

## ORIGINAL ARTICLE

# Individual optimal dose of amrubicin to prevent severe neutropenia in Japanese patients with lung cancer

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## Abstract

This study determined individual optimal amrubicin doses for Japanese patients with lung cancer after platinum-based treatment. We carried out population pharmacokinetic and pharmacodynamic modeling incorporating gene polymorphisms of metabolizing enzymes and transporters. Fifty patients with lung cancer, who were given 35–40 mg/m<sup>2</sup> amrubicin on days 1–3 every 3–4 weeks, were enrolled. Mechanism-based modeling described relationships between the pharmacokinetics of amrubicin and absolute neutrophil counts. A population pharmacokinetic and pharmacodynamic model was developed for amrubicin and amrubicinol (active metabolite), connected by a delay compartment. The final model incorporated body surface area as a covariate of amrubicin and amrubicinol clearance and distribution volume. *SLC28A3* single nucleotide polymorphism (rs7853758) was also incorporated as a constant covariate of the delay compartment of amrubicinol. Performance status was considered a covariate of pharmacokinetic (amrubicinol clearance) and pharmacodynamic (mean maturation time) parameters. Twenty-nine patients with grade 4 neutropenia showed higher amrubicinol area under the plasma concentration-time curve from 0 to 72 hours (AUC<sub>0–72</sub>, *P* = .01) and shorter overall survival periods than other patients did (*P* = .01). Using the final population pharmacokinetic and pharmacodynamic model, median optimal dose to prevent grade 4 neutropenia aggravation was estimated at 22 (range, 8–40) mg/m<sup>2</sup> for these 29 patients. We clarified correlations between area under the plasma concentration-time curve from 0 to 72 hours of amrubicinol and severity of neutropenia and survival of patients given

**Abbreviations:** AAG,  $\alpha$ 1-acid glycoprotein; ALB, serum albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; AUC, area under the plasma concentration-time curve; BSA, body surface area; BUN, blood urea nitrogen; BW, body weight; C<sub>AMR</sub>, plasma concentration of amrubicin; C<sub>AMROH</sub>, plasma concentration of amrubicinol; Circ, a compartment of circulating observed blood cells; Circ0, baseline value of a compartment of circulating observed blood cells; CL2, intercompartment clearance of peripheral-1 of amrubicin; CL3, intercompartment clearance of peripheral-2 of amrubicin; CLm, clearance of amrubicinol; CLp, metabolic clearance from amrubicin to amrubicinol; Comp, compartment; Cov, covariate; CV, coefficient of variation; ED, extensive disease; G-CSF, granulocyte colony stimulating factor; Hgb, hemoglobin; HGT, height; Ht, hematocrit; Kdc, rate constant of delay compartment of amrubicinol; K<sub>prol</sub>, proliferation rate constant determining the rate of cell division; K<sub>tr</sub>, rate constant of transit compartment; LCNEC, large-cell neuroendocrine carcinoma; LD, limited disease; LDH, lactate dehydrogenase; MMT, mean maturation time; NLME, nonlinear mixed-effects; OFV, objective function value; OS, overall survival; PD, pharmacodynamics; PK, pharmacokinetics; PLT, platelet count; Pop, population; PS, performance status; SCLC, small-cell lung cancer; SCr, serum creatinine; SNP, single nucleotide polymorphism; SQ, squamous cell carcinoma; T-Bil, total bilirubin; T-CHO, total cholesterol; tv, typical value; V2, peripheral-1 volume of distribution of amrubicin; V3, peripheral-2 volume of distribution of amrubicin; Vm, central volume of distribution of amrubicinol; Vp, central volume of distribution of parent amrubicin; VPC, visual predictive check; WBC, white blood cell count.

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amrubicin after platinum chemotherapy. This analysis revealed important amrubicin pharmacokinetic-pharmacodynamic covariates and provided useful information to predict patients who would require prophylactic granulocyte colony stimulating factor.

**KEYWORDS**

amrubicin, amrubicinol, lung cancer, pharmacodynamics, pharmacokinetics

## 1 | INTRODUCTION

A third-generation anthracycline, amrubicin, and its active metabolite, amrubicinol, markedly inhibit topoisomerase II activity.<sup>1</sup> Amrubicin is approved only in Japan, and its single agent-based regimen is a promising second-line chemotherapy for SCLC, after platinum-containing chemotherapy.<sup>2,3</sup> Despite the high response rate to amrubicin in a majority of SCLC patients in previous phase II studies, which included sensitive relapses or refractory cases,<sup>4-8</sup> a randomized phase III study showed that the overall survival with amrubicin was not superior to that of topotecan, which is the only standard regimen for the second-line treatment of SCLC.<sup>9</sup> In these studies, several participants treated with amrubicin required dose reduction and treatment delays because of severe hematological toxicities, including febrile neutropenia. Furthermore, 60%-80% of the patients required treatment with G-CSF. Based on the result of the phase III study, von Pawel et al<sup>9</sup> reported an increased infection rate during amrubicin treatment, which led to an amendment of the protocol requiring prophylactic growth factor support. Daily prophylactic use of G-CSF was recommended according to an approved guideline of the Japan Lung Cancer Society.<sup>10</sup>

We recently evaluated the relationship between the severity of neutropenia and the AUC of amrubicinol.<sup>11</sup> However, an optimal AUC that avoids severe neutropenia has not been elucidated, and the covariate factors that govern the PK and PD of amrubicin and amrubicinol have not been analyzed. Therefore, we carried out a Pop-PK-PD analysis with the following primary objectives: (i) to develop a Pop-PK model of amrubicinol connected with amrubicin, and to define the covariates of the Pop-PK parameters, incorporating gene polymorphisms of the metabolizing enzymes and transporters; and (ii) to develop a final Pop-PK-PD model of the relationship between the PK profiles of amrubicin and/or amrubicinol and the time course of ANC in the first course of amrubicin treatment and define the covariates of the PD parameters. The secondary objective was to apply the results generated from the Pop-PK-PD modeling to simulate clinically feasible dosage regimens. We clarify the precision dosing of amrubicin for PK-PD modeling to prevent severe neutropenia in Japanese patients with lung cancer.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients and treatment

The prospective clinical study (UMIN000002970) was approved by the ethical review boards of the National Cancer Center

Hospital (Tokyo, Japan) and Showa University (Tokyo, Japan). Patients (20 years of age or older) diagnosed with lung cancer and who had received amrubicin monotherapy in the National Cancer Center Hospital were enrolled after obtaining written informed consent. Patients with hepatitis B, hepatitis C, or HIV infection and patients considered ineligible by physicians, including those who refused blood sampling or who had poor health, were excluded.

Amrubicin was given as a 5-minute i.v. infusion at a dose of 35-40 mg/m<sup>2</sup> on days 1-3, and subsequently every 3 or 4 weeks. Prophylactic use of serotonin type 3 (5-HT<sub>3</sub>) receptor antagonists was allowed before amrubicin was given. The use of G-CSF was only allowed for patients who had developed grade 4 neutropenia or grade 3 febrile neutropenia in accordance with the guidelines of the national health insurance coverage of Japan.

### 2.2 | Safety, tumor response, and survival assessment

Body temperature monitoring and laboratory tests (eg, tests for blood counts, electrolytes, and liver and renal function) were routinely carried out during the first cycle of amrubicin treatment. Toxicity was graded according to the CTCAE version 3.0. There was no protocol restriction for response; however, we obtained information from the medical records retrospectively. Tumor response to treatment was classified according to RECIST version 1.1.

Time to treatment failure was defined as the duration from enrollment to the first clinical evidence of progressive disease, early discontinuation of treatment because of amrubicin toxicity or other reasons (ie, patient's request or physician's discretion), or death from any cause. Overall survival was defined as the duration from enrollment to death, or to loss to follow-up.

### 2.3 | Blood sampling and DNA extraction

Plasma samples were obtained to determine the PK of amrubicin and amrubicinol. For the first 21 patients, blood samples were obtained on day 1 before infusion, at the end of amrubicin infusion (0 minutes), at 5, 15, and 30 minutes, and at 1, 2, 4, 8, and 24 hours after the end of infusion. On days 2 and 3, blood samples were collected before infusion, and at 0 minute and 8 hours after infusion. For the other 29 patients who were enrolled in the expanded study, blood samples were obtained at 15 minutes and

2, 4, and 8 hours after the end of infusion on day 1. The plasma concentrations of amrubicin and amrubicinol were estimated using an HPLC method previously reported by us.<sup>12</sup> The lower limit of quantification of the assay was 2.5 ng/mL, for both amrubicin and amrubicinol. For all the 50 enrolled patients, blood was obtained before amrubicin treatment, and buffy coat samples were used for DNA extraction (QIAamp DNA blood kit; Qiagen) and genotyping.

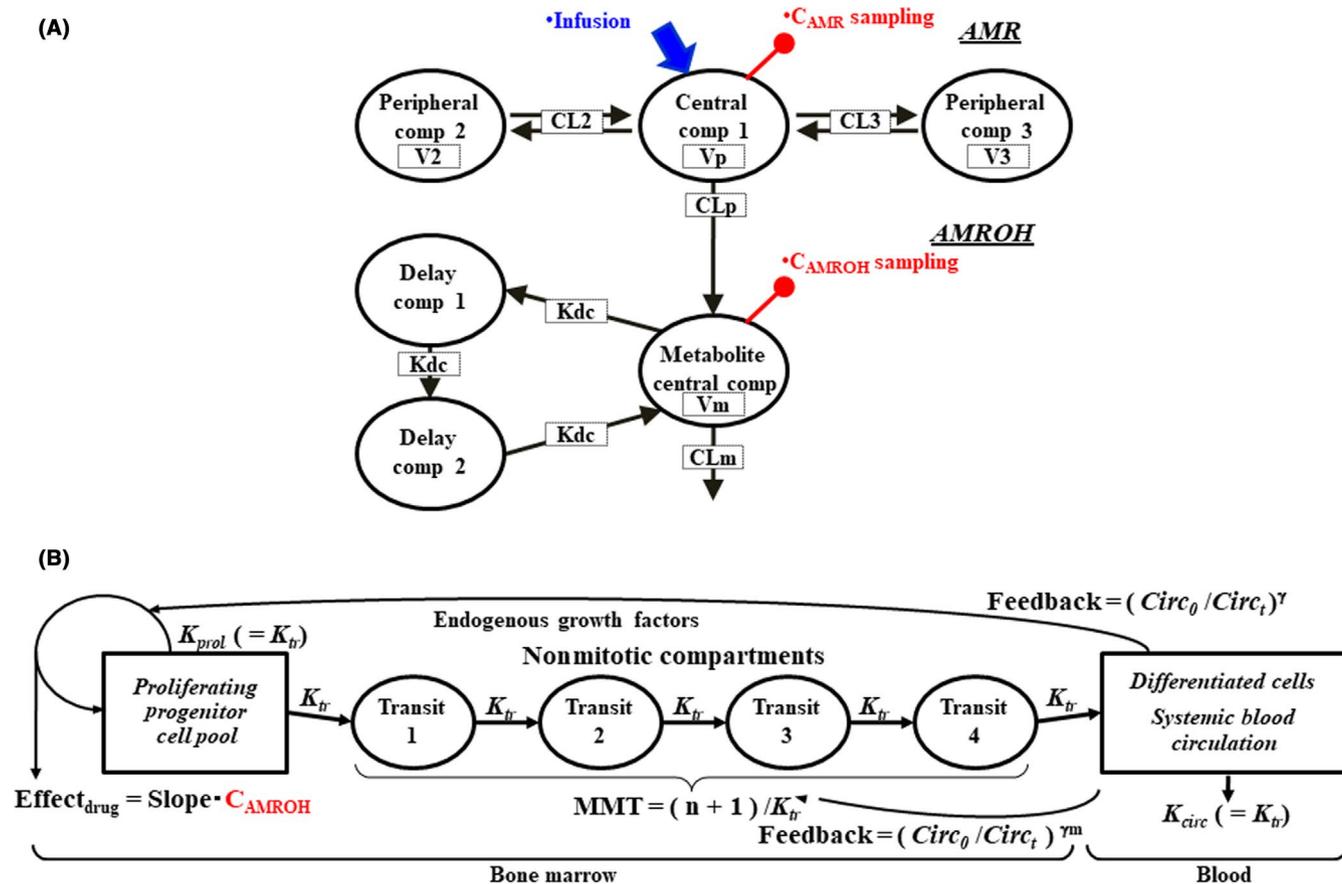
### 2.4 | Genotyping

DNA processing and genotyping of most of the drug-metabolizing enzymes and transporters in each patient sample were carried out using the DNA chip DMET plus platform (Affymetrix). This system is capable of analyzing 1936 SNPs in 225 genes associated with drug metabolism and transport. Genotypes were determined for each SNP site, and reported as homozygous WT, heterozygous, homozygous variant, or “no-call.”

### 2.5 | Population PK and semimechanistic myelosuppression model development and evaluation

In total, 388 plasma samples were obtained for the population PK analysis. The PK parameters were determined by nonlinear mixed-effects modeling, using Phoenix NLME 1.3 (Certara). The first order, conditional estimation-extended least squares estimation method was used.

First, to develop an amrubicin-amrubicinol-linked Pop-PK model (base model), amrubicin and amrubicinol plasma concentrations were converted to molar concentrations. The recorded amrubicin dosing times on day 2 or 3 were used for modeling. The amrubicin-amrubicinol-linked model, which is a parent-metabolite model, was then developed (Figure 1). Briefly, the PK of parent amrubicin was described as a 3-compartment model, and that of amrubicinol was modeled as a 1-compartment model, connected to amrubicin's central compartment by a first-order metabolic process with 2 delay compartments (Figure 1A). The amrubicin-amrubicinol modeling involved 9 structural parameters using 11 differential equations. The parameters



**FIGURE 1** Schematics of the population pharmacokinetic (PK) and pharmacodynamic (PD) models consisting of the PK components of amrubicin (AMR) and amrubicinol (AMROH) (A), and the PD component for time profile of absolute neutrophil count at the first cycle for amrubicin treatment (B) in patients with small-cell lung carcinoma.  $C_{AMR}$ , plasma concentration of amrubicin;  $C_{AMROH}$ , plasma concentration of amrubicinol; Circ, a compartment of circulating observed blood cells; CL2, intercompartmental clearance of amrubicin peripheral-1; CL3, intercompartmental clearance of amrubicin peripheral-2; CLm, clearance of amrubicinol; CLp, metabolic clearance from amrubicin to amrubicinol; Comp, compartment; Kdc, rate constant of amrubicinol delay compartment;  $K_{prol}$ , proliferation rate constant determining the rate of cell division;  $K_{tr}$ , rate constant of transit compartment; MMT, mean maturation time; V2, peripheral-1 volume of distribution of amrubicin; V3, peripheral-2 volume of distribution of amrubicin; Vm, central volume of amrubicinol distribution; Vp, central volume of parent amrubicin distribution

included were: central volume of parent amrubicin distribution, peripheral-1 volume of distribution of amrubicin, peripheral-2 volume of distribution of amrubicin, CLp, intercompartmental clearance of amrubicin peripheral-1 and peripheral-2, Vm, CLm, and Kdc.

For the base model, interpatient variability was modeled using an exponential function. For instance, CLp was estimated from the equation:

$$CLp_i = tvCLp \times e^{\eta_i}$$

where,  $CLp_i$  represents the CL of the  $i$ th individual,  $tvCLp$  is the population (typical) CLp value, and  $\eta$  is the interindividual random effect with mean 0, and variance  $\omega^2$ . The residual error between the  $j$ th measured concentration ( $C_{obsij}$ ) and predicted concentration ( $C_{predij}$ ) for the patient was modeled with a proportional error model:

$$C_{obsij} = C_{predij}(1 + \varepsilon)$$

where,  $\varepsilon$  is an independent random variable with mean 0, and variance  $\sigma^2$ .

Second, the effects of covariates, such as age, BW, BSA, sex, PS, serum albumin, alanine aminotransferase, serum creatinine, total bilirubin, and gene polymorphisms of metabolic enzymes and transporters were evaluated for the final model. The patients' characteristics, not including gene polymorphisms, were first visually examined to determine whether they had potential covariate effects on the PK parameters of interest. At this time, an x-y plot was used when the patient characteristic was a continuous scale, and a box plot was used when the patient characteristic was a nominal scale. We determined that BSA, BW, and PS were correlated with several PK parameters, including those shown in Table 1. Because BW is associated with BSA, we decided to include only BSA.

Separately, gene polymorphisms (SNPs) of metabolic enzymes and transporters that correlated with the PK profile of amrubicin were identified by a 2-step strategy. In the first step, an association analysis between genotypes and the amrubicinol  $AUC_{0-24}$  was carried out for all 50 patients whose PK was analyzed by the developed Pop-PK model. The Kruskal-Wallis test was used, and  $P < .05$  was considered significant (Table S1). To develop a robust Pop-PK model, selection and filtering criteria that only considered SNPs with minor allele frequencies of 0.2 or more were applied. Among these gene polymorphisms of enzymes or transporters, *SLC28A3* (rs7853758) was the only SNP, that has been reported to be related to PK and PD of anthracyclines and for which the frequency of each allele was 20% or higher (8 cases), which was required for incorporation into the model. Therefore, *SLC28A3* (rs7853758) was selected as a covariant candidate.

Continuous covariates were centered at the mean values and were included in the model using a power model.

The continuous covariates were modeled according to the following general equation:

$$P_i = tvP \left( \frac{COV_i}{COV_{mean}} \right)^{cov\theta(P_i)} \cdot e^{\eta_i}$$

The categorical covariates were modeled according to the following general equation:

$$P_i = tvP \cdot e^{cov\theta} \cdot e^{\eta_i}$$

where  $P_i$  is the individual PK parameters of a patient,  $tvP$  is the typical value of PK parameters for patients,  $cov_i$  is the individual's value of the covariate,  $cov_{mean}$  is the population mean value of the covariate, and  $cov\theta$  is the magnitude of the covariate effect.

Finally, we examined whether BSA, PS, and SNPs of *SLC28A3* (rs7853758) could be covariates and completed the final model. Stepwise forward addition followed by a backward deletion method was used to identify these covariates. The  $\chi^2$  test was used to compare the OFVs of the nested models (likelihood ratio test). A covariate was considered statistically significant in this analysis when its addition to the model reduced the  $-2$  log-likelihood by at least 6.63 units ( $P < .01$ ), based on the  $\chi^2$  test for the difference in the  $-2$  log-likelihood between 2 hierarchical models that differ by 1 degree of freedom. If more than 10% of the patients were missing covariate data, the covariate was excluded from analysis (Table S2).

Third, a semimechanistic-physiological Pop-PD model was built using the time courses of neutrophil counts after amrubicin administration (Figure 1B), based on previously established models.<sup>13,14</sup> A total of 357 ANC observations from 50 patients were used for Pop-PK-PD modeling. This PD model was constructed to mimic physiological processes and consisted of 6 compartments that mimicked the maturation of bone marrow progenitor cells to circulating neutrophils: 1 stem/progenitor cell, 4 maturation, and 1 circulation compartment. The drug effect (ie, the inhibitory effect of the drug on progenitor cell growth) was expressed as a linear equation:

$$Effect_{drug} = Slope \cdot C_{AMROH}$$

where Slope is the parameter that describes the drug effect in a linear correlation with  $C_{AMROH}$  (ie, the plasma concentration of amrubicinol predicted by the Pop-PK model).

The data were Box-Cox transformed with a factor of 0.2. The residual error was an additive error on the Box-Cox scale. Furthermore, in accordance with a previous report,<sup>14</sup> we successfully characterized a second feedback mechanism of endogenous G-CSF, which reduced the maturation time of neutrophils when their blood levels were below the baseline. Therefore, the combined PK-PD model comprised a total of 12 compartments, 6 each assembling the PK portion (including 2 delay compartments) and the PD portion of the model. The covariate PD model building was also a stepwise process. If a chosen covariate did not reasonably explain the PD variation, it was excluded from the covariate analysis.

## 2.6 | Model evaluation and other statistical analyses

To evaluate the model, simulations were undertaken in Phoenix NLME using the dataset obtained in this study. Simulated percentiles (5th, 50th, and 95th) were calculated, and VPCs were carried

**TABLE 1** Population pharmacokinetic (PK) parameters of amrubicin and amrubicinol, and pharmacodynamic (PD) parameters of myelosuppression in patients with small-cell lung carcinoma

Fixed effects	Base model		Final model		Bootstrap
	estimate	CV%	estimate	CV%	estimate
Estimation of population PK parameters					
-2 Log likelihood	-2677.8		-2745.4		-2745.4
tvVp, L	10.1	17.0	9.8	7.8	9.8
tvV2, L	28.5	27.3	28.5	10.0	28.5
tvCL2, L/h	9.1	43.1	9.2	15.8	9.2
tvV3, L	32.7	28.0	32.3	9.7	32.3
tvCL3, L/h	50.5	20.7	49.5	6.9	49.5
tvVm, L	1032.2	13.5	1050.0	4.9	1050.0
tvCLp, L/h	19.4	7.3	19.5	2.4	19.5
tvCLm, L/h	97.0	35.4	121.3	6.4	121.3
tvKdc, L/h	0.1	33.5	0.1	7.8	0.1
ωVp, %	32.7	75.2	33.3	34.6	33.3
ωV2, %	17.5	88.2	17.1	41.1	17.1
ωV3, %	30.7	94.1	30.3	22.7	30.3
ωCL3, %	33.7	85.1	33.3	13.4	33.3
ωVm, %	32.1	47.0	27.6	24.0	27.5
ωCLp, %	20.0	44.7	14.3	20.1	14.3
ωCLm, %	35.1	93.1	18.3	46.2	18.3
ωKdc, %	30.0	180.9	-	-	-
ωCLp-V3,	0.05	73.2	0.03	18.7	0.03
ωCLp-CL3,	0.05	97.7	0.04	11.4	0.04
ωV3-CL3,	0.10	81.1	0.10	14.0	0.10
Cov BSA (Vm)			1.5	28.2	1.5
Cov BSA (CLp)			1.0	16.6	1.0
Cov BSA (CLm)			1.8	27.5	1.8
Cov SLC28A3 (Kdc)			-2.0	-39.1	-2.0
Cov PS (CLm)			-0.3	-26.3	-0.3

$$CL_p = tvCL_p \cdot \left( \frac{BSA_i}{BSA_{mean}} \right)^{covBSA} \cdot e^{\eta_{CL_p}}$$

$$V_m = tvV_m \cdot \left( \frac{BSA_i}{BSA_{mean}} \right)^{covBSA(V_m)} \cdot e^{\eta_{V_m}}$$

$$CL_m = tvCL_m \cdot \left( \frac{BSA_i}{BSA_{mean}} \right)^{covBSA(CL_m)} \cdot e^{covPS(CL_m)(PS)} \cdot e^{\eta_{CL_m}}$$

$$K_{dc} = tvK_{dc} \cdot e^{covSLC28A3(K_{dc})(SLC28A3)} \cdot e^{\eta_{K_{dc}}}$$

Estimation of population PD parameters					
-2 Log Likelihood	754.4		746.5		746.5
tvCirc0	3.7	5.9	3.7	5.91	3.7
tvMMT	157.0	4.4	172.6	4.6	172.5

(Continued)

**TABLE 1** (Continued)

Fixed effects	Base model		Final model		Bootstrap
	estimate	CV%	estimate	CV%	estimate
tvGamma, $\gamma$	0.4	16.6	0.4	16.5	0.4
tvGamma-m, $\gamma_m$	0.2	27.3	0.2	27.4	0.2
tvSlope	31.1	11.5	30.6	11.2	30.6
$\omega_{Circ0}$ , %	31.7	34.3	14.7	28.4	14.7
$\omega_{MMT}$ , %	16.2	28.4	31.4	35.1	31.4
$\omega_{Slope}$ , %	51.7	23.7	52.4	22.8	52.4
Cov PS (MMT)			-0.2	-39.2	-0.2

$$MMT = tvMMT \cdot e^{covPS \cdot (PS)} \cdot e^{\eta_{MMT}}$$

$\omega$ , interindividual variability; BSA, body surface area; Circ0, baseline value of a compartment of circulating observed blood cells; CL2, intercompartmental clearance of peripheral-1 of amrubicin; CL3, intercompartmental clearance of peripheral-2 of amrubicin; CLm, clearance of amrubicinol; CLp, metabolic clearance from amrubicin to amrubicinol; Cov, covariate; CV, coefficient of variation; Kdc, rate constant of delay compartment of amrubicinol; MMT, mean maturation time; PS, performance status; tv, typical value; V2, peripheral-1 volume of distribution of amrubicin; V3, peripheral-2 volume of distribution of amrubicin; Vm, central volume of distribution of amrubicinol; Vp, central volume of distribution of parent amrubicin.

out to compare the observed plasma concentrations of amrubicinol and ANC data, over the simulated predictions based on the model. Bootstrap analysis was carried out to assess the stability of PK and PK-PD models and to determine the precision of the parameter estimates. For the bootstrap analysis, 300 bootstrap runs were performed. In this technique, the final model developed from the original dataset was fitted to each bootstrap dataset to obtain bootstrap parameter estimates. The median of the parameter estimates was computed from the bootstrap runs and compared with the point estimates.

We used the 1-tailed Student's *t* test for the 1-way plots of the amrubicinol  $AUC_{0-72}$ , with or without grade 4 neutropenia.

## 2.7 | Simulation

Simulations were carried out using the final Pop-PK-PD model, to explore the optimal dosage for preventing grade 4 neutropenia (less than 500 cells/mm<sup>3</sup> ANC) during the first course of amrubicin treatment. The simulation method was as follows: (i) individual PK parameters of each patient with grade 4 neutropenia were calculated by the Bayesian method using the final Pop-PK model, including the amrubicin/amrubicinol concentration data of each patient and covariates of PK parameters; (ii) the amrubicinol plasma concentration of each patient was predicted every hour, from 0 to 72 hours; (iii) the PK profile of amrubicinol was substituted for each patient for the final Pop-PK-PD model with the covariate of PD parameter (PS), and PD parameters of each patient were calculated; and (iv) the optimal dose of amrubicin on days 1-3 in the first course of amrubicin treatment was calculated considering no fewer than 500 cells/mm<sup>3</sup> ANC in that course. The final Pop-PK-PD model and PK-PD parameters of individual patients with a dose reduction of 1 mg per interval were used.

## 3 | RESULTS

### 3.1 | Demography and clinical characteristics of patients

Between May 2008 and January 2012, 50 patients with a median age of 63.5 (range, 39-81) years were enrolled. Forty-six and 4 patients received 40 and 35 mg/m<sup>2</sup>, respectively, on days 1-3 as the initial amrubicin dose. The demographic and baseline characteristics are summarized in Table 2. Forty-two patients were men and 8 were women; 30 and 20 patients had a PS of 1 and 0, respectively. A total of 214 treatment cycles were administered, and the number of treatment cycles per patient ranged from 1 to 10 (median, 4). Fifteen patients (30.0%) had 1 or more dose reductions and 40 cycles (18.7%) with a prolonged treatment interval over 4 weeks.

### 3.2 | Toxicity

The most common grade 4 adverse events for hematological toxicity were neutropenia (58%), and thrombocytopenia (28%). Severe anemia of grade 3 or higher occurred at 14% frequency. The majority of nonhematological toxicities were of grade 1 and 2. Fatigue, nausea, vomiting, anorexia, diarrhea, and constipation were common, but mild. One patient experienced grade 3 anorexia, and 9 patients had grade 3 febrile neutropenia.

### 3.3 | Final Pop-PK and semimechanistic myelosuppression model

The final Pop-PK model used a 3-compartment model for amrubicin, and a 1-compartment model with a delay compartment for amrubicinol, connected to amrubicin by a first-order metabolic process (Table 1, Figure 1A). In the final model, BSA was added

**TABLE 2** Demographic and clinical characteristics of patients with small-cell lung carcinoma at baseline

Characteristics	All patients		500 cells/mm <sup>3</sup> ≤ ANC		ANC < 500 cells/mm <sup>3</sup>	
	n	%	n	%	n	%
All	50	100	21	42	29	58
≥70 y old	12	24	6	29	6	21
≥75 y old	5	10	3	14	2	7
Sex, male	42	84	20	95	22	76
ECOG PS						
0	20	40	11	52	9	31
1	30	60	10	48	20	69
Disease, stage						
SCLC, LD	13	26	6	29	7	24
SCLC, ED	29	58	11	52	18	62
LCNEC, IV	6	12	4	19	2	7
SQ, IV	1	2	0	—	1	3
Other, IV	1	2	0	—	1	3
Dose						
40 mg/m <sup>2</sup>	46	92	20	95	26	90
35 mg/m <sup>2</sup>	4	8	1	5	3	10
Prior chemotherapy						
1 regimen	35	70	17	81	18	62
2 regimens	10	20	4	19	6	21
3 or more	5	10	0	—	5	17
Relapse (SCLC)						
Sensitive	19	45	10	59	9	36
Refractory	22	53	7	41	15	60
Unknown	1	2	0	—	1	4
Prior radiotherapy						
Yes, thoracic	11	22	7	33	4	14
Yes, brain	12	24	4	19	8	28
Yes, others	6	12	2	10	4	14
No	21	42	8	38	13	45
Prior surgery						
Yes, primary	9	18	5	24	4	14
Yes, others	9	18	2	10	7	24
No	32	64	14	67	18	62
	Median	Range	Median	Range	Median	Range
Pretreatment laboratory data						
Age, y	63.5	39-81	62	39-76	65	42-81
BSA, m <sup>2</sup>	1.65	1.21-1.97	1.72	1.38-1.97	1.62	1.21-1.93
BW, kg	59.2	34.9-80.3	66.1	43.5-78.2	55.8	34.9-80.3
HGT, cm	166.1	145.4-179.4	167.3	145.4-179.4	165.0	146.9-172.6
AAG, mg/dL	107	50-292	94	63-133	116	50-292
ALB, g/dL	4.0	2.5-4.9	4.1	3.5-4.9	3.9	2.5-4.8
ALP, U/L	254	9-918	258	98-517	231	9-918
ALT, IU/L	18	7-152	18	8-92	18	7-152

**TABLE 2** (Continued)

	Median	Range	Median	Range	Median	Range
AST, IU/L	24	11-155	24	13-58	23	11-155
BUN, mg/dL	17	5-34	17	11-34	16	5-30
Ht, %	35.7	23.0-47.2	37.6	26.9-47.2	33.9	23.0-42.0
LDH, U/L	232	133-1286	22	144-774	234	133-1286
SCr, mg/dL	0.8	0.3-1.5	0.9	0.6-1.5	0.8	0.3-1.1
T-Bil, g/dL	0.5	0.3-1.5	0.4	0.3-1.3	0.5	0.3-1.5
T-CHO, mg/dL	188	128-280	188	151-246	189	128-280
WBC, cells/mm <sup>3</sup>	5.5	2.5-15.2	5.6	3.4-10.6	5.2	2.5-15.2
ANC, cells/mm <sup>3</sup>	3.7	1.4-11.9	3.8	2.3-7.4	3.6	1.4-11.9
Hgb, g/dL	11.6	7.2-16.3	12.5	8.6-16.3	11.4	7.2-14.0
PLT, cells/mm <sup>3</sup>	22.1	9.3-43.9	21.9	14.2-37.3	22.6	9.3-43.9

–, not applicable; AAG,  $\alpha$ 1-acid glycoprotein; ALB, serum albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BSA, body surface area; BUN, blood urea nitrogen; BW, body weight; ED, extensive disease; Hgb, hemoglobin; HGT, height; Ht, hematocrit; LCNEC, large-cell neuroendocrine carcinoma; LD, limited disease; LDH, lactate dehydrogenase; PLT, platelet count; PS, performance status; SCLC, small-cell lung cancer; SCr, serum creatinine; SQ, squamous cell carcinoma; T-Bil, total bilirubin; T-CHO, total cholesterol; WBC, white blood cell count.

as a covariate of metabolic clearance from amrubicin to amrubicinol (CL<sub>p</sub>) of amrubicin, as well as the CL<sub>m</sub> and V<sub>m</sub> of amrubicinol. Furthermore, PS and 1 *SLC28A3* variant (rs7853758) were added to the covariate of CL<sub>m</sub> and K<sub>dc</sub>, respectively. Participants with the rs7853758 minor allele had a higher AUC<sub>0-72</sub> of amrubicinol ( $P = .02$ ). The OFV of the final model after inclusion of BSA, PS, and *SLC28A3* SNP (rs7853758) (OFV = -2745.4) was significantly lower than that of the base model (OFV = -2677.8), which did not include covariates.

The final Pop-PK-PD model was previously well described by Friberg et al.<sup>13</sup> and Quartino et al.<sup>14</sup> Our model was further refined by increasing the number of transit compartments from 3 to 4, and by including a PS as a covariate. The OFV of the final model after covariate inclusion (OFV = 746.5) was significantly lower than that of the base model (OFV = 754.4). A linear model was better than the E<sub>max</sub> model to explain the drug effect (Table 1, Figure 1B).

The VPC plots for the Pop-PK of amrubicin and amrubicinol, and the Pop-PK-PD models are presented in Figures 2A, 2B, and 2C, respectively. The VPC plots showed that both PK and PD models adequately describe the overall trend and variability of the data. No systematic deviation was observed between the observed and the simulated data. The percentage of observations outside the 90% confidence interval for the Pop-PK of amrubicin and amrubicinol, and the Pop-PK-PD data were 93.3, 94.3, and 91.0%, respectively. In the bootstrap analysis, 100% of the Pop-PK and Pop-PK-PD model runs successfully converged. The median bootstrap parameter estimates were similar to the NLME model estimates, based on the original dataset (Table 1).

### 3.4 | Relationship between PK-PD and survival

The 1-way plots of the amrubicinol AUC<sub>0-72</sub>, with or without grade 4 neutropenia, are presented in Figure 3A. Patients ( $n = 29$ ) with grade 4 neutropenia experienced higher amrubicinol AUC<sub>0-72</sub> than

the other patients (1385.9  $\mu$ g/h/L vs 1208.5  $\mu$ g/h/L,  $P = .01$ ). The median OS for all patients was 255 days. However, the OS for patients with grade 4 neutropenia was significantly shorter than those without (228 days with grade 4 vs 376 days without grade 4, log-rank test,  $P = .01$ , Figure 3B).

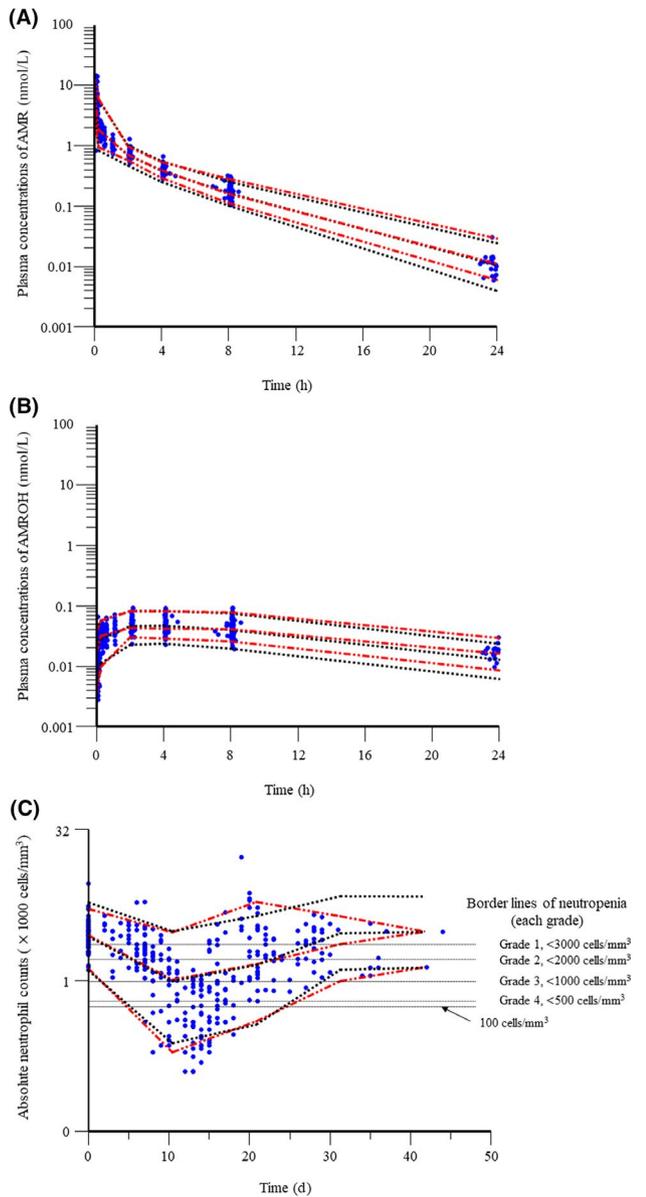
### 3.5 | Model-based simulations for estimating amrubicin optimal doses in patients with severe hematological toxicity

By undertaking simulations using the final Pop-PK-PD model, the median optimal dose for preventing grade 4 neutropenia (29 patients) was predicted to be 22 (range, 8-40) mg/m<sup>2</sup>. With the optimal dose, the amrubicinol mean  $\pm$  SD AUC<sub>0-72</sub> was calculated as 805.5  $\pm$  337.0  $\mu$ g/h/L (Table S3).

## 4 | DISCUSSION

A reduction in the dose of amrubicin from the recommended dose is necessary to reduce hematological toxicity. This reduction is usually based on the physician's experience; however, severe neutropenia is often observed, and in some patients, delay of treatment interval or reduction of secondary dose is required.

The primary objectives of this study were to develop a Pop-PK model for connecting amrubicin to amrubicinol and to identify the covariates of various PK parameters. Only amrubicinol concentration was incorporated into the effect equation (slope  $\cdot$  C<sub>AMROH</sub>) because the OFV of the model did not improve when C<sub>AMR</sub> was added to C<sub>AMROH</sub>, or when C<sub>AMR</sub> was used alone (Figure S1). Thus, we only included the amrubicinol concentration data in the subsequent analyses and simulations. To the best of our knowledge, this is the first study to demonstrate a Pop-PK model with delay

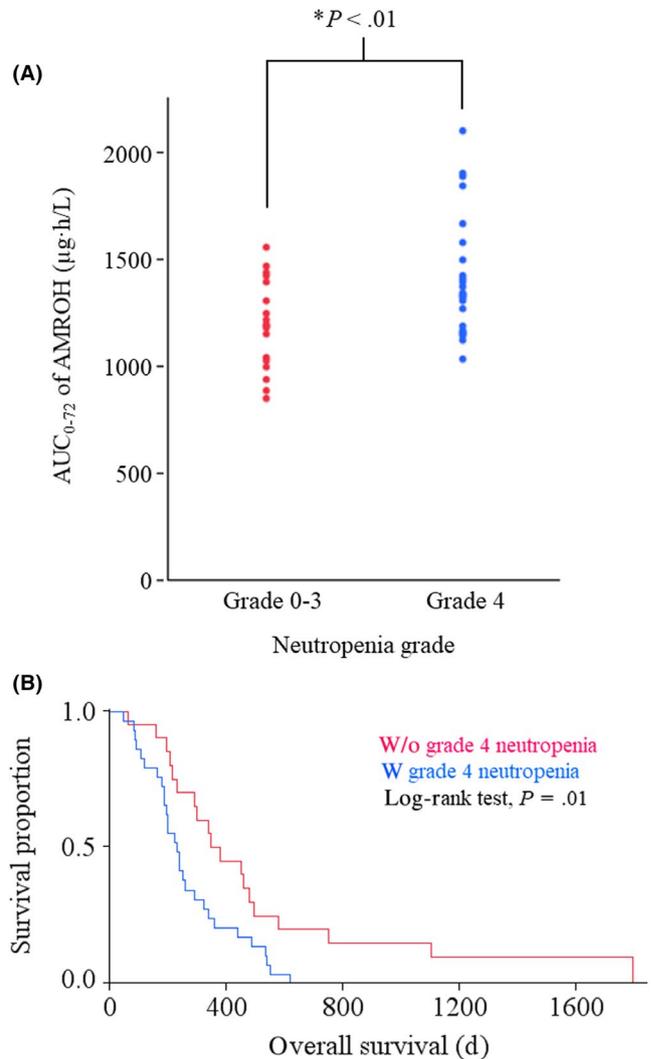


**FIGURE 2** Visual predictive check for plasma amrubicin (AMR) (A) and amrubicinol (AMROH) (B) concentrations, and absolute neutrophil counts in patients with small-cell lung carcinoma after the first cycle of amrubicin treatment on days 1-3 (C). The data were Box-Cox-transformed with a factor of 0.2 (C). Closed circles represent observed data. Dash-dot-dot lines represent the 5th, 50th, and 95th percentiles of observed data. Dotted lines represent the 5th, 50th, and 95th percentiles of the simulated data

compartment for amrubicinol. As shown in Figure S2, a second peak in the amrubicinol concentration profile was observed after ~8 hours in some of the first 21 patients.

In the present study, the Pop-PK final model was improved by including a delay compartment pathway for the clearance of amrubicinol. This second peak might be attributable to a time-lag caused by a transfer of amrubicinol to blood cells, and subsequent reentry from blood cells to plasma.

The *SLC28A3* SNP rs7853758, a synonymous coding variant (L461L), was a significant covariate of *K<sub>dc</sub>* in the final Pop-PK model.



**FIGURE 3** A, One-tailed Student's *t* test for comparison between amrubicinol area under the plasma concentration-time curve (AUC<sub>0-72</sub> of AMROH) and neutropenia grade (grade 4 vs nongrade 4) in patients with small-cell lung carcinoma. B, Kaplan-Meier curves of overall survival for all the participants with or without grade 4 neutropenia. Overall survival results from the patients with or without grade 4 neutropenia are shown by blue solid curves and red, respectively

Subjects with the minor rs7853758 allele showed reduced *SLC28A3* mRNA expression in the monocytes.<sup>15</sup> Concentrative Na<sup>+</sup>-nucleoside cotransporter protein, which is an *SLC28* concentrative nucleoside transporter, can transport several anthracyclines into the cells.<sup>16</sup> Although patients with this minor allele showed a higher AUC<sub>0-72</sub> for amrubicinol than the other patients, the former cohort did not show more severe neutropenia or higher response rates. Further functional studies are required to determine the exact mechanisms by which *SLC28A3* affects the *K<sub>dc</sub>*.

Performance status is a covariate of both PK and PD parameters and could be an important marker for successful amrubicin treatment. Thirty patients with PS1 showed lower amrubicinol CL (91.4 vs 125.8 L/h, *P* < .0001) and shorter mean maturation time (148.3 vs

172.9 h,  $P < .0001$ ) relative to the other 20 patients with PS0. The CL of amrubicinol was lower in patients with higher amrubicinol AUC. However, patients with PS1 tend to show faster transit of progenitor cells into the systemic circulation, resulting in increased cell death or higher toxicity. Thus, these results explained the relationship between the severity of neutropenia and PS.

We successfully predicted the effects of amrubicin-amrubicinol PK (plasma profiles) on the ANC-time profiles. Furthermore, we showed that amrubicinol  $AUC_{0-72}$  was related to the severity of neutropenia and short survival times (Figures 3B and S3). Contrarily, the efficacy (overall response rate) was not related to various PK profiles (Figure S4). Simulations using the final Pop-PK-PD model revealed that the median optimal dose for 29 patients who experienced grade 4 neutropenia was 22 (range, 8-40) mg/m<sup>2</sup>, with a predicted mean  $AUC_{0-72}$  of  $805.5 \pm 337.0$  µg/h/L (Table S3). However, clinical situations would determine if this dose is effective because the mean  $AUC_{0-72}$  of 21 responsive patients (CR + PR) was 1283.0 µg/h/L, which is higher than the predicted  $AUC_{0-72}$  for preventing grade 4 neutropenia in the other 29 patients. Even if physicians measure the blood levels of amrubicinol during amrubicin treatment and predict neutropenia grade using our developed PK-PD model, amrubicin dose reduction is not recommended. Recent studies have shown that low doses of amrubicin (35 mg/m<sup>2</sup>) might be sufficient to produce desirable effects.<sup>5,17,18</sup> However, in this study, we could not determine the optimal therapeutic range for amrubicinol, despite examination using receiver operating characteristic curve analysis (data not shown). Thus, a higher probability of increased antitumor effects at higher doses must be carefully balanced against the increased risk of severe neutropenia.

In general, G-CSF is used to treat severe or febrile neutropenia in patients with SCLC receiving amrubicin. Use of G-CSF reduces the risk, severity, and duration of febrile neutropenia, but owing to its high cost, its routine use is restricted. The present study provides a model-based strategy for understanding the differences in PD outcomes among patient populations and quantifying these differences based on scientific mechanisms. The Pop-PK-PD modeling strategy, therefore, is a valuable method for rationalizing the use of G-CSF as prophylactic agents.

The present study has some limitations. First, the sample size was small because of the difficulty of collecting the plural blood samples per patient. Second, our results have not been validated in different patients. We consider the final Pop-PK-PD model to be useful to simulate the ANC time courses for particular doses and patients; however, the 95% confidence interval of the ANC prediction by this model appeared to be relatively broad. Therefore, to increase the predictability of the Pop-PK-PD model, future prospective studies in a large population with external validation are required to explore the target AUC of amrubicinol and to use for the precision dosing of amrubicin after platinum chemotherapy in practice. In addition, the sensitivity of tumor cells to amrubicin warrants further study.

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## DISCLOSURE

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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