Plasma concentration of amrubicinol in plateau phase in patients treated for 3 days with amrubicin is correlated with hematological toxicities

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Amrubicinol (AMR-OH) is an active metabolite of amrubicin (AMR), a novel synthetic 9-aminoanthracycline derivative. The time–concentration profile of AMR-OH exhibits a continuous long plateau slope in the terminal phase. To determine the relationships between the steady-state plasma concentration of AMR-OH and treatment effects and toxicities associated with AMR therapy, we carried out a pharmacokinetic/pharmacodynamic study in patients treated with AMR alone or the combination of AMR + cisplatin (CDDP). AMR was given at a dose of 30 or 40 mg/m^2 on days 1–3. Plasma samples were collected 24 h after the third injection (day 4). Plasma concentrations of AMR-OH or total CDDP were determined by a high-performance liquid chromatography or an atomic absorption spectrometry. Percent change in neutrophil count (dANC) and the plasma concentration of AMR-OH were evaluated using a sigmoid E_{max} model. A total of 35 patients were enrolled. Significant relationships were observed between AMR-OH on day 4 and the toxicity grades of leukopenia, neutropenia, and anemia ($P=0.018$, $P=0.012$, and $P=0.025$, respectively). Thrombocytopenia grade exhibited a tendency toward relationship with AMR-OH on day 4 ($P=0.081$). The plasma concentration of AMR-OH on day 4 was positively correlated with dANC in the group of all patients, as well as in patients treated with AMR alone and in patients coadministered with CDDP. In conclusion, the plasma concentration of AMR-OH on day 4 was correlated with hematological toxicities in patients treated with AMR. The assessment of plasma concentration of AMR-OH at one timepoint might enable the prediction of hematological toxicities. ***Anti-Cancer Drugs*** **20:513–518 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Amrubicin (AMR) was recently approved in Japan for treatments of small-cell lung cancer (SCLC) and non-SCLC (NSCLC). It is a novel synthetic 9-aminoanthracycline derivative, and is converted to an active metabolite, amrubicinol (AMR-OH), through reduction of its C-13 ketone group to a hydroxy group [1]. AMR and AMR-OH are inhibitors of DNA topoisomerase II, which exert cytotoxic effects by stabilizing a topoisomerase II-mediated cleavable complex [1]. The in-vitro cytotoxic activity of AMR-OH was found to be 18–220 times more potent than that of its parent compound, AMR [2].

Some phase I/II studies of AMR have been conducted in chemotherapy-naive or relapsed patients with advanced SCLC or NSCLC, and the recommended schedule of administration for AMR is daily treatment for 3 consecutive days every 3 weeks at a dose of 35–45 mg/m^2 as a single agent [3–5], or at a dose of 30–40 mg/m^2 for combination therapy with cisplatin (CDDP) [6,7]. In these studies, the major grade 3/4 toxicities were neutropenia, leukopenia, anemia, thrombocytopenia, anorexia, and nausea/vomiting. Pharmacokinetic (PK) and pharmacodynamic (PD) profiles in a phase I trial showed that the area under the concentration–time curve (AUC) and C_{max} of plasma AMR were related to duration of grade 4 neutropenia [8]. Another pharmacological study reported a significant relationship between grade of toxicity for leukopenia and the AUC of AMR-OH [9]. These pharmacological studies obtained blood samples at 5–7 timepoints within 24 h after the end of injection of AMR. It is advisable that PK/PD profiles of AMR and AMR-OH be monitored to assess unexpected adverse effects. However, in clinical settings, it may be difficult to perform blood sampling frequently enough for calculation of the AUC.

In a study examining the time–concentration profiles of AMR and AMR-OH, the plasma concentration curves

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fit a three-compartment open model [10]. Although the plasma concentration curve of AMR exhibited a high peak in α/β phase and down slope in γ phase, that of AMR-OH exhibited a slight or low peak in α/β phase and a continuous long plateau slope in γ phase. Indeed, the half-lives in the terminal phase ($T_{1/2}\gamma$) of AMR and AMR-OH after administration of 30 mg/m^2 AMR on day 3 were 2.2 ± 0.19 and 23.2 ± 18.26 h, respectively [11]. For these reasons, we hypothesized that a steady-state plasma concentration of AMR-OH in the γ phase can predict frequencies and severities of toxicities associated with AMR therapy. To test this hypothesis, a PK/PD analysis was performed on patients with lung cancer during their first cycle of AMR treatment administered on days 1–3. Plasma sampling was performed at pretreatment, 24 h after the first AMR injection, and 24 h after the third AMR injection.

Patients and methods

Patient eligibility

Patients were eligible for inclusion if they were willing to undergo treatment with AMR alone or with the combination of AMR and CDDP, with histologically or cytologically confirmed NSCLC or SCLC, stage IV or IIIB. Eligibility criteria included the following, as well: Eastern Cooperative Oncology Group performance status of 0–2; adequate organ function, such as white blood cell count of at least 4000×10^6/l, hemoglobin level of 9.5 g/dl or greater, platelet count of at least 100×10^9/l, aspartate aminotransferase and alanine aminotransferase less than 100 IU/l, bilirubin level 1.5 mg/dl or less, creatinine concentration 1.2 mg/dl or less, electrocardiographic findings within normal range, and left ventricular ejection fraction on echocardiography of 60% or greater.

Exclusion criteria included the following: symptomatic brain metastasis that required radiation treatment; accumulation of pleural fluid that required treatment such as drainage; continuous serious infectious disease; serious medical conditions (heart disease, interstitial pneumonitis, or uncontrollable diabetes); a history of drug allergy; other active concurrent malignancies; or other problems judged by the investigators to make patients inappropriate for inclusion in this study. Patients were also ineligible who were pregnant or those who wish to conceive.

Written informed consent was obtained from all patients. This study was approved by the Institutional Review Board of Osaka City University, and conducted in accordance with the Japanese Good Clinical Practice guidelines.

Treatment assessment and follow-up

AMR was dissolved in 20 ml physiological saline and administered once intravenously as a 5-min injection at a dose of 30 or 40 mg/m^2/day on days 1–3, every 3 weeks. Before treatment, all patients underwent a medical history, physical examination, hematology and serum biochemistry tests, urinalysis, electrocardiographic findings, left ventricular ejection fraction measurement, and baseline tumor measurements, (chest radiography, computed tomography scans, and bone scintigraphy). Physical examination and toxicity assessment, including hematology tests, serum biochemistry tests, and urinalysis, were repeated at least weekly during chemotherapy. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria Version 3.0 criteria. Response was assessed according to the Response Evaluation Criteria In Solid Tumors [12].

Pharmacokinetic analysis

Blood sampling for pharmacokinetic analysis was performed in the first cycle of AMR treatment. Seven milliliter blood samples were obtained in tubes containing EDTA as an anticoagulant at 3 timepoints: berore AMR injection; 24 h after the first AMR injection (AMR on day 2, AMR-OH on day 2), and, thus, just before the second AMR injection; and 24 h after the third AMR injection (AMR on day 4, AMR-OH on day 4). Blood samples were immediately centrifuged and aliquots of plasma were frozen at −80°C until analyzed. The concentrations of AMR and AMR-OH in plasma were determined by a high-performance liquid chromatography (HPLC) method, as reported earlier [10]. The elemental platinum concentration (total CDDP) was measured in the plasma sample by atomic absorption spectrometry (Perkin-Elmer, model SIMAA 6000 spectrometer, Norwalk, Colorado, USA) that monitored at 265.9 nm [13]. The lower limit of quantification for AMR and AMR-OH in plasma was 8.00 ng/ml, using a sample volume of 100 µl, whereas that of CDDP was 25 ng/ml.

Pharmacodynamic analysis

Correlations between drug concentrations and toxicity grades or responses were determined. The relationship between percent change in neutrophil count (dANC) and the plasma concentration of AMR-OH on day 4 was investigated using a sigmoid E_{max} model [14]. Neutrophil counts were monitored at least weekly, and the nadir count during the first course was recorded. A dANC was defined as:

$$\mathrm{dANC} = (\mathrm{Pretreatment\ count} - \mathrm{Nadir\ count})/(\mathrm{Pretreatment\ count}) \times 100$$

The sigmoid E_{max} model was defined as:

$$\mathrm{dANC} = [E_{max} \times (\mathrm{AMR{-}OH})^{\gamma}]/[(\mathrm{AMR{-}OH})^{\gamma} + \mathrm{EC}_{50}\gamma] \times 100$$

E_{max} represents the maximum effect, and EC_{50} is the plasma concentration of AMR-OH at which the effect is 50% of maximum effect. The exponent gamma (γ) is

a shape factor that determines the steepness of the response curve. These values were determined using WinNonlin Professional Version 5.2 (Pharsight Corporation, Mountain View, California, USA).

Statistical methods

Differences between two dose levels and timepoints of measurement of plasma concentrations of AMR and AMR-OH were evaluated by Mann–Whitney's *U* test. Correlations between PK parameters were assessed with Spearman's rank test. PD analyses were performed with the nonparametric Kruskal–Wallis test. A *P* value of less than 0.05 was regarded as significant, and all reported *P* values are two-tailed.

Results

Patient characteristics

Between November 2004 and January 2007, 35 patients were enrolled in this PK/PD study, and a total of 107 blood samples was obtained. The patient population profile is provided in Table 1. Ten patients had SCLC, 23 patients had NSCLC, and two patients had other tumors, including thymic cancer and neuroblastoma. First-line treatment was performed for a total of 20 patients, including four with SCLC, 15 with NSCLC, and one with neuroblastoma. Second-line treatment was performed for 13 patients, including six with SCLC, six with NSCLC, and one with thymic cancer. Treatment regimens are shown in Table 2. A total of 15 patients, including six with SCLC, eight with NSCLC, and one with thymic cancer, were treated with AMR alone at a dose of 40 mg/m^2 on days 1–3. A total of 20 patients, including four with SCLC, 15 with NSCLC, and one with neuroblastoma, underwent combined treatment with CDDP and AMR. The regimen for SCLC was CDDP 20 mg/m^2 on days 1–3 + AMR 30 mg/m^2 on days 1–3, whereas that for NSCLC was CDDP 80 mg/m^2 on day 1 + AMR 30 mg/m^2 on days 1–3. These schedules were used for phase I/II studies in our hospital and reported previously [15,16].

Table 1 Patient characteristics

Age (years)			
Median		65	
Range		(40–78)	
Sex			
Male		26	
Female		9	
Histology			
SCLC		10	
NSCLC		23	
Others		2	
Treatment	SCLC	NSCLC	Others
First line	4	15	1
Second line	6	6	1
More		2	
Monotherapy	6	8	1
Platinum doublet	4	15	1

NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

Table 2 Plasma concentration levels

Regimens	Dose	*N*		Day 2	Day 4
AMR alone	AMR 40 mg/m^2 (ng/ml ± SD)	15	AMR	10.49 ± 3.89	18.35 ± 9.95
			AMR-OH	8.03 ± 2.35	16.18 ± 6.17
CDDP + AMR	AMR 30 mg/m^2 (ng/ml ± SD)	20	AMR	6.96 ± 4.89	13.18 ± 9.86
			AMR-OH	6.81 ± 4.49	11.02 ± 3.82
	CDDP 20 mg/m^2 days 1–3 (μg/ml ± SD)	5		0.30 ± 0.03	0.75 ± 0.37
	CDDP 80 mg/m^2 day 1 (μg/ml ± SD)	15		1.14 ± 0.40	0.88 ± 0.29

AMR, amrubicin; AMR-OH, amrubicinol; CDDP, cisplatin.

Pharmacokinetics

The plasma concentrations of each drug are summarized in Table 2. As some values were beneath the lower limit of the assay, the concentrations of plasma AMR on day 2 and AMR on day 4 could be measured directly in only 20 of 35 patients and 25 of 35 patients, respectively. The concentrations of plasma AMR-OH on day 2 and AMR-OH on day 4 could be measured directly in only nine of 35 patients and 28 of 35 patients, respectively. Values beneath the lower limit were extrapolated using HPLC data. Mean plasma concentrations (mean ± standard deviation) of AMR on day 2, AMR on day 4, AMR-OH on day 2, and AMR-OH on day 4 were 8.52 ± 4.63, 16.55 ± 11.92, 7.28 ± 3.56, and 13.35 ± 5.56 ng/ml, respectively, with significant increases from day 2 to day 4 in both AMR ($P < 0.0001$) and AMR-OH ($P < 0.0001$). The dose of 30 mg/m^2 of AMR was administered to 20 patients and the dose of 40 mg/m^2 of AMR to 15 patients, and mean plasma concentrations of AMR-OH on day 4 were 12.2 ± 4.89 and 14.8 ± 6.19 ng/ml, respectively. These plasma concentrations of AMR-OH on day 4 increased in a dose-dependent manner, and statistical significance was reached ($P = 0.026$). Of the patients receiving 30 mg/m^2 of AMR, five patients were coadministered CDDP 20 mg/m^2 on days 1–3, whereas 15 patients were coadministered CDDP 80 mg/m^2 on day 1. No significant difference in plasma concentration of AMR-OH on day 4 was observed between the patient group receiving combination treatment with CDDP at a dose of 20 mg/m^2 and the group receiving it at 80 mg/m^2 ($P = 0.407$).

Responses and toxicities

Results of assessment indicated 14 (40%) partial responses, 13 (34%) patients with stable disease, and six (16%) with disease progression. For two patients, response could not be evaluated. The most frequent toxicities were leukopenia and neutropenia. Grade 4 hemotological toxicities and grade 3/4 nonhematological toxicities included the following: hematological toxicities: leukopenia in seven patients (20%), neutropenia in 16 patients (46%), thrombocytopenia in one patient (3%), and anemia in one patient (3%); nonhematological toxicities: nausea/vomiting in four patients (11%), loss of

appetite in six patients (17%), and febrile neutropenia in five patients (14%). No cardiotoxicity was observed during this study. One toxicity-related death was reported in a patient with SCLC who received combination chemotherapy with CDDP 20 mg/m^2 on days 1–3 + AMR 30 mg/m^2 on days 1–3. The plasma concentrations of AMR on day 4, AMR-OH on day 4, and CDDP on day 4 in this patient were 17.4, 10.8 ng/ml and 0.47 μg/ml, respectively.

Pharmacodynamics

Correlations between plasma concentration of AMR-OH on day 4 and toxicity grades and responses were determined, because statistical significance increase was reached from day 2 to day 4 in AMR-OH. No significant differences were observed in plasma concentration of AMR-OH on day 4 between responders and non-responders (P=0.751). Significant Relationships were observed between the plasma concentration of AMR-OH on day 4 and hematological toxicity grades for leukopenia, neutropenia, and anemia ($P = 0.018$, $P = 0.012$, and $P = 0.025$, respectively) (Fig. 1a–c). The toxicity grade of thrombocytopenia exhibited a tendency toward a relationship with AMR-OH on day 4 ($P = 0.081$) (Fig. 1d). There was no relationship between nonhematological toxicities and the plasma concentration of AMR-OH on day 4. The plasma levels of CDDP, including those on day 2 and day 4, were correlated with none of hematological toxicity grades, nonhematological toxicity grades, and responses.

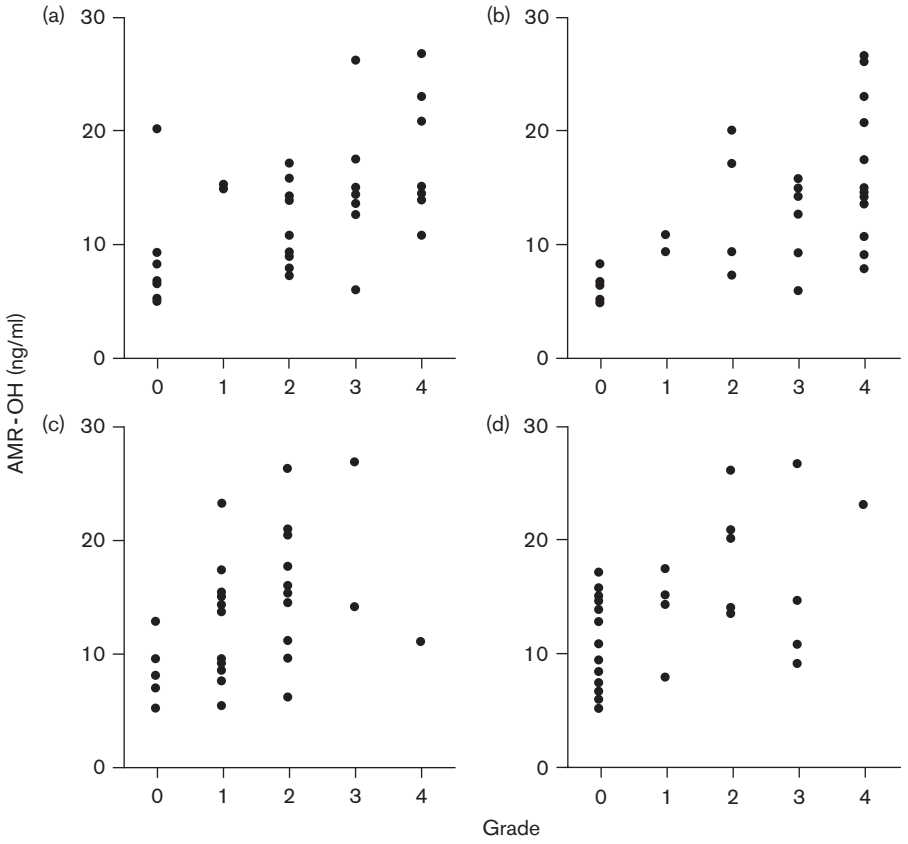
The relationship between percentage change in neutrophil count and plasma concentration of AMR-OH on day 4 was evaluated using a sigmoid E_{max} model. The plasma concentration of AMR-OH on day 4 was positively correlated with dANC in the group of all patients, as well as in those treated with AMR alone and those coadministered with CDDP (Fig. 2). The relationships were as follows:

All patients: E_{max}=96.80, EC_{50}=4.98, and γ=2.30

AMR alone: E_{max}=98.37, EC_{50}=5.19, and γ=1.99

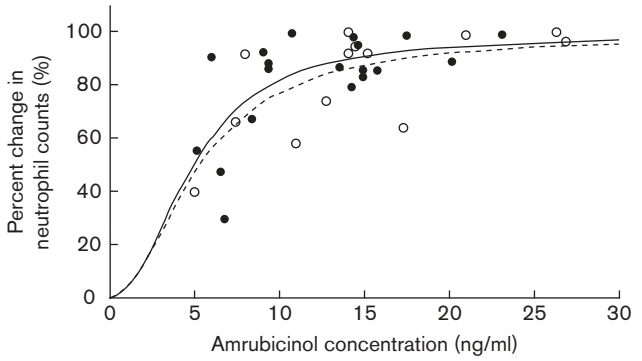
AMR with CDDP: E_{max}=99.01, EC_{50}=4.91, and γ=2.21

Fig. 1



Distributions of plasma concentration of amrubicinol (AMR-OH) on day 4 as a function of hematological toxicity grades in patients treated with the first cycle of amrubicin. (a) leukopenia, (b) neutropenia, (c) anemia, (d) thrombocytopenia.

Fig. 2



Sigmoid E_{max} modeling of percentage change in neutrophil count versus plasma concentration of amrubicinol on day 4 for subgroups of patients treated with amrubicin alone (open circles and dotted lines) or those with coadministration with cisplatin (closed circles and solid lines).

In contrast, no sigmoid relationship between the plasma level of dANC and CDDP on day 2 or CDDP on day 4 was observed, and parameters in the sigmoid E_{max} model could not be calculated.

It is worthy in clinical practice for using the plasma concentration of AMR-OH as a predictive factor of severe toxicities. We calculated a cut-off point of AMR-OH on day 4 to predict 80% or more decrease of neutrophil counts. When a cut-off point was determined to 13.0 ng/ml, the positive predictive value and negative predictive value were 91 and 63%, respectively.

Discussion

We carried out a PK/PD study of AMR in patients with lung cancer to examine the relationships between the steady-state plasma concentration of AMR-OH in the γ phase and toxicity associated with AMR therapy. We showed that the plasma concentration of AMR-OH on day 4, but not AUC, was significantly correlated with hematological toxicities. When a cut-off point was determined as 13.0 ng/ml, the positive predictive value and negative predictive value of 80% or more decrease of neutrophil counts were 91 and 63%, respectively. This cut-off point may useful to predict severe neutropenia associated with AMR therapy in clinical practice.

Pharmacological parameters including AUC, C_{max}, $T_{1/2}$, clearance, and others could not be calculated because plasma sampling was performed only at one timepoint, 24 h after the third injection. Fortunately, we had an opportunity to communicate with researchers who joined the phase I study of AMR, and who noted the finding of a significant linear correlation between plasma concentration of AMR-OH at 8 h after injection and the $AUC_{0–8}$ of AMR-OH (personal communication). Taken together, our findings suggest that the plasma concentration of AMR-OH at 24 h after injection may be related to the $AUC_{0–24}$ of AMR-OH in patients with AMR treatment.

The major metabolic pathway of AMR is known to involve reduction of the C-13 carbonyl group to a hydroxyl group by cytoplasmic carbonyl reductase [1]. The conversion of anthracycline derivatives, including doxorubicin, epirubicin, and daunorubicin, to their 13-hydroxy metabolites is generally regarded as an inactivation pathway for elimination [2]. However, AMR has the unique pharmacological profile that its metabolite AMR-OH has more potent antitumor activities than its parent compound, AMR. In in-vitro studies, AMR was found to be metabolized to AMR-OH by human tumor cells, and substantial amounts of AMR-OH were found in cells after 5-h drug incubation in several cancer cell lines tested [2]. AMR-OH is less susceptible to further metabolism or is retained in tissues for a longer period of time [10]. It was also found that the ratio of AMR-OH level to AMR level in plasma was about 0.1 from 1 h after administration [10]. These findings may explain the unique time–concentration profiles of AMR-OH, which include a slight or low peak in α/β phase and a continuous long plateau slope in γ phase in the three-compartment open model.

Relationships between AMR-OH and hematological toxicities were established for treatment with AMR alone, as well as for coadministration of CDDP, using a sigmoid E_{max} model for pharmacodynamic analysis. The sigmoid curve for coadministration with CDDP was shifted to the left compared with that for AMR alone. This shift may indicate that patients treated with AMR and CDDP experienced neutropenia more often that would be expected in the case of AMR alone. This mild additive effect in hematological toxicity is in agreement with clinical observations noted in many previous reports; patients with combined treatment with AMR and CDDP experienced more profound myelotoxicity than those treated with AMR alone, and the dose of AMR for combined treatment with CDDP was decreased compared with that for AMR alone [6,7].

In this study, no relationship between plasma level of CDDP and hematological toxicity was apparent. In general, the toxic effects of CDDP include nephrotoxicity, nausea and vomiting, ototoxicity, neurotoxicity, and relatively minor hematological toxicities [17]. Other reports have noted that the pharmacokinetic parameters of CDDP were significantly higher in patients with nausea and vomiting or nephrotoxicity than in those without these conditions [18]. In addition, in a PK/PD study of combined treatment with CDDP and docetaxel, neutropenia was positively correlated with area under the AUC–time curve for docetaxel but not that for CDDP [19].

Our findings have certain limitations. First, plasma sampling was performed only at 24 h after AMR injection. At this timepoint, some samples could not be measured because AMR or AMR-OH was beneath the lower limit of the assay used. Values beneath the lower limit were extrapolated using HPLC data. These extrapolated values are not accurate, though it is clear that they were each under 8 ng and related to mild neutropenia. Measurements at early timepoints of 4 or 8 h after injection may thus be worthwhile for this type of analysis. Second, factors associated with metabolism of AMR and AMR-OH were not examined. Some factors may affect the toxicities of AMR directly or indirectly. Third, the combined regimens involving CDDP that we used differed between SCLC and NSCLC. In SCLC patients, the plasma concentrations of CDDP on day 4 were higher than those on day 2, because of consecutive administration of CDDP for 3 days. In contrast, NSCLC patients had higher concentrations of CDDP on day 2 than on day 4, because CDDP was administered only on day 1. Both regimens were restricted to patients with adequate organ function. Good clearance might explain why use of different doses of CDDP administration did not alter the pharmacokinetics of AMR-OH.

In conclusion, we found that the plasma concentration of AMR-OH on day 4 was significantly correlated with hematological toxicities. This relationship can be used to predict the degree of neutropenia in an individual patient. AMR is one of the most attractive agents for use against lung cancer, because of the unique pharmacological profile of its active metabolite AMR-OH. The assessment at one timepoint of plasma concentration might enable prediction of the pharmacological parameter of AUC as well as hematological toxicities.

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References

1 Hanada M, Mizuno S, Fukushima A, Saito Y, Noguchi T, Yamaoka T. A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. *Jpn J Cancer Res* 1998; **89**:1229–1238.

2 Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y. Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. *Jpn J Cancer Res* 1998; **89**:1067–1073.

3 Okamoto I, Hamada A, Matsunaga Y, Sasaki J, Fujii S, Uramoto H, *et al*. Phase I and pharmacokinetic study of amrubicin, a synthetic 9-aminoanthracycline, in patients with refractory or relapsed lung cancer. *Cancer Chemother Pharmacol* 2006; **57**:282–288.

4 Onoda S, Masuda N, Seto T, Eguchi K, Takiguchi Y, Isobe H, *et al*. Phase II trial of amrubicin for treatment of refractory or relapsed small-cell lung cancer: Thoracic Oncology Research Group Study 0301. *J Clin Oncol* 2006; **24**:5448–5453.

5 Igawa S, Yamamoto N, Ueda S, Ono A, Nakamura Y, Tsuya A, *et al*. Evaluation of the recommended dose and efficacy of amrubicin as second- and third-line chemotherapy for small cell lung cancer. *J Thorac Oncol* 2007; **2**:741–744.

6 Ohe Y, Negoro S, Matsui K, Nakagawa K, Sugiura T, Takada Y, *et al*. Phase I-II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer. *Ann Oncol* 2005; **16**:430–436.

7 Ikeda J, Maruyama R, Okamoto T, Shoji F, Wataya H, Ichinose Y. Phase I study of amrubicin hydrochloride and cisplatin in patients previously treated for advanced non-small cell lung cancer. *Jpn J Clin Oncol* 2006; **36**:12–16.

8 Shibayama T, Hotta K, Takigawa N, Tada A, Ueoka H, Harita S, *et al*. A phase I and pharmacological study of amrubicin and topotecan in patients of small-cell lung cancer with relapsed or extensive-disease small-cell lung cancer. *Lung Cancer* 2006; **53**:189–195.

9 Matsunaga Y, Hamada A, Okamoto I, Sasaki J, Moriyama E, Kishi H, *et al*. Pharmacokinetics of amrubicin and its active metabolite amrubicinol in lung cancer patients. *Ther Drug Monit* 2006; **28**:76–82.

10 Noguchi T, Ichii S, Morisada S, Yamaoka T, Yanagi Y. Tumor-selective distribution of an active metabolite of the 9-aminoanthracycline amrubicin. *Jpn J Cancer Res* 1998; **89**:1061–1066.

11 Yanaihara T, Yokoba M, Onoda S, Yamamoto M, Ryuge S, Hagiri S, *et al*. Phase I and pharmacologic study of irinotecan and amrubicin in advanced non-small cell lung cancer. *Cancer Chemother Pharmacol* 2007; **59**:419–427.

12 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, *et al*. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**:205–216.

13 Johnsson A, Olsson C, Nygren O, Nilsson M, Seiving B, Cavallin-Stahl E. Pharmacokinetics and tissue distribution of cisplatin in nude mice: platinum levels and cisplatin-DNA adducts. *Cancer Chemother Pharmacol* 1995; **37**:23–31.

14 Beelen AP, Lewis LD. Clinical pharmacology overview. In: Figg WD, McLeod HL, editors. *Handbook of anticancer pharmacokinetics and pharmacodynamics*. Totowa: Humana Press Inc.; 2004. pp. 111–127.

15 Kudoh S, Yoshimura N, Kimura T, Mitsuoka S, Hirata K. A phase II trial of amrubicin (AMR) for recurrent or refractory small cell lung cancer (SCLC). *Proc Am Soc Clin Oncol* 2006; **24**:abstr 17053.

16 Yoshimura N, Kudoh S, Kimura T, Mitsuoka S, Yana T, Hirata K. Phase I study of amrubicin and cisplatin in previously untreated patients with advanced non-small-cell lung cancer. *Proc Am Soc Clin Oncol* 2008; **26**:abstr 19119.

17 Einhorn LH, Williams SD. The role of cis-platinum in solid-tumor therapy. *N Engl J Med* 1979; **300**:289–291.

18 Johnsson A, Hoglund P, Grubb A, Cavallin-Stahl E. Cisplatin pharmacokinetics and pharmacodynamics in patients with squamous-cell carcinoma of the head/neck or esophagus. *Cancer Chemother Pharmacol* 1996; **39**:25–33.

19 Minami H, Ohe Y, Niho S, Goto K, Ohmatsu H, Kubota K, *et al*. Comparison of pharmacokinetics and pharmacodynamics of docetaxel and Cisplatin in elderly and non-elderly patients: why is toxicity increased in elderly patients? *J Clin Oncol* 2004; **22**:2901–2908.