Spironolactone is associated with reduced mitotane levels in adrenocortical carcinoma patients

Linus Haberbosch[1], Lukas Maurer[1], Arvid Sandforth[2,3], Charlotte Wernicke[1], Joachim Spranger[1,4,5], Knut Mai[1,4,5]+*, Reiner Jumpertz von Schwartzenberg[1,2,3]+

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health; Department of Endocrinology and Metabolism; 10117 Berlin, Germany.

[2]University of Tübingen, Department of Internal Medicine IV, Division of Diabetology, Endocrinology and Nephrology, 72076 Tübingen, Germany.

[3]Institute for Diabetes Research and Metabolic Diseases, Helmholtz Center Munich, University of Tübingen, Partner of the German Center for Diabetes Research (DZD), Germany

[4]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health; Charité Center for Cardiovascular Research; 10117 Berlin, Germany.

[5]DZHK (German Centre for Cardiovascular Research), partner site Berlin

+authors contributed equally to this work

***Correspondence**

Prof. Dr. Knut Mai

Department of Endocrinology & Metabolism,

Charité-Universitätsmedizin Berlin

Chariteplatz 1

10117 Berlin

Germany

25 ph: +49/30/450514252

26 fax: +49/30/450514950

27 e-mail: knut.mai@charite.de

28 **Keywords:** Mitotane, o,p-DDD, Adrenocortical carcinoma, Spironolactone, Hypokalemia

29 **Word Count:** 3218

Abstract

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a poor prognosis. Mitotane, a derivative of the pesticide DDT, has been used successfully as first line chemotherapy since the 1960s, if maintained within a narrow therapeutic window. Spironolactone (SPL) is frequently used to treat glucocorticoid excess-associated adverse effects such as severe hypokalemia. Although data of a previous case report indicate a link, valid data regarding SPL use and mitotane plasma concentrations in a human cohort are lacking.This retrospective analysis includes data from 54 mitotane-receiving ACC patients (14 co-administered with SPL) treated between January 2005 and April 2020 (20 male, mean age 54.1 ± 2.2 yrs.). All available mitotane concentrations, treatment doses, tumor stage and evidence of hormone activity were collected. Primary outcomes included mitotane levels and concentration/dose-ratios as well as time-in-range in patients with and without additional SPL treatment. The SPL group was characterized by higher glucocorticoid secretion. Other features such as tumor stage, size and anthropometrics were similar between groups. Interestingly, the SPL group had significantly lower mitotane levels despite higher doses. Mitotane time-in-range was significantly reduced in the SPL group, as was time-in-range to progression. These data provide first evidence in a human cohort for potential SPL-mitotane interactions (beyond mentioned case report), which affect dose response and may modulate treatment outcomes. This should caution clinicians to carefully adjust mitotane doses during SPL treatment in ACC patients or choose alternative therapeutic options.

Introduction

Adrenocortical carcinoma (ACC) is a rare endocrine tumor characterized by high recurrence rates and often-fatal prognosis. The overall 5-year survival of patients undergoing tumor surgery is believed to be less than 40 % (Bilimoria et al., 2008). Beside surgical removal of the tumor, mitotane is the most widely used treatment option until today (Terzolo et al., 2007). Mitotane is an isomer of 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane (DDD), the insecticide analogue of DDT which was first shown to induce adrenal atrophy in dogs in 1948 (Nelson and Woodard, 1948). Its first use in humans as a chemotherapy of adrenocortical cancer was described by Bergenstal et al. (1960). Numerous publications have documented its use as a chemotherapeutic drug ever since (Luton et al., 1990, Barzon et al., Haak et al., 1994). Long-lasting treatment experience over many decades and its proven efficacy (compared to other treatment options) makes mitotane an important component in the management of ACC patients, although a high variability of the effect is reported (Fassnacht et al., 2018b). Mitotane yielded especially encouraging results in the prognostically poor subgroup of steroid secreting tumors (Abiven et al., 2006). However, due to gastrointestinal and specifically neurotoxic adverse effects, the therapeutic window for this drug is narrow (14-20 mg/l) and frequent monitoring of mitotane levels is mandatory (Allolio and Fassnacht, 2006, Kerkhofs et al., 2013, Kerkhofs et al., 2014, Terzolo et al., 2013). Its long half-life (18-159 days), the prolonged time to achieve effective plasma mitotane concentrations and enhanced glucocorticoid metabolism, which increases the required glucocorticoid replacement dosage, make mitotane treatment rather complex. Moreover, mitotane is a potent inductor of cytochrome P450 CYP3A4 activity causing numerous interactions with other drugs such as warfarin, midazolam or the thyrosine kinase inhibitor sunitinib

(van Erp et al., 2011, Baudin et al., 2001, Kroiss et al., 2011). However, literature on the metabolism of mitotane or other drugs that may affect mitotane levels is surprisingly scarce. Since not all ACC patients respond to mitotane therapy, it is important to define potential drug interactions that may affect mitotane efficacy (Hahner and Fassnacht, 2005).

Steroid excess, frequently seen in ACCs (Koschker et al., 2006), may cause hypertension and hypokalemia. Due to its antihypertensive and potassium retention effects, the mineralocorticoid receptor antagonist spironolactone (SPL) is therefore frequently used in those patients.

Interestingly, a potential inhibitory effect of SPL on mitotane-induced adrenal atrophy in dogs was presented at a scientific meeting in the 1970s (Menard et al., 1977). Human data suggesting such a potential interaction are limited to one case report in 1977 (Wortsman and Soler), in which a patient with pituitary-dependent Cushing´s syndrome was treated with 3 g of mitotane per day (no mitotane plasma concentrations were documented), however never experienced any known adverse effects of mitotane until the discontinuation of SPL. Given the sparsity of convincing data on clinically relevant interactions between SPL and mitotane, until today the use of SPL is warranted in the management of endocrine manifestations of ACC (Veytsman et al., 2009). Considering the frequent combination of mitotane and SPL, the dismal prognosis of patients with ACC and the toxicity of mitotane, it is pivotal to understand the effects of SPL on mitotane concentration and thereby efficacy which may have clinical consequences in ACC patients. We therefore conducted a retrospective data analysis of 54 ACC patients treated with mitotane in our center between January 2005 and April 2020 . We provide first evidence that additional SPL

103 treatment is associated with lower mitotane plasma concentrations despite higher
104 dosage. Preliminary survival data support an association with clinical outcomes.

Methods

This retrospective data analysis was approved by the Ethics Committee of the Charité—Universitätsmedizin Berlin ("Ethikkommission der Charité—Universitätsmedizin Berlin"; EA2/021/20).

Patients

We included 54 ACC patients undergoing mitotane treatment with available data on mitotane concentrations in our center between January 1, 2005 and April 1, 2020. The inclusion criteria were age > 18, pathologically confirmed diagnosis of ACC and treatment with mitotane. The standard surgical therapy was adrenalectomy. Beside mitotane treatment, patients received adjuvant chemo-, radio-, and hormone replacement therapy. Patients' charts were reviewed and the following information was retrieved for the study: sex, age, body mass index (BMI), hormone secretion status of the tumor, ACC stage at start of mitotane treatment, start date of mitotane therapy, first progression under therapy, loss to follow-up or death. Hormone secretion was determined by respectively elevated androgen precursors (dehydroepiandrosterone, 17-hydroxyprogesterone and/or androstenedione), aldosterone-to-renin ratio and/or a pathologic 1 mg dexamethasone suppression test. Tumor stage was assessed according to the ENSAT classification (Fassnacht et al., 2009).

Mitotane and SPL doses as well as mitotane concentrations were documented at monthly intervals (concurrent with clinical visits) if available. Plasma samples were collected and sent to Lysosafe (a free of charge testing service of Laboratoire HRA Pharma, Paris, France) for measurement of mitotane concentrations. For this,

plasma samples were extracted by precipitation with ethanol, and tested by a standardized gas chromatography/mass spectrometry method (Inouye et al., 1987). Mitotane plasma concentrations, a concentration/dose ratio, time in range (TR) and time in range to (first) progression (TRP) of mitotane (both defined as percentage of total time of mitotane treatment, starting one month after treatment start) were calculated. The concentration/dose ratio was calculated by dividing the serum mitotane concentration (in mg/l) by the total daily dose (in g) taken by the patient at that time, concordant with similar analyses in drug monitoring studies (Rudberg et al., 2006, Westin et al., 2008, Fukumoto et al., 2006, Thölking et al., 2016).

Statistics

All analyses were performed using IBM SPSS Statistics, Version 19.0.0.1 (IBM, United States). Group comparisons with normally distributed variables were analyzed with t-tests. If normal distribution was not given, we used Mann-Whitney-U tests. Categorical variables were compared via Chi-squared tests or Fisher's exact test, depending on the number of categories. A correlation between mitotane dose and concomitant concentration was calculated via Spearman's rho. $P < 0.05$ was considered statistically significant. Scatter plots were created in SPSS, Boxplots and jitter plots were created using GraphPad Prism version 7.02 for Windows (GraphPad Software, United States). All plots were colorized and arranged in Adobe Illustrator (Adobe Inc., United States).

Results

Cohort Characteristics

Out of the 54 ACC patients who met the inclusion criteria, 14 patients were treated with SPL at any time during the study interval. We found no significant differences between the SPL group and the no-SPL group regarding age, sex or BMI (**Table 1**). For the overall cohort (20 male, mean age 54.1 ± 2.2), the most common ENSAT stage was IV (42.6%), with an average maximum tumor diameter of 10.5 cm (± 0.7) and an average Ki67-expression of 24.4% (± 3.0). In two patients, we were not able to clearly differentiate the ENSAT stage at treatment start between stage 3 and stage 4 due to lacking or conflicting pathological data. Both patients were removed from all further analyses including ENSAT stage as a covariate. Of note, maximum tumor diameter and Ki67-expression were similar between groups, albeit the SPL-group contained more patients with ENSAT stage IV tumors. In the overall cohort, 65% of carcinomas showed hormonal activity. While 13 of 14 tumors in the SPL group were hormone secreting, only 22 out of 40 in the control group were hormonally active. The complete cohort characteristics are provided in **Table 1**. We found no statistically significant differences regarding adjuvant therapies, albeit with a higher percentage of patients from the spironolactone group receiving etoposide, doxorubicin and cisplatin (EDP). The full list of additional therapies is provided in **Suppl. Table 1**.

Mitotane concentrations

We documented a total of 909 mitotane concentrations, with an average of 17 per patient (Median: 13, ranging from 2-80). Including all available mitotane concentrations, patients in the SPL treated group had significantly lower mitotane levels compared to non-SPL treated subjects (7.4 ± 1.4 vs. 11.8 ± 0.8 mg/l, $p<0.001$; **Fig. 1A**), despite higher mitotane doses (3.7± 0.4 vs. 2.4 ± 0.2 g/day, $p<0.001$; **Fig.**

1B). The mean concentration/dose ratio was 2.3 (± 0.4) for the SPL group and 6.2 (± 0.7) for the no-SPL group (**Fig. 1C**).

To validate these findings and to exclude the possibility of these results only reflecting different starting regimen, we repeated this analysis after the exclusion of any data from the first 6 months of treatment, concordant with the expected time to reach therapeutic range (Puglisi et al., 2019, Terzolo et al., 2013, Terzolo et al., 2000). Although 11 patients were additionally excluded by this approach, the findings of significantly lower mitotane concentration (12.2 ± 1.2 vs. 14.7 ± 0.2 mg/l, p = 0.018), higher mitotane doses (2.6 ± 0.3 vs. 1.8 ± 0.04 g/day, p = 0.020) and a lower concentration/dose ratio (5.8 ± 0.9 vs. 9.5 ± 0.2) in the SPL group could be replicated (**Supplemental Figure 1**).

We then calculated non-parametric correlations between mitotane dose and concentration at each available timepoint via Spearman's rho. While we noted a significant, albeit weak correlation in the no-SPL group (r_s = 0.09, p = 0.011), we found no correlation in the SPL group (r_s = -0.28, p = 0.773) (**Supplemental Figure 2**).

To further characterize these findings, we visualized mitotane concentrations and concentration/dose ratios in a scatter plot against time after the start of mitotane treatment (**Fig. 1D,E**). The concentration scatter decreases with time after the initial start of the therapy in both groups.

Finally, we calculated the overall time in range (defined as percent of mitotane concentration measurements in the therapeutic range per individual (Puglisi et al., 2020)) as well as the time in range to (first) progression. Time in range was overall low in the no-SPL treated group (29.3 ± 3.5%), however even lower in the SPL-

198 treated group with only 7.9% (± 0.3) of measurements per individual that were within
199 the therapeutic range (p=0.001 vs. no-SPL group). The same was true for time in
200 range from mitotane treatment start to first progression (time in range to
201 progression), with a mean value of 28.8% (± 4.0) in the no-SPL group compared to
202 4.7% (± 2.5) in the SPL group (p=0.001 for between-group comparison). The results
203 are visualized in **Figure 1F**.

Discussion

While radical resection remains the first line therapy of ACC, adjuvant mitotane is recommended in high risk cases (ENSAT II/IV or Ki-67 > 10%) even after complete resection. In cases of Rx or R1 resection as well as ACCs not amenable to radical resection, mitotane therapy is started in addition to possible local therapies and/or chemotherapy. This includes several regimes like etoposide, doxorubicine and cisplatin (EDP) or streptozotozine or gemcitabine, all of moderate value (Fassnacht et al., 2018a). Small studies also indicate a potential impact of immunotherapy in some of those patients (Fassnacht et al., 2015, Raj et al., 2020, Carneiro et al., 2019, Le Tourneau et al., 2018, Habra et al., 2019).

While mitotane has been shown to significantly prolong recurrence-free survival in patients with advanced ACC (Terzolo et al., 2007), its plasma concentration must be closely monitored and managed to ensure treatment effectivity (Fassnacht et al., 2013) and avoid major adverse effects. In this retrospective analysis we provide first clinical evidence in a human cohort that SPL treatment in ACC patients receiving mitotane chemotherapy is closely linked with reduced mitotane concentrations, concentration/dose ratios and time in range. This aggravates the known clinical difficulties in adequately dosing ACC patients to achieve therapeutic concentrations over long intervals. Recently, a study in 110 ACC patients found time in target range of plasma mitotane to be a significant predictor of recurrence-free survival independent of the Ki67 index (Puglisi et al., 2019, Puglisi et al., 2020). This underscores the importance of stable and therapeutic mitotane concentrations in ACC patients, especially in those with steroid hypersecretion. In this context, effects of SPL on mitotane concentrations may have adverse effects on clinical outcomes in ACC patients who already bear a high mortality risk.

Albeit, adequately mitotane dosing is complicated by the low oral bioavailability and lipophilicity of mitotane, the latter leading to a large distribution volume and slow accumulation (Arshad et al., 2018, Corso et al., 2021). A further complicating factor is the complex and not fully understood metabolism of mitotane, leading to a considerable influence of individual pharmacogenetics (Yin et al., 2021, Altieri et al., 2020). Current integrative pharmacokinetic models strive towards predicting mitotane levels in individual patients, moving towards individualized mitotane dosing (Kerkhofs et al., 2015, Cazaubon et al., 2019).

Mitotane is itself a potent inhibitor of steroidogenesis and used for this specific effect as a treatment for Cushing's syndrome at lower doses (Daniel and Newell-Price, 2015). But due to the aforementioned challenges, Mitotane treatment alone is often insufficient to rapidly control severe steroid hypersecretion and the resulting hypokalemia in a relevant percentage of ACC patients (Allolio and Fassnacht, 2006). This often necessitates additional anti-hormonal treatment, which can be initiated together with mitotane. Metyrapone, an 11β-hydroxylase inhibitor, is the first therapeutic choice for the management advanced ACC patients with severe Cushing's syndrome, as it is well tolerated and has no reported interactions with mitotane (Fassnacht et al., 2018b). Recently, the similarly acting osilodrostat was approved as a therapeutic alternative. Ketoconazole blocks several enzymes of the adrenal steroidogenesis, but the hepatotoxicity often contraindicates the co-administration with mitotane, especially at start of the treatment. Levoketokonazole might improve on this, although clinical data is currently lacking (Castinetti et al., 2021). Mifepristone as a glucocorticoid receptor antagonist is also an option, but it is difficult to monitor and the high circulating cortisol levels induce mineralocorticoid effects, including hypertension and hypokalemia.

Nevertheless, a fraction of ACC patients, particularly with glucocorticoid- and mineralocorticoid secreting tumors, suffer from severe hypokalemia (Fassnacht et al., 2009) requiring specific treatment or replacement beyond hypercortisolism treatment (Allolio and Fassnacht, 2006). Therefore, supporting pharmacological therapy with either mineralocorticoid receptor antagonists (MRA, eplerenone, SPL) or epithelial sodium channel (ENaC) blockers (amiloride, triamterene) is frequently required.

The most commonly used and most ubiquitously available of all these pharmacological agents is SPL, now amassing over 60 years of clinical use (Kolkhof and Barfacker, 2017). Aside from the well-known adverse effects related to its affinity to the androgen receptor, a possible interaction with mitotane has been discussed for some time, with only anecdotal evidence amounting to only one published case report (Wortsman and Soler, 1977). Still, due to lack of evidence, the administration of SPL is recommended in educational articles, reviews and guidelines for the clinical management of patients with hormone-secreting adrenocortical cancer (Lacroix, 2010, Rao and Habra, 2016, Veytsman et al., 2009). Considering our observations indicating potential pharmacological interactions of SPL and mitotane treatment, which may lead to difficulties to achieve effective mitotane concentrations and may thereby have potential deleterious effects on clinical outcomes, we would like to caution clinicians regarding the use of SPL in mitotante receiving ACC patients. ENaC-blockers, with preferential use of amiloride, might be the favored first line treatment of hypokalemia in these patients (Fassnacht et al., 2018a, Quinkler and Stewart, 2010). At therapy failure or if MRAs are strongly indicated for other reasons, eplerenone rather than SPL should be used, although possibly higher doses of eplerenone are needed due to CYP3A4 interactions. If SPL is unavoidable,

close monitoring of mitotane levels is essential, and the patient will likely require higher doses than usually administered.

The mechanism of action behind this potential interaction remains unknown. One possible way of interaction is SPL interfering with mitotane metabolism via the cytochrome P450-system. Mitotane (o,p'-DDD) is primarily transformed and metabolized in adrenocortical mitochondria (Hahner and Fassnacht, 2005). The transformation requires β-hydroxylation followed by rapid dechlorination to a reactive acyl chloride thought to bind adrenocortical binucleophiles in target cells to exert an adrenolytic effect via enhanced oxygen activation (Waszut et al., 2017). The metabolic reaction is known to be inhibited by ketoconazole but not by aminoglutethimide, metyrapone, or other steroids (Veytsman et al., 2009). SPL, like ketoconazole, is a broad cytochrome P450-inhibitor and its metabolites canrenone and canrenoate-k have been shown to inhibit 11β- and 18-hydroxylation in human adrenal cortical mitochondria (Lund and Lund, 1995). Moreover, inhibition of 2β, 6β, 15β and 18-hydroxylation was also demonstrated by other metabolites in the rat model (Decker et al., 1989, Colby et al., 1991). Although, it seems not implausible that SPL could inhibit those initial hydroxylation steps, induction of microsomal elimination might also be involved. This is supported by animal studies hinting towards a faster elimination of DDT-metabolites when administered with pentobarbital (Fries et al., 1971), with a similar effect described for SPL (Solymoss et al., 1969). This may additionally contribute to lower mitotane concentrations during SPL treatment.

And while unchanged mitotane appears to be eliminated largely through biliary excretion (but can also undergo enterohepatic circulation) (Kroiss et al., 2011), its

less hydrophobic metabolite o,p′-DDA is subject to rapid renal elimination (Corso et al., 2021).

These data underscore the further need for exploring the metabolism of mitotane, as well as the interaction of mitotane and SPL, since other pharmacological agents may also interfere with the pharmacokinetics of this key drug for ACC therapy.

This study is limited by its retrospective character and thus does not provide direct proof that SPL treatment reduces mitotane concentrations. Considering ACC being a rare disease the cohort size seems well dimensioned, however the overall number of patients is rather low, which limits the analysis with further confounders. Additionally, the mechanism for the interaction of SPL and mitotane remains unknown and might include further mediators or moderators. Confirmatory studies are needed to adequately address confounders and analyze survival data in a larger cohort.

Still, considering these limitations, we find that this study contributes important first clinical evidence in a human cohort indicating potential deleterious SPL-mitotane interactions.

We believe that these data should caution clinicians to consider this potential interaction when treating hypokalemia in ACC patients, and to carefully adjust mitotane doses if spironolactone treatment is unavoidable or prefer alternative therapeutic agents such as amiloride. Future studies should address the mechanism of action behind this interaction and investigate other possible interaction candidates.

Acknowledgments

We would like to thank Kathrin Zopf for her help with data collection.

Conflicts of Interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

ABIVEN, G., COSTE, J., GROUSSIN, L., ANRACT, P., TISSIER, F., LEGMANN, P., DOUSSET, B., BERTAGNA, X. & BERTHERAT, J. 2006. Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients. *J Clin Endocrinol Metab,* 91**,** 2650-5.

ALLOLIO, B. & FASSNACHT, M. 2006. Clinical review: Adrenocortical carcinoma: clinical update. *J Clin Endocrinol Metab,* 91**,** 2027-37.

ALTIERI, B., SBIERA, S., HERTERICH, S., DE FRANCIA, S., DELLA CASA, S., CALABRESE, A., PONTECORVI, A., QUINKLER, M., KIENITZ, T. & MANNELLI, M. 2020. Effects of germline CYP2W1* 6 and CYP2B6* 6 single nucleotide polymorphisms on mitotane treatment in adrenocortical carcinoma: a multicenter ENSAT study. *Cancers,* 12**,** 359.

ARSHAD, U., TAUBERT, M., KURLBAUM, M., FRECHEN, S., HERTERICH, S., MEGERLE, F., HAMACHER, S., FASSNACHT, M., FUHR, U. & KROISS, M. 2018. Enzyme autoinduction by mitotane supported by population pharmacokinetic modeling in a large cohort of adrenocortical carcinoma patients. *European journal of endocrinology,* 179**,** 287-297.

BARZON, L., FALLO, F., SONINO, N., DANIELE, O. & BOSCARO, M. 1997. Adrenocortical carcinoma: experience in 45 patients. *Oncology,* 54**,** 490-6.

BAUDIN, E., PELLEGRITI, G., BONNAY, M., PENFORNIS, A., LAPLANCHE, A., VASSAL, G. & SCHLUMBERGER, M. 2001. Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'DDD) levels on the treatment of patients with adrenocortical carcinoma. *Cancer,* 92**,** 1385-92.

BERGENSTAL, D. M., HERTZ, R., LIPSETT, M. B. & MOY, R. H. 1960. Chemotherapy of adrenocortical cancer with o, p′ DDD. *Annals of Internal Medicine,* 53**,** 672-682.

BILIMORIA, K. Y., SHEN, W. T., ELARAJ, D., BENTREM, D. J., WINCHESTER, D. J., KEBEBEW, E. & STURGEON, C. 2008. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. *Cancer,* 113**,** 3130-6.

CARNEIRO, B. A., KONDA, B., COSTA, R. B., COSTA, R. L., SAGAR, V., GURSEL, D. B., KIRSCHNER, L. S., CHAE, Y. K., ABDULKADIR, S. A. & RADEMAKER, A. 2019. Nivolumab in metastatic adrenocortical carcinoma: results of a phase 2 trial. *The Journal of Clinical Endocrinology & Metabolism,* 104**,** 6193-6200.

CASTINETTI, F., NIEMAN, L. K., REINCKE, M. & NEWELL-PRICE, J. 2021. Approach to the Patient Treated with Steroidogenesis Inhibitors. *The Journal of Clinical Endocrinology & Metabolism,* 106**,** 2114-2123.

CAZAUBON, Y., TALINEAU, Y., FELIU, C., KONECKI, C., RUSSELLO, J., MATHIEU, O. & DJERADA, Z. 2019. Population pharmacokinetics modelling and simulation of mitotane in patients with adrenocortical carcinoma: an individualized dose regimen to target all patients at three months? *Pharmaceutics,* 11**,** 566.

COLBY, H. D., O'DONNELL, J. P., FLOWERS, N. L., KOSSOR, D. C., JOHNSON, P. B. & LEVITT, M. 1991. Relationship between covalent binding to microsomal protein and the destruction of adrenal cytochrome P-450 by spironolactone. *Toxicology,* 67**,** 143-54.

CORSO, C. R., ACCO, A., BACH, C., BONATTO, S. J. R., DE FIGUEIREDO, B. C. & DE SOUZA, L. M. 2021. Pharmacological profile and effects of mitotane in adrenocortical carcinoma. *British journal of clinical pharmacology,* 87**,** 2698-2710.

DANIEL, E. & NEWELL-PRICE, J. D. 2015. Therapy of endocrine disease: steroidogenesis enzyme inhibitors in Cushing's syndrome. *European Journal of Endocrinology,* 172**,** R263-R280.

DECKER, C. J., RASHED, M. S., BAILLIE, T. A., MALTBY, D. & CORREIA, M. A. 1989. Oxidative metabolism of spironolactone: evidence for the involvement of electrophilic thiosteroid species in drug-mediated destruction of rat hepatic cytochrome P450. *Biochemistry,* 28**,** 5128-36.

FASSNACHT, M., BERRUTI, A., BAUDIN, E., DEMEURE, M. J., GILBERT, J., HAAK, H., KROISS, M., QUINN, D. I., HESSELTINE, E. & RONCHI, C. L. 2015. Linsitinib (OSI-906) versus placebo for patients with locally advanced or metastatic adrenocortical carcinoma: a double-blind, randomised, phase 3 study. *The lancet oncology,* 16**,** 426-435.

FASSNACHT, M., DEKKERS, O. M., ELSE, T., BAUDIN, E., BERRUTI, A., DE KRIJGER, R., HAAK, H. R., MIHAI, R., ASSIE, G. & TERZOLO, M. 2018a. European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol,* 179**,** G1-G46.

FASSNACHT, M., DEKKERS, O. M., ELSE, T., BAUDIN, E., BERRUTI, A., DE KRIJGER, R. R., HAAK, H. R., MIHAI, R., ASSIE, G. & TERZOLO, M. 2018b. European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors. *European journal of endocrinology,* 179**,** G1-G46.

FASSNACHT, M., JOHANSSEN, S., QUINKLER, M., BUCSKY, P., WILLENBERG, H. S., BEUSCHLEIN, F., TERZOLO, M., MUELLER, H. H., HAHNER, S., ALLOLIO, B., et al. 2009. Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification. *Cancer,* 115**,** 243-50.

FASSNACHT, M., KROISS, M. & ALLOLIO, B. 2013. Update in adrenocortical carcinoma. *J Clin Endocrinol Metab,* 98**,** 4551-64.

FRIES, G. F., MARROW, G. S., JR., LESTER, J. W. & GORDON, C. H. 1971. Effect of microsomal enzyme inducing drugs on DDt and dieldrin elimination from cows. *J Dairy Sci,* 54**,** 364-8.

FUKUMOTO, K., KOBAYASHI, T., TACHIBANA, K., KATO, R., TANAKA, K., KOMAMURA, K., KAMAKURA, S., KITAKAZE, M. & UENO, K. 2006. Effect of amiodarone on the

serum concentration/dose ratio of metoprolol in patients with cardiac arrhythmia. *Drug metabolism and pharmacokinetics,* 21**,** 501-505.

HAAK, H. R., HERMANS, J., VAN DE VELDE, C. J., LENTJES, E. G., GOSLINGS, B. M., FLEUREN, G. J. & KRANS, H. M. 1994. Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. *Br J Cancer,* 69**,** 947-51.

HABRA, M. A., STEPHEN, B., CAMPBELL, M., HESS, K., TAPIA, C., XU, M., AHNERT, J. R., JIMENEZ, C., LEE, J. E. & PERRIER, N. D. 2019. Phase II clinical trial of pembrolizumab efficacy and safety in advanced adrenocortical carcinoma. *Journal for immunotherapy of cancer,* 7**,** 1-9.

HAHNER, S. & FASSNACHT, M. 2005. Mitotane for adrenocortical carcinoma treatment. *Curr Opin Investig Drugs,* 6**,** 386-94.

HAINSWORTH, A. J. & GATENBY, P. A. 2008. Oral potassium supplementation in surgical patients. *Int J Surg,* 6**,** 287-8.

INOUYE, M., MIO, T. & SUMINO, K. 1987. Use of GC/MS/SIM for rapid determination of plasma levels of o,p'-DDD, o,p'-DDE and o,p'-DDA. *Clin Chim Acta,* 170**,** 305-14.

KERKHOFS, T., BAUDIN, E., TERZOLO, M., ALLOLIO, B., CHADAREVIAN, R., MUELLER, H., SKOGSEID, B., LEBOULLEUX, S., MANTERO, F. & HAAK, H. 2013. Comparison of two mitotane starting dose regimens in patients with advanced adrenocortical carcinoma. *The Journal of Clinical Endocrinology & Metabolism,* 98**,** 4759-4767.

KERKHOFS, T., DERIJKS, L., ETTAIEB, M., EEKHOFF, E., NEEF, C., GELDERBLOM, H., DEN HARTIGH, J., GUCHELAAR, H. & HAAK, H. 2014. Short-term variation in plasma mitotane levels confirms the importance of trough level monitoring. *European journal of endocrinology,* 171**,** 677-683.

KERKHOFS, T. M., DERIJKS, L. J., ETTAIEB, H., DEN HARTIGH, J., NEEF, K., GELDERBLOM, H., GUCHELAAR, H.-J. & HAAK, H. R. 2015. Development of a

pharmacokinetic model of mitotane: toward personalized dosing in adrenocortical carcinoma. *Therapeutic drug monitoring,* 37**,** 58-65.

KOLKHOF, P. & BARFACKER, L. 2017. 30 YEARS OF THE MINERALOCORTICOID RECEPTOR: Mineralocorticoid receptor antagonists: 60 years of research and development. *J Endocrinol,* 234**,** T125-T140.

KOSCHKER, A. C., FASSNACHT, M., HAHNER, S., WEISMANN, D. & ALLOLIO, B. 2006. Adrenocortical carcinoma -- improving patient care by establishing new structures. *Exp Clin Endocrinol Diabetes,* 114**,** 45-51.

KROISS, M., QUINKLER, M., LUTZ, W. K., ALLOLIO, B. & FASSNACHT, M. 2011. Drug interactions with mitotane by induction of CYP3A4 metabolism in the clinical management of adrenocortical carcinoma. *Clin Endocrinol (Oxf),* 75**,** 585-91.

KRUSE, J. A. & CARLSON, R. W. 1990. Rapid correction of hypokalemia using concentrated intravenous potassium chloride infusions. *Arch Intern Med,* 150**,** 613-7.

LACROIX, A. 2010. Approach to the patient with adrenocortical carcinoma. *J Clin Endocrinol Metab,* 95**,** 4812-22.

LE TOURNEAU, C., HOIMES, C., ZARWAN, C., WONG, D. J., BAUER, S., CLAUS, R., WERMKE, M., HARIHARAN, S., VON HEYDEBRECK, A. & KASTURI, V. 2018. Avelumab in patients with previously treated metastatic adrenocortical carcinoma: phase 1b results from the JAVELIN solid tumor trial. *Journal for immunotherapy of cancer,* 6**,** 1-9.

LUND, B. O. & LUND, J. 1995. Novel involvement of a mitochondrial steroid hydroxylase (P450c11) in xenobiotic metabolism. *J Biol Chem,* 270**,** 20895-7.

LUTON, J. P., CERDAS, S., BILLAUD, L., THOMAS, G., GUILHAUME, B., BERTAGNA, X., LAUDAT, M. H., LOUVEL, A., CHAPUIS, Y., BLONDEAU, P., et al. 1990. Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. *N Engl J Med,* 322**,** 1195-201.

MENARD, R., CUTLER, G. & RIFKA, S. Spironolactone and adrenal cytochrome P-405: Inhibition of op'DDD (mitotane) induced adrenal atrophy and necrosis. Read before the 59th Annual Meeting of the Endocrine Society, Chica-go, 1977.

NELSON, A. A. & WOODARD, G. 1948. Adrenal cortical atrophy and liver damage produced in dogs by feeding 2,2-bis-(parachloro-phenyl)-1,1-dichloroethane. *Fed Proc,* 7**,** 277.

PUGLISI, S., CALABRESE, A., BASILE, V., CECCATO, F., SCARONI, C., ALTIERI, B., DELLA CASA, S., LOLI, P., PIVONELLO, R., DE MARTINO, M. C., et al. 2020. Mitotane Concentrations Influence Outcome in Patients with Advanced Adrenocortical Carcinoma. *Cancers (Basel),* 12.

PUGLISI, S., CALABRESE, A., BASILE, V., CECCATO, F., SCARONI, C., SIMEOLI, C., TORLONTANO, M., CANNAVO, S., ARNALDI, G., STIGLIANO, A., et al. 2019. Mitotane Concentrations Influence the Risk of Recurrence in Adrenocortical Carcinoma Patients on Adjuvant Treatment. *J Clin Med,* 8.

QUINKLER, M. & STEWART, P. M. 2010. Treatment of primary aldosteronism. *Best Pract Res Clin Endocrinol Metab,* 24**,** 923-32.

RAJ, N., ZHENG, Y., KELLY, V., KATZ, S. S., CHOU, J., DO, R. K., CAPANU, M., ZAMARIN, D., SALTZ, L. B. & ARIYAN, C. E. 2020. PD-1 blockade in advanced adrenocortical carcinoma. *Journal of Clinical Oncology,* 38**,** 71.

RAO, S. N. & HABRA, M. A. 2016. 5th International ACC Symposium: Old Syndromes with New Biomarkers and New Therapies with Old Medications. *Horm Cancer,* 7**,** 17-23.

ROSENTHAL, T., ADAR, R., MILITIANU, J. & DEUTSCH, V. 1974. Esophageal ulceration and oral potassium chloride ingestion. *Chest,* 65**,** 463-5.

RUDBERG, I., HENDSET, M., UTHUS, L. H., MOLDEN, E. & REFSUM, H. 2006. Heterozygous mutation in CYP2C19 significantly increases the concentration/dose ratio of racemic citalopram and escitalopram (S-citalopram). *Therapeutic drug monitoring,* 28**,** 102-105.

SOLYMOSS, B., CLASSEN, H. G. & VARGA, S. 1969. Increased hepatic microsomal activity induced by spironolactone and other steroids. *Proc Soc Exp Biol Med,* 132**,** 940-2.

TERZOLO, M., ANGELI, A., FASSNACHT, M., DAFFARA, F., TAUCHMANOVA, L., CONTON, P. A., ROSSETTO, R., BUCI, L., SPERONE, P., GROSSRUBATSCHER, E., et al. 2007. Adjuvant mitotane treatment for adrenocortical carcinoma. *N Engl J Med,* 356**,** 2372-80.

TERZOLO, M., BAUDIN, E., ARDITO, A., KROISS, M., LEBOULLEUX, S., DAFFARA, F., PEROTTI, P., FEELDERS, R., DEVRIES, J. & ZAGGIA, B. 2013. Mitotane levels predict the outcome of patients with adrenocortical carcinoma treated adjuvantly following radical resection.

TERZOLO, M., PIA, A., BERRUTI, A., OSELLA, G., ALÌ, A., CARBONE, V., TESTA, E., DOGLIOTTI, L. & ANGELI, A. 2000. Low-dose monitored mitotane treatment achieves the therapeutic range with manageable side effects in patients with adrenocortical cancer. *The Journal of Clinical Endocrinology & Metabolism,* 85**,** 2234-2238.

THÖLKING, G., SIATS, L., FORTMANN, C., KOCH, R., HÜSING, A., CICINNATI, V. R., GERTH, H. U., WOLTERS, H. H., ANTHONI, C. & PAVENSTÄDT, H. 2016. Tacrolimus concentration/dose ratio is associated with renal function after liver transplantation. *Annals of transplantation,* 21**,** 167-179.

VAN ERP, N. P., GUCHELAAR, H. J., PLOEGER, B. A., ROMIJN, J. A., HARTIGH, J. & GELDERBLOM, H. 2011. Mitotane has a strong and a durable inducing effect on CYP3A4 activity. *Eur J Endocrinol,* 164**,** 621-6.

VEYTSMAN, I., NIEMAN, L. & FOJO, T. 2009. Management of endocrine manifestations and the use of mitotane as a chemotherapeutic agent for adrenocortical carcinoma. *J Clin Oncol,* 27**,** 4619-29.

WASZUT, U., SZYSZKA, P. & DWORAKOWSKA, D. 2017. Understanding mitotane mode of action. *J Physiol Pharmacol,* 68**,** 13-26.

WESTIN, A. A., REIMERS, A., HELDE, G., NAKKEN, K. O. & BRODTKORB, E. 2008. Serum concentration/dose ratio of levetiracetam before, during and after pregnancy. *Seizure,* 17**,** 192-198.

WORTSMAN, J. & SOLER, N. G. 1977. Mitotane: spironolactone antagonism in Cushing's syndrome. *JAMA,* 238**,** 2527-2527.

YIN, A., ETTAIEB, M. H., SWEN, J. J., VAN DEUN, L., KERKHOFS, T. M., VAN DER STRAATEN, R. J., CORSSMIT, E. P., GELDERBLOM, H., KERSTENS, M. N. & FEELDERS, R. A. 2021. Population Pharmacokinetic and Pharmacogenetic Analysis of Mitotane in Patients with Adrenocortical Carcinoma: Towards Individualized Dosing. *Clinical pharmacokinetics,* 60**,** 89-102.

Figure Legends

Figure 1

Mitotane Concentrations During Spironolactone Treatment

Mitotane concentrations (A,D), mitotane dose (B), mitotane concentration/dose ratios (C, E) and time in range to progression (TRP) and time in range (TR) as mean % of measure mitotane concentrations per patient that were in the therapeutic range (plotted as boxplots with whiskers representing minimal and maximal values) (F). The grey area between the dotted lines in (A and D) represents the therapeutic range of 14-20 mg/l. Error bars represent interquartile range (A-C). *** p<0·001

Supplemental Figure 1

Mitotane Concentrations During Spironolactone Treatment After 6 Months

Mitotane concentrations (A), mitotane dose (B) and mitotane concentration/dose ratios (C), excluding all data fro 0-6 months after treatment start. The grey area between the dotted lines in (A) represents the therapeutic range of 14-20 mg/l. Error bars represent interquartile range (A-C). *** p<0·001, * p<0·05

Supplemental Figure 2

Correlation between Mitotane Doses and Concentrations

Scatter plot of mitotane concentrations plotted against mitotane doses. Lines represent linear interpolations for each group.