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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 5α-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 5α-reductase (12) and hence activation of testosterone to 5α-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.

METHODS

Urinary steroid metabolome profiling at baseline and during mitotane treatment was carried out by gas chromatography-mass spectrometry, utilizing selected-ion-monitoring analysis for identification and quantification of 32 distinct steroid metabolites reflective of 24-h net steroid output, as previously described (13). Serum steroid measurements were carried out in the routine clinical biochemistry setting, using established and validated tandem mass spectrometry (androstenedione, testosterone) and immunoassays (DHEAS, 17β-oestradiol), respectively.

We carried out immunohistochemistry for sterol-O-acyl transferase 1 (SOAT1) as described previously (14), using antibodies against SOAT1 (1:1000; ab39327; Abcam). The intensity of staining was scored as described by Sbiera et al (15).

CASE REPORTS

Case 1: A 51-year-old patient presented with severe restlessness, insomnia, impaired concentration, increased aggressiveness, redness of the face and body hair growth, all gradually developing over the last six months. Fifteen years previously, he had undergone an orchidectomy for LCT and thirteen years later excision of a retroperitoneal mass, confirmed on histology as LCT metastasis. Imaging revealed multiple lesions consistent with liver, lung and retroperitoneal metastases. Immunohistochemistry of a tissue biopsy confirmed vimentin positive, inhibin negative metastatic LCT. Serum testosterone was very high at 93nmol/L (normal male reference range 7-27nmol/L). Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) showed increased androgen metabolite excretion (sum of androsterone and etiocholanolone 101,476µg/24h; adult male reference range <8000µg/24h) as well as increased excretion of DHEA, metabolites of pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, and cortisol (230 µg/24h; normal <130) (**Fig. 1A**). Prognosis was assessed as poor and the patient declined chemotherapy. However, he agreed to the initiation of mitotane treatment in an attempt to improve the clinical signs and symptoms of tumour-related androgen excess that was significantly limiting his quality of life. Mitotane dose was gradually titrated to 3g per day, with concurrent hydrocortisone

replacement (20mg tid). Within a few weeks, androgen excretion decreased from 101,476 to 12,827 µg/24h, with evidence of significant inhibition of 5α-reductase activity and normalization of other steroids that were increased at baseline (**Table 1**). Plasma mitotane concentrations considered therapeutic (anti-proliferative) in the context of adrenocortical carcinoma (14-20mg/L) (16) were reached after 5 months of treatment (**Suppl. Table 1**). Follow-up imaging still showed progressive disease at two months, but stable disease according to RECIST 1.1 criteria after six months of mitotane treatment (**Suppl. Fig. 1**). Alongside the decrease in androgens, the patient reported a significant improvement of his previously debilitating clinical signs and symptoms. He returned to full-time work and enjoyed good quality of life. After 10 months of mitotane treatment, he died suddenly of a suspected myocardial infarction; no post-mortem examination was carried out.

Case 2: A previously fit-and-well 59-year-old man presented with a right testicular mass and underwent orchidectomy; histopathology revealed malignant LCT. Three years later he presented with lower back pain and imaging showed a large retroperitoneal mass, confirmed as disease recurrence by transcutaneous biopsy. He underwent laparoscopic removal of the mass together with retroperitoneal lymph node dissection. One year later, follow-up imaging revealed disseminated metastases, including liver, kidney and peritoneal deposits. He was unwell, with agitation, anxiety and insomnia. Biochemical work-up showed increased serum testosterone (88.5 nmol/L, normal 7-27), oestradiol (744 pmol/L, normal <156), androstenedione (7.0 nmol/L, normal 0.8-3.1), and DHEAS (> 27 µmol/L, normal 0.91-6.76). GC-MS profiling showed increased steroid excretion including androgen metabolites (69,108 µg/24h, normal <8000) and cortisol (414 µg/24h, normal <130) (**Fig. 1A**). He rejected chemotherapy and agreed to palliative mitotane treatment with concurrent hydrocortisone replacement; mitotane was administered employing the high-dose saturation regimen (Day 1 500mg tds, Day 2 1000mg tds, and from day 3 onwards 1500 mg tds; therapeutic plasma mitotane levels were reached after 4 months (**Suppl. Table 1**). Mitotane decreased serum androgen production within four weeks. Six months after treatment initiation, plasma testosterone had decreased to 29.1 nmol/L and oestradiol to 177 pmol/L, while androstenedione and DHEAS had normalized. Urinary steroid profiling 4 months after initiation of miotane showed a decline in all previously raised steroid

metabolites and decreased 5α-reductase activity. This was paralleled by significant clinical improvement in signs and symptoms, specifically reduced restlessness, aggressiveness and insomnia. Imaging four months after initiation of mitotane revealed a mixed response, with regression of some previous lesions but emergence of new metastatic deposits in lung and abdomen. The patient passed away 12 months after his second recurrence, i.e. six months after the start of mitotane treatment.

DISCUSSION

Here we used mitotane, an established drug in adrenocortical carcinoma, in two patients with metastatic testicular LCT associated with severe androgen excess, clinically manifesting with severe restlessness, insomnia, irritability and impaired concentration. Both patients experienced significant improvement in signs and symptoms with mitotane therapy, swift normalization of steroid excess and some stabilization of radiologically quantified tumour load.

In a comprehensive PubMed search (search terms: Leydig cell tumour, malignant Leydig cell tumour, metastatic Leydig cell tumour, mitotane, lysodren, and o,p'DDD) we identified eight cases of LCT treated with mitotane (**Table 2**). Four patients received mitotane as second- or third-line treatment for metastatic LCT for a very short time only (3 days to 8 weeks); none of them showed a biochemical, clinical or radiological response. The remaining four cases received mitotane as first-line treatment for metastatic LCT, with treatment duration varying between 10 weeks and 33 months (**Table 2**). All four patients experienced significant radiological tumour response and reduction in steroid excess during mitotane treatment. Azer and Braunstein (17) used mitotane to treat a patient with metastatic LCT for six months, resulting in a dramatic response with complete remission of multiple pulmonary metastases, which lasted three months prior to relapse. Radiological reduction in tumour load for several months was observed in two cases (18, 19). Abelson et al (20) noted a significant reduction in 17-ketosteroid excretion and clinical improvement in a metastatic LCT patient treated with mitotane for 18 months, while his disseminated metastases progressed. Adding the experience of our cases, mitotane can be considered a worthwhile palliative option in metastatic LCT, particularly when the disease is associated with steroid excess.

During human fetal development, adrenals and gonads both arise from the urogenital ridge and they both develop steroidogenic capacity, albeit with distinct features, i.e. sex steroid synthesis in the gonads and glucocorticoid, mineralocorticoid and androgen precursor synthesis in the adrenal glands. Benign testicular adrenal rest tumours, which are regularly found in men with congenital adrenal hyperplasia, have been shown to display features of both adrenal and gonadal steroidogenesis (21, 22). Using mass spectrometry, we observed that our two LCT patients showed not only androgen excess, but also increased production of glucocorticoid precursors and cortisol, without clinical signs of Cushing's syndrome. Two previous case reports in patients with malignant LCTs have described ectopic production of steroids normally produced by the adrenal cortex, including cortisol and aldosterone (23, 24). In our two cases, both androgen excess and glucocorticoid overproduction responded well to mitotane treatment. Comprehensive steroid metabolome mapping by GC-MS has been used successfully to differentiate malignant from benign adrenocortical tumours (13). It will be useful to test in future studies whether steroid metabolome profiling would also help differentiate benign from malignant LCT and could have a role in follow-up monitoring.

Recent studies have implicated sterol-A-acyl transferase 1 (SOAT1), previously also termed ACAT-1 for Acyl-coenzyme A cholesterol acyltransferases, as a target of mitotane action (14). SOAT1 is located in the endoplasmic reticulum and involved in intracellular esterification of free cholesterol. John Achermann's group has shown that this enzyme operates downstream of SF-1 and is important for regulation of adrenal steroidogenesis (25). A recent study (14) has provided evidence of inhibition of SOAT1 by mitotane in an adrenocortical cell model, by demonstrating an increase in free cholesterol, oxysterols and fatty acids after treatment with mitotane. We had access to formalin-fixed paraffin-embedded tissue from the tumour recurrence in Patient 2 and used it for carrying out immunohistochemistry for SOAT1 (**Fig. 1B**), which demonstrated predominantly high and moderate expression, detected in 60% and 30% of the cells, respectively. Thus, it is likely that both the steroid-ameliorating and anti-proliferative effects of mitotane are mediated by SOAT not only in adrenocortical carcinoma but also in LCT.

Based on our current findings, the use of mitotane in the palliative treatment of metastatic LCTs of the testes appears feasible and useful, with effective control of tumour-related steroid excess and possible beneficial effects on disease progression, a viable treatment option in a rare endocrine cancer that is not responsive to cytotoxic chemotherapy or radiotherapy.

Declaration of interest: The authors have nothing to disclose.

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References

1. Risk MC, Porter CR. Management of non-germinal testicular tumors. World J Urol. 2009;27(4):507-12.
2. Leonhartsberger N, Ramoner R, Aigner F, Stoehr B, Pichler R, Zangerl F, et al. Increased incidence of Leydig cell tumours of the testis in the era of improved imaging techniques. BJU Int. 2011;108(10):1603-7.
3. Kim I, Young RH, Scully RE. Leydig cell tumors of the testis. A clinicopathological analysis of 40 cases and review of the literature. Am J Surg Pathol. 1985;9(3):177-92.
4. Grem JL, Robins HI, Wilson KS, Gilchrist K, Trump DL. Metastatic Leydig cell tumor of the testis. Report of three cases and review of the literature. Cancer. 1986;58(9):2116-9.
5. Hassan HC, Cullen IM, Casey RG, Rogers E. Gynaecomastia: an endocrine manifestation of testicular cancer. Andrologia. 2008;40(3):152-7.
6. Suardi N, Strada E, Colombo R, Freschi M, Salonia A, Lania C, et al. Leydig cell tumour of the testis: presentation, therapy, long-term follow-up and the role of organ-sparing surgery in a single-institution experience. BJU Int. 2009;103(2):197-200.
7. Conkey DS, Howard GC, Grigor KM, McLaren DB, Kerr GR. Testicular sex cord-stromal tumours: the Edinburgh experience 1988-2002, and a review of the literature. Clin Oncol (R Coll Radiol). 2005;17(5):322-7.
8. Heer R, Jackson MJ, El-Sherif A, Thomas DJ. Twenty-nine Leydig cell tumors: histological features, outcomes and implications for management. Int J Urol. 2010;17(10):886-9.
9. Acar C, Gurocak S, Sozen S. Current treatment of testicular sex cord-stromal tumors: critical review. Urology. 2009;73(6):1165-71.
10. Jou P, Maclennan GT. Leydig cell tumor of the testis. J Urol. 2009;181(5):2299-300.
11. Hahner S, Fassnacht M. Mitotane for adrenocortical carcinoma treatment. Curr Opin Investig Drugs. 2005;6(4):386-94.
12. Chortis V, Taylor AE, Schneider P, Tomlinson JW, Hughes BA, O'Neil DM, et al. Mitotane therapy in adrenocortical cancer induces CYP3A4 and inhibits 5alpha-reductase, explaining the need for personalized glucocorticoid and androgen replacement. J Clin Endocrinol Metab. 2013;98(1):161-71.
13. Arlt W, Biehl M, Taylor AE, Hahner S, Libe R, Hughes BA, et al. Urine steroid metabolomics as a biomarker tool for detecting malignancy in adrenal tumors. J Clin Endocrinol Metab. 2011;96(12):3775-84.
14. Sbiera S, Leich E, Liebisch G, Sbiera I, Schirbel A, Wiemer L, et al. Mitotane Inhibits Sterol-O-Acyl Transferase 1 Triggering Lipid-Mediated Endoplasmic Reticulum Stress and Apoptosis in Adrenocortical Carcinoma Cells. Endocrinology. 2015;156(11):3895-908.
15. Sbiera S, Schmull S, Assie G, Voelker HU, Kraus L, Beyer M, et al. High diagnostic and prognostic value of steroidogenic factor-1 expression in adrenal tumors. J Clin Endocrinol Metab. 2010;95(10):E161-71.
16. Haak HR, Hermans J, van de Velde CJ, Lentjes EG, Goslings BM, Fleuren GJ, et al. Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. Br J Cancer. 1994;69(5):947-51.

17. Azer PC, Braunstein GD. Malignant Leydig cell tumor: objective tumor response to o,p'-DDD. Cancer. 1981;47(6):1251-5.
18. Schwarzman MI, Russo P, Bosl GJ, Whitmore WF, Jr. Hormone-secreting metastatic interstitial cell tumor of the testis. J Urol. 1989;141(3):620-2.
19. van der Hem KG, Boven E, van Hennik MB, Pinedo HM. Malignant Leydig cell tumor of the testis in complete remission on o,p'-dichlorodiphenyl-dichloroethane. J Urol. 1992;148(4):1256-9.
20. Abelson D, Bulaschenko H, Trommer PR, Valdes-Dapena A. Malignant interstitial-cell tumor of the testis treated with o,p'-DDD. Metabolism. 1966;15(3):242-56.
21. Puar T, Engels M, van Herwaarden AE, Sweep FC, Hulsbergen-van de Kaa C, Kamphuis-van Ulzen K, et al. Bilateral Testicular Tumors Resulting in Recurrent Cushing Disease After Bilateral Adrenalectomy. J Clin Endocrinol Metab. 2017;102(2):339-44.
22. Smeets EE, Span PN, van Herwaarden AE, Wevers RA, Hermus AR, Sweep FC, et al. Molecular characterization of testicular adrenal rest tumors in congenital adrenal hyperplasia: lesions with both adrenocortical and Leydig cell features. J Clin Endocrinol Metab. 2015;100(3):E524-30.
23. Papadimitris C, Alevizaki M, Pantazopoulos D, Nakopoulou L, Athanassiades P, Dimopoulos MA. Cushing syndrome as the presenting feature of metastatic Leydig cell tumor of the testis. Urology. 2000;56(1):153.
24. Jain SH, Sadow PM, Nose V, Dluhy RG. A patient with ectopic cortisol production derived from malignant testicular masses. Nat Clin Pract Endocrinol Metab. 2008;4(12):695-700.
25. Ferraz-de-Souza B, Hudson-Davies RE, Lin L, Parnaik R, Hubank M, Dattani MT, et al. Sterol O-acyltransferase 1 (SOAT1, ACAT) is a novel target of steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) in the human adrenal. J Clin Endocrinol Metab. 2011;96(4):E663-8.

Figure Legend

Fig. 1:

Panel A, Steroid synthesis in the two patients with metastatic testicular Leydig cell tumour as assessed by mass spectrometry-based 24-h urinary steroid profiling before initiation of mitotane treatment (log scale; closed circles, patient 1; open triangles, patient 2). Box plots represent medians and interquartile ranges from a group of 24 healthy male volunteers (age 40-60 years); whiskers represent the full range.

Panel B, Immunohistochemical staining for Sterol-O-acyltransferaase 1 (SOAT1) using formalin-fixed paraffin-embedded tissue from the recurrent tumour of patient 2, demonstrating high (60% of 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									

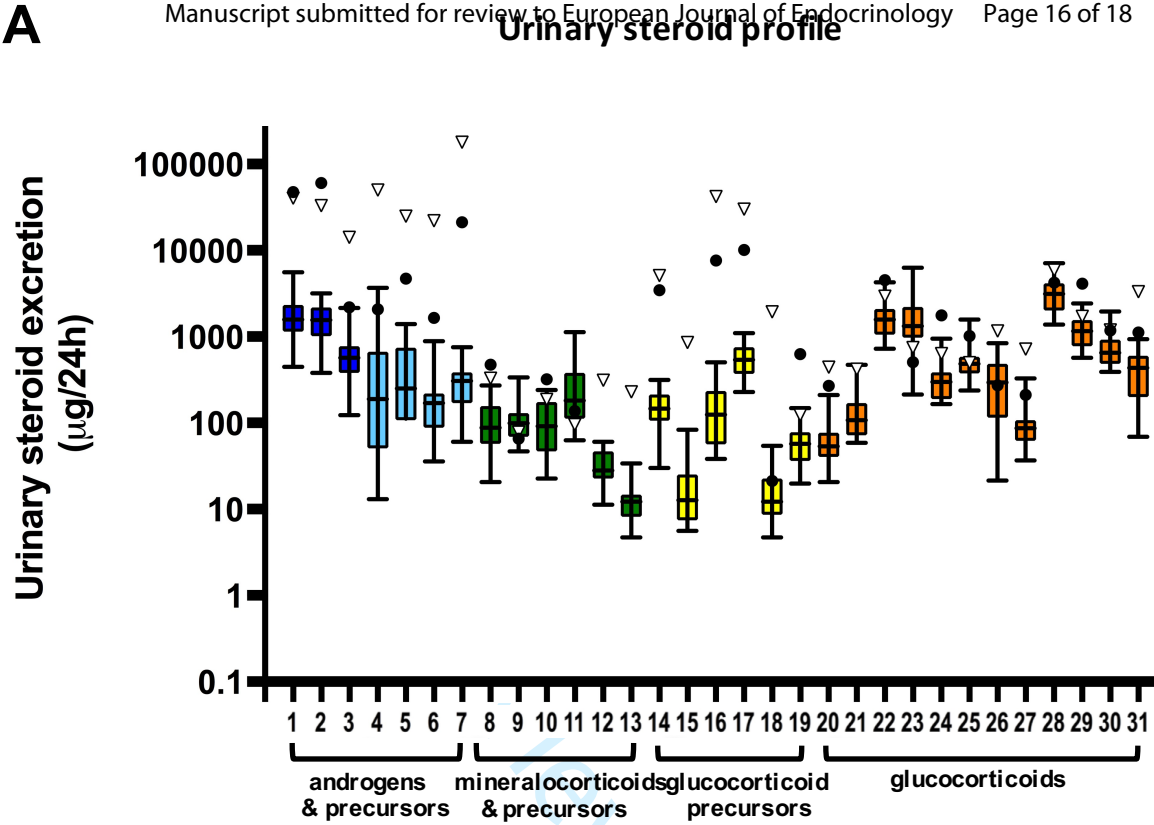
14	Pregnanediol	157 (32-336)	**3,249**	1,857	1,474	1,171	455	646	**4,832**	199
15	3α,5α-17-hydroxy-pregnanolone	14 (6-89)	**n.m.**	n.m.	n.m.	n.m.	13	18	**809**	8
16	17-hydroxypregnanolone	133 (41-537)	**7,163**	1,940	1,589	1,538	817	998	**39,306**	551
17	Pregnanetriol	576 (243-1,175)	**9,562**	5,701	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.

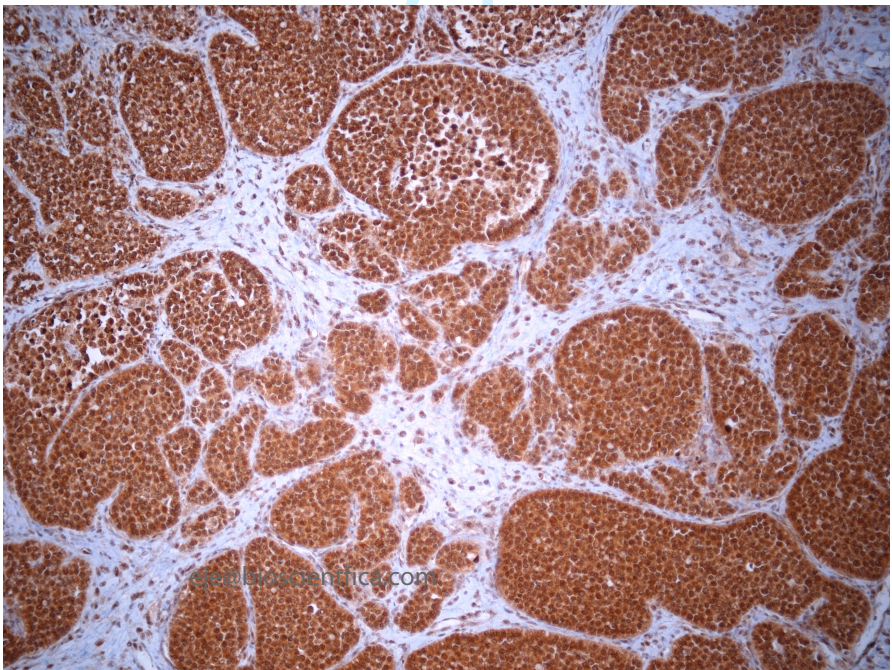
Reference	Patient age (years)	Length of mitotane treatment	Mitotane dose (plasma mitotane levels)	Gluco-corticoid replace-ment	Documented steroid excess	Patient outcome	Additional information whilst on mitotane therapy
Second- to third-line treatment (treatment duration 3 days – 8 weeks)							
Tamoney 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
Urinary steroid excretion (µg/24h)
100000
10000
1000
100
10
1
0.1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
androgens & precursors
mineralocorticoids & precursors
glucocorticoid precursors
glucocorticoids

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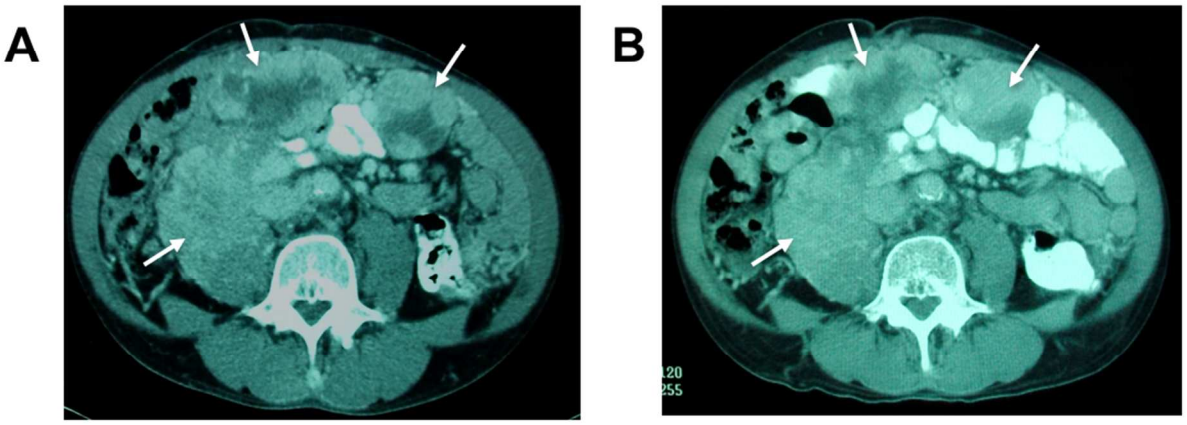
Supplemental Appendix **Chortis V, Johal N et al.** **EJE 17-0542**

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.



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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.