研究成果の刊行に関する一覧表

雑誌

| | | | | | | |
|---|---|---|---|---|---|---|
| 62 | Okabe, T., Okamoto, I., Tsukioka, S., Uchida, J., Hatashita, E., Yamada, Y., Yoshida, T., Nishio, K., Fukuoka, M., Janne, PA., Nakagawa, K. | Addition of S-1 to the epidermal growth factor receptor inhibitor gefitinib overcomes gefitinib resistance in non-small cell lung cancer cell lines with MET amplification. | Clin Cancer Res. | 15(3) | 907-913 | 2009 |
| 63 | Tanaka,K., Arao,T., Maegawa, M., Matsumoto, K., Kaneda, H., Kudo, K., Fujita, Y., Yokote, H., Yanagihara, K., Yamada, Y., Okamoto, I., Nakagawa, K., Nishio, K. | SRPX2 is overexpressed in gastric cancer and promotes cellular migration and adhesion. | Int J Cancer | 124 | 1072-1080 | 2009 |
| 64 | Morinaga, R., Okamoto, I., Fujita,Y., Arao,T., Sekijima, M., Nishio, K., Ito, H, Fukuoka, M., Kadota, J., Nakagawa, K. | Association of epidermal growth factor receptor (EGFR) gene mutations with EGFR amplification in advanced non-small cell lung cancer. | Cancer Sci. | 99(2) | 2455-2460 | 2008 |
| 65 | Iwasa, T., Okamoto, I., Suzuki, M., Nakahara,T., Yamanaka, K., Hatashita, E., Yamada, Y., Fukuoka, M., Ono, K., Nakagawa, K. | Radiosensitizing effect of YM155, a novel small molecule urvivin suppressant, in non−small cell lung cancer cell lines. | Clinical Cancer Res. | 14 (20) | 6496-6504 | 2008 |
| 66 | Okabe, T., Okamoto, I., Tsukioka, S., Uchida, J., Iwasa, T., Yoshida, T., Hatashita, E., Yamada, Y., Satoh, T., Tamura, K., Fukuoka, M., Nakagawa, K. | Synergistic antitumor effect of S-1 and the epidermal growth factor receptor inhibitor gefitinib in non-small cell lung cancer cell lines:role of gefitinib-induced down-regulation of thymidylate synthase. | Mol Cancer Ther. | 7(3) | 599-606 | 2008 |
| 67 | Akashi, Y., Okamoto, I., Iwasa, T., Yoshida, T., Suzuki, M., Hatashita, E., Yamada, Y., Satoh, T., Fukuoka, M., Ono, K., Nakagawa, K. | Enhancement of the antitumor activity of ionising radiation by nimotuzumab, a humanised monoclonal antibody to the epidermal growth factor receptor, in non-small cell lung cancer cell lines of differing epidermal growth factor receptor status. | Br J Cancer | 98(4) | 749-755 | 2008 |

研究成果の刊行に関する一覧表

雑誌

| | | | | | | |
|---|---|---|---|---|---|---|
| 68 | Yoshida, T., Okamoto, I., Okabe, T., Iwasa, T., Satoh, T., Nishio, K., Fukuoka, M., Nakagawa, K. | Matuzumab and cetuximab activate the epidermal growth factor receptor but fail to trigger downstream signaling by Akt or Erk. | Int J Cancer | 122(7) | 1530-1538 | 2008 |
| 69 | Okabe, T., Okamoto, I., Tamura, K., Terashima, M., Yoshida, T., Satoh, T., Takada, M., Fukuoka, M., Nakagawa, K. | Differential Constitutive Activation of the Epidermal Growth Factor Receptor in Non-Small Cell Lung Cancer Cells Bearing EGFR Gene Mutation and Amplification. | Cancer Res. | 67(5) | 2046-2053 | 2007 |
| 70 | Tanei, T., Morimoto, K., Shimazu, K., Kim, S. J., Tanji, Y., Taguchi, T., Tamaki, Y., Noguchi, S. | Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential Paclitaxel and epirubicin-based chemotherapy for breast cancers. | Clin Cancer Res | 15 | 4234-4241 | 2009 |
| 71 | Okishiro, M., Taguchi, T., Jin Kim, S., Shimazu, K., Tamaki, Y., Noguchi, S. | Genetic polymorphisms of CYP2D6 10 and CYP2C19 2, 3 are not associated with prognosis, endometrial thickness, or bone mineral density in Japanese breast cancer patients treated with adjuvant tamoxifen. | Cancer | 115 | 952-961 | 2009 |
| 72 | Okishiro, M., Taguchi, T., Kim, S. J., Tanji, Y., Shimazu, K., Tamaki, Y., Noguchi, S. | Incidence of joint symptoms and bone fractures in Japanese postmenopausal breast cancer patients treated with adjuvant anastrozole. | J Cancer Res Clin Oncol. | 135 | 823-827 | 2009 |
| 73 | Kim, S. J., Taguchi, T., Shimazu, K., Tanji, Y., Tamaki, Y., Noguchi, S. | Good Response to Paclitaxel Predicts High Rates of Pathologic Complete Response for Breast Cancer Patients Treated Preoperatively with Paclitaxel Followed by 5-Fluorouracil, Epirubicin and Cyclophosphamide. | Oncology | 77 | 134-139 | 2009 |
| 74 | Nakayama, S., Miyoshi, Y., Ishihara, H., Noguchi, S. | Growth-inhibitory effect of adiponectin via adiponectin receptor 1 on human breast cancer cells through inhibition of S-phase entry without inducing apoptosis. | Breast Cancer Res Treat. | 112 | 405-410 | 2008 |

研究成果の刊行に関する一覧表

雑誌

| | | | | | | |
|---|---|---|---|---|---|---|
| 75 | Miyoshi, Y., Kurosumi, M., Kurebayashi, J., Matsuura, N., Takahashi, M., Tokunaga, E., Egawa, C., Masuda, N., Kim, S. J., Okishiro, M., Yanagisawa, T., Ueda, S., Taguchi, T., Tamaki, Y., Noguchi, S. | Low nuclear grade but not cell proliferation predictive of pathological complete response to docetaxel in human breast cancers. | J Cancer Res Clin Oncol. | 134 | 561-567 | 2008. |
| 76 | Miyoshi, Y., Kurosumi, M., Kurebayashi, J., Matsuura, N., Takahashi, M., Tokunaga, E., Egawa, C., Masuda, N., Kim, S. J., Okishiro, M., Yanagisawa, T., Ueda, S., Taguchi, T., Tamaki, Y., Noguchi, S. | Topoisomerase IIalpha-positive and BRCA1-negative phenotype: Association with favorable response to epirubicin-based regimens for human breast cancers. | Cancer Lett. | 264 | 44-53 | 2008 |
| 77 | Kim, S. J., Nakayama, S., Miyoshi, Y., Taguchi, T., Tamaki, Y., Matsushima, T., Torikoshi, Y., Tanaka, S., Yoshida, T., Ishihara, H., Noguchi, S. | Determination of the specific activity of CDK1 and CDK2 as a novel prognostic indicator for early breast cancer. | Ann Oncol. | 19 | 68-72 | 2008 |
| 78 | Arai, T., Miyoshi, Y., Kim, S. J., Akazawa, K., Maruyama, N., Taguchi, T., Tamaki, Y., Noguchi, S. | Association of GSTP1 expression with resistance to docetaxel and paclitaxel in human breast cancers. | Eur J Surg Oncol. | 34 | 734-738 | 2008 |
| 79 | Takahashi, Y., Miyoshi, Y., Morimoto, K., Taguchi, T., Tamaki, Y., Noguchi, S. | Low LATS2 mRNA level can predict favorable response to epirubicin plus cyclophosphamide, but not to docetaxel, in breast cancers. | J Cancer Res Clin Oncol. | 133 | 501-509 | 2007 |
| 80 | Ooe, A., Kato, K., Noguchi, S. | Possible involvement of CCT5, RGS3, and YKT6 genes up-regulated in p53-mutated tumors in resistance to docetaxel in human breast cancers. | Breast Cancer Res Treat. | 101 | 305-315 | 2007 |
| 81 | Maruyama, N., Miyoshi, Y., Taguchi, T., Tamaki, Y., Monden, M., Noguchi, S. | Clinicopathologic Analysis of Breast Cancers with PIK3CA Mutations in Japanese Women. | Clin Cancer Res. | 13 | 408-414 | 2007 |

研究成果の刊行に関する一覧表

雑誌

| | | | | | | |
|---|---|---|---|---|---|---|
| 82 | Matsumoto, K., Arao, T., Tanaka, K., Kaneda, H., Kudo, K., Fujita, Y., Tamura, D., Aomatsu, K., Tamura, T., Yamada, Y., Saijo, N., Nishio, K. | mTOR signal and hypoxia-inducible factor-1 alpha regulate CD133 expression in cancer cells. | Cancer Res. | 69(18) | 7160-7164 | 2009 |
| 83 | Maegawa, M., Arao, T., Yokote, H., Matsumoto, K., Kudo, K., Tanaka, K., Kaneda, H., Fujita, Y., Ito, F., Nishio, K. | EGFR mutation up-regulates EGR1 expression through the ERK pathway. | Anticancer Res. | 29(4) | 1111-1117 | 2009 |
| 84 | Matsumoto, K., Shimizu, C., Arao, T., Andoh, M., Katsumata, N., Koho, T., Yonemori, K., Koziumi, F., Yokote, H., Aogi, K., Tamura, K., Nishio, K., Fujiwara, Y. | Identification of predictive biomarkers for response to Trastuzumab using plasma FUCA activity and N-Glycan identified by MALDI-TOF-MS. | J Proeome Res. | 8(2) | 457-462 | 2009 |
| 85 | Maegawa, M., Arao, T., Yokote, H., Matsumoto, K., Kudo, K., Tanaka, K., Kaneda, H., Fujita, Y., Ito, F., Nishio, K. | Epidermal growth factor receptor lacking C-terminal autophosphorylation sites retains signal transduction and high sensitivity to epidermal growth factor receptor tyrosine kinase inhibitor. | Cancer Sci. | 100(3) | 552-557 | 2009 |
| 86 | Takeuchi, K., Shin-Ya, T., Nishio, K., Ito, F. | Mitogen-activated protein kinase phosphatase-1 modulated JNK activation is critical for apoptosis induced by inhibitor of epidermal growth factor receptor-tyrosine kinase. | FEBS J. | 276(5) | 1255-1265 | 2009 |
| 87 | Honma, K., Iwao-Koizumi, K., Takeshita, F., Yamamoto, Y., Yoshida, T., Nishio, K., Nakagawa, S., Nagahara, S., Kato, K., Ochiya, T. | RPN2 gene congers docetaxel resistance in breast cancer. | Natuer Med. | 14(9) | 939-948 | 2008 |

研究成果の刊行に関する一覧表

雑誌

| | | | | | | |
|---|---|---|---|---|---|---|
| 88 | Yanagihara, K., Takigahira, M., Tanaka, H., Arao, T., Aoyagi, Y., Oda, T., Ochiai, A., Nishio, K. | Establishment and molecular profiling of a novel human pancreatic cancer panel for 5-FU. | Cancer Sci. | 99(9) | 1859-1864 | 2008 |
| 89 | Matsumoto, K., Yokote, H., Arao, T., Maegawa, M., Tanaka, K., Fujita, Y., Shimizu, C., Hanafusa, T., Fujiwara, Y, Nishio, K. | N-Glycan fucosylation of EGFR modulates receptor activity and sesitivity to EGFR tyrosine kinase inhibitors. | Cancer Sci. | 99(8) | 1611-1617 | 2008 |
| 90 | Sakai, K., Yokote, H., Murakami-Murofushi, K., Tamura, T., Saijo, N., Nishio, K. | Pertuzumab, a novel HER dimerization inhibitor, inhibits the growth of human lung cancer cells mediated by the HER3 signaling pathway. | Cancer Sci. | 98(9) | 1498-1503 | 2007 |
| 91 | Kimura, H., Sakai, K., Arao, T., Shimoyama, T., Tamura, T., Nishio, K. | Antibody-dependent cellular cytotoxicity of cetuximab against tumor cells with wild-type of mutant epidermal growth factor receptor. | Cancer Sci. | 98(8) | 1275-1280 | 2007 |
| 92 | Takeda, M., Arao, T., Yokote, H., Komatsu, T., Yanagihara, K., Sasaki, H., Yamada, Y., Tamura, T., Fukuoka, K., Kimura, H., Saijo, N., Nishio, K. | AZD2171 shows potent antitumor activity against gastric cancer over-expressing FGFR2/KGFR. | Clin Cancer Res . | 13(10) | 3051-3057 | 2007 |

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第３次対がん総合戦略研究事業

新しい薬物療法の導入とその最適化に関する研究

研究成果の刊行物・別刷


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# Simple and sensitive HPLC method for determination of amrubicin and amrubicinol in human plasma: application to a clinical pharmacokinetic study

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**ABSTRACT: A simple and sensitive high-performance liquid chromatographic (HPLC) method was developed for determination of amrubicin and its metabolite amrubicinol in human plasma. After protein precipitation with methanol without evaporation procedure, large volume samples were injected and separated by two monolithic columns with a guard column. The mobile phase consisted of tetrahydrofuran–dioxane–water (containing 2.3 mM acetic acid and 4 mM sodium 1-octanesulfonate; 2:6:15, v/v/v). Wavelengths of fluorescence detection were set at 480 nm for excitation and 550 nm for detection. Under these conditions, linearity was confirmed in the 2.5–5000 ng/mL concentration range of both compounds. The intra- and inter-day precision and intra- and inter-day accuracy for both compounds were less than 10%. The method was successfully applied to a clinical pharmacokinetic study of amrubicin and amrubicinol in cancer patients. Copyright © 2009 John Wiley & Sons, Ltd.**

**Keywords:** amrubicin; amrubicinol; cancer; protein precipitation; monolithic column

## Introduction

Amrubicin, a completely synthetic 9-aminoanthracycline, is an active anticancer agent. Both amrubicin and amrubicinol, the C-13 hydroxy active metabolite of amrubicin, are inhibitors of the DNA topoisomerase II mediated cleavable complex. The antitumor activity of amrubicinol is 10–100 times greater than that of the parent compound *in vitro* (Yamaoka *et al.*, 1998). In phase I/II trials conducted in Japan the recommended dose of amrubicin was determined to be 45 mg/m$^2$ for three consecutive days every 3 or 4 weeks. In phase II trials of amrubicin monotherapy the response rate of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) was found to be 75.8% (Yana *et al.*, 2007) and 27.9% (Sawa *et al.*, 2006), respectively. Amrubicin was approved in Japan for the treatment of SCLC and NSCLC in December 2002. The major problem with amrubicin is hematological toxicity. An incidence of grade 3 or 4 toxicity of 76.8% has been found for neutropenia, 54.7% for leucopenia, 26.0% for anemia, 22.1% for thrombocytopenia and 35% for the more serious toxicity, febrile neutropenia (Kato *et al.*, 2006). The severity of these toxicities varies from individual to individual. Neutropenia has been reported to be associated with the area under the curve of the plasma amrubicinol concentration, which is one of the major pharmacokinetic (PK) parameters (Matsunaga *et al.*, 2006). A prospective PK and pharmacodynamic (PD) study was planned in our institution, the National Cancer Center Hospital (Tokyo, Japan), to evaluate the PK and PD parameters of amrubicin and amrubicinol and to develop an individualized dosing strategy for amrubicin.

Development of a simple and sensitive HPLC method for determination of amrubicin and amrubicinol in human plasma was required to conduct the PK/PD study. Four methods, including two HPLC methods (Noguchi *et al.*, 1998; Matsunaga *et al.*, 2006), an HPLC-MS-MS method (Yanaihara *et al.*, 2007) and a UPLC-MS-MS method (Li *et al.*, 2008), have already been reported. The two HPLC methods (Noguchi *et al.*, 1998; Matsunaga *et al.*, 2006) involve preparation by liquid–liquid extraction and solid-phase extraction, respectively, and they lack sensitivity because of low recovery and loss during processing. The HPLC-MS-MS method (Yanaihara *et al.*, 2007), on the other hand, involves preparation by solid-phase extraction, but the lower limits of quantification (LOQ) of amrubicin and amrubicinol is 20 ng/mL, which is higher than the plasma concentration of amrubicin 24 h after an intravenous bolus and higher than the concentration of amrubicinol, stated in the application for the approval of amrubicin. However,

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**Abbreviations used:** NSCLC, non-small cell lung cancer; PD, pharmacodynamic; PK, pharmacokinetic; SCLC, small cell lung cancer.

although an UPLC-MS-MS method by protein precipitation (Li *et al.*, 2008) improved the LOQ and preparation procedure, it is not general measurement equipment in hospitals because the equipment is too expensive.

A monolithic column, which has applied for bioanalysis with in the past 10 years, has a lower back-pressure than packed beds (Nguyen *et al.*, 2006). Many applications of analysis using monolithic column have been investigated in μ-HPLC and capillary electrochromatography. Otherwise, few methods refer to the use of a monolithic column by HPLC. The advantage of using monolith columns is that sensitivity can be improved by a long column and large volume samples can be injected.

There are many methods of sample preparation, including protein precipitation, liquid–liquid extraction and solid-phase extraction. Protein-precipitation is the simplest method in terms of procedure and technique. Moreover, recovery with protein precipitation is higher than that with liquid–liquid extraction or solid-phase extraction because amrubicin and amrubicinol have both hydrophilic sites and hydrophobic sites.

The aim of this study was to use monolithic columns to develop a simple and sensitive HPLC method for determination of amrubicin and amrubicinol in human plasma.

## Experimental

### Chemicals

Amrubicin and amrubicinol were provided by Dainippon Sumitomo Pharmaceuticals Co. Ltd (Osaka, Japan). The chemical structure of amrubcin and amrubicinol are shown in Fig. 1. All other chemicals and reagents used were of analytical reagent grade or HPLC grade and were purchased from Wako (Osaka, Japan).

### Chromatographic Instrumentation and Conditions

The chromatographic system consisted of an LC-20AD pump, a SIL-20AC auto sampler, an RF-10AXL fluorescence detector and C-R8A Chromatopac integrator with a CTO-10A oven (Shimadzu, Kyoto, Japan). Two connected Onyx Monolithic $C_{18}$ (100 × 4.6 mm) columns were used with an Onyx Monolithic $C_{18}$ Guard Cartridge (10 × 4.6 mm; Phenomenex, Torrance, CA, USA). Effluent was monitored with a fluorescence detector set at an excitation wavelength of 480 nm and a detection wavelength of 550 nm. The mobile phase consisted of tetrahydrofuran–dioxane–water (containing 2.3 mM acetic acid and 4 mM sodium 1-octanesulfonate; 2:6:15, v/v/v) pumped at flow rate of 0.9 mL/min at a column temperature of 35°C.

### Preparation of Standards and Plasma Samples

Stock solutions of amrubicin and amrubicinol were stored in plastic microtubes to avoid adsorption to glassware, and stored at −80°C. Working solutions were obtained by diluting the stock solutions with acetonitrile. Blank plasma samples for use in validating the method were obtained from healthy volunteers. The patient plasma samples were obtained from the National Cancer Center Hospital. Both the blank plasma samples and patient plasma samples were stored at −80°C until analyzed. Heparin sodium salt was added to patient blood samples to prevent coagulation. Then the blood samples were centrifuged at 5000 rpm for 10 min.

### Extraction Procedure

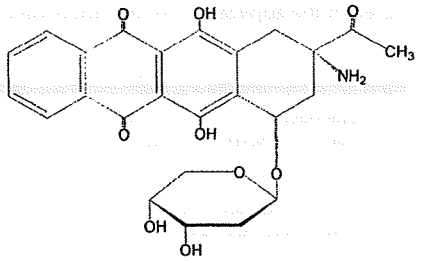
Plasma (100 μL) in a 1.5 mL screw-capped tube was diluted with 20 μL of 16 mM citric acid–16 mM $Na_2HPO_4$–0.9% NaCl solution, and after adding 480 μL of methanol the tube was shaken for 30 min. The mixture was then centrifuged for 10 min at 12,000 rpm. The supernatant was filtered through an UltraFree-MC filter (Millipore, Tokyo, Japan), and 250 μL of the solution was transferred into auto sampler vials and vortex-mixed with a 500 μL of 16 mM citric acid–16 mM $Na_2HPO_4$–0.9% NaCl solution. A 450 μL volume of the solution was injected into the HPLC system for analysis.

### Validation

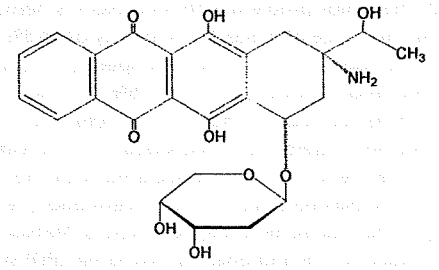
**Specificity.** The specificity of the method was evaluated by comparing different blank plasma samples and plasma samples spiked with amrubicin and amrubicinol. The blank plasma samples were collected from nine volunteers.

**Accuracy, precision and recovery.** Accuracy and precision were determined by replicate analysis ($n = 6$) of plasma samples spiked with three concentrations of amrubicin and amrubicinol: 10, 100 and 1000 ng/mL. Accuracy was evaluated as relative error (RE), and precision was evaluated as coefficient of variation (CV). Recovery was assessed by comparing the results of analyses of extracted plasma samples and unextracted standards containing the same concentrations.

**Calibration curve.** The LOQ was determined from the peak and the standard deviation of the noise level (SN). The LOQ was defined as the concentration of amrubicin and amrubicinol resulting in a peak height of 10 times SN. The calibration curve was generated by linear regression of the peak areas ($y$) of amrubicin and amrubicinol against the corresponding concentrations ($x$) of amrubicin and amrubicinol in plasma.




amrubicin




amrubicinol

**Figure 1.** Chemical structures of amrubicin and amrubicinol.



### Analysis of Patient Samples

For the analysis of plasma concentration of amrubicin and amrubicinol, plasma samples were obtained from lung cancer patients treated with 40 mg/m$^2$ of amrubicin. All patients were enrolled in the prospective PK/PD study, which was aimed to evaluate the correlation between PK and PD of amrubicin and amrubicinol. Written informed consent was obtained from all patients. This study was approved by the Ethical Review Board of National Cancer Center Hospital and is ongoing. The plasma samples were obtained from blood samples collected immediately before injection, and immediately after the injection, and 5, 15 and 30 min, and 1, 2, 4, 8 and 24 h after the end of injection. Each sample was determined in triplicate.

## Results

### Specificity

No endogenous interference was observed at the retention times of amrubicin and amrubicinol. The retention time of amrubicin and amrubicinol was approximately 8.5 and 10.2 min, respectively. Representative chromatograms of the blank plasma sample, the plasma sample spiked and the patient plasma sample are shown in Fig. 2. The capacity factors ($k'$) of amrubicin and amrubicinol were 1 and 1.4, respectively.

### Accuracy, Precision and Recovery

The results for intra- and inter-day accuracy, precision and recovery are shown in Table 1. Intra-day accuracy ranged between −4.1 and 0.8% for amrubicin and between −9.8 and −2.1% for amrubicinol. Inter-day accuracy was between −3.1 and 3.0% for amrubicin and between −4.0 and 2.3% for amrubicinol. Intra-day precision was 1.4–8.8% for amrubicin and 1.3–4.2% for amrubicinol. Inter-day precision was 2.7–8.8% for amrubicin and 5.3–5.5% for amrubicinol. Recovery was greater than 95% at all concentrations (10, 100 and 1000 ng/mL) of amrubicin and amrubicinol.

### Lower Limit of Quantitation

The LOQ was 2.5 ng/mL for both amrubicin and amrubicinol. At that level the coefficient of variation (CV) was 8.3% for amrubicin and 3.2% for amrubicinol ($n = 6$).

### Linearity

Under the chromatographic conditions described, linearity and the appropriate correlation coefficient were achieved for amrubicin within the concentration range from 2.5 to 5000 ng/mL. The linear regression equation for amrubicin was $y = 526.3x + 6156.3$, and the correlation coefficient ($r$) was 0.999. Similar results were obtained for amrubicinol with the concentration range from 2.5 to 5000 ng/mL. The linear regression equation for amrubicinol was $y = 662.9x + 2947.7$, and the correlation coefficient ($r$) was 0.996.

### Analysis of Patient Plasma Samples

The amrubicin and amrubicinol in the patient plasma samples were separated well under the optimal chromatographic conditions. Figure 2(C) shows a chromatogram of amrubicin and amrubicinol in a plasma sample from a patient who was treated at dose of 40 mg/m$^2$ of amrubicin. Figure 3 shows the concentration–time profiles for amrubicin and amrubicinol after an intravenous bolus. The amrubicin concentrations ranged between 4.3 and 2504 ng/mL, and the amrubicinol concentrations ranged between 3.0 and 18.5 ng/mL. These concentrations were similar to the concentrations stated in the application for approval of amrubicin.

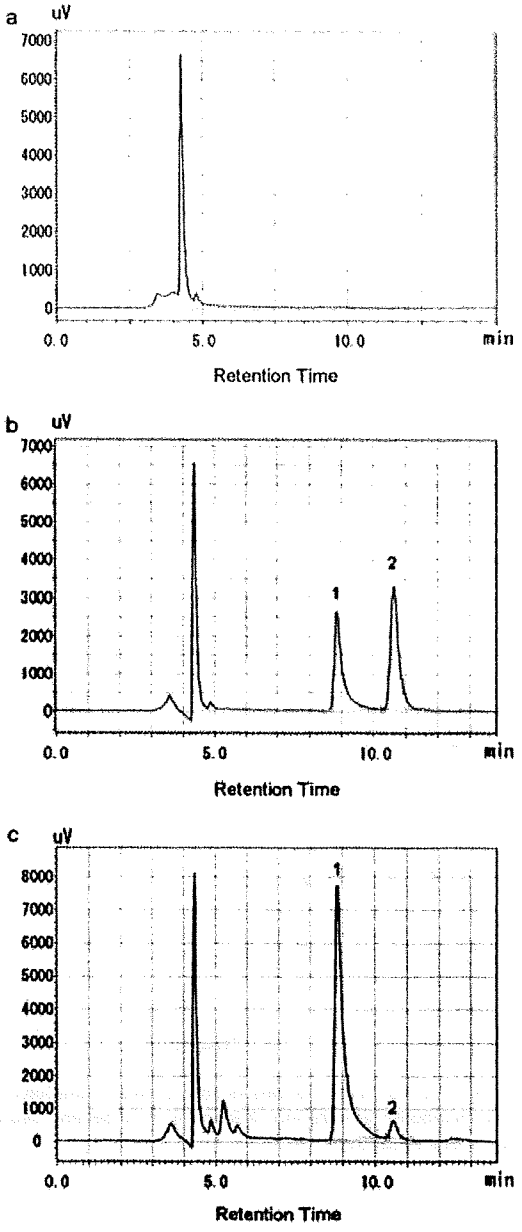



**Figure 2.** Representative HPLC chromatogram. (a) Blank plasma sample; (b) plasma sample spiked with 100 ng/mL amrubicin and amrubicinol; (c) patient plasma sample obtained 2 h after anintravenous bolus dose of 40 mg/m$^2$ of amrubicin, Peaks:1 = amrubicin; 2 = amrubicinol. For chromatographic condition see Experimental section.

## Discussion

We developed a simple and sensitive method of determination for amrubicin and amrubicinol by HPLC. In our method protein



**Table 1.** Intra-day and inter-day accuracy, precision and recovery of the HPLC method for amrubicin and amrubicinol

| | Nominal concentration (ng/mL) | *n* | Experimental concentration (mean ± SD, ng/mL) | Precision (% CV) | Mean recovery (%) | Accuracy (%RE) |
|---|---|---|---|---|---|---|
| *Intra-day* | | | | | | |
| Amrubicin | 10 | 6 | 9.9 ± 0.6 | 5.9 | 98.9 | −1.1 |
| | 100 | 6 | 95.9 ± 1.3 | 1.4 | 100.0 | −4.1 |
| | 1000 | 6 | 1007.6 ± 88.2 | 8.8 | 100.7 | 0.8 |
| Amrubicinol | 10 | 6 | 9.0 ± 0.4 | 4.2 | 98.5 | −9.8 |
| | 100 | 6 | 97.8 ± 1.3 | 1.3 | 97.8 | −2.1 |
| | 1000 | 6 | 954.9 ± 14.6 | 1.5 | 95.5 | −4.5 |
| *Inter-day* | | | | | | |
| Amrubicin | 10 | 6 | 9.7 ± 0.3 | 2.7 | 98.0 | −3.1 |
| | 100 | 6 | 102 ± 8.6 | 8.4 | 100.0 | 2.0 |
| | 1000 | 6 | 1029.8 ± 90.9 | 8.8 | 100.7 | 3.0 |
| Amrubicinol | 10 | 6 | 10.0 ± 0.5 | 5.3 | 99.3 | 0.1 |
| | 100 | 6 | 96.0 ± 5.5 | 5.7 | 99.3 | −4.0 |
| | 1000 | 6 | 1023.2 ± 56.4 | 5.5 | 100.4 | 2.3 |

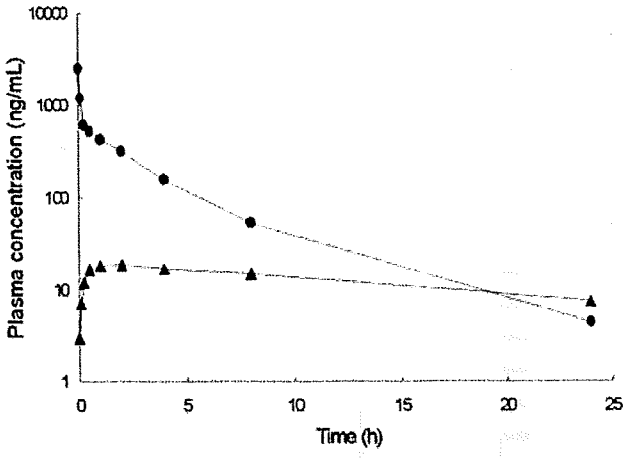



**Figure 3.** Plasma concentrations vs time curves of amrubicin (circles) and its metabolite amrubicinol (triangles) in a patient treated with 40 mg/m$^2$ of amrubicin.

precipitation is used to prepare the samples, and monolithic columns are used to make determination.

During the past decade monolithic columns have emerged as an alternative to traditional packed-bed columns. Monolithic columns are structurally very different from packed-bed columns. The most interesting characteristic of monoliths is their high external porosity resulting from the structure of the network of through-macropores. Another interesting characteristic is the structure of the stationary phase skeleton, which consists of a network of small, thin threads of porous silica. These structural characteristics allow the combination of the low hydraulic resistance of the column to the stream of mobile phase and an enhancement of the column of the rate of the mass transfer of the sample molecules through the column. In this way, the monolithic column improves back-pressure. Yunsheng *et al.* (2003) investigated the utility of monolithic column for direct HPLC-MS-MS analysis. Although access to the matrix in biological samples was prevented in analysis by packed-bed columns, the monolith column with high porosity could remove matrix macromolecules.

In this study, we evaluated packed-bed columns, such as Sumipax ODS A-212 (5 μm, 6 mm × 15 cm), Synergi Hydro-RP (4 μm, 4.6 mm × 15 cm), Luna $C_{18}$ (4 μm, 4.6 mm × 15 cm) and Luna $C_{18}$ (3 μm, 4.6 mm × 15 cm). The LOQ using Sumipax ODS A-212 was 5 ng/mL for both amrubicin and amrubicinol. Using the other three columns, we could not obtain sufficient result (data not shown). We considered the reason why small particles prevented the access of macromolecules in biological samples.

We connected two monolithic columns in tandem like a long column, which made it possible to determine low concentrations of amrubicin and amrubicinol without high pressure. As a result, the sensitivity of our method is equal or superior to that of other methods, including methods that use HPLC-MS-MS or UPLC-MS-MS, and its sensitivity is adequate for performance of the analyses in the PK study.

The sample preparation procedure in this method is based on protein precipitation, because it is simple. Moreover, recovery by protein precipitation is higher than by liquid–liquid extraction or solid-phase extraction, because amrubicin and amurbicinol have both hydrophilic sites and hydrophobic sites. Methanol was selected for protein precipitation, because methanol makes shaper peaks and enables better separation than other organic solvent (data not shown). We added an appropriate amount of buffer, which prevents broad peaks, to the samples after protein precipitation.

The fluorescence detector was set at an excitation wavelength of 480 nm and a detection wavelength of 550 nm. In the excitation wavelength, the highest energy should be obtained in the 480 nm region at the level of excitation lamps, according to the proposal for determination of anthracyclines (Sepaniak and Yeung, 1980). We set the excitation wavelength at 480 nm. Since the most sensitive detection wavelength for amrubicin and amrubicinol was 550 nm, we used it as the detection wavelength in our method. The mobile phase was a modification of a previous report (Noguchi *et al.*, 1998). 1-Octanesulfonate improved separation compared with other ion-pair agents including 1-heptanesulfonate, which used Noguchi's method (data not



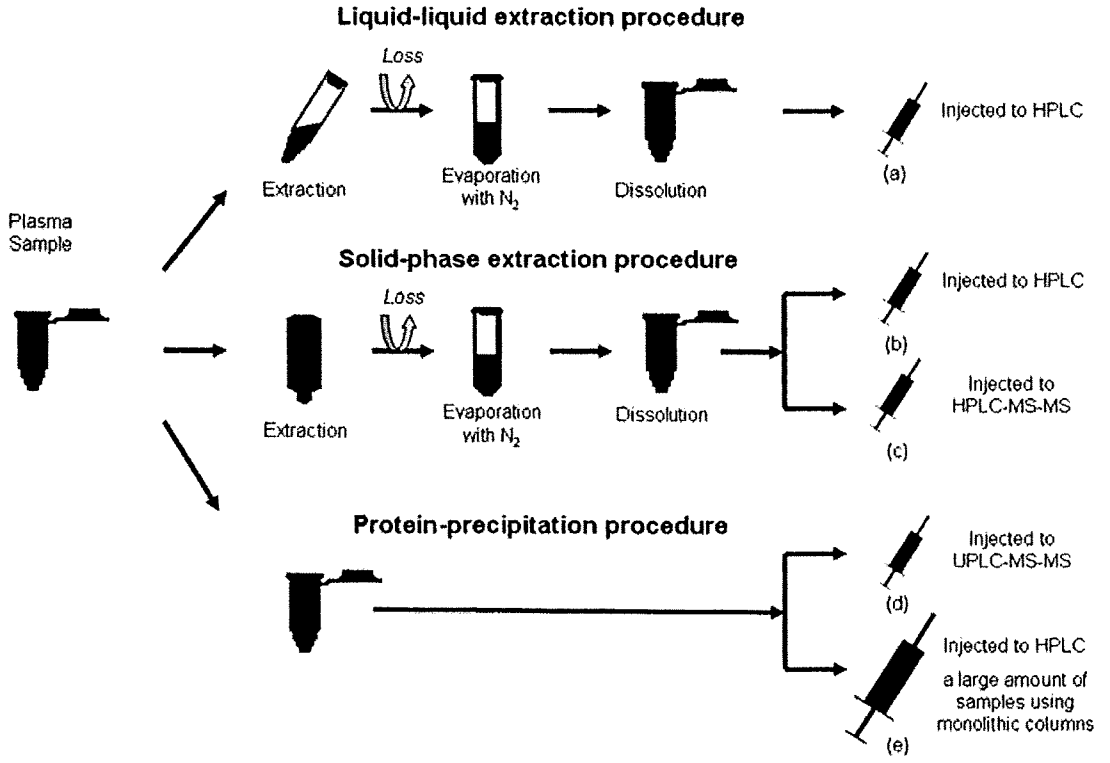



**Figure 4.** Four methods previously described methods for determination of amrubicin and amrubicinol in plasma; (a) HPLC method (Noguchi *et al.*, 1998) with sample preparation by liquid–liquid extraction; (b) HPLC method (Matsunaga *et al.*, 2006) with sample preparation by solid-phase extraction; (c) HPLC-MS-MS method (Yanaihara *et al.*, 2007) with sample preparation by solid-phase extraction; (d) UPLC-MS-MS method (Li *et al.*, 2008) with sample preparation by protein precipitation; (e) HPLC method with sample preparation by protein precipitation in this paper.

shown). We added 1-octanesulfonate to mobile phase as an ion-pair agent.

Previous reports have described four methods for determination of amrubicin and amrubicinol. The UPLC-MS-MS method (Li *et al.*, 2008) is the most sensitive of the four; however, UPLC-MS-MS is not widely available in hospitals. The other methods involve problems in relation to application to PK studies, such as low recovery or loss during processing (Fig. 4). A more simple and sensitive method that can be performed with equipment that is generally available was needed for analysis in hospitals.

We validated our method under Guidance for Industry of the Food and Drug Administration in Bioanalytical Method Validation, with regard to specificity, accuracy, precision, recovery and calibration curve for concentrations ranging from 2.5 to 5000 ng/mL, which were thought to be clinically relevant range for amrubicin and amrubicinol concentrations in plasma. Both the inter-day and intra-day accuracy and precision of the method were adequate. Our method provides good sensitivity, and was able to detect all points in our PK study.

## Conclusion

A simple and sensitive HPLC method was developed for determination of amrubicin and amrubicinol in human plasma. In our method, we selected a monolithic column for determination and protein precipitation for preparation, and it was validated sufficiently. This method can be used clinically because the required equipment and technique are simple. The PK/PD study of amrubicin is ongoing, and a therapeutic drug monitoring study by this HPLC method is in the planning stage.

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## References

Kato T, Nokihara N, Ohe Y, Yamamoto N, Sekine I, Kunitoh H, Kubota K, Nishiwaki Y, Saijo N and Tamura T. Phase II trial of amrubicin in patients with previously treated small cell lung cancer (SCLC). *Proceedings of the American Society for Clinical Oncology* 2006; **24**: 379s.

Li Y, Sun Y, Du F, Yuan K and Li C. Pulse gradient, large-volume injection, high-throughput ultra-performance liquid chromatographic/tandem mass spectrometry bioanalysis for measurement of plasma amrubicin and its metabolite amrubicinol. *Journal of Chromatography A* 2008; **1193**: 109–116.

Matsunaga Y, Hamada A, Okamoto I, Sasaki J, Moriyama E, Kishi H, Matsumoto M, Hira A, Watanabe H and Saito H. Pharmacokinetics of amrubicin and its active metabolite amrubicinol in lung cancer patients. *Therapeutic Drug Monitoring* 2006; **28**: 76–82.

Nguyen DT, Guillarme D, Rudaz S and Veuthey JL. Fast analysis in liquid chromatography using small particle size and high pressure. *Journal of Separation Science* 2006; **29**: 1836–1848.

Noguchi T, Ichii S, Morisada S, Yamaoka T and Yanagi Y. Tumor-selective distribution of an active metabolite of the 9-aminoanthracycline amrubicin. *Japanese Journal of Cancer Research* 1998; **89**: 1061–1066.

Sawa T, Yana T, Takada M, Sugiura T, Kudoh S, Kamei T, Isobe T, Yamamoto H, Yokota S, Katakami N, Tohda Y, Kawakami A, Nakanishi Y and Ariyoshi Y. Multicenter phase II study of amrubicin, 9-amino-anthracycline, in patients with advanced non-small-cell lung cancer (Study 1): West Japan Thoracic Oncology Group (WJTOG) trial. *Investigation of New Drugs* 2006; **24**: 151–158.

Sepaniak MJ and Yeung ES. Determination of adriamycin and daunorubicin in urine by high-performance liquid chromatography with laser fluorometric detection. *Journal of Chromatography* 1980; **190**: 377–383.

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

Yana T, Negoro S, Takada M, Yokota S, Takada Y, Sugiura T, Yamamoto H, Sawa T, Kawahara M, Katakami N, Ariyoshi Y and Fukuoka M. Phase II study of amrubicin in previously untreated patients with extensive-disease small cell lung cancer: West Japan Thoracic Oncology Group (WJTOG) study. *Investigation of New Drugs* 2007; **25**: 253–258.

Yanaihara T, Yokoba M, Onoda S, Yamamoto M, Ryuge S, Hagiri S, Katagiri M, Wada M, Mitsufuji H, Kubota M, Arai S, Kobayashi H, Yanase N, Abe T and Masuda N. Phase I and pharmacologic study of irinotecan and amrubicin in advanced non-small cell lung cancer. *Cancer Chemotheraphy and Pharmacology* 2007; **59**: 419–427.

Yunsheng H, Ganfeng W, Yuguang W, Samuel C and Walter AK. Direct plasma analysis of drug compounds using monolithic column liquid chromatography and tandem mass spectrometry. *Analytical Chemistry* 2003; **75**: 1812–1818.

  

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ORIGINAL ARTICLE

# A dose-finding and pharmacokinetic study of nedaplatin in elderly patients with advanced non-small cell lung cancer

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**Abstract**
*Purpose* Nedaplatin is a second-generation platinum showing favorable activity against non-small cell lung cancer (NSCLC). Dose-limiting toxicity (DLT) is thrombocytopenia, predicted by creatinine clearance (Ccr). This study was conducted to determine the recommended dose, and evaluate the toxicities, pharmacokinetics and efficacy for elderly NSCLC patients.
*Methods* Patients ≥70 years were stratified into two groups based on renal functions: Group A, Ccr ≥ 60 and Group B, 40 ≤ Ccr < 60. The initial doses were 80 and 60 mg/m$^2$ in Groups A and B, respectively. The doses were escalated in 20-mg/m$^2$ increments to 100 mg/m$^2$ until DLT.
*Results* Chemotherapy-naïve 39 elderly patients (Group A/Group B: 22/17) received a total of 83 cycles. Major toxicities were hematological. In Group A, one of the 15 patients at 100 mg/m$^2$ experienced DLT (neutropenia) and the recommended dose was determined at 100 mg/m$^2$. In Group B, three of the five patients had DLTs (leukopenia, neutropenia, thrombocytopenia and febrile neutropenia) at 100 mg/m$^2$, and the recommended dose was determined at 80 mg/m$^2$. The percentage decreases of neutrophil were well correlated with total and free-Pt AUCs. Partial responses were observed in 13 (33%) of the 39 patients, and 12 of the 13 patients who responded had a squamous cell carcinoma.
*Conclusions* Nedaplatin was administered simply and feasibly by stratifying renal function and exerted favorable antitumor activity for elderly patients with NSCLC, especially on squamous cell carcinoma.

**Keywords** Nedaplatin · Dose-finding study · Pharmacokinetics · NSCLC · Elderly patient

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## Introduction

The proportion of elderly patients with non-small cell lung cancer (NSCLC) is increasing [1]. At present, the first-line standard chemotherapy for non-elderly patients with advanced NSCLC is a platinum-based doublet regimen. The efficacy and feasibility of this strategy have been demonstrated in several randomized trials in patients with a good performance status and aged ≤70 years [2–4]. However, platinum-based doublet regimens are not always feasible for elderly patients. Age-related comorbidity and physiologic changes increase inter-individual pharmacokinetic variability, possibly leading to unacceptable severe toxicities. In particular, application of a cisplatin-based regimen to elderly patients is substantially restricted because of the risk of emesis, neurotoxicity and nephrotoxicity.

Oshita et al. [5] prospectively evaluated the feasibility of cisplatin-based chemotherapy in patients aged 75 years or older. Only 10 (29%) out of the 34 patients fulfilled the eligibility criteria for the cisplatin-based regimen. Furthermore, the majority of these eligible patients had grade 4 neutropenia and infectious episodes requiring antibiotics. In another analysis of cisplatin pharmacokinetics, the area under the plasma concentration versus time curve (AUC) of the ultrafilterable and total plasma platinum increased with age, and this was an independent predictor of cisplatin pharmacokinetics [6]. Therefore, the administration of cisplatin is restricted to highly select elderly patients.

(Glycolate-*O,O'*)-diammine platinum (II) (nedaplatin) is a second-generation platinum analog synthesized by Shionogi & Co., Ltd. (Osaka, Japan). In the preclinical studies, nedaplatin is highly active against solid tumors and has higher aqueous solubility than cisplatin [7–9]. The emesis and nephrotoxicity of nedaplatin are substantially reduced, compared with those of cisplatin, and multiple days of hydration for renal protection are not required [10]. Dose-limiting toxicity (DLT) is thrombocytopenia, and recommended dose in Japanese patient ≤70 years is 100 mg/m$^2$ every 4 weeks. This agent is active against NSCLC, with a response rate of 20.5% for previously untreated patients [10]. In a pharmacokinetic analysis, thrombocytopenia was significantly correlated with renal function (i.e., creatinine clearance [Ccr]), and nadir platelet count could be predicted from the following formula [11]:

$$[\text{Nadir platelet count}]\left(/\text{mm}^3\right) = -64{,}264.7 + 2{,}783.4 \times [\text{Ccr}](\text{mL/min})$$

We conducted a dose-finding and pharmacokinetic study of nedaplatin in elderly patients with NSCLC, stratified into two groups based on renal function. This study was conducted to determine the recommended dose, and evaluate the toxicity profiles, pharmacokinetics and antitumor activity.

## Patients and methods

### Eligibility

Patients with histologically and cytologically confirmed chemotherapy-naïve advanced or metastatic non-small cell lung cancer were eligible for this study. Other eligibility criteria included the following: (1) age ≥70 years; (2) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; (3) adequate bone marrow (white blood cell [WBC] count ≥4,000/mm$^3$, absolute neutrophil count [ANC] ≥2,000/mm$^3$, hemoglobin level ≥9.0 g/dL and platelet [PLT] count ≥100,000/mm$^3$), hepatic (serum total bilirubin level ≤1.5 mg/dL, serum asparatate aminotransferase [AST] level ≤100 IU/L and serum alanine aminotransferase [ALT] level ≤100 IU/L), renal (serum creatinine [Cr] level ≤1.5 mg/dL, creatinine clearance [Ccr] ≥40 mL/min) and pulmonary ($PaO_2$ ≥60 torr) functions.

The exclusion criteria were as follows: (1) symptomatic brain metastasis; (2) pleural or pericardial effusions and ascites requiring drainage; (3) serious pre-existing medical conditions such as uncontrolled infections, severe heart disease, uncontrolled diabetes and psychogenic disorders; and (4) hepatic B or C virus or human immunodeficiency virus infection.

Written informed consent was obtained from all the patients. This study was approved by the Institutional Review Board of the National Cancer Center.

### Study design, dosage and dose escalation

This study was designed to determine the recommended dose of nedaplatin for elderly patients with advanced NSCLC, stratified into two groups based on renal function. The primary objective was to determine the recommended dose, and the secondary objectives were to evaluate toxicity profiles, pharmacokinetics and antitumor activity.

Patients were stratified into two groups based on their renal function at the time of study entry: Group A, Ccr ≥60 mL/min; and Group B, 40 ≤ Ccr < 60 mL/min. Ccr was measured on three consecutive days, and the mean value was used for stratification. Each Ccr was calculated using the following formula:

$$\text{Ccr}\,(\text{mL/min}) = [\text{urine volume}\,(\text{mL/min}) \times \text{urine creatinine}\,(\text{mg/dL})]/\text{serum creatinine}\,(\text{mg/dL})$$

In Group A, the initial dose of nedaplatin was 80 mg/m$^2$, and this was escalated to 100 mg/m$^2$. In Group B, the initial dose was 60 mg/m$^2$, and this was escalated to 80 and 100 mg/m$^2$. At least three to six patients were enrolled at each dose level, and the unacceptable dose was defined as the dose level at which >50% of the patients experienced DLT. The definition of DLT was as follows: (1) ≥grade 3 leukopenia, neutropenia or thrombocytopenia; (2) ≥grade 3 non-hematological toxicities except for alopecia, nausea and vomiting; (3) ≥grade 3 nausea and vomiting for ≥5 days. The recommended dose was defined as one dose level below the unacceptable dose level in each treatment arm.

### Nedaplatin administration

Nedaplatin (Aqupla, (glycolate-*O,O'*)-diammine platinum (II); Shionogi Pharmaceutical Company, Osaka, Japan) was obtained commercially. Premedication, consisting of

3 mg of granisetron and 16 mg of dexamethasone diluted in 100 mL of 0.9% saline, was administered via a 30-minute intravenous (IV) infusion. The calculated doses of nedaplatin in both treatment groups were diluted in 300 mL of 0.9% saline and were administered using a 1-h IV infusion every 4 weeks. Following the nedaplatin administration, 500 mL of 0.9% saline was administered intravenously to provide minimal hydration.

### Pretreatment and follow-up evaluation

On enrollment into the study, history and physical examination was performed. Complete differential blood cell count (including WBC count, ANC, hemoglobin and PLT), and clinical chemistry analysis (including serum total protein, albumin, bilirubin, Cr, AST, ALT, gamma-glutamyltransferase, and alkaline phosphatase) were performed. These above were performed at least twice a week throughout the study. Tumor measurement was planned every cycle, and antitumor response was assessed using the WHO standard response criteria. Toxicity was evaluated according to the National Cancer Institute common toxicity criteria (version 2.0).

### PK study

Pharmacokinetic (PK) evaluations were performed in all patients during the initial cycle of treatment. Heparinized venous blood samples (7 mL) were taken before infusion, at 30 min and just before the end of infusion, as well as at 15 and 30 min and 1, 2, 3, 5, 7, 11, 23 and 47 h after the end of infusion.

Blood samples were centrifuged immediately at 4,000 rpm for 10 min. One milliliter of plasma was stored at −20°C or below in a polyethylene tube until the measurement of total plasma platinum (total-Pt) concentration. Residual plasma was transferred to an Amicon Centrifree tube (Amicon, Inc., Beverly, MA, USA) and centrifuged at 4,000 rpm for 20 min. Ultrafiltrate of the plasma was taken and stored at −20°C or below in a polyethylene tube until the measurement of the plasma-free platinum (free-Pt) concentration. The total-Pt and free-Pt concentrations were measured using flameless atomic absorption spectrometry, as previously reported [12].

The PK parameters were estimated using a nonlinear least-squares regression analysis (WinNonlin, Version 5.2; Bellkey Science, Inc., Chiba, Japan) with a weighting factor of $1/year^2$. The individual plasma concentration–time data were fitted to one-, two- and three-exponential equations using a zero-order infusion input and first-order elimination (corresponding to a one-, two- and three-compartment PK model). The model was chosen on the basis of Akaike's information criteria [13]. Fitted parameters (coefficients and exponent of exponential equations) were permitted in the computation of the following PK parameters: half life ($t_{1/2}$), area under the plasma concentration versus time curve (AUC), systemic clearance (CL), and volume of distribution at steady state ($V_{dss}$).

To assess the pharmacodynamic effect, percentage decrease was calculated in WBC, ANC or PLT according to the following formula:

$$\text{Percentage decrease} = [(\text{pretreatment count} - \text{nadir count})/(\text{pretreatment count})] \times 100.$$

These percentages were related to the AUC according to the sigmoid $E_{max}$ model, as follows:

$$\text{Effect}(\%) = [E_{max}(\text{AUC})^k]/[\text{AUC}_{50}^k + \text{AUC}^k] \times 100.$$

A nonlinear least-squares regression using WinNonlin was used to estimate the AUC that produces 50% of the maximum effect ($AUC_{50}$) and the sigmoidicity coefficient ($k$).

## Results

### Patient characteristics

Between June 1996 and July 2001, 39 patients were stratified into two groups (22 in Group A and 17 in Group B) based on their renal functions at entry into the study (Table 1). They received a total of 83 cycles of therapy. The patients comprised 35 males and 4 females with good performance status, and the median age was 76 years in both treatment groups. All the patients were included in the toxicity evaluation. A total of 28 (72%) patients were included in the PK analysis and the remaining 11 (28%) were excluded because of insufficient PK samplings. Eight patients (two from Group A and six from Group B) had stage IIIA disease, but were not candidates for thoracic radiotherapy because of their poor pulmonary function. Six patients (five from Group A and one from Group B) received surgical resections for primary tumors. As much as 21 patients (54%, 12 from Group A and 9 from Group B) had squamous cell carcinoma. Nine patients (4 from Group A and 5 from Group B) received only one cycle of therapy because of progressive disease (PD) and 22 patients (12 from Group A and 10 from Group B) received two cycles of treatment. Among these 22 patients, partial response (PR), stable disease (SD) and PD were observed in 8, 10 and 4 patients, respectively. Five of eight patients with PR, two of ten with SD and one of four with PD received sequential thoracic radiotherapy for primary lesion following two cycles of treatment. Two of ten patients with SD and one of four with PD received palliative

radiotherapy for metastatic lesion. Two of four patients with PD received second-line chemotherapy. The remaining nine patients received supportive care according to the patients' request.

## Toxicity

All the 39 patients were included in the toxicity evaluation. Major toxicities were hematological, such as leukopenia, neutropenia and thrombocytopenia, in both groups, and these hematological toxicities increased in severity with increased dose level of nedaplatin. In Group A, 1 (6.7%) out of the 15 patients treated at a dose level of 100 mg/m$^2$ had grade 3 neutropenia; this dose level was considered to be acceptable (Table 2). In Group B, three (50%) out of six patients treated at a dose level of 80 mg/m$^2$ had ≥grade 3 hematological toxicities (one with grade 3 neutropenia, another with grade 4 neutropenia and febrile neutropenia, and the other with grade 3 leukopenia, anemia and grade 4 thrombocytopenia). The patient with grade 4 thrombocytopenia required a platelet transfusion. At a dose level of 100 mg/m$^2$, three (60%) out of five patients had ≥grade 3 hematological toxicities (one with grade 3 leukopenia and neutropenia, another with grade 3 thrombocytopenia and grade 4 neutropenia, and the other with grade 3 leukopenia, thrombocytopenia and grade 4 neutropenia). These three patients had also febrile neutropenia. In Group B, a dose level of 100 mg/m$^2$ was considered to be unacceptable (Table 2).

Non-hematological toxicities, mainly nausea and anorexia, were generally mild in severity and were not dose limiting in either group (Table 3). Renal toxicity,

**Table 1** Patient characteristics

| | Group A (Ccr ≥60 mL/min) | | Group B (40 ≤ Ccr < 60 mL/min) | |
|---|---|---|---|---|
| | No. of patients | Percentage | No. of patients | Percentage |
| Total patients enrolled | 22 | 100 | 17 | 100 |
| Assessable for toxicity | 22 | 100 | 17 | 100 |
| Assessable for PK analysis | 15 | 68 | 13 | 76 |
| Age, median (range), years | 76 (70–82) | | 76 (70–78) | |
| Sex | | | | |
| Male | 19 | 86 | 16 | 94 |
| Female | 3 | 14 | 1 | 6 |
| ECOG PS | | | | |
| 0 | 6 | 27 | 1 | 6 |
| 1 | 16 | 73 | 15 | 88 |
| 2 | 0 | 0 | 1 | 6 |
| Stage | | | | |
| IIIA | 2 | 9 | 6 | 35 |
| IIIB | 4 | 18 | 6 | 35 |
| IV | 11 | 50 | 4 | 24 |
| Postoperative recurrence | 5 | 23 | 1 | 6 |
| Pathological subtype | | | | |
| Squamous cell carcinoma | 12 | 54 | 9 | 53 |
| Adenocarcinoma | 9 | 41 | 8 | 47 |
| P/D carcinoma | 1 | 5 | 0 | 0 |
| Dose of nedaplatin (mg/m$^2$) | | | | |
| 60 | – | – | 6 | 35 |
| 80 | 7 | 32 | 6 | 35 |
| 100 | 15 | 68 | 5 | 30 |
| Treatment cycle | | | | |
| Median (range) | 2 (1–5) | | 2 (1–4) | |
| 1 cycle | 4 | 18 | 5 | 29 |
| 2 cycles | 12 | 55 | 10 | 59 |
| ≥3 cycles | 6 | 27 | 2 | 12 |

*PK* pharmacokinetics, *ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *P/D carcinoma* poorly differentiated carcinoma

**Table 2** Hematological toxicity

| Group A (Ccr ≥60 mL/min) | Dose level (mg/m$^2$), (number of patients) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 80 ($n$ = 7) Grade | | | | | 100 ($n$ = 15) Grade | | | | |
| Event | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| Leukopenia | 6 | 1 | 0 | 0 | 0 | 12 | 1 | 2 | 0 | 0 |
| Neutropenia | 6 | 1 | 0 | 0 | 0 | 8 | 4 | 2 | 1[a] | 0 |
| Anemia | 4 | 2 | 1 | 0 | 0 | 5 | 7 | 3 | 0 | 0 |
| Thrombocytopenia | 7 | 0 | 0 | 0 | 0 | 12 | 2 | 1 | 0 | 0 |
| No. of patients with febrile neutropenia | 0 | | | | | 0 | | | | |
| No. of patients with DLT | 0 | | | | | 1 | | | | |

| Group B (40 ≤ Ccr < 60 mL/min) | Dose level (mg/m$^2$), (number of patients) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 60 ($n$ = 6) Grade | | | | | 80 ($n$ = 6) Grade | | | | | 100 ($n$ = 5) Grade | | | | |
| Event | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| Leukopenia | 5 | 1 | 0 | 0 | 0 | 2 | 1 | 2 | 1[a] | 0 | 2 | 0 | 1 | 2[a] | 0 |
| Neutropenia | 5 | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 1[a] | 1[a] | 1 | 1 | 0 | 1[a] | 2[a] |
| Anemia | 4 | 1 | 1 | 0 | 0 | 3 | 1 | 1 | 1[a] | 0 | 1 | 2 | 2 | 0 | 0 |
| Thrombocytopenia | 6 | 0 | 0 | 0 | 0 | 3 | 1 | 1 | 0 | 1[a] | 2 | 1 | 0 | 2[a] | 0 |
| No. of patients with febrile neutropenia | 0 | | | | | 1 | | | | | 3 | | | | |
| No. of patients with DLT | 0 | | | | | 3 | | | | | 3 | | | | |

[a] DLT

characterized as an increase in Cr, was also mild, and only one out of five patients treated at a dose level of 100 mg/m$^2$ in Group B had a grade 2 Cr increase. Considering the toxicity profiles, the recommended doses in Groups A and B were determined to be 100 and 80 mg/m$^2$, respectively.

## Response and survival

The antitumor response was assessed in all the 39 patients (Table 4). Of the 39 patients who achieved PR, 13 had an overall response rate of 33%. Similar antitumor responses were observed in both treatment groups; that is, 6 (27%) of 22 and 7 (41%) of 17 patients had PRs in Groups A and B, respectively. Furthermore, 12 of the 13 patients with PRs in both groups had squamous cell carcinoma, and the response rate among patients with squamous cell carcinoma was 57%. Survival follow-up was completed in all the enrolled patients. The median survival time was 11.2 months (95% confidence interval: 7.7–14.6 months), and the 1-, 2- and 5-year survival rates were 46, 23 and 5%, respectively.

## Pharmacokinetics

Pharmacokinetic analysis was performed using data from 28 (72%) of the 39 patients. The first patient enrollment in both treatment groups was started in 1996, and techniques of the sample centrifuging and measurement were not fully developed at the beginning of this pharmacokinetic study. Therefore, the remaining 11 patients (28%) were excluded for pharmacokinetic analysis. The mean plasma concentration–time profiles of total-Pt and free-Pt of nedaplatin are illustrated in Fig. 1. The plasma disappearances of total-Pt and free-Pt were biphasic, and the mean terminal half lives in all the assessable patients averaged 6.28 and 3.57 h, respectively. The $C_{max}$ and AUC of the total-Pt and free-Pt tended to increase with the dose of nedaplatin. The AUCs of the total- and free-Pt at a dose of 100 mg/m$^2$ in Group A seemed similar to those at a dose of 80 mg/m$^2$ in Group B (Table 5), and there were no significant differences between these two treatment subgroups ($P$ = 0.293 for total-Pt AUC and $P$ = 0.336 for free-Pt AUC). Furthermore, the AUCs of free-Pt at the recommended doses in both groups (i.e., 100 mg/m$^2$ in Group A and 80 mg/m$^2$ in Group B) seemed also similar to that in patients aged 70 years or under who had been treated with 100 mg/m$^2$ of nedaplatin [14]. In the sigmoid Emax model assessing the pharmacodynamic effect of nedaplatin, the percentage decrease in the neutrophil counts were well correlated with the total-Pt ($r$ = 0.652) and free-Pt ($r$ = 0.723; Fig. 2).

**Table 3** Non-hematological toxicity

| Group A (Ccr ≥60 mL/min) | Dose level (mg/m$^2$), (number of patients) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 80 ($n$ = 7) Grade | | | | | 100 ($n$ = 15) Grade | | | | |
| Event | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| Nausea | 5 | 1 | 1 | 0 | 0 | 3 | 9 | 3 | 0 | 0 |
| Vomiting | 6 | 1 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| Anorexia | 5 | 1 | 1 | 0 | 0 | 7 | 4 | 4 | 0 | 0 |
| Diarrhea | 6 | 1 | 0 | 0 | 0 | 14 | 1 | 0 | 0 | 0 |
| Stomatitis | 7 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| Hyperbilirubinemia | 6 | 0 | 1 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| AST increase | 6 | 1 | 0 | 0 | 0 | 13 | 2 | 0 | 0 | 0 |
| ALT increase | 6 | 1 | 0 | 0 | 0 | 13 | 2 | 0 | 0 | 0 |
| ALP increase | 7 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| Cr increase | 7 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |

| Group B (40 ≤ Ccr < 60 mL/min) | Dose level (mg/m$^2$), (number of patients) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 60 ($n$ = 6) Grade | | | | | 80 ($n$ = 6) Grade | | | | | 100 ($n$ = 5) Grade | | | | |
| Event | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| Nausea | 1 | 4 | 1 | 0 | 0 | 1 | 3 | 2 | 0 | 0 | 1 | 1 | 3 | 0 | 0 |
| Vomiting | 6 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Anorexia | 4 | 2 | 0 | 0 | 0 | 1 | 3 | 2 | 0 | 0 | 1 | 1 | 3 | 0 | 0 |
| Diarrhea | 6 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Stomatitis | 6 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Hyperbilirubinemia | 6 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 0 |
| AST increase | 4 | 2 | 0 | 0 | 0 | 5 | 0 | 1 | 0 | 0 | 4 | 0 | 1 | 0 | 0 |
| ALT increase | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 1 | 0 | 0 | 4 | 0 | 1 | 0 | 0 |
| ALP increase | 6 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Cr increase | 6 | 0 | 0 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 0 |

*AST* asparatate aminotransferase, *ALT* serum alanine aminotransferase, *ALP* alkaline phosphatase, *Cr* creatinine

## Discussion

In this dose-finding study, we evaluated the toxicities, pharmacokinetics as well as antitumor activity, and determined the recommended doses of nedaplatin for elderly patients with advanced NSCLC based on renal function. The predominant toxicities were hematological, such as leukopenia, neutropenia and thrombocytopenia, in both groups. These hematological toxicities tended to increase in severity with the increased dose level of nedaplatin. Non-hematological toxicities were acceptable and those were not dose limiting in either group. The recommended dose was determined as 100 mg/m$^2$ every 4 weeks in elderly patients with a renal function of Ccr ≥ 60 mL/min, which is the same dose recommended for patients aged ≤70 years. On the other hand, for elderly patients with a renal function of 40 ≤ Ccr < 60 mL/min, the recommended dose was 80 mg/m$^2$ every 4 weeks. In this study,

**Table 4** Response

| Group | Dose level (mg/m$^2$) | No. of patients | Response | | | | PR | |
|---|---|---|---|---|---|---|---|---|
| | | | CR | PR | SD | PD | Sq. | Non-sq. |
| Group A (Ccr ≥60 mL/min) | 80 | 7 | 0 | 2 | 3 | 2 | 2 | 0 |
| | 100 | 15 | 0 | 4 | 6 | 5 | 4 | 0 |
| Group B (40 ≤ Ccr < 60 mL/min) | 60 | 6 | 0 | 3 | 2 | 1 | 2 | 1 |
| | 80 | 6 | 0 | 3 | 1 | 2 | 3 | 0 |
| | 100 | 5 | 0 | 1 | 1 | 3 | 1 | 0 |
| Total | | 39 | 0 | 13 | 13 | 13 | 12 | 1 |

*CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *Sq.* squamous cell carcinoma, *Non-sq.* non-squamous cell carcinoma

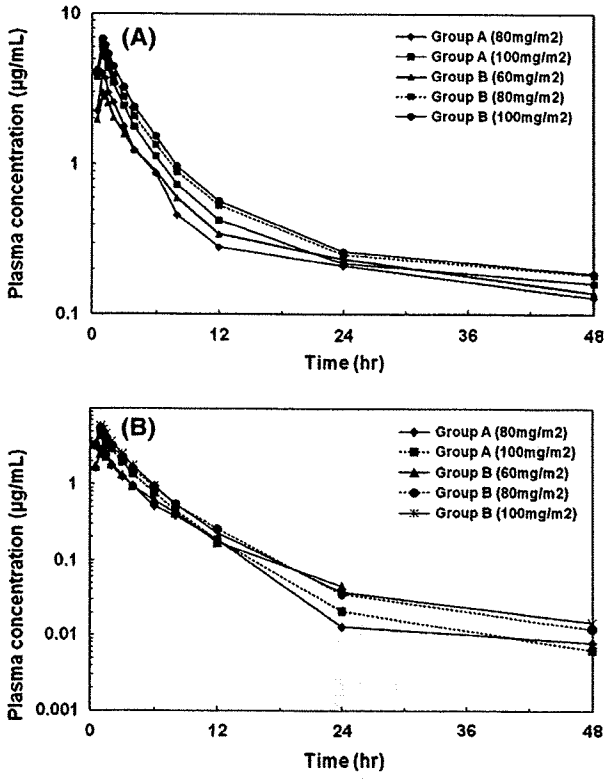


**Fig. 1** Mean plasma concentration–time profiles for: **a** total-Pt and **b** free-Pt of nedaplatin

an additional nine patients were enrolled at the dose level of 100 mg/m$^2$ in Group A. First, the favorable antitumor response was observed in squamous cell carcinoma and we intended to evaluate the antitumor response mainly for squamous cell carcinoma. Then, five of nine additional patients enrolled had squamous cell carcinoma. Second, the recommended dose was determined as 100 mg/m$^2$ in Group A, which was the same dose in younger patients. We intended to confirm the toxicity and pharmacokinetic profiles in this elderly subgroup.

In the development of chemotherapy for elderly patients, the selection of appropriate agents is extremely important. Candidate agents must have confirmed antitumor activities and acceptable toxicity profiles in younger patients (e.g., aged ≤70 years). In this study, we investigated nedaplatin as it had a lower incidence of associated emesis and nephrotoxicity, compared with cisplatin, and favorable antitumor activity in NSCLC patients aged ≤70 years. Furthermore, the current standard treatment for elderly patients with advanced NSCLC, that is, third-generation single-agent chemotherapy such as vinorelbine, gemcitabine or docetaxel, had not been established at the time of planning of the study [15–17]. The DLT of nedaplatin in patients aged ≤70 years was reported to be thrombocytopenia, which is correlated with renal function; therefore, we expected that nedaplatin could be safely administered to elderly patients by stratifying the patients according to renal function. Patients with a Ccr ≥40 mL/min were eligible for inclusion in this study based on the results of a previous PK analysis examining the correlation between the nadir platelet count and renal function (described in "Introduction") [11]. When younger patients with a Ccr ≥40 mL/min were treated with 100 mg/m$^2$ of nedaplatin, the predicted nadir platelet count was ≥50,000/mm$^3$. Therefore, the initial doses of nedaplatin in Group A (Ccr ≥60 mL/min) and Group B (40 ≤ Ccr < 60 mL/min) were determined to be 80 and 60 mg/m$^2$, respectively. The dose escalation over 100 mg/m$^2$ was not planned, because the recommended dose in younger patients (aged ≤70 years) had already been determined at 100 mg/m$^2$.

In this study, milder criteria of DLT was applied, compared with that used in conventional phase I studies. In this developmental strategy, we pursued "the recommended dose with moderate and acceptable toxicities for the majority of elderly patients", instead of "the recommended dose with the severe toxicities in a small and limited number of patients, as per most conventional phase I studies", because the physiological and pharmacological function of elderly patients is highly variable.

**Table 5** Pharmacokinetic parameters of total-Pt and free-Pt

| Group | Dose level ($mg/m^2$) | No. of patients | No. of assessables for PK analysis | $C_{max}$ (μg/mL) | AUC (μg/mL h) | $V_{dss}$ (L) | $T_{1/2}$ (h) | CL (L/h) |
|---|---|---|---|---|---|---|---|---|
| PK parameters of total-Pt | | | | | | | | |
| Group A (Ccr ≥60 mL/min) | 80 | 7 | 2[a] | 4.02 (3.49, 4.57) | 22.58 (13.46, 31.69) | 64.24 (35.27, 93.21) | 14.15 (3.25, 25.04) | 6.00 (3.60, 8.40) |
| | 100 | 15 | 13 | 5.94 ± 1.38 | 21.65 ± 4.54 | 31.50 ± 13.40 | 3.28 ± 1.35 | 7.63 ± 1.74 |
| Group B (40 ≤ Ccr < 60 mL/min) | 60 | 6 | 2[a] | 3.02 (2.91, 3.12) | 19.78 (14.87, 24.68) | 57.05 (33.21, 80.89) | 10.77 (4.08, 17.46) | 5.21 (4.16, 6.25) |
| | 80 | 6 | 6 | 6.35 ± 1.11 | 25.99 ± 9.68 | 29.29 ± 13.18 | 7.88 ± 8.97 | 6.10 ± 1.13 |
| | 100 | 5 | 5 | 6.83 ± 1.20 | 32.11 ± 7.86 | 32.84 ± 22.00 | 6.62 ± 4.55 | 5.01 ± 1.57 |
| PK parameters of free-Pt | | | | | | | | |
| Group A (Ccr ≥60 mL/min) | 80 | 7 | 2[a] | 2.72 (2.13, 3.31) | 10.56 (7.05, 14.06) | 42.30 (37.98, 46.62) | 3.49 (2.70, 4.28) | 12.08 (8.11, 16.04) |
| | 100 | 15 | 13 | 5.11 ± 1.51 | 16.20 ± 3.34 | 32.26 ± 11.17 | 3.51 ± 4.02 | 10.26 ± 2.46 |
| Group B (40 ≤ Ccr < 60 mL/min) | 60 | 6 | 2[a] | 2.55 (2.46, 2.64) | 11.59 (11.38, 11.79) | 49.33 (33.22, 65.43) | 6.16 (2.98, 9.34) | 8.45 (7.89, 9.01) |
| | 80 | 6 | 6 | 5.52 ± 1.25 | 18.53 ± 7.12 | 29.51 ± 9.11 | 3.40 ± 0.65 | 7.25 ± 2.21 |
| | 100 | 5 | 5 | 5.91 ± 1.21 | 20.69 ± 5.52 | 29.63 ± 12.32 | 2.92 ± 0.66 | 7.87 ± 2.71 |
| Patients ≤70 years [14] | 100 | 5 | 5 | | 15.9 | | | |

Data are shown as mean ± SD excepting the dose level of 80 $mg/m^2$ in Group A and 60 $mg/m^2$ in Group B

*PK* pharmacokinetics, *total-Pt* total platinum, *free-Pt*, free platinum, $C_{max}$ maximum plasma concentration, *AUC* area under the plasma concentration versus time curve, $V_{dss}$ volume of distribution at steady-state, $T_{1/2}$ terminal half life, *CL* systemic clearance

[a] Data are shown as mean (actual data)