# Mitotane treatment in patients with metastatic testicular Leydig cell tutor associated with severe androgen excess

Chortis, Vasileios; Johal, Nicholas J.; Bancos, Irina; Evans, Matthew; Skordilis, Kassiani; Guest, Peter; Cullen, Michael H.; Porfiri, Emilio; Arlt, Wiebke

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1    **Mitotane treatment in patients with metastatic testicular Leydig cell tumour <mark>associated</mark>**

2                                  **<mark>with severe androgen excess</mark>**

3    Vasileios Chortis[1,2*], Nicholas J. Johal[1*], Irina Bancos[1,3], Matthew Evans[4], Kassiani Skordilis[4],

4                    Peter Guest[5], Michael H. Cullen[6], Emilio Porfiri[6], Wiebke Arlt[1,2]

5    [1]Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, B15 2TT,

6    UK

7    [2]Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham,

8    B15 2TH, UK

9    [3]Division of Endocrinology, Metabolism and Nutrition, Mayo Clinic, Rochester, MN, 55905, USA

10   [4]Department of Radiology, [5]Department of Pathology, and [6]Cancer Centre, Queen Elizabeth Hospital,

11   University Hospitals Birmingham NHS Foundation Trust, Birmingham, B15 2TH, UK

12   * equal first authors

13   **Address all correspondence and request for reprints to:**

14   Professor Wiebke Arlt

15   Institute of Metabolism and Systems Research

16   University of Birmingham

17   Birmingham, B15 2TT, UK

18   Phone: +441214158716          Fax: +441214158712                email: w.arlt@bham.ac.uk

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## ABSTRACT

Mitotane (o,p'DDD) is established in the adjuvant and advanced stage treatment of adrenocortical carcinoma and counteracts both tumour growth and tumour-related steroid production. Both the adrenal glands and the gonads are steroidogenically active organs and share a common embryogenic origin. Here we describe the effects of mitotane in two patients with metastatic Leydig cell tumour (LCT) of the testes and associated severe androgen excess (serum testosterone 93 and 88 nmol/l, respectively; male reference range 7-27 nmol/L). Both men suffered from severe restlessness, insomnia and irritability, which they described as intolerable and disrupting normal life activities. Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) confirmed excess androgen production and revealed concurrent overproduction of glucocorticoids and glucocorticoid precursors, which under physiological conditions are produced only by the adrenal glands but not by the gonads. In a palliative approach, they were commenced on mitotane, which achieved swift control of the hormone excess and the debilitating clinical symptoms, restoring normal quality of life. GC-MS demonstrated normalization of steroid production and decreased $5\alpha$-reductase activity, resulting in decreased androgen activation, and imaging demonstrated disease stabilization for 4-10 months. In conclusion, mitotane can be highly effective in controlling steroid excess in metastatic LCTs, with anti-tumour activity in some cases.

**INTRODUCTION**

Testicular Leydig cell tumours (LCTs) are rare stromal tumours, comprising 1-3% of all testicular neoplasms (1, 2). LCTs result in precocious puberty in 10% of affected children due to excess androgen secretion (3). Affected adult men most commonly present with a painless testicular mass and significant androgen excess (4) and can also have tumour-related oestrogen excess, manifesting with gynecomastia in 10-30% of cases (4-6). An estimated 10-15% of testicular LCTs are malignant (3, 7), although the true proportion remains debated (6, 8). The primary approach to malignant LCTs is surgical, usually involving orchidectomy, retroperitoneal lymph node dissection and lifelong surveillance (9). LCT metastases are rare and are detected on average 10 years after primary surgery (7), but therapeutic options are very limited, with no known role for radiotherapy and lack of efficacy of cytotoxic chemotherapy (7, 9). Therefore, prognosis for this rare endocrine cancer is poor, with an approximate median survival of two years (3, 4, 10).

During human fetal development, gonads and adrenal glands both derive from the urogenital ridge and after separation they develop distinct steroidogenic features, with gonadal sex steroid production and adrenal production of glucocorticoids, mineralocorticoids and adrenal androgen precursors. Mitotane (o,p'DDD) is routinely used in the treatment of adrenocortical cancer, where it has been shown to control adrenal steroid excess and, to a degree, tumour proliferation (11). Mitotane also diminishes androgen action by inhibiting $5\alpha$-reductase (12) and hence activation of testosterone to $5\alpha$-dihydrotestosterone. Thus, we considered mitotane as a potentially useful drug in patients with metastatic Leydig cell tumour, in particular in patients with tumour-associated androgen excess. Here we describe the effects of mitotane treatment in two patients with metastatic LCT, leading to a significant biochemical and clinical amelioration of the signs and symptoms of tumour-related steroid excess, and in one of them also to radiological stabilization of previously rapid disease progression.

62 **METHODS**

63      Urinary steroid metabolome profiling at baseline and during mitotane treatment was carried

64 out by gas chromatography-mass spectrometry, utilizing selected-ion-monitoring analysis for

65 identification and quantification of 32 distinct steroid metabolites reflective of 24-h net steroid output,

66 as previously described (13). Serum steroid measurements were carried out in the routine clinical

67 biochemistry setting, using established and validated tandem mass spectrometry (androstenedione,

68 testosterone) and immunoassays (DHEAS, 17β-oestradiol), respectively.

69      We carried out immunohistochemistry for sterol-O-acyl transferase 1 (SOAT1) as described

70 previously (14), using antibodies against SOAT1 (1:1000; ab39327; Abcam). The intensity of staining

71 was scored as described by Sbiera et al (15).

72 **CASE REPORTS**

73      **Case 1:** A 51-year-old patient presented with severe restlessness, insomnia, impaired

74 concentration, increased aggressiveness, redness of the face and body hair growth, all gradually

75 developing over the last six months. Fifteen years previously, he had undergone an orchidectomy for

76 LCT and thirteen years later excision of a retroperitoneal mass, confirmed on histology as LCT

77 metastasis. Imaging revealed multiple lesions consistent with liver, lung and retroperitoneal

78 metastases. Immunohistochemistry of a tissue biopsy confirmed vimentin positive, inhibin negative

79 metastatic LCT. Serum testosterone was very high at 93nmol/L (normal male reference range 7-

80 27nmol/L). Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) showed

81 increased androgen metabolite excretion (sum of androsterone and etiocholanolone 101,476μg/24h;

82 adult male reference range <8000μg/24h) as well as increased excretion of DHEA, metabolites of

83 pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, and cortisol (230

84 μg/24h; normal <130) (**Fig. 1A**). Prognosis was assessed as poor and the patient declined

85 chemotherapy. However, he agreed to the initiation of mitotane treatment in an attempt to improve the

86 clinical signs and symptoms of tumour-related androgen excess that was significantly limiting his

87 quality of life. Mitotane dose was gradually titrated to 3g per day, with concurrent hydrocortisone

88    replacement (20mg tid). Within a few weeks, androgen excretion decreased from 101,476 to 12,827

89    μg/24h, with evidence of significant inhibition of 5α-reductase activity and normalization of other

90    steroids that were increased at baseline (**Table 1**). Plasma mitotane concentrations considered

91    therapeutic (anti-proliferative) in the context of adrenocortical carcinoma (14-20mg/L) (16) were

92    reached after 5 months of treatment (**Suppl. Table 1**). Follow-up imaging still showed progressive

93    disease at two months, but stable disease according to RECIST 1.1 criteria after six months of

94    mitotane treatment (**Suppl. Fig. 1**). Alongside the decrease in androgens, the patient reported a

95    significant improvement of his previously debilitating clinical signs and symptoms. He returned to

96    full-time work and enjoyed good quality of life. After 10 months of mitotane treatment, he died

97    suddenly of a suspected myocardial infarction; no post-mortem examination was carried out.

98        **Case 2**: A previously fit-and-well 59-year-old man presented with a right testicular mass and

99    underwent orchidectomy; histopathology revealed malignant LCT. Three years later he presented with

100   lower back pain and imaging showed a large retroperitoneal mass, confirmed as disease recurrence by

101   transcutaneous biopsy. He underwent laparoscopic removal of the mass together with retroperitoneal

102   lymph node dissection. One year later, follow-up imaging revealed disseminated metastases,

103   including liver, kidney and peritoneal deposits. He was unwell, with agitation, anxiety and insomnia.

104   Biochemical work-up showed increased serum testosterone (88.5 nmol/L, normal 7-27), oestradiol

105   (744 pmol/L, normal <156), androstenedione (7.0 nmol/L, normal 0.8-3.1), and DHEAS (> 27

106   μmol/L, normal 0.91-6.76). GC-MS profiling showed increased steroid excretion including androgen

107   metabolites (69,108 μg/24h, normal <8000) and cortisol (414 μg/24h, normal <130) (**Fig. 1A**). He

108   rejected chemotherapy and agreed to palliative mitotane treatment with concurrent hydrocortisone

109   replacement; mitotane was administered employing the high-dose saturation regimen (Day 1 500mg

110   tds, Day 2 1000mg tds, and from day 3 onwards 1500 mg tds; therapeutic plasma mitotane levels

111   were reached after 4 months (**Suppl. Table 1**). Mitotane decreased serum androgen production within

112   four weeks. Six months after treatment initiation, plasma testosterone had decreased to 29.1 nmol/L

113   and oestradiol to 177 pmol/L, while androstenedione and DHEAS had normalized. Urinary steroid

114   profiling 4 months after initiation of miotane showed a decline in all previously raised steroid

115    metabolites and decreased 5α-reductase activity. This was paralleled by significant clinical

116    improvement in signs and symptoms, specifically reduced restlessness, aggressiveness and insomnia.

117    Imaging four months after initiation of mitotane revealed a mixed response, with regression of some

118    previous lesions but emergence of new metastatic deposits in lung and abdomen. The patient passed

119    away 12 months after his second recurrence, i.e. six months after the start of mitotane treatment.


120    **DISCUSSION**

121         Here we used mitotane, an established drug in adrenocortical carcinoma, in two patients with

122    metastatic testicular LCT associated with severe androgen excess, clinically manifesting with severe

123    restlessness, insomnia, irritability and impaired concentration. Both patients experienced significant

124    improvement in signs and symptoms with mitotane therapy, swift normalization of steroid excess and

125    some stabilization of radiologically quantified tumour load.

126         In a comprehensive PubMed search (search terms: Leydig cell tumour, malignant Leydig cell

127    tumour, metastatic Leydig cell tumour, mitotane, lysodren, and o,p'DDD) we identified eight cases of

128    LCT treated with mitotane (**Table 2**). Four patients received mitotane as second- or third-line

129    treatment for metastatic LCT for a very short time only (3 days to 8 weeks); none of them showed a

130    biochemical, clinical or radiological response. The remaining four cases received mitotane as first-line

131    treatment for metastatic LCT, with treatment duration varying between 10 weeks and 33 months

132    (**Table 2**). All four patients experienced significant radiological tumour response and reduction in

133    steroid excess during mitotane treatment. Azer and Braunstein (17) used mitotane to treat a patient

134    with metastatic LCT for six months, resulting in a dramatic response with complete remission of

135    multiple pulmonary metastases, which lasted three months prior to relapse. Radiological reduction in

136    tumour load for several months was observed in two cases (18, 19). Abelson et al (20) noted a

137    significant reduction in 17-ketosteroid excretion and clinical improvement in a metastatic LCT patient

138    treated with mitotane for 18 months, while his disseminated metastases progressed. Adding the

139    experience of our cases, mitotane can be considered a worthwhile palliative option in metastatic LCT,

140    particularly when the disease is associated with steroid excess.

141　　　　　During human fetal development, adrenals and gonads both arise from the urogenital ridge

142　and they both develop steroidogenic capacity, albeit with distinct features, i.e. sex steroid synthesis in

143　the gonads and glucocorticoid, mineralocorticoid and androgen precursor synthesis in the adrenal

144　glands. Benign testicular adrenal rest tumours, which are regularly found in men with congenital

145　adrenal hyperplasia, have been shown to display features of both adrenal and gonadal steroidogenesis

146　(21, 22). Using mass spectrometry, we observed that our two LCT patients showed not only androgen

147　excess, but also increased production of glucocorticoid precursors and cortisol, without clinical signs

148　of Cushing's syndrome. Two previous case reports in patients with malignant LCTs have described

149　ectopic production of steroids normally produced by the adrenal cortex, including cortisol and

150　aldosterone (23, 24). In our two cases, both androgen excess and glucocorticoid overproduction

151　responded well to mitotane treatment. Comprehensive steroid metabolome mapping by GC-MS has

152　been used successfully to differentiate malignant from benign adrenocortical tumours (13). It will be

153　useful to test in future studies whether steroid metabolome profiling would also help differentiate

154　benign from malignant LCT and could have a role in follow-up monitoring.

155　　　　　Recent studies have implicated sterol-A-acyl transferase 1 (SOAT1), previously also termed

156　ACAT-1 for Acyl-coenzyme A cholesterol acyltransferases, as a target of mitotane action (14).

157　SOAT1 is located in the endoplasmic reticulum and involved in intracellular esterification of free

158　cholesterol. John Achermann's group has shown that this enzyme operates downstream of SF-1 and is

159　important for regulation of adrenal steroidogenesis (25). A recent study (14) has provided evidence of

160　inhibition of SOAT1 by mitotane in an adrenocortical cell model, by demonstrating an increase in free

161　cholesterol, oxysterols and fatty acids after treatment with mitotane. We had access to formalin-fixed

162　paraffin-embedded tissue from the tumour recurrence in Patient 2 and used it for carrying out

163　immunohistochemistry for SOAT1 (**Fig. 1B**), which demonstrated predominantly high and moderate

164　expression, detected in 60% and 30% of the cells, respectively. Thus, it is likely that both the steroid-

165　ameliorating and anti-proliferative effects of mitotane are mediated by SOAT not only in

166　adrenocortical carcinoma but also in LCT.

167      Based on our current findings, the use of mitotane in the palliative treatment of metastatic

168   LCTs of the testes appears feasible and useful, with effective control of tumour-related steroid excess

169   and possible beneficial effects on disease progression, a viable treatment option in a rare endocrine

170   cancer that is not responsive to cytotoxic chemotherapy or radiotherapy.

171   **Declaration of interest:** The authors have nothing to disclose.

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244

245　**Figure Legend**

246　**Fig. 1:**

247　**Panel A,** Steroid synthesis in the two patients with metastatic testicular Leydig cell tumour as

248　assessed by mass spectrometry-based 24-h urinary steroid profiling before initiation of mitotane

249　treatment (log scale; closed circles, patient 1; open triangles, patient 2). Box plots represent medians

250　and interquartile ranges from a group of 24 healthy male volunteers (age 40-60 years); whiskers

251　represent the full range.

252　**Panel B,** Immunohistochemical staining for Sterol-O-acyltransferaase 1 (SOAT1) using formalin-

253　fixed paraffin-embedded tissue from the recurrent tumour of patient 2, demonstrating high (60% of

254　cells) to moderate (30% of cells) expression of SOAT1 in the tumour tissue.

**Table 1: 24-h urine steroid metabolite excretion (μg/24h) in the two patients with metastatic Leydig Cell Tumour before (=baseline) and during mitotane treatment.**
The male reference range is derived from the 24-h urine steroid excretion observed in 24 healthy men aged 40-60 years. The numbers of the steroid metabolites relate to the numbers in Fig. 1A.  The total glucocorticoid metabolites were calculated as the sum of metabolites 20, 22-25 and 27-30. n.m., not measured

| | | Median (min-max) steroid excretion in healthy men (μg/24h) | Patient 1 | | | | | | Patient 2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Baseline | Mitotane | | | | | Baseline | Mitotane |
| | | | | Month 1 | Month 2 | Month 4 | Month 6 | Month 9 | | Month 4 |
| | **Androgen and androgen precursor metabolites** | | | | | | | | | |
| 1 | Androsterone | 1,684 (477-5,915) | **44,744** | 13,833 | 11,823 | 9,790 | 4,448 | 4,440 | **38,092** | 597 |
| 2 | Etiocholonaolone | 1,668 (404-3,393) | **56,732** | 30,283 | 37,617 | 22,408 | 9,798 | 8,387 | **31,016** | 2,697 |
| 3 | 11β-hydroxy-androsterone | 609 (131-2,302) | **2,066** | 1,414 | 2318 | 301 | 169 | 174 | **13,351** | 643 |
| 4 | Dehydroepiandrosterone (DHEA) | 202 (14-3,948) | **1,939** | 1,034 | 434 | 294 | 194 | 311 | **47,344** | 359 |
| 5 | 16α-hydroxy-DHEA | 269 (0-1,492) | **4,404** | 9,179 | 4,098 | 3,510 | 2,533 | 6,250 | **23,569** | 1,126 |
| 6 | 5-pregnenetriol | 181 (38-951) | **1,558** | 2,574 | 2,484 | 2,600 | 1,575 | 2,483 | **20,891** | 1,053 |
| 7 | 5-pregnenediol | 326 (64-801) | **19.972** | 27,844 | 24,732 | 25,548 | 9,545 | 15,450 | **168,192** | 11,304 |
| | **Mineralocorticoids and mineralocorticoid precursor metabolites** | | | | | | | | | |
| 8 | Tetrahydro-11-deoxycorticosterone | 94 (22-290) | **445** | 195 | 162 | 188 | 29 | 128 | **308** | 22 |
| 9 | 5α-Tetrahydro-11-deoxycorticosterone | 107 (50-360) | **62** | 100 | 87 | 52 | 32 | 49 | **74** | 18 |
| 10 | Tetraydrocorticosterone | 97 (24-258) | **300** | 154 | 263 | 169 | 41 | 105 | **177** | 18 |
| 11 | 5α-Tetrahydrocorticosterone | 193 (67-1,197) | **130** | 261 | 489 | 0 | 0 | 0 | **90** | 0 |
| 12 | 3α,5β-Tetrahydroaldosterone | 30 (12-64) | **n.m.** | n.m. | n.m. | n.m. | 13 | 28 | **293** | 25 |
| 13 | Tetrahydrodeoxycorticosterone | 13 (5-36) | **n.m.** | n.m. | n.m. | n.m. | 93 | 343 | **216** | 37 |
| | **Glucocorticoid precursor metabolites** | | | | | | | | | |

| # | Metabolite | Reference (range) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Pregnanediol | 157 (32-336) | **3,249** | 1,857 | 1,474 | 1,171 | 455 | 646 | **4,832** | 199 |
| 15 | 3α,5α-17-hydroxy-pregnanolone | 14 (6-89) | **n.m.** | n.m. | n.m. | n.m. | 13 | 18 | **809** | 8 |
| 16 | 17-hydroxypregnanolone | 133 (41-537) | **7,163** | 1,940 | 1,589 | 1,538 | 817 | 998 | **39,306** | 551 |
| 17 | Pregnanetriol | 576 (243-1,175) | **9,562** | 5,701 | 4,295 | 4,366 | 2,128 | 2,677 | **28,349** | 1,495 |
| 18 | Pregnanetriolone | 13 (5-58) | **20** | 2 | 0 | 3 | 5 | 0 | **1,822** | 10 |
| 19 | Tetrahydro-11-deoxycortisol | 61 (21-159) | **594** | 525 | 690 | 492 | 314 | 911 | **116** | 322 |
| | **Glucocorticoid metabolites** | | | | | | | | | |
| 20 | Cortisol | 57 (22-224) | **252** | 735 | 497 | 495 | 399 | 813 | **414** | 201 |
| 21 | 6β-hydroxy-cortisol | 114 (63-504) | **n.m.** | n.m. | n.m. | n.m. | 7,657 | 23,193 | **393** | 3,578 |
| 22 | Tetrahydrocortisol | 1,694 (772-4,534) | **4,260** | 9,578 | 7,936 | 5,087 | 2,115 | 3,001 | **2,779** | 1,391 |
| 23 | 5α-Tetrahydrocortisol | 1,408 (229-6,744) | **477** | 344 | 161 | 114 | 37 | 66 | **702** | 40 |
| 24 | α-cortol | 319 (177-1,005) | **1,665** | 2,566 | 1,880 | 1,256 | 524 | 831 | **597** | 286 |
| 25 | β-cortol | 513 (255-1,678) | **957** | 378 | 306 | 207 | 108 | 153 | **467** | 60 |
| 26 | 11β-hydroxy-etiocholanolone | 315 (23-899) | **257** | 147 | 91 | 95 | 37 | 50 | **1092** | 89 |
| 27 | Cortisone | 92.5 (39-348) | **198** | 400 | 389 | 286 | 309 | 480 | **671** | 102 |
| 28 | Tetrahydrocortisone | 3,333 (1,465-7,597) | **3,978** | 2,391 | 2,113 | 1,564 | 763 | 1,124 | **5,597** | 807 |
| 29 | α-cortolone | 1,228 (605-2,599) | **3,892** | 2,661 | 2,222 | 1,166 | 410 | 539 | **1,623** | 479 |
| 30 | β-cortolone | 696 (417-2,075) | **1,110** | 300 | 303 | 216 | 108 | 213 | **1130** | 42 |
| 31 | 11-oxo-etiocholanolone | 464 (74-997) | **1,059** | 734 | 736 | 868 | 844 | 1,196 | **3,144** | 267 |
| | **Total glucocorticoid metabolite excretion** | 9,66 (5,467-15,426) | **16,789** | 19,353 | 15,807 | 10,391 | 4,773 | 7,220 | **13,980** | 3,408 |
| | **Steroid ratios indicative of 5α-reductase:** | | | | | | | | | |
| | Androsterone/Etiochaolanolone | 1.13 (0.05-3.00) | **0.79** | 0.46 | 0.31 | 0.44 | 0.45 | 0.53 | **1.23** | 0.22 |
| | 5α-tetrahydrocortisol/tetrahydrocortisol | 0.92 (0.05-2.27) | **0.11** | 0.04 | 0.02 | 0.02 | 0.02 | 0.02 | **0.25** | 0.03 |

**Table 1:** Previously reported cases of patients with widespread metastases from testicular Leydig cell carcinoma treated with mitotane, presented in the order of duration of treatment.

| Reference | Patient age (years) | Length of mitotane treatment | Mitotane dose (plasma mitotane levels) | Gluco-corticoid replace-ment | Documented steroid excess | Patient outcome | Additional information whilst on mitotane therapy |
|---|---|---|---|---|---|---|---|
| **Second- to third-line treatment (treatment duration 3 days – 8 weeks)** | | | | | | | |
| Tamoney et al., Cancer 1969 (23) | 64 | 3 days | 10g/day (not done) | not reported | Increased urinary 17-ketosteroids, increased urinary estrogen | Died – no effect | First-line radiotherapy (40000 rads cobalt therapy); Died 3 days after commencing mitotane therapy |
| Grem et al., Cancer 1986 (4) | 37 | 7 weeks | 1.5g/day (not done) | not reported | Increased urinary 17-KS, increased serum testosterone, androstenedione, DHEAS | Survived another 5 years on alternative treatment (Lonidamine) | First-line therapy cisplatin; mitotane stopped after 7 weeks due to abdominal discomfort and increasing nausea |
| Davis et al., Cancer 1981 (24) | 61 | 8 weeks | 12g/day (not done) | not reported | Normal urinary 17-KS and 17-OHCS | Died 8 weeks after commencing mitotane | First line therapy cisplatin/vinblastin/bleomycin; Second line therapy cyclophosphamide/doxo-rubicin/vincristine); Was concurrently on chemotherapy and radiotherapy. |

| Bertram et al., Cancer 1991 (25) | 60 | 8 weeks | 6-12g/day (not done) | Dexamet ha-sone 1mg/day | Normal 17-KS and 17-OHCS; normal serum E1, E2, Aldo | Died after 8 weeks from widespread metastatic disease | 2nd line therapy (1st line doxorubicin); No response to mitotane |
|---|---|---|---|---|---|---|---|
| **First line treatment (treatment duration 10 weeks-33 months)** | | | | | | | |
| Schwarzmann et al., 1989 (15) | 59 | 10 weeks | 9g/day (not done) | none | Increased serum testosterone, estradiol, aldosterone and cortisol | Died 6 months after commencing mitotane therapy | Reduction in abdominal tumor size and reduction in testosterone and estradiol to normal levels lasting 2 months. Treatment stopped on patient's wish following sudden deterioration and increase in tumor size. |
| Azer&Braunst ein, Cancer 1981 (14) | 63 | 6 months | 4-14g/day during first four weeks, followed by 2.4g/day | Dexamet ha-sone 1 mg/day | Normal urinary 17-KS + 17-OHCS; normal serum aldosterone, testosterone, DHEAS, cortisol | Died after deterioration and continuing metastatic spread of disease. Clinical improvement with mitotane | Complete disappearance of pulmonary metastasis and clinical improvement after 14 weeks on mitotane. 3 months later pulmonary metastasis reappeared, mitotane was stopped and chemotherapy commenced. |
| Abelson et al., Metabolism 1966 (17) | 58 | 18 months | 10g initially, then 4-6g/day (not done) | Dexamet ha-sone 0.375mg twice daily | Increased urinary 17-KS and estrogens | Died after clinical and biochemical improvement with mitotane but radiological progression | Believed to be clinically improving, with reduction in urinary 17-ketosteroids from 1462 to 100 mg/day |
| Van der Hem et al., J Urol 1992 (16) | 56 | 6 months + 27 months (9 months break in between) | 4-10g/day (15-20 mg/L), | Cortisone acetate, no dose recorded | Normal urinary 17OHCS, An, Et, DHEA; normal serum T and DHEAS | Died – metastatic disease stabilized for 18 months on mitotane treatment before disease slowly deteriorated | decrease in retroperitoneal tumor, liver lesions, ascites along with stable disease for 18 months. Once disease deteriorated mitotane dose was escalated to 10g/day with no effect. |

**A**

## Urinary steroid profile
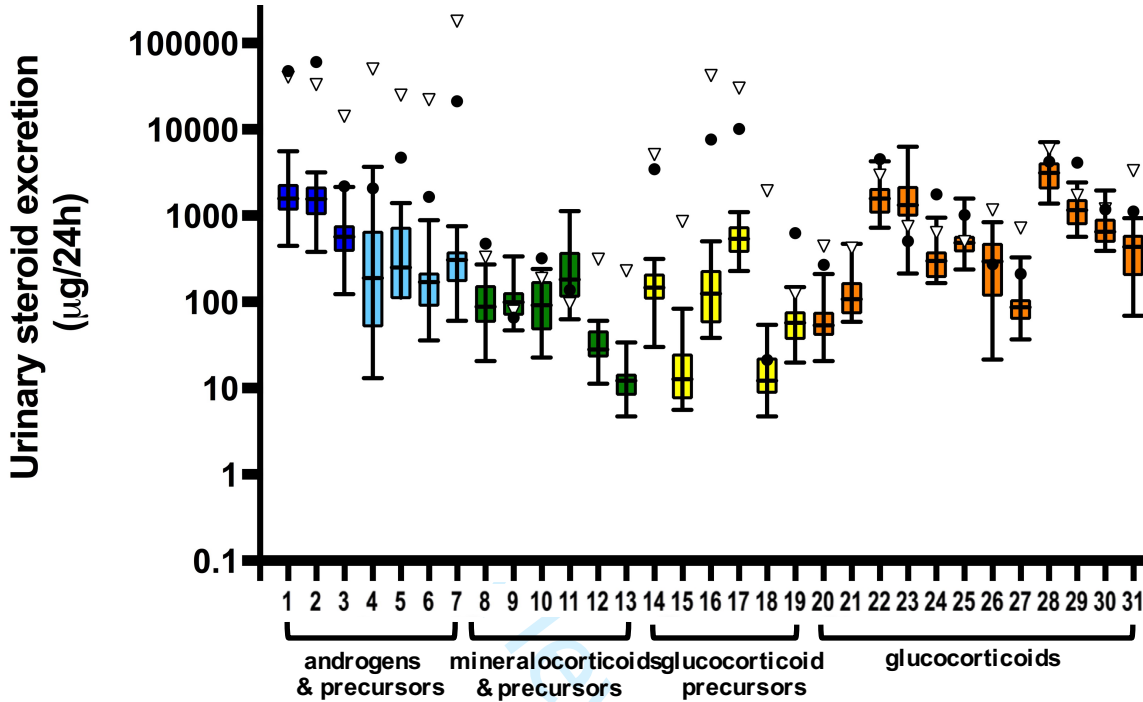


**B**
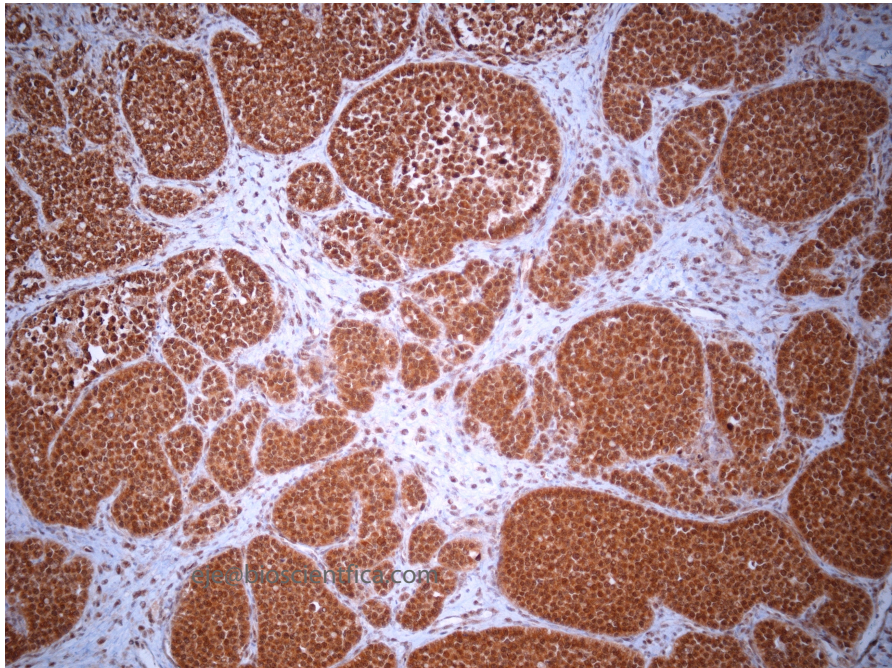
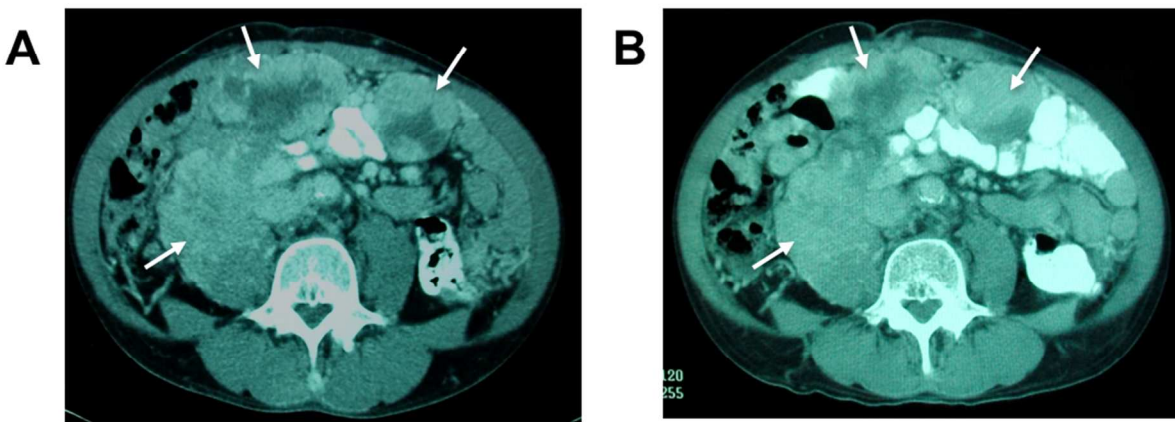**Supplemental Appendix**          **Chortis V, Johal N et al.**          **EJE 17-0542**

**Mitotane treatment in patients with metastatic testicular Leydig cell tumor
associated with severe androgen excess**

**Suppl. Fig. 1:** Computed tomography scans taken in Patient 1. **A**, Abdominal CT after two months of mitotane treatment showing metastatic Leydig cell tumor deposits, with heterogeneous omental masses and a large retroperitoneal mass. **B,** Abdominal CT after six months of mitotane treatment, demonstrating stable disease according to RECIST 1.1 criteria.

**Mitotane treatment in patients with metastatic testicular Leydig cell tumor associated with severe androgen excess**

**Suppl. Table 1:** Mitotane daily dose and plasma concentrations in the two Leydig cell tumor patients. Therapeutic range for plasma mitotane in adrenal cancer is accepted as 14-20 mg/L

| | Patient 1 | | Patient 2 | |
|---|---|---|---|---|
| Duration of mitotane treatment (months) | Mitotane Dose (g/d) | Plasma Mitotane (mg/L) | Mitotane Dose (g/d) | Plasma Mitotane (mg/L) |
| 1 | 1.5 | 4.0 | 4.5. | n.m. |
| 2 | 3 | 5.9 | 4.5 | 9.1 |
| 4 | 3 | 9.1 | 4.5 | 14.2 |
| 5 | 3 | 23.5 | 4.5 | n.m. |
| 6 | 2 | 17.6 | | |
| 7 | 3 | 9.2 | | |

n.m., not measured.