



# Population Pharmacokinetic and Pharmacogenetic Analysis of Mitotane in Patients with Adrenocortical Carcinoma: Towards Individualized Dosing

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## Abstract

**Background** Mitotane is the only approved treatment for patients with adrenocortical carcinoma (ACC). A better explanation for the variability in the pharmacokinetics (PK) of mitotane, and the optimization and individualization of mitotane treatment, is desirable for patients.

**Objectives** This study aims to develop a population PK (PopPK) model to characterize and predict the PK profiles of mitotane in patients with ACC, as well as to explore the effect of genetic variation on mitotane clearance. Ultimately, we aimed to facilitate mitotane dose optimization and individualization for patients with ACC.

**Methods** Mitotane concentration and dosing data were collected retrospectively from the medical records of patients with ACC taking mitotane orally and participating in the Dutch Adrenal Network. PopPK modelling analysis was performed using NONMEM (version 7.4.1). Genotypes of drug enzymes and transporters, patient demographic information, and clinical characteristics were investigated as covariates. Subsequently, simulations were performed for optimizing treatment regimens.

**Results** A two-compartment model with first-order absorption and elimination best described the PK data of mitotane collected from 48 patients. Lean body weight (LBW) and genotypes of *CYP2C19*\*2 (rs4244285), *SLCO1B3* 699A>G (rs7311358) and *SLCO1B1* 571T>C (rs4149057) were found to significantly affect mitotane clearance (CL/F), which decreased the coefficient of variation (CV%) of the random inter-individual variability of CL/F from 67.0 to 43.0%. Fat amount (i.e. body weight – LBW) was found to significantly affect the central distribution volume. Simulation results indicated that determining the starting dose using the developed model is beneficial in terms of shortening the period to reach the therapeutic target and limit the risk of toxicity. A regimen that can effectively maintain mitotane concentration within 14–20 mg/L was established.

**Conclusions** A two-compartment PopPK model well-characterized mitotane PK profiles in patients with ACC. The *CYP2C19* enzyme and *SLCO1B1* and *SLCO1B3* transporters may play roles in mitotane disposition. The developed model is beneficial in terms of optimizing mitotane treatment schedules and individualizing the initial dose for patients with ACC. Further validation of these findings is still required.

## 1 Introduction

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy (1 per million per year) with a poor prognosis and limited treatment options [1]. Mitotane, a highly lipophilic compound, is the only treatment approved by the US FDA and the European Medicines Agency for ACC [1]. Mitotane is developed as an orally administered treatment and its absorption is improved by concomitant intake of fat-rich food [2]. The bioavailability of mitotane is around 35–40% [3]. Mitotane has a high volume of distribution and the

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### Key Points

A two-compartment population pharmacokinetic (PK) model with first-order absorption and elimination was developed for mitotane based on PK data collected from 48 adrenocortical carcinoma patients.

The pharmacogenetic variation of *CYP2C19*\*2 (rs4244285), *SLCO1B3* 699A>G (rs7311358) and *SLCO1B1* 571T>C (rs4149057) was found to have a significant effect on mitotane clearance. Fat amount, which was defined as the difference between total body weight and lean body weight, had a significant effect on the central distribution volume.

With the help of the model, mitotane treatment can be guided and optimized for individual patients. Further validation of the findings is warranted to confirm the results. .

primary distribution site is fat [3, 4]. The half-life of mitotane elimination ranges from 18 to 159 days, with a median of 53 days [2, 3].

The efficacy and toxicity of mitotane are related to the plasma concentration [1, 3]. In order to ensure efficacy and avoid increased toxicity, the mitotane plasma concentration should be between the therapeutic range of 14 and 20 mg/L, which requires therapeutic drug monitoring (TDM) [1].

However, due to the large distribution volume and long half-life of mitotane, a long-time interval (around 3–5 months [1]) is usually required for patients to reach the effective concentration [3], which limits the clinical utility of mitotane. The inability to reliably predict mitotane plasma concentrations may result in a prolonged time to reach the target value, hence causing a significant delay in tumour treatment, or may give rise to drug toxicity. In addition, it has been demonstrated that only half of the patients who received a high-dose regimen for 3 months achieved the target [5], suggesting a demand for individualized treatment and a presence of high inter-individual variability (IIV) in the pharmacokinetics (PK) of mitotane. Currently, the dosage titration is largely expert-based, making it prone to errors. Therefore, a tool enabling mitotane concentration prediction and an optimized treatment regimen for individual patients, which shortens the period required to reach the target concentration while limiting the toxicity, would be desirable for patients with ACC.

A population PK (PopPK) modelling approach with mixed-effect models enables quantitative characterization and prediction of drug PK profiles for both the study population and individuals [6]. The development of a PopPK model of mitotane would be beneficial for the characterization and

understanding of mitotane PK, as well as for the optimization and personalization of mitotane treatment. Until now, two studies have performed PopPK modelling analysis on mitotane in patients with ACC [3, 7]; one-compartment models were developed in these two studies. One study assuming a self-induced clearance and a body mass index (BMI) was found to be a covariate of mitotane distribution volume [3], while the other study identified the effects of triglyceride and high-density lipoprotein on mitotane clearance [7]. Another model-based PK study of mitotane developed a three-compartment model and showed weak correlations of age, sex, body weight, height, and body surface area with model parameters [8].

In order to further elucidate the variability of mitotane PK, it would be beneficial to explore the effect of pharmacogenetic polymorphisms [8]. Although the exact PK pathway of mitotane and the enzymes involved in mitotane metabolism remain unknown [9], two studies suggested possible roles for cytochrome P450 (CYP) 2B6 and CYP2C9 [10, 11]. One study demonstrated that the genotype of *CYP2B6*\*6 (rs3745274) was significantly correlated with mitotane plasma concentrations at 3 and 6 months after the initiation of treatment [10]. The other study showed that one patient with high mitotane concentration was a CYP2C9 intermediate metabolizer [11]. Further analysis of the relationship between genes encoding for PK enzymes and transporters and mitotane PK profiles, and incorporating these variables into a PopPK model, may allow better explanation of mitotane PK variability.

In the current study, a PopPK analysis was performed for mitotane in patients with ACC utilizing the retrospectively collected PK data. The effect of genes encoding drug absorption, distribution, metabolism, and elimination (ADME), patient demographic information, and clinical characteristics on mitotane PK were investigated as covariates. We aimed to develop a PopPK model to describe and predict the PK of mitotane in patients with ACC, as well as to explore the effect of genetic variation on mitotane clearance. Moreover, we intended to better explain mitotane PK variability using the developed model and to facilitate treatment optimization and individualization for patients with ACC.

## 2 Methods

### 2.1 Patients

Forty-nine adult patients diagnosed with ACC ( $\geq 18$  years of age) who were enrolled in the Dutch Adrenal Network Registry, had been treated with mitotane, had provided consent, and had available mitotane dosing information as well

as concentration data were included in this PopPK analysis. One patient was eventually excluded because of missing information regarding starting dose.

The study was approved by the Medical Ethical Committee of the Máxima Medical Center, Veldhoven (2015), and approval for the inclusion of patients in other institutes was obtained from the local boards. The required informed consents were obtained from all patients. All procedures performed in this study were in accordance with the ethical standards of the institutional Medical Ethical Committee and the 1964 Helsinki Declaration.

## 2.2 Pharmacokinetic (PK) Data

Data on mitotane plasma concentrations, including concentrations from routine TDM, data sampled during one treatment interval, and data collected after treatment discontinuation, as well as all mitotane dosing data, were collected retrospectively from patients' medical records. Patients administered mitotane orally were advised to take mitotane with fat-rich food. Concomitant medication information was not included in the current analysis since the data were not complete. The mitotane plasma concentrations were determined by a validated gas-chromatography/mass spectrometry assay at the Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center (LUMC) [12]. The lower limit of quantification (LLOQ) was 2 mg/L. In addition, patients' demographic information, including age, sex, and body weight (WT) and height (HT) at the start of treatment, were collected. Furthermore, levels of serum aspartate transaminase (ASAT), alanine transaminase (ALAT), gamma-glutamyltransferase ( $\gamma$ GT), total cholesterol, and estimated glomerular filtration rate (GFR; recorded as 0 if the result was  $\geq 60$  mL/min/1.73 m<sup>2</sup>, otherwise 1) were also collected in our analysis.

Lean body weight (LBW) and fat amount (FAT) were also calculated for each patient. LBW was estimated using the *Boer* formula [13] and FAT was obtained by subtracting LBW from WT.

## 2.3 Genotyping Method

The DNA of included patients was isolated from EDTA blood samples using Maxwell (Promega, Leiden, The Netherlands) or MagNAPure compact (Roche, Almere, The Netherlands). Genotyping of patients was performed using the Drug Metabolizing Enzymes and Transporters (DMET™) Plus array (Affymetrix UK Ltd, High Wycombe, UK), which contains 1936 genetic variants (1931 single nucleotide polymorphisms [SNPs] and 5 copy number variations [CNVs]) of ADME-related enzymes and transporters [14], according

to the manufacturers' protocol. The method has been previously described in detail [15, 16].

A preset selection was performed using the DMET™ console software that generates fully annotated marker reports based on a translation file as recommended by Affymetrix® [17]. The reports include commonly recognized, haplotype-based allele calls commonly cited in Medline reference studies [18–20]. The DMET™ Plus allele translation software produces a comprehensive genotyping report containing pharmacogenomic reference data on all probes. This step leads to the selection of 959 SNPs from the total of 1931 SNPs present on the DMET™ platform. Subsequently, the SNPs that deviated from Hardy–Weinberg equilibrium ( $p < 0.0001$ ), with a call rate below 97% or with a minor allele frequency (MAF)  $< 0.1$ , as well as tri-allelic SNPs and SNPs of genes located on the X chromosome, were excluded from further analysis.

## 2.4 Population PK Model Development

Based on the obtained mitotane concentration data, a non-linear mixed-effects model was developed. Parameters were estimated using the first-order conditional estimation method with interaction (FOCEI) implemented in NONMEM software version 7.4.1 (ICON Development Solutions, Ellicott City, MD, USA). One-, two- and three-compartment models with first-order absorption and first-order elimination were explored as the structural model. Data points below the LLOQ were omitted since they only contributed to 3.6% of the observations [21, 22].

Since the majority of collected data were trough concentrations, and data regarding the absorption phase were limited, the absorption rate constant ( $K_A$ ) was first estimated based on a sub-dataset containing data of the patients who contributed multiple data points during one treatment interval at steady state. The  $K_A$  estimate was then fixed to analyze the full dataset. Inter-occasion variability (IOV) was incorporated on apparent systemic clearance ( $CL/F$ ) and every 200 days of treatment was defined as an occasion. In addition, to simplify the situation, all patients were assumed to receive a single dose once daily at 8:00 am, with the dose amount being equal to the total daily dose.

A further detailed description of the PopPK modelling methods is shown in Online Resource 1.

## 2.5 Identify Potential Correlated Single Nucleotide Polymorphisms and Covariate Analysis

Since knowledge regarding the relationship between mitotane clearance and pharmacogenetic polymorphisms is limited, an exploratory analysis was first performed to find potential SNPs that were correlated with mitotane

clearance. The estimates of random IIV of  $CL/F$  ( $\eta_{IIVi_{CL}}$ ) from the basic model and the genotyping results were utilized. For each SNP, when the number of patients in a minor homozygous group was  $< 4$ , the results of these patients were combined with the corresponding heterozygote group for the association analysis assuming a dominant allele effect. Additionally, when the number of patients with genotype results of ‘zero copy number’ or ‘possible rare allele’ was less than four, or when patients had ‘NoCall’ results, the results were not included for statistical analysis. A one-way analysis of variance (ANOVA) test and a two-sided  $t$  test were performed using R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria) to evaluate the difference in  $\eta_{IIVi_{CL}}$  across genotype groups for each SNP. Selection of the test method depended on the number of genotype groups of each SNP after the combination. The SNPs were considered to correlate with mitotane clearance if the  $p$ -value was  $< 0.05$ . Correction for multiple testing was not performed due to the exploratory characteristics of the current analysis.

The identified SNPs, as well as patient demographic information and clinical characteristics, were considered in the covariate analysis. The stepwise covariate modelling (SCM) function implemented with Perl-Speaks-NONMEM (version 4.7.0) [23] was applied. Both forward inclusion ( $p < 0.05$ ) and backward elimination processes ( $p < 0.01$ ) were performed to identify significant covariates. A more detailed description of the covariates analysis is shown in Online Resource 1.

## 2.6 Model Evaluation

The predictability and stability of the final model was evaluated using goodness-of-fit (GOF) plots, prediction-corrected virtual predicted checks (pcVPC) [24], and non-parametric bootstrap. Normalized prediction distribution errors (NPDEs) were also applied for evaluation. All figures were created using R (The R Foundation for Statistical Computing). A detailed description of the evaluation methods is shown in Online Resource 1.

## 2.7 Simulations for Treatment Optimization

Based on the final model, simulations were performed to optimize mitotane dosing regimen and starting dose determination, in order to shorten the target-reaching time while limiting the risk of toxicity. The simulation was performed for patients included in this study, as they are considered to be able to represent the corresponding adult patient population. The individual parameters of each patient were used to simulate the ‘real’ mitotane concentrations ( $C_{sim\_real}$ ) under each regimen. The residual errors were not considered. Different

strategies of adjusting the dose according to  $C_{sim\_real}$  are shown in Fig. 1. All simulations were performed using R (The R Foundation for Statistical Computing) and the differential equations were solved using the RxODE package (version 0.6-1) [25]. A detailed description of the regimens and simulation methods are shown in Online Resource 1.

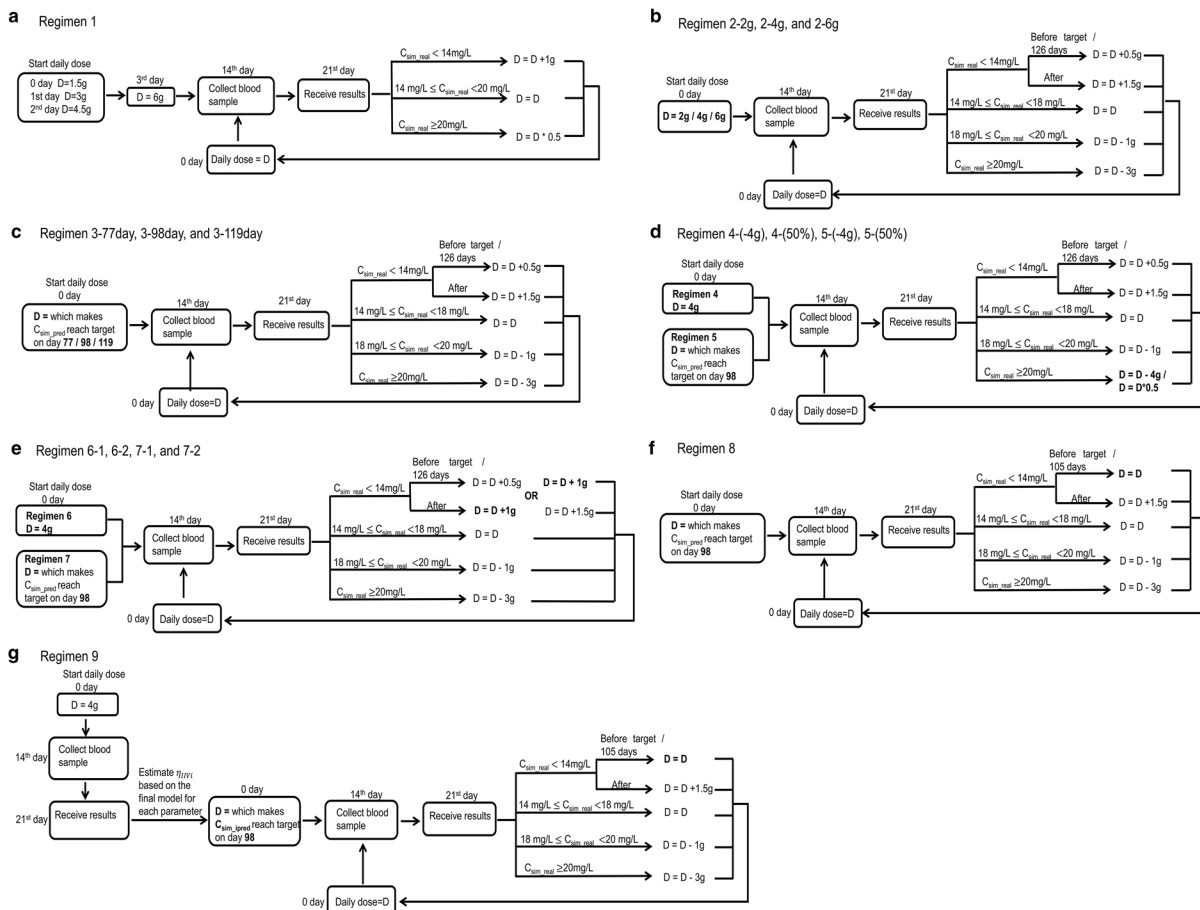
On the basis of the simulated PK curves, for patients who originally reached the target, the mean and maximum time needed to reach the target ( $T_{target}$ , the first day when  $C_{sim\_real} \geq 14$  mg/L), the mean percentage of days when  $C_{sim\_real}$  was higher than 20 mg/L in the first 200 days ( $P_{toxicity}$ ) and the mean percentage of  $C_{sim\_real}$  located outside the therapeutic window after reaching the target ( $P_{o.window}$ ), were calculated and compared across different strategies.  $P_{toxicity}$  represents the probability of causing toxicity in the early phase of treatment, and  $P_{o.window}$  represents the ability to maintain the concentration within the therapeutic window. Meanwhile, the median maximum and minimum  $C_{sim\_real}$ , as well as the range of determined starting doses, were also collected and evaluated. As an optimized regimen is expected to be able to ensure a shorter target-reaching time and well-maintain the concentration within the therapeutic window while not causing much toxicity, the optimization target was defined as the mean  $T_{target} \leq 90$  days (3 months), the mean  $P_{toxicity} \leq 10\%$ , and the mean  $P_{o.window} \leq 15\%$ .

Using the optimized regimen, a Shiny application was created based on the Shiny package (version 1.4.0) and the RxODE package in R (The R Foundation for Statistical Computing) in order to perform simulation for a random patient and to elucidate an option of providing treatment advice for a new patient based on the model. A detailed description is shown in Online Resource 2.

## 3 Results

### 3.1 Patients and Data

Data from 48 patients with ACC (21 males and 27 females) were included in the PopPK analysis. The characteristics of patients are summarized in Table 1. Patients received mitotane treatment between 2002 and 2017, and the median duration of treatment was 713.5 days (range 90–2856). The total daily dosage ranged from 0.5 to 16 g/day and was divided into one to four doses. Five (two patients), six (one patient), and eight (one patient) daily dosages were also applied occasionally. Forty-one patients reached the concentration target during treatment, among whom 16 patients reached the target after 150 days. In total, 914 concentration data points were collected from patients’ electronic hospital records, 33 of which were below the LLOQ. The time-course of collected mitotane concentrations is shown in Fig. 2. Nine patients contributed multiple sampling data within one



**Fig. 1** Designed treatment regimens that were evaluated by simulation. **a** A previously reported dosing regimen (Regimen 1), where the dose started as 1.5 g/day and increased up to 6 g/day in 4 days and continued until the next dose adjustment. The dosage was adjusted each time according to the monitored mitotane concentration level. **b** Regimens where all patients started with 2 g (Regimen 2–2 g), 4 g (Regimen 2–4 g) or 6 g (Regimen 2–6 g) per day. The dosage increased by 0.5 g every 21 days until the target was reached, or 126 days if  $C_{sim\_real} < 14$  mg/L. Thereafter, the dosage increased by 1.5 g if  $C_{sim\_real} < 14$  mg/L, remained unchanged if  $14 \text{ mg/L} \leq C_{sim\_real} < 18 \text{ mg/L}$ , decreased by 1 g if  $18 \text{ mg/L} \leq C_{sim\_real} < 20 \text{ mg/L}$ , and decreased by 3 g if  $C_{sim\_real} \geq 20 \text{ mg/L}$ . **c** Regimens where patients started with an individualized dose that allowed  $C_{sim\_pred}$  on day 77 (Regimen 3–77 day), 98 (Regimen 3–98 day) or 119 (Regimen 3–119 day) to reach the target. The remaining dose-adjustment strategies were the same as Regimen 2. **d** Regimens where patients started with 4 g/day (Regimen 4) or an individualized dose (Regimen 5) and the dosage decreased by 4 g, or 50%, if  $C_{sim\_real} \geq 20 \text{ mg/L}$ . The remaining dose-adjustment strategies were the same as Regi-

men 2. **e** Regimens where patients started with 4 g/day (Regimen 6) or an individualized dose (Regimen 7) and the dosage increased by 1 g after reaching target or 126 days if  $C_{sim\_real} < 14 \text{ mg/L}$  (Regimens 6-1 and 7-1), or increased by 1 g until reaching target or 126 days if  $C_{sim\_real} < 14 \text{ mg/L}$  (Regimens 6-2 and 7-2). The remaining dose-adjustment strategies were the same as Regimen 2. **f** A regimen where patients started with an individualized dose that remained unchanged until reaching target or 105 days if  $C_{sim\_real} < 14 \text{ mg/L}$ . The remaining dose-adjustment strategies were the same as Regimen 2. **g** A regimen where patients started with 4 g/day for the first 21 days and the next dosage was determined that allowed  $C_{sim\_ipred}$  on day 98 to reach the target (Regimen 9). The remaining dose-adjustment strategies were the same as Regimen 8.  $C_{sim\_real}$  simulated ‘real’ mitotane concentrations based on individual parameters,  $C_{sim\_pred}$  model predictions based on patient characteristics,  $C_{sim\_ipred}$  model predictions using individual parameters, i.e. incorporating the inter-individual variability ( $\eta_{IVi}$ ) estimated based on the first monitored concentration

treatment interval and 13 patients had more than one data point collected after treatment discontinuation. The median number of data points contributed by each patient was 16.5, ranging from 2 to 47.

### 3.2 The Basic Model

Based on the sub-dataset containing data from the nine patients with multiple sampling data within one treatment interval, the KA was estimated as 22.1 (/day) and 15.0 (/day)

**Table 1** Patient characteristics ( $n=48$ )

Characteristic	Value/mean	SD	Range
<i>Patient characteristics</i>			
No. of patients	48		
Sex, male [ $n$ (%)]	21 (43.8)		
Age, years <sup>a</sup>	52.0	12.1	22.6–76.8
Weight, kg <sup>a</sup> ( $n=2$ no record)	80.0	15.9	52.5–120
Height, cm <sup>a</sup> ( $n=5$ no record)	172	10.0	154–193
BMI, kg/m <sup>2a</sup> ( $n=5$ no record)	27.1	4.48	18.2–38.3
LBW, kg <sup>a</sup> ( $n=5$ no record)	55.8	10.0	39.7–78.5
ASAT, IU/L <sup>b</sup> ( $n=1$ no record)	45.15	35.3	16–185
ALAT, IU/L <sup>b</sup> ( $n=1$ no record)	42.68	35.6	9–197
$\gamma$ GT, IU/L <sup>b</sup> ( $n=1$ no record)	278.70	215.9	55–898
GFR, > 50% of records were normal [ $n$ (%)] ( $n=7$ no record)	39 (95.1)		
Cholesterol, mmol/L <sup>b</sup> ( $n=11$ no record)	6.54	1.56	3.6–11.6
<i>Disease characteristics [<math>n</math> (%)]</i>			
ENSAT I, patients	2 (4.2)		
ENSAT II, patients	19 (39.6)		
ENSAT III, patients	10 (20.8)		
ENSAT IV, patients	17 (35.4)		
<i>Target-reaching characteristics</i>			
No. of patients who reached the target	41		
> 150 days [ $n$ (%)]	16 (39.0)		
$\leq$ 90 days [ $n$ (%)]	19 (46.3)		
Target-reaching time, days	142	113.9	24–579
Duration of treatment, days	742	553.2	90–2856

SD standard deviation, BMI body mass index, LBW lean body weight, ASAT aspartate transaminase, ALAT alanine transaminase,  $\gamma$ GT gamma-glutamyltransferase, GFR glomerular filtration rate, ENSAT European Network for the Study of Adrenal Tumors

<sup>a</sup>At the start of treatment

<sup>b</sup>Mean record of each patient

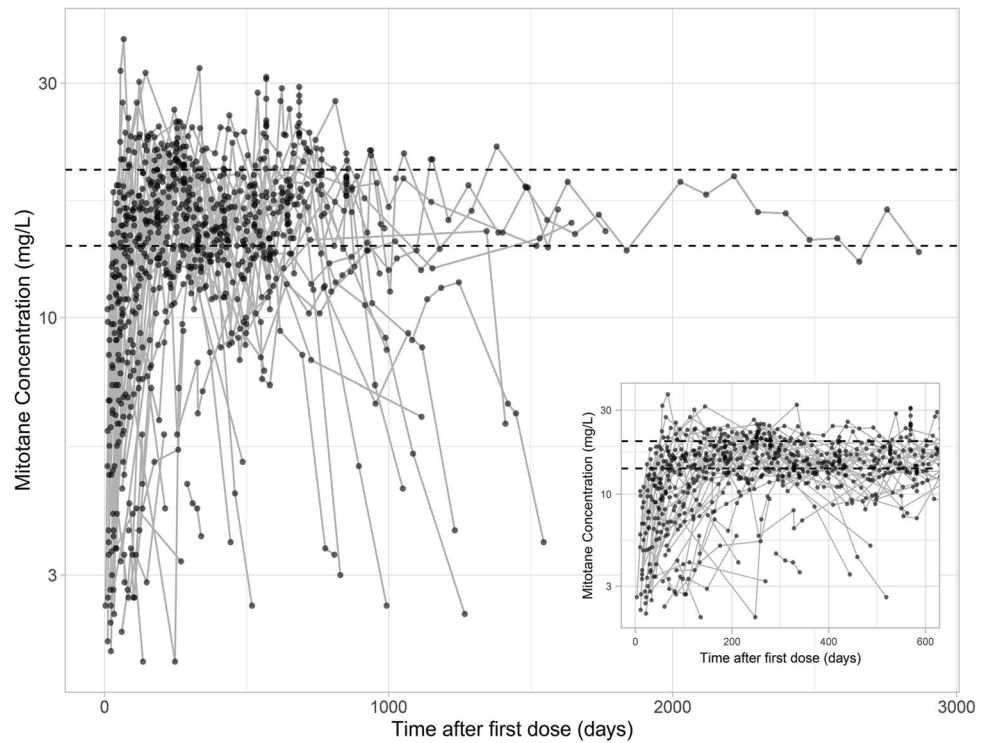
under a one-compartment and a two-compartment model structure, respectively. A three-compartment model could not be identified, since (1) the time-course of mitotane concentration did not meet the characteristics of a three-compartment model; and (2) when running the three-compartment model, the parameters were shown to be unidentifiable. The basic models were then developed by fitting the full dataset with fixed KA and incorporating IOV on CL/F. The relative standard error (RSE) parameter estimates of both two-model structures were all within the acceptable range (<30%). The objective function value (OFV) of the two-compartment model was reduced by 92.13 compared with that of the one-compartment model ( $p < 0.001$ , degree of freedom = 4), suggesting an improvement on the model fitness. Therefore, the two-compartment model was ultimately selected for describing mitotane PK profiles in patients with ACC in this study. The model structure is shown in Online Resource 1, Fig. S1. The parameter estimates of the basic model are shown in Table 2. The high percentage coefficient of variation (CV%) of IIV for all parameters was identified,

and the CV% of IIV for the apparent distribution rate constant (Q/F) was even higher than 100%.

### 3.3 Pharmacogenetic Analysis

For each patient, the genotyping results of the 959 SNPs from the DMET™ platform were obtained. A list of these SNPs can be found in Online Resource 3. All SNPs were in Hardy–Weinberg equilibrium ( $p \geq 0.0001$ ). A flow diagram of the selection of genetic variants is shown in Fig. 3. Eventually, 172 SNPs were included for further investigation. Among these 172 SNPs, 55 had less than four patients belonging to the minor homozygous group. The ‘NoCall’ result was reported in one patient in 19 SNPs and the ‘Possible Rare Allele’ result was reported in one patient in one SNP. The results of these patients were thus not included in the association analysis of corresponding SNPs. In contrast, the ‘Zero Copy Number’ result occurred in three SNPs in 8, 24, and 24 patients, respectively. Thus, patients with a ‘Zero

**Fig. 2** Mitotane concentration–time curve collected from patients on the logarithmic scale. Inserts show the data during the first 600 days of treatment



**Table 2** Parameter estimates of both the basic and final models

Parameters	Basic model			Final model			Bootstrap	
	Estimate (RSE %)	IIV (CV%) [shrinkage, %]	IOV <sup>c</sup> (CV%)	Estimate (RSE %)	IIV (CV%) [shrinkage, %]	IOV <sup>c</sup> (CV%)	Median	95% CI
KA (/day)	15.0 fixed	–	–	15.0 fixed	–	–	15	–
CL/F (L/day) <sup>a</sup>	217 (11)	67.0 [8]	30.5	298 (13)	43.0 [16]	31.6	281.6	200.5–398.4
CL_SNP1 (GA/AA)	–	–	–	0.551 (15)	–	–	0.573	0.385–0.881
CL_SNP2 (AG/GG)	–	–	–	0.601 (19)	–	–	0.613	0.419–0.949
CL_SNP3 (CC)	–	–	–	0.753 (10)	–	–	0.784	0.550–1.07
CL_SNP3 (TT)	–	–	–	2.49 (29)	–	–	2.67	0.991–6.16
CL_LBW (power)	–	–	–	1.10 (16)	–	–	1.07	0.205–2.13
V <sub>c</sub> /F (L) <sup>b</sup>	4790 (20)	68.1 [53]	–	6210 (18)	47.2 [55]	–	6795	3281–10,752
V <sub>c</sub> _FAT (power)	–	–	–	1.22 (19)	–	–	1.29	0.450–2.18
V <sub>p</sub> /F (L)	19,300 (13)	76.9 [17]	–	18,100 (12)	88.8 [15]	–	17882	11,341–25,709
Q/F (/day)	1100 (21)	102 [34]	–	883 (20)	97.3 [34]	–	785.4	337.4–1502
<i>Residual errors</i>								
PRO (CV%)	16.6 (7)	–	–	16.6 (6)	–	–	16.8	14.2–18.9
ADD (mg/L)	0.931 (28)	–	–	0.920 (17)	–	–	0.871	0.373–1.384

SNP1 CYP2C19\*2 (rs4244285), SNP2 SLCO1B3 699A>G (rs7311358), SNP3 SLCO1B1 571T>C (rs4149057), LBW lean body weight, FAT fat amount, RSE relative standard error, CV coefficient of variation, IIV interindividual variability, IOV interoccasion variability, PRO proportional residual error, ADD additive residual error, CL/F apparent systemic clearance, KA absorption rate constant, V<sub>c</sub>/F apparent distribution volume of the central compartment, V<sub>p</sub>/F apparent distribution volume of the peripheral compartment, Q/F apparent distribution rate constant, CI confidence interval

$$^a\text{CL}/F = \text{CL}/F_t \times \text{CL\_SNP1} \times \text{CL\_SNP2} \times \text{CL\_SNP3} * \left(\frac{\text{LBW}}{56.6}\right)^{\text{CL\_LBW}}$$

$$^bV_c/F = V_c/F_t \times \left(\frac{\text{FAT}}{23.6}\right)^{V_c\_FAT}$$

<sup>c</sup>Every 200 days of dosing was defined as an occasion

Copy Number' were treated as a different genotype group in the association analysis of these three SNPs.

Finally, the result of the association test showed that 11 SNPs, as shown in Online Resource 1, Table S1, were potentially related to mitotane clearance ( $p \leq 0.05$ ). Among these 11 SNPs, the genotyping results of *CYP2C18* 1154C>T (rs2281891) and *CYP2C19\*2* (rs4244285) were shown to be 100% in linkage disequilibrium in our dataset, which was the same as the genotyping results of *SLCO1B3* 334G>T (rs4149117), 699A>G (rs7311358), and 1557G>A (rs2053098) and that of the three SNPs located on *VKORC1* (283+124G>C, 174-136C>T, and -1639G>A). The results of the 11 identified SNPs were subsequently combined into the full dataset for stepwise covariate analysis.

### 3.4 The Final Model

The parameter estimates of the final model are shown in Table 2. The *CYP2C19\*2* (rs4244285), *SLCO1B3* 699A>G (rs7311358) and *SLCO1B1* 571T>C (rs4149057) genotypes, and LBW at the start of treatment, with power

relation, were found to have a significant effect on the CL/F of mitotane (Table 2). Carrying the 'A' variant in *CYP2C19\*2* reduced the CL/F by 44.9%, and carrying 'G' variant in *SLCO1B3* 699A>G resulted in a 39.9% reduction in CL/F (Table 2). As for *SLCO1B1* 571T>C, the CL/F of patients carrying one 'C' variant decreased to 40.2% that of wild-type patients, while the CL/F of patients carrying two 'C' variants decreased to 30.2%. The distribution of  $\eta_{IVi_{CL}}$  derived from the basic model in each genotype group of the above three SNPs is shown in Online Resource 1, Fig. S2. In addition, FAT at the start of treatment with power relation was found to significantly influence the apparent distribution volume of the central compartment ( $V_c/F$ ). The inclusion of these covariates decreased the CV% of CL/F and  $V_c/F$  from 67.0% and 68.1% to 43.0% and 47.2%, respectively. Overall, the parameter estimates were shown to be in good agreement with the bootstrap results (Table 2).

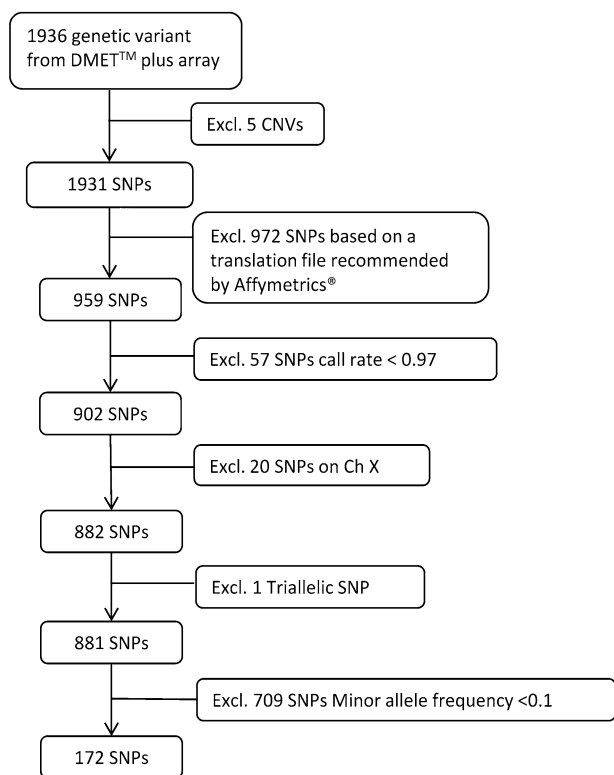
The GOF plots (Fig. 4) show that the individual predictions of the final model are in good accordance with the observations, while the population predictions are slightly deviated from the observations. The conditional weighted residual errors (CWRES) randomly distributed around zero, without obvious trends over time or across population predictions. The pcVPC plot (Fig. 5) shows that the 5th, 50th, and 95th percentiles of prediction-corrected concentrations can be mostly adequately covered by the 95% confidence interval (CI) of the corresponding percentiles of simulations, although a few large prediction-corrected concentrations are present. The NPDE results are shown in Online Resource 1, Fig. S3.

### 3.5 Simulation Results

The simulation results of different regimens in included patients who originally reached the target ( $n=41$ ) are summarized in Table 3.

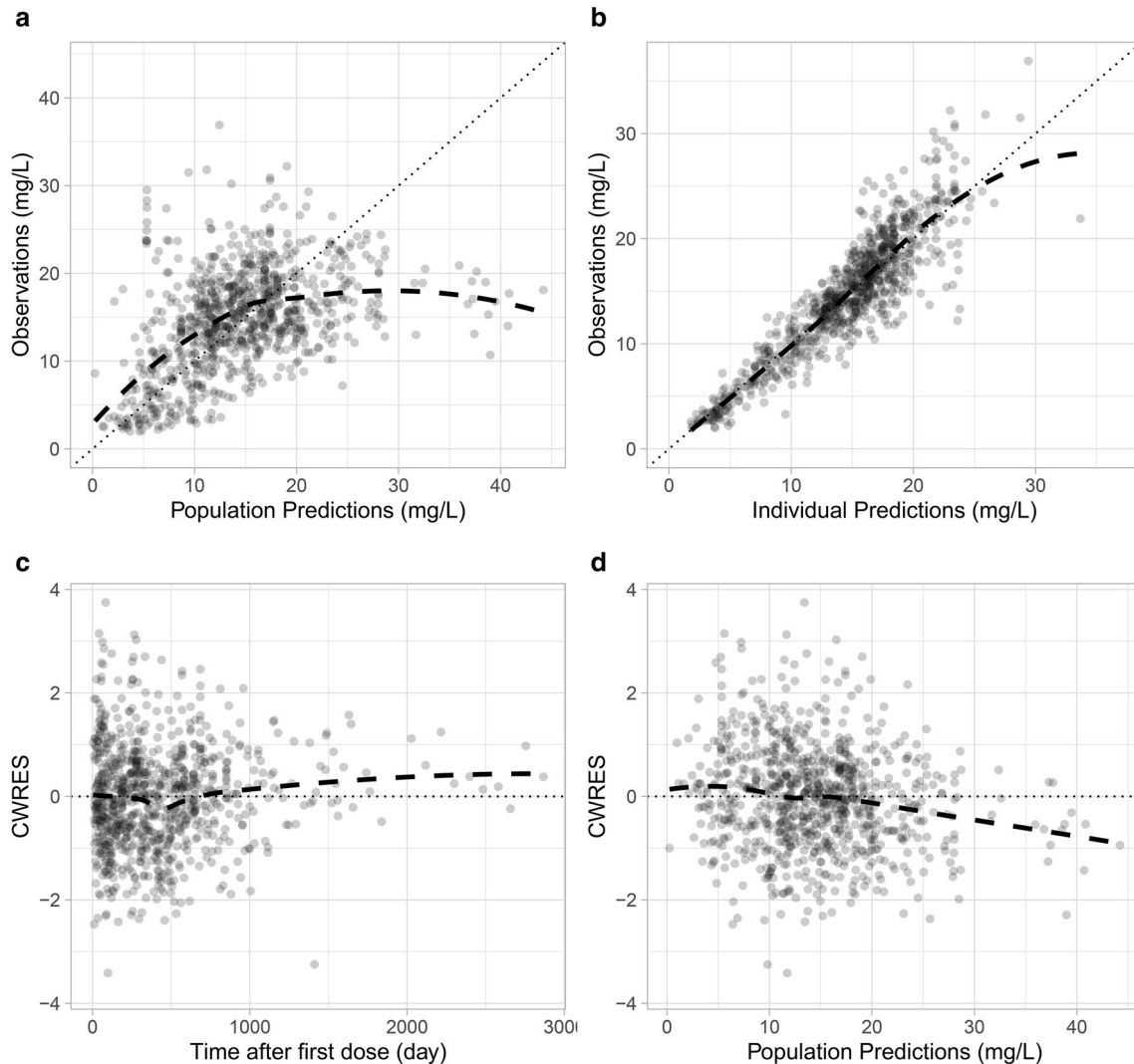
The previously suggested high-dose regimen (Regimen 1) resulted in the lowest  $T_{target}$  but the highest  $P_{toxicity}$ . The  $C_{sim\_real}$  can also not be well-maintained within the therapeutic range.

As for the newly designed strategies, if all patients started with the same dosage (Regimen 2–2 g, 2–4 g and 2–6 g), the increase in the starting dosage reduced the  $T_{target}$  but increased  $P_{toxicity}$  and weakened the ability of maintaining  $C_{sim\_real}$  within the therapeutic range. When determining the starting dose individually (Regimen 3–77 day, 3–98 day and 3–119 day), Regimen 3–98 day fulfilled the optimization target and resulted in a lower  $T_{target}$  but higher  $P_{toxicity}$  and  $P_{o\_window}$  compared with Regimen 2–4 g. The range of determined starting dose was in accordance with what is currently recommended (Table 3) [26].



**Fig. 3** Selection of the genetic variants. *Excl.* excluding, *Ch X* chromosome X, *DMET™* Drug Metabolizing Enzymes and Transporters, *CNVs* copy number variations, *SNPs* single nucleotide polymorphisms





**Fig. 4** Goodness-of-fit plots of the final population pharmacokinetic model of mitotane in patients with adrenocortical carcinoma, including observations versus **a** population predictions and **b** individual predictions, and CWRES versus **c** time and **d** populations predictions.

The *black dotted lines* represent  $y=x$  (**a, b**) and  $y=0$  (**c, d**), and the *black dashed lines* represent the corresponding LOESS regressions. CWRES conditional weighted residual errors, LOESS locally estimated scatterplot smoothing

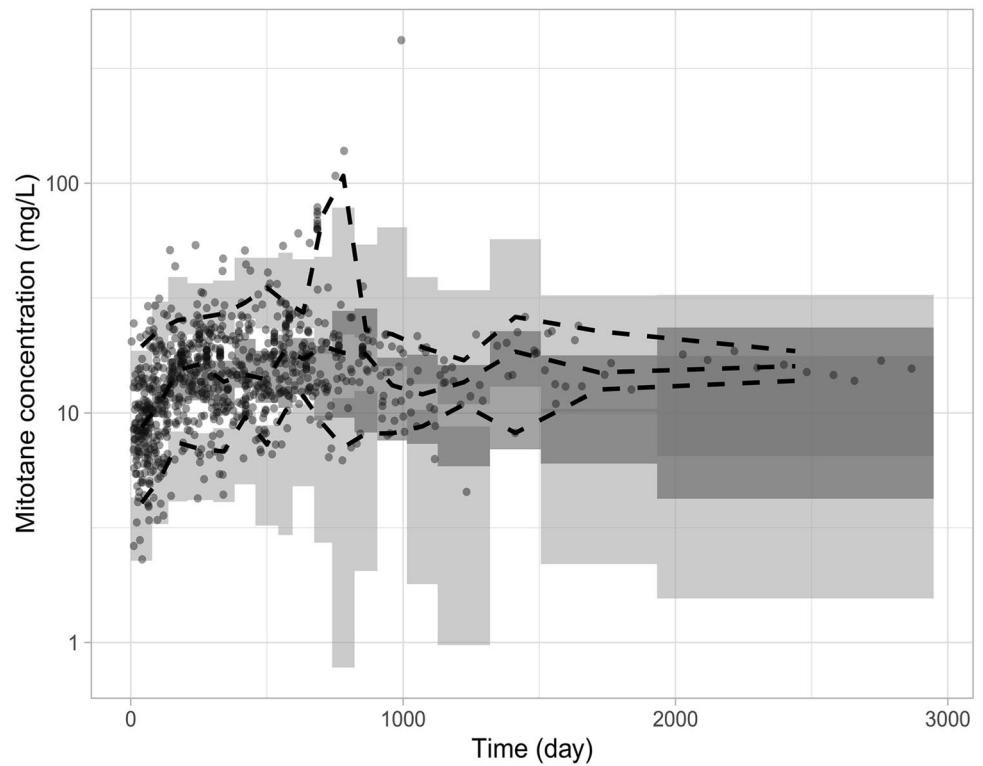
Compared with Regimens 2–4 g and 3–98 day, increasing the dose reduction amount to 4 g when  $C_{\text{sim\_real}} > 20$  mg/L reduced the  $P_{\text{toxicity}}$  and  $P_{\text{o.window}}$ , whereas setting a 50% deduction when  $C_{\text{sim\_real}} > 20$  mg/L reduced the  $P_{\text{o.window}}$  but increased the  $P_{\text{toxicity}}$  (Regimens 4 and 5). Both of these changes did not affect  $T_{\text{target}}$ . In contrast, when adjusting the dose change amount when  $C_{\text{sim\_real}} < 14$  mg/L, the evaluated regimens did not provide better results (Regimens 6 and 7).

Regimen 8, where a constant starting dose determined by the model was applied, provided generally better results compared with starting with 4 g/day for all patients (Regimen 2–4 g) in terms of  $T_{\text{target}}$ ,  $P_{\text{toxicity}}$ , and the ability to maintain concentration within the therapeutic range. The suggested starting dose range (3–7 g, median 5 g) was slightly beyond the current recommended range but was

considered to be acceptable. In comparison, when determining a constant dose using individual PK parameters (incorporating IIV estimates) [Regimen 9], the  $P_{\text{o.window}}$  and maximum  $T_{\text{target}}$  decreased. Although  $P_{\text{toxicity}}$  increased, it was still low enough. The suggested doses under Regimen 9 were relatively higher (3–10 g) since IIV was taken into account.

Overall, Regimens 2–4 g, 3–98 day, 4–(–4 g), 4–(–50%), 5–(–4 g), 7-1, 8, and 9 fulfilled the optimization target. The individualized starting dose resulted in a lower  $T_{\text{target}}$  but higher  $P_{\text{toxicity}}$  compared with the fixed starting dose. Regimens 3–98 day and 5–(–4 g) provided the lowest mean  $T_{\text{target}}$ , while regimen 5–(–4 g) resulted in lower  $P_{\text{toxicity}}$ . Regimen 8 provided the lowest  $P_{\text{toxicity}}$  and Regimen 9 provided the lowest maximum  $T_{\text{target}}$  and mean  $P_{\text{o.window}}$ . Based on these results, Regimen 5–(–4 g) and Regimen 8

**Fig. 5** Prediction-corrected visual predictive check plot of the final model on the logarithmic scale. Black dashed lines represent the 50th, 95th and 5th percentiles of the prediction-corrected observations; light-grey shading represents the 95% confidence interval of the 95th and 5th percentiles of the simulations; and dark-grey shading represents the 95% confidence interval of the 50th percentiles of the simulations



**Table 3** Simulation results of different treatment regimens for included patients who originally reached the target ( $n=41$ )

Regimen (Fig. 1)	Mean $T_{\text{target}}$ (day)	Max $T_{\text{target}}$ (day)	Mean $P_{\text{toxicity}}$ (%)	Mean $P_{\text{o.window}}$ (%)	Median max/min $C_{\text{sim.real}}$ (mg/L)	Starting dose range (g)	
1	54.22	125	23.6	18.35	22.3/13.11	–	
2-2 g	133.98	236	4.16	12.6	20.65/13.14	2	
2-4 g	89.8	182	7.01	13.15	20.90/13.20	4	*
2-6 g	60.61	149	13.85	15.13	21.13/13.09	6	
3-77 day	73	173	10.63	12.7	21.07/13.29	3.5–7	
3-98 day	85.07	182	9.26	14.35	21.03/13.16	3–6	*
3-119 day	97.9	191	6.44	12.22	20.96/13.21	2.5–5	
4-(4 g)	89.8	182	5.96	12.66	20.91/13.22	4	*
4-50%	89.8	182	8.82	12.37	20.91/13.22	4	*
5-(4 g)	85.07	182	7.92	13.01	20.84/13.14	3–6	*
5-50%	85.07	182	11.13	12.21	20.84/13.22	3–6	
6-1	91.12	194	6.61	13.37	20.84/12.91	4	
6-2	74.32	151	14.34	16.26	21.57/13.02	4	
7-1	86.12	194	8.52	14.69	21.03/12.96	3–6	*
7-2	80.27	160	14	15.53	21.46/12.87	2.5–5	
8	87.85	191	5.05	11.26	20.34/13.30	3.5–7	*
9	87.8	161	5.56	10.72	20.33/13.09	3–10	*

$T_{\text{target}}$  target reaching time (the day when simulated mitotane concentration  $\geq 14$  mg/L),  $P_{\text{toxicity}}$  percentage of days when simulated mitotane concentrations were higher than the upper limit of mitotane therapeutic window (20 mg/L) in the first 200 days,  $P_{\text{o.window}}$  percentage of simulated mitotane concentrations located outside the therapeutic window after reaching the target,  $\text{max}$  maximum,  $\text{min}$  minimum, \* indicates the regimen fulfills the optimization target,  $C_{\text{sim.real}}$  simulated 'real' mitotane concentrations based on individual parameters

were considered to be more beneficial, while Regimen 9 could also be applied, considering the patients' tolerance to the level of dose increase.

The Shiny app was established based on the final model, and the treatment strategy 5-(− 4 g) was applied since this regimen provided the lowest mean  $T_{\text{target}}$ . A reduced model where the effect of pharmacogenetic variation was not included was also built in to serve as an alternative option for patients when genotyping results were not available. The results are shown in Online Resource 2.

## 4 Discussion

In the current study, a two-compartment PopPK model was developed that adequately described the PK profile of mitotane in patients with ACC. The covariates identified explained 24% and 20.9% of random variability in mitotane clearance and distribution volume, respectively. As mitotane distributes in most body tissues, predominantly in the fat [1], the two-compartment model structure is considered to also be in line with the PK characteristics of mitotane, although the wide 95% CI of the  $Q/F$  parameter still indicates uncertainty in the estimation. A three-compartment model structure, which has been previously applied on mitotane [8], could not be identified in this study as the time-course of mitotane concentration did not meet the characteristics of a three-compartment model and parameter estimates for the three-compartment model were found to be unidentifiable.

Because of the limited data in the absorption phase,  $K_A$  was first estimated based on a sub-dataset and then fixed to analyze the full dataset. Precise  $K_A$  estimation was unidentifiable if estimating based on the full dataset. The estimates of  $V_c/F$  and  $V_p/F$  were relatively large, which is in accordance with previous reports and the fact that mitotane distributes in many body tissues [1, 3]. The separate effects of LBW and FAT on mitotane distribution volumes were of interest in this study as they are more realistic covariates physiologically [3, 4]. As a result, FAT was found to be a significant covariate on the  $V_c/F$ . The estimated half-life of mitotane in the included patients ranged from 16.4 to 700.6 days, with a median of 101.5 days. This range is wider than what has been previously reported [1, 2], which may be explained by the larger number of patients included in the current study than in the original study [2]. Incorporating IOV on  $CL/F$  in the current study explained the intrasubject variability. The estimates of IOV indicate an overall increasing clearance during the first 500 days, followed by a decrease thereafter (Online Resource 1, Fig. S4). This dynamic indicates that a self-induction in mitotane clearance, which has been suggested previously [3], may exist temporarily.

For the first time, the current study explored and quantified the potential effect of pharmacogenetic variation on

mitotane clearance in patients with ACC. Due to the lack of knowledge regarding the PK pathway of mitotane, a wide range of SNPs from the DMET™ Plus array were considered. However, because of the limited number of patients, it was decided to focus on the SNPs with known functionality by adopting a preset selection [17], although an exploratory analysis based on all genetic variants from the DMET™ Plus array was also performed. The flow diagram of the SNP selection and the nine additional SNPs that are potentially correlated to mitotane clearance if the preset selection was not considered are shown in Online Resource 1, Table S2 and Fig. S5. Genes located on the X chromosome were excluded since only the general influence of sex on mitotane PK was considered.

Eventually, three SNPs, i.e. *CYP2C19*\*2 (rs4244285), *SLCO1B3* 699A>G (rs7311358) and *SLCO1B1* 571T>C (rs4149057), were included in the final model and were considered as the pharmacogenetic polymorphisms that should be considered for mitotane dose selection. This result also suggests that the *CYP2C19* enzyme and *SLCO1B3* and *SLCO1B1* transporters for drug uptake in the liver might be involved in the mitotane PK pathways, but further confirmation is required.

In fact, in our dataset, *CYP2C19*\*2 was in 100% linkage disequilibrium with *CYP2C18* 1154C>T (rs2281891), which was the same as *SLCO1B3* 699A>G with *SLCO1B3* 334G>T (rs4149117) and *SLCO1B3* 1557G>A (rs2053098). Comparable high linkage disequilibrium was also found in 1000 Genomes CEU population (Utah residents with Northern and Western European ancestry). Compared with *CYP2C18* 1154C>T, for which no sufficient evidence has been found regarding the effect on the drug PK, the 'A' variant of *CYP2C19*\*2 is known to be a nonfunctioning variant and has been demonstrated to decrease the activity of *CYP2C19* [27, 28]. Similarly, the variants of *SLCO1B3* 699A>G with *SLCO1B3* 334G>T have been reported to be associated with a decrease in drug clearance, and *SLCO1B3* 699A>G has a stronger level of clinical annotations [29, 30]. Therefore, *CYP2C19*\*2 and *SLCO1B3* 699A>G were included in the final model.

*CYP2B6*\*6, which has been reported to be related to mitotane plasma concentrations detected at 3 and 6 months [10], was not found to have a significant effect on mitotane clearance in the current study. Among the five SNPs located on *CYP2B6* that were included in the association analysis, none were significantly related to mitotane clearance ( $p > 0.05$ ). This discrepancy may be due to the much longer observation period in the present study. One SNP located on *CYP2C9*, *CYP2C9*\*2 (rs1799853), was also not found to be significant; however, the evidence of the involvement of *CYP2C9* is in fact insufficient.

The predictability and stability of the final model were confirmed to be acceptable. In the pcVPC plot, a few

prediction-corrected concentrations are inadequately covered by the simulations. A possible explanation is that the observations at corresponding time points are from a single patient and the population prediction of this patient is much smaller than real observations. The deviation of population predictions from observations can also be seen in the GOF plots. Patients' adherence and other unknown factors may also introduce additional bias. Identification of additional covariates, such as the effect of co-medication and food intake, might improve the population predictions.

Based on the final PopPK model, several mitotane treatment strategies were designed and evaluated by simulations. A regimen with a bolus dose followed by a maintenance dose was not considered as this regimen requires a high dosage, which is not tolerable for some patients. Among the regimens that fulfilled the optimization target, applying the individual starting dose determined by the model was demonstrated to shorten the time to achieve the therapeutic window compared with starting with a fixed dose for all patients. Under the setting of individualized starting dose, the regimens with a stepwise increasing dose at start required less time to reach the therapeutic target, while the regimen with a constant starting dose demonstrated the lowest risk of having toxicity. The determined individual starting dose was also acceptable. In addition, the newly designed dose-adjustment strategies were able to satisfactorily keep the mitotane concentrations within the therapeutic range. Therefore, determining the starting dose using the developed model is considered to be most beneficial in terms of shortening the time to reach the therapeutic target and limit the risk of toxicity. However, due to the fact that a shorter  $T_{\text{target}}$  is normally paired with a higher  $P_{\text{toxicity}}$ , it is suggested to consider, based on a patient's condition, whether the increased risk of having toxicity can be tolerated in order to gain the benefit of a shorter time to reach the therapeutic target when selecting a dosing regimen.

Obtaining individual parameters based on one (or more) TDM result with the PopPK model, and determining the dose amount accordingly, can also decrease the risk of toxicity while providing a satisfactory target reaching time; thus, this is also a promising strategy. However, patients' tolerance to the high level of dose increase needs to be considered when applying this strategy. This method can also be useful to estimate an adequate dose for the drug concentration level maintenance after reaching the therapeutic window, thereby decreasing the frequency of dose adjustment.

Simulation results also indicate that in order to reduce the risk of having toxicity and to effectively maintain mitotane concentration within the therapeutic range, a better strategy is to set the concentration boundary of dose decreases at 18 mg/L instead of 20 mg/L. This early dose adjustment takes into consideration the 7-day period when the monitoring result is unknown and the dose is not adjusted. The concentration boundary of dose increases needs to be 14 mg/L

since it affects the adequacy of maintaining the plasma concentration above 14 mg/L. The frequency of TDM was set at once every 21 days, as suggested by the guideline in the simulation. If TDM is performed less frequently, a larger dose change step will be required.

The current study has some limitations. First, the small number of patients included in this study and the exploratory characteristics of this analysis may influence the power of covariate analysis, especially for pharmacogenetic analysis. However, as the dataset consisted of concentrations on different occasions for each patient, which enabled differentiation between IIV and intrasubject variability (i.e. IOV) in clearance, the certainty of the possible genotype effect on clearance, which is more likely to be covered by IIV since genotype is a constant factor in patients, was increased. Nonetheless, further validation with an external dataset to replicate the findings is warranted to confirm the identified associations and to translate the findings into a clinical recommendation. However, since ACC is a very rare disease (1 per million per year), the collection of another comparable or even larger dataset will be challenging. Therefore, an in vitro assay might be more feasible in future studies to substantiate the activity of the suggested enzymes in mitotane PK. Second, the model lacks a strong ability to accurately predict high concentrations (e.g., peak concentrations) due to the limited data input in the absorption and distribution phase. Furthermore, the accuracy of parameter estimates may be affected by our simplification of multiple daily dosing to a single dose. However, the prediction of mitotane trough concentrations and the suggestion of daily dose based on the model will not be significantly affected. Therefore, we believe this model is still fit for the current application. Third, the impact of coadministered drugs and food intake on mitotane PK was not taken into account in this study due to the lack of data.

## 5 Conclusions

The current study presents a two-compartment PopPK model that well-characterizes mitotane PK profiles in patients with ACC. The polymorphisms of *CYP2C19\*2* (rs4244285), *SLCO1B3* 699G>A (rs7311358) and *SLCO1B1* 571T>C (rs4149057) were found to be correlated to mitotane PK. Further external or in vitro evaluation is suggested to confirm the results. Moreover, optimized mitotane treatment schedules for patients with ACC were identified by simulation and the developed model can be of help to individualize the initial dose. These strategies should be confirmed in a prospective study.

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## Compliance with Ethical Standards

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**Conflicts of interest** Anyue Yin, Madeleine H.T. Ettaieb, Jesse J. Swen, Liselotte van Deun, Thomas M.A. Kerkhofs, Robert J.H.M van der Straaten, Eleonora P.M. Corssmit, Hans Gelderblom, Michiel N. Kerstens, Richard A. Feelders, Marelise Eekhoff, Henri J.L.M. Timmers, Antonio D'Avolio, Jessica Cusato, Henk-Jan Guchelaar, Harm R. Haak, and Dirk Jan A.R. Moes declare they have no conflicts of interest.

**Ethical Approval** This study was approved by the Medical Ethical Committee of the Máxima Medical Center Veldhoven (2015), and approval for the inclusion of patients in other institutes was obtained from the local boards. All procedures performed in this study were in accordance with the ethical standards of the institutional Medical Ethical Committee and the 1964 Helsinki Declaration.

**Consent to Participate** The required informed consents were obtained from all patients.

**Availability of Data and Material** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Code Availability** PopPK analysis was performed using the FOCEI method implemented in NONMEM software version 7.4.1 (ICONDevelopment Solutions). Statistical analysis, plot generation, and simulations were performed using R version 3.6.1 (The R Foundation for Statistical Computing). The R script of the Shiny application established in this study for simulation can be found at <https://github.com/AnyueYin/Shiny-app-script-for-model-simulation---Population-PK-and-PG-analysis-of-mitotane>.




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