesterol concentration, and bodyweight), as well as clinical signs of Cushing's syndrome. Levoketoconazole was generally well tolerated, with no unexpected safety signals identified during the study.

Implications of all the available evidence

The medical needs of patients with Cushing's syndrome remain high, despite the availability of approved treatments. This debilitating condition is associated with increased mortality, often related to cardiovascular complications. The findings from this study show the sustained clinical benefits of twice-daily oral levoketoconazole, including improvements in cardiovascular risk factors as well as normalisation of cortisol concentrations in some patients. Levoketoconazole might represent a useful therapeutic option for the medical treatment of Cushing's syndrome.

not susceptible to other antifungals).[10] Ketoconazole reduces adrenal cortisol production by inhibiting various enzymes involved in steroidogenesis.[11,12] To our knowledge, the efficacy of oral ketoconazole for Cushing's syndrome has never been previously investigated in a clinical trial; the available clinical evidence for its use has been derived from retrospective and prospective observational data.[13,14] The most common adverse effects associated with ketoconazole include nausea, headache, diarrhoea, and abdominal pain; of greater concern is the potential for rare but serious hepatotoxicity, for which weekly monitoring is recommended by the FDA.[10,13] Ketoconazole is also associated with prolongation of the QT interval, a risk factor for ventricular arrhythmia.[15,16] Because ketoconazole inhibits various drug-metabolising enzymes and xenobiotic transporters, many drug–drug interactions have been reported.[10,13,17] Sufficient gastric acidity is required for absorption of oral ketoconazole.[18]

See Online for appendix

Levoketoconazole, the 2S,4R enantiomer of ketoconazole, is an orally administered investigational drug in development for the treatment of Cushing's syndrome. Levoketoconazole has been previously studied in patients with type 2 diabetes.[19] Based on in-vitro studies, levoketoconazole is substantially more potent in inhibiting 17α-hydroxylase, 11β-hydroxylase, and the cholesterol side-chain cleavage enzymes than its dextroisomer (dextroketoconazole),[12] which might allow for a lower dose of levoketoconazole to achieve the same efficacy as currently utilised doses of racemic ketoconazole. In vitro, levoketoconazole is about twice as potent an inhibitor of these enzymes as ketoconazole, suggesting that essentially all of the cortisol production-inhibition activity of ketoconazole in vivo derives from its 2S,4R enantiomer (ie, levoketoconazole).[12]

We aimed to investigate the efficacy and safety of levoketoconazole in patients with endogenous Cushing's syndrome.

Methods

Study design and patients

SONICS is a phase 3, single-arm, non-randomised, open-label study done at 60 hospital and community sites in 19 countries (15 countries in Europe, and Canada, Israel, Turkey, and the USA; appendix pp 1–2). There were three phases: dose titration (2–21 weeks to achieve an effective and tolerable therapeutic dose), maintenance (6 months of treatment at the therapeutic dose), and extended evaluation (6 months of continued treatment). Results from the first two phases, which include the primary efficacy outcome, are the subject of this report; data from the extended evaluation phase will be reported elsewhere. The study protocol was approved by an institutional review board or independent ethics committee at each site, and the study was done in

accordance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation Guideline for Good Clinical Practice. All patients provided written informed consent to participate.

Key inclusion criteria were an age of 18 years or older, confirmed diagnosis of persistent, recurrent, or de novo Cushing's disease or endogenous Cushing's syndrome of other causes (if not considered to be candidates for surgery or radiotherapy within 18 months after enrolment), mean 24-h urinary free cortisol concentration (mUFC) of at least 1·5 times the upper limit of normal (ULN; calculated from nominally four or more and no fewer than two samples, considered adequate by volume and creatinine excretion rate criteria), and either abnormal dexamethasone suppression test results (morning serum cortisol ≥50 nmol/L [1·8 µg/dL] after oral administration of 1 mg of dexamethasone between 2300 h and 0000 h the previous evening) or raised (greater than the ULN) late-night salivary cortisol (from saliva samples collected between 2300 h and 0000 h) concentrations (from at least two samples). Patients with previous radiation therapy were eligible, provided treatment had occurred at least 4 years previously and no improvement was observed during the past 6 months; patients who had undergone surgery and been deemed surgical failures (≥6 weeks since surgery) were also eligible. Key exclusion criteria were pseudo-Cushing's syndrome; cyclic Cushing's syndrome; exogenous hypercortisolism; malignancy-associated hypercortisolism (apart from ectopic adrenocorticotropic hormone from an unidentified source); history of malignancy (other than thyroid, early-stage prostate, skin squamous, or basal cell carcinoma) within 3 years of screening; optic chiasm compression; QT prolongation or abnormal electrocardiogram requiring medical intervention; pre-existing hepatic disease (apart from mild-to-moderate non-alcoholic fatty liver disease); alanine aminotransferase or aspartate aminotransferase more than three times the ULN or total bilirubin more than twice the ULN; persistent, uncontrolled hypertension; and poorly controlled diabetes. Additionally, patients with hypercortisolism caused by a known inherited syndrome (eg, multiple endocrine neoplasia type 1) were excluded because of the potential drug interactions between levoketoconazole and treatments required for these conditions. A complete list of inclusion and exclusion criteria is provided in the appendix (pp 3–5). Specific medications were not allowed to be used during the study (eg, strong CYP3A4 inducers; appendix p 6).

Procedures

In the dose-titration phase, levoketoconazole was initially dosed as 150 mg oral tablets twice daily. Subsequent dose increases of 150 mg per day increments no more frequently than every other week were driven by assessments of mUFC concentration, with a goal of normalisation, as well as safety and tolerability. mUFC was based on one to four adequate 24-h urine samples (two to four adequate samples at screening, baseline, and month 6). During dose titration, 24-h urine samples were collected about 10 days after starting each dose level, and as soon as was practical after an mUFC at or below the ULN was observed. UFC and late-night salivary cortisol were assayed at a central laboratory by high-performance liquid chromatography–tandem mass spectroscopy (normal range for UFC 11–138 nmol/24 h or 4–50 µg/24 h; late-night salivary cortisol [taken at 2200–2300 h ≤2·50 nmol/L or ≤0·09 µg/dL). A therapeutic dose was considered established when either of the following criteria were met: mUFC at or below the ULN or 600 mg twice daily or a maximum-tolerated dose was reached in addition to a clinically meaningful partial response in the opinion of the investigator. Patients for whom a therapeutic dose was established were eligible to continue to the maintenance phase, during which the therapeutic dose was to be held stable, unless change was needed to maintain control of hypercortisolism or in response to safety or tolerability issues. During maintenance, scheduled in-clinic assessments and 24-h urine sample collections occurred monthly.

Outcomes

The primary outcome was the complete response rate, defined as the proportion of patients with mUFC at or below the ULN at the completion of 6 months of therapy in the maintenance phase, without an increase in dose at any time during maintenance. Improvements in hypercortisolism were also assessed as secondary outcomes after each month of maintenance as the proportion of patients with mUFC at or below the ULN without a dose increase; the proportion of patients with mUFC at or below the ULN or at least a 50% reduction from baseline in mUFC without a dose increase; shifts in UFC normality category from baseline, irrespective of dose increase; as change from baseline in mUFC, irrespective of dose increase; and as change from baseline in late-night salivary cortisol.

Cushing's syndrome comorbidity biomarkers related to cardiovascular risk were assessed as key secondary outcomes: changes from baseline after each month of maintenance in fasting glucose concentration, HbA_{1c} (at months 1, 3, and 6), systolic or diastolic blood pressure, total cholesterol concentration, LDL cholesterol concentration, HDL cholesterol concentration, bodyweight, and C-reactive protein (CRP) concentration. Other secondary outcomes were clinical signs and symptoms of Cushing's syndrome (investigator-rated, assessed monthly) and patient-rated quality of life (Cushing's Quality-of-Life questionnaire[20]) and severity of depression (Beck Depression Inventory II [BDI-II][21]), assessed after months 3 and 6 of maintenance. BDI-II was added as an outcome after the study was initiated (part of a formal amendment and early-recruited patients have BDI-II data missing) to provide additional patient-reported outcome data. Several secondary outcomes are not reported here:

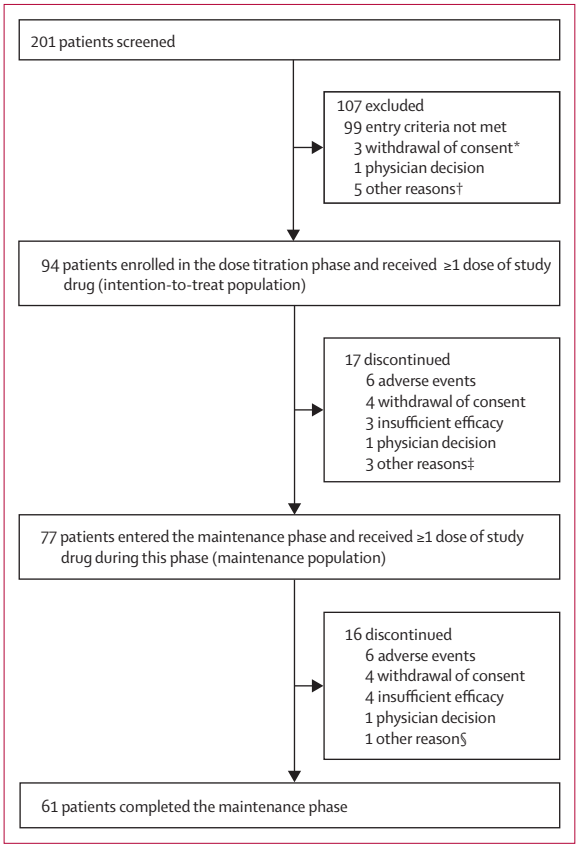


Figure 1: **Patient disposition**
*One patient had two reasons for screen failure (entry criteria not met and withdrawal of consent). †Sponsor decision (n=2), sponsor closed the recruitment (n=2), and patient with fracture before treatment (n=1). ‡Adenocarcinoma of the colon (diagnosed before the first dose of study medication), funder request, patient request (because of poor clinical conditions). §Pituitary surgery.

serum cortisol levels (collected for safety monitoring and not timed), oral glucose tolerance test findings (done only in nine patients with prediabetes in the maintenance population, of whom only five had data at month 6), urine spot albumin-to-creatinine ratio (done only in 14 patients in the maintenance population with an abnormal ratio at baseline, of whom only five had data at month 6), and data for tumour size (to be reported separately along with the long-term data from the extension phase). Exploratory outcomes based on data from the extension phase and exploratory pharmacokinetic and pharmacodynamic analyses will be reported elsewhere.

Safety evaluations included adverse event reports, vital signs, electrocardiograms (ECGs), routine laboratory tests, adrenocorticotropic hormone concentration, and free testosterone concentration (derived from total testosterone [measured with liquid chromatography–tandem mass spectroscopy], serum albumin, and sex hormone binding globulin concentrations [immunoassay] by the Sodergard method[22]). ECGs were recorded over 5 min at baseline, at each dose level during dose titration, and at each monthly visit during the maintenance phase, within about 1–2 h after levoketoconazole administration; these readings were centrally interpreted. Adverse events were coded in accordance with the Medical Dictionary for Regulatory Activities version 20.1 and graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Treatment-emergent adverse events (onset or worsening after one or more doses of levoketoconazole) were reported for the dose-titration and maintenance phases combined, unless otherwise indicated.

Statistical analysis

The intention-to-treat population, which included all patients who received at least one dose of levoketoconazole, was used for assessment of the primary efficacy outcome. The prespecified per-protocol population included intention-to-treat population patients who completed the maintenance phase with no major protocol deviations that might have affected the primary efficacy assessment. The maintenance population, which consisted of all patients who entered the maintenance phase of the study, was used for supportive analyses of the primary outcome and for most secondary outcomes.

For the primary efficacy analysis, the proportions of intention-to-treat and per-protocol responders at the end of month 6 of the maintenance phase were estimated by generalised linear models with repeated measurements for nominal visit, participant as a random effect, and adjustment for baseline covariates: region (US or non-US), diagnoses of diabetes or hypertension, age, sex, Cushing's syndrome duration, previous Cushing's syndrome medication, and previous radiation treatment for Cushing's syndrome. The null hypothesis was that the proportion of responders at the end of the maintenance phase (EoM) was 20% or less. We calculated that an intention-to-treat sample size of 90 patients provided 90% power, with a two-sided type I error of 0·05, assuming an observed rate of 35%. Patients who did not provide an EoM (month 6) mUFC value for any reason (ie, discontinued before EoM, month 6 outside of visit window, fewer than two adequate 24-h urine samples) were considered non-responders, as were patients who had an increase in dose (transient or permanent) during maintenance and patients with a history of radiation who exhibited no rebound increase in mUFC following brief withdrawal of levoketoconazole immediately after EoM. Sensitivity analyses of the primary outcome assessed mUFC normalisation at month 6, irrespective of dose increase, with or without imputation for missing data in the intention-to-treat population; and mUFC normalisation at month 6, irrespective of dose increase in patients who completed the maintenance phase.

Key secondary outcomes of Cushing's syndrome comorbidity biomarker changes from baseline at month 6 (including only those patients with both baseline and month 6 data) were estimated using a generalised linear

model with repeated measurements for nominal visit, participant as a random effect, and baseline covariates as per the primary analysis, apart from inclusion of the corresponding baseline comorbidity biomarker value; p values were assessed for statistical significance and adjusted for multiplicity by use of the Hochberg method (excluding CRP concentration as a separate secondary endpoint and not as a comorbidity biomarker to be included in the multiple testing adjustment), maintaining a family-wise error rate of 0·05. In a post-hoc sensitivity analysis, we also assessed change in LDL cholesterol concentrations after excluding patients on cholesterol-lowering medications. Safety assessments were summarised with descriptive statistics. SAS version 9.1.3 (or higher) was used for statistical analyses. This trial is registered with ClinicalTrials.gov, NCT01838551.

Role of the funding source

The funder contributed to the study design and the analysis and interpretation of the data, provided financial support for editorial assistance, and was involved in the writing of this report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

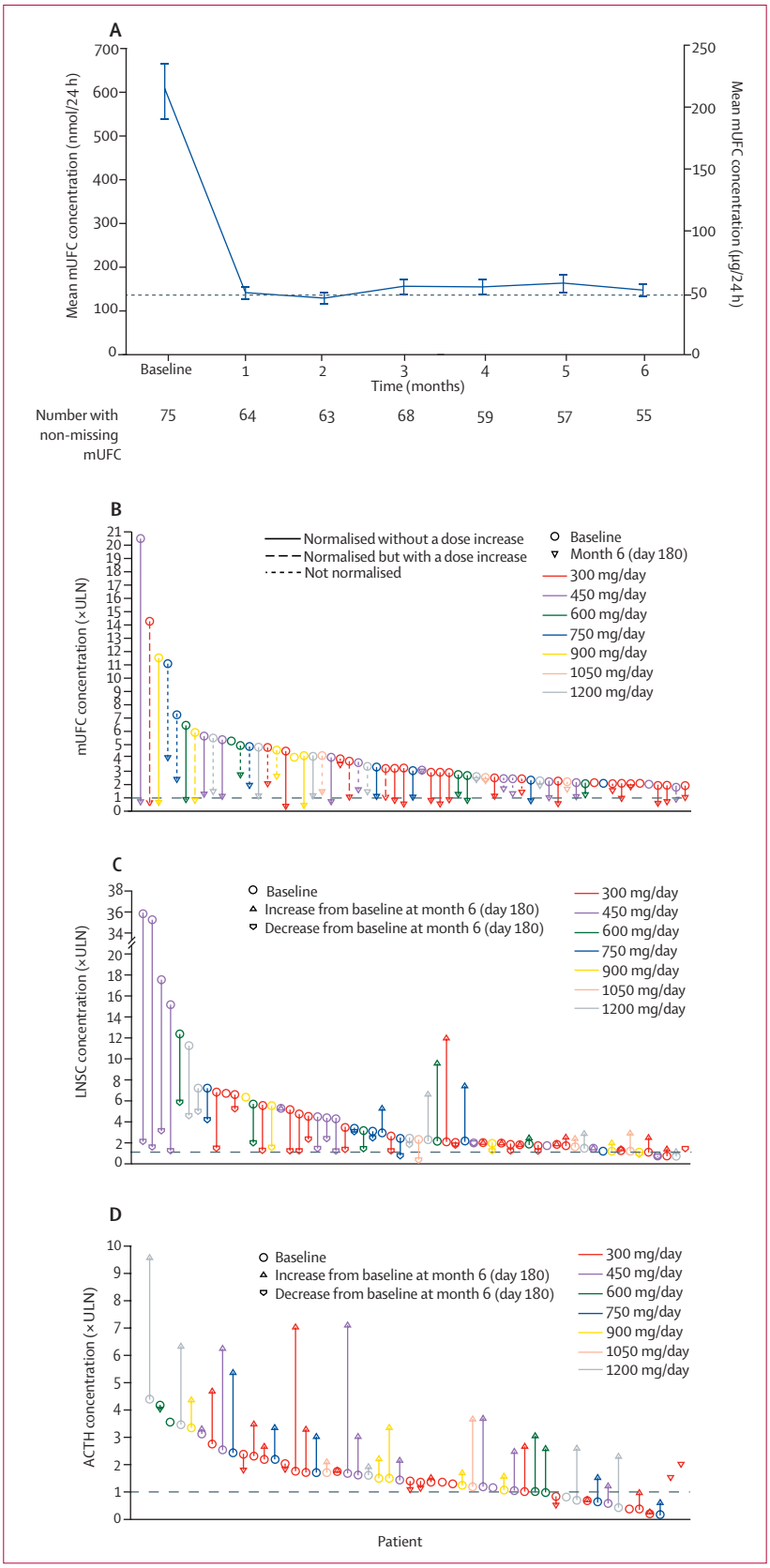
Between July 30, 2014, and June 30, 2017, 201 individuals were screened; 94 were enrolled and received at least one dose of study medication (intention-to-treat population; figure 1, table 1), 80 (85%) of whom had pituitary Cushing's syndrome. 17 (18%) of 94 patients discontinued from the dose-titration phase, therefore, 77 patients entered the maintenance phase. 16 (17%) of 94 patients discontinued from the maintenance phase, and 61 patients completed the maintenance phase. The most common reasons for discontinuation in all phases combined were adverse events (n=12), withdrawal of consent (n=8), and insufficient efficacy (n=7).

In the intention-to-treat population, mean mUFC at baseline was 671·4 nmol/24 h (243·3 µg/24 h; 4·9×ULN). Of the 92 patients with baseline mUFC data, 69 (75%) had mUFC of 2·0 or higher than the ULN. Mean mUFC concentrations were reduced during the dose-titration phase; 62 (81%) of 77 patients entering the maintenance phase had a complete response (mean mUFC ≤ULN) by the end of dose titration (appendix p 7). Mean mUFC remained at approximately the ULN from month 1 through month 6 of maintenance (figure 2A). At EoM, 29 (31%) of 94 patients were responders; the least-squares mean estimate of the proportion of responders was 0·30 (95% CI 0·21–0·40; p=0·0154 *vs* null hypothesis of ≤0·20; table 2). In the prespecified per-protocol analysis, 27 (45%) of 60 patients were responders (one patient was excluded due to a major protocol deviation that might have affected the primary efficacy assessment); the least-squares mean estimate of the proportion of responders was 0·45 (95% CI 0·32–0·59; p=0·0001). The crude proportion of patients with normalised mUFC was 34 (36%) of 94 when five patients who required a dose increase during the maintenance phase were included; therefore, 34 (62%) of the 55 patients who completed the maintenance phase with both baseline and EoM mUFC data had normalised mUFC (figure 2B).

	Patients (n=94)
Age (years)	
Mean	43·7 (13·4)
Median	44·0 (18–75)
Sex	
Women	77 (82%)
Men	17 (18%)
Ethnicity	
White	90 (96%)
Black	1 (1%)
Other	1 (1%)
Unknown	2 (2%)
Mean bodyweight (kg)	84·0 (23·4)
Mean BMI (kg/m²)*	30·8 (8·2)
Time since Cushing's syndrome diagnosis (months)	
Mean	68·0 (80·4)
Median	33·7 (0·7–434·0)
Biological cause	
Cushing's disease	80 (85%)
Ectopic ACTH secretion	1 (1%)
Adrenal dependent	8 (9%)
Unknown	5 (5%)
Diabetes	36 (38%)
Hypertension	67 (71%)
Hypercholesterolaemia	34 (36%)
Baseline mUFC†	
Molar concentration (nmol/24 h)	
Mean	671·4 (743·1)
Median	407·9 (162·0–4168·0)
Mass concentration (µg/24 h)	
Mean	243·3 (269·3)
Median	147·8 (58·7–1510·1)
Baseline mUFC × ULN‡	
Mean	4·9 (5·4)
Median	3·0 (1·2–30·2)§
Previous treatment¶	
Surgery	65 (69%)
Medication	11 (12%)
Radiotherapy	9 (10%)
None	26 (28%)

Data are mean (SD), n (%), or median (range). ACTH=adrenocorticotropic hormone. mUFC=mean urinary free cortisol. ULN=upper limit of normal. *n=93; one patient had missing BMI data because of missing height information. †For each patient, the average of the UFCs from the adequate samples at baseline were calculated; two patients had missing baseline mUFC because of inadequate urine collection. ‡ULN for UFC=138 nmol/24 h (50 µg/24 h). §One patient with mUFC <1·5 × ULN was excluded because of inadequate urine collection. ¶Patients can be included in more than one category.

Table 1: **Baseline characteristics (intention-to-treat population)**



The response rate was higher in patients with lower mUFC concentrations at baseline; conversely, the required dose of levoketoconazole was lower. The final maintenance phase mUFC value was in the normal range for 12 (71%) of 17 patients with baseline mUFC from one and a half times to less than twice the ULN, with a median levoketoconazole dose of 300 mg (range of 300 mg to 600 mg); 19 (49%) of 39 patients with baseline mUFC from twice to less than five times the ULN, with a median dose of 600 mg (300 mg to 1200 mg); and nine (56%) of 16 patients with a baseline mUFC of at least five times the ULN, with a median dose of 750 mg (300 mg to 900 mg).

More patients in the mUFC non-responder group had an initial mUFC greater than the ULN (as assessed at the last visit of the dose-titration phase) than patients in the responder group (mUFC ≤ULN at month 6 irrespective of dose increase; appendix p 8). However, the two groups had qualitatively similar maintenance of their initial mUFC response over time.

Of the nine patients who had received radiation therapy, one was considered a responder in the primary outcome analysis. This patient's mUFC values rebounded and became abnormal 2 weeks after discontinuing the drug at EoM, thus suggesting that UFC normalisation was not due to radiation therapy. Of the eight patients who had received radiation therapy and were considered non-responders, three had month 6 mUFC above ULN, three did not have month 6 mUFC data, and two had a dose increase during maintenance treatment.

Mean late-night salivary cortisol decreased from baseline (11·9 nmol/L [0·43 µg/dL]) to month 6 of maintenance (6·1 nmol/L [0·22 µg/dL]); however, only four patients had normal late-night salivary cortisol levels at EoM (figure 2C), of whom three also had normalised mUFC. In patients with both baseline and EoM late-night salivary cortisol data, the mean change in late-night salivary cortisol from baseline to EoM was –5·8 nmol/L (–0·21 µg/dL [95% CI –10·7 to –0·79 nmol/L; –0·39 to –0·03 µg/dL]; p=0·0239).

Significant mean improvements from baseline at EoM were observed in several of the Cushing's syndrome comorbidity biomarkers: fasting blood glucose concentration, HbA_{1c}, total and LDL cholesterol, and bodyweight (table 3). Of 21 patients with HbA_{1c} values in the range of 5·7% to less than 6·5% (38·8 mmol/mol to

Figure 2: **Cortisol and ACTH responses**
(A) mUFC concentration from baseline of the dose titration phase through to the end of the maintenance phase (month 6; maintenance population). Dotted line represents the ULN for urinary free cortisol (138 nmol/24 h [50 µg/24 h]). Error bars in panel A represent ±1 SE. Baseline mUFC data were missing for two patients in the maintenance population. (B–D) Change in individual concentrations from baseline of the dose titration phase to the end of maintenance phase (month 6; maintenance completers population) for mUFC (B), LNSC (C), and ACTH in a subset of patients with Cushing's disease (D). (B–D) Doses are from day 1 of the maintenance phase; horizontal line represents ULN. ACTH=adrenocorticotrophic hormone. LNSC=late-night salivary cortisol. mUFC=mean urinary free cortisol. ULN=upper limit of normal.

<47·5 mmol/mol) at baseline, ten (48%) had values below 5·7% (38·8 mmol/mol) at month 6 (or last visit in the maintenance phase); and of 16 with baseline HbA_{1c} values of 6·5% (47·5 mmol/mol) or higher, seven (44%) ended with values less than 6·5% (appendix pp 9–11). Of 32 patients with a BMI 30 kg/m² or higher at baseline, nine (28%) ended with a BMI below 30 kg/m². For LDL cholesterol, 22 (46%) of 48 patients with baseline LDL cholesterol concentration greater than or equal to 2·59 mmol/L (100 mg/dL) ended with concentrations below 2·59 mmol/L. Mean serum triglycerides increased slightly from 1·59 mmol/L (140·7 mg/dL) at baseline to 1·63 mmol/L (144·2 mg/dL) at end of maintenance, with a mean change of 0·110 mmol/L (9·7 mg/dL). A significant mean reduction in HDL cholesterol occurred; no significant effect was noted in CRP or in systolic or diastolic blood pressure (table 3). Overall, there was effectively no net change in medications used to treat diabetes (appendix p 12). Six patients started taking cholesterol-lowering medications during maintenance therapy (appendix p 12); however, a post-hoc analysis showed that exclusion of these patients did not affect the mean reduction of cholesterol concentration from baseline to EoM (data not shown).

Significant mean improvements in clinician-rated acne, hirsutism (among women), and peripheral oedema scores were noted (table 3). Other assessed signs and symptoms did not change significantly (table 3). Patient-reported outcomes of quality of life significantly improved from baseline to month 6 (mean change in Cushing's Quality-of-Life score 10·6 points; p<0·0001), as did depression severity (mean change in BDI-II score, –4·3 points; p=0·0043; appendix p 13).

The mean treatment duration for the combined phases was 232·5 (SD 82·3) days (median 237·8 days; range 17–439 days). Overall, 92 (98%) of 94 patients had one or more adverse events. The most commonly reported adverse events were nausea and headache (table 4). 15 adverse events of special interest were reported among 14 patients (15%): liver-related adverse events (n=7), QT prolongation (n=5), and adrenal insufficiency (n=3), with one patient experiencing both QT prolongation and adrenal insufficiency. Most adverse events were of mild or moderate intensity (grade 1–2). The most common severe (grade 3) events were hypertension and increased alanine aminotransferase (ALT; three patients each); one life-threatening, grade 4 event was reported (depression). One person died due to colon carcinoma (detected on study day 45), which was deemed unrelated to levoketoconazole treatment. 12 (13%) of 94 patients discontinued levoketoconazole because of an adverse event; the most common events leading to discontinuation were liver related (six patients). No patients discontinued the study due to an adrenal insufficiency event. Serious adverse events were reported in 14 (15%) patients. Four patients had serious events that were considered probably or definitely related to study drug: abnormal liver function test results (one patient), prolonged QT interval (two patients), and adrenal insufficiency (one patient). The most common serious adverse events (two patients each) were chronic pyelonephritis, muscle weakness, and QT interval prolongation.

	Response rate	Response rate LS mean
mUFC normalisation without a dose increase*†		
Month 1	41/85	0·48 (0·37–0·59)
Month 2	44/88	0·50 (0·39–0·61)
Month 3	41/92	0·44 (0·34–0·55)
Month 4	31/90	0·35 (0·25–0·46)
Month 5	32/90	0·36 (0·26–0·47)
Month 6 (primary endpoint)	29/94	0·30 (0·21–0·40)
mUFC normalisation at month 6 (irrespective of dose increase)*‡	34/94	0·36 (0·26–0·46)
mUFC normalisation at month 6 (irrespective of dose increase, with imputation)*‡§	36/94	0·38 (0·28–0·49)
Analysis of observed rate at month 6 with imputation for missing mUFC after month 3‡¶	40/94	0·43 (0·32–0·53)
≥50% mUFC decrease or normalisation at month 6 (irrespective of dose increase)*‡	43/94	0·46 (0·35–0·56)
≥50% mUFC decrease or normalisation at month 6 (irrespective of dose increase, with imputation)*‡§	45/94	0·48 (0·37–0·58)
Participants who completed the maintenance phase with mUFC data and mUFC normalisation at month 6 (irrespective of dose increase)‡‖	34/55 (62%)	..
Participants who completed the maintenance phase with mUFC data and ≥50% mUFC decrease or normalisation at month 6 (irrespective of dose increase)‡‖	43/55 (78%)	..

Data are n/N, n/N (%), or LS mean (95% CI). UFC=urinary free cortisol. LS=least-squares. mUFC=mean urinary free cortisol. EoM=end of maintenance (month 6). *Based on mixed-effects, repeated-measures model with underlying binomial distribution and logit link function, adjusted for baseline covariates. †Patients who had discontinued were considered non-responders at all timepoints after discontinuation; patients with missing mUFC data were not included in the analyses for months 1–5 but were considered non-responders in the month 6 analysis. ‡Sensitivity analysis. §Imputed mUFC as normal for a missing value at EoM if mUFC was normal at preceding and subsequent visits. ¶Imputed missing value at EoM as last non-missing mUFC from latest of months 3, 4, or 5. CI is from the Clopper-Pearson two-sided 95% CI for the one-sample binomial proportion. ||Data based on 55 maintenance phase completers with both baseline and month 6 mUFC data available.

Table 2: **UFC responder analyses (intention-to-treat population)**

Routine laboratory assessments showed ALT increases above the ULN in 39 (41%) of 94 patients at any time before EoM, including seven patients with an ALT concentration of between more than three times and five times the ULN and three patients with ALT more than five times the ULN. Of the 90 patients with normal ALT concentrations at baseline, 36 (40%) had an abnormal value at least once during maintenance phase. Among the ten (11%) patients with an ALT value of more than three times the ULN, the highest ALT increases occurred by the month 2 (day 60) visit of the maintenance phase and were fully reversible; eight of these patients discontinued from the study (four because of ALT increase, four for other reasons) and two completed maintenance, with no clinical sequelae in any patient.

Mean adrenocorticotropic hormone concentrations in the subset of 80 patients diagnosed with Cushing's disease increased during dose titration and remained raised through the maintenance phase. Mean adrenocorticotropic

	Baseline mean (SD; n)	LS mean or mean change from baseline to EoM (SE; 99% CI or 95% CI; n)	p value
Comorbidity biomarker*†			
Fasting blood glucose	..	..	<0·0001
Molar concentration (mmol/L)	5·8 (2·0; 76)	−0·68 (0·09; −0·93 to −0·43; 50)	..
Mass concentration (mg/dL)	103·6 (35·5; 76)	−12·3 (1·7; −16·8 to −7·7; 50)	..
HbA_{1c} (%)	6·0 (1·1; 77)	−0·39 (0·08; −0·61 to −0·18; 55)	<0·0001
Systolic blood pressure (mm Hg)	133·7 (15·8; 77)	0·34 (2·02; −5·1 to 5·7; 54)	0·87
Diastolic blood pressure (mm Hg)	83·6 (12·8; 77)	−0·89 (1·32; −4·4 to 2·6; 54)	0·87
Total cholesterol	..	..	<0·0001
Molar concentration (mmol/L)	5·6 (1·4; 75)	−1·1 (0·09; −1·4 to −0·9; 53)	..
Mass concentration (mg/dL)	218·1 (52·7; 75)	−43·0 (3·4; −52·1 to −33·9; 53)	..
LDL cholesterol	..	..	<0·0001
Molar concentration (mmol/L)	3·3 (1·2; 75)	−0·97 (0·08; −1·2 to −0·8; 53)	..
Mass concentration (mg/dL)	127·3 (45·7; 75)	−37·3 (2·9; −45·1 to −29·6; 53)	..
HDL cholesterol	..	..	<0·0001
Molar concentration (mmol/L)	1·6 (0·5; 75)	−0·20 (0·04; −0·30 to −0·10; 53)	..
Mass concentration (mg/dL)	63·1 (17·4; 75)	−7·7 (1·5; −11·6 to −3·9; 53)	..
Bodyweight (kg)	82·1 (22·5; 77)	−5·1 (0·81; −7·3 to −3·0; 54)	<0·0001
C-reactive protein	..	..	0·15
Molar concentration (nmol/L)	37·9 (43·4; 74)	10·1 (6·8; −8·3 to 28·5; 50)	..
Mass concentration (mg/L)	4·0 (4·6; 74)	1·1 (0·7; −0·9 to 3·0; 50)	..
Clinical signs and symptoms*‡			
Acne global score§	2·8 (5·8; 75)	−1·8 (0·7; −3·1 to −0·5; 51)	0·0063
Hirsutism total score (women only)¶	7·8 (5·7; 60)	−2·6 (0·7; −4·1 to −1·1; 44)	0·0008
Peripheral oedema total score‖	1·0 (1·8; 75)	−0·4 (0·2; −0·8 to 0·0; 49)	0·030
Total score for seven other clinical signs and symptoms**††	4·3 (3·8; 61)	−0·8 (0·5; −1·9 to 0·2; 44)	0·11

LS=least squares. EoM=end of maintenance (month 6). *Analysis is based on the 77 patients who entered the maintenance phase. †LS mean change from baseline from a generalised linear model with repeated measurements adjusted for baseline covariates; 99% CI for the LS mean change of biomarkers of Cushing's syndrome comorbidities outcomes; p values based on LS mean change from baseline to EoM (Hochberg adjustment applied to p values to control type 1 error at 0·05, apart from C-reactive protein, which was not identified as a Cushing's syndrome comorbidity biomarker in the statistical analysis plan). ‡95% CI information for the mean change of clinical signs and symptoms outcomes; p values from the paired t test performed on the change from baseline to EoM. §Acne global score: range from 0 to 44, where 0=none, 1–18=mild, 19–30=moderate, 31–38=severe, and ≥39=very severe. ¶Hirsutism total score: range from 0 (none) to 36 (worst). ‖Peripheral oedema total score: range from 0 (none) to 12 (worst). **The seven clinical signs and symptoms are moon facies, facial plethora, striae, bruising, supraclavicular fat, irregular menstruation (women only), and dysmenorrhoea (women only); each symptom was graded as none (score=0), mild (1), moderate (2), or severe (3); since only five of seven signs and symptoms apply to men, their total score is multiplied by seven and divided by five to standardise it to the same scale as the total score for women; total score can range from 0 (none) to 21 (worst). ††Only patients who consented to study participation under protocol amendments 5 and 6 had this assessment.

Table 3: **Change from baseline to month 6 of maintenance phase in Cushing's syndrome comorbidity biomarkers and clinical signs and symptoms (maintenance population)**

	Patients (n=94)
Any adverse event	92 (98%)
Serious adverse event	14 (15%)
Drug-related adverse event*	40 (43%)
Adverse event leading to discontinuation	12 (13%)
Intensity of adverse events	
Mild	21 (22%)
Moderate	54 (57%)
Severe	15 (16%)
Life-threatening	1 (1%)
Death	1 (1%)
Most common adverse events†	
Nausea	30 (32%)
Headache	26 (28%)
Peripheral oedema	18 (19%)
Hypertension	16 (17%)
Fatigue	15 (16%)
Diarrhoea	14 (15%)
ALT increased‡	14 (15%)
GGT increased‡	12 (13%)
AST increased‡	11 (12%)
Nasopharyngitis	11 (12%)
Urinary-tract infection	11 (12%)
Arthralgia	10 (11%)
Dizziness	10 (11%)
Dry skin	10 (11%)
Hypokalaemia	10 (11%)
Myalgia	10 (11%)
Vomiting	10 (11%)

Data are n (%). ALT=alanine aminotransferase. AST=aspartate aminotransferase. GGT=γ-glutamyltransferase. *Assessed by the investigator as probably or definitely related to study drug. †Reported in ≥10% of patients. ‡Includes all ALT, AST, GGT increases reported as an adverse event irrespective of concentration recorded or relation to drug.

Table 4: **Summary of adverse events (both phases combined; intention-to-treat population)**

hormone concentration was 10·1 pmol/L (46·0 pg/mL; 1·5×ULN) at baseline and 18·5 pmol/L (83·9 pg/mL; 2·9×ULN) at EoM (mean change 8·1 pmol/L [36·7 pg/mL]). The largest increase at EoM was to 9·6 times the ULN; 28 (61%) of 46 patients with available data had adrenocorticotropic hormone concentrations of more than twice the ULN at EoM (figure 2D). There was no correlation between change (from baseline to month 6) in adrenocorticotropic hormone concentrations and change in mUFC (r=0·22; p=0·1701).

Mean free testosterone concentrations decreased significantly in women between baseline and EoM (from 0·011 to 0·004 nmol/L [0·3 ng/dL to 0·1 ng/dL]; p<0·0001) and increased numerically (but not significantly) in men (from 0·177 to 0·202 nmol/L [5·1–5·8 ng/dL]; p=0·38).

Nine (10%) of 94 patients had one or more Fridericia-corrected QT value representing an increase of more than 60 ms from baseline, and two patients (2%) had one or more confirmed Fridericia-corrected QT intervals of more than 500 ms, both predefined thresholds. In five patients (5%) for whom QT prolongation was an adverse event of special interest, the QT increase was reversed with temporary drug interruption, and all patients affected were able to resume study medication at the same or lower dose. No arrhythmias or changes in conduction intervals other than QT were reported. Potassium values were often less than 4·0 mmol/L among patients with a corrected QT interval of more than 470 ms (experienced by 67% of patients). The majority of cases of QT prolongation were considered by

the investigator as probably related to or definitely related to levoketoconazole treatment.

Discussion

The results of the phase 3 SONICS trial showed the efficacy and safety of levoketoconazole, the 2S,4R enantiomer of ketoconazole, in a representative population of patients with endogenous Cushing's syndrome. The primary efficacy outcome of mUFC normalisation without a preceding dose increase at EoM was achieved in 30% of the patients. Although the study was open-label and uncontrolled, the design included key elements that strengthen the efficacy conclusion. There was no upper limit of mUFC concentration at enrolment, and most participants had moderate-to-severe baseline hypercortisolism (69 [75%] of 92 with baseline data had mUFC ≥2·0×ULN). Furthermore, patients with cyclic Cushing's syndrome were excluded, inadequate urine samples were not used in the calculation of mUFC, and measures of key outcomes were done monthly and analysed with a repeated-measures model on the basis of intention-to-treat principles. For these reasons, the sustained reductions in cortisol production, as measured by 24-h mUFC and late-night salivary cortisol, might be inferred as largely, if not entirely, drug related. Further support from sensitivity (table 2) and secondary analyses of mUFC response were directionally consistent with and implied a greater efficacy than the primary outcome analysis, indicative of a conservative primary analysis inference.

Initial normalisation during dose titration and sustained normalisation at month 6 of the maintenance phase seemed to be at least partly a reflection of the baseline UFC concentration. All doses studied (300–1200 mg per day) were effective at lowering mUFC without a clear dose-response relation; however, efficacy, as determined by normalisation of mUFC at month 6, diminished at the higher end of the dose range. This diminished efficacy was presumably related to the higher average baseline mUFC in the higher-dose groups (ie, greater reductions in mUFC were needed to achieve and maintain normalisation). Even so, levoketoconazole was effective in patients with severe hypercortisolism: mUFC was normalised at the last maintenance phase assessment in nine (56%) of 16 of patients who had baseline mUFC levels at least five times the ULN.

Investigators were instructed to titrate doses slowly, with no more than 150 mg added every 2 weeks, which might help to account for the low incidence (three patients [3%]) of adrenal insufficiency over the combined dose-titration and maintenance phases. They were also instructed to advance patients into the maintenance phase immediately on the first confirmation of mUFC within the normal range, rather than requiring all four samples to be within the normal range and waiting a month or more to determine if mUFC was stable. It is not yet known whether a more aggressive titration scheme would be more effective, as well tolerated, or safe.

Secondary outcomes indicative of subjective clinical benefits included improvements in signs and symptoms of Cushing's syndrome, such as those associated with androgen excess and reductions in fluid retention, depression, and collectively, those associated with impaired quality of life. These improvements were observed despite baseline scores indicating that symptoms affecting appearance, daily functioning, and quality of life were generally of mild to moderate severity. This finding suggests the need for a study of the effects of therapy on patients with more severe subjective impairments.

Cardiovascular disease is believed to be responsible for most of the excess mortality and substantial morbidity in Cushing's syndrome, and some studies have shown that cardiovascular risk persists even after biochemical cure.[4,23,24] In view of the rarity of Cushing's syndrome, cardiovascular outcome studies are infeasible. Thus, observing changes in established cardiovascular risk markers is a practical means by which various therapies can be judged as to their potential ability to prevent major adverse cardiovascular events. Treatment with levoketoconazole produced notable improvements in biomarkers of cardiovascular risk, including significant mean reductions in total and LDL cholesterol, HbA_{1c}, fasting blood glucose concentration, and bodyweight, accompanied by shifts in the population towards healthy ranges. The cardiovascular risk marker improvements observed do not seem to have been due to changes in concomitant medication use. In opposition to these improvements, a small but significant mean decrease in HDL cholesterol was seen, and mean serum triglycerides increased slightly. The mechanism underlying the decrease in HDL cholesterol is unknown but might be related to ketoconazole-like effects on bile acid synthesis and cholesterol homeostasis, or to effects on cortisol (the glucocorticoid antagonist mifepristone has also been shown to reduce serum HDL cholesterol concentrations[25]). No improvement in blood pressure was identified.

An important limitation of our study is that we did not do a direct efficacy comparison between levoketoconazole and ketoconazole, and indirect comparison is problematic because of the absence of prospective studies assessing the efficacy of ketoconazole in Cushing's syndrome. Based on retrospective data collected from patients who had received long-term treatment with ketoconazole, in some cases for many years, efficacy has been reported as mUFC normalisation in 45–50% of patients.[26,27] However, retrospective chart reviews of patients being treated clinically are prone to bias, as an unknown proportion of non-responders might not be counted, and the methods used to ascertain mUFC normalisation were not standardised. Additionally, in clinical practice a single UFC (and not average of repeated samples) is typically measured at any one time. Likewise, we are unaware of data in a similar population that shows salutary effects of ketoconazole therapy on Cushing's syndrome

comorbidities, signs and symptoms, or patient-reported outcomes, as were shown in the present study.

Levoketoconazole inhibits several adrenal enzymes, including 17α-hydroxylase and the cholesterol side-chain cleavage enzyme,[11,12] and does not result in excess testosterone concentrations in women, by contrast with medications that preferentially block 11β-hydroxylase.[28] Mean free testosterone concentrations decreased significantly in women with Cushing's syndrome in this study, with concomitant improvements in hirsutism and acne. By contrast with racemic ketoconazole, which can induce hypogonadism in men, mean free testosterone concentration increased (numerically, but not significantly) in men in this study. As expected with any adrenal steroidogenesis inhibitor administered to patients with Cushing's disease, mean adrenocorticotropic hormone concentrations increased in this study; 80 patients (85% of the study population) had Cushing's disease, and more than half of those with an adrenocorticotropic hormone assessment at EoM had concentrations of at least twice the ULN.

No unexpected safety signals were observed. Nausea and headache were the most common adverse events, but they rarely affected treatment, and there was little evidence that any adverse event was dose related. Some cases of nausea and headache might possibly have been a result of relative cortisol deficiency (ie, cortisol withdrawal). Potentially important side-effects that were anticipated at the outset of the study, including adrenal insufficiency, QT interval prolongation, and liver enzyme increases, were observed at frequencies and severities that were manageable, as evidenced by the relatively low proportion of participants who discontinued the study because of adverse events (12 patients [13%]). The liver test abnormalities suggest uncommon, idiosyncratic (ie, not clearly dose related), drug-related effects that were usually of mild to moderate severity and fully reversible by stopping the study drug, without any clinical sequelae. The most relevant comparison data with ketoconazole come from a prospective, observational, compassionate-use registry study in Cushing's syndrome in France.[14] In that study, the incidence of ALT at least five times the ULN in patients with Cushing's syndrome previously untreated with ketoconazole, assessed over a similar mean period of follow-up time as SONICS, was 13% (four of 31 patients), as compared with 3% (three of 94 patients) in SONICS.

Ketoconazole prolongs the QT interval, presumably at least partly through inhibition of the *hERG* (human ether-a-go-go related gene; encoding Kv11.1, the rapidly activating component of the cardiac delayed rectifier potassium channel) IKr channel.[29,30] Levoketoconazole has a similar effect on the inhibition of the IKr channel in vitro as ketoconazole, but with less potency (unpublished data). In SONICS, nine patients had an increase in Fridericia-corrected QT value from baseline that exceeded 60 ms, a threshold used for signal detection, and two patients exceeded an interval duration of 500 ms, which has been associated with an increased risk of arrhythmia in long QT syndromes.[16] Some cases were confounded by hypokalaemia and other drugs that can prolong QT; however, most were considered by the investigator as probably related to levoketoconazole treatment, and in some cases the T-wave morphology suggested IKr inhibition.[31] No arrhythmias or changes in conduction intervals other than QT were observed.

As with ketoconazole, the potential for drug interactions must be considered with levoketoconazole. Patients taking specific medications were excluded from this study, and careful examination of patients' medication lists for possible drug–drug interactions, as well as gastric acid inhibition, will be necessary when considering levoketoconazole for the treatment of Cushing's syndrome.

Limitations to this study include the open-label design and absence of a control group, which can introduce observer bias in subjective measures such as Cushing's syndrome clinical scores and quality-of-life measures. Use of a concurrent placebo control was considered unethical in a long-term study, in view of the known risks of withholding therapy in moderate-to-severe Cushing's syndrome. Although inclusion of an active comparator (specifically ketoconazole) would have provided important information, this design was considered impractical, as a large sample size would be needed. Additionally, in many countries where this study was done, ketoconazole is not approved for Cushing's syndrome and, as such, could not be used as a comparator in this clinical trial. As this was not a comparative study, the results cannot be used as the basis for any comparative or cost-effectiveness discussion. Notably, this study is the first prospective trial of any ketoconazole-derived drug for treatment of Cushing's syndrome and, thus, there are no similar studies of ketoconazole available for comparison. Strict inclusion and exclusion criteria in this study limited patient heterogeneity and, since most patients were white, generalisability of the findings to patients of other races and ethnic groups might be limited.

The medical needs in Cushing's syndrome are very high despite the availability of approved treatments.[32] In this relatively large, prospective, international study, in which 28% of patients had no previous therapy, levoketoconazole was effective for reducing and normalising mUFC concentrations and biomarkers of cardiovascular risk, as well as improving clinical signs and quality of life in patients with endogenous Cushing's syndrome. Since oral levoketoconazole was also generally well tolerated, and had risks that were manageable with appropriate monitoring, the drug has the potential to be an important therapeutic option for patients with Cushing's syndrome.

Contributors

The study was designed by MF and BMKB in collaboration with the study funder. The investigators enrolled patients in the study. All investigators collected data using data management systems provided by a contract research organisation. Data were analysed by statisticians at a separate contract research organisation or by the funder's statistician. All authors contributed to interpreting the data and writing and revising the report.

Declaration of interests
MF reports research grants to Oregon Health & Science University from Millendo, Novartis, and Strongbridge Biopharma; and serving as an occasional consultant to Novartis and Strongbridge Biopharma. RP reports research grants to Università di Napoli Federico II from Corcept Therapeutics, Novartis, and Strongbridge Biopharma; and receiving consulting honoraria from Novartis and Strongbridge Biopharma. AE reports receiving consulting honoraria from Novartis. RS reports research grants to Johns Hopkins University from Novartis and Strongbridge Biopharma. RJA reports research grants to the University of Michigan from Corcept Therapeutics, Novartis, and Strongbridge Biopharma; and serving as a consultant for Corcept Therapeutics, Novartis, and Strongbridge Biopharma. RAF reports research grants from Novartis; and serving on speakers' bureaux for HRA Pharma and Novartis. EBG reports research grants to Memorial Sloan Kettering Cancer Center from Ionis, Novartis, and Strongbridge Biopharma; and serving as an occasional consultant to Novartis and Strongbridge Biopharma. YG reports research grants to Tel Aviv Sourasky Medical Center from Chiasma, Novartis, and Strongbridge Biopharma; and receiving lecture fees from Medison, Novartis, and Pfizer. PW reports receiving travel grants from Ipsen and Novartis; and receiving personal fees as a clinical investigator from Ipsen, Novartis, Novo Nordisk, and Strongbridge Biopharma. FC is an employee of Strongbridge Biopharma. BMKB reports research grants to Massachusetts General Hospital from Millendo, Novartis, and Strongbridge Biopharma; and serving as an occasional consultant to Novartis and Strongbridge Biopharma.

Data sharing
There are no plans to share the data from this trial publicly.

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References

1 Biller BMK, Grossman AB, Stewart PM, et al. Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. *J Clin Endocrinol Metab* 2008; **93:** 2454–62.
2 Creemers SG, Hofland LJ, Lamberts SW, Feelders RA. Cushing's syndrome: an update on current pharmacotherapy and future directions. *Expert Opin Pharmacother* 2015; **16:** 1829–44.
3 Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. *Lancet Diabetes Endocrinol* 2016; **4:** 611–29.
4 Ragnarsson O, Olsson DS, Papakokkinou E, et al. Overall and disease-specific mortality in patients with Cushing's disease: a Swedish nationwide study. *J Clin Endocrinol Metab* 2019; **104:** 2375–84.
5 Pivonello R, De Leo M, Cozzolino A, Colao A. The treatment of Cushing's disease. *Endocr Rev* 2015; **36:** 385–486.
6 Nieman LK, Biller BMK, Findling JW, et al. Treatment of Cushing's syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2015; **100:** 2807–31.
7 Fleseriu M. Medical treatment of Cushing disease: new targets, new hope. *Endocrinol Metab Clin North Am* 2015; **44:** 51–70.
8 Feelders RA, Newell-Price J, Pivonello R, Nieman LK, Hofland LJ, Lacroix A. Advances in the medical treatment of Cushing's syndrome. *Lancet Diabetes Endocrinol* 2019; **7:** 300–12.
9 European Medicines Agency. Ketoconazole HRA 200 mg tablets. London: European Medicines Agency, 2015.
10 Ketoconazole tablets USP, 200 mg [package insert]. Haifa Bay: Taro Pharmaceutical Industries, 2017.
11 Fleseriu M, Castinetti F. Updates on the role of adrenal steroidogenesis inhibitors in Cushing's syndrome: a focus on novel therapies. *Pituitary* 2016; **19:** 643–53.
12 Auchus RJ, Wu Y, Liu J. 2S,4R-ketoconazole is the relevant enantiomer of ketoconazole for cortisol synthesis inhibition: steroidogenic P450s inhibition involves multiple mechanisms. *Endocr Rev* 2018; **39** (2 suppl): SUN-410 (abstr).
13 European Medicines Agency, Committee for Medicinal Products for Human Use. Ketoconazole HRA: assessment report. London: European Medicines Agency, 2014.
14 Young J, Bertherat J, Vantyghem MC, et al. Hepatic safety of ketoconazole in Cushing's syndrome: results of a compassionate use programme in France. *Eur J Endocrinol* 2018; **178:** 447–58.
15 Boyce MJ, Baisley KJ, Warrington SJ. Pharmacokinetic interaction between domperidone and ketoconazole leads to QT prolongation in healthy volunteers: a randomized, placebo-controlled, double-blind, crossover study. *Br J Clin Pharmacol* 2012; **73:** 411–21.
16 Heist EK, Ruskin JN. Drug-induced arrhythmia. *Circulation* 2010; **122:** 1426–35.
17 Vermeer LM, Isringhausen CD, Ogilvie BW, Buckley DB. Evaluation of ketoconazole and its alternative clinical CYP3A4/5 inhibitors as inhibitors of drug transporters: the in vitro effects of ketoconazole, ritonavir, clarithromycin, and itraconazole on 13 clinically-relevant drug transporters. *Drug Metab Dispos* 2016; **44:** 453–59.
18 Chin TW, Loeb M, Fong IW. Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrob Agents Chemother* 1995; **39:** 1671–75.
19 Schwartz SL, Rendell M, Ahmann AJ, Thomas A, Arauz-Pacheco CJ, Welles BR. Safety profile and metabolic effects of 14 days of treatment with DIO-902: results of a phase IIa multicenter, randomized, double-blind, placebo-controlled, parallel-group trial in patients with type 2 diabetes mellitus. *Clin Ther* 2008; **30:** 1081–88.
20 Webb SM, Badia X, Barahona MJ, et al. Evaluation of health-related quality of life in patients with Cushing's syndrome with a new questionnaire. *Eur J Endocrinol* 2008; **158:** 623–30.
21 Beck A, Steer R, Brown G. Beck Depression Inventory. 2nd edn. San Antonio, TX: Psychological Corporation, 1996.
22 Södergård R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem* 1982; **16:** 801–10.
23 Clayton RN, Jones PW, Reulen RC, et al. Mortality in patients with Cushing's disease more than 10 years after remission: a multicentre, multinational, retrospective cohort study. *Lancet Diabetes Endocrinol* 2016; **4:** 569–76.
24 van Haalen FM, Broersen LH, Jorgensen JO, Pereira AM, Dekkers OM. Management of endocrine disease: mortality remains increased in Cushing's disease despite biochemical remission: a systematic review and meta-analysis. *Eur J Endocrinol* 2015; **172:** R143–49.
25 Page ST, Krauss RM, Gross C, et al. Impact of mifepristone, a glucocorticoid/progesterone antagonist, on HDL cholesterol, HDL particle concentration, and HDL function. *J Clin Endocrinol Metab* 2012; **97:** 1598–605.
26 Castinetti F, Guignat L, Giraud P, et al. Ketoconazole in Cushing's disease: is it worth a try? *J Clin Endocrinol Metab*. 2014; **99:** 1623–30.
27 Castinetti F, Morange I, Jaquet P, Conte-Devolx B, Brue T. Ketoconazole revisited: a preoperative or postoperative treatment in Cushing's disease. *Eur J Endocrinol* 2008; **158:** 91–99.
28 Fleseriu M, Pivonello R, Young J, et al. Osilodrostat, a potent oral 11β-hydroxylase inhibitor: 22-week, prospective, phase II study in Cushing's disease. *Pituitary* 2016; **19:** 138–48.
29 Dumaine R, Roy ML, Brown AM. Blockade of HERG and Kv1.5 by ketoconazole. *J Pharmacol Exp Ther* 1998; **286:** 727–35.
30 Takemasa H, Nagatomo T, Abe H, et al. Coexistence of hERG current block and disruption of protein trafficking in ketoconazole-induced long QT syndrome. *Br J Pharmacol* 2008; **153:** 439–47.
31 Vicente J, Johannesen L, Mason JW, et al. Comprehensive T wave morphology assessment in a randomized clinical study of dofetilide, quinidine, ranolazine, and verapamil. *J Am Heart Assoc* 2015; **4:** e001615.
32 Geer EB, Shafiq I, Gordon MB, et al. Biochemical control during long-term follow-up of 230 adult patients with Cushing disease: a multicenter retrospective study. *Endocr Pract* 2017; **23:** 962–70.

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.

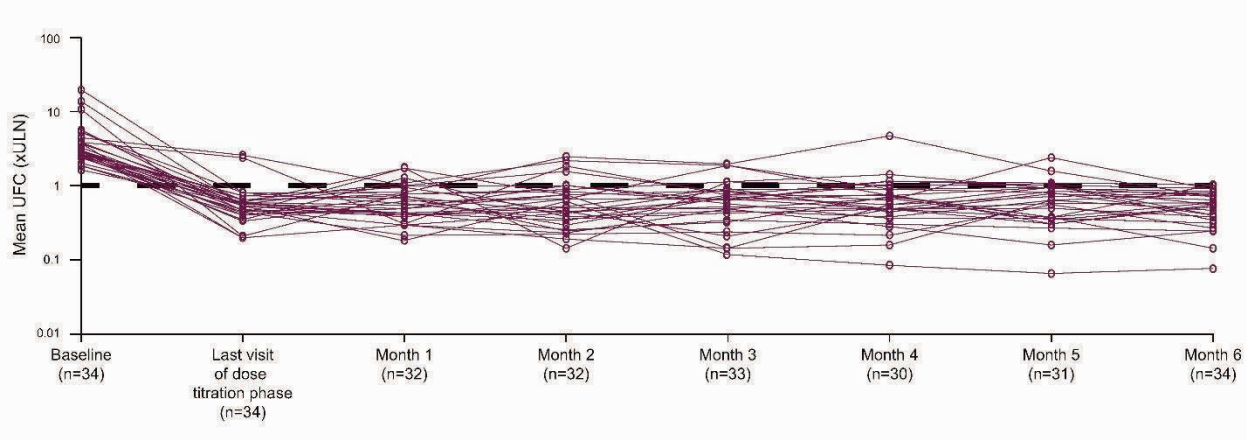
Table S4. Clinical responses for patients entering the maintenance phase

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)

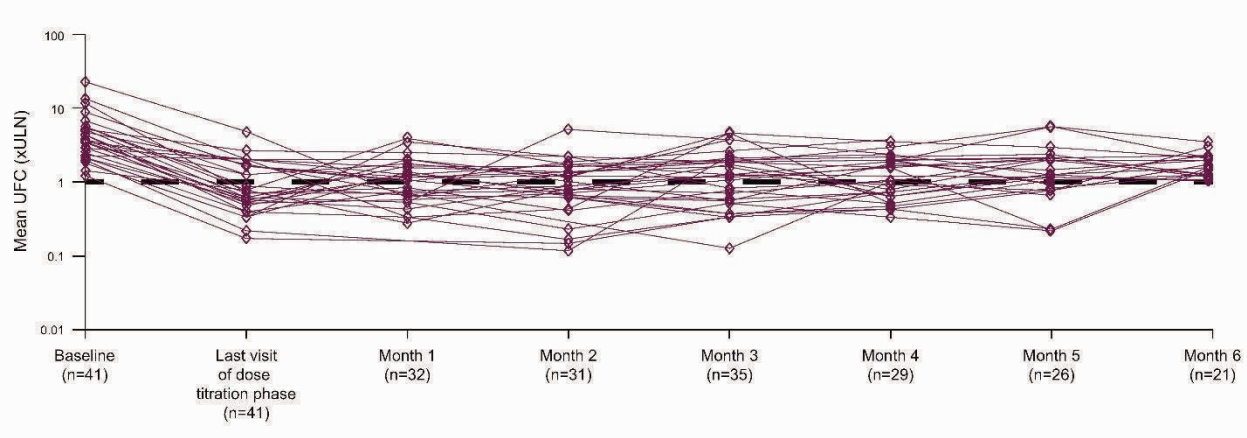
*Three patients were switched to another dose at the start of the maintenance phase. one patient was switched from 300 to 450 mg/day; one patient from 450 to 300 mg/day; and one from 600 to 450 mg/day. Therefore, the number of patients in the maintenance phase are 24, 11, 12, 10, 8, 3, and 9 for 300, 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%)

	≥1·55 mmol/L (60 mg/dL) (n=40)	<1·03 mmol/L 1·03–<1·55 mmol/L ≥1·55 mmol/L	3 (8%) 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%)

		25·0–<30·0 kg/m^2	11 (42%)
		30·0–<40·0 kg/m^2	1 (4%)
		≥40 kg/m^2	0
	30·0–<40·0 kg/m^2 (n=25)	<18·5 kg/m^2	0
		18·5–<25·0 kg/m^2	2 (8%)
		25·0–<30·0 kg/m^2	7 (28%)
		30·0–<40·0 kg/m^2	15 (60%)
		≥40 kg/m^2	1 (4%)
	≥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	0
		30·0–<40·0 kg/m^2	2 (29%)
		≥40 kg/m^2	5 (71%)

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.

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