Journal of Endocrinological Investigation
https://doi.org/10.1007/s40618-024-02439-7

ORIGINAL ARTICLE

Prognostic value of total, free and lipoprotein fraction-bound plasma mitotane levels in advanced adrenocortical carcinoma: a prospective study of the ENDOCAN-COMETE-Cancer network

M. Faron[1,2] · A. Naman[3] · J. Delahousse[4] · S. Hescot[5] · J. Hadoux[3] · F. Castinetti[6] · D. Drui[7] · P. Renoult-Pierre[8] · R. Libe[9] · L. Lamartina[3] · S. Leboulleux[3] · A. Al-Ghuzlan[10] · M. Lombès[11] · A. Paci[4] · E. Baudin[3] · For Endocan-Comete Network

Received: 26 March 2024 / Accepted: 3 August 2024

Abstract

Purpose Mitotane is the only approved treatment for metastatic adrenocortical carcinoma (ACC). Monitoring plasma levels is recommended, but its predictive value is insufficient.

Methods This prospective study of the French ENDOCAN-COMETE network aimed to investigate the prognostic role of plasma mitotane levels pharmacokinetics and free or bound to lipoprotein fraction measurements during six consecutive months. Lipoprotein fractions were isolated by ultracentrifugation, and mitotane level was determined by HPLC–UV. Total, free, and lipoprotein fraction bound plasma mitotane were monitored every two months for six months with morphological assessment. The primary endpoint was overall survival (OS).

Results 21 patients with metastatic ACC were included. Median overall survival was 23 months. The median free mitotane level per patient was 12% (±7%), and the majority (88%) was bound to lipoprotein fractions. Several pharmacokinetics measures of total mitotane were related to OS: first level at one month ($p=0.026$), mean level ($p=0.055$), and area under the curve (AUC) ($p=0.048$), with higher exposure associated to longer OS. Free mitotane (not bounded) and mitotane bounded to lipoprotein subfraction added no prognostic values. The relationship between the mitotane level and OS suggested a minimum "effective" threshold of 10–15 mg/L or an area under the curve above 100 mg/L/month with no individualized maximum value.

Conclusion This prospective study did not identify any added prognostic value of free mitotane level over the total level. Early total mitotane level measurements (before 3–6 months) were related to OS with a higher and faster exposure related to more prolonged survival.

Keywords Adrenocortical carcinoma · Mitotane · Pharmacokinetics · Lipoprotein

Introduction

Adrenocortical Carcinoma (ACC) is a rare tumor with a poor prognosis, as highlighted by a five year-survival rate below 15% for metastatic ACC [1]. In the context of metastatic or locally non-resectable ACC, mitotane (Op'DDD) remains the standard of care, whether used alone or in combination [2]. Despite ongoing exploration into new therapeutic options, including targeted therapies, results have been largely disappointing. Furthermore, the potential role of immunotherapy is still under investigation [2, 3]. Mitotane remains the only approved agent for treating ACC to date [4]. Mitotane exerts both anti-secretory and anti-tumor actions providing 11–33% partial response rates but no robust demonstration of its benefit on overall survival has been established [5–9].

Recent studies have proposed that proteins related to mitochondrial and/or endoplasmic reticulum functions could serve as critical targets [10, 11]. Considering the debatable data on efficacy and the poor tolerance, the investigation for predictors of responses to mitotane is a pivotal issue. International guidelines for ACC management recommend monitoring and targeting a therapeutic window range of plasma mitotane between 14 and 20 mg/L

M. Faron and A. Naman have contributed equally to this paper.

Extended author information available on the last page of the article

Published online: 22 August 2024

[4]. Indeed, the relationship between total plasma mitotane peak above 14 mg/L and tumor response and/or survival have been reported in several studies both in the adjuvant or palliative setting [5, 6, 8]. In addition, the 20 mg/L upper threshold level has been established based on a higher rate of adverse events, especially neurotoxicity [5, 6, 12–16]. Recent investigations underline the importance of maintaining the target range over time [13, 14, 17], proposing an expanded therapeutic mitotane level window between 10 and 30 mg/L, suggesting enhanced efficacy in patients with smaller tumor sizes [8]. However, significant criticisms remain on the potential prognostic value of plasma mitotane due to the retrospective nature of almost of the available studies and survival bias resulting from the delayed peak of plasma mitotane following initiation. One recent study suggests the prognostic role of early plasma mitotane level measured before 3 months [8]. Finally, taking into account three prospective and one retrospective studies, the estimated positive predictive value of the 14 mg/L cutoff was only 45% [5–7, 12].

Mitotane exhibits high lipophilicity; consequently, the majority is bound to lipoprotein fractions while a minor portion remains free or bound to albumin [18, 19]. Recent preclinical studies reported improved efficacy in terms of response/survival of free mitotane (associated with albumin) [20, 21]. Based on these findings, we hypothesized that evaluating free plasma mitotane concentrations or bound to lipoprotein fractions could potentially predict tumor response more accurately.

A prospective multicenter study (MITOLIPO study) was conducted within the French Endocan-Comete network which main objective was to evaluate the prognostic value of plasma mitotane levels bound to lipoprotein fractions during six consecutive months (MITOLIPO period) as compared to total plasma mitotane level.

Patients and methods

Inclusion criteria

Patients treated for metastatic ACC in one of four French specialized centers within the ENDOCAN COMETE- network (Villejuif, Marseille, Tours, Nantes) were prospectively enrolled between March 2014 and July 2017. Inclusion criteria were: histologically confirmed ACC, ENS@T stage III or IV [2] not amenable to complete resection (i.e. inoperable primary lesion without metastasis or inoperable metastases), measurable disease according to RECIST 1.1 criteria, and mitotane initiation within the previous year with at least two plasma mitotane measurements available.

Mitotane therapy

Mitotane was given using a regimen specific to each center/physician. According to international guidelines, a common aim was to target a plasma mitotane between 14 to 20 mg/mL. According to guidelines, the first measurement of total plasma mitotane was performed one month after the beginning of the treatment and then every two months, with a clinical examination. Dose adjustments were based on total plasma level and adverse events monitoring at each visit. Overnight fasting routine biochemical tests and imaging work-up (neck-thorax-abdomen-pelvis CT-scan) were also performed (recommended every two to three months).

Additional therapies (cytotoxic chemotherapy, locoregional therapy, steroidogenic inhibitors, and lipid-lowering drugs) were allowed based on local and national multidisciplinary meeting decisions. Adverse events were graded according to the Common Toxicity Criteria of the National Cancer Institute version 5.

Study design

MITOLIPO is a prospective, observational study. Given the rarity of this disease, patients were incluable in this study, either at the time of mitotane initiation or within the first year of mitotane initiation. During the study period of 6 months (March 2014 to July 2017), four additional measurements every two months (besides the total plasmatic mitotane level, e.g. relation to lipoproteins subfraction) were performed but results were not disclosed to the physician and thus had no impact on the therapeutic decisions (observational study only). At the end of the study period, patients were followed up according to local practices. MITOLIPO is an exploratory study; thus, no sample size was calculated a priori.

For patients who had the treatment initiated during the study period, the first measurement of total plasma mitotane was performed one month after the beginning of the treatment; during the MITOLIPO period (March 2014 to July 2017), 4 consecutive measurements were required (every two months) for all patients. For patients who had the treatment initiated prior to the study period, total plasmatic mitotane levels, including early plasma level at one month were retrospectively collected.

The local Ethics committee approved the study. Written informed consent was collected for each patient before

inclusion. This study complies with French law (Jarde type 3) for an observational study.

Measurement of total plasma and lipoprotein-bound mitotane level

All samples were centrally processed. Total plasma mitotane level was determined by high-performance liquid chromatography at Atlanbio laboratory (Saint Nazaire, France) using the Lysosafe service offered by HRA Pharma (Paris, France).

Analyses of plasma mitotane within the lipoprotein were determined by HPLC combined with HPLC–UV detection as previously described [22] and conducted by the Pharmacology and Drug Analysis Department at Gustave Roussy.

Density gradient ultracentrifugation using iodixanol (OptiprepTM, Sigma-Aldrich) was used to isolate lipoprotein fractions in plasma samples. A saline solution with HEPES buffer was added to a mixed solution of 60% (m/v) iodixanol in water (d = 1.32 g/mL) and plasma in Optiseal Polyallimer Centrifuge tubes (Beckman Coulter). This final solution was ultracentrifuged at 350 000*g* at 16°C (60°F) for 3.5 h. After ultracentrifugation, each lipoprotein fraction corresponding to LDL subfractions (density = 1.063 g/mL) and HDL subfractions (density = 1.063–1.179 g/mL), VLDL subfractions (density < 1.017 g/mL) and free mitotane or bound to albumin, was collected with syringe and needle systems.

Endpoints

The primary endpoint was the relationship between mitotane pharmacokinetics and distribution among lipoproteins and overall survival (OS) calculated from mitotane initiation until death or last follow-up. Secondary endpoints were disease control rate (DCR), as defined by the complete response, partial response, or stable disease, at 6 and 12 months after mitotane initiation and best objective responses (complete response and partial response) according to the RECIST 1.1 criteria [23].

Statistical analysis

Considered "predictors" were (i) early total plasma mitotane, (ii) standard parameters describing its kinetic, and (iii) repartition among the lipoproteins.

The first mitotane plasma level was the first measurement performed one month ± 7 days after mitotane initiation, whether during the MITOLIPO period or in the year before. The mean mitotane level was defined as the mean of all total mitotane plasma levels available between the initiation of mitotane and the end of the observation period. The dose intensity was defined as the total mitotane value at each time point multiplied by the time in days spent at this level and is, therefore, equivalent to the area under the curve (AUC). The unit chosen was mg/L*month, with a higher value indicating a higher exposure to mitotane (longer duration and/or higher values). The mitotane growth rate was calculated by dividing the total mitotane plasma level obtained at the second measurement after mitotane initiation by the number of days between the treatment initiation and the 2nd measurement. Therefore, its unit is mg/L/month. A higher value indicated a steeper increase in blood mitotane levels. Supplementary Fig. 1 illustrates this measures.

Quantitative variables were presented as median (interquartile range) and compared by Wilcoxon test. Qualitative variables were presented as count (percentage) and compared by the Chi2 test or Fisher's exact test as appropriate.

Survival curves were calculated using the Kaplan–Meier method and compared with the Log-Rank test for qualitative variables. A single-variable Cox Proportional Hazard model was used for quantitative variables to study the relationship between the (log) hazard and the variable. For the disease control rate, a binary logistic regression was used. To respect the log-linearity assumptions of all these models, continuous variables were coded by polynomials when necessary.

For each continuous variable, at the first step, the variable was entered in its linear form. In the second step, choosing the best functional form was based on Akaike Information Criterion (AIC) to identify nonlinear relationships [24]. No multivariable analysis was performed due to the low number of cases.

A sensitivity analysis was performed after excluding patients who started mitotane before study initiation.

All tests are bilateral. A value of $p < 0.1$ was chosen to define statistical significance due to a small observational study of a rare disease [25]. *P*-values were not adjusted to account for multiple testing. All analyses were performed using R 3.5 software (The R Core Team, Vienna, Austria).

Results

Characteristics of the included population

From March 2014 to July 2017, 30 patients were enrolled in the study. Nine patients were excluded due to premature death before the first MITOLIPO measurement (4 patients), absence of available blood samples for centralized assessment (4 patients), and poor compliance (1 patient). Finally, data from the remaining 21 patients were analyzed in this study (Fig. 1).

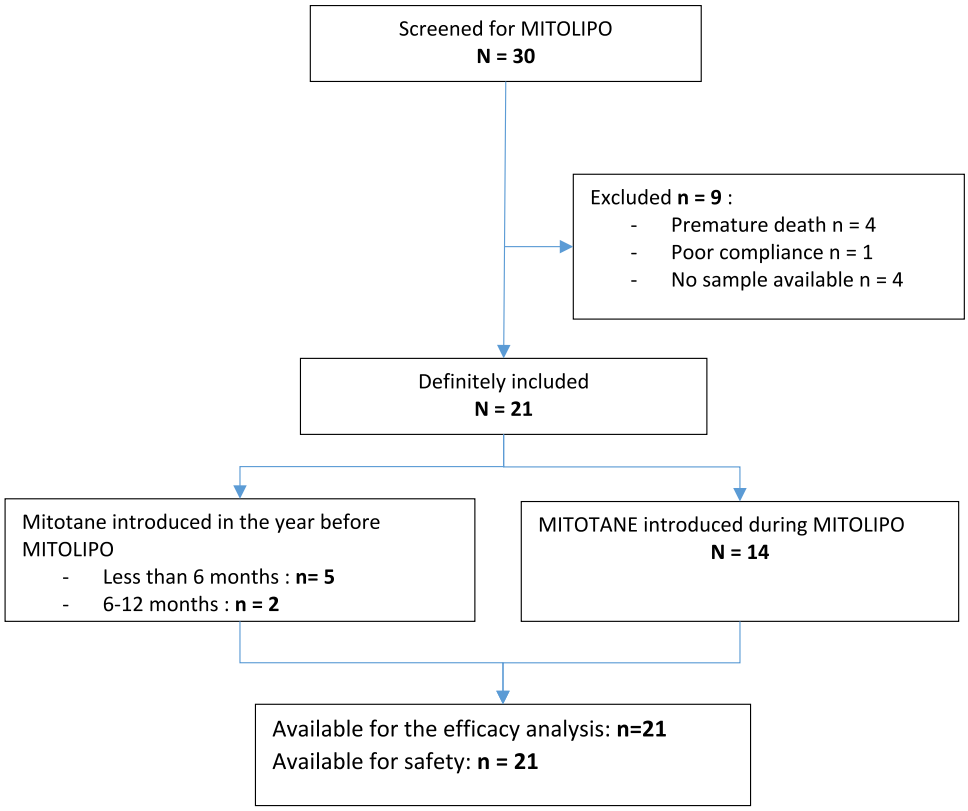

Fig. 1 Flow chart of the MITOLIPO study

Patients

Table 1 describes the patient population: a slight predominance of woman gender was found (57%), and the median age was 52 years [IQR 43–54]; ENSAT stage was stage IV in all patients, and a median Ki-67 index of 40% (10–80) was observed. Most patients (86%) had hormone-related symptoms.

Treatment with mitotane and other therapies

The median time between initial diagnosis and mitotane initiation was one month (0–9 months). The median time between mitotane initiation and the first MITOLIPO measurement was two months (0–11 months) and within the first three months in 67% of the cases (14 patients) (supplementary Figs. 2 and 3). Five patients had first dosage before the MITOLIPO period.

The median dose of mitotane at treatment initiation and first MITOLIPO measurement were 4 (1.5–6.5) grams and 3 (1.5–7) grams per day, respectively.

Sixteen patients (76%) received Cisplatin-Etoposide combined with mitotane as the first-line treatment during the MITOLIPO study (Table 1). Patients receiving this concomitant treatments tend to have lower mitotane measurements without reaching statistical significance (supplementary Figs. 4 and 5).

Only one patient had a statin at baseline, and three patients started this treatment during the study period (two at first dosage and one at third dosage).

Distribution of mitotane among lipoproteins

A median of 4 (2–8) plasma mitotane levels and 3 (2–4) plasma mitotane levels within lipoproteins were measured per patient.

Over all the measurements, most of the circulating mitotane was bound to LDL with a median of 41.1% (IQR 32.7–42.5), then HDL 21.3% (14.8–13.3), then VLDL mitotane 17.5% (10.6–22.8) and finally free 12.3% (6.04–16.9) (Note that these medians of individual measurements do not necessarily sum to 100%) (Fig. 2). Little inter- and intra-patient variations were seen (Supplementary Fig. 4).

Primary endpoint

After a median follow-up of 38 (95% CI 32–Not reached) months, 17 events of OS were observed. Median overall survival was 23 months (95% CI 4–61). OS at 12 months was 71% (54–93%) (Fig. 3).

Table 1 Characteristics of the included patients

	N = 21
Sex	
Male	9 (43%)
Female	12 (57%)
Median age in years (IQR)	52(43–54)
Median baseline body mass index, kg/m^2(IQR)	26.2(22.9–27.9)
Secreting tumor	18 (86%)
Cortisol	12 (57%)
Precursors	8 (38%)
Androgen	1 (4.7%)
R status of the primary surgery	
R0	11(52%)
RX	4 (20%)
R1	3(14%)
No surgery	3(14%)
Weiss Score >6	17 (81%)
Ki67 (%) (range)	40(10–80)
ENSAT Stage IV at T0	21 (100%)
Metastatic status	
Synchronous	13(62%)
Metachronous	8 (38%)
Median time (month (range))	8 (3–25)
Metastatic site	
Lung	11 (52%)
Liver	14 (66%)
Bones	2 (10%)
Peritoneal carcinomatosis	3(14%)
Associated treatment during the Mitolipo study	
Cisplatin-Etoposide	16 (76%)
Others chemotherapies	10 (48%)
Tyrosine Kinase Inhibitors	1 (4.7%)
Local therapies	10 (48%)

Numbers are n(%) unless otherwise stated

ENS@T European Network for the Study of Adrenal Tumours

Relationship with mitotane pharmacokinetics

In the models with variables in the linear form, we observed that higher total mitotane level at first measurement ($p = 0.026$), mean total mitotane level ($p = 0.055$), and the area under the curve ($p = 0.048$), were associated with better overall survival (Table 2) whereas the speed of rise was not ($p = 0.17$).

In the second step, non-linear relationships were found better to represent the underlying relation for most of these parameters. Figure 4 illustrates the best functional form selected for each variable. For instance, for the first total plasma mitotane level the inflection point near 9–15 mg/L suggested that a minimum level of total plasma mitotane should be reached to expect an effect (around 10 mg/L of plasma mitotane level) and the absence of a plateau for higher value suggested that the maximum dose according to patient's tolerance should be sought (Supplementary Fig. 7). Similar results were observed for the mean mitotane level. For the total mitotane's AUC was best represented as a quadratic function ($p = 0.059$) with an inflection point near 100 mg/L*month suggesting that at least 10 mg/L for 10 months or 14 mg/L for 7.1 months) should be maintained to get an impact on overall survival.

Speed of rise during the first two measurements of plasma mitotane was best represented as a linear function ($p = 0.068$) with a negative sign suggesting that the more the rise of mitotane level is high, the more significant benefit on survival was seen.

Relationship with mitotane repartition in lipoprotein subfraction

The model only identified that a higher LDL-mitotane area under the curve ($p = 0.047$) was associated with better overall survival (Table 2). The linear relation was the best functional form. The mitotane measurements in other subfractions were not statistically associated with OS (Table 2).

Secondary endpoints

The disease control rate (DCR) at six months was 52.4% (11 out of 21 patients) (Supplementary Table 1). A positive association was found between disease control rate at six months and the first total plasma mitotane level (5.5 mg/L *vs.* 9 mg/L $p = 0.19$), total plasma mitotane AUC (38 mg/L*month *vs.* 130 mg/L*month $p = 0.16$), HDL mitotane AUC (7 mg/L *vs.* 19 mg/L $p = 0.065$) and LDL mitotane AUC (13 mg/L *vs* 40 mg/L $p = 0.1$) with Chi 2 test. There were only two patients with a disease controlled at 12 months, therefore no statistical analysis was performed for this endpoint.

For the best response analysis, according to RECIST 1.1, there was no complete response, partial response was seen in three patients (14%), stable disease in 16 patients (76%), and progression in 2 patients (10%). Due to the low number of partial responses, the predictive analysis was not performed.

Safety

During the study, adverse events were mainly grade I and II (Table 3). Neurological toxicity was present in all patients but one patient with mitotane plasma level > 20 mg/L (4 patients). Grade III and IV toxicities were observed in 14

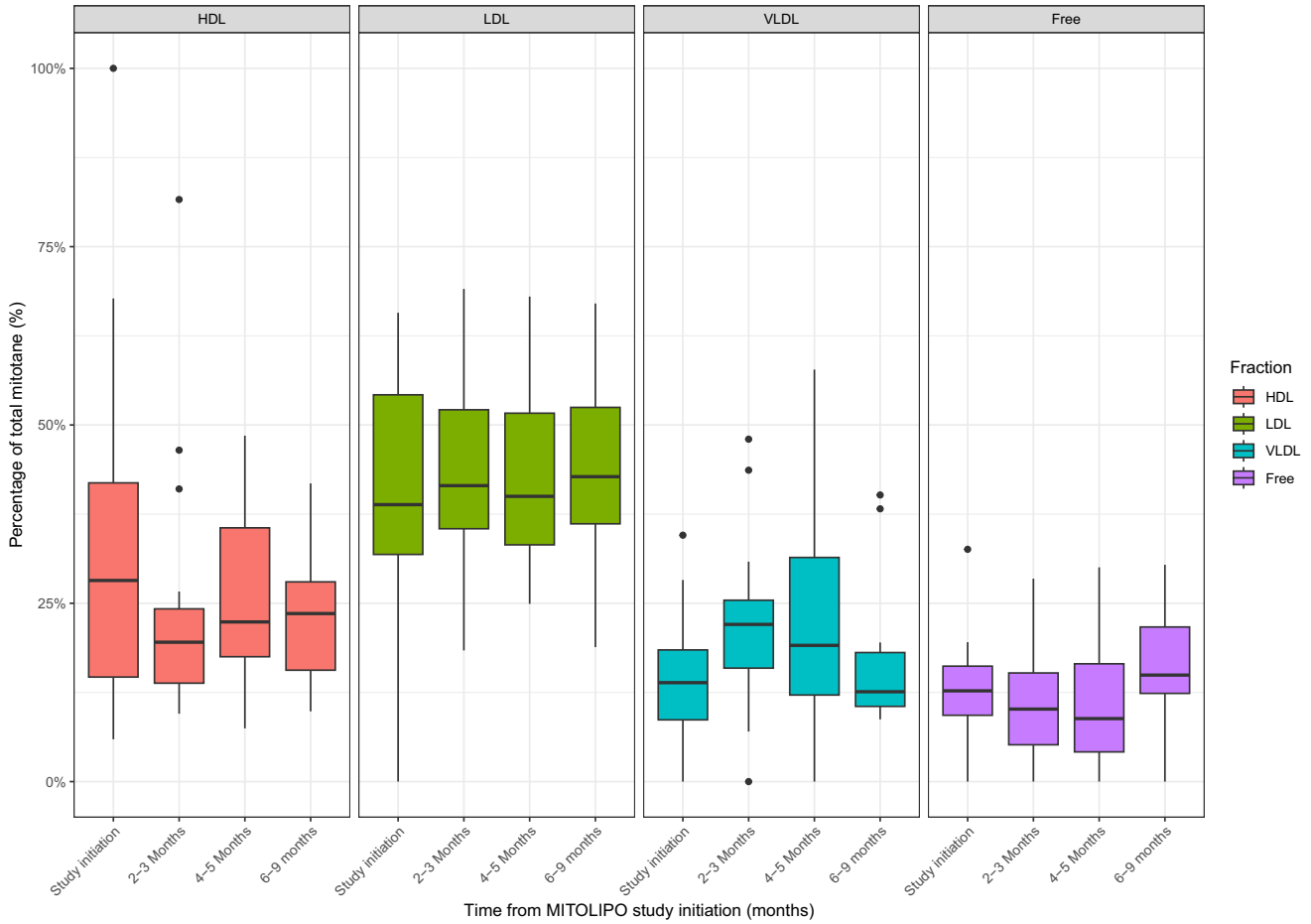

Fig. 2 Mitotane repartition across lipoprotein in 21 patients. Values are expressed as the percentage of total mitotane. *HDL* High Density Lipoprotein, *LDL* Low Density Lipoprotein, *VLDL* Very Low Density Lipoprotein, *Free* mitotane not bounded to lipoprotein

(67%) patients and required dose adjustment. One patient had anemia requiring a blood transfusion. Two patients (9.5%) discontinued mitotane treatment for intolerance: persisting asthenia grade II or neurological event grade II. All adverse events were consistent with those described in the literature and considered related to the treatment.

Discussion

Monitoring total mitotane concentrations is recommended by international guidelines due to their prognostic and safety relevance [4]. However, in real-life practice, even if early measurements are performed the peak mitotane level is the most used measurement to guide mitotane therapy, and this information becomes available only late after mitotane initiation. Therefore, it could be considered a consequence of prolonged survival rather than a prognostic marker, and there is an urgent need for earlier and more reliable biomarkers. Also, while it is known that mitotane is bound to lipoproteins, the exact mechanism, and therefore which fraction of mitotane is effective, remains unknown. This study is one of the few prospective studies on mitotane administration that evaluated the relationship between survival and two new markers: early mitotane pharmacokinetics and distribution of mitotane among lipoproteins.

The first total plasma mitotane level measured at one month from treatment initiation was found to be associated with survival, along with total exposure as measured by the AUC and the speed of rise showed a trend toward significance. From a clinical point of view, these prospective data suggest that early and prolonged exposure to a high mitotane level may have a favourable impact, as seen in previous series [8, 13, 17]. Also, the curvilinear form of

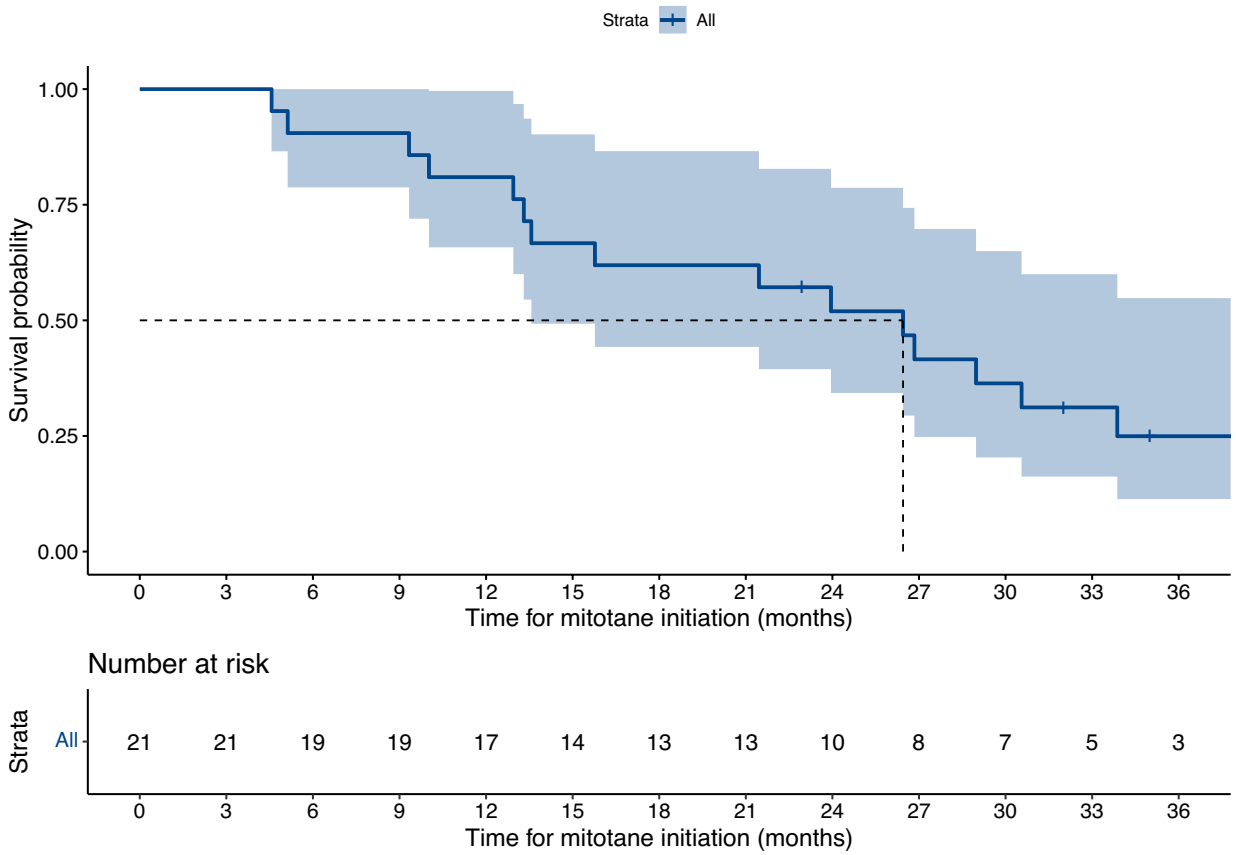

Fig. 3 Overall survival since mitotane introduction. Time unit in months

the relationship suggests that there was little to no effect until a certain point then, after which higher levels were associated with greater efficacy without reaching a plateau. These exploratory findings should be confirmed in future studies.

Only three old prospective studies have been conducted so far to analyze the antitumor activity of mitotane monotherapy in 13–36 patients with metastatic ACC, including measuring plasma mitotane levels in one of them [6, 26, 27]. Partial response rates ranged from 13 to 33%, and a correlation between total plasma mitotane peak level and tumor response was found in only one study [28]. In the palliative setting, five studies had reported a higher rate of response rate (ranging from 28 to 55%) in patients who had a peak of total plasma mitotane > 14 mg/L as compared to 0–15% when this peak was not reached [5–8, 29], suggesting that the 14 mg/L cutoff should be seen as a compromise rather than a strict cutoff to be achieved in all patients. More precisely, tumor response were observed in patients with plasma mitotane levels of at least 10 mg/L up to >20 mg/L when tolerance allowed to reach such levels [7]. In addition, progressive increased in median progression free survival were reported when subcategories of plasma mitotane levels of 10–14, 14–20 or >20 mg/L were considered suggesting again a progressive continuous antitumor impact of Mitotane. Impact on overall survival of the peak >14 mg/L was observed in most [5, 7, 8, 14, 30], but not all, studies [31, 32]. In the adjuvant setting, the benefit of reaching a peak above 14 mg/L on recurrence-free survival has been reported [13, 30, 33], and some impact on overall survival was found [34]. Importantly, the peak of plasma mitotane level has been reported to occur after 80 days to several months and the >14 mg/L level after 46 days or several months after mitotane initiation [35], emphasizing the need to also look at earlier marker of efficacy. Current guidelines recommend to closely monitor mitotane by frequent measurements.

In this prospective study, for the first time in advanced ACC patients, we also analyzed whether mitotane antitumor was related to its repartition among lipoproteins. Indeed, as previously described, the lipophilic nature of mitotane

Table 2 Effect of total mitotane kinetics and distribution in lipoprotein subfraction on overall survival

Variable	HR [95% CI]	*P value*
Total mitotane pharmacokinetics		
First plasma mitotane level	0.92 [0.85–0.99]	0.026
Mean mitotane	0.93 [0.86–1.00]	0.055
Mitotane AUC	0.99 [0.99–1]	0.048
Speed of rise	0.92 [0.82–1.04]	0.17
Distribution in subfraction		
Free mitotane		
Mean	0.83 [0.61–1.12]	0.21
AUC	0.97 [0.92–1.02]	0.21
HDL-bounded mitotane		
Mean	0.87 [0.65–1.17]	0.36
AUC	0.97 [0.93–1.02]	0.2
LDL-bounded mitotane		
Mean	0.91 [0.80–1.05]	0.18
AUC	0.98 [0.95–1]	0.037
VLDL-bounded mitotane		
Mean	0.99 [0.78–1.26]	0.94
AUC	0.99 [0.95–1.03]	0.53

HR Hazard ratio, *CI* confidence interval, *AUC* Area Under the Curve, *HDL* High Density Lipoprotein, *LDL* Low Density Lipoprotein, *VLDL* Very Low Density Lipoprotein, *Free* mitotane not bounded to lipoprotein

For these continuous variables, HRs are for a variation of one unit of the variable

explains its high affinity for lipoproteins which warrants further exploration. However, we were unable to find a specific lipoprotein-bound mitotane measurement that was superior to the total count. Free plasma mitotane levels were not associated with survival, while mitotane within LDL fractions was, probably, as they better correlate with the total plasma mitotane level. As a matter of fact, we observed minor variations in the partitioning of plasma mitotane into lipoprotein fractions between patients and over time. This result suggests that even if mitotane increases LDL, HDL cholesterol, or triglyceride levels, the partition of mitotane within each lipoprotein fraction remains stable. These data suggest that the total mitotane levels should remain the standard.

Various alternative biomarkers have been investigated to improve the predictive value of plasma mitotane measurements. These biomarkers include Mitotane metabolites (op'DDA, op'DDE), the study of different polymorphism of cytochrome P450, or gene expression of ribonucleotide large subunit 1 (RRM1), but none has been validated so far [7, 36, 37]. Two recent preclinical studies suggested that free plasma mitotane level could be the active antitumor compound [20, 21]. Yet, our data demonstrated no prognostic role of free plasma mitotane measurements.

We prospectively confirmed that, as previously suggested, maintenance of high plasma mitotane levels over time is critical for patient outcome and not only one high total plasma measurement [13, 14, 17, 37]. More in detail, the models suggested that (i) a minimum level of total plasma mitotane (estimated around 10 mg/L but subject to variability) should be reached to expect an impact on survival and (ii) higher levels (even above 20 mg/L if the patient can tolerate) were associated to higher benefits. Concerning the kinetics, our data suggest that besides the mean level, mitotane levels should rise fast (as seen by the first level >10–15 mg/L and speed of rise importance) and be maintained at a high level during a long period (as seen with the AUC >100 mg/L/day) to expect the greater impact on survival. Thus, an early adaptation of mitotane concentrations in treated patients is probably advisable. Based on AUC results, our results suggest that plasma Mitotane levels of 14 or 10 mg/L should be maintained for 7.1 or 10 months to expect a survival impact, respectively. These figures provide pragmatic markers expected to guide clinicians in managing this drug with unfavorable pharmacokinetic properties.

We acknowledge that our study has several limitations. Our cohort was relatively small due to the rarity of the disease; thus, the cut-off should be interpreted with caution and need to be validated by future studies. In addition, as experienced in real life, not all patients received mitotane only, and other treatments may have added confounding biases. In this study patients receiving concomitant chemotherapy tend to have lower levels but without reaching statistical significance in this restricted sample. Lastly, a survival bias cannot be excluded as, because of the rarity of the disease, not all patients were included at treatment initiation in order to facilitate enrollment of patients. Nevertheless, the first measurements were performed in 71% of patients before three months from mitotane initiation and 91% within the first six months and all patients had first plasma mitotane levels measured at one month from Mitotane initiation. Sensitivity analysis excluding patients with mitotane initiation more than six months before inclusion in MITOLIPO was consistent with the principal analysis (supplementary Table 2). Because of mitotane therapy's well-known delayed antitumor action and uncertainties regarding the progression-free survival information, we choose overall survival

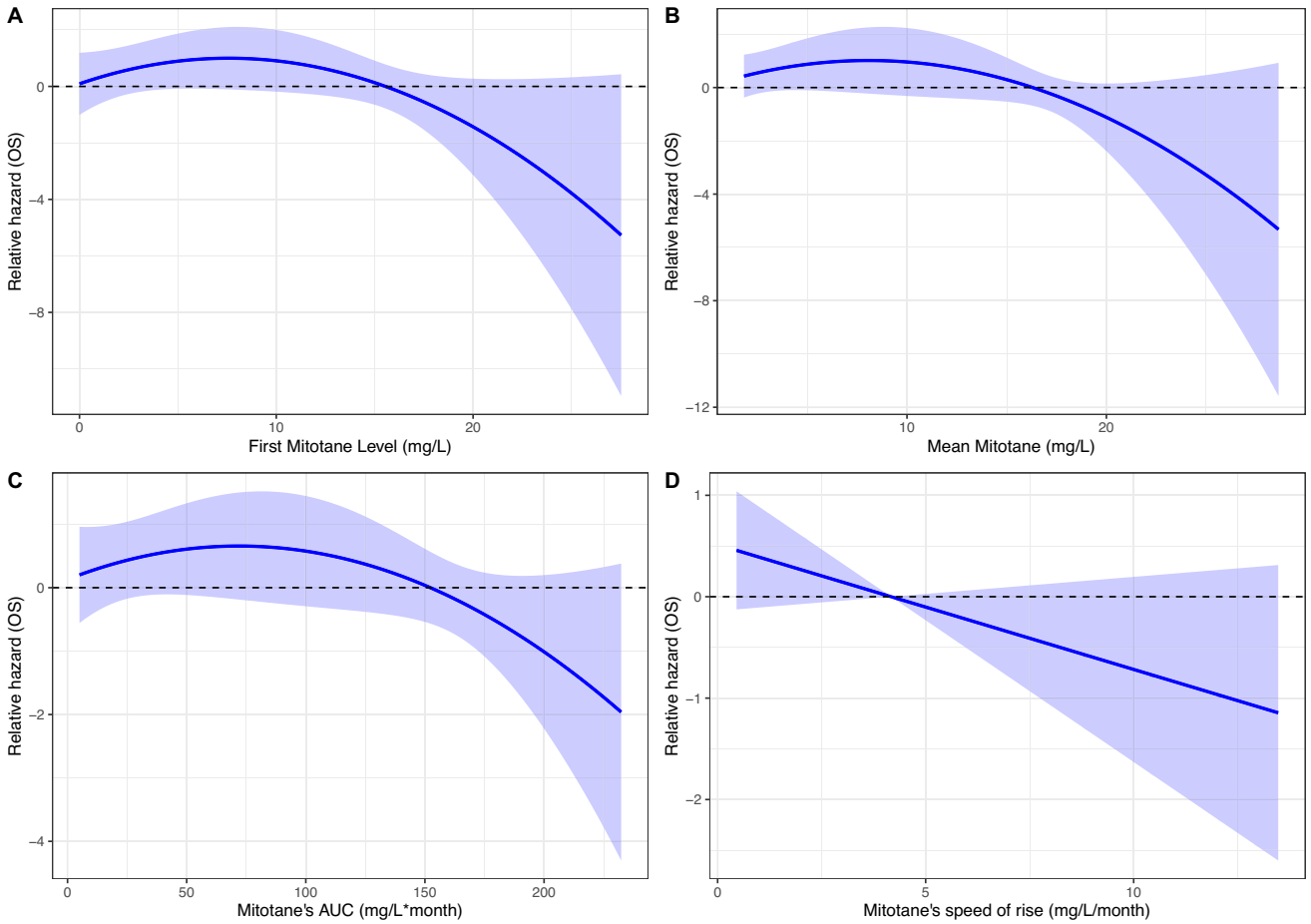

Fig. 4 Variation in the relative Hazard for death and **A** First mitotane measurement, **B** Mean mitotane level, **C** Mitotane's Area under the Curve, and **D** Speed of rise, according to the Cox model. The dotted line represents the average risk in the population, negative values below this line indicate a lower hazard for death. The blue line is the relation, and the shaded line is its 95% confidence interval

Table 3 Adverse events related to mitotane during the study period

	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal: nausea, vomiting, anorexia, diarrhea	*4* (19%)			
Central nervous system	*5* (24%)	*1 (4.7%)*		
Fatigue	*8* (38%)	*1 (4.7%)*		
Hyperlipidemia	*12* (57%)	*4* (19%)	*1* (4.7%)	*1* (4.7%)
Hypercholesterolemia	*11* (52%)	*4* (19%)		*1* (4.7%)
Elevated liver enzymes	*4* (19%)	*5* (24%)	*8* (38%)	*2* (9.5%)
Anemia			*1* (4.7%)	

Grade according to CTCAE v5

as the primary endpoint, even though it may not be a perfect outcome. Finally, our results only applies to patients with advanced disease, most of them being treated with concurrent chemotherapy.

In conclusion, in this prospective study, the measurement of free mitotane did not add prognostic value over total mitotane. Our data shows a prognostic value of early measurement of plasma mitotane.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40618-024-02439-7.

Acknowledgements The authors acknowledge Atmane Seck (laboratory technician of the pharmacology department of Gustave Roussy) and HRA Pharma (Paris, France) for providing access to Atlanbio laboratory (Saint Nazaire, France) using the Lysosafe service and a MITOLIPO grant.

Funding "The authors declare that no funds, grants, or other support were received during the preparation of this manuscript."

Data availability The data on which this study is based can be accessed on request from the corresponding author (MF). The data are not publicly available because they contains information that could potentially compromise the privacy of the patients.

Declaration

Conflict of interest EB and RL: received honoraria from HRA Pharma.
MF: received honoraria from VIFOR Pharma and travel grant from Novartis.
LL: received honoraria from BAYER, EISAI, LILLY, IPSEN and ROCHE and travel grant from AAA Novartis.
JH: received honoraria from BIARD.

Ethics approval The local Ethics committee approved the study. This study complies with French law (Jarde type 3) for an observational study.

Consent to participate Written informed consent was collected for each patient before inclusion.

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Authors and Affiliations

M. Faron[1,2] · A. Naman[3] · J. Delahousse[4] · S. Hescot[5] · J. Hadoux[3] · F. Castinetti[6] · D. Drui[7] · P. Renoult-Pierre[8] · R. Libe[9] · L. Lamartina[3] · S. Leboulleux[3] · A. Al-Ghuzlan[10] · M. Lombès[11] · A. Paci[4] · E. Baudin[3] · For Endocan-Comete Network

✉ M. Faron
matthieu.faron@gustaveroussy.fr

1 Department of Surgical Oncology, Institut Gustave Roussy, 114 Rue Edouard Vaillant, 94800 Villejuif, France

2 INSERM 1018 CESP ONCOSTAT Team, Institut Gustave Roussy, 114 Rue Edouard Vaillant, 94800 Villejuif, France

3 Nuclear Medicine and Endocrine Unit, Institut Gustave Roussy, 114 Rue Edouard Vaillant, 94800 Villejuif, France

4 Pharmacology Department, Gustave Roussy, 114 Rue Edouard Vaillant, 94805 Villejuif, France

5 Nuclear Medicine Unit, Institut Curie, 35 Rue Dailly, 92210 Saint Cloud, France

6 Department of Endocrinology, Assistance Publique-Hôpitaux de Marseille (AP-HM), Hôpital de La Conception, 147 Boulevard Baille, 13005 Marseille, France

7 Department of Endocrinology, L'institut du Thorax, CHU Nantes, Bd J Monod Saint Herblain, 44093 Nantes Cedex 1, France

8 CHRU de Tours Hopital Bretonneau, 2 Boulevard Tonnellée, 37000 Tours, France

9 Endocan-Comete Network Coordinator, Assistance Publique Hôpitaux de Paris, Hôpital Cochin, 27 Rue du Faubourg Saint Jacques, 75014 Paris, France

10 Department of Biology and Pathology, Institut Gustave Roussy, 94805 Villejuif, France

11 INSERM UMR_S U1185, Fac Med Paris Sud, Université Paris-Saclay, 63 Rue Gabriel Péri, 94276 Le Kremlin Bicêtre, France