

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Influence of the CYP2B6 polymorphism on the pharmacokinetics of mitotane.

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/132522> since 2021-04-29T12:47:38Z

Published version:

DOI:10.1097/FPC.0b013e3283606cb2

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

D'Avolio A; De Francia S; Basile V; Cusato J; De Martino F; Pirro E; Piccione F; Ardito A; Zaggia B; Volante M; Di Perri G; Terzolo M. Influence of the CYP2B6 polymorphism on the pharmacokinetics of mitotane.. PHARMACOGENETICS AND GENOMICS. 23 (6) pp: 293-300.
DOI: 10.1097/FPC.0b013e3283606cb2

The publisher's version is available at:

<http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=01213011-201306000-00001>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/132522>

Influence of the CYP2B6 polymorphism on the pharmacokinetics of mitotane

D'Avolio, Antonio; De Francia, Silvia; Basile, Vittoria; Cusato, Jessica; De Martino, Francesca; Pirro, Elisa; Piccione, Francesca; Ardito, Ariannac Zaggia, Barbara; Volante, Marco; Di Perri, Giovanni; Terzolo, Massimo

Abstract

Objective: The aim of this study was to assess the potential impact of the pharmacogenetic variability of CYP2B6 and ABCB1 genes on the pharmacokinetics of mitotane.

Methods: A retrospective analysis was carried out on 27 patients with adrenocortical carcinoma on postoperative adjunctive mitotane. CYP2B6 and ABCB1 polymorphisms were genotyped and tested for an association with plasma trough concentration after 3, 6, 9, and 12 months of therapy.

Results: Patients with the GT/TT genotype had higher mitotane plasma concentrations compared with patients with GG at 3 months (14.80 vs. 8.01 µg/ml; P=0.008) and 6 months (17.70 vs. 9.75 µg/ml; P=0.015). Multivariate logistic regression analysis showed that only the CYP2B6 rs3745274GT/TT genotype (odds ratio=10.7; P=0.017) was a predictor of mitotane plasma concentrations of at least 14 µg/ml after 3 months of treatment. Mitotane concentrations were not influenced by the polymorphisms of the ABCB1 gene.

Conclusion: Evaluation of the CYP2B6 polymorphism enabled prediction of the individual response to adjuvant mitotane treatment.

Introduction

Adrenocortical carcinoma (ACC) is a rare tumor characterized by a poor prognosis as the survival rate after diagnosis is less than 40% (1–3). To date, the only antineoplastic agent specifically approved by the Food and Drug Administration and the European Medicine Executive Agency for treatment of ACC is mitotane (o,p'-DDD), a chemical congener of the insecticide dichlorodiphenyltrichloroethane.

The mechanism of action of mitotane is not yet fully understood. It is generally considered that mitotane is able to destroy the adrenal glands in animal models (4) and to inhibit different enzymatic steps of adrenal steroidogenesis (5). Mitotane inhibits the cytochrome cholesterol side chain cleavage enzyme (P450), which converts cholesterol into pregnenolone (6) as well as other CYP-450-dependent enzymes, such as 11-hydroxylase and 18-b-hydroxylase, and CYP450 independent enzymes, for example 3-b-hydroxysteroid-dehydrogenase (7,8). More than 40% of a mitotane dose is absorbed from the gastrointestinal tract and the absorption rate increases with food. There is a significant distribution of mitotane and its metabolites in fatty tissue and only 6% of mitotane is bound to protein (9). The metabolism of mitotane is mainly performed by enzymes of the CYP-450 family, and its metabolites, p,p'-DDA and o,p'-DDE, are excreted in urine and bile. The elimination half-life of the parent compound ranges between 18 and 159 days (9).

Over the past 40 years, mitotane has been used widely for the medical treatment of advanced ACC as a monotherapy and also in combination with other cytotoxic agents (1,10). The concept of a therapeutic interval for mitotane plasma levels emerged from studies showing that concentrations between 14 and 20 µg/ml are required to attain maximal antineoplastic activity while minimizing risks for unwanted effects (11). Although these target concentrations have been defined arbitrarily and retrospectively (11,12), a recent study has confirmed that mitotane concentrations above 14 µg/ml predict tumor response and

better survival in patients with advanced ACC (13).

The high propensity of ACC to recur even after apparent complete resection (up to 75–85% in high-risk tumors) (14) is a strong argument for considering an adjunctive treatment with mitotane. A retrospective analysis involving a large cohort of patients showed that adjuvant mitotane was associated with a significant advantage in recurrence-free and overall survival (15). Recently, other studies have shown evidence of mitotane efficacy as an adjuvant measure, but these studies are also not exempt from limitations (16,17). Overall, the role of adjuvant mitotane in the treatment plan of ACC remains controversial (5,18).

The most important limit to the use of mitotane is its narrow therapeutic index and its potential for toxicity (19,20). The monitoring of circulating mitotane level, to maintain target concentrations, is key for achieving compliance to treatment and is also beneficial in an adjuvant setting. We have shown recently that maintaining mitotane plasma levels higher than 14 µg/ml was associated with prolonged recurrence-free survival 21.

Mitotane levels correlate roughly with the dose administered, although wide individual differences are observed, thus suggesting that other factors may influence achievement of target concentrations (22).

Thus, genetic polymorphisms of proteins involved in the metabolism of mitotane may play a key role. Besides CYP3A4, which shows limited genetic variability in the Caucasian population (23–25), the most important and polymorphic cytochrome involved in mitotane metabolism is CYP2B6 (8,25). P-glycoprotein, a product of the multidrug resistance (MDR-1/ABCB1) gene, is a 130–180 kDa plasma membrane phosphoglycoprotein that mediates multidrug resistance in cell culture and in vivo by increasing efflux of the natural product chemotherapeutic agents (26). In-vitro studies have shown that mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (ABCB1) and modulates the activity and expression of p-glycoprotein (26,27).

Then, the aim of this study was to assess the potential impact of the genetic variability of the CYP2B6 gene on pharmacokinetics of mitotane in the first year of adjuvant therapy. We also investigated whether polymorphisms of the ABCB1 transporter could influence mitotane plasma concentrations.

Methods

Patients

We recruited 56 patients with ACC who were treated adjuvantly with mitotane at the San Luigi Gonzaga Hospital between May 2005 and October 2011, but only 27 patients had a complete set of samplings over the first year of treatment and were considered eligible for this retrospective analysis. Follow-up for this study was closed in August 2012. Demographic and clinical data of the patients are presented in Table 1.

Table 1. Main characteristics of the 27 patients

Variables	Value
Age (years)	45 (38–58)
Male sex (%)	44.4
Body weight (kg)	71 (63–78)
Caucasian ethnicity (%)	100
Mitotane dose (g/day) in the first 3 months (%)	
1.5	3.7
2.0	11.1
2.5	7.4
3.0	44.4
3.5	7.4
4.0	11.1
4.5	3.7
5.0	7.4
6.0	3.7

Data are expressed as median and IQR if not otherwise specified.
IQR, interquartile range.

To be eligible for this study, the patients had to fulfill the following inclusion criteria: age 18 years or older, histologically confirmed diagnosis of ACC, complete tumor resection, availability of postoperative follow-up information including the results of imaging tests (computed tomography or MRI scans), and regular determinations of plasma mitotane concentration. Exclusion criteria were macroscopically incomplete resection, incomplete tumor staging, concomitant cancers within the previous 5 years, except for nonmelanoma skin cancer treated radically, clinically significant concomitant disease, incomplete follow-up information or follow-up duration of less than 6 months, initiation of mitotane treatment longer than 6 months after surgery, and concomitant postoperative adjuvant therapies (chemotherapy or radiotherapy).

All data were obtained by reviewing patient history, discharge summaries, medical records, and source documents. Data were retrieved by trained medical personnel using specifically tailored data forms. We collected data on clinical and demographical characteristics, the date of diagnosis, the stage at diagnosis, the hormonal work-up, the date and type of surgery, the pathology report, the date of recurrence, and either the date and cause of death or the date of the last follow-up visit. The following information on mitotane treatment was collected: treatment dose and duration, adverse effects, reasons for discontinuation, and results of monitoring of plasma concentrations. The institutional ethics committee of the San Luigi Gonzaga Hospital approved the study and all patients provided written informed consent.

Complete resection was defined as no evidence of macroscopic residual disease on the basis of surgical reports, histopathological analysis, and postoperative imaging. All diagnoses were confirmed by an experienced pathologist according to the Weiss criteria with a score of 3, or more, identifying malignancy (28,29). Staging at diagnosis was carried out on the basis of imaging studies and was corroborated by the findings at surgery. Staging was reported according to the ENS@T staging system (13). Disease recurrence

was defined as radiologic evidence of a new tumor lesion during follow-up.

During the study period, monitored mitotane treatment aimed at achieving and maintaining a target plasma concentration of 14–20 µg/ml (11,26,30) was a standard of care following removal of ACC at our center. All patients received the same mitotane formulation (Lysodren, 500 mg tablets), which was purchased from Laboratoire HRA Pharma (Paris, France). Mitotane was administered orally following a schedule of progressive dose increments according to a local protocol and patient compliance aiming to achieve concentrations between 14 and 20 µg/ml (11,31,32).

At our center, a low-dose protocol is followed (33,34), based on a starting mitotane dose of 1 g daily, with further dose increase every 4–7 days, up to 8–10 g daily or the maximum tolerated dose, aiming to attain concentrations between 14 and 20 mg/l.

When these or even higher concentrations were attained, doses were tapered with further individual dose adjustments guided by the results of mitotane measurement and toxicity assessment. In the event of unacceptable side effects, the patients were allowed to return to a lower dose or discontinue mitotane temporarily, restarting with a lower dose. All patients received glucocorticoid replacement, whereas thyroxine and sex steroid hormone replacement was provided if deemed appropriate (11,26,30). Follow-up visits were performed every 3 months during the study period. At each visit, the patients underwent physical examination, routine laboratory evaluation, monitoring of mitotane concentrations and hormonal work-up, and computed tomography of chest and abdomen. Mitotane-related toxicity was graded using the Common Terminology Criteria for Adverse Event V3.0 (35).

Genotyping

Patients were genotyped at polymorphic sites shown in Table 2 using the ABI TaqMan allelic discrimination kit (Life Technologies, Monza, Italy) by real-time PCR using standard methodology (36). All primers, probes, and PCR conditions are available on request.

Table 2. Single nucleotide polymorphisms evaluated and their frequency

Gene	Protein	Rs numbers and ID	Allelic combinations (from PubMed website)	Minor allele (from PubMed website)	Minor allele frequency (from PubMed website)	Minor allele frequency (observed in our population)
CYP2B6	2B6 enzyme	rs3745274 (G516T)	G/G, G/T, T/T	T	0.26	0.29
ABCB1	P-gp	rs1045642 (C3435T)	C/C, C/T, T/T	T	0.39	0.55
ABCB1	P-gp	rs1128503 (C1236T)	C/C, C/T, T/T	T	0.42	0.51
ABCB1	P-gp	rs2032582 (G2677T)	G/G, G/T, T/T	T	0.34	0.50

Measurement of plasma mitotane concentration

Plasma samples were collected ~12 h after the dose administration of mitotane, just before the subsequent administration (trough value). Samples were centrifuged at 1400 g for 10 min to separate plasma and then stored at -80°C until analysis. Mitotane concentrations were measured using a previously validated high-performance liquid chromatography method (37). The calibration curve ranged from 0.078 up to 40 µg/ml. Accuracy and precision standard errors were below 10%.

Statistical analysis

For descriptive statistics, continuous variables were summarized as median (25–75th percentile,

interquartile range). Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro–Wilk test and categorical data were compared using a Mann–Whitney or Kruskal–Wallis statistical test. To determine continuous data, a Spearman's Rank correlation was utilized.

The mean mitotane Ctrough was calculated as the mean of all available Ctrough determinations for each patient between the first and the third month of therapy. The association between individual single nucleotide polymorphism (SNP) and plasmatic concentrations was tested using a basic allelic test and calculated using the [chi] 2-test.

Multivariate logistic regression analysis with stepwise forward selection was carried out with P-values of less than 0.05 (<0.200 for univariate analysis) as the criteria for model inclusion. Furthermore, for the univariate analysis, P-values were corrected according to Benjamini–Hochberg rules when appropriate. Statistical analyses were carried out using SPSS software package, version 18.0 (SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

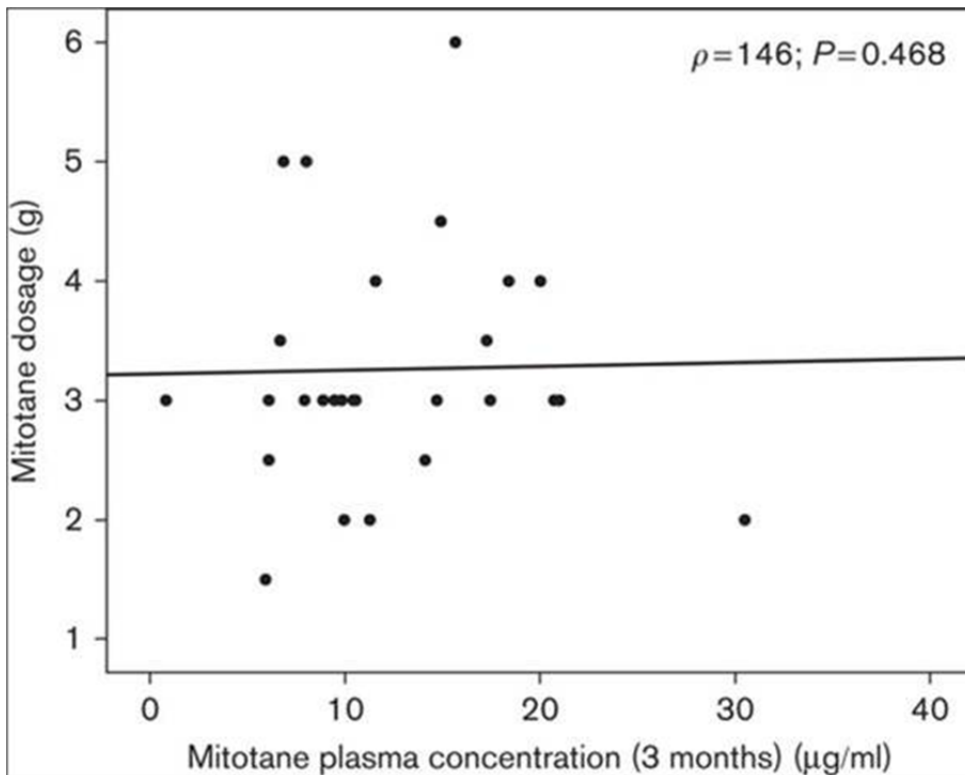
As shown in Table 1, the dose of mitotane administered in the first 3 months was highly variable, but most patients received 3 g daily (n=12). Thereafter, the dosing of the drug was adjusted on the basis of drug monitoring and patient compliance.

The allele frequencies for all polymorphisms observed in our population are listed in Table 2. All SNPs are in Hardy–Weinberg equilibrium. As the ABCB1 SNPs rs1045642, rs1128503, and rs2032582 were in strong linkage disequilibrium (38,39), only the SNP variant for rs1045642 (3435 C>T) was included in the following analyses. No differences between demographic, physical characteristics, and biochemical parameters were observed among genetic groups.

The median mitotane plasma concentrations after the first 3 months of treatments were 10.55 µg/ml (7.94–17.26), 15.39 µg/ml (9.75–18.89) after 6 months, 13.55 µg/ml (10.56–18.76) after 9 months, and 13.09 µg/ml (10.78–15.24) after 12 months of therapy. The percentage of patients achieving target mitotane concentrations of 14 µg/ml, or greater, was 40.7% at 3 months, 66.7% at 6 months, 44.4% at 9 months, and 33.3% at 12 months.

There was a significant ([rho]=550; P=0.003) correlation between CYP2B6 SNP and mitotane plasma levels (after 3 months). Conversely, no correlation ([rho]=146; P=0.468) was observed between the daily dose of mitotane and mitotane plasma levels in the first 3 months (Fig. 1).

Figure 1.

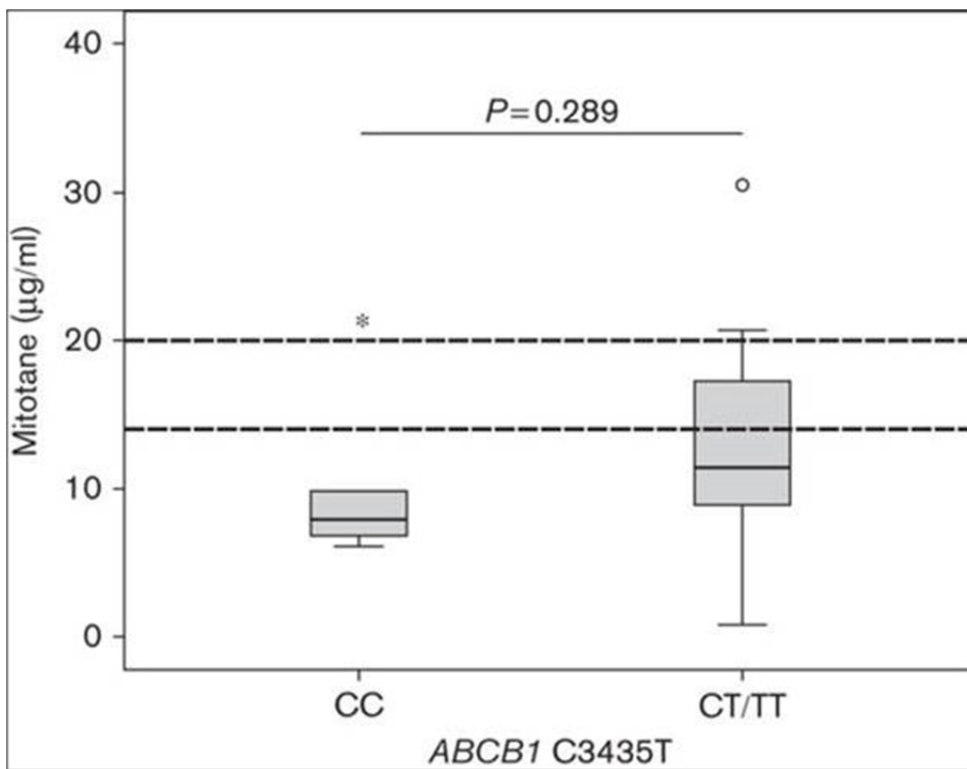


Absence of correlation between mitotane dosage and plasma concentration after 3 months of therapy

Moreover, no correlation could be found between patient age and weight at baseline, and the mean mitotane concentrations after the first 3 months of treatment.

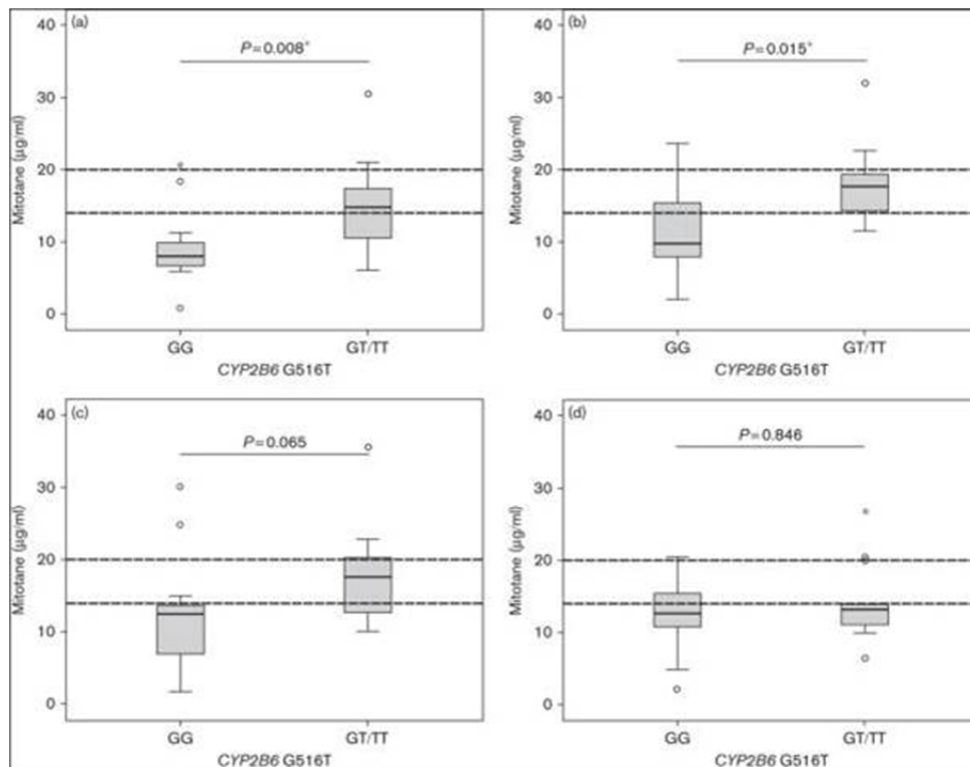
Similarly, mitotane concentrations were not influenced by the three SNPs of the ABCB1 gene as shown in Fig. 2. For the 3435 SNP, mitotane levels were not significantly different between CC (n=5) and CT/TT genotypes (n=22) [7.94 µg/ml (6.46–15.41) vs. 11.42 µg/ml (8.67–17.31); P=0.289]. Rather, plasma concentrations were influenced by the CYP2B6 polymorphism as patients with the GT/TT genotype had higher mitotane plasma concentrations after 3 months of treatment compared with patients with GG, the wild-type genotype [14.80 µg/ml (10.50–18.08) vs. 8.01 µg/ml (6.37.6–10.61); P=0.008] (Fig. 3a). Patients with the GT/TT genotype had higher mitotane plasma concentrations also after 6 months of treatment compared with patients with the GG genotype [17.70 µg/ml (14.27–19.42) vs. 9.75 µg/ml (7.66–15.69); P=0.015] (Fig. 3b), whereas differences were not significant after 9 and 12 months (Fig. 3c and d).

Figure 2.



Mitotane plasma concentrations after 3 months of therapy in patients with the 3435 polymorphism in the ABCB1 gene. Median values (horizontal line), IQR (bars), patient values (black square), and highest and lowest values (whiskers) are shown. The two horizontal dotted lines represent the target range of mitotane plasma concentrations (14-20 [micro]g/ml). Outliers are represented by little circles, and extreme outliers are represented by little stars. Specifically, star represents an extreme outlier (a value more than three times the interquartile range from a quartile). A circle is used to mark other outliers with values between 1.5 and 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range. IQR, interquartile range.

Figure 3.



Mitotane plasma concentrations after 3 (a), 6 (b), 9 (c), and 12 (d) months of therapy in patients with the 516 polymorphism in the CYP2B6 gene. Median values (horizontal line), IQR (bars), patient values (black square), and highest and lowest values (whiskers) are shown. The two horizontal dotted lines represent the target range of mitotane plasma concentrations (14-20 [micro]g/ml). Significant differences (*) are shown. Outliers are represented by little circles, and extreme outliers are represented by little stars. Specifically, star represents an extreme outlier (a value more than three times the interquartile range from a quartile). A circle is used to mark other outliers with values between 1.5 and 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range. IQR, interquartile range.

A multivariate logistic regression analysis showed that only the CYP2B6 516-GT/TT genotype (odds ratio=10.7, P=0.017) was a predictor of mitotane plasma concentrations of at least 14 µg/ml after 3 months of treatment (Table 3).

Table 3. Predictors of mitotane plasma concentrations ≥ 14 [micro]g/ml after 3 months of treatment in univariate and multivariate logistic regression analysis.

Factors	Univariate			Multivariate	
	OR (95% CI)	P-value	P-value*	OR (95% CI)	P-value
Sex (male)	0.93 (0.72-3.7)	0.930	1.116	-	-
Weight (10 kg)	0.96 (0.89-1.04)	0.376	0.565	NS	NS
Age (10 years)	0.7 (0.5-1)	0.948	5.688	NS	NS
Mitotane dose above 3 g/day	2.50 (0.48-12.8)	0.173	0.519	2.9 (0.42-20.9)	0.274
CYP2B6 rs3745274GT/TT	9.90 (1.53-63.6)	0.016	0.096	10.7 (1.53-75.0)	0.017
ABCB1 rs1045642C/TT	3.33 (0.13-34.8)	0.315	0.630	-	-

In bold are the values or factors statistically significant.
 CI, confidence interval; NS, nonsignificant; OR, odds ratio.
 *Corrected (Benjamini-Hochberg - false discovery rate) P-value for univariate analyses.

Mitotane has been used to treat ACC for more than 40 years. A number of studies have shown the importance of monitoring circulating concentrations of mitotane during the course of treatment, as they correlate with the therapeutic efficacy and occurrence of side effects (11,12,40). The concept of a monitored mitotane treatment has been established in the treatment strategy of advanced disease but has

also been introduced in the postoperative adjuvant setting (18,41).

It remains uncertain as to which is the better starting regimen of adjuvant mitotane treatment. A high-dose regimen may be preferable to ensure a rapid achievement of the therapeutic mitotane concentrations, but the trade-off is a higher incidence of side effects (42,43). In contrast, a low-dose regimen, even if requiring a longer period for the achievement of the therapeutic concentrations, is better tolerated (42,44). An important point emerging from these studies, which has been confirmed by clinical experience, is that the mitotane dose is not the only determinant of plasma mitotane concentrations (22). Only a paper by Faggiano et al. (42) showed a significant linear correlation between mitotane dose and plasma level, but based on a very small cohort (four patients), whereas previous studies have shown that mitotane levels correlate poorly with the dose administered, suggesting that other factors may influence attainment of target concentrations (22).

Conversely, there is a wide interindividual variability in drug concentrations for the same dose received.

As mitotane accumulates in adipose tissue, the plasma elimination half-life is very long (18–159 days) (9,45). Consequently, it can take months to reach steady-state pharmacokinetic and the different interindividual variability half-life is probably related to CYP metabolic activity.

Moreover, we have to underline the high number of medications concomitantly taken by patients.

However, to our knowledge, it is presently unclear whether interactions between mitotane and these drugs do exist. Even if interaction of these drugs with mitotane has not been established, we cannot exclude that some interference was operative in our patients. As distribution of comedication was similar in the two genetic groups (GG vs. GT/TT), it is unlikely that this factor may have affected the observed difference.

The present study confirmed the huge variability in the dose–concentration relationship and shed light on possible mechanisms that may influence mitotane pharmacokinetics. We found that a SNP of the CYP2B6 enzyme, which is involved in mitotane metabolism (8), was associated independently with mitotane concentrations at 3 months of treatment, whereas the dose administered during this period was less important. The patients with the wild-type ‘G’ allele showed significantly lower mitotane concentrations than the patients carrying the mutant ‘T’ allele.

These findings support the view that mitotane metabolism is genetically determined and may vary among patients, thus contributing toward the fluctuations in mitotane levels. Our observation is in agreement with the concept that the mutant ‘T’ allele confers a lower enzyme activity, therefore a reduced ability to metabolize certain drugs (46,47). Differences in mitotane concentrations were more evident at the beginning of treatment (after 3 and 6 months), whereas they became less apparent during the course of treatment because of our active attitude to target the therapeutic concentrations through adjustments of mitotane dose. Notwithstanding our efforts, limited number of patients were able to achieve and maintain target mitotane concentrations, a problem that has been reported previously (11–13,21,42).

Our observation, associated with drug lipophilicity and other still unknown factors, may explain the low bioavailability (40%) of the drug with a potential first-pass metabolism by CYP2B6 in the intestinal mucosa.

There is evidence that mitotane influences the CYP3A4 enzyme (45,48,49), but there is no evidence, to our knowledge, that mitotane could also influence the expression/activity of CYP2B6. However, it is logical to

assume that mitotane may have an inductive capacity on CYP2B6, as shown on CYP3A4 and as shown by other drugs, probably mediated by PXR (36,50–52).

The impact of the main p-glycoprotein ABCB1 polymorphism on mitotane concentrations was less evident, although having at least one variant allele 'T' of the 3435 polymorphism in the ABCB1 gene was associated with nonsignificantly higher mitotane concentrations compared with CC carriers. However, we cannot exclude that the effect may attain statistical significance with a larger sample size. To date, there are no data linking this p-glycoprotein to the pharmacokinetics of mitotane, but some studies showed that mitotane could enhance the cytotoxicity of chemotherapy in ACC cell lines expressing the ABCB1 gene (26,32).

In conclusion, we have identified a CYP2B6 genetic profile predictive of the circulating concentrations of mitotane. Our findings show that CYP2B6 is a key cytochrome for mitotane metabolism and provide the first demonstration of the role of pharmacogenetic in mitotane pharmacokinetic. A limitation of the study is the relatively low patient numbers and the retrospective nature of the study.

The prospective evaluation of this polymorphism at the initiation of adjuvant mitotane therapy could enable prediction of the individual pharmacokinetic response and to tailor an individual dose regimen. A high-dose regimen may be best suited for patients with wild-type 516-GG genotype who are actively metabolizing the drug, whereas a low-dose regimen may be applicable to 516-TG/TT mutant carrier patients. Further studies are required to explore this potential application of CYP2B6 genotyping and the relationship with patient outcome.

References

1. Allolio B, Fassnacht M. Clinical review: adrenocortical carcinoma: clinical update. *J Clin Endocrinol Metab* 2006; 91: 2027–2037.
2. Dackiw AP, Lee JE, Gagel RF, Evans DB. Adrenal cortical carcinoma. *World J Surg* 2001; 25: 914–926.
3. Wajchenberg BL, Albergaria Pereira MA, Medonca BB, Latronico AC, Campos Carneiro P, Alves VA, et al.. Adrenocortical carcinoma: clinical and laboratory observations. *Cancer* 2000; 88: 711–736.
4. Nelson AA, Woodard G. Severe adrenal cortical atrophy (cytotoxic) and hepatic damage produced in dogs by feeding 2,2-bis(parachlorophenyl)-1,1-dichloroethane (DDD or TDE). *Arch Pathol (Chic)* 1949; 48: 387–394.
5. Veytsman I, Nieman L, Fojo T. Management of endocrine manifestations and the use of mitotane as a chemotherapeutic agent for adrenocortical carcinoma. *J Clin Oncol* 2009; 27: 4619–4629.
6. Schteingart DE. Conventional and novel strategies in the treatment of adrenocortical cancer. *Braz J Med Biol Res* 2000; 33: 1197–1200.
7. Hart MM, Reagan RL, Adamson RH. The effect of isomers of DDD on the ACTH-induced steroid output, histology and ultrastructure of the dog adrenal cortex. *Toxicol Appl Pharmacol* 1973; 24: 101–113.
8. Kitamura S, Shimizu Y, Shiraga Y, Yoshida M, Sugihara K, Ohta S. Reductive metabolism of p,p'-DDT and

o,p'-DDT by rat liver cytochrome P450. *Drug Metab Dispos* 2002; 30: 113–118.

9. . MICROMEDEX healthcare series. 2009. New York, NY USA: Thomson Reuters.

10. Fassnacht M, Terzolo M, Allolio B, Baudin E, Haak H, Berruti A, et al.. Combination chemotherapy in advanced adrenocortical carcinoma. *N Engl J Med* 2012; 366: 2189–2197.

11. 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.

12. Baudin E, Pellegriti G, Bonnay M, Penfornis A, Laplanche A, Vassal G, et al.. Impact of monitoring plasma 1,1-dichlorodiphenildichloroethane (o,p'DDD) levels on the treatment of patients with adrenocortical carcinoma. *Cancer* 2001; 92: 1385–1392.

13. Hermsen IG, Fassnacht M, Terzolo M, Houterman S, den Hartigh J, Leboulleux S, et al.. 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.

14. Stojadinovic A, Ghossein RA, Hoos A, Nissan A, Marshall D, Dudas M, et al.. Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. *J Clin Oncol* 2002; 20: 941–950.

15. Terzolo M, Angeli A, Fassnacht M, Daffara F, Tauchmanova L, Conton PA, et al.. Adjuvant mitotane treatment for adrenocortical carcinoma. *N Engl J Med* 2007; 356: 2372–2380.

16. Fassnacht M, Johanssen S, Fenske W, Weismann D, Agha A, Beuschlein F, et al.. Improved survival in patients with stage II adrenocortical carcinoma followed up prospectively by specialized centers. *J Clin Endocrinol Metab* 2010; 95: 4925–4932.

17. Wangberg B, Khorram-Manesh A, Jansson S, Nilsson B, Nilsson O, Jakobsson CE, et al.. The long-term survival in adrenocortical carcinoma with active surgical management and use of monitored mitotane. *Endocr Relat Cancer* 2010; 17: 265–272.

18. Terzolo M, Berruti A. Adjunctive treatment of adrenocortical carcinoma. *Curr Opin Endocrinol Diabetes Obes* 2008; 15: 221–226.

19. Pommier RF, Brennan MF. An eleven-year experience with adrenocortical carcinoma. *Surgery* 1992; 112: 963–970 discussion 70–71.

20. Schteingart DE, Motazed A, Noonan RA, Thompson NW. Treatment of adrenal carcinomas. *Arch Surg* 1982; 117: 1142–1146.

21. Ardito A, Al Ghuzian A, Fassnacht M, Daffara F, Leboulleux S, Wortmann S, et al.. . Do mitotane levels impact on the outcome of patients treated adjuvantly following radical resection of adrenocortical cancer (ACC)? 13th European Congress of Endocrinology. 2011. Rotterdam, The Netherlands: Bristol Bioscientifica; 66.

22. Daffara F, De Francia S, Reimondo G, Zaggia B, Arosio E, Porpiglia F, et al.. Prospective evaluation of

mitotane toxicity in adrenocortical cancer patients treated adjuvantly. *Endocr Relat Cancer* 2008; 15: 1043–1053.

23. Turolo S, Tirelli AS, Ferrareso M, Ghio L, Belingheri M, Groppali E, et al.. Frequencies and roles of CYP3A5, CYP3A4 and ABCB1 single nucleotide polymorphisms in Italian teenagers after kidney transplantation. *Pharmacol Rep* 2010; 62: 1159–1169.

24. Zhou X, Barber WH, Moore CK, Tee LY, Aru G, Harrison S, et al.. Frequency distribution of cytochrome P450 3A4 gene polymorphism in ethnic populations and in transplant recipients. *Res Commun Mol Pathol Pharmacol* 2006; 119: 89–104.

25. Solus JF, Arietta BJ, Harris JR, Sexton DP, Steward JQ, McMunn C, et al.. Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics* 2004; 5: 895–931.

26. Bates SE, Shieh CY, Mickley LA, Dichek HL, Gazdar A, Loriaux DL, et al.. Mitotane enhances cytotoxicity of chemotherapy in cell lines expressing a multidrug resistance gene (mdr-1/P-glycoprotein) which is also expressed by adrenocortical carcinomas. *J Clin Endocrinol Metab* 1991; 73: 18–29.

27. Shabbir A, DiStasio S, Zhao J, Cardozo CP, Wolff MS, Caplan AJ. Differential effects of the organochlorine pesticide DDT and its metabolite p,p'-DDE on p-glycoprotein activity and expression. *Toxicol Appl Pharmacol* 2005; 203: 91–98.

28. Papotti M, Libe R, Duregon E, Volante M, Bertherat J, Tissier F. The Weiss score and beyond – histopathology for adrenocortical carcinoma. *Horm Cancer* 2011; 2: 333–340.

29. Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am J Surg Pathol* 1984; 8: 163–169.

30. Khan TS, Imam H, Juhlin C, Skogseid B, Grondal S, Tibblin S, et al.. Streptozocin and o,p'DDD in the treatment of adrenocortical cancer patients: long-term survival in its adjuvant use. *Ann Oncol* 2000; 11: 1281–1287.

31. Abraham J, Bakke S, Rutt A, Meadows B, Merino M, Alexander R, et al.. A phase II trial of combination chemotherapy and surgical resection for the treatment of metastatic adrenocortical carcinoma: continuous infusion doxorubicin, vincristine, and etoposide with daily mitotane as a P-glycoprotein antagonist. *Cancer* 2002; 94: 2333–2343.

32. Villa R, Orlandi L, Berruti A, Dogliotti L, Zaffaroni N. Modulation of cytotoxic drug activity by mitotane and lonidamine in human adrenocortical carcinoma cells. *Int J Oncol* 1999; 14: 133–138.

33. Schteingart DE. Adjuvant mitotane therapy of adrenal cancer – use and controversy. *N Engl J Med* 2007; 356: 2415–2418.

34. Terzolo M, Ardito A, Zaggia B, Laino F, Germano A, De Francia S, et al.. Management of adjuvant mitotane therapy following resection of adrenal cancer. *Endocrine* 2012; 42: 521–525.

35. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al.. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003; 13:

176–181.

36. Calcagno A, D'Avolio A, Simiele M, Cusato J, Rostagno R, Libanore V, et al.. Influence of CYP2B6 and ABCB1 SNPs on nevirapine plasma concentrations in Burundese HIV-positive patients using dried sample spot devices. *Br J Clin Pharmacol* 2012; 74: 134–140.

37. De Francia S, Pirro E, Zappia F, De Martino F, Sprio AE, Daffara F, et al.. A new simple HPLC method for measuring mitotane and its two principal metabolites tests in animals and mitotane-treated patients. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006; 837: 69–75.

38. Jeannesson E, Albertini L, Siest G, Gomes AM, Ribeiro V, Aslanidis C, et al.. Determination of ABCB1 polymorphisms and haplotypes frequencies in a French population. *Fundam Clin Pharmacol* 2007; 21: 411–418.

39. Jeannesson E, Siest G, Bastien B, Albertini L, Aslanidis C, Schmitz G, et al.. Association of ABCB1 gene polymorphisms with plasma lipid and apolipoprotein concentrations in the STANISLAS cohort. *Clin Chim Acta* 2009; 403: 198–202.

40. Haak HR, Hermans J, van de Velde CJ, Lentjes EG, Goslings BM, Fleuren GJ, et al.. Optimal treatment of adrenocortical carcinoma with mitotane: results in a consecutive series of 96 patients. *Br J Cancer* 1994; 69: 947–951.

41. Fassnacht M, Libe R, Kroiss M, Allolio B. Adrenocortical carcinoma: a clinician's update. *Nat Rev Endocrinol* 2011; 7: 323–335.

42. 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 (Oxf)* 2006; 64: 110–113.

43. Mauclere-Denost S, Leboulleux S, Borget I, Paci A, Young J, Al Ghuzlan A, et al.. High-dose mitotane strategy in adrenocortical carcinoma: prospective analysis of plasma mitotane measurement during the first 3 months of follow-up. *Eur J Endocrinol* 2012; 166: 261–268.

44. Terzolo M, Pia A, Berruti A, Osella G, Ali A, Carbone V, et al.. 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.

45. Van Erp NP, Guchelaar HJ, Ploeger BA, Romijn JA, Hartigh J, Gelderblom H. Mitotane has a strong and a durable inducing effect on CYP3A4 activity. *Eur J Endocrinol* 2011; 164: 621–626.

46. Haas DW. Human genetic variability and HIV treatment response. *Curr HIV/AIDS Rep* 2006; 3: 53–58.

47. Hofmann MH, Blievernicht JK, Klein K, Saussele T, Schaeffeler E, Schwab M, et al.. Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of CYP2B6*6, is responsible for decreased expression and activity of CYP2B6 in liver. *J Pharmacol Exp Ther* 2008; 325: 284–292.

48. Chortis V, Taylor AE, Schneider P, Tomlinson JW, Hughes BA, O'Neil DM, et al.. Mitotane therapy in adrenocortical cancer induces CYP3A4 and inhibits 5alpha-reductase, explaining the need for personalized

glucocorticoid and androgen replacement. *J Clin Endocrinol Metab* 2012; 98: 161–171.

49. Kroiss M, Quinkler M, Lutz WK, Allolio B, Fassnacht M. Drug interactions with mitotane by induction of CYP3A4 metabolism in the clinical management of adrenocortical carcinoma. *Clin Endocrinol (Oxf)* 2011; 75: 585–591.

50. Ngaimisi E, Mugusi S, Minzi OM, Sasi P, Riedel KD, Suda A, et al.. Long-term efavirenz autoinduction and its effect on plasma exposure in HIV patients. *Clin Pharmacol Ther* 2010; 88: 676–684.

51. Svard J, Spiers JP, Mulcahy F, Hennessy M. Nuclear receptor-mediated induction of CYP450 by antiretrovirals: functional consequences of NR1I2 (PXR) polymorphisms and differential prevalence in whites and sub-Saharan Africans. *J Acquir Immune Defic Syndr* 2010; 55: 536–549.

52. Siccardi M, D'Avolio A, Baietto L, Gibbons S, Sciandra M, Colucci D, et al.. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir. *Clin Infect Dis* 2008; 47: 1222–1225.