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Efficacy and safety of osilodrostat in patients with Cushing's disease (LINC 3): a Phase III multicentre study with a double-blind, randomised withdrawal phase

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Abstract

Background: Cushing's disease is a rare endocrine disorder characterised by cortisol overproduction, with severe complications. Therapies for cortisol reduction are often necessary. Outcomes from the pivotal Phase III study of osilodrostat (new, potent oral 11β-hydroxylase inhibitor) in Cushing's disease patients (LINC 3; NCT02180217) are reported.

Methods: LINC 3 (prospective, multicentre study) comprised four periods. Cushing's disease patients with mean urinary free cortisol (mUFC)>1·5xULN were enrolled. Period 1: open-label osilodrostat was initiated and adjusted every 2 weeks (1–30mg twice daily) based on mUFC and safety until week (W)12. Period 2 (W13–24): osilodrostat was continued at the therapeutic dose determined during period 1. Period 3 (W26): 71 eligible participants (mUFC≤ULN at W24 without up-titration after W12) were randomised (1:1 via interactive-response technology; double blind, stratified by osilodrostat dose at W24 and history of pituitary irradiation) to continue osilodrostat (n=36) or switch to placebo (n=35) for 8 weeks. Ineligible participants continued open-label osilodrostat. Period 4: all participants then received open-label osilodrostat until core-study end (W48). Primary objective: compare osilodrostat efficacy versus placebo at end of period 3. Primary endpoint: proportion of randomised participants with mUFC≤ULN at end of randomised withdrawal (W34), without up-titration during this period.

Findings: 137 patients were enrolled. The primary objective was met: more patients maintained mUFC≤ULN with osilodrostat versus placebo at W34 (86·1% vs 29·4%; OR 13·7 [95%CI 3·7,53·4], *P*<0·001). At W24, 72/137 (52·6%; 95%CI 43·9,61·1) patients maintained mUFC≤ULN without up-titration after W12. At W48, 66·4% of enrolled patients had mUFC≤ULN. Most common adverse events (>25% of all patients): nausea (n=57, 41·6%), headache (n=46, 33·6%), fatigue (n=39, 28·5%), and adrenal insufficiency (n=38, 27·7%). Hypocortisolism and adverse events related to adrenal hormone precursors occurred in 70 (51·1%) and 58 (42·3%) patients, respectively.

Interpretation: Osilodrostat is an effective new treatment option for Cushing's disease patients.

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Introduction

Cushing's disease is a rare, serious disorder caused by an adrenocorticotropic hormone (ACTH)-secreting pituitary tumour, which stimulates the adrenal glands to overproduce cortisol.[1] Excess cortisol leads to an increased mortality risk largely driven by cardiovascular disease and infections.[2,3] First-line treatment for most patients is transsphenoidal surgery.[4] However, additional second-line treatments are often needed as persistent or recurrent Cushing's disease after surgery has been documented in approximately one-third of patients.[2] For these patients or in cases where surgery or radiotherapy are not possible, medical therapies are used. Several options with various mechanisms of action are available, including a multireceptor-targeted somatostatin analogue, dopamine receptor agonists, steroidogenesis inhibitors, adrenolytic agents, and glucocorticoid receptor antagonists.[5] Despite the availability of multiple medical therapies, Cushing's disease remains a difficult-to-treat disorder that often necessitates a multimodal treatment approach.[7-9] Additional effective and well-tolerated medical therapy options with alternative mechanisms of action are, therefore, needed to improve long-term outcomes for patients.[10,11]

Osilodrostat (LCI699) is a potent, oral, reversible inhibitor of 11β-hydroxylase (CYP11B1), the enzyme that catalyses the final step of cortisol synthesis in the adrenal cortex. In a 22-week, prospective, Phase II study of osilodrostat in patients with Cushing's disease (LINC 2),[12,13] osilodrostat rapidly reduced mean urinary free cortisol (mUFC), leading to normalised mUFC in most patients (79%; n=15/19) at week 22. Treatment was generally well tolerated; the most common adverse events (AEs) included nausea, diarrhoea, asthenia, and adrenal insufficiency, mostly mild or moderate in severity.[12]

The current manuscript reports the outcomes of a multicentre, open-label, Phase III study that assessed the efficacy and safety of osilodrostat in patients with Cushing's disease (LINC 3; clinicaltrials.gov identifier: NCT02180217) and included a double-blind randomised withdrawal phase. The study consisted of four phases to allow identification of effective osilodrostat dose within a narrow therapeutic window, to demonstrate the sustained safety and efficacy of osilodrostat (without the need for

further dose escalation), and to assess osilodrostat against placebo while minimising the duration of placebo treatment. To our knowledge, this is the first Phase III study to include a placebo-control arm in patients with Cushing's disease.

Methods

Study design and procedures

This was a prospective, multicentre (66 centres across 19 countries), open-label study with a double-blind randomised withdrawal phase following a 24-week, open-label, single-arm treatment period (Figure 1). There were four study periods in the core phase. All participants initiated open-label oral osilodrostat 2 mg twice daily (bid [roughly every 12 hours]; 1, 5, 10, or 20 mg film-coated tablets for oral administration; Novartis Pharma AG, Basel, Switzerland), with dose adjustments every 2 weeks (range 1–30 mg bid) up to week 12 (study period 1) based on efficacy and tolerability. Dose was increased according to a 2 mg bid, 5 mg bid, 10 mg bid, 20 mg bid, and 30 mg bid escalation sequence if mUFC (mean of three 24-hour samples) exceeded the upper limit of normal (ULN). Throughout the study, osilodrostat dose was reduced if mUFC was below the lower limit of normal (LLN), or if mUFC was in the lower part of the normal range in patients with symptoms of hypocortisolism or adrenal insufficiency. From weeks 13 to 24 (study period 2), osilodrostat was continued at the therapeutic dose determined during study period 1. In participants whose mUFC became elevated (>ULN), osilodrostat dose was increased (managed by a phone call to the patient between visits or by an unscheduled visit as soon as possible after receipt of mUFC results), if tolerated, but these participants were not randomised to the next study period. Participants were eligible to enter the randomised withdrawal phase at week 26 if they had mUFC ≤ULN at week 24 without a dose increase after week 12; patients were randomised (1:1) in a double-blind manner to continue osilodrostat at the same therapeutic dose or receive matching placebo for 8 weeks without further dose increases (study period 3; as requested by regulatory authorities). Dose reductions/interruptions were permitted for safety reasons and did not preclude the possibility of a complete response at week 34. Participants not eligible for randomisation continued open-label osilodrostat. After week 34, all participants received open-label osilodrostat until week 48 (study period 4). Osilodrostat dose could be adjusted throughout, depending on efficacy and tolerability. The dose schedule for osilodrostat was based, in part, on modelling data that estimated that a dose of 4–5 mg bid would achieve a plasma concentration above the *in vitro* 50% inhibitory concentration (IC_{50})

for 11β-hydroxylase (2·5 nM) for a full 24-hour period (Novartis Pharma AG, unpublished data). Furthermore, results of a Phase II study demonstrated that the dose of osilodrostat required to normalise mUFC ranged from 2 mg bid to 50 mg bid after individual dose titration.[13] The lower starting dose of 2 mg bid and progressive up-titration to a maximal dose of 30 mg bid (based on individual clinical response and tolerability) were chosen to reduce potential risks of hypocortisolism and/or adrenal insufficiency.

Participants could enter an optional, open-label extension phase for up to week 72 as a minimum (ongoing in 2019). The study was conducted in accordance with the Declaration of Helsinki, with an independent ethics committee/institutional review board at each site approving the study protocol.

Participants

Adult patients (18–75 years of age) with confirmed persistent/recurrent Cushing's disease after pituitary surgery and/or irradiation, or *de novo* patients who refused surgery or were not deemed to be surgical candidates, were eligible for inclusion. Confirmed active Cushing's disease was evidenced at screening by a mean of two or three 24-hour UFC levels of ≥1·5 x ULN and morning plasma ACTH above the LLN. Inclusion criteria also required evidence of a pituitary origin for the excess ACTH based on one or more criteria: a pituitary tumour >6 mm by magnetic resonance imaging (MRI); a central-to-peripheral bilateral inferior petrosal sinus sampling gradient >2 pre- or >3 post-stimulation with either corticotrophin-releasing hormone or desmopressin acetate stimulation; and/or histopathological and immunohistochemical confirmation of an ACTH-producing pituitary tumour in patients with prior pituitary surgery. Patients receiving other medical therapies for Cushing's disease could be included following a washout period of: 1 week for ketoconazole (n=36) and metyrapone (n=1); 4 weeks for cabergoline (n=18), rosiglitazone, pioglitazone, and mifepristone (n=4); 1 week for short-acting pasireotide/8 weeks for long-acting pasireotide (n=24); and 6 months for mitotane (n=1), followed by rescreening if required. Exclusion criteria included: stereotactic radiosurgery in the past 2 years; conventional radiotherapy in the past 3 years;

pituitary surgery in the past 29 days; treatment with other investigational drugs within 30 days or five half-lives (whichever was longer); a history of hypersensitivity to osilodrostat or therapies of a similar chemical class; and presence or high risk of compression of optic chiasm. Although not a specific exclusion criterion, no participant had previously received osilodrostat. All patients provided written informed consent prior to participation.

Randomisation and blinding

For the double-blind randomised withdrawal phase starting at week 26, randomisation was performed via interactive-response technology (IRT). The investigator employed the IRT after confirming that the patient had fulfilled all randomisation criteria. A randomisation number was then assigned by the IRT to link the patient to a treatment group and unique medication number. Randomisation was stratified by osilodrostat dose at week 24 (≤5 or >5 mg bid) and history of pituitary irradiation (yes or no). Treatment identity was concealed by using identical packaging, labelling, schedule of administration, and tablet appearance and odour. To ensure that randomisation was concealed, a randomisation list was produced by the IRT provider using a validated system that automated the random assignment of patient numbers to randomisation numbers. Participants, investigators, and study sponsor as required were blinded from the time of randomisation until completion of the core study (week 48).

Study endpoints and assessments

The primary endpoint was the proportion of randomised participants who maintained a complete response to osilodrostat therapy or matching placebo (mUFC ≤ULN) without any dose increase during the randomised withdrawal period, at the end of the 8-week randomised withdrawal period (week 34). Patients discontinued the randomised withdrawal period and were considered non-responders for the primary endpoint if their mUFC increased to >1·5 x ULN and they had at least two urine samples with UFC >1·5 x ULN at a single visit. These patients resumed open-label osilodrostat. The key secondary endpoint was the proportion of participants with mUFC ≤ULN at the end of the single-arm, open-label period (week 24) without up-

titration during weeks 13–24. Other secondary endpoints included: rates of complete response (mUFC ≤ULN) and partial response (mUFC >ULN but ≥50% reduction from baseline) at weeks 12, 24, and 48; change from baseline in mUFC, cardiovascular-related parameters (fasting plasma glucose, glycated haemoglobin [HbA_{1c}], fasting lipid profile, sitting systolic and diastolic blood pressure, body weight, body mass index [BMI], and waist circumference), and patient-reported outcomes (health-related quality of life [HRQoL] assessed using the Cushing's quality-of-life questionnaire [CushingQoL] and the Beck Depression Inventory [BDI]); maximum plasma concentration of osilodrostat (C_{max}); and safety. A minimal important difference (MID; the smallest change in treatment outcome that an individual patient would identify as important) in CushingQoL score of 10·1 was defined based on the distribution method of a 0·5 standard deviation (SD) unit change using baseline data, with a 1-week recall period. An MID for improvement in BDI score was a 17·5% reduction in scores from baseline, as described previously.[14] UFC was measured at a central laboratory (Q^2 Solutions, Global Laboratory Services, Morrisville, NC, USA) using liquid chromatography–tandem mass spectrometry (LC-MS/MS; normal range 11–138 nmol/24h [4–50 µg/24h]). Safety and tolerability were assessed by the investigators throughout the study, as required, by monitoring AEs according to Common Terminology Criteria for Adverse Events v4·03, which were coded using Medical Dictionary for Regulatory Activities (MedDRA) terminology. AEs of special interest (anticipated AEs) were those related to increase in adrenal hormone precursors, hypocortisolism, pituitary tumour enlargement, QT prolongation, and arryhthmogenic potential on electrocardiogram (ECG; assessed regularly throughout the study). Clinical and laboratory evaluations included total testosterone (LC-MS/MS), plasma ACTH (Immulite 2000 ACTH kit), early-morning serum cortisol (LC-MS/MS), serum 11-deoxycortisol (LC-MS/MS), late-night salivary cortisol (LC-MS/MS), plasma aldosterone (LC-MS/MS), dehydroepiandrosterone sulfate (Chemiluminescent Immunoassay), 11-deoxycorticosterone (LC-MS/MS), active renin (Chemiluminescent Immunoassay), serum oestradiol (LC-MS/MS), and oestrone (LC-MS/MS). For normal ranges, see Supplementary Table 1. Tumour volume was assessed by MRI with gadolinium enhancement at baseline, week 24, and week 48 and was assessed centrally (Supplementary Methods). Mean percentage changes in tumour volume from baseline to week 48 were assessed for

all patients with evaluable measurements and by maximum tumour diameter at baseline (<6, 6–<10, or ≥10 mm). For efficacy and safety evaluations, the last available pre-dose assessment within 35 days prior to or on the first day of osilodrostat treatment (before the first dose) was taken as the baseline assessment.

Pharmacokinetic parameters for osilodrostat were determined by Phoenix WinNonlin v6·2 using non-compartmental analysis. Plasma samples from all participants were assayed for osilodrostat concentration using LC-MS/MS (lower limit of quantification for osilodrostat was 0·10 ng/mL).

Statistical analyses

Sample-size calculation was based on the primary endpoint. To detect a clinically meaningful difference of 40% in complete response rate (mUFC ≤ULN) between 70% of patients in the osilodrostat arm and 30% in the placebo arm, a sample size of 33 participants per arm was required based on a two-sided Cochran–Mantel–Haenszel (CMH) test at the two-sided 0·05 level of statistical significance with 87% power. Assuming that ≥50% of participants enrolled would be eligible for randomisation, 132 participants needed to be enrolled. The primary analysis was based on the comparison of the proportion of participants with mUFC ≤ULN without a dose increase at the end of the 8-week randomised withdrawal period (ie at week 34) between participants randomised to continue osilodrostat versus placebo. The statistical null hypothesis was that there would be no difference in the complete response rates between the two randomised arms. For the primary endpoint, testing was performed using the randomised analysis set composed of all randomised participants who received at least one dose of randomised treatment (osilodrostat or placebo) during period 3, following the intent-to-treat principle. If the CMH exact test two-sided *P* value was <0·05 and the odds ratio was >1, the null hypothesis would be rejected and the complete response rate in the osilodrostat arm considered higher than that in the placebo arm. For the key secondary objective, the statistical null hypothesis was that the complete response rate at the end of the 24-week open-label period of osilodrostat treatment was ≤30%. Analysis of the key secondary objective was based on the two-sided 95% exact confidence interval (95% CI;

Clopper–Pearson method). If the lower bound of this 95% CI was ≥30%, the secondary null hypothesis would be rejected, and a complete response rate of ≥30% after 24 weeks of treatment with osilodrostat would be concluded. Testing on the key secondary objective was only carried out if the null hypothesis for the primary objective was rejected to ensure preservation of the overall two-sided type 1 error at 5%. Analysis of the key secondary endpoint was performed using the full analysis set composed of all enrolled participants who received at least one dose of osilodrostat. All statistical analyses were performed using SAS v9·4. Changes from baseline in secondary objective parameters are summarised descriptively. Safety was assessed using all data from first patient, first visit until the time the last patient completed or discontinued the core study (ie safety is reported beyond 48 weeks for some participants). The number and percentage of patients with AEs was tabulated by preferred term and CTCAE grade. For patients who discontinued the randomised withdrawal period and resumed open-label osilodrostat, AEs that occurred after withdrawal of randomised treatment were reported as part of the open-label osilodrostat phase.

Role of funding source

The funder of the study (Novartis Pharma AG) contributed to study design, data collection, data analysis, data interpretation, writing of the report, and decision to submit the report for publication. The funder also paid for the services of professional medical writers, who provided editorial assistance in developing the outline and subsequent drafts of the manuscript. All authors had full access to all the study data and were responsible for interpreting the data, writing the manuscript, and the decision to submit for publication.

Results

Patient population

A total of 202 patients were screened and 137 were enrolled between 12 November 2014 and 22 March 2017 to receive open-label osilodrostat (Figure 2). At week 26, 71 patients (51·8%) were randomised to continue osilodrostat (n=36) or matching placebo (n=35; Figure 2). Nineteen patients discontinued treatment prior to the randomised withdrawal phase (AEs, n=12; patient withdrew consent, n=4; physician decision, n=2; patient/guardian decision, n=1), and one patient met randomisation criteria but was not randomised in accordance with the investigator's decision. Of the remaining 46 patients not randomised, 20 did not meet the mUFC normalisation criteria at week 24, and 26 had dose increases beyond that established at week 12 (end of the dose-titration period), although 19 of these 26 patients later met the mUFC normalisation criteria at week 24. Among the enrolled patients, 106 (77·4%) entered the ongoing extension phase.

Baseline patient characteristics were generally balanced between randomised groups, although median mUFC was higher in patients randomised to osilodrostat versus placebo (457 vs 358 nmol/24h; Table 1). Median mUFC was similar between patients randomised to osilodrostat and placebo at the start of the randomised withdrawal period (week 26; 57·6 vs 57·0 nmol/24h, respectively).

Exposure to osilodrostat

By week 24, the majority of patients (62·0%) were receiving an osilodrostat dose of ≤5 mg bid, irrespective of severity of mUFC elevation at baseline, with only 5·8% of patients requiring a dose of >10 mg bid. During the first 26 weeks, mean (SD) dose received was 10·0 (7·3) mg/day, and mean (SD) highest dose was 17·8 (13·6) mg/day; 100 patients (73·0%) had a dose reduction during the first 26 weeks. During the randomised withdrawal phase, mean (SD) dose was 10·0 (9·6) mg/day. In total, 121 patients (88·3%) were treated for >24 weeks, with 105 patients (76·6%) exposed to osilodrostat for at least 48 weeks (core phase). Median (IQR) exposure was 74·7 (48·1–117·0) weeks (including the 48-week core phase plus extension

data up to the data cut-off date, when all patients had completed the core period of the study or discontinued early). In total, 42/137 (30·7%) patients received at least one dose of osilodrostat different to that planned in the protocol.

Pharmacokinetic exposure of osilodrostat

Mean C_{max} increased with increasing doses of osilodrostat during the initial 12-week dose-titration period. At the end of the dose-titration period (week 12), mean plasma osilodrostat concentration was 4·5 ng/mL and 54·0 ng/mL, respectively, 2 hours after a 1 mg (n=16) and 10 mg (n=20) incident dose.

Efficacy of osilodrostat

Primary efficacy endpoint

The primary efficacy endpoint was met and the null hypothesis rejected. At the end of the randomised withdrawal phase (week 34; study period 3), mUFC ≤ULN was maintained without any dose increase in statistically significantly more patients who continued osilodrostat treatment versus placebo (86·1% [n=31/36] vs 29·4% [n=10/34]; odds ratio: 13·7 [95% CI 3·7, 53·4], *P*<0·001; Figure 3a and Table 2); intrapatient changes in mUFC levels are shown in Supplementary Figure 1. One patient randomised to the placebo arm did not receive treatment because of an AE (glucocorticoid deficiency), which required drug interruption. A consistent treatment effect was observed irrespective of randomisation stratum (week 24 dose ≤5 or >5 mg bid and history of pituitary irradiation; Table 2).

Of the 10 patients randomised to placebo who maintained mUFC ≤ULN at the end of the randomised withdrawal period, seven had study baseline mUFC <2 x ULN, two had mUFC 2–5 x ULN, and one had mUFC ≥5 x ULN. All 10 patients had undergone prior neurosurgery (most recently ≥6 months prior to enrolment), and one patient had received prior pituitary irradiation. Of the three patients with baseline mUFC >2 x ULN, all were receiving osilodrostat ≤2 mg bid at week 24.

Key secondary endpoint

The key secondary endpoint was also met. At week 24 (end of study period 2), 52·6% (n=72/137; 95% CI 43·9, 61·1) of all patients maintained mUFC ≤ULN without a dose increase after week 12 (end of study period 1). The lower bound of the 95% CI exceeded the prespecified threshold for a statistically significant clinical benefit (30%). Irrespective of dose increase, in total, 93/137 patients (67.9%) normalised mUFC by week 24. Individual changes in mUFC from baseline to week 24 for all enrolled patients are shown in Figure 3b.

Other secondary endpoints

Overall, mean mUFC decreased rapidly during the initial 12-week osilodrostat dose-titration period (study period 1), then remained below baseline values throughout the study (Supplementary Figure 2a). Mean serum and salivary cortisol also decreased rapidly in the first 12 weeks, then remained below baseline values and the ULN (Supplementary Figure 2b–d); however, slight increases were seen in patients randomised to placebo between weeks 28 and 34.

Most enrolled patients (96·4%) achieved mUFC ≤ULN at least once during the study, with no differences observed in males versus females; median time to first complete response was 41 days. In addition, 64/97 (66·0%) patients maintained normal mUFC (mUFC ≤ULN) for at least 6 months after the first mUFC normalisation. At the end of the core phase (week 48), 66·4% of all enrolled patients had controlled mUFC (Supplementary Table 2).

Changes in clinical and laboratory parameters

Overall, improvements were observed from baseline in most evaluated cardiovascular-related metabolic parameters associated with hypercortisolism, including weight, BMI, glucose, systolic/diastolic blood pressure, and total cholesterol (Supplementary Table 3). Mean (SD) CushingQoL score improved by 52·4% (107·4), and BDI score improved by 31·8% (65·0), by week 48. Changes in CushingQoL score reached the distribution-based MID of a 10·1-point change from baseline at weeks 26, 30, 32, 34, and 48. Changes in BDI score reached the MID of

a 17·5% reduction from baseline at weeks 24, 26, 28, 30, and 48. Most improvements in clinical and laboratory parameters were evident during the dose-titration period (up to week 12, study period 1) and were generally maintained throughout the study (Supplementary Table 4), including the 8-week randomised withdrawal period, although this was a short time frame.

Safety and tolerability

All patients experienced at least one investigator-reported AE during the study, most frequently nausea (41·6%), headache (33·6%), fatigue (28·5%), and adrenal insufficiency (27·7%; Supplementary Table 5). The most frequently reported grade 3/4 AEs were adrenal insufficiency (4·4%), glucocorticoid deficiency (3·6%), headache (2·9%), and vomiting (2·9%).

A similar proportion of patients randomised to continue osilodrostat treatment or switch to placebo had AEs during the randomised withdrawal period (72·2% and 65·7% in the osilodrostat and placebo groups, respectively). The most commonly reported AEs in the osilodrostat group during the randomised withdrawal were nausea (11·1% [n=4] vs 0% for placebo), anaemia (8·3% [n=3] vs 8·6% [n=3]), arthralgia (8·3% [n=3] vs 0%), and headache (8·3% [n=3] vs 0%; Table 3). Arthralgia was often considered by the treating investigator to be related to the underlying disease or other pathologies (including injuries).

Hypocortisolism-related AEs were clinically assessed and reported by the investigators in 51·1% of all patients at any point during the study (Supplementary Table 5), most commonly being classified as adrenal insufficiency (27·7%) or glucocorticoid deficiency (21·2%), which reflect the same condition. They mostly occurred and resolved during the dose-titration period (study period 1), were typically single episodes of grade 1–2, and were managed by dose reductions/interruptions and corticosteroid supplementation when clinically indicated. In total, 25 patients with ≥1 hypocortisolism-related AE required treatment with glucocorticoids. In general, UFC values closest to the occurrence of these events did not fall below the LLN (ie the AE occurred concurrently with a rapid decrease in pathologically high cortisol

levels, which were still >ULN or within the normal range). No relationship was observed between the dose of osilodrostat or baseline UFC and the occurrence of hypocortisolism-related AEs.

AEs potentially related to increases in adrenal steroid precursors occurred in 42·3% of patients during the study (mostly grade 1–2; Supplementary Table 5), most commonly reported as hypokalaemia (13·1%) and hypertension (12·4%). Although serum potassium was generally maintained within the normal range, there was a trend for greater decreases in mean serum potassium levels with increasing osilodrostat C_{max} (Supplementary Figure 3). The lowest reported serum potassium value observed in the seven patients who experienced newly occurring grade 3/4 hypokalaemia was 2·4 mmol/L (normal range 3·5–5·3 nmol/L). Episodes were treated with potassium supplements, spironolactone, and/or dose reduction/interruption.

No male patients experienced signs or symptoms related to increases in androgens or oestrogens during the study. In female patients, AEs of hirsutism (8·8%; n=12/137), acne (8·8%; n=12/137), and hypertrichosis (0·7%; n=1/137) were reported; all were grade 1–2 and none led to study discontinuation. An AE of QT prolongation (defined as notable ECG abnormalities [eg QTcF >480 ms or >60 ms increase from baseline]) was reported in five (3·6%) patients (QTcF values <480 ms at all time points); all were reported as non-serious, which resulted in dose adjustment or interruption in three patients and discontinuation in one patient.

In total, 18 (13·1%) patients discontinued treatment because of an AE by the time of the data cut-off, most commonly adrenal insufficiency (n=4, 2·9%) and pituitary tumour (n=4, 2·9%), reported as ‘pituitary tumour enlargement’, ‘pituitary tumour volume increase’, ‘increased adenoma size’, or ‘pituitary tumour growth’. Of the four patients with pituitary tumour, two had macroadenomas and two had microadenomas at baseline.

Overall, 81 (59·1%) patients had a measurable pituitary tumour at baseline (n=13/81 [16·0%] macroadenoma [≥10 mm], n=68/81 [84·0%] microadenoma [<10 mm]).

Seventy-nine patients (57·7%) had a measurable tumour at both baseline and at least one post-baseline assessment. A similar proportion of patients had either a ≥20% decrease or ≥20% increase from baseline in tumour volume at week 24 (30·3% and 28·8%, respectively) and week 48 (37·5% and 32·8%, respectively). Similar results were observed irrespective of maximum tumour diameter at baseline (<10 or ≥10 mm). At data cut-off, four patients with a ≥20% increase from baseline in tumour volume and 13 with a ≥20% decrease had received prior pituitary irradiation. In those patients with no tumour identifiable at baseline, none had evidence of a newly measurable pituitary tumour identified by MRI during the study.

One death occurred, which was not considered to be related to the study drug – the patient died by suicide during the extension phase (day 551) in the context of an extensive psychiatric history. The patient, who was randomised to placebo, had mUFC within the normal range during osilodrostat treatment.

All enrolled patients received concomitant medications and significant non-drug therapies during the study. Concomitant medications were mainly prescribed to manage AEs. During the study, 97 (70·8%) patients received one or more antihypotensive or antihypertensive medications (72/137 [52·6%] patients were newly started during the study), 50 (36·5%) received antidiabetic medications (23/137 [16·8%] patients newly started), and 34 (24·8%) received lipid-lowering treatments (13/137 [9·5%] patients newly started; most commonly statins).

Effect of osilodrostat on other hormones

In the overall population, mean (SD) ACTH levels were >ULN (18·4 [35·5] pmol/L) at baseline and increased during the study to 50·0 (69·7) pmol/L at week 48. No association was observed between ACTH and cortisol levels (Pearson's correlation coefficient at week 48: –0.10) [Supplementary Table 6]. The increase was reversed during the randomised withdrawal phase in patients randomised to placebo (Supplementary Figure 4). Mean (SD) 11-deoxycortisol also increased over time from 3·4 (2·6) nmol/L at baseline in male patients and 6·3 (20·1) nmol/L in female patients to 23·3 (24·8) and 36·6 (36·9) nmol/L, respectively, at week 48. Increases in

mean 11-deoxycortisol were also reversed during the randomised withdrawal phase in patients randomised to placebo (Supplementary Figure 5a and 5b). An increase in mean testosterone was seen in both male and female patients during the first 12 weeks, which stabilised thereafter (Supplementary Figure 6a and 6b). Mean (SD) testosterone levels in males increased from 9·5 (5·8) nmol/L at baseline to 17·7 (8·0) nmol/L at week 48; for individual patients, testosterone levels tended to increase from the low to the middle of the normal range, with no values above the ULN reported for last available values. For some hypogonadal patients, testosterone increased from <LLN into the normal range during osilodrostat treatment. In females, mean (SD) testosterone increased from 1·3 (1·2) nmol/L at baseline to 2·6 (2·4) nmol/L at week 48. In male and female patients, a mild increase in gonadotrophin levels was seen from baseline, although we cannot exclude effects of spontaneous recovery of post-surgical damage of the gonadotropic pituitary cells. Compared with baseline, gradual reductions were seen in plasma aldosterone and dehydroepiandrosterone sulphate, and gradual increases were seen in 11-deoxycorticosterone, renin, serum oestradiol, and oestrone (Supplementary Figure 7a–f). No clear trend was observed between absolute 11-deoxycorticosterone levels and systolic blood pressure or serum potassium levels, or between changes from baseline in these parameters (data not shown).

Discussion

This large, prospective study is, to our knowledge, the first Phase III trial of a medical therapy for patients with Cushing's disease to include a randomised, double-blind, placebo-controlled period. The study met both its primary and key secondary endpoints. At the end of the 8-week, blinded, randomised withdrawal, placebo-controlled phase (study period 3), a statistically significant higher proportion of patients in the osilodrostat arm maintained normal mUFC versus placebo. These findings were seen irrespective of randomisation stratum, confirming that prior irradiation and higher osilodrostat dose were not driving factors for mUFC response.

For the patients receiving placebo who maintained a complete response, the last mUFC value during the withdrawal phase was higher than that at randomised withdrawal baseline in 9/10 patients but still within the normal range, with all patients showing a progressive increase in mUFC during this period; a longer withdrawal period would likely have resulted in a higher proportion of patients receiving placebo experiencing elevated mUFC. These results are consistent with findings from a 10-week proof-of-concept study of osilodrostat in patients with Cushing's disease (N=12), in which mUFC levels remained below ULN in some patients following a 2-week washout of osilodrostat.[13] It is possible that mild cases of Cushing's disease, as evident in the majority of these patients with mUFC <2 x ULN at study baseline, and/or fluctuations in mUFC may have influenced our findings. However, the statistically significant difference in complete response between the two arms strongly indicates a benefit with osilodrostat. A delay in cortisol recovery following osilodrostat withdrawal in some patients is also a possibility. No association was observed between ACTH and cortisol levels in our study. In addition, a delay in cortisol increase above the ULN in some patients is not fully explained by the reversible inhibitory effect of osilodrostat on 11β-hydroxylase, while there is no evidence supporting a cytolytic effect of osilodrostat on adrenal tissue or prolonged action on the hypothalamic–pituitary–adrenal axis. As such, the reason underlying a delayed increase in cortisol above the ULN in some patients after osilodrostat withdrawal is unknown and further investigation would be of interest.

Patients experienced a rapid reduction in mUFC during the initial 24-week open-label phase, which included the dose-titration (study period 1) and therapeutic-dose periods (study period 2). More than half (52.6%) of the patients enrolled achieved the key secondary endpoint of mUFC ≤ULN at week 24 without dose up-titration after week 12. Furthermore, most patients (96·4%) achieved mUFC ≤ULN at some point during osilodrostat treatment, with 66·0% of patients maintaining mUFC ≤ULN for at least 6 months after first mUFC normalisation, indicating that osilodrostat provides sustained control of mUFC levels in a majority of patients. Although most patients achieved mUFC ≤ULN at some point during the study, a lower proportion of all patients (66·4%) had mUFC ≤ULN at the end of the core phase. This may be explained by day-to-day fluctuations in mUFC measurements, but also because patients who discontinued before week 48 were counted as non-responders, affecting the overall proportion of patients with mUFC ≤ULN by the end of the core study. Reductions in mean morning serum and late-night salivary cortisol accompanied the reductions in mean mUFC, further strengthening our conclusion of a benefit with osilodrostat treatment for patients with Cushing's disease. In a recent Phase III study of the investigational agent levoketoconazole, a steroidogenesis inhibitor, 29/94 (31%) patients with Cushing's syndrome achieved mUFC ≤ULN following an initial dose-titration period and 6 months of maintenance therapy (without a dose increase during the maintenance phase).[11]

Reductions in mUFC during 48 weeks of osilodrostat treatment were accompanied by improvements in weight, BMI, glucose, systolic/diastolic blood pressure, and total cholesterol. Improvements occurred soon after osilodrostat initiation and were sustained until the end of the study. Given the known clinical burden of cardiovascular risk associated with Cushing's disease,[2] demonstrated by the abnormal baseline values of many participants in this study, the improvement in clinical features shown here represent important benefits of osilodrostat. By improving multiple cardiovascular risk factors, our findings are likely to be clinically relevant. The slight decrease in HDL cholesterol observed at week 48 was similar to that described in a study of mifepristone (a glucocorticoid receptor antagonist) for the treatment of Cushing's syndrome;[15] further investigation concluded that such a reduction would not adversely affect cardiovascular risk.[16] Osilodrostat also led to

clinically meaningful improvements in HRQoL throughout the study from week 12, including CushingQoL score, despite considerable impairments at baseline associated with prolonged hypercortisolism.[17] Clinically meaningful improvements in depression as assessed by BDI score were also evident throughout the study, representing clinical benefit following osilodrostat treatment. Future evaluation of other manifestations of cortisol excess, including menstrual abnormalities, which often resolve following normalisation of cortisol levels,[18] as well as facial rubor, striae, muscle wasting, and bone loss, would be of considerable interest given the potential deleterious effects they can exert on quality of life and morbidity.[3]

Osilodrostat was generally well tolerated; commonly reported AEs were as expected based on its mechanism of action. They were consistent with those reported during the 22-week Phase II study.[12] The majority of patients (82·5%) completed the 48-week study, with a low rate of discontinuations due to AEs (13·1%) and one death, which was not attributed to osilodrostat. The most commonly reported AEs of special interest (associated with osilodrostat) were related to hypocortisolism, highlighting the potency of osilodrostat; these were generally managed by dose adjustments and/or corticosteroid supplementation. These AEs were mostly mild to moderate in severity and mainly occurred during the initial dose-titration period, as was also found when using either metyrapone[19] or ketoconazole.[20] As most AEs occurred during the rapid dose up-titration period (forced dose increases every 2 weeks if mUFC >ULN), we anticipate that smaller dose increases and/or more gradual dose up-titration (except in cases of severely ill patients who require rapid cortisol control) might reduce the rate of AEs related to hypocortisolism in clinical practice. Indeed, in the interests of safety, the rate of mUFC decrease was considered by many investigators when making dose-titration decisions during the study and, as a result, some patients with mUFC >ULN did not receive a dose increase as specified in the protocol.

Elevations in liver transaminases were infrequent, typically mild, and reversed spontaneously or following dose adjustment. AEs of hypertension and low serum potassium, potential outcomes of inhibiting 11β-hydroxylase, which causes the accumulation of precursor mineralocorticoids such as 11-deoxycorticosterone, were

reported in only a few patients; there was no clear indication of a causal relationship (data not shown). However, it is possible that changes in concomitant blood pressure control medications could have contributed to the lack of correlation between 11-deoxycorticosterone levels and blood pressure. These data suggest that the inhibition of 11β-hydroxylase with osilodrostat is, therefore, not a phenocopy of classic congenital 11β-hydroxylase deficiency.[21-25] In addition to inhibiting 11β-hydroxylase, osilodrostat is known to inhibit aldosterone synthase in a dose-dependent manner, as well as basal and ACTH-stimulated cortisol secretion at specific doses.[26] Increases in precursors to cortisol and aldosterone, as well as ACTH, were observed. The expected physiological rise in ACTH that is associated with a reduction in cortisol levels likely contributed to the observed increases in adrenal steroid precursors; however, resultant AEs rarely led to drug discontinuation and were generally managed with conventional medications. Generally, no specific action was required for increases in ACTH, 11-deoxycortisol or 11-deoxycorticosterone. Furthermore, increases were reversible upon discontinuation of osilodrostat, as seen in the placebo arm during the randomised withdrawal period. Owing to the increase in serum 11-deoxycortisol induced by osilodrostat therapy, it is important that assays measuring cortisol have no cross-reactivity with this precursor, and this is most readily achieved by using a mass spectrometry assay for cortisol, as was done here.[27] Mean testosterone levels increased in male and female patients, but the increases were mild to moderate and did not lead to study discontinuation in any patient. Osilodrostat treatment did not adversely affect pituitary tumour size, with a small proportion of patients experiencing ≥20% decrease or ≥20% increase from baseline in tumour volume. Longer-term follow-up would be needed to further evaluate changes in tumour volume during osilodrostat treatment.

Although the study permitted the inclusion of patients aged 18–75 years, enrolled participants were aged 19–70 years; as such, our study provides no data for patients aged >70 years and therefore further evaluation of osilodrostat in older patients may be of interest. Our data are also limited by the short, randomised withdrawal period. The 8-week withdrawal period was not long enough to confirm whether withdrawal of osilodrostat would have resulted in worsening clinical signs and features of hypercortisolism. Longer-term follow-up to assess both duration of effectiveness and

long-term safety of osilodrostat will be important. Assessment of serum and/or salivary cortisol levels across a 24-hour period would be valuable in order to further explore the pharmacodynamic effect of twice-daily osilodrostat, including on the restoration of cortisol diurnal rhythm, which is frequently disrupted in patients with Cushing's disease.[28] It should also be noted that nearly all patients received concomitant medications during the study, including antihypertensive and antidiabetic medications, and we are unable to exclude the possibility that they may have influenced some findings. Further examination of the effects of osilodrostat on the clinical signs of Cushing's disease, as well as the reasons for changes in concomitant medications and the relationship between such medications and associated clinical outcomes, would be valuable.

In conclusion, the results of this prospective, Phase III study, which included a double-blind randomised withdrawal phase, demonstrate that osilodrostat rapidly reduces mUFC as well as serum cortisol and sustains these reductions alongside improvements in clinical signs of hypercortisolism, CushingQoL score, and depression without unexpected side effects. Alongside careful dose adjustments and monitoring of known risks associated with osilodrostat, our findings indicate a positive benefit–risk consideration of treatment for most patients with Cushing's disease.

Research in context

Evidence before this study

In June 2014, during the development of the study protocol, we reviewed the literature using PubMed (no date restrictions; search terms “Cushing’s disease”, “pituitary gland”, and “adrenocorticotropic hormone”), which highlighted that despite advances and recent approval of medical therapies to treat Cushing’s disease (eg pasireotide and mifepristone), a substantial proportion of patients with Cushing’s disease do not achieve and maintain normalisation of mean urinary free cortisol (mUFC), a key treatment goal in such patients. As such, there remained an unmet medical need to develop additional new and effective drugs, as patients often require multimodal treatment and more options may allow better tailoring of treatment. Based on earlier Phase I and Phase II studies in patients with Cushing’s disease, osilodrostat, a potent oral inhibitor of 11β-hydroxylase, showed promise in fulfilling this unmet need.

Added value of this study

This is the first Phase III study of any medical therapy to include a placebo-controlled period in patients with Cushing’s disease. LINC 3 was a prospective, multicentre, open-label study with a double-blind randomised withdrawal phase following a 24-week, open-label, single-arm treatment period. Osilodrostat was superior to placebo (statistically significant) at maintaining mUFC ≤ULN after randomised withdrawal and normalised mUFC in two-thirds of enrolled patients by the end of the study (week 48). Osilodrostat was shown to be generally well tolerated, with no unexpected side effects and few patients discontinuing treatment because of adverse events.

Implications of all the available evidence

This study demonstrates that osilodrostat rapidly reduces elevated mUFC and cortisol production and sustains this reduction alongside improvements in clinical signs of hypercortisolism, CushingQoL score, and depression without unexpected side effects. As such, osilodrostat is a promising new treatment option for patients

with Cushing's disease. Further evaluation of osilodrostat in patients with other causes of Cushing's syndrome is also of interest.

Author contributions

The study was designed by the academic investigator steering committee (RP, MF, JN-P, XB, JF, AS, FG, and BMKB) and the funder. All academic investigators enrolled patients in the study (RP, MF, JN-P, XB, JF, AS, FG, RA, RL, EJL, JHK, ALac, and BMKB). Data were collected by investigators of the LINC 3 Study Group using the funder's data management systems and analysed by the funder's statistical team and POC. A data-sharing and kick-off meeting was held with all authors and an outline prepared by a professional medical writer based on interpretation provided by the authors. Each new draft of the manuscript subsequently prepared by the medical writer was reviewed and revised in line with author direction/feedback. All authors approved the final version of the manuscript and made the final decision to submit.

Declaration of interests

RP reports grants and personal fees from Novartis, Pfizer, HRA Pharma, Viropharma, Shire, and Ipsen, personal fees from Ferring and Italfarmaco, and grants from Corcept Therapeutics, Cortendo AB, and IBSA outside the submitted work. MF reports grants to her university and personal consulting fees from Strongbridge and Novartis and grants to her university from Millendo during the conduct of the study. JN-P reports grants from Novartis during the conduct of the study, as well as grants from Novartis, HRA Pharma, and Diurnal and research grants and consultancy payments to his university from Novartis, HRA Pharma, and Diurnal outside the submitted work. XB reports personal fees from Novartis during the conduct of the study and personal fees from Idorsia and Ipsen outside the submitted work. JF reports grants and investigator and consulting fees from Novartis during the conduct of the study and consulting fees from Corcept Therapeutics

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Data sharing

Novartis is committed to sharing with qualified external researchers access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided are anonymised to respect the privacy of patients who have participated in the trial, in line with applicable laws and regulations. This

trial data availability is in accordance with the criteria and process described on www.clinicalstudydatarequest.com.

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Tables

Table 1. Summary of patient demographics and baseline characteristics, overall (full analysis set) and by randomised treatment group

Demographic variable	Randomised treatment group (randomised withdrawal phase)			All patients
	Osilodrostat n=36	Placebo* n=35	Non-randomised n=66	N=137
Median age, years (IQR)	41·0 (37·5–51·5)	40·0 (31·0–55·0)	37·5 (28·0–47·0)	40·0 (31·0–49·0)
Male:female, n:n	6:30	13:22	12:54	31:106
Race, n (%)				
Caucasian	27 (75·0)	23 (65·7)	39 (59·1)	89 (65·0)
Black	0	3 (8·6)	1 (1·5)	4 (2·9)
Asian	7 (19·4)	7 (20·0)	25 (37·9)	39 (28·5)
Other	2 (5·6)	2 (5·7)	1 (1·5)	5 (3·6)
Median time since diagnosis, months (IQR)	53·6 (25·9–94·3)	76·8 (39·3–133·7)	34·7 (14·1–65·7)	47·2 (19·0–88·3)
Previous pituitary surgery, n (%)	32 (88·9)	33 (94·3)	55 (83·3)	120 (87·6)
Previous medical therapy for Cushing's disease, n (%)	26 (72·2)	24 (68·6)	52 (78·8)	102 (74·5)
Previous pituitary irradiation, n (%)	6 (16·7)	5 (14·3)	11 (16·7)	22 (16·1)
mUFC, nmol/24h				
Mean (SD) [x ULN]	890 (1276) [6·4]	560 (549) [4·1]	1306 (2012) [9·5]	1006 (1590) [7·3]
Median (IQR) [x ULN]	457 (268–777) [3·3]	358 (210–652) [2·6]	557 (348–1246) [4·0]	476 (314–919) [3·4]

*One patient in the placebo group (25-year-old female with persistent/recurrent Cushing's disease after previous pituitary surgery; mUFC level at screening: 2037·2 nmol/24h [14·8 x ULN]) was randomised but did not receive any allocated treatment during the randomised withdrawal period. ULN for mUFC is 138 nmol/24h. IQR, interquartile range

Table 2. Proportion of primary efficacy responders at week 34 by randomised treatment and stratum

			Cochran–Mantel–Haenszel exact test	
	Responders/N (%)	**95% CI***	**Odds ratio (95% CI)**	**Two-sided *P* value**
All randomised participants				
Osilodrostat	31/36 (86·1)	70·5, 95·3	13·7	<0·001
Placebo	10/34 (29·4)	15·1, 47·5	(3·7, 53·4)	
Osilodrostat dose at week 24 ≤5 mg bid and with history of pituitary irradiation				
Osilodrostat	5/5 (100·0)	47·8, 100·0	NE	
Placebo	1/5 (20·0)	0·51, 71·6	(1·5, NE)	
Osilodrostat dose at week 24 ≤5 mg bid and without history of pituitary irradiation				
Osilodrostat	17/21 (81·0)	58·1, 94·6	8·5	
Placebo	7/21 (33·3)	14·6, 57·0	(1·7, 46·2)	
Osilodrostat dose at week 24 >5 mg bid and with history of pituitary irradiation				
Osilodrostat	–			
Placebo	–			
Osilodrostat dose at week 24 >5 mg bid and without history of pituitary irradiation				
Osilodrostat	9/10 (90·0)	55·5, 99·8	27·0	
Placebo	2/8 (25·0)	3·2, 65·1	(1·5, 1374)	

NE, not evaluable

Table 3. Summary of adverse events (all grades and grade 3/4) in either treatment group during the randomised withdrawal phase

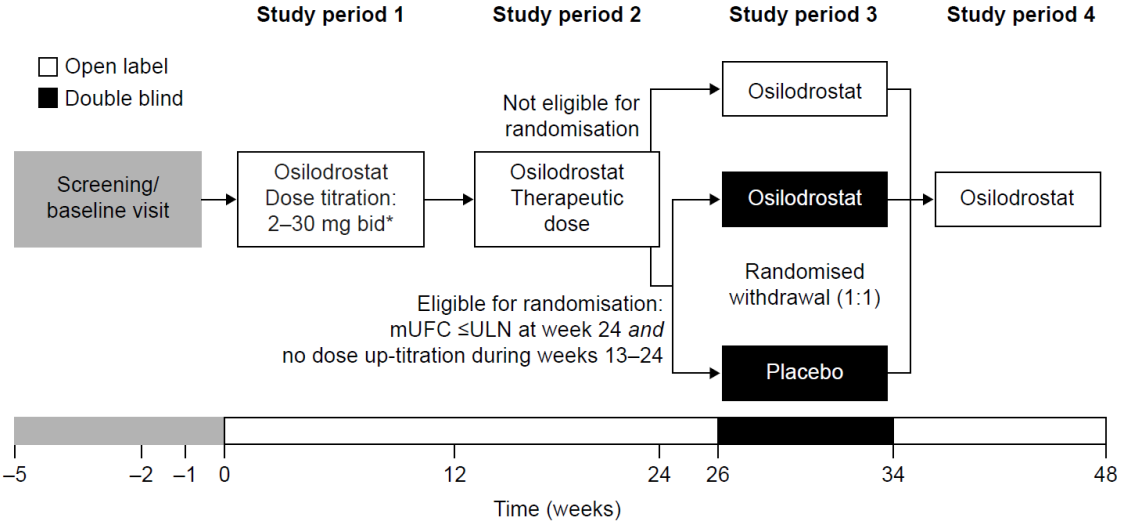
	Randomised treatment group (randomised withdrawal phase)			
	Osilodrostat n=36		Placebo n=35	
	All grades, n (%)	Grade 3/4, n (%)	All grades, n (%)	Grade 3/4, n (%)
Any AE	26 (72·2)	2 (5·6)	23 (65·7)	3 (8·6)
Any serious AE	2 (5·6)	1 (2·8)	1 (2·9)	1 (2·9)
AEs requiring dose adjustment	7 (19·4)	NA	5 (14·3)	NA
Anticipated AEs of special interest				
Adrenal hormone precursor accumulation related	2 (5·6)	0	1 (2·9)	0
Hypocortisolism related	3 (8·3)	0	1 (2·9)	0
Pituitary tumour enlargement related	0	0	0	0
QT prolongation related	0	0	0	0
Arrhythmogenic potential	0	0	0	0
Most common study-emergent AEs (occurring in >5% of patients in either group)				
Nausea	4 (11·1)	0	0	0
Anaemia	3 (8·3)	0	3 (8·6)	0
Arthralgia	3 (8·3)	0	0	0
Headache	3 (8·3)	0	0	0
Asthenia	2 (5·6)	0	0	0
Blood corticotrophin increased	2 (5·6)	0	1 (2·9)	1 (2·9)
Constipation	2 (5·6)	0	0	0
Depression	2 (5·6)	0	1 (2·9)	0
Dizziness	2 (5·6)	0	1 (2·9)	0
Fatigue	2 (5·6)	0	3 (8·6)	1 (2·9)
Hirsutism	2 (5·6)	0	1 (2·9)	0
Nasopharyngitis	2 (5·6)	0	1 (2·9)	0
UFC decreased	2 (5·6)	0	1 (2·9)	0
Cough	1 (2·8)	0	2 (5·7)	0
Insomnia	1 (2·8)	0	2 (5·7)	0
Urinary tract infection	1 (2·8)	0	2 (5·7)	0

Diarrhoea	0	0	2 (5·7)	0
Gastro-oesophageal reflux disease	0	0	2 (5·7)	0

Patients with multiple events in the same category are counted only once in that category. NA, not assessed

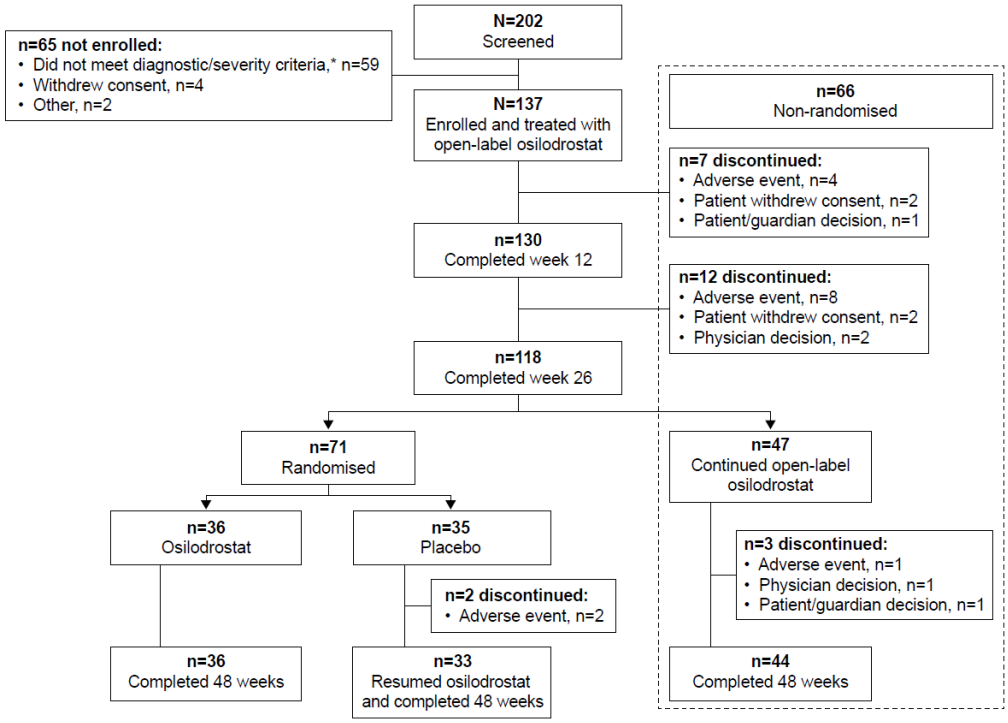
Figures

Figure 1. Study design and dosing schedule



*Based on efficacy and tolerability

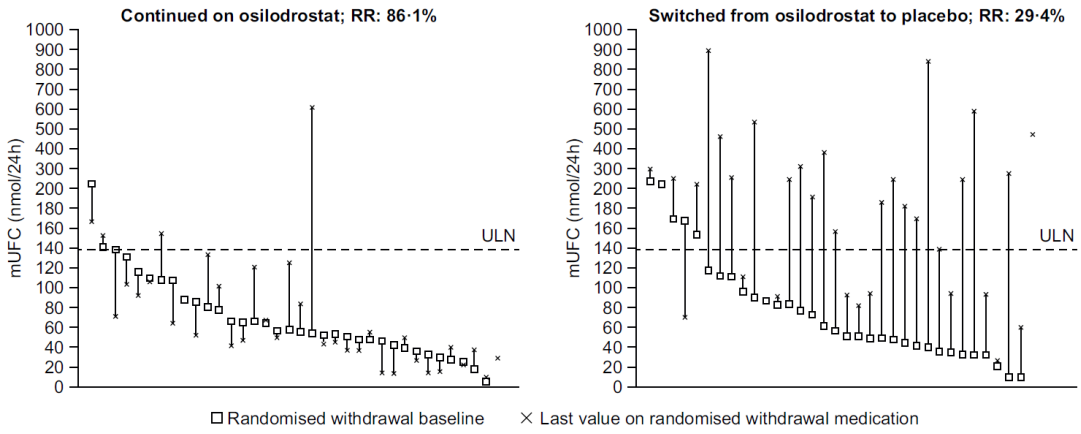
Figure 2. Patient disposition flow chart



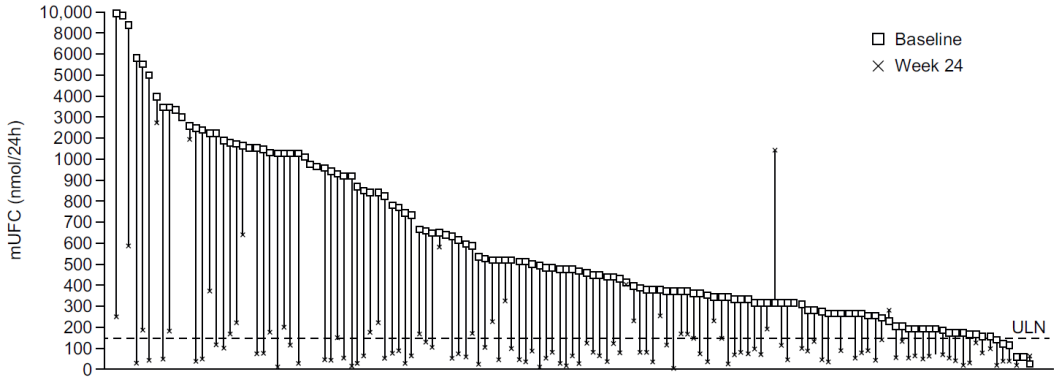
*Including unacceptable test procedure results, laboratory values, past medical history, and use of excluded medications; †(Before week 12) rash (n=1), visual impairment (n=1), headache/paresis cranial nerve/pituitary tumour benign (n=1), AE not recorded (n=1); ‡(Weeks 12–26) adrenal insufficiency (n=2), hypokalaemia/adrenal insufficiency (n=1), pain in extremity/fatigue (n=1), systolic/diastolic blood pressure increased (n=1), asthenia (n=1), pituitary tumour benign (n=1), malignant pituitary tumour (n=1); §(Randomised withdrawal, placebo – week 48) hyponatraemia (n=1), increased ACTH/pituitary tumour (n=1; occurred during the open-label period following randomised withdrawal); ¶(Non-randomised) pituitary tumour (n=1)

Figure 3. Individual changes in mUFC a) during the randomised withdrawal phase, by randomised treatment group and b) from baseline to week 24

a)



b)



mUFC ULN = 138 nmol/24h. For (b), patients are shown in order of decreasing baseline mUFC value. Five patients had mUFC ≤ULN at baseline; however, mUFC was ≥1·5 x ULN at screening, thus the patients met the eligibility criterion. RR, response rate

Supplementary appendix

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Supplementary methods

Tumour volume assessment

Pituitary magnetic resonance imaging (MRI) with gadolinium enhancement (unless contraindicated) was performed at each study site according to standardised image acquisition guidelines and the images assessed centrally. If MRI could not be conducted, then computed tomography (CT) of the pituitary was performed. The modality of imaging remained consistent to that used at baseline unless a

contraindication developed. The readings were performed by an independent review committee of neuroradiologists who were blinded to randomised treatment and the time point at which the image was taken. The outer boundaries of the pituitary tumour/gland (region of interest) were semi-automatically delineated using the post-contrast T1 MRI sequence (preferred) or post-contrast coronal reconstruction CT images, as applicable. The total combined volume of pituitary tumour/gland was automatically calculated by adding the measured cross-sectional areas and the slice thickness. The percentage change in volume at each follow-up time point relative to baseline was automatically calculated and recorded in the background but not displayed to the neuroradiologist.

Supplementary Table 1. Summary of reference ranges

Parameter	Units	Gender	Age, years	Reference range
Urinary free cortisol	nmol/24h	Male and female	≥18	11–138
Testosterone	nmol/L	Male	≥18	8·36–28·67
		Female	≥18	0·69–2·63
Plasma ACTH	pmol/L	Male	≥18	1·6–11·1 (7–10 am)
		Female	≥18	1·1–6·0 (7–10 am)
Early-morning serum cortisol	nmol/L	Male and female	≥18	127–567
Serum 11-deoxycortisol	nmol/L	Male	18–29	≤3·45
		Male	30–39	≤3·92
		Male	40–49	≤2·2
		Male	50–59	≤1·22
		Female	18–29	≤3·1
		Female	30–39	≤1·48
		Female	40–49	≤1·8
		Female	50–59	≤1·07
Late-night salivary cortisol	nmol/L	Male and female	≥18	≤2·5 (10–11 pm)
Plasma aldosterone	pmol/L	Male and female	≥18	≤777 (upright 8–10 am) ≤583 (upright 4–6 pm) 83–444 (supine 8–10 am)
Dehydroepiandrosterone sulphate	µmol/L	Male	18–21	0·7–14·6
		Male	22–30	2·3–18·8
		Male	31–40	2·9–12·6
		Male	41–50	1·9–13·5
		Male	51–60	1·0–8·5
		Male	61–70	0·7–6·6
		Male	≥71	0·1–6·9
		Female	18–21	1·4–8·7
		Female	22–30	0·5–10·6
		Female	31–40	0·6–7·2
		Female	41–50	0·5–6·3
		Female	51–60	0·2–5·1

		Female	61–70	0·3–3·6
		Female	≥71	0·2–4·8
11-deoxycorticosterone	pmol/L	Male	≥18	≤455
		Female	≥18	≤545 (mid-follicular) ≤696 (surge) ≤575 (mid-luteal)
Renin	μIU/L	Male and female	≥18	4·4–46·1
Serum oestradiol	pmol/L	Male	≥18	≤106
		Female	≥18	143–1377 (follicular) 345–2797 (mid-cycle) 176–1615 (luteal) ≤37 (postmenopausal)
Oestrone	pmol/L	Male	≥18	≤255
		Female	≥18	37–510 (follicular) 181–991 (mid-cycle) 59–640 (luteal) <244 (postmenopausal)

ACTH, adrenocorticotropic hormone

Supplementary Table 2. Proportion of mUFC responders at time points up to week 48, overall and by randomised treatment group

n (%) [95% CI]	Treatment during randomised withdrawal phase			All patients N=137
	Osilodrostat n=36	Placebo n=35	Non-randomised n=66	
Week 12				
All responders	32 (88·9) [73·9, 96·9]	34 (97·1) [85·1, 99·9]	51 (77·3) [65·3, 86·7]	117 (85·4) [78·4, 90·9]
Complete responders	31 (86·1) [70·5, 95·3]	32 (91·4) [76·9, 98·2]	35 (53·0) [40·3, 65·4]	98 (71·5) [63·2, 78·9]
Partial responders	1 (2·8) [0·07, 14·5]	2 (5·7) [0·7, 19·2]	16 (24·2) [14·5, 36·4]	19 (13·9) [8·6, 20·8]
Week 24				
All responders	36 (100) [90·3, 100]	34 (97·1) [85·1, 99·9]	43 (65·2) [52·4, 76·5]	113 (82·5) [75·1, 88·4]
Complete responders	36 (100) [90·3, 100]	34 (97·1) [85·1, 99·9]	23 (34·8) [23·5, 47·6]	93 (67·9) [59·4, 75·6]
Partial responders	0 [0, 9·7]	0 [0, 10·0]	20 (30·3) [19·6, 42·9]	20 (14·6) [9·2, 21·6]
Week 48				
All responders	34 (94·4) [81·3, 99·3]	31 (88·6) [73·3, 96·8]	39 (59·1) [46·3, 71·1]	104 (75·9) [67·9, 82·8]
Complete responders	32 (88·9) [73·9, 96·9]	27 (77·1) [59·9, 89·6]	32 (48·5) [36·0, 61·1]	91 (66·4) [57·9, 74·3]
Partial responders	2 (5·6) [0·7, 18·7]	4 (11·4) [3·2, 26·7]	7 (10·6) [4·4, 20·6]	13 (9·5) [5·2, 15·7]

All responders = complete + partial responders; complete responder = mUFC ≤ULN; partial responder = mUFC >ULN but >50% reduction from baseline. mUFC, mean urinary free cortisol; ULN, upper limit of normal

Supplementary Table 3. Change from baseline in clinical signs and features of hypercortisolism at week 48 in all patients

Parameter	Mean value at baseline (SD)	Mean value at week 48 (SD)	Mean absolute change from baseline (95% CI)	Mean percentage change from baseline (95% CI)
Weight, kg	80·8 (22·4)	75·5 (20·7)	–3·8 (–4·8, –2·7)	–4·6 (–5·8, –3·3)
BMI, kg/m^2	30·3 (7·8)	28·4 (7·1)	–1·4 (–1·8, –1·0)	–4·6 (–5·8, –3·3)
Systolic blood pressure, mmHg	132·2 (15·1)	121·7 (13·7)	–9·8 (–12·7, –6·9)	–6·8 (–8·9, –4·7)
Diastolic blood pressure, mmHg	85·3 (10·6)	78·9 (10·1)	–6·3 (–8·4, –4·2)	–6·6 (–9·0, –4·2)
Fasting plasma glucose, mg/dL	99·2 (29·8)	87·2 (18·9)	–9·5 (–14·3, –4·8)	–7·1 (–10·4, –3·8)
HbA_{1c}, %	6·0 (1·0)	5·6 (0·8)	–0·4 (–0·5, –0·2)	–5·4 (–7·2, –3·6)
Total cholesterol, mmol/L	5·3 (1·2)	4·8 (1·0)	–0·5 (–0·7, –0·3)	–8·8 (–11·8, –5·8)
LDL cholesterol, mmol/L	3·0 (1·0)	2·8 (1·0)	–0·2 (–0·4, –0·1)	–5·4 (–10·4, –0·4)
HDL cholesterol, mmol/L	1·6 (0·5)	1·3 (0·3)	–0·3 (–0·3, –0·2)	–14·4 (–17·5, –11·4)
Triglycerides, mmol/L	1·5 (1·3)	1·4 (0·9)	–0·1 (–0·2, 0·1)	5·4 (–14·0, 24·9)
CushingQoL score	42·2 (19·1)	58·3 (21·3)	14·1 (10·9, 17·3)	52·4 (32·2, 72·7)
BDI score	16·8 (10·6)	10·7 (10·7)	–5·8 (–7·6, –4·1)	–31·8 (–44·3, –19·3)

Reference ranges: plasma glucose 70–125 mg/dL; HbA_{1c} ≤6·4%; total cholesterol 0–5·2 mmol/L; HDL cholesterol >0·89 mmol/L; LDL cholesterol 0–3·4 mmol/L; triglycerides 0–2·2 mmol/L. BDI, Beck's Depression Inventory; BMI, body mass index; CushingQoL, Cushing's Quality-of-Life Questionnaire; HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation

Supplementary Table 4. Percentage change from baseline in cardiovascular-related metabolic parameters associated with Cushing's disease at selected visits during the core period, in all patients

Parameter	Mean value at baseline (SD)	Mean percentage change from baseline (95% CI)		
		Week 12	Week 24	Week 48
Weight, kg	80·8 (22·4)	–0·9 (–1·6, –0·2)	–3·0 (–3·9, –2·1)	–4·6 (–5·8, –3·3)
Body mass index, kg/m^2	30·3 (7·8)	–0·9 (–1·6, –0·2)	–3·0 (–3·9, –2·1)	–4·6 (–5·8, –3·3)
Systolic blood pressure, mmHg	132·2 (15·1)	–4·8 (–7·0, –2·6)	–4·1 (–6·2, –2·0)	–6·8 (–8·9, –4·7)
Diastolic blood pressure, mmHg	85·3 (10·6)	–4·7 (–7·0, –2·5)	–3·8 (–6·2, –1·4)	–6·6 (–9·0, –4·2)
Fasting plasma glucose, mg/dL	99·2 (29·8)	–7·0 (–10·4, –3·6)	–10·0 (–13·0, –7·1)	–7·1 (–10·4, –3·8)
HbA_{1c}, %	6·0 (1·0)	–5·0 (–6·4, –3·5)	–4·6 (–6·2, –3·0)	–5·4 (–7·2, –3·6)
Total cholesterol, mmol/L	5·3 (1·2)	–8·9 (–11·8, –6·0)	–9·0 (–12·1, –6·0)	–8·8 (–11·8, –5·8)
LDL cholesterol, mmol/L	3·0 (1·0)	–5·0 (–10·0, 0·0)	–3·5 (–9·0, 2·0)	–5·4 (–10·4, –0·4)
HDL cholesterol, mmol/L	1·6 (0·5)	–19·9 (–22·8, –16·9)	–14·3 (–17·0, –11·6)	–14·4 (–17·5, –11·4)
Triglycerides, mmol/L	1·5 (1·3)	15·2 (5·6, 24·8)	–1·8 (–8·1, 4·5)	5·4 (–14·0, 24·9)
CushingQoL score	42·2 (19·1)	29·1 (18·8, 39·5)	42·4 (23·5, 61·4)	52·4 (32·2, 72·7)
BDI score	16·8 (10·6)	–14·5 (–28·4, –0·5)	–19·2 (–31·8, –6·5)	–31·8 (–44·3, –19·3)

Normal ranges: plasma glucose 70–115 mg/dL; HbA_{1c} ≤6·4%; total cholesterol ≤5·2 mmol/L; HDL cholesterol 0·9–99·9 mmol/L; LDL cholesterol ≤3·4 mmol/L; triglycerides ≤2·2 mmol/L

Supplementary Table 5. Summary of adverse events in all patients (all grades and grade 3/4)

All patients	All grades, n (%)	Grade 3/4, n (%)
Summary of AEs		
Any AE	137 (100)	78 (56·9)
Any serious AE	50 (36·5)	39 (28·5)
AEs requiring dose interruption and/or change	106 (77·4)	39 (28·5)
Death	1 (0·7)	1 (0·7)
Most common study emergent AEs (occurring in >15% of patients overall)		
Nausea	57 (41·6)	3 (2·2)
Headache	46 (33·6)	4 (2·9)
Fatigue	39 (28·5)	3 (2·2)
Adrenal insufficiency*	38 (27·7)	6 (4·4)
Nasopharyngitis	31 (22·6)	1 (0·7)
Vomiting	30 (21·9)	4 (2·9)
Glucocorticoid deficiency†	29 (21·2)	5 (3·6)
Arthralgia	27 (19·7)	3 (2·2)
Back pain	27 (19·7)	0 (0)
Diarrhoea	25 (18·2)	1 (0·7)
Influenza	24 (17·5)	0 (0)
Asthenia	23 (16·8)	1 (0·7)
Blood ACTH increased	23 (16·8)	1 (0·7)
Oedema peripheral	21 (15·3)	0 (0)
Anticipated AEs of special interest		
Adrenal hormone precursor accumulation related	58 (42·3)	22 (16·1)
Hypocortisolism related	70 (51·1)	14 (10·2)
Pituitary tumour enlargement related	3 (2·2)	0
QT prolongation related	5 (3·6)	1 (0·7)
Arrythmogenic potential	1 (0·7)	1 (0·7)

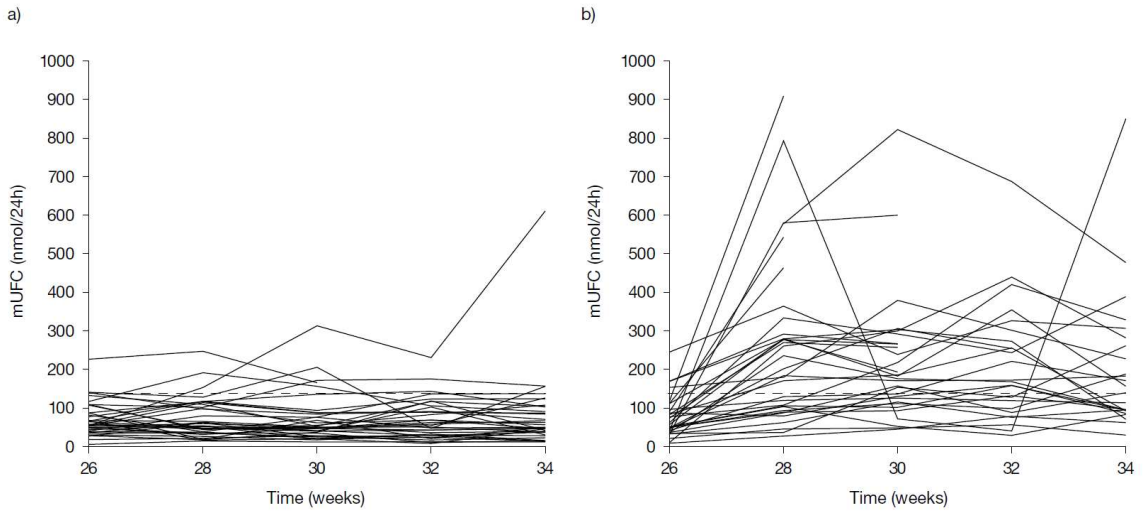
Patients with multiple events in the same category are counted only once in that category. *Adrenal insufficiency includes 'relative adrenal insufficiency', 'adrenocortical insufficiency', 'hypoadrenalcorticism', 'suspected hypoadrenalism', 'mild adrenal insufficiency', and 'adrenal deficiency'; †Glucocorticoid deficiency includes 'hypocortisolism', 'symptoms of hypocortisolism', 'relative hypocortisolism', 'suspicion of hypocortisolism', 'asymptomatic/symptomatic

hypocortisolism', and 'subjective symptoms of hypocortisolism'; clinical signs and serum cortisol measurements were not systematically collected. AE, adverse event

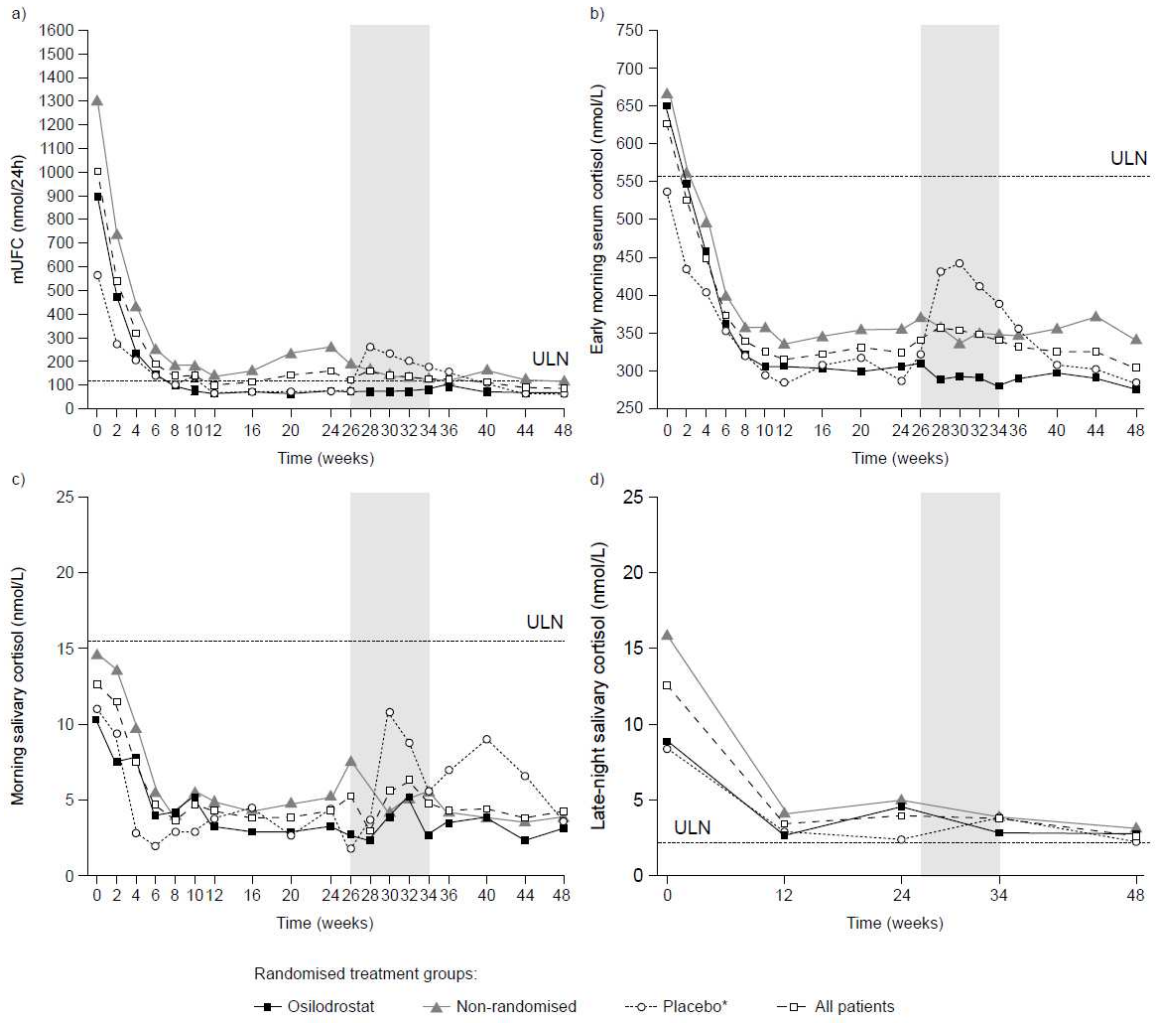
Supplementary Table 6. Pairwise correlation between ACTH (pmol/L) and mUFC (nmol/24h) over time in all patients

	n	Pearson's correlation, r
Baseline	137	0.56
Week 12	124	0.10
Week 24	124	0.12
Week 48	108	–0.10

Supplementary Figure 1. Intrapatient changes in mUFC levels during the randomised withdrawal period for patients randomised to a) osilodrostat and b) placebo

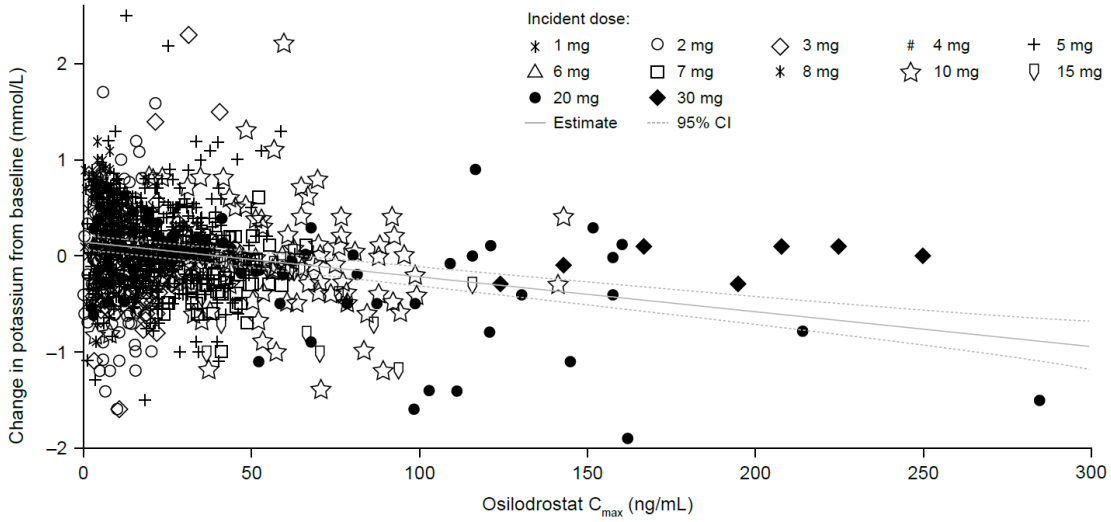


Supplementary Figure 2. Mean a) mUFC, b) serum cortisol, c) morning salivary cortisol, and d) late-night salivary cortisol at time points up to week 48, overall and by randomised treatment group



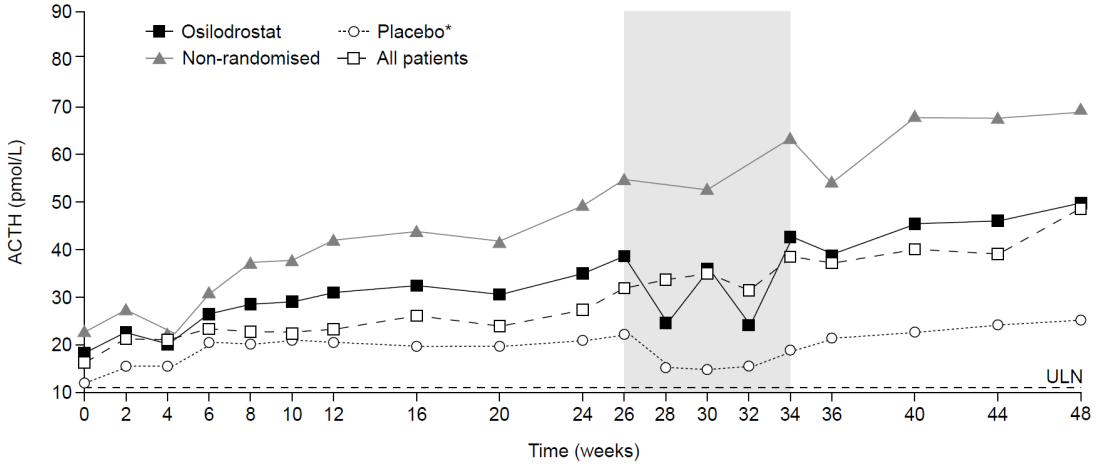
Includes scheduled visits only. Shaded areas indicate the randomised withdrawal period, starting at week 26 and ending at week 34. Normal ranges are as follows: UFC, 11–138 nmol/24h; serum cortisol, 127–567 nmol/L; morning salivary cortisol, 1·1–15·5 nmol/L; late-night salivary cortisol, ≤2·5 nmol/L. *For patients randomised to placebo during the randomised withdrawal period and including all data while on either treatment (ie including data for patients who were randomised to placebo but restarted open-label osilodrostat before the end of the randomised withdrawal period)

Supplementary Figure 3. Scatter plot of plasma osilodrostat C_{max} versus change in serum potassium from baseline up to data cut-off



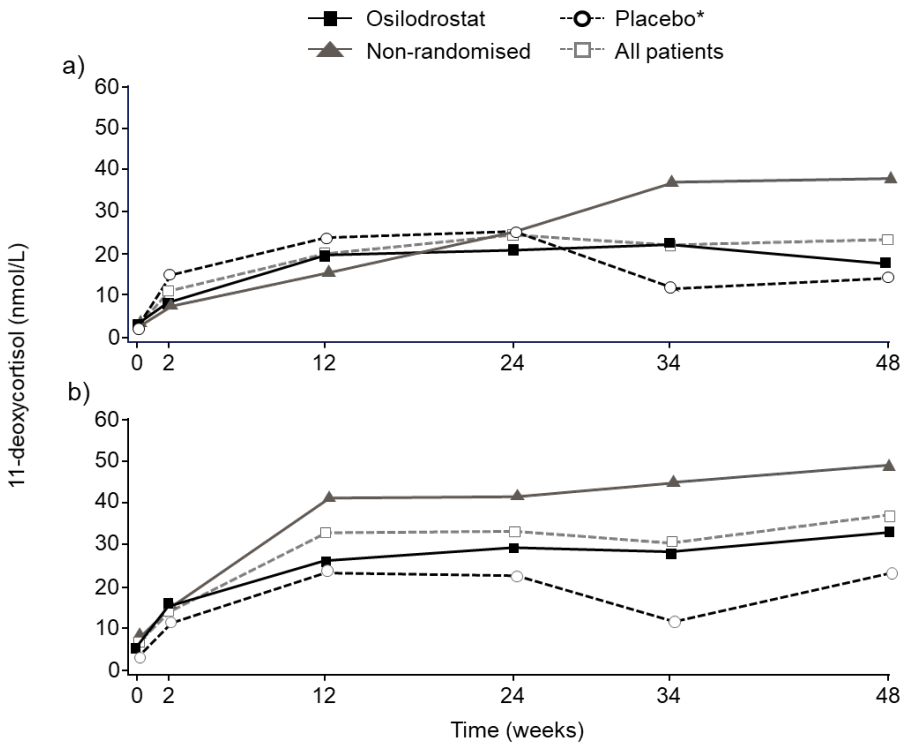
Intercept = 0·1425; estimate (95% CI) for C_{max} = –0·0036 (–0·0044, –0·0028). C_{max}, maximum plasma concentration

Supplementary Figure 4. Mean plasma ACTH levels at time points up to week 48 by treatment group



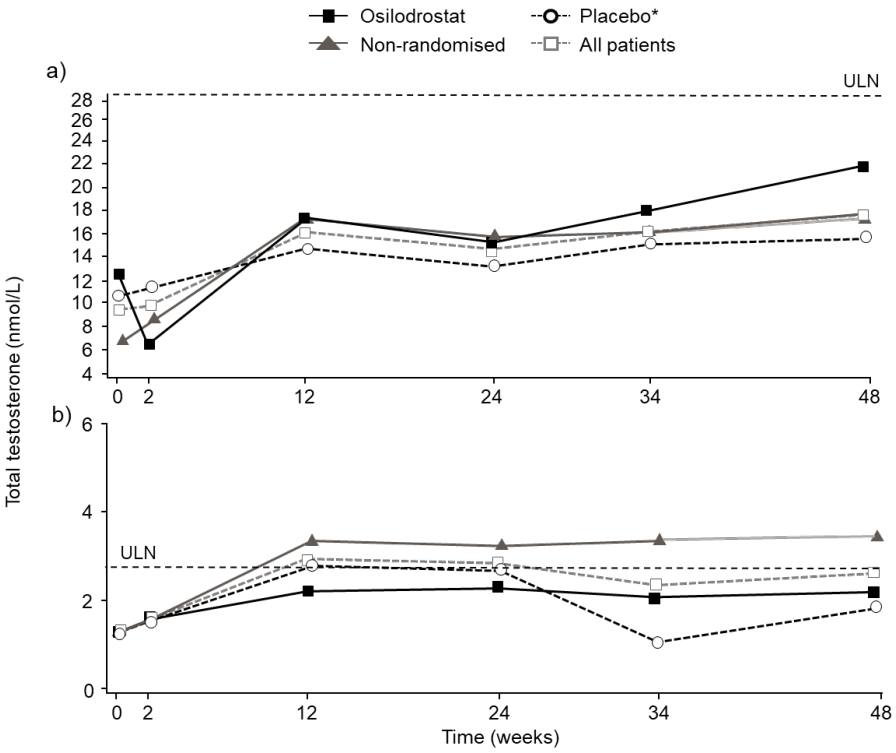
ACTH was assessed using the Immulite 2000 ACTH kit; ULN = 11·1 pmol/L (males), 6·0 pmol/L (females). *For patients randomised to placebo during the randomised withdrawal period and including all data while on either osilodrostat or placebo. Shaded areas indicate the randomised withdrawal period, starting at week 26 and ending at week 34

Supplementary Figure 5. Mean plasma 11-deoxycortisol levels at time points up to week 48, by treatment group, in a) males and b) females



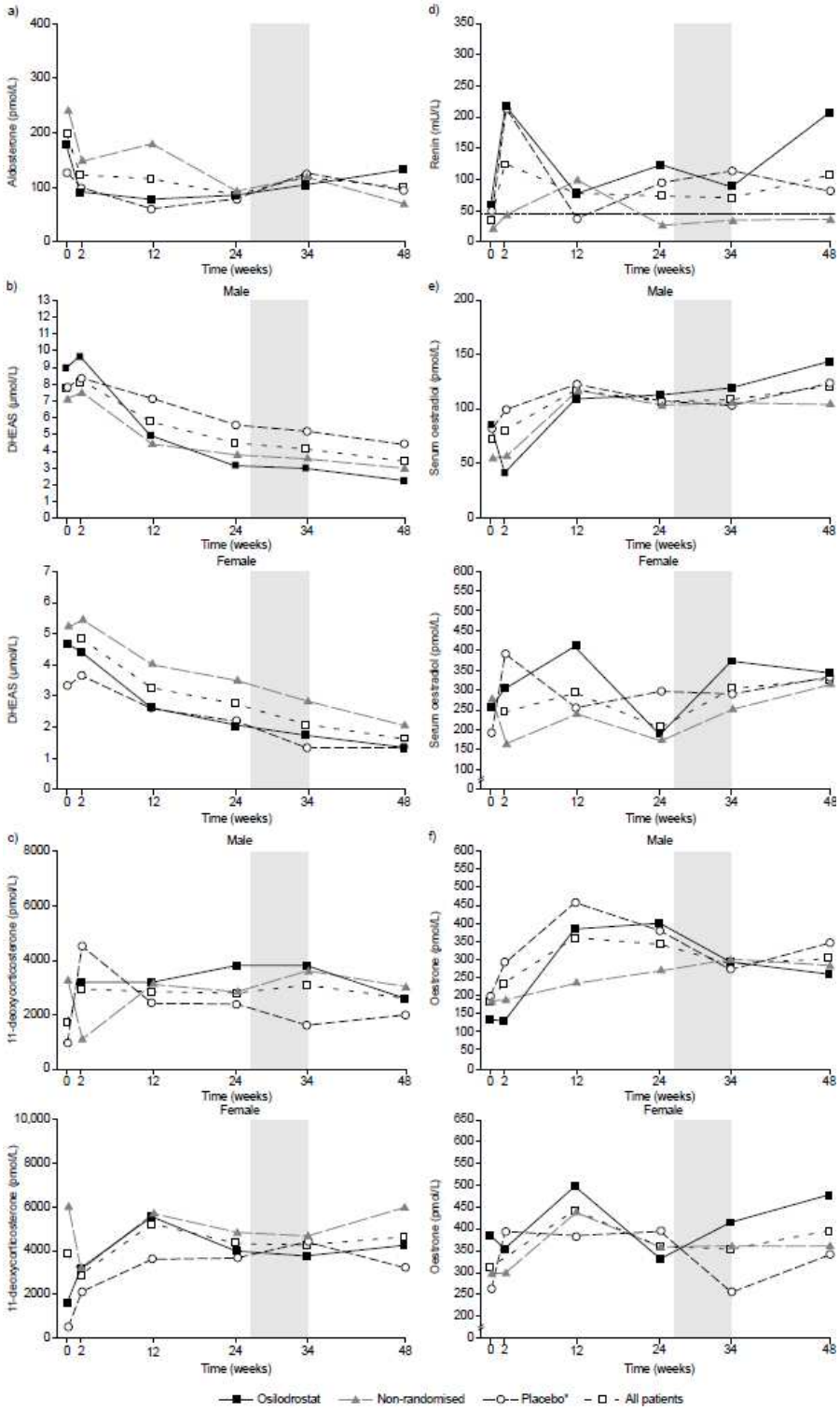
*For patients randomised to placebo during the randomised withdrawal period and including all data while on either osilodrostat or placebo. Shaded areas indicate the randomised withdrawal period, starting at week 26 and ending at week 34.

Supplementary Figure 6. Mean serum testosterone levels at time points up to week 48, by treatment group, in a) males and b) females



Testosterone reference range: 8.4–28·7 nmol/L (males), 0.7–2·6 nmol/L (females). *For patients randomised to placebo during the randomised withdrawal period and including all data while on either osilodrostat or placebo. Shaded areas indicate the randomised withdrawal period, starting at week 26 and ending at week 34. LLN, lower limit of normal

Supplementary Figure 7. Mean hormone levels at time points up to week 48 by treatment group for a) plasma aldosterone, b) serum dehydroepiandrosterone sulphate (DHEAS), c) serum 11-deoxycorticosterone, d) plasma renin, e) serum oestradiol, and f) serum oestrone



Shaded areas indicate the randomised withdrawal period, starting at week 26 and ending at week 34.