Enzyme autoinduction model of mitotane

Enzyme autoinduction by mitotane supported by population pharmacokinetic modelling in a large cohort of adrenocortical carcinoma patients

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Abstract

Objective: Mitotane is used for the treatment of adrenocortical carcinoma. High oral daily doses of typically 1-6 g are required to attain therapeutic concentrations. The drug has a narrow therapeutic index and patient management is difficult because of a high volume of distribution, very long elimination half-life, and drug interaction through induction of metabolizing enzymes. The present evaluation aimed at the development of a population pharmacokinetic model of mitotane to facilitate therapeutic drug monitoring.

Methods: Appropriate dosing information, plasma concentrations (1137 data points) and covariates were available from therapeutic drug monitoring (TDM) of 76 adrenocortical carcinoma patients treated with mitotane. Using nonlinear mixed effects modeling, a simple structural model was first developed, with subsequent introduction of metabolic autoinduction. Covariate data were analyzed to improve overall model predictability. Simulations were performed to assess the attainment of therapeutic concentrations with clinical dosing schedules.

Results: A one-compartment pharmacokinetic model with first order absorption was found suitable to describe the data, with an estimated central volume of distribution of 6086 L related to a high interindividual variability of 81.5%. Increase in clearance of mitotane during treatment could be modeled by a linear enzyme autoinduction process. Body mass index was found to have an influence upon disposition kinetics of mitotane. Model simulations favor a high dose regimen to rapidly attain therapeutic concentrations, with the first TDM suggested on day 16 of treatment to avoid systemic toxicity.

Conclusion: The proposed model describes mitotane pharmacokinetics and can be used to facilitate therapy by predicting plasma concentrations.
1. Introduction

The adrenolytic drug mitotane (1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl) ethyl]benzene) is the only approved treatment of the orphan malignant disease adrenocortical carcinoma (ACC). ACC has a high rate of recurrence after complete tumor resection and a dismal prognosis in advanced stages (1, 2). Mitotane is used both as an adjuvant treatment after complete tumor resection (3, 4) and for palliative treatment of advanced disease (5). Clinically used drug effects of mitotane include reduction of tumor related steroid hormone excess and a direct cytotoxic effect leading to objective treatment response in ~20% of cases (6) which appears to be relatively specific to cells of the adrenal cortex. Several molecular mechanisms for mitotane action appear to contribute to mitotane efficacy (7, 8, 9). We recently found sterol-O-acyl transferase 1 to be inhibited by mitotane which leads to impaired steroidogenesis and lipid induced endoplasmic reticulum stress (10). Published data on the pharmacokinetics of mitotane are scarce and have been conducted in small patient series only. It has been shown that mitotane has a low oral bioavailability (F) of 35-40% (11) and a high volume of distribution which is likely due to its lipophilic nature and extensive accumulation in adipose tissue (12). The majority of mitotane has been found to be bound to lipoprotein particles in circulation with pharmacological activity limited to the unbound fraction (13, 14, 15).

Efficacy of mitotane treatment is associated with plasma concentrations >14 mg/l which could be demonstrated in several retrospective series both in adjuvant and palliative treatment with mitotane monotherapy (6, 16, 17, 18, 19, 20) but also in combination with cytotoxic drugs in advanced disease (21, 22). Adverse effects, including CNS toxicity are associated with plasma concentrations exceeding 20 mg/L (23). However, the time interval to achieve therapeutic plasma concentrations of mitotane limits the clinical utility
of the drug regardless of the dosing regimen applied (24, 25, 26). Accordingly, therapeutic drug monitoring (TDM) is needed for continuous treatment evaluation and decision making. There is a poor correlation of mitotane dose with plasma concentrations, which suggests the involvement of other factors influencing the attainment of therapeutic concentrations (27).

Main metabolites of mitotane are o,p'-dichlorodiphenyl-ethene (o,p'-DDE) and –acetate (o,p’-DDA) (28, 29). o,p'-DDA can be detected at ten-fold higher concentration in blood than mitotane itself whereas o,p'-DDE is barely detectable in most cases (19, 30). Small amounts of these derivatives apparently undergo aromatic hydroxylation and glycine conjugation (28). The compound is a strong inducer of hepatic CYP3A4 in vitro and in vivo, which causes interactions with co-administered drugs such as sunitinib (31, 32, 33, 34). Orally administered mitotane is excreted in urine and bile and has a long elimination half-life ranging from 18-159 days (35).

In vitro, drug metabolizing enzymes and transporters beyond CYP3A4 were induced by mitotane, probably via the pregnane X receptor (PXR) (32). PXR ligands transcriptionally induce the activity of a broad range of processes in drug metabolism, which in turn often also accelerate the metabolism of PXR ligands, a phenomenon called autoinduction (36, 37). It is therefore conceivable that mitotane metabolism may be affected by autoinduction, and variability in autoinduction may contribute to differences in clinical toxicity and efficacy among patients. Thus, it might be helpful to account for enzyme induction in order to appropriately describe the pharmacokinetics of mitotane during a long-term treatment. A quantitative description of enzyme induction by mitotane in patients may also be useful to predict drug interactions that may limit the exposure to co-administered chemotherapeutic or targeted agents (3). Previous modeling efforts have not considered autoinduction (38).
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The objective of the present evaluation is thus to develop a model describing mitotane pharmacokinetics incorporating enzyme autoinduction, which should contribute to optimizing mitotane dosing schedules.

2. Subjects and methods

2.1 Patients characteristics and data preparation

Clinical and demographical data were retrieved from records of patients participating in the German ACC Registry and the European Network for the Study of Adrenal Tumors (ENSAT) at a single reference center. Both registries have been approved by the ethics committee of the University of Würzburg (approval number 86/03 and 88/11) and all patients provided written informed consent to participate in the study. The following parameters were collected: age, sex, weight, height, body mass index, ENSAT tumor stage, treatment intention, concomitant systemic therapy, albumin, triglyceride, high and low-density lipoprotein, cholesterol, creatinine, and γ-glutamyltransferase (γ-GT) plasma concentrations. Mitotane plasma concentrations were measured within the Lysosafe® TDM provided on behalf of the manufacturer, HRA-Pharma (Paris, France) using HPLC. A total number of 103 patients with adrenocortical carcinoma were treated with oral mitotane doses (0.5-10g per day, with interruptions).

R (version 3.2.3) with ‘dplyr’, ‘tidyr’ ‘lubridate’ and ‘ggplot2’ packages was used for data manipulation, cleaning and visualization (39, 40, 41, 42). All patients treated with mitotane over the age of 18 were eligible. Only patients with missing dosing at the initiation of therapy were excluded from analysis. Data from patients with missing dosing information during the treatment course was excluded partly by evaluating only data
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gathered during the period prior to the missing information. Exploratory data analysis
was performed to judge general trends in the data.

2.2 Data analysis and pharmacokinetic model development

Nonlinear mixed effect modeling was performed for data analysis. Estimation of
pharmacokinetic parameters was performed by first order conditional estimation with
interaction (FOCE-I) using NONMEM 7.4.1 (ICON, Development Solutions, Elliot City,
MD, USA) (43). Model development was aided by Pearl-speaks-NONMEM toolkit
-Version 4.7.0) (44). Graphical user interface Pirana (Version 2.9.6) was used for model
management and execution, output generation and interpretation of results (45). Xpose4
package with R was used for visualizing output data, post processing and analyzing
NONMEM output (46).

A compartmental approach was adapted in a stepwise manner to develop a
pharmacokinetic model. In the first step, pharmacokinetic parameters representing a
typical individual of the population were estimated using the basic structural model,
followed by estimation of interindividual variability (IIV). Subsequently, a hypothetical
mitotane metabolizing enzyme compartment was introduced.

Change in amount of drug in the central compartment ($A_{D,cent}$) was described by equation
1.

$$\frac{dA_{D,cent}}{dt} = ka \cdot A_{D,gut} - C_p \cdot CL_{baseline} \cdot A_{enz} \quad (1)$$

Where,

$ka$ is the absorption rate constant,

$A_{D,gut}$ is the amount of drug in gut compartment,

$C_p$ is the mitotane plasma concentration,
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$CL_{\text{baseline}}$ is the baseline mitotane clearance,

$A_{\text{enz}}$ is the relative amount of enzyme in hypothetical enzyme compartment.

Mitotane plasma concentrations influenced enzyme turnover rate and enhanced enzyme synthesis as described in equation 2.

$$\frac{dA_{\text{enz}}}{dt} = K_{\text{in}} \cdot (1 + \text{Slope} \cdot C_p) - K_{\text{out}} \cdot A_{\text{enz}} \quad (2)$$

The enzyme induction model assumes that the rate of enzyme synthesis ($K_{\text{in}}$) follows zero-order kinetics, while the rate of degradation ($K_{\text{out}}$) follows first-order kinetics dependent upon relative amount of enzyme.

At steady state enzyme concentrations,

$$K_{\text{in}} = K_{\text{out}} \quad (3)$$

Parameters which could not be estimated from the data because of insufficient information were fixed according to published values or to arbitrary and/or physiologically plausible values which were subsequently evaluated by sensitivity analysis.

Other models tested included: (i) a two-compartment model; (ii) models with mitotane being eliminated by two distinct inducible and uninducible pathways; and (iii) models incorporating both gut wall and hepatic enzyme induction. A more complex physiologically based approach was also tested including (iv) a minimal physiologically based pharmacokinetic model (47); and (v) a semiphysiological well stirred liver model to incorporate the first pass effect (48). Both linear and nonlinear relationships ($E_{\text{max}}$ and sigmoidal $E_{\text{max}}$ models) were tested to describe the effect of mitotane on enzyme formation.
IIV was introduced to volumes of distribution and slope (Equation 4) assuming a normal distribution of \( \eta \) with mean zero and variance \( \omega^2 \).

\[
\phi_{ij} = \theta_j \cdot \exp(\eta_{ij})
\] (4)

Where \( \phi_{ij} \) is the \( j \)th individual pharmacokinetic parameter of the \( i \)th subject, \( \theta_j \) the population estimate of the respective pharmacokinetic parameter and \( \eta_{ij} \) the deviation of the subject’s individual parameter from the population point estimate.

Additive, proportional and combined error models were scrutinized to obtain estimates for residual unexplained variability (RUV). For nested (hierarchical) models, the likelihood ratio test was used which assumes that the difference in objective function values (OFV) (representing an overall prediction error) between two models is chi-squared distributed. Decisions regarding model preference were based on a preselected level of significance \( (p=0.05) \), degrees of freedom (difference in total number of parameters), and a critical chi-square value (for the chosen level of significance and degrees of freedom). Nested models with fewer parameters and an OFV lower by an amount larger than the critical chi-square value was finally given a preference. For non-nested (non-hierarchical) models, the Akaike Information Criterion (AIC: OFV plus two times the number of parameters) was used and the model with a lower AIC value was preferred. Goodness of fit (GOF) plots were evaluated to assess the discrepancy between the observed and predicted data and included individual/population predicted concentrations (IPRED/PRED) vs. the observed concentrations and conditional weighted residuals (CWRES) vs. observed concentrations and vs. time range. Physiological plausibility and precision of parameter estimates assessed via bootstrap statistics with 1,000 samples were further criteria of model selection.
2.3 Covariate analysis

After successful development of a basic structural model, covariates were analyzed to provide an explanation for IIV and to improve overall model performance. Covariate pre-selection was based primarily on physiological plausibility. Graphical screening for potential covariates was performed including CWRES and individual pharmacokinetic parameters estimates versus covariates. Correlated covariates were avoided to be tested together and preference among those was given to the covariate with greater scientific plausibility if they provided a similar improvement of the model. Mitotane is reported to alter γ-GT (49) and triglyceride levels in patients and it is suggested to closely monitor the lipid profile during mitotane treatment (50). As the drug is known to be accumulated in the adipose tissue, greater body fat proportion in women might have an impact influence upon its volume of distribution (51). Considering these facts, parameter covariate relationships were tested on volume of distribution (BMI and sex) and Slope (CL\textsubscript{CR}, plasma γ-GT and triglyceride levels).

Categorical covariate relationship (sex) was tested as a fractional change ($\theta_{COV}$) from the typical value of a parameter estimate ($\theta_1$)

\[
TVP \text{ (typical value of parameter)} = \theta_1 \cdot [1 + \theta_{COV} \cdot (COV)]
\]  

(5)

Whereas, continuous covariates (BMI, CL\textsubscript{CR}, plasma γ-GT and triglyceride levels) were analyzed as linear relationships,

\[
TVP = \theta_1 \cdot [1 + \theta_{COV} \cdot (COV - COV_{median})]
\]  

(6)

Final covariate inclusion in the model was mainly based upon the decrease in OFV and IIV.
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2.4 Simulation study

Relative change in clearance over time was evaluated graphically by designing stochastic simulations with model estimates. Both high dose (day1: 1.5 g, day2: 3g, day3: 4.5g, day4-onwards: 6g daily) and low dose (day1-2: 1g, day3-5: 1.5g, day6-8: 2g, day 9-11: 2.5g, day12-onwards: 3g daily) regimens used in clinical practice (24, 25) were tested in simulated population over a period of 3 months. Statistical and graphical evaluation was performed with the aim to attain therapeutic mitotane concentrations (14-20mg/L), avoiding toxic concentrations and to define appropriate timing of first TDM.

3. Results

Data regarding 76 patients out of 103, 45 females and 31 males, could finally be included in the model development process. These patients were aged between 17 and 75 years, the body weight was between 44 and 129 kg. 1137 observations of concentration data were part of the analysis. Descriptive statistics of patient, disease and treatment characteristics are presented in Table 1.

Absorption from gut compartment was modeled as a first order process. A one-compartment model was given preference over a two-compartment model, apparently because there was not sufficient information available in the data to precisely estimate peripheral volume of distribution and intercompartmental clearance. Although an empirical two-compartment model provided a lower OFV, this was at the expense of highly imprecise parameter estimates for volume of distribution and intercompartmental clearance. Attempt to fix these parameters according to published fat to plasma concentration ratios (12) resulted in even a higher IIV with regard to central volume of
distribution and therefore conflicted with the model selection criteria. Fig. 1 provides a schematic representation of the model.

The parameter value for $F$ was fixed to 0.35 (11) and value for $k_a$ (49.9 day$^{-1}$) was also taken from the literature because of insufficient information available during the absorption phase of mitotane in the present data (52). Model simulations demonstrated that parameter estimate for $K_{\text{out}}$ did not have any substantial impact upon concentration time profile. Therefore, the parameter value for $K_{\text{out}}$ was fixed to 0.23 day$^{-1}$ according to literature (33). $CL_{\text{baseline}}$ was a priori assumed to be not estimable and sensitivity analysis using different $CL_{\text{baseline}}$ values of up to 60 L/day did not exhibit any changes in OFV, hence a value of 1 was used to describe the relative change over time. The drug was estimated to have a high central volume of distribution of 6086 L (4743-7676L; bootstrap 95% CI). Clearance of the drug was found to increase over time due to the enzyme induction process. A relative linear increase of 3.97 L/day (“Slope”, 3.22-4.80; bootstrap 95% CI) per day per mg/L mitotane plasma concentration was observed. A simple linear model was preferred over $E_{\text{max}}$ and sigmoidal $E_{\text{max}}$ models because of implausible estimates for $EC_{50}$ and $E_{\text{max}}$.

High IIV was associated with the volume of distribution (81.5%; point estimate). Extent of induction (Slope) was also found to be highly variable among the population (78.8%; point estimate). Estimates for RUV were adequately obtained with a combined error model. BMI was found to be a significant covariate for the volume of distribution, with an objective function value (OFV) decrease by 11 points. IIV was marginally reduced by 4.1% upon volume of distribution and 3.5% upon Slope. The comparison of the basic model and the covariate model in terms of decrease in IIV and OFV is represented in table 2. Table 3 presents bootstrap parameter estimates.
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Fig. 2 displays the basic goodness-of-fit (GOF) plots for the developed model. The IPRED were symmetrically distributed along the line of unity without any major outlier trends and PRED were adequate. CWRES were evenly distributed around zero depicting an adequate model performance over the concentration and time range. Individual plots (Fig. 3) exhibit the concentration time profiles for the observed and model predicted concentrations for four individuals exposed to mitotane treatment over different time periods ranging from 150 to 600 days. These plots illustrate that the model appropriately describes the diverse pharmacokinetic profiles across the studied population.

**Monte-Carlo simulations**

Temporal changes in clearance over time in a simulated population are depicted in Fig. 4. Median simulated plasma mitotane concentrations and respective percentiles (5\textsuperscript{th} and 99\textsuperscript{th}) with the high and the low dose regimen, respectively, are shown in Fig. 5. Persistent increase in plasma concentrations was observed. The 99\textsuperscript{th} percentile reached the upper range of the therapeutic window at around day 16 for the high dose regimen and on day 55 for the low dose regimen.

**4. Discussion**

In this by far largest and well characterized series of ACC patients on mitotane treatment, we investigate the pharmacokinetics of mitotane by implementing a nonlinear mixed effect modeling approach. The approach not only considers the fixed effects (descriptors of a process e.g., pharmacokinetic parameters and respective covariates) but also estimates the random variability (reflected by IIV and RUV) across the population by making use of nonlinear regression techniques. Mitotane showed a large and highly variable volume of distribution, partly explained by interindividual differences in BMI.
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Pronounced variability was also found for concentration dependent mitotane clearance attributable to autoinduction of mitotane metabolism.

Sparsity of data points as well as the complex pharmacokinetics were well handled by the population pharmacokinetic model as indicated by the GOF plots. A high volume of distribution is compatible with the extensive distribution of mitotane drug to adipose tissue (53). Moreover, high IIV associated with volume of distribution suggests a significant influence of individual patient characteristics. Individual patient demographics and lipid profiles were analyzed as possible sources of variability. While BMI was found to be a significant covariate for the volume of distribution, the reduction in IIV was marginal and individualization of therapy based on BMI seems not useful.

Our study has the particular strength of a uniform mode of measurement in regular time intervals and a large individual number of data points and corresponding clinical and laboratory data available. In comparison with a previous modeling effort (38) we come to a similar conclusion regarding high volume of distribution. Nevertheless, the published study has some important limitations although the data were informative enough to develop a three-compartmental model. Thus, the estimate for absorption rate constant (0.005 hr\(^{-1}\)) corresponds to a physiologically implausible absorption half-life of 138 hours in that study. Importantly, this model does not take enzyme autoinduction into account.

Empirical modeling approaches of metabolic autoinduction are frequently based on either of the two following approaches. The drug may be assumed to increase the rate of enzyme synthesis as demonstrated in case of rifampin, or it may decrease the rate of enzyme degradation as modeled in the case of ifosfamide (55, 56). Studies elucidating the mechanism of enzyme induction responsible for drug interactions concluded that mitotane increases gene expression of a number of transporters and enzymes, including CYP3A4 (57). Therefore, we assumed an increase in enzyme synthesis as an approach for
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modeling enzyme induction. An attempt was made to estimate a baseline value for mitotane clearance prior to any enzyme induction process but limited information in the initial phase of dosing precluded a reliable estimate. The sparsity of initial TDM data reflects the current practice of monitoring mitotane 3-4 weeks after the first dose. Therefore, the enzyme compartment which represented a time changing clearance of the drug was initialized to a baseline value of 1 (100%). With regard to the magnitude of mitotane induction of drug metabolizing enzymes, a limited parallel group comparison study revealed 18.3 fold and 5.0 fold decrease in midazolam (a CYP3A probe drug) and sunitinib AUCs (both administered orally) by mitotane, respectively (31). As a comparison, the very potent known CYP3A inducer rifampin caused a 4-fold reduction in oral sunitinib plasma exposure (58), while it decreased AUC of orally and intravenously administered CYP3A probe substrates by 10-20 fold and 1.9-3.5 fold respectively (33). The effect on intravenously administered CYP3A probe substrates reflects hepatic CYP3A induction. A reasonable estimate for maximal induction of hepatic CYP3A activity by mitotane may therefore be about 5-fold. The larger changes in mitotane clearance according to our empirical model (Fig. 4) suggest an additional induction of gut wall enzymes.

In order to quickly establish antitumor efficacy, it is imperative to attain target concentrations as early as possible. Previously, efforts have been made to develop an appropriate dosing regimen with mitotane considering ≥14 mg/L as the target concentration. Appropriate plasma levels can be ultimately achieved with a chronic dose from the beginning of treatment but at the expense of a lag time to achieve therapeutic concentrations (24). This period may be shortened with high loading dose regimens but at the cost of a higher risk to attain potentially toxic concentrations (25). Studies indicating high variation in plasma concentration buildup suggest the involvement of factors other
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than the dosage regimen such as enzyme induction and differences in intestinal absorption due to dietary variation (26). Our model was not able to identify major sources of interindividual variability; therefore, an exact prediction of the individual required dose is not possible. Hence TDM remains essential for mitotane treatment. Simulations based on our model however were supportive of using the high dose regimen for the rapid attainment of therapeutic concentrations (Fig 5).

The current model is expected to be helpful for decision making in clinical management of ACC with mitotane. It can be used for *a posteriori* dose adjustments in patients using Maximum *a posteriori* (MAP) Bayesian methods (59) based on limited TDM measurements (two or three) to estimate individual pharmacokinetic parameters. MAP Bayesian approaches have proven their benefit in TDM of a number of anticancer drugs including methotrexate and carboplatin (60, 61). Another important aspect regarding decision making is the addition of cytotoxic chemotherapy in patients who are not predicted to attain therapeutic concentrations within a clinically useful time frame (e.g. 90 days) when using their individual maximum tolerated dose. Also, in a palliative setting where response to mitotane monotherapy is limited (6), optimal dosing may be supported when drug exposure related parameters are taken into account in addition to tissue based markers of response (10). From a research perspective, the model may be used to link mitotane pharmacokinetics to pharmacodynamic endpoints such as tumor growth inhibition.

The limitations of the evaluation related to retrospective nature of the present study suggest to design a prospective study for a more physiological and a more detailed description of mitotane pharmacokinetics. It would be desirable to (i) take more samples (e.g., 1 per day) during the initial build-up of plasma concentration; (ii) occasionally apply a dense sampling scheme including several samples within the first hour after dose
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during one dosing interval to describe mitotane absorption kinetics; (iii) evaluate the
effect of additional covariates such as food intake, disease state and co-medications on
mitotane pharmacokinetics, and (iv) to quantify enzyme induction during therapy by
separate CYP3A probe drugs such as midazolam.

5. Conclusion

The proposed model appropriately describes plasma concentrations during chronic
treatment with mitotane. It includes concentration dependent induction of metabolizing
enzymes that considerably accelerates mitotane elimination. If tolerated, using the high
dose regimen with a first TDM on day 16 of treatment might be a good treatment
strategy. The model is a next important step to use pharmacokinetic modeling to improve
personalized dose selection as well as establishing the timing of TDM, while more data
are urgently needed.

6. Declaration of interest

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

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levels of mitotane in a patient with stage IV adrenocortical carcinoma (ACC). 


10. Figure legends

**Fig 1.** A schematic representation of one compartment pharmacokinetic model linked to the enzyme induction model. Mitotane plasma concentrations increasing the enzyme formation rate and enzyme amount is in turn affecting the mitotane clearance.

**Fig 2.** Goodness of fit plots; observed vs individual predicted concentration (A) observed vs population predicted concentrations (B) conditional weighted residuals vs population predicted concentrations (C) conditional weighted residuals (CWRES) vs time after first dose (D).

**Fig 3.** Individual plots of 4 patients treated with mean mitotane doses of 4.12g (A), 4.57g (B), 3.91g (C) and 4.77g over different periods of time (TAFD, time after first dose; -●-observed concentrations; -▲-predicted concentrations). Doses (rectangular boxes with associated lines) represent actual daily doses.

**Fig 4.** Change in clearance over time in a simulated population of 500 virtual subjects (median with 5th and 95th percentiles).

**Fig 5.** Concentration vs time plot for a simulated population of 500 virtual subjects administered with the clinically used highy dose (A) and low dose (B) mitotane regimens (median, 5th and 99th percentiles). The region between the dashed lines represents the therapeutic window. The vertical lines identify the time points proposed for the first TDM sampling for the two dosing regimens.
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11. Table legends

Table 1. Patient, disease and treatment characteristics

Table 2. Comparison of base and covariate model

Table 3. Model parameter estimates
Gut compartment

ka

Central compartment (V)

CL

Enzyme compartment

\( K_{in}(1 + \text{Slope} \cdot C_p) \)

\( K_{out} \)
### Patient characteristic

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<th>Characteristic</th>
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<td>Age, years [mean ± sd]</td>
<td>48.6 ± 11.8</td>
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<td>Sex, female [n (%)]</td>
<td>45 (59.2%)</td>
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<td>Body height, m [mean ± sd]</td>
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<td>Body weight, kg [mean ± sd]</td>
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<td>BMI, kg/m² [mean ± sd]</td>
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<td>Plasma cholesterol, mg/dL [mean ± sd]</td>
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<td>Plasma creatinine, mg/dL [mean ± sd]</td>
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### Disease characteristics

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### Treatment characteristics

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<td>5 (1-5)</td>
</tr>
<tr>
<td>EDP</td>
<td>34 (45)</td>
</tr>
<tr>
<td>Streptozotocin</td>
<td>29 (38)</td>
</tr>
<tr>
<td>Gemcitabine/Capecitabine</td>
<td>13 (17)</td>
</tr>
<tr>
<td>other</td>
<td>16 (21)</td>
</tr>
<tr>
<td>unknown</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

*Treatment initiation in adjuvant intention, later continued as palliative treatment

BMI: Body Mass Index, ENSAT: European Network for the Study of Adrenal Tumors, EDP: Etoposide, Doxorubicin, Cisplatin regimen.
Table 2: Comparison of base and covariate model

<table>
<thead>
<tr>
<th>Model</th>
<th>Objective function value</th>
<th>Interindividual variability (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base model</td>
<td>4764.4</td>
<td>81.5 (V), 78.8 (Slope)</td>
</tr>
<tr>
<td>Covariate model</td>
<td>4753.3</td>
<td>77.3 (V), 75.2 (Slope)</td>
</tr>
</tbody>
</table>

CV: coefficient of variation, V: volume of distribution
Table 3: Model parameter estimates

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Parameter</th>
<th>median estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ka (day⁻¹)</td>
<td>49.9</td>
<td>Fixed</td>
</tr>
<tr>
<td></td>
<td>F (%)</td>
<td>35</td>
<td>Fixed</td>
</tr>
<tr>
<td></td>
<td>V/F (L)</td>
<td>6086</td>
<td>4743-7673</td>
</tr>
<tr>
<td></td>
<td>Kᵢᵣ (day⁻¹)</td>
<td>0.23</td>
<td>Fixed</td>
</tr>
<tr>
<td></td>
<td>Kᵢᵢᵣ (day⁻¹)</td>
<td>0.23</td>
<td>Fixed</td>
</tr>
<tr>
<td></td>
<td>slope (L day⁻¹ day⁻¹)</td>
<td>3.97</td>
<td>3.22-4.80</td>
</tr>
<tr>
<td></td>
<td>BMI covariate effect upon V (fractional change from typical value of V per unit of BMI)</td>
<td>0.055</td>
<td>0.01-0.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interindividual variability</th>
<th>Parameter</th>
<th>median estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ηᵢ (ω²)</td>
<td>0.54</td>
<td>0.28-0.87</td>
</tr>
<tr>
<td></td>
<td>ηᵢ (ω²)</td>
<td>0.56</td>
<td>0.35-0.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residual variability (combined error model)</th>
<th>Type</th>
<th>median estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Additive (σ²)</td>
<td>0.24</td>
<td>0.18-0.29</td>
</tr>
<tr>
<td></td>
<td>Proportional (σ²)</td>
<td>2.28</td>
<td>1.50-3.13</td>
</tr>
</tbody>
</table>

ka: absorption rate constant, F: bioavailability, V: volume of distribution, Kᵢᵣ: rate of enzyme synthesis, Kᵢᵢᵣ: rate of enzyme degradation, ηᵢ: interindividual variability in V, ηᵢ (ω²): interindividual variability in slope, ω² and σ²: variance, BMI: body mass index as kg m⁻², CI: confidence interval.