



**Number of patients planned and analysed:**

It was planned to screen 70 patients in order to have 55 patients included (52 patients were planned to be treated for 12 weeks and a provision of 3 patients were added to allow for drop-out before week 12). A total of 89 patients were screened and 50 patients were included.

**Objectives:****Primary objective:**

To assess the efficacy of metyrapone (MTP) to normalize cortisol levels (Urinary Free Cortisol – UFC) after 12 weeks of treatment in patients with endogenous Cushing's syndrome (CS).

**Secondary objectives:**

The secondary objectives are:

- To assess the effects of MTP after 12 weeks of treatment on:
  - mUFC decrease of  $\geq 50\%$
  - Salivary and serum cortisol levels
  - Hormonal and biochemical parameters that are associated with Cushing's syndrome or represent safety measurements.
  - Clinical signs of Cushing's syndrome
  - Quality of life as judged by Cushing's Quality of Life (CushingQoL) questionnaire and Tuebingen Cushing's disease quality of life inventory.
  - Safety and tolerability
- To assess the effects of long-term MTP treatment on efficacy and safety parameters (up to 36 weeks of treatment).

**Main inclusion criteria:**

1. Any men and women  $\geq 18$  years
2. Patients with endogenous Cushing's syndrome for whom the following criteria apply:
  - Newly diagnosed Cushing's disease patients who are unsuitable for early surgery or wish to defer surgery;
  - Or recurrent or persistent Cushing's disease after pituitary surgery.
  - Or patients with ectopic ACTH syndrome either occult or after surgery failure or inoperable or metastatic.
  - Or patients with Cushing's syndrome from adrenal causes who are unsuitable for early surgery or wish to defer surgery.
3. For patients receiving previous medical therapy, the following wash-out periods should be completed:
  - Steroidogenesis inhibitors excluding mitotane (e.g. ketoconazole), 1 week
  - Dopamine agonists (bromocriptine, cabergoline), 4 weeks
  - Pasireotide S/C, 1 week
  - Pasireotide LAR (formulated for once-monthly dosing), 12 weeks
  - Mifepristone, 4 weeks
4. UFC  $\geq 1.5$ -fold ULN on each of the three 24-hour urinary sampling measurements (after previous treatment withdrawal if applicable or in non-treated patients) provided that the diagnosis of Cushing's syndrome has been confirmed. Urine collections for UFC measurements was done within 5 weeks before the baseline visit.
5. Female patients should not be at risk of pregnancy (could be included if sterilized, post-menopausal, sexually inactive or using methods of contraception throughout the study).
6. Able and willing to give voluntary, written informed consent to participate in the study.
7. Agree to observe all study requirements and be available for all planned study visits.

### **Main Exclusion Criteria**

1. Pseudo Cushing's syndrome
2. Cyclic Cushing's syndrome defined by at least one normal UFC value among at least three 24-hour urinary sampling measurements over the previous 2 months.
3. Advanced adrenocortical carcinoma or ectopic ACTH secretion (EAS) secondary to a small cell lung carcinoma
4. Life expectancy less than 3 months
5. Pituitary or adrenal surgery or pituitary irradiation or surgery of the ACTH-secreting ectopic tumor or bilateral adrenalectomy planned before the week 12 visit.
6. Pituitary irradiation within the previous 5 years (for Cushing's disease patients)
7. Enlarged pituitary adenoma (greater than 1 cm in vertical diameter and leaving less than 2 mm from the chiasma) or compression of the optic chiasma on the pituitary MRI for patients with Cushing's disease.
8. Severe uncontrolled hypertension (>180/110 mmHg) despite anti-hypertensive therapy (for otherwise eligible patients, blood pressure medication may be adjusted to meet this criterion)
9. Severe hypokalemia (< 2.5 mmol/L) despite corrective measures
10. White blood cell count <3 x 10<sup>9</sup> /L; hemoglobin <10 g/dL; platelets <100 x 10<sup>9</sup> /L
11. Any other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that in the judgment of the investigator, would present excess risk associated with study participation or study drug administration, or which, in the judgment of the investigator, would make the patient inappropriate for entry into this study.
12. Pregnant or positive pregnancy test at entrance or breast-feeding women
13. Current alcohol or drug abuse
14. Acute or chronic severe uncontrolled infections
15. Known hypersensitivity to metyrapone or to any of its excipients namely glycerol, disodium edentate, sodium hydroxide and phosphoric acid
16. Patients with mitotane (Lysodren®) plasma concentration > 3 mg/L
17. Participation in another treatment study or receiving any investigational treatment (drug, biological agent or device) within 30 days
18. Prohibited treatments (see section 6.2.1 Prohibited treatments)

### **Test product: dose and mode of administration, batch number**

Treatments were dispensed in an open-label fashion. At the visit V3 (or V2 when V2 and V3 visits are done on the same day), the investigator provided the patient with the study medication, a bottle of 50 capsules of 250 mg each. At each visit, the patient brought back the bottle(s) of study medication and the investigator/designee counted the remaining capsules for drug accountability and made sure that the patient had enough capsules until the next visit. A new bottle was given to the patient when needed.

Two possible initiation doses were used depending on the severity of hypercortisolism (based on the mUFC levels calculated from three 24-hour urinary samples collected at baseline and analysed by a central laboratory) with the following initial dosage:

1. For patients with moderate hypercortisolism, i.e. baseline mUFC levels  $\leq$  5-fold the ULN, study treatment started with administration of MTP at 750 mg/day.
2. For patients with severe hypercortisolism, i.e. baseline mUFC levels > 5-fold the ULN, the initial MTP dose was 1,500 mg/day.

The total daily dose had to be divided in 3 or 4 doses per day. If the daily dose cannot be divided equally (i.e. in 250 mg increments), the highest dose was given at night.

Dose was then adjusted (up or down-titrated) during the first month on an individual basis at least once a week  $\pm$  2 days according to clinical tolerance and cortisol levels achieved (i.e. UFC and/or morning serum cortisol, for patients taking an oral contraception, dose titration was performed only according to UFC levels).

The following dose titration had to be followed until normal UFC or serum cortisol is achieved:

- If UFC concentrations are  $>$  ULN using 1 adequate 24-hour urine collection or if morning serum cortisol level before MTP intake is above 12  $\mu$ g/dL (330 nmol/L), MTP dose should be increased.
- If morning serum cortisol levels before MTP intake decrease below 7  $\mu$ g/dL (193 nmol/L) OR if the patient develops signs and/or symptoms suggesting adrenal insufficiency, MTP should be withdrawn for at least one day and then restarted at a lower dose.
- If UFC concentrations are  $\leq$  ULN in the absence of symptoms of adrenal insufficiency, OR if morning serum cortisol levels are between 7 (193 nmol/L) and 12  $\mu$ g/dL (330 nmol/L), the optimal dose is reached, and the patient can continue with this dose until the next visit.

For patients with moderate Cushing's syndrome who are initiated with MTP 750 mg/day: the daily dose can be decreased or increased by 250 to 500 mg/day.

For patients with severe Cushing's syndrome who started with MTP 1500 mg/day: the daily dose can be decreased or increased by 500 to 1000 mg/day. The maximum daily dose is 6 g/day (SmPC).

The batch numbers used in the study are listed in the following table:

	<b>Batch numbers</b>	<b>expiry date</b>
Multilingual - Vial(s) of 50 capsules of Metyrapone 250mg	MM050C	28/02/2021
	MM032A	30/09/2019
	MM025A	31/10/2018
	MM012A	30/09/2017

**Statistical Analysis:**

The primary analysis consisted in calculating the exact 95% CI (Confidence Interval) of the percentage of patients attaining normalization of 24-h UFC levels (based on the mean of three UFC collections) in the modified Intent to Treat (mITT) population after 12 weeks of treatment.

The mITT population is all included patients who have signed the informed consent, have received at least one study drug dose of MTP (exposed population) and have performed at least one UFC collection post-baseline including patients prematurely withdrawn will be included in the mITT set.

Patients were considered to have normalized UFC levels if the mean of three 24-hour UFC collections is at or below the upper limit of the normal range (ULN).

Main secondary analysis consisted of:

- Percentage of responders defined as patients who either reached a  $mUFC \leq ULN$  or had  $\geq 50\%$  decrease from baseline of UFC levels, up to 12 weeks and then up to 36 weeks,
- Time to eucortisolemia ( $mUFC \leq ULN$ ) up to 12 weeks,  
The time course of raw data of serum cortisol (before MTP dose and 2h after MTP dose) and of salivary cortisol at all 6 time points of the day, up to 12 weeks and then up to 36 weeks,
- The time course of the percentage of patients with normalization of late night salivary cortisol (LNSC) will be presented as well as the exact CI of the estimation, up to 12 weeks and then up to 36 weeks,
- Time course of the mean improvement, stabilization or worsening of the clinical signs related to hypercortisolism, up to 12 weeks and then up to 36 weeks,
- Time course of the mean improvement of biochemical parameters, up to 12 weeks and then up to 36 weeks,
- The time course of the mean improvement, stabilization or worsening of the clinical signs related to hypercortisolism as assessed by investigator (blood pressure, body mass index, waist circumference, facial plethora, bruising, buffalo hump, supraclavicular fullness) and specific QoL questionnaires (Cushing's QoL questionnaire and Tuebingen CD QoL) was provided up to 12 weeks and then up to 36 weeks,

All patients were assessed for occurrence of adverse events. Adverse events were graded according to three severities: mild, moderate and severe.

Analysis of adverse events was performed on the basis of Treatment Emergent Signs and Symptoms (TESS) considering those effects that were not seen at baseline together with those present at baseline that worsened later on. Adverse events of special interest (AESI) were also identified. Adverse events were coded according to MedDRA version 18.1.

**Patient Disposition:**

A total of 89 patients signed informed consent in 26 sites and 8 countries between April 14<sup>th</sup>, 2015 and May 31<sup>st</sup> 2019. The recruitment period lasted 4 years and 2 months.

Of the 89 patients selected, 50 (56%) patients were included in 21 sites in 8 countries and treated with metyrapone between May 11<sup>th</sup> 2015 and April 29<sup>th</sup>, 2020. Of the remaining 39 patients, one patient withdrew his consent. Thirty-eight (38) patients were not included because they did not meet selection criteria, including inclusion criterion  $n^{\circ}4$   $mUFC \geq 1.5$  fold ULN on each of three 24h urine samples,  $n=36$ , exclusion criterion  $n^{\circ}7$  enlarged pituitary adenoma larger than 1 cm (vertical diameter),  $n=2$ , exclusion criterion  $n^{\circ}11$ : Any other risky medical or psychiatric condition  $n=1$ .

Between baseline and week 12, 3 patients discontinued the study drug, one for SAE (pneumonia and septic shock) and 2 because of physician's decision to perform surgery (a transsphenoidal surgery in a patient diagnosed CD and a bilateral adrenalectomy in a patient diagnosed EAS) despite improvement under metyrapone treatment.

At week 12, among the 50 treated patients 86% (43/50) were eligible to enter the 6-month extension period (normal mUFC levels or mUFC levels above normal range but not exceeding 2-fold ULN at week 12). Of these 43 patients, 41 patients entered the extension period as two patients eligible for the extension period declined to continue.

Between week 12 and week 24, one patient decided to discontinue the study drug to undergo pituitary surgery. Between week 24 and 36, five (5) patients discontinued the study drug: one physician's decision because patient wished surgery and there was no clinical improvement, one patient's decision to undergo pituitary surgery despite improvement, one because of AE: hirsutism, two because of SAEs: one patient had bilateral acute angle glaucoma and one patient had hypotension, respiratory arrest, suspicion of pulmonary embolism, cellulitis and renal failure.

A total of 35 patients completed the extension period.

**Baseline Characteristics of mITT set:**

**Age: median (min-max), years, and sex (M/F) and BMI - mITT set:**

	<b>Total (N=49)</b>
<b>Age (years)</b>	
Mean (SD)	46.4 (13.3)
Median [Min; Max]	47.0 [22 ; 73]
<b>Age in classes (years)</b>	
< 65	43 (87.8%)
≥ 65	6 (12.2%)
<b>Male/female</b>	
Female	34 (69.4%).
Male	15 (30.6%).
<b>BMI (Kg/m<sup>2</sup>)</b>	
Mean (SD)	30.5 (7.3)
Median	27.7
Min. ; Max.	22.5 ; 55.4

Among the 34 women in the mITT, 17 (50%) were menopausal for a mean of 7 years. Of note, the patient treated but not included in the mITT was a woman of 73 years old with a diagnostic of EAS.

**Disease characteristics - mITT set:**

	<b>Total(N=49)</b>
<b>Time since first symptoms of CS (years)</b>	
Mean (SD)	5.8 (5.6)
Median [Min; Max]	3.9 [0.3; 21.8]
<b>Type of CS n(%)</b>	
Cushing's disease	44 (89.8%)
Ectopic ACTH syndrome	4 (8.2%)
Adrenal cause	1 (2.0%)

**Number of patients with prior radiotherapy and/or surgery for CD – mITT set:**

Among the 49 patients, 60.0% (30/49) had prior surgery of pituitary adenoma. Among these 30 patients, 60.0% (18/30) had 1 prior surgery, 30.0% (9/30) had 2 prior surgeries and 10.0% (3/30) had 3 prior surgeries. Five (5) patients had prior radiotherapy. The median [Min.; Max] time since radiotherapy was 7 [5.2 ; 18.3] months.

**Prior medical therapies for CS- mITT set:**

Among the 49 patients, 51.0% (25/49) were previously treated with at least one medical therapy. Among these 25 patients, 80.0% (20/25) took ketoconazole, 48% (12/25) took pasireotide, 20.0% (5/25) took

cabergoline, 16.0% (4/25) took metyrapone, 4.0% (1/25) took bromocriptine, 4.0% (1/25) took osilodrostat and 4.0% (1/25) took one investigational drug.

#### **Comorbidities at baseline -mITT set:**

At baseline, the following comorbidities associated to Cushing's syndrome were ongoing: hypertension in 69.0% (34/49), dyslipidemia in 41.0% (20/49), osteoporosis in 41.0% (20/49), diabetes mellitus in 39.0% (21/49), insulin resistance in 2 (4.0%) treated with metformin and glucose tolerance impaired in 4.0% (2/49) of patients, treated both with diet.

#### **Severity of mUFC and morning serum cortisol at baseline - mITT set:**

At baseline, the mean (SD) mUFC was 1041.7 (1337.0) nmol/24h and the median mUFC was 570.3 nmol/24h (3.5-fold ULN) [range:291.0 (1.8 x ULN) to 8476.2 (51.4 x ULN)]. ULN is 165 nmol/24h.

Categorisation of individual mUFC at baseline in three groups according to severity: mild ( $\geq 1.5$  ULN and  $\leq 2$ xULN), moderate ( $> 2$ xULN and  $\leq 5$ xULN) and severe ( $>5$ xULN) showed that the moderate category was the most represented with 63.0% (31/49) of patients with mUFC at baseline between 330 nmol/24h and 825 nmol/24h. The severe category represented 33% (16/49) of patients (mUFC  $> 825$  nmol/24h) and the mild category represented only 4% (2/49) patients.

At baseline, the mean (SD) and median [Min.; Max] morning serum cortisol were 607.8 nmol/L (222.2) and 578.0 [137.0 to 1286.0] nmol/L, respectively. The ULN is 500 nmol/L.

#### **Salivary cortisol day curve:**

Baseline mean and median values of salivary cortisol at the 6 time points assessed over the day were quite similar showing the loss of the normal cortisol circadian rhythm of cortisol secretion (cortisol peaks one hour after waking up falls throughout the day and drops to its lowest point within an hour of sleep). At baseline, median [Min;Max] salivary cortisol (of all time points) was 14.7 [3.2 ; 96.6] nmol/L and median [Min;Max] LNSC was 15.0 [3.2 ; 96.6] nmol/L.

Normal ranges of salivary cortisol were 5.0 to 46.0 nmol/L in the morning (8 to 9 a.m) and  $<2.6$  nmol/L for evening salivary cortisol (10 to 12 p.m).

#### **Efficacy results:**

##### **Datasets analysed:**

From the 89 patients screened, 50 patients were eligible: meeting inclusion and exclusion criteria and were included in the study and treated with metyrapone.

The mITT dataset includes all patients having received at least one study drug dose of MTP (exposed population) and having performed at least one UFC collection post-baseline including patients prematurely withdrawn. mITT set is considered as the primary population for efficacy analysis. From the 50 patients enrolled and treated, 49 were included in the mITT set. One patient was excluded from mITT as no post baseline evaluation was available for the primary efficacy criterion: UFC.

The safety dataset includes all the 50 patients having received at least one study drug dose of MTP.

##### **Primary endpoint:**

In the mITT population (Clopper Pearson 95% CI), using LOCF for missing data/dropouts, 47% (23/49) (95% CI [32.5%;61.7%]) patients normalized 24h mUFC at week 12. Last Observation Carried Forward (LOCF) was used for the 2 patients discontinuing the study drug before week 12, considered as not normalized for this analysis.

The median [Min;Max] of mUFC at week 12 was 171.3 [5.0; 816.0] nmol/24h, corresponding to a median decrease of -74.0% .

Among the 24 patients not normalized at week 12, categorization of mUFC by severity showed that 19 patients were in the mild category with mUFC below 2-fold ULN (330 nmol/24h) corresponding to a clinically significant improvement. The other 5 patients were in the moderate category with mUFC above 2 fold ULN and less than 5 fold ULN (825 nmol/L).

##### **Secondary endpoints:**

**Time to reach 50% decrease of mUFC and time to first eucortisolemia (UFC  $\leq$  ULN):**

The following results confirmed the fast action of metyrapone. The median time to reach a 50% reduction in mUFC was 14 days [IQR: 8-35] for 94% (46/49) of patients. And the median time to first eucortisolemia was 34 days [IQR: 14-92] for 71.0% (35/49) of patients. Fourteen (14) patients did not normalize mUFC during the 12-week period either because of premature withdrawal (n= 3) or because eucortisolism was not met before week 12 (n=11).

### **Secondary endpoints:**

#### **Normalisation of mUFC at weeks 24 and 36:**

At weeks 24 and 36, 53.0% [95% CI: 37.5%;67.1%] and 49.0% [95% CI:33.0%;64.4%] of patients normalized 24h mUFC (mean of 2 measures), respectively. For patients not normalized, the mild and moderate categories comprised 30.0 and 31.0% patients, respectively at week 24 and 17 and 20%, respectively, at week 36.

Median mUFC [ranges] were 160.2 [5.0; 710.0] and 173.0 [5.0; 810.0] nmol/24h at weeks 24 and 36, respectively corresponding to a median decrease from baseline of - 73% and - 70% at weeks 24 and 36, respectively.

#### **Rate of responders at weeks 12, 24 and 36:**

The rate of responders (complete responders: mUFC  $\leq$ ULN and partial responders: 50% decrease of mUFC from baseline) was similar over time, 80% (39/ 49) [95%CI: 66-89], 78% (31/40) [95% CI: 62.5%;87.7%) and 71.0% (25/35) [95% CI: 54.9%;83.7%) of patients at weeks 12, 24 and 36, respectively.

#### **Morning serum cortisol before metyrapone intake:**

A median decrease in morning serum cortisol was observed over time of - 29% at weeks 12 and 24 and of - 35% at week 36.

#### **Serum cortisol 2 h after metyrapone intake:**

Serum cortisol measured 2 h after metyrapone intake in the morning confirmed the rapid decrease of cortisol following intake. The median decrease was - 43% at week 1 and achieved - 67% at week 12. This median decrease was maintained at weeks 24 and 36: - 67% and- 71%, respectively.

#### **Salivary cortisol day curve:**

A median decrease in salivary cortisol was observed (of all time points of the day) starting at week 1, maximal at week 12 and maintained at week 36. Median decrease from baseline were - 4.4, -6.8 and - 7.7 nmol/L at weeks 1, 12 and 36, respectively.

#### **Late night salivary cortisol (11.pm) (LNSC):**

The median [range] of LNSC at baseline was 12.4 [1.2; 64.7] nmol/L. A median decrease of -36%, -55% and - 72% was observed at weeks 1, 12 and 36, respectively. Normalisation of LNSC was observed in 18% (8/44) [95% CI: 9.5%;32.0%], 22% (10/46) [95% CI: 12.3%;35.6%] and 27% (9/33) [95% CI: 15.1%;44.2%] of patients at weeks 1, 12 and 36, respectively.

#### **Laboratory tests:**

The median [range] of fasting glucose at baseline was 5.1 [3.9; 11.3] mmol/L. A median decrease of -5.3% and - 6.1 % was observed from baseline at weeks 12 and 36, respectively. At baseline 27% (12/45) of patients had fasting glucose above ULN, decreasing to 21% (9/44) of patients at week 12. At week 36, 26% (9/35) had fasting glucose above ULN.

The median [range] of fasting insulin at baseline was 93.4 [13.9; 287.8] pmol/L. A median decrease of -8.7% and - 15.4 % was observed from baseline at weeks 12 and 36, respectively. Patients with value above the ULN were 20% (9/46), 21% (8/38) and 20% (6/30) at weeks 12, 24 and 36, respectively.

The median [range] of HbA1C at baseline was 5.8 % [3.2; 8.1]. A median decrease of -2.0% and -4.0% was observed from baseline at weeks 12 and 36, respectively. At baseline 45% of patients had HbA1C above ULN and a decrease was observed over time with 28% and 31% of patients at weeks 12 and 36, respectively.

The median [range] of total cholesterol at baseline was 5.4 [3.5; 9.2] mmol/L. A median decrease of -14.0 % and - 19.0 % was observed from baseline at weeks 12 and 36, respectively. The number of patients with total cholesterol above ULN was 58% at baseline and decreased to 41% and 39% at weeks 12 and 36, respectively.

The median [range] of HDL cholesterol at baseline was 1.5 [0.9; 2.5] mmol/L. A median decrease of -17.5 % and - 22 % was observed from baseline at weeks 12 and 36, respectively. The number of patients with HDL cholesterol under LLN at baseline was 12.5% and increased to 34% and 33% at weeks 12 and 36, respectively.

The median [range] of LDL cholesterol at baseline was 3.4 [1.8; 7.1] mmol/L. A median decrease of -12 % and - 11 % was observed from baseline at weeks 12 and 36, respectively. The number of patients with value above ULN at baseline of 61% of patients decreased to 45% of patients at weeks 12 and 36.

The median [range] of triglycerides at baseline was of 1.5 [0.5; 6.6] mmol/L. A median decrease of -0.2% and - 4 % was observed from baseline at weeks 12 and 36, respectively. At baseline 33% of patients had triglycerides above ULN and 37% at week 12 decreasing to 28% at week 36.

In conclusion, slight improvements were observed in metabolic parameters over time. Exploratory analysis in subgroup of patients with mUFC and/or LNSC normalisation may provide data with further improvements.

#### **Testosterone and androstenedione level:**

Among the 32 women patients with values at baseline, testosterone level above the ULN was observed in 2 patients (6%) increasing to 81% and 88% at weeks 12 and 36, respectively.

At baseline, mean testosterone level was under the ULN and increased above ULN at weeks 12 and 36, respectively, corresponding to an increase of 1.9-fold and 2.8-fold baseline value at weeks 12 and 36.

The mean androstenedione increased from baseline value by 3-fold at week 12 and by 4-fold at week 36.

The mean and median androstenedione were above ULN at baseline. Among the 32 women analyzed, baseline value was above ULN in 13 (41%) patients. This proportion increased to more than 90% of patients at weeks 12 and 36.

#### **Blood pressure:**

The median of systolic blood pressure (SBP) was 134 mmHg range: [87.5; 172.0] at baseline. A median decrease of SBP from baseline of -3%, - 1% and - 3% was observed at weeks 12, 24 and 36, respectively.

The median of diastolic blood pressure (DBP) was of 86.2 mmHg range: [52.5; 113.0] at baseline. A median decrease of DBP from baseline of -6%, -1% and -6% was observed at weeks 12, 24 and 36, respectively.

#### **Signs and symptoms of CS:**

Improvement in CS signs and symptoms started at week 2 with 49% (23/47) of patients having improvement or normalization and increased over time with 66% (31/47) of patients having improvement or normalization of signs and symptoms at week 12. During the extension period, improvement or normalization was maintained in 63% (25/40) and in 78% (28/36) of patients at week 24 and 36 respectively.

The mean (SD) weight was 84 (20.1) kg at baseline. A mean decrease of -1.8 (6.0) kg was observed at week 36. Baseline waist circumference was 106.2 (14.2) cm. No change was observed between baseline and week 12. A mean decrease of - 3.7 (7.4) cm was observed at week 36. The mean (SD) baseline BMI 30.5 (7.3) kg/m<sup>2</sup> showed a mean decrease of - 0.65 (2.2) kg/m<sup>2</sup> at week 36.

#### **Modified Ferriman and Gallwey score (hirsutism):**

In the 33 women with a score at baseline, the mFerriman and Gallwey score showed at week 12 an improvement from baseline in 23% (7/35) of patients and a worsening in 26% (8/35) of patients. While in 52% (16/35) of patients there was no change. At week 36, the mFerriman and Gallwey showed an improvement from baseline in 26% (6/35) of patients and a worsening in 26% (6/35) of patients. **QoL:**

**Cushing QoL:** The mean (SD) total score at baseline of 41.5 (19.4) increased to 51.0 (18.0) points at week 12. There was a further increase in total score at weeks 24 and 36, with a mean of 51.6 (20.2) and 53.1 (18.2), respectively. A mean increase of Cushing QoL score from baseline of 10.0 points (13.8) was observed at week 12. The mean increase at week 12 is close to the minimal clinically relevant difference of 10.1. The mean (SD) increase further improved at weeks 24 and 36 by 11.3 (13.2) and 10.4 (13.1) from baseline, respectively.

**Tuebingen CD-25 QoL:** The mean total score showed a decrease over time from baseline to week 12. This decrease improved further during extension period. Mean Total score of 41 at baseline decreased to 35 and 30 at weeks 12 and 36, respectively. The mean (SD) decrease of the total score from baseline was of -5.3 (13.0) at week 12 and of -10.4 (13.7) and - 8.7 (16.3) at weeks 24 and 36, respectively. Improvement was reported in

all the domains, including depression, sexual activity, environment, eating behavior, bodily restrictions and cognition.

### **Safety Results:**

#### Extent of exposure:

Among the 50 patients enrolled and treated, during the first period of 12 weeks, 47 patients received metyrapone for 12 weeks, the 3 other patients discontinued before week 12. During the extension period, a total of 41 patients were treated with metyrapone for 6 months, except 6 patients who discontinued before the end of the study. One between week 12 and 24 and 5 between weeks 24 and 36.

#### Dosing:

Median duration of the treatment was 36 weeks. Median dose of metyrapone at week 12 was 1500 mg/day, with a range of 250 to 5550 mg/day. During the extension period, median dose of metyrapone was maintained at 1500 mg/day, with a range of 250 to 5750 mg/day at week 36.

#### Adverse Events:

Overall, 47 (94%) patients experienced 322 AEs, with any relationship to study drug, during the whole study period. Among the 322 AEs, 74% (237/322) AEs were reported during the first 3 months of exposure. During the 6-month extension period, fewer AEs were observed, as only 85 AEs were reported.

Up to week 12, a total of 84% experienced at least one AE regardless of any relationship to metyrapone. For the great majority of patients 70% (35/50), AEs were of mild intensity, whereas only 8% (4/50) of patients had AEs of severe intensity. Comparing the number of patients experiencing at least one AE regardless of the relationship with metyrapone at week 12 (n=42) to week 36 (n=47) only 5 additional patients experienced at least one AE during the 6-month extension period.

Adverse events related to hypocortisolism were the most frequent reported AEs and included:

Gastrointestinal disorders: nausea and abdominal pain were the most frequent adverse events reported in 19 (38%) and 7 (14%) patients, respectively and were experienced mostly during the initiation and up-titration period of the initial 12 weeks. General disorders: fatigue/asthenia in 18 (36%) patients. Metabolism and nutrition disorders: decreased appetite in 9 (18%) patients. Nervous system disorders: headache/head discomfort/migraines in 13 (26%) patients and dizziness in 8 (16%) patients. Vascular disorders: hypotension in 3 (6%) patients. They were less frequent during the extension period as dose adjustments were less frequent.

Incidence of adrenal insufficiency (symptoms of hypocortisolism with biochemical confirmation of very low cortisol level) was 12% (6/50) and observed only during the first period of titration of the drug. The majority (5) were of moderate severity and only one was severe. Episodes were managed at home, with dose decrease or temporary discontinuation of metyrapone and supplementation with hydrocortisone,

for the majority of patients. Episodes were reversible and metyrapone was restarted at a lower dose and then again uptitrated. All episodes were reported as SAEs as per protocol recommendation.

Other main AEs reported and possibly related to study drug due to increase in hormone precursors were:

Hypertension was reported in 14% (7/50) of patients of mild (n=5) or moderate (n=2) severity, corresponding to worsening of existing hypertension and related to an increase in mineralocorticoid precursors. They were reported mainly during the first 12-week period, resolving spontaneously in 4 of them and after change in antihypertensive medications in 3 of them.

Peripheral oedema was observed in 12% (6/50) of patients, during the first treatment period, and were of mild (n=3) to moderate (n=3) severity. Origin was probably of vascular origin in 5 patients, resolving spontaneously or with the addition of diuretics. The last patient was treated with a non-steroidal anti-inflammatory drug as oedema was probably related to concomitant joint pain.

Asymptomatic hypokalemia was reported as AE in 8% (4/50) patients of mild to moderate severity, each for 2 of them. Three of them had already hypokalemia at baseline treated with oral supplementation of potassium. Episodes resolved after either adding oral supplementation or increasing existing oral supplementation or by removal of a diuretic (furosemide). One patient was hospitalized with temporary interruption of metyrapone and IV potassium supplementation followed by oral supplementation and resolution.

Incidence of hirsutism was reported in 9% (3/34) of women and only during the 6-month extension period; one case led to study drug discontinuation. This AE is related to the increase in testosterone observed in women following blockade of cortisol synthesis by metyrapone. Acne is also related to the same mechanism and was reported in 2 (4%) patients.

Muskuloskeletal events, such as arthralgia and/or myalgia, were observed in 18% (9/50) of patients. The majority (n=6) were of mild severity untreated and resolved spontaneously for 4 of them. Two were of moderate severity. One patient had a worsening of existing rheumatoid arthritis and was hospitalized for corrective treatment.

Dose reductions related to AEs were observed in 12% (6/50) of patients during the study and were mainly related to gastrointestinal disorders (nausea, anorexia), general disorders (fatigue, headache), and adrenal insufficiency. Five patients were affected by dose reductions related to AEs before week 12, while only one patient after week 12.

Temporary dose interruption related to AEs occurred once in 18% (9/50) of patients during the study; 7 (14%) during the first period of 12 weeks and 2 (4%) during the extension period. For 7 patients AEs were study drug related: hypocortisolism symptoms (nausea, vomiting, fatigue, dizziness, migraines) (n=4), AI (n=2) and hypokalemia (n=1). For 2 patients, AEs were not study drug related, according to the investigator: anxiety and respiratory viral infection. The severity of AEs was moderate except for one patient: severe (an adrenal insufficiency).

Permanent study drug discontinuation related to AEs/SAEs occurred in 8% (4/50) of patients. one patient up to week 12 (pneumonia and septic shock) and 3 after week 12 (hirsutism in one; angle closure glaucoma in one and cellulitis, pulmonary embolism, respiratory arrest, renal failure, and hypotension in the last one).

Overall, 24% (12/50) of patients had 21 SAEs between baseline and week 36, with any relationship to study drug. Half (6) experienced an adrenal insufficiency, occurring during the first 12-week period of titration. All SAEs resolved without sequelae. Among these 12 patients, 8 experienced 12 SAEs related to the study drug, according to the investigator.

The majority of SAEs experienced by 14% (7/50) of patients were of moderate intensity.

Severe SAEs occurred in 10% (5/50 patients). Four of them had severe SAEs related to study drug, which were: adrenal insufficiency, angle closure glaucoma, pneumonia and septic shock experienced by one patient, and cellulitis, pulmonary embolism, respiratory arrest, renal failure, and hypotension experienced by one patient. One patient had a severe unrelated SAE, which was a pituitary adenoma for which surgery was already planned and occurred before week 12.

SAEs led to premature discontinuation for one patient during the first 12-week period: pneumonia and septic shock; and for two patients between week 12 and 36: angle closure glaucoma for one patient and cellulitis, respiratory arrest, pulmonary embolism, renal failure and hypotension for the last patient.

#### Clinical Laboratory Parameters:

With respect to biochemistry tests, mild increases of hepatic enzymes less than 2-fold ULN for ASAT and ALAT, and less than 1.2-fold ULN for GGT, were observed in 6 patients during the study, which were reversible in the majority of cases without dose reduction or discontinuation of metyrapone.

Mild increase above ULN in conjugated bilirubin was observed in 3 patients during the study less than 1.5-fold ULN. (One at week 1 (1.2-fold ULN), one at week 12 (1.1-fold ULN) and one at week 36 (1.4 fold-ULN). Spontaneous resolution was observed in 2 patients at the subsequent visits, for the last one at week 36 the evolution was unknown.

ECGs: Three (3) patients had an asymptomatic increase from baseline of QTcF above 60 ms but no patient had a QTcF of or above 480 ms. Relationship to study drug of QTc increase observed is unassessable in the context of this single arm study without a control arm that included patients with many other risk factors (e.g., chronic illnesses, hypothyroidism, hypokalemia).

**Conclusion**

This single arm 9-month study confirms the efficacy previously shown over 5 decades by short and long term metyrapone treatment of all forms of CS. The study also confirms the rapid onset of action of metyrapone leading to a clinically significant reduction in mUFC, with a median decrease of -74% at week 12, which was maintained at weeks 24 and 36 (-73% and -70%, respectively). The proportion of responders (complete responders: mUFC  $\leq$  ULN and partial responders: 50% decrease of mUFC from baseline) was similar over time: 80%, 78% and 71% at weeks 12, 24 and 36, respectively. The frequency of mUFC normalization at weeks 12, 24 and 36 was 47%, 53% and 49%. It is likely that additional patients may have normal results if results are assessed more frequently and the uptitration algorithm adapted to each patient. Titration is based on individual response and tolerability, which differ among patients due to inter -and intra -variability in steroidogenesis. An improvement of cortisol circadian rhythm also was observed based on cortisol salivary day curve with a decrease of cortisol at all time points and a lower late night salivary cortisol (LNSC) than the prebreakfast one, with normalization of LNSC in 27% of patients at week 36. This rate also may be improved by optimizing the distribution and amount of metyrapone over the day.

The safety of short and long-term use of metyrapone also was confirmed based on analysis of adverse events, laboratory tests and ECG. Health-related quality of life was assessed for the first time; a sustained improvement was observed beginning at week 12.

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