Mitotane monotherapy in patients with advanced adrenocortical carcinoma

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Abstract:	Context While mitotane is the only approved drug for the treatment of adrenocortical carcinoma (ACC), data on monotherapy in advanced disease is still scarce. Objective To assess the efficacy of mitotane in advanced ACC in a contemporary setting and to identify predictive factors Design/Setting Multicenter cohort study of three German referral centers Patients 127 patients with advanced ACC treated with mitotane monotherapy Outcome measures RECIST evaluation. Progression-free and overall survival (PFS, OS) by Kaplan-Meier method. Predictive factors by Cox-regression. Results Twenty-six patients (20.5%) experienced objective response including three with complete remission. Overall, median PFS was 4.1 months (range 1.0-73) and median OS 18.5 months (range 1.3-220). Multivariate analysis indicated two main predictive factors: low tumor burden (<10 tumoral lesions): hazard ratio (HR) for progression of 0.51(p=0.002) and for death of 0.59(p=0.017), and initiation of mitotane at delayed advanced recurrence: HR 0.35(p<0.001) and 0.34(p<0.001), respectively. Accordingly, 67% of patients with low tumor burden and mitotane initiation ≥360 days after primary diagnosis experienced a clinical benefit (stable disease >180 days). Patients who achieved mitotane levels >14 mg/l had significantly better OS (HR 0.42; p=0.003). Conclusions With 20.5% the objective response rate was slightly lower than previously reported. However, more than 20% of patients experienced a long-term disease control >1 year.

	In general, patients with late diagnosis of advanced disease and low tumor burden might especially benefit from mitotane monotherapy, whereas patients with early advanced disease and high tumor burden are probably better candidates for combined therapy of mitotane and cytotoxic drugs.
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PRECIS: The precis is a brief description of your paper that will appear on the Table of Contents underneath your article title, should your paper be accepted (see the current issue of JCEM for examples). The description should be **no longer than 200**	We evaluated the efficacy of mitotane monotherapy in 127 patients with advanced adrenocortical carcinoma in a contemporary setting and identify the first time factors that predict response to mitotane

characters, including spaces, and should briefly explain what was done in your study and what was concluded. Please ensure that the precis does not simply repeat the article title.	
INVITED SUBMISSION: Is this an invited submission?	No
SPECIAL REQUESTS: Enter specific comments or requests to the editors here.	

Dear Dr. Robertson,

we are grateful for the constructive comments of the reviewers, which helped us to improve our manuscript significantly.
Below you find our answers to the criticisms of the reviewers. We have addressed their queries in a step by step manner. All changes made in the manuscript are marked by the track function.
We hope that, the revised version is now acceptable for publication in *"The Journal of Clinical Endocrinology & Metabolism"*

Yours sincerely

Martin Fassnacht

Reviewers' Comments:

Reviewer #1: The management of adrenocortical carcinoma (ACC) remains a major challenge and although mitotane has been used for many years, there are still a number of questions around its use and often the outcomes remain dismal. The only data we have to work from is, as noted by the authors, limited studies and/or retrospective studies. The present study involves a substantial number of patients from three centres in Germany; it is "a large clinical audit" or a "multicentre experience". The authors highlight one of the weaknesses, being the lack of either a control group or an appropriate comparator. There are, however, points of clinical relevance that would be of interest to those challenged by the management of these patients. The need for careful attention to serum mitotane levels is clearly reinforced by this study. Similarly, the lack of any meaningful efficacy in a very aggressive disease is also highlighted. One is certainly left with a clear impression that we still need improved therapeutics for this condition. That patients with a late recurrence do better on mitotane seems hardly surprising, by definition they are likely to be less aggressive tumours. I was not entirely clear why the authors seemed a little surprised by this?

Response: We are grateful that the reviewer came to same key conclusions as we did and that he/she appreciated the value of our work. We agree with the reviewer that "surprise" is probably the wrong wording and we revised the corresponding parts of the article (line 232ff). What was indeed not clear to us in advance (and had never really been demonstrated) is the fact that mitotane seems to be more effective in less aggressive tumors.

The other confounder in this type of study is that not only does it not have a control group, but the patients are subject to a range of selection biases, reflecting the Centre's and clinicians different approaches and to the selection of patients for treatment.

Response: We fully agree with the reviewer and have emphasized this aspect even further (line 295ff).

The paper would benefit from careful proofing, in some places the English is a little disjointed e.g.
line 80 "Overall response rate is reported …… highly desirable" - needs to be rephrased.
Line 93 "Treatment have not finally been clarified" - that sentence needs rephrasing.

Line 215 "There are no doubts that these trials…." is a colloquial mistranslation.
Line 216 ".. the patients of our sample are not ….." - rephrase.
Line 255 "..immortal time bias" - I have no idea what this means.

Response: We thank the reviewer for these helpful hints and corrected the English as mentioned above and supplemented an explanation of immortal time bias (264ff).

Overall, the discussion is arguably too long and carries a little repetition. Given the limitations of this study and the relatively simple message that comes from it, it may be better substantially condensed perhaps even, if the Editors allow, with a merge of the Results and Discussion sections?

Response: As suggested the Discussion has significantly shortened (old version 1565 words vs. 1079 words in the new version), although we added several new arguments (as suggested by the reviewers).

In conclusion, although there is a sense in which this is by its nature not formally scientifically rigorous, it does represent the reality of this condition and the information is of value and importance for training clinicians.

Response: Again we would like to express our thanks to the reviewer for this supportive conclusion.

Reviewer #2: In the manuscript by Megerle and colleagues, a retrospective study was conducted on mitotane efficacy for the treatment of advanced adrenocortical carcinomas. As mentioned by the authors, this is the largest study gathering clinical data from 1997 to 2016. Due to this long interval (19 years), several issues must be addressed.
- The year 2005 appears to be a boundary in the lysodren dose that the patients received and the way mitotane concentration is determined. Is the starting dose before and after 2005 has any influence on the objective response rate claimed by the authors?

Response: We agree with the reviewer that the long study period and modifications in mitotane monitoring are relevant issues. However, only 19 patients have been treated before 2005 with quite similar outcome. In this subgroup objective responses were found in 31.6%, stable disease in 5.2%, and progressive disease in 63.2% of patients, whereas after 2005 (n=108) the rate of objective responses was 18.5%; of stable disease 28.7%, and of progressive disease 65%. Due to the small sample size before 2005, we suggest not to add these results to the paper. Nevertheless, we mention now the number of patients before 2015 (line 136) and discuss the issue with the long study period and difference in mitotane management over time as limitation in the Discussion (line 309ff).

Moreover is there any potential impact on the mitotane concentration determined by different laboratories? Is there possibility that the measurement might be variable and should be taken into account?

Response: We are sorry, but the fact that only 19 patients were treated before 2015 and that 3 different labs had performed the analysis prevented a meaningful analysis. However, at the time we switched to the Lysosafe service we performed in several patients a cross-validation and did not find relevant differences.

How does the mitotane dose used at initiation influence the time interval to reach plasma significance?

Response: The reviewer is certainly right that the initial dosage should have some impact on the time interval to reach the target plasma level. Unfortunately, in this retrospective analysis it was not possible to have the exact daily dose of all patients throughout the treatment period. However, in 2012 we published a prospective study on exactly this topic (Kerkhofs et al. JCEM 2012). In this study with 40 patients we have seen some trend to a shorter median time to reach a plasma mitotane level of 14 mg/l in the group with the higher starting dose, but this was not statistically significant (46 days (range 18-81 days) vs. 55 days (range 46-74 days); p= 0.29). Furthermore, in our current analysis and the multicenter study from 2012 there was a high inter-individual variability. We comment on this aspect now in the Discussion (line 268ff).

In the subgroup of cortisol-secreting tumors, Cushing's syndrome does cause significant morbidity and mortality due to edema, infections, and metabolic derangements. Mitotane should improve hormone overproduction, but if not, had those patients been treated for hypercortisolism?

Response: We agree with the reviewer that Cushing's syndrome can significantly influence morbidity and mortality. However, in this retrospective analysis we were not able to retrieve exact data on Cushing-related morbidity (this is often even difficult in prospective studies). Regarding mortality, we performed two analyses: one defined cortisol-producing ACC by biochemical evidence and here are the results given in Table 2 (lower panel): There was only a small trend for an increased mortality (HR for death for the not cortisol-producing tumors 0.94 (95% CI 0.56-1.57)). Following the comment of the reviewer, we now performed an additional analysis, in which we compared only patients with overt Cushing's syndrome with all other patients. However, again we did not found a significant difference (HR 0.95 (95% CI 0.62 – 1.57). Of note, only 34 patients had an overt Cushing's syndrome, and only 5 of them were treated with metyrapone or ketoconazole, respectively. This important aspect is now discussed (line 283ff).

Line 199: "Index" should be changed for "index".
Line 238: the colon should be changed for a full stop.
Line 293: the sentence should be changed because it is confusing. The Ki67 index is not an easy marker to measure in patients with ACC.

Response: We thank the reviewer for these helpful hints, which we corrected as suggested in the 3 comments above.

Mitotane monotherapy in patients with advanced adrenocortical carcinoma

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Précis: We evaluated the efficacy of mitotane monotherapy in 127 patients with advanced adrenocortical carcinoma in a contemporary setting and identify for the first time factors that predict response to mitotane.

Short Title: Mitotane in advanved adrenocortical carcinoma

Keywords: Adrenal Cancer, Mitotane, o,p'-DDD, objective response

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Abstract

Context

While mitotane is the only approved drug for the treatment of adrenocortical carcinoma (ACC), data on monotherapy in advanced disease is still scarce.

Objective

To assess the efficacy of mitotane in advanced ACC in a contemporary setting and to identify predictive factors

Design/Setting

Multicenter cohort study of three German referral centers

Patients

127 patients with advanced ACC treated with mitotane monotherapy

Outcome measures

RECIST evaluation. Progression-free and overall survival (PFS, OS) by Kaplan-Meier method. Predictive factors by Cox-regression.

Results

Twenty-six patients (20.5%) experienced objective response including three with complete remission. Overall, median PFS was 4.1 months (range 1.0-73) and median OS 18.5 months (range 1.3–220). Multivariate analysis indicated two main predictive factors: low tumor burden (<10 tumoral lesions): hazard ratio (HR) for progression of 0.51(p=0.002) and for death of 0.59(p=0.017), and initiation of mitotane at delayed advanced recurrence: HR 0.35(p<0.001) and 0.34(p<0.001), respectively. Accordingly, 67% of patients with low tumor burden and mitotane initiation ≥360 days after primary diagnosis experienced a clinical benefit (stable disease >180 days). Patients who achieved mitotane levels >14 mg/l had significantly better OS (HR 0.42; p=0.003).

Conclusions

With 20.5% the objective response rate was slightly lower than previously reported. However, more than 20% of patients experienced a long-term disease control >1 year. In general, patients with late diagnosis of advanced disease and low tumor burden might especially benefit from mitotane monotherapy, whereas patients with early advanced disease and high tumor burden are probably better candidates for combined therapy of mitotane and cytotoxic drugs.

1. Introduction

Adrenocortical carcinoma (ACC) is a rare and aggressive disease with dismal prognosis and limited therapeutic options in advanced tumor stages [1, 2]. Mitotane is the only drug approved for treatment of ACC and has been in clinical use both in an adjuvant and palliative setting for many years [3-5]. However, data on the benefit of mitotane treatment are limited.

Although the approval of mitotane in most countries is restricted to ACC not amenable to complete resection, data on mitotane monotherapy in advanced ACC are scarce [2]. In fact, only 11 series with >10 patients have reported a total number of 395 patients treated with mitotane monotherapy in advanced disease. ~~In~~Of these studies (3 prospective [6-8], 8 retrospective [3, 9-15]) the largest series ~~of patients enrolled~~included just 67 patient in ~~a single~~an retrospective evaluation ~~was 67~~. Overall response rate is reported only at about 25~~% clearly indicating that~~%. Therefore, factors of response prediction would ~~be highly desirable~~help clinicians to choose the right treatment for each individual patient. Furthermore, the relevance of these studies for contemporary medicine is likely to be limited since most of the studies had been performed in or even before the 1990's. Accordingly, in the majority of studies tumor response assessment was very heterogeneous and criteria are mostly not comparable with RECIST criteria, which are now standard of care and major ~~outcome~~endpoint in ~~oncology studies.~~cancer trials. Of note, according to the labeling of mitotane in several countries (e.g. Europe Union) the effects of mitotane in non-functioning tumors has not been established. However, data supporting or disproving this statement are actually completely lacking. While the interest in mitotane as a sole first-line therapy in advanced ACC has increased recently, the lack of convincing data on monotherapy has unsettled clinicians. One reason for this "revival" of mitotane monotherapy comes indirectly from the FIRM-ACT trial. The results of this first randomized trial in ACC suggested that the most effective therapy, the combination of etoposide, doxorubicin, cisplatin, and mitotane, is as effective as second-line therapy as it is as first-line therapy [16]. Therefore, it seems to be justified to test other drugs (e.g. mitotane) first without risking the lives of the patients.

~~The~~Despite efforts from several groups, the mechanisms of action of mitotane ~~treatment~~ have not finally been clarified ~~despite efforts from several groups~~. Although we had recently demonstrated that mitotane induces endoplasmatic reticulum stress specifically in adrenocortical carcinoma cell lines and identified inhibition of sterol O-acyltransferase 1 (SOAT1) as a key molecular event [17], other mechanisms are likely to be relevant and may overlap when considering the high mitotane concentrations required for efficacy. In addition, pharmacokinetic properties including basic aspects such as intestinal resorption and metabolic transformation have only been partially elucidated.

Several small studies suggested the importance of drug monitoring in the management of patients with mitotane. First, in 1984 van Slooten and colleagues had measured mitotane blood levels in 34 patients and found a relation to the response rate [14]. This concept was then confirmed in a larger retrospective series (n=58) [11] and a

small prospective study (n=13) [{8}]. Since that time most authors recommend aiming at plasma mitotane levels between 14 and 20 mg/l to improve response rate and to limit toxicity [{1, 2}].

In the current large cohort study we analyze 127 patients with advanced ACC treated at three German centers with the aim to provide efficacy data on mitotane monotherapy based on contemporary imaging methods and to identify for the first time predictive factors for treatment response to mitotane.

2. Subjects and Methods

A. Study population

Patients and clinical and histological parameters (sex, age at diagnosis, tumor size, evidence of hormone excess, and tumor stage according to the European Network for the Study of Adrenal Tumors (ENSAT) classification [{18}], date of documented unresectability, Weiss score, Ki67 index, presence, site, size and number of tumor lesions, mitotane plasma concentration, and follow-up information) were retrieved from the German ACC Registry and the ENSAT Registry (www.ensat.org/registry). Both registries have been approved by the ethics committee of the University of Würzburg (approval number 86/03 and 88/11). The study is part of the German Adrenocortical Carcinoma Study group (ClinicalTrials.gov Identifier: NCT00453674). Written informed consent was obtained from all patients. Patients from three German centers (Würzburg, Berlin, Munich) were included who fulfilled the following eligibility criteria: age ≥18 years, histologically confirmed ACC, advanced (i.e. not completely resectable) disease at initial diagnosis or during the course of the disease; mitotane monotherapy for at least 30 days, cross sectional imaging (abdominal and thoracic CT, MRI or FDG-PET/CT) before start of mitotane therapy (≤30 days) and regularly during mitotane treatment (at least every 4 months in the first year; median 93 days). To ensure use of contemporary imaging methods and sufficient follow-up, inclusion was restricted to patients with a start of mitotane treatment between 01. Jan 1997 and 31. Dec 2016. Exclusion criteria were: incomplete information on primary diagnosis and/or follow-up, concomitant therapies such as radiotherapy or cytotoxic chemotherapy, and previous therapy with mitotane.

Field Code Changed

B. Mitotane dosage and drug monitoring

Mitotane was given as tablets (Lysodren®, Bristol Myers Squibb, Princeton, U.S.A. before 2004, then HRA Pharma, Paris, France). Before 2005, the drug was administered at doses usually not exceeding 3.5 g per day [{19}]. After 2005, in most cases a high-dose starting schedule introduced by Baudin and colleagues [{20, 21}] was employed with a median maximal dosage of 7.5 g mitotane per day. Starting from 2005 mitotane plasma levels were measured centrally using the Lysosafe ® service (www.lysosafe.com). Before this service was available mitotane was analyzed in three different German laboratories that offered blood concentration assessment of mitotane. However, only 19 of our 127 patients did start mitotane before 2005.

C. Response assessment

Treatment response was recorded according to routine radiologic assessment and qualified as complete response, partial response, progressive disease, and stable disease in analogy to the Response Evaluation Criteria In Solid Tumors Version 1.1 (RECIST 1.1, [{22}]). In uncertain cases (n=12), RECIST 1.1 was applied by reviewing all images by a blinded radiologist (W.S.).

D. Statistical analysis

Progression-free survival (PFS) was defined as the interval between start of mitotane therapy and first documentation of progressive disease during follow-up or censored at last follow-up. Overall survival (OS) was calculated as the time between start of mitotane therapy and death, with censoring at last follow-up otherwise. PFS and OS were evaluated by the Kaplan-Meier method. We defined meaningful clinical benefit as disease control of more than 180 days, because in a recent placebo-controlled trial none of the patients without active treatment experienced disease stabilization for more than 150 days (23). The following potential prognostic and predictive factors were defined prior to the analysis: age (dichotomized at the median), sex, endocrine activity, Ki67 index of the primary tumor, tumor burden, pattern of affected organs at start of mitotane therapy, peak mitotane blood concentration during monotherapy or within the first 3 months of treatment, and timing of the mitotane initiation. In this context patients were classified according to the question whether mitotane treatment was initiated for advanced disease at time of initial diagnosis or at recurrence at a certain time during follow-up. In the latter case (treatment for recurrent advanced disease) we differentiated patients according the time between primary diagnosis and start of mitotane (< 360 days, 360-999 days, or ≥ 1000 days). All factors were investigated by univariate analysis using Cox regression. In case of p-value of below 0.1 in univariate analysis multivariate analyses were performed. Tumor burden was assessed in the multivariate analysis only as the sum of tumoral lesions. Association between variables and PFS/OS was expressed as hazard ratio (HR) and 95% confidence interval (CI). Statistical significance was set at $p<0.05$. For calculation of possible differences in response rate dependent on mitotane blood level “Fisher Exact Test” was used. Results are presented as median and range if not stated otherwise. For statistical calculation SPSS 24.0 (IBM Corp., Armonk, NY) was used.

3. Results

At time of analysis (March 2017) 127 patients (female:male 77:50) were included. 49 patients (38.6%) suffered from advanced ACC at initial diagnosis and did start mitotane therapy within 3 weeks after establishment of the diagnosis. 78 patients (61.4%) started mitotane after experiencing recurrence with unresectable disease during follow-up. Median age was 58.6 years with a range of 19.8 to 85.8 years. Other baseline characteristics are given in Table 1.

A. Best objective response, progression-free and overall survival

Best response was complete response in three patients (2.4 %), partial response in 23 (18.1 %) and stable disease (SD) in 32 patients (25.2 %). Of the patients with SD, disease was controlled in 23 of them for at least 180 days. 69 patients (54.3 %) had progressive disease at the time of first tumor evaluation. PFS was 4.1 months (range 1 – 73 months) and OS was 18.5 months (1.3 – 220 months) after initiation of mitotane (Figure 1). Overall, 50 patients (40.9%) experienced clinical benefit (disease control > 180 days), including 28 (22.0%) with a long-term benefit of more than 12 months.

B. Predictive factors of progression-free and overall survival

To identify possible predictive factors univariate and multivariate analyses (adjusted by age, sex, tumor burden, timing of mitotane initiation) were performed. Results are given in Table 2 and Figure 2. In short, univariate analyses showed better prognosis regarding PFS and OS for patients with Ki67 index <10%, mitotane initiation at delayed advanced recurrence (≥360d after initial diagnosis) and low tumor burden represented by <10 tumoral lesions. Multivariate analyses only indicated a better outcome for low tumor burden (PFS: HR 0.51, p=0.002, CI 0.33-0.79; OS: HR 0.59, p=0.0017, CI 0.39-0.91) and timing of mitotane initiation at delayed advanced recurrence (HR for PFS: 0.35, p<0.001, CI 0.23-0.55 and HR for OS: 0.34, p<0.001, CI 0.22-0.52). For detailed analysis including subgroups see Table 2.

C. Influence of mitotane drug levels on efficacy

Patients who reached mitotane blood levels ≥14 mg/l had both superior PFS and OS as compared to patients in whom this blood concentration was not achieved (Table 3). Given that the association with response might be confounded by the long time interval required to reach a significant plasma concentration, we performed a subgroup analysis in which only mitotane levels in the first 3 months were considered. Here, the effect was statistically not significant. Remarkably, the 15 patients, who reached a level >20 mg/l within these first 3 months had a median PFS of 277 days (Table 3). Objective response rate in patients with mitotane level ≥14 mg/l was also significantly higher compared to <14 mg/l (31.9% vs. 11.1%; p=0.041). However, there were two patients with partial and one patient with complete response, whose peak level never was above 13 mg/l, including one patient with partial response and a maximum documented level below 10 mg/l.

D. Response rate in different subgroups

In a next step we checked response rates to mitotane monotherapy in subgroups defined by potential predictive factors suggested by the present study or previous studies (9, 24-26). We found objective response rates (PR, CR) were highest (30%) in patients with both low tumor burden and mitotane initiation at delayed advanced recurrence (≥ 360 days after initial diagnosis) (Table 4). In contrast no objective response was seen in patients with both high tumor burden and mitotane initiation at initial diagnosis or early recurrence (< 360 days after primary diagnosis). Furthermore, in this subgroup only 6.3% experienced a clinical benefit (> 180 days), whereas such a benefit was present in more than 67% of patients in the complementary group (Table 4). Additionally, in 54% patients with low Ki67 ~~Index~~index (≤ 10 %) long-term disease control was achieved in comparison to only 24 % with Ki67 > 20%.

4. Discussion

Although mitotane is the only approved drug for the treatment of ACC patients, reliable data on mitotane monotherapy are surprisingly scarce. Here, we present the by far largest study analyzing the effects of mitotane monotherapy in 127 patients with advanced disease. We demonstrate that mitotane leads to an objective response rate of 21% (including 3 patients with complete response). Furthermore, additional 25 % of patients experienced stable disease translating to a median progression-free survival of 4.1 months and overall survival of 18.5 months.

~~In addition to providing contemporary efficacy data on mitotane, a further purpose of this study was to investigate potential prognostic and predictive factors in patients at mitotane therapy. In fact, we were able to identify a subgroup of patients (e.g. with late diagnosis of non-resectable disease and low tumor burden) that might benefit especially from mitotane monotherapy. Probably even more important from a clinical perspective, we did not observe any objective response and almost no clinical benefit in the group of patients who had advanced ACC within 12 months of the primary diagnosis and more than 10 tumoral lesions. Thus, in this subgroup additional therapeutic measures (e.g. cytotoxic chemotherapy with EDP [16]) seem to be justified or even necessary.~~

Although a median progression-free survival of four months is not impressive, it compares favorably to other drugs investigated in ACC (23, 27-30) ~~({~~for review see ~~[{~~2~~]}~~). However, ~~there are no doubts that~~without a doubt these trials are not readily comparable (e.g. for different imaging intervals). ~~Furthermore, the patients of our sample are not necessarily representative for a cohort of advanced ACC, because patients that were judged as harboring aggressive tumors might have been selected for the immediate start of adjunctive cytotoxic chemotherapy. Moreover, this study lacks an untreated comparator group, which renders demonstration of a direct causal relationship between treatment and disease stabilization impossible. However~~ Nevertheless, this study clearly shows that mitotane is capable to induce objective response in a relevant percentage of patients and can control advanced disease ~~at least~~ in a subgroup for a long time.

~~Despite the limitations of several earlier studies on mitotane monotherapy in the literature, the objective response rate in our study of 21% is surprisingly close to those from previously reported results [3,6-14], which had a mean response rate of 27% [2].~~

~~One of the more surprising results~~A key result of our study is the observation that patients that started mitotane after late recurrence did much better than those patients who had advanced disease at the time of the initial diagnosis. An important and known aspect to consider ~~might be~~is that patients with late recurrence have a ~~generally~~ better prognosis, because these tumors are usually less aggressive (31). However, this ~~does not~~doesn't necessarily explain the higher objective response rate in this cohort (Table 4). High tumor burden, described by maximal tumor diameter and/or number of tumoral lesions or organs, is associated with poor prognosis in literature (2, 32). Here, we could confirm that tumor load is an important prognostic factor. ~~There is a significant~~

~~better PFS and OS in multivariate analysis in patients with advanced ACC and low tumor burden treated with mitotane.~~ Of note, our data seems to suggest that low tumor burden is also a predictive factor for response to mitotane treatment, as 71% of patients with less than 10 tumoral lesions experienced disease control for more than 6 months, whereas this was the case in only 18% of patients with higher tumor load.

~~Regarding Ki67 index, there was a slightly earlier progression in patients with Ki67 of ≥ 20 % in comparison to patients with Ki67 index ≤10 % in the primary tumor. However, this trend disappeared in multivariate analysis. Furthermore, patients~~Patients with Ki67 index ≤10 % had a better overall survival than patients with Ki67 index ≥ 20 ~~%: With this endpoint, the effect was only visible as trend after multivariate analysis (HR 0.52; p=0.08).~~%. These results are in line with previous studies ~~[24]~~ that described Ki67 as an important prognostic marker in ACC, which however appears to have less discriminative value in advanced disease (33). Again, tumors with low Ki67 seem to respond slightly better than tumors with high proliferative activity. However, one has to acknowledge that Ki67 staining of the primary tumor is most likely not the ideal parameter to judge the clinical behavior of a tumor that recurred in almost half of the patients more than 1 year after the initial surgery.

~~Due to the fact that the disease can be controlled for more than 6 months in only 40% of patients, additional markers that could predict response to mitotane would be highly desirable. Expression of SOAT1 has been suggested as such a marker in a small cohort of patients [17].~~

~~Taking these key findings together, our hypothesis is that less aggressive tumors might respond better to mitotane. Therefore, it could be reasonable to offer patients with late recurrence, low grade tumor and limited tumor burden mitotane monotherapy. In contrast, patients with aggressive disease probably benefit more from early administration of cytotoxic drugs.~~

Mitotane therapy and mitotane blood levels have shown to be correlated with objective response rate and also ~~PFS~~progression-free and/or ~~OS~~overall survival in patients with advanced or recurrent ACC (8, 9, 11, 34). In our study a trend towards better ~~PFS~~progression-free survival with higher peak mitotane blood levels during therapy could be seen in multivariate analysis. Regarding ~~OS~~overall survival, results show a significant correlation between higher mitotane levels and longer ~~OS~~survival. However, the so called immortal time bias may partially explain this finding. meaning that higher mitotane levels correlate with treatment duration and this certainly comes with longer survival. If we used only the mitotane measurements in the first three months of therapy, there was no significant correlation with progression-free or overall survival. However, the number of patients in this sub-analysis might have been too small to draw strong conclusions. Furthermore initial dosage of mitotane might influence the time interval to reach relevant plasma levels, although an earlier analysis suggested no significant difference between two starting regimens in the first 12 weeks (21). Therefore, further studies on mitotane blood levels in the first weeks after initiation are warranted to investigate its value as predictive marker for outcome. ~~Of note~~Interestingly, we have seen objective response in three patients, who never reached the "therapeutic

concentration" of 14mg/l. Thus, a level ≥ 14mg/l seems to us still desirable, but lower levels do not preclude clinical benefit.

~~Female to male ratio in our cohort matched previous reports, but median age was higher than shown in earlier reports [2,33]. This might be due to the fact that in the enrolled cohort there was a notable amount of patients with recurrence, which occurred partially years after first diagnosis. Another and more important reason is that young patients with advanced ACC might be often treated more aggressively with mitotane plus cytotoxic chemotherapy, which excluded them from this study.~~

~~Regarding secretory status of ACC, several~~Several studies have shown worse prognosis for cortisol-producing tumors (26, 35, 36). In our cohort we could not show any difference in progression-free and overall survival between patients with and without cortisol-producing ACC. This might be due to the small number of patients (n = 80) of which we had sufficient information about the endocrine function of their ACC. Furthermore, only 34 patients had overt Cushing's syndrome, but only 5 had to be treated with inhibitors of steroidogenesis suggesting that only a minority of the cohort had severe hypercortisolism not controlled by mitotane. However, our study provides important additional information for the official labeling of mitotane in several countries (e.g. in the European Union), where it is mentioned that the effects of mitotane in non-functioning tumors is not established. Our study clearly suggests that mitotane is effective independently of endocrine activity of the tumor. Therefore, we recommend - as it is already clinical practice in most expert centers - to administer mitotane in both, functioning and non-functioning tumors.

Our study has obvious limitations: (i) Its retrospective design and the lack of a control group hinders a proper separation of the effect of mitotane from other known and unknown prognostic factors in ACC. However, a placebo-controlled trial might be even unethical, because it is well known that almost all ACC progress rapidly, if they are left untreated, as just recently prospectively demonstrated in the GALACCTIC trial (23). ~~We also think that despite~~ (ii) Furthermore, patients in our cohort are probably not representative for the ~~lack of a formal control~~entire group~~, we can draw relevant conclusions regarding the effectiveness of mitotane. The reason is that complete or partial response will not occur in ACC in the absence of treatment. This means that the numbers on response, whether partial or complete, provide valuable estimates of mitotane effect. Of course this does not beyond doubt answer the question what the optimal treatment regimen in~~ of advanced ACC~~ is. (ii~~, because patients with aggressive tumors might have been selected for the immediate start of adjunctive cytotoxic chemotherapy. (iii) Another weakness is of course the relative small number of cases ~~presented here~~ that ~~might have~~has prevented ~~statistically significant results in some interesting~~detailed subgroup analyses. On the other hand, 127 patients can be considered a very large number in comparison to earlier reports. Most likely a cohort of more than 500 would be required to allow for a better statistical power and such a cohort size is currently unlikely to be achievable~~ even in a worldwide effort. Furthermore, it seems to be unrealistic to gather funding for a large prospective observational trial for a drug that is already approved for many years.~~. Thus, it is likely that these

results are the most reliable results for the time being. (~~iii) Moreover, a complete endocrine workup was lacking in several of our patients, thereby hampering an in-depth analysis on the impact of hormonal hypersecretion in this situation. (iiii~~iv) The ~~same is true for certain other factors (like Ki67)~~long study period and the modification of the mitotane ~~blood level in the first three months~~management in 2005 might have influenced the results. However, the fact that only 19 patients were recruited before 2005 suggests that ~~were just not available for all patients. (iiiii~~the overall influence is limited. (v) Finally, we did not report adverse effects. However, it is well established that reliable data on adverse events require prospective data collection and to avoid underreporting we preferred not to elaborate on this issue.

5. Conclusions

This largest study on mitotane monotherapy demonstrated that this drug is able to achieve relevant clinical benefit for patients with advanced ACC. Although the objective response rate was slightly lower than reported previously, the fact that a fifth of the cohort had clear tumor shrinkage proves efficacy of the drug. This is further substantiated by the 20% of patients, who experienced a long-term disease control >1 year. Our study suggests that patients with less aggressive tumors (e.g. low grade tumors with low tumor burden and a long interval between initial diagnosis and necessity to start systemic therapy) might be especially good candidates for mitotane monotherapy. In contrast, patients with advanced disease at primary diagnosis and high tumor load probably benefit more from early administration of cytotoxic drugs.

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Author Contributions.

FM contributed to the data collection, performed the statistical analyses, and has written the first draft of the manuscript. WH contributed to the data collection and performed the statistical analyses. WS has re-evaluated the radiological images. CLR, AP, MQ, FB, SH, MK contributed to the data collection and data interpretation. MF conceived the design of the study, contributed to the data collection and data interpretation, and supervised the entire study. All authors edited the manuscript and approved the final version and its submission.

7. References

1. Berruti A, Baudin E, Gelderblom H, Haak HR, Porpiglia F, Fassnacht M, Pentheroudakis G, Group EGW. Adrenal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7:vii131-138
2. Else T, Kim AC, Sabolch A, Raymond VM, Kandathil A, Caoili EM, Jolly S, Miller BS, Giordano TJ, Hammer GD. Adrenocortical carcinoma. Endocr Rev 2014; 35:282-326
3. Luton JP, Cerdas S, Billaud L, Thomas G, Guilhaume B, Bertagna X, Laudat MH, Louvel A, Chapuis Y, Blondeau P, et al. Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. N Engl J Med 1990; 322:1195-1201
4. Schteingart DE, Doherty GM, Gauger PG, Giordano TJ, Hammer GD, Korobkin M, Worden FP. Management of patients with adrenal cancer: recommendations of an international consensus conference. Endocr Relat Cancer 2005; 12:667-680
5. Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, Rossetto R, Buci L, Sperone P, Grossrubatscher E, Reimondo G, Bollito E, Papotti M, Saeger W, Hahner S, Koschker AC, Arvat E, Ambrosi B, Loli P, Lombardi G, Mannelli M, Bruzzi P, Mantero F, Allolio B, Dogliotti L, Berruti A. Adjuvant mitotane treatment for adrenocortical carcinoma. N Engl J Med 2007; 356:2372-2380
6. Decker RA, Elson P, Hogan TF, Citrin DL, Westring DW, Banerjee TK, Gilchrist KW, Horton J. Eastern Cooperative Oncology Group study 1879: mitotane and adriamycin in patients with advanced adrenocortical carcinoma. Surgery 1991; 110:1006-1013
7. Williamson SK, Lew D, Miller GJ, Balcerzak SP, Baker LH, Crawford ED. Phase II evaluation of cisplatin and etoposide followed by mitotane at disease progression in patients with locally advanced or metastatic adrenocortical carcinoma: a Southwest Oncology Group Study. Cancer 2000; 88:1159-1165
8. Baudin E, Pellegriti G, Bonnay M, Penfornis A, Laplanche A, Vassal G, Schlumberger M. Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'DDD) levels on the treatment of patients with adrenocortical carcinoma. Cancer 2001; 92:1385-1392
9. Gonzalez RJ, Tamm EP, Ng C, Phan AT, Vassilopoulou-Sellin R, Perrier ND, Evans DB, Lee JE. Response to mitotane predicts outcome in patients with recurrent adrenal cortical carcinoma. Surgery 2007; 142:867-875; discussion 867-875
10. Barzon L, Fallo F, Sonino N, Daniele O, Boscaro M. Adrenocortical carcinoma: experience in 45 patients. Oncology 1997; 54:490-496
11. Haak HR, Hermans J, van de Velde CJ, Lentjes EG, Goslings BM, Fleuren GJ, Krans HM. Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. Br J Cancer 1994; 69:947-951
12. Pommier RF, Brennan MF. An eleven-year experience with adrenocortical carcinoma. Surgery 1992; 112:963-970; discussion 970-961
13. Venkatesh S, Hickey RC, Sellin RV, Fernandez JF, Samaan NA. Adrenal cortical carcinoma. Cancer 1989; 64:765-769
14. van Slooten H, Moolenaar AJ, van Seters AP, Smeenk D. The treatment of adrenocortical carcinoma with o,p'-DDD: prognostic implications of serum level monitoring. Eur J Cancer Clin Oncol 1984; 20:47-53
15. Reidy-Lagunes DL, Lung B, Untch BR, Raj N, Hrabovsky A, Kelly C, Gerst S, Katz S, Kampel L, Chou J, Gopalan A, Saltz LB. Complete Responses to Mitotane in Metastatic Adrenocortical Carcinoma-A New Look at an Old Drug. Oncologist 2017; 22:1102-1106
16. Fassnacht M, Terzolo M, Allolio B, Baudin E, Haak H, Berruti A, Welin S, Schade-Brittinger C, Lacroix A, Jarzab B, Sorbye H, Torpy DJ, Stepan V, Schteingart DE, Arlt W, Kroiss M, Leboulleux S, Sperone P, Sundin A, Hermsen I, Hahner S, Willenberg HS, Tabarin A, Quinkler M, de la Fouchardiere C, Schlumberger M, Mantero F, Weismann D, Beuschlein F, Gelderblom H, Wilmink H, Sender M, Edgerly M, Kenn W, Fojo T, Muller HH, Skogseid B, Group F-AS. Combination chemotherapy in advanced adrenocortical carcinoma. N Engl J Med 2012; 366:2189-2197
17. Sbiera S, Leich E, Liebisch G, Sbiera I, Schirbel A, Wiemer L, Matysik S, Eckhardt C, Gardill F, Gehl A, Kendl S, Weigand I, Bala M, Ronchi CL, Deutschbein T, Schmitz G, Rosenwald A, Allolio B, Fassnacht M, Kroiss M. Mitotane Inhibits Sterol-O-Acyl Transferase 1 Triggering Lipid-Mediated Endoplasmic Reticulum Stress and Apoptosis in Adrenocortical Carcinoma Cells. Endocrinology 2015; 156:3895-3908
18. Fassnacht M, Johanssen S, Quinkler M, Bucsky P, Willenberg HS, Beuschlein F, Terzolo M, Mueller HH, Hahner S, Allolio B, German Adrenocortical Carcinoma Registry G, European Network for the Study of

Adrenal T. Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification. Cancer 2009; 115:243-250

19. Terzolo M, Pia A, Berruti A, Osella G, Ali A, Carbone V, Testa E, Dogliotti L, Angeli A. Low-dose monitored mitotane treatment achieves the therapeutic range with manageable side effects in patients with adrenocortical cancer. J Clin Endocrinol Metab 2000; 85:2234-2238

20. Faggiano A, Leboulleux S, Young J, Schlumberger M, Baudin E. Rapidly progressing high o,p'DDD doses shorten the time required to reach the therapeutic threshold with an acceptable tolerance: preliminary results. Clin Endocrinol 2006; 64:110-113

21. Kerkhofs TM, Baudin E, Terzolo M, Allolio B, Chadarevian R, Mueller HH, Skogseid B, Leboulleux S, Mantero F, Haak HR, Fassnacht M. Comparison of two mitotane starting dose regimens in patients with advanced adrenocortical carcinoma. J Clin Endocrinol Metab 2013; 98:4759-4767

22. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45:228-247

23. Fassnacht M, Berruti A, Baudin E, Demeure MJ, Gilbert J, Haak H, Kroiss M, Quinn DI, Hesseltine E, Ronchi CL, Terzolo M, Choueiri TK, Poondru S, Fleege T, Rorig R, Chen J, Stephens AW, Worden F, Hammer GD. Linsitinib (OSI-906) versus placebo for patients with locally advanced or metastatic adrenocortical carcinoma: a double-blind, randomised, phase 3 study. Lancet Oncol 2015; 16:426-435

24. Beuschlein F, Weigel J, Saeger W, Kroiss M, Wild V, Daffara F, Libe R, Ardito A, Al Ghuzlan A, Quinkler M, Osswald A, Ronchi CL, de Krijger R, Feelders RA, Waldmann J, Willenberg HS, Deutschbein T, Stell A, Reincke M, Papotti M, Baudin E, Tissier F, Haak HR, Loli P, Terzolo M, Allolio B, Muller HH, Fassnacht M. Major prognostic role of Ki67 in localized adrenocortical carcinoma after complete resection. T J Clin Endocrinol Metab 2015; 100:841-849

25. Abiven G, Coste J, Groussin L, Anract P, Tissier F, Legmann P, Dousset B, Bertagna X, Bertherat J. 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 2006; 91:2650-2655

26. Berruti A, Fassnacht M, Haak H, Else T, Baudin E, Sperone P, Kroiss M, Kerkhofs T, Williams AR, Ardito A, Leboulleux S, Volante M, Deutschbein T, Feelders R, Ronchi C, Grisanti S, Gelderblom H, Porpiglia F, Papotti M, Hammer GD, Allolio B, Terzolo M. Prognostic role of overt hypercortisolism in completely operated patients with adrenocortical cancer. Eur Urol 2014; 65:832-838

27. Lerario AM, Worden FP, Ramm CA, Hesseltine EA, Stadler WM, Else T, Shah MH, Agamah E, Rao K, Hammer GD. The combination of insulin-like growth factor receptor 1 (IGF1R) antibody cixutumumab and mitotane as a first-line therapy for patients with recurrent/metastatic adrenocortical carcinoma: a multi-institutional NCI-sponsored trial. Hormones & cancer 2014; 5:232-239

28. O'Sullivan C, Edgerly M, Velarde M, Wilkerson J, Venkatesan AM, Pittaluga S, Yang SX, Nguyen D, Balasubramaniam S, Fojo T. The VEGF inhibitor axitinib has limited effectiveness as a therapy for adrenocortical cancer. J Clin Endocrinol Metab 2014; 99:1291-1297

29. Kroiss M, Deutschbein T, Schlotelburg W, Ronchi CL, Neu B, Muller HH, Quinkler M, Hahner S, Heidemeier A, Fassnacht M, German Adrenocortical Carcinoma Study G. Salvage Treatment of Adrenocortical Carcinoma with Trofosfamide. Hormones & cancer 2016; 7:211-218

30. Henning JEK, Deutschbein T, Altieri B, Steinhauer S, Kircher S, Sbiera S, Wild V, Schlötelburg W, Kroiss M, Perotti P, Rosenwald A, Berruti A, Fassnacht M, Ronchi CL. Gemcitabine-Based Chemotherapy In Adrenocortical Carcinoma: A Multicentric Study On Efficacy and Predictive Factors. J Clin Endocrinol Metab 2017:jc.2017-01624-jc.02017-01624

31. Erdogan I, Deutschbein T, Jurowich C, Kroiss M, Ronchi C, Quinkler M, Waldmann J, Willenberg HS, Beuschlein F, Fottner C, Klose S, Heidemeier A, Brix D, Fenske W, Hahner S, Reibetanz J, Allolio B, Fassnacht M, German Adrenocortical Carcinoma Study G. The role of surgery in the management of recurrent adrenocortical carcinoma. J Clin Endocrinol Metab 2013; 98:181-191

32. Assie G, Antoni G, Tissier F, Caillou B, Abiven G, Gicquel C, Leboulleux S, Travagli JP, Dromain C, Bertagna X, Bertherat J, Schlumberger M, Baudin E. Prognostic parameters of metastatic adrenocortical carcinoma. J Clin Endocrinol Metab 2007; 92:148-154

33. Libe R. Adrenocortical carcinoma (ACC): diagnosis, prognosis, and treatment. Front Cell Dev Biol 2015; 3:45

34. Hermsen IG, Fassnacht M, Terzolo M, Houterman S, den Hartigh J, Leboulleux S, Daffara F, Berruti A, Chadarevian R, Schlumberger M, Allolio B, Haak HR, Baudin E. Plasma concentrations of o,p'DDD, o,p'DDA, and o,p'DDE as predictors of tumor response to mitotane in adrenocortical carcinoma: results of a retrospective ENS@T multicenter study. J Clin Endocrinol Metab 2011; 96:1844-1851

35. Ayala-Ramirez M, Jasim S, Feng L, Ejaz S, Deniz F, Busaidy N, Waguespack SG, Naing A, Sircar K, Wood CG, Pagliaro L, Jimenez C, Vassilopoulou-Sellin R, Habra MA. Adrenocortical carcinoma: clinical outcomes and prognosis of 330 patients at a tertiary care center. Eur J Endocrinol 2013; 169:891-899

36. Kim Y, Margonis GA, Prescott JD, Tran TB, Postlewait LM, Maithel SK, Wang TS, Evans DB, Hatzaras I, Shenoy R, Phay JE, Keplinger K, Fields RC, Jin LX, Weber SM, Salem AI, Sicklick JK, Gad S, Yopp AC, Mansour JC, Duh QY, Seiser N, Solorzano CC, Kiernan CM, Votanopoulos KI, Levine EA, Poultsides GA, Pawlik TM. Nomograms to Predict Recurrence-Free and Overall Survival After Curative Resection of Adrenocortical Carcinoma. JAMA Surg 2016; 151:365-373

8. Tables

Table 1. Clinical characteristics of the study cohort

Table 2. Prognostic factors on progression-free and overall survival

Table 3. Influence of Mitotane blood level on progression-free and overall survival

Table 4. Response to therapy in different subgroups

9. Figures

Fig. 1. Kaplan-Meier curve for progression-free (A) and overall (B) survival

Fig. 2. Influence of timing of mitotane initiation and tumor burden on progression-free survival (A + B) and overall survival (C + D)

Mitotane monotherapy in patients with advanced adrenocortical carcinoma

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Table 1. Clinical characteristics of the study cohort

Parameter/Subgroup	
Entire cohort	127
Age at start mitotane (yr)	
Median	58.6
Range	19.8 – 85.8
≤ 58 yrs	63 (49.6 %)
>58 yrs	64 (50.4 %)
Sex	
Female	77 (60.6 %)
Male	50 (39.4 %)
BMI (kg/m^2) (n=99)	
Median	25.1
Range	17.2 – 42.9
Endocrine activity of the primary tumor	
Cortisol (+/- others)	49 (38.6 %)
Pure sex-hormones and precursors	9 (7.1 %)
Pure Aldosterone	2 (1.6 %)
No hypersecretion	20 (15.7 %)
Not determined	47 (37 %)
Predefined subgroup: endocrine activity (n=80)	
Hypersecretion of cortisol	49 (61.2 %)
No hypersecretion of cortisol	31 (38.8 %)
Ki67 index (%) (n=90)	
Median	10
Range	1 – 70
Ki67 index subgroups (n=90)	
≤ 10 %	46 (51.1 %)
10.1 – 20 %	22 (24.4 %)
> 20 %	22 (24.4 %)
Number of tumoral lesions at mitotane initiation	
≤ 2 lesions	28 (22.0 %)
3-4 lesions	23 (18.1 %)
5-9 lesions	21 (16.5 %)
≥ 10 lesions	55 (43.3 %)
Sum of diameter of all tumoral lesions at start mitotane (n=124)	
≤ 3 cm	19 (15.3 %)
3.1 - <10 cm	46 (37.1 %)
≥ 10 cm	59 (47.6 %)
Affected organs	
Lung only	30
Liver only	10
Local recurrence only	8
Others	14
Tumoral lesions at multiple localizations	65
Timing of mitotane initiation	
at initial diagnosis (with advanced disease)	49 (38.6%)
at early advanced recurrence (< 360 days since initial diagnosis)	22 (17.3%)
at delayed advanced recurrence (360-999 days since initial diagnosis)	33 (26%)
at delayed advanced recurrence (≥ 1000 days since initial diagnosis)	23 (18.1%)
Peak mitotane blood level during mitotane monotherapy (mg/l) (n=96)	
Median	19.6
Range	2.5 – 66.4
Peak mitotane blood level (n=96)	
< 10 mg/l	13 (13.5 %)
10-13.9 mg/l	14 (14.6 %)
14-20 mg/l	33 (34.3 %)
> 20 mg/l	36 (37.5 %)
Peak mitotane blood level after 3 months of therapy (mg/l) (n=74)	
Median	13.05
Range	2.46 – 66.40
Peak mitotane blood level after 3 months of therapy (n=74)	
< 10 mg/l	24 (32.4 %)
10-13.9 mgl/l	13 (17.6%)
14-20 mg/l	22 (29.7 %)
> 20 mg/l	15 (20.2 %)

All subgroups with less than 127 patients have been marked. Otherwise the complete cohort is included. Mitotane blood level was measured in total with 489 blood samples (Median 4 blood samples per patient, range 1-12)

Table 2. Prognostic factors on progression-free and overall survival

Prognostic factors on progression-free survival

	n	Median PFS in days	range	Univariate analysis HR	95% CI	P	Multivariate analysis* HR	95% CI	P
Sex	127								
Female	77	109	28-1518	**1**			**1**		
Male	50	181	34-2196	**0.69**	0.46-1.03	*0.07*	**0.85**	0.56-1.29	*0.45*
Age at start mitotane	127								
≤ 58 yr	63	117	28-1518	**1.09**	0.74-1.61	*0.65*			
>58 yr	64	165	32-2196	**1**					
Endocrine activity	80								
Cortisol-producing	49	102	28-1518	**1**					
Not cortisol-producing	31	122	33-964	**1.07**	0.66-1.72	*0.79*			
Ki67 index	90								
≤ 10 %	46	197	46-948	**0.63**	0.36-1.1	*0.10*	**0.80**	0.43-1.5	*0.50*
10.1 % - 20 %	22	90	28-2196	**1.06**	0.54-2.06	*0.86*	**1.07**	0.52-2.2	*0.85*
> 20 %	22	92	33-644	**1**			**1**		
Number of tumoral lesions	127								
≤ 2	28	171	35-1079	**0.57**	0.35-0.94	*0.027*	**0.53**	0.31-0.92	*0.023*
3-4	23	139	35-746	**0.52**	0.29-0.92	*0.026*	**0.48**	0.26-0.91	*0.025*
5-9	21	185	52-2196	**0.56**	0.32-0.98	*0.042*	**0.50**	0.28-0.91	*0.023*
≥ 10	55	83	28-1518	**1**			**1**		
Timing of mitotane initiation	127								
at initial diagnosis	49	92	28-644	**1**			**1**		
at early advanced recurrence (< 360 days since initial diagnosis)	22	99	52-1079	**0.56**	0.32-0.98	*0.041*	**0.65**	0.35-1.2	*0.17*
at delayed advanced recurrence (360-999 days since initial diagnosis)	33	232	32-1518	**0.40**	0.24-0.66	*<0.001*	**0.33**	0.19-0.56	*<0.001*
at delayed advanced recurrence (≥ 1000 days since initial diagnosis)	23	269	49-2196	**0.26**	0.14-0.48	*<0.001*	**0.27**	0.14-0.50	*<0.001*

Prognostic factors on overall survival

	n	Median OS in days	range	Univariate analysis HR	95% CI	P	Multivariate analysis HR	95% CI	P
Sex	127								
Female	77	553	39-6611	**1**					
Male	50	556	70-3848	**0.78**	0.52-1.18	*0.24*			
Age at start mitotane	127								
≤58 yr	63	621	39-6611	**0.88**	0.60-1.31	*0.55*			
>58 yr	64	511	40-4033	**1**					
Endocrine activity	80								
Cortisol-producing	49	555	39-3603	**1**					
Not cortisol-producing	31	531	40-6611	**0.94**	0.56-1.57	*0.82*			
Ki67 index	90								
≤10 %	46	754	72-4033	**0.36**	0.20-0.67	*0.001*	**0.52**	0.25-1.08	*0.08*
10.1 % - 20 %	22	512	70-2572	**0.75**	0.40-1.42	*0.38*	**0.77**	0.37-1.57	*0.47*
>20 %	22	374	39-2432	**1**			**1**		
Number of tumoral lesions	127								
≤ 2	28	790	81-4033	**0.63**	0.38-1.06	*0.08*	**0.55**	0.32-0.96	*0.035*
3-4	23	711	64-3820	**0.65**	0.38-1.13	*0.13*	**0.55**	0.31-0.99	*0.047*
5-9	21	553	251-2660	**0.78**	0.44-1.38	*0.39*	**0.70**	0.39-1.29	*0.26*
≥10	55	433	39-6611	**1**			**1**		
Timing of mitotane initiation	127								
at initial diagnosis	49	389	39-3848	**1**			**1**		
at early advanced recurrence (< 360 days since initial diagnosis)	22	415	64-3820	**0.69**	0.40-1.2	*0.18*	**0.78**	0.44-1.38	*0.39*
at delayed advanced recurrence (360-999 days since initial diagnosis)	33	874	59-6611	**0.38**	0.23-0.63	*<0.001*	**0.33**	0.2-0.57	*<0.001*
at delayed advanced recurrence (≥ 1000 days since initial diagnosis)	23	863	72-4033	**0.31**	0.17-0.56	*<0.001*	**0.27**	0.14-0.51	*<0.001*

Only possible prognostic factors that showed at least a trend ($p \leq 0.10$) in univariate analysis were further investigated by multivariate analysis (adjusted by age, sex, tumor burden, timing of mitotane initiation). Subgroups with less than 127 patients in total were analyzed separately (adjusted by age, sex, tumor burden, timing of mitotane initiation)

Table 3. Influence of Mitotane blood level on progression-free and overall survival

Influence of Mitotane blood level on progression-free survival

	n	Median PFS in days	range	Univariate analysis HR	95% CI	P	Multivariate analysis* HR	95% CI	P
Peak mitotane blood level	96								
<10 mg/l	13	77	34-667	**1**			**1**		
10 – 13.9 mg/l	14	146	32-509	**0.73**	0.32-1.66	*0.46*	**0.71**	0.29-1.74	*0.45*
14 – 20 mg/l	33	208	39-2196	**0.40**	0.19-0.83	*<0.05*	**0.55**	0.24-1.27	*0.16*
>20 mg/l	36	181	28-1518	**0.42**	0.20-0.85	*<0.05*	**0.49**	0.22-1.2	*0.09*
Peak mitotane blood level within 3 months	74								
<10 mg/l	24	125	32-1079	**1**					
10 – 13.9 mg/l	13	179	38-644	**0.82**	0.37-1.81	*0.62*			
14 – 20 mg/l	22	144	39-2196	**0.85**	0.45-1.64	*0.64*			
>20 mg/l	15	277	52-1518	**0.64**	0.31-1.34	*0.23*			

Influence of Mitotane blood level on overall survival

	n	Median OS in days	range	Univariate analysis HR	95% CI	P	Multivariate analysis* HR	95% CI	P
Peak mitotane blood level	96								
<10 mg/l	13	262	70-1599	**1**			**1**		
10 – 13.9 mg/l	14	502	46-2577	**0.60**	0.28-1.30	*0.2*	**0.35**	0.15-0.83	*0.017*
14 – 20 mg/l	33	814	106-3820	**0.29**	0.14-0.58	*0.001*	**0.26**	0.12-0.59	*0.001*
>20 mg/l	36	770	84-6611	**0.25**	0.12-0.51	*<0.001*	**0.18**	0.08-0.42	*<0.001*
Peak mitotane blood level within 3 months	74								
<10 mg/l	24	685	70-2660	**1**					
10 – 13.9 mg/l	13	535	46-1803	**1.52**	0.67-3.44	*0.31*			
14 – 20 mg/l	22	857	90-2422	**0.83**	0.41-1.67	*0.60*			
>20 mg/l	15	679	84-6611	**0.62**	0.28-1.38	*0.24*			

PFS: progression-free survival; OS: overall survival. Only possible prognostic factors that showed at least a trend ($p \leq 0.10$) in univariate analysis were further investigated by multivariate analysis (adjusted by age, sex, tumor burden, timing of mitotane initiation)

Table 4. Response to therapy in different subgroups

	CR	PR	CR+PR	SD	Benefit>180d	PD
Timing of mitotane initiation						
At initial diagnosis or at early recurrence (< 360 d since initial diagnosis)	0	7 (9.9%)	7 (9.9%)	17 (24%)	16 (22.5%)	47 (66.2%)
At delayed advanced recurrence (≥ 360 d since initial diagnosis)	3 (5.3 %)	16 (28.6%)	19 (33%)	15 (26.7%)	34 (60.7%)	22 (39.3%)
Number of tumoral lesions						
<10	2 (2.7%)	6 (8.3%)	8 (15.7%)	22 (30.5%)	36 (70.6%)	21 (41.2%)
≥10	1 (1.8%)	17 (30.9%)	18 (23.7%)	10 (18.1%)	14 (18.4%)	48 (63.2%)
Ki67 (n=90)						
≤ 10 %	1 (2.2%)	11 (23.9%)	12 (26%)	15 (32.6%)	25 (54.3%)	19 (41.3 %)
10.1 % - 20 %	0	4 (18.1%)	4 (18.1%)	4 (18.1%)	2 (8.7%)	15 (65.2 %)
> 20 %	0	3 (13.6%)	3 (13.6%)	3 (13.6%)	5 (23.8%)	15 (71.4 %)
Endocrine activity (Cortisol, n=80)						
Yes	1 (2%)	8 (16.3%)	9 (18.4%)	6 (12.2%)	16 (32.6%)	16 (51.6%)
No	0	10 (32.2%)	10 (32.2%)	10 (32.2%)	13 (41.9%)	29 (59.0%)
Cohort who fulfilled the following criteria:						
At initial diagnosis or at early recurrence (<360 d since initial diagnosis) + ≥10 tumoral lesions (n=32)	0	0	0	3 (9.3%)	2 (6.3%)	29 (90.6%)
At delayed advanced recurrence (≥ 360 d since initial diagnosis) + <10 tumoral lesions (n=33)	2 (6%)	8 (24.2%)	10 (30.3%)	11 (33.3%)	22 (66.7%)	12 (36.4%)

CR=complete response, PR=partial response, SD=stable disease, PD=progressive disease, Benefit = SD, PR or CR for >180 days

Figure 1

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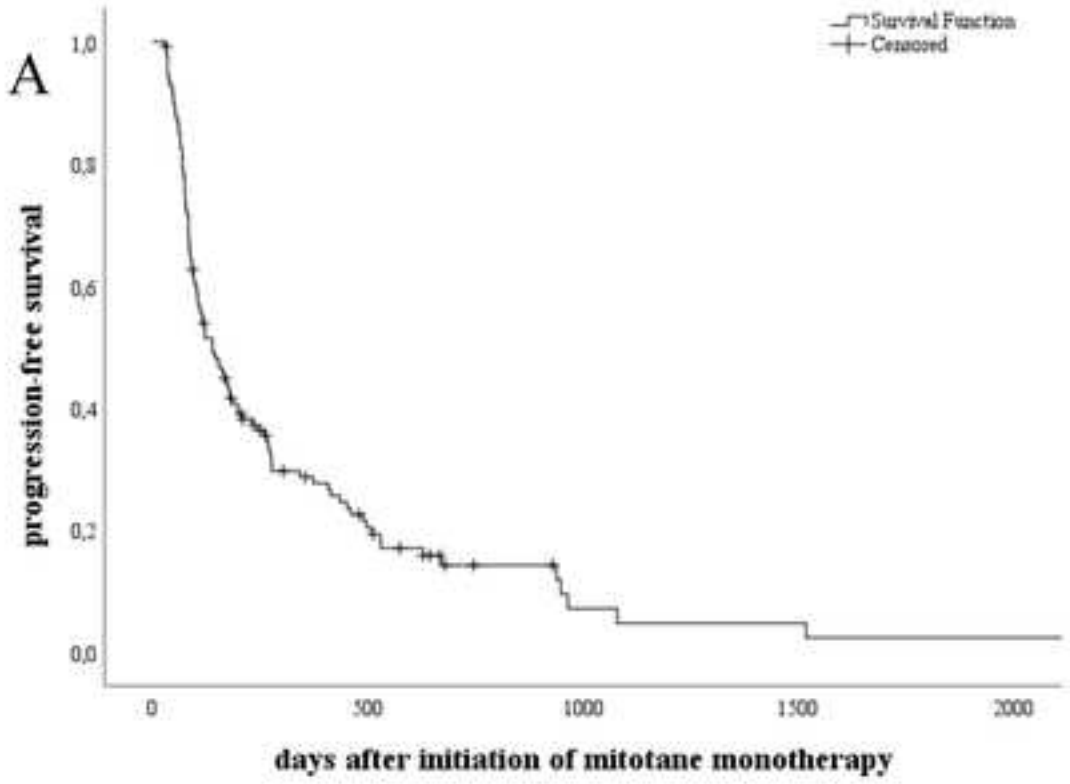

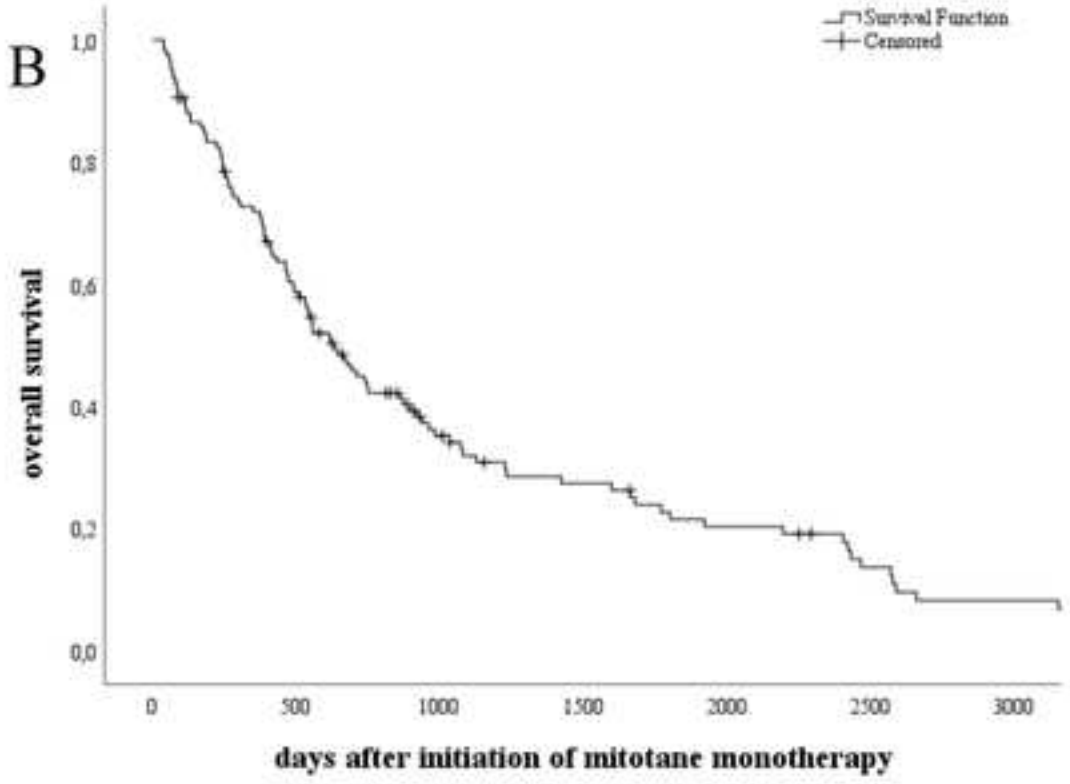

Figure 2