Anticancer activity of methoxymorpholinyl doxorubicin (PNU 152243) on human hepatocellular carcinoma

Shengtao Yuan[a], Xiongwen Zhang[a], Lijuan Lu[a], Chenghui Xu[a], Weiyi Yang[a] and Jian Ding[a]

Methoxymorpholinyl doxorubicin (PNU 152243) is a morpholinyl analog possessing a methoxymorpholinyl group at the 3′ position of the sugar moiety, which, compared with doxorubicin, appears to be less cardiotoxic and more cytotoxic against multidrug-resistant tumor cells. In this study, we report the anticancer activity of PNU 152243 on human hepatocellular carcinoma (HCC) *in vitro* and *in vivo*. The average IC_{50} value of PNU was 0.08 μM. In contrast, the average IC_{50} values of adriamycin (ADM), 4′-epidoxorubicin (EDR), mitomycin C (MMC), cisplatin and vepesid (VP-16) were 0.96, 0.74, 2.81, 7.27 and 26.66 μM, respectively. PNU 152243 was 13.7, 10.6, 40.1, 103.8 and 380.8 times more potent than ADM, EDR, MMC, cisplatin and VP-16 against HCC *in vitro*. In nude mice, the T/C (%) were 43.8 at the dose of 25 μg/kg and 41.2 at the dose of 50 μg/kg on BEL-7402 xenograft, the T/C (%) were 41.7 at the dose of 25 μg/kg and 54.6 at the dose of 50 μg/kg on Zip-177 xenograft. The results showed that PNU 152243 had growth inhibition of HCC *in vitro* and *in vivo* and may be useful in HCC chemotherapy. *Anti-Cancer Drugs* 15:641–646 © 2004 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2004, **15**:641–646

Keywords: anticancer activity, doxorubicin, hepatocellular carcinoma, *in vitro*, *in vivo*, methoxymorpholinyl doxorubicin, PNU 152243

[a]Division of Anti-tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.

Sponsorship: Supported by high tech research and development (863) program (2002AA2Z346A).

Correspondence to J. Ding, Division of Anti-tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Shanghai 201203, China.
Tel: +86 21 50806079; fax: +86 21 50807088;
e-mail: jding@mail.shcnc.ac.cn

Received 6 January 2004 **Revised form accepted** 23 March 2004

Introduction

Since its introduction into clinical medicine in the early 1970s, doxorubicin has become an important anticancer drug in the treatment of a variety of solid tumors [1]. However, its clinical uses are limited by the dose-related cardiomyopathy [2] and emergence of drug resistance, particularly multidrug resistance (MDR) [3]. Over the last 20 years, several anthracycline analogs have been synthesized in an attempt to circumvent the cardiotoxicity and drug resistance associated with doxorubicin. One promising series of analogs is the morpholinyl analogs, which, compared with doxorubicin, appear to be less cardiotoxic at the therapeutic doses [4,5] and more cytotoxic against multidrug-resistant tumor cells [6–8].

Methoxymorpholinyl doxorubicin (PNU 152243) is a morpholinyl analog possessing a methoxymorpholinyl group at the 3′ position of the sugar moiety (Fig. 1). PNU 152243 is at least 80- to 150-fold more potent than doxorubicin when administered to mice *in vivo*. Furthermore, its maximum tolerated dose, as defined on the basis of drug-induced myelosuppression in phase I trials, is 50-fold lower than that of doxorubicin. In contrast, PNU 152243 is only 3- to 4-fold more potent than doxorubicin *in vitro* [9,10]. This compound also maintains *in vitro* and *in vivo* cytotoxic activity against P388 leukemia resistant to doxorubicin and some human solid tumors, such as colon adenocarcinoma, mammary carcinoma, lung adenocarcinoma and small cells lung carcinoma [11]. Primary hepatocellular carcinoma (HCC) is one of the most common malignancies in China and there were no reports on the anticancer activity of PNU 152243 on HCC up to now. In this communication, we report the *in vitro* and *in vivo* anticancer activity of PNU 152243 on HCC.

Materials and methods

Chemicals

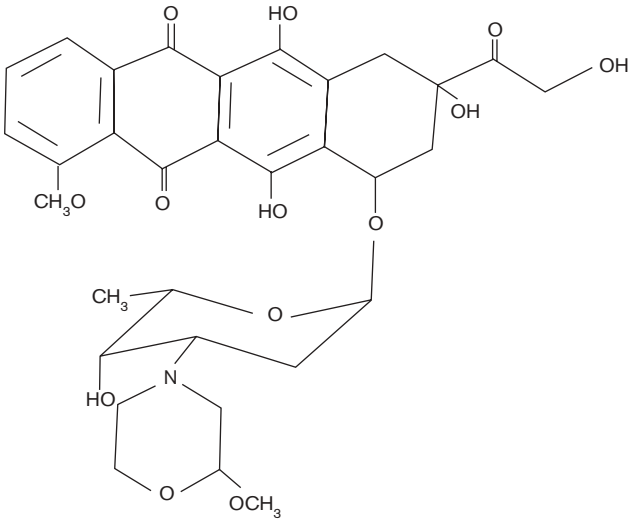
PNU 152243, doxorubicin–HCl (ADM) and 4′-epidoxorubicin (EDR) were supplied by Pharmacia & Upjohn (Shanghai, China). Mitomycin (MMC) was purchased from Kyowa Hakko Kogyo (Tokyo, Japan), cisplatin was from QiLu Pharmaceutical Factory (Jinan, China) and vepesid (VP-16) was from the Experimental Pharmaceutical Factory of Shanghai Institute of Medicine Industry (Shanghai, China). All drug solutions were prepared immediately with normal saline before use. Unless indicated otherwise, all other chemicals were purchased from Sigma (St Louis, MO).

Animals and tumor cell lines

KM adult mice of both sexes supplied by Shanghai Medical University were used to evaluate the acute

0959-4973 © 2004 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000132238.23050.f5

Fig. 1



Structure of PNU 152243.

toxicity of PNU 152243 and ADM. KM mice were 5–6 weeks of age, weighted 18–22 g and were kept under standard laboratory conditions. Adult male BALB/cA nude mice supplied by Shanghai Institute of Materia Medica, Chinese Academy of Sciences were employed in experiments with human tumor xenografts. The nude mice were 4–5 weeks of age, weighted 18–22 g and were maintained under specific pathogen-free conditions. Food and bedding were sterilized and water was acidified (pH 2.5–3).

The study was performed on five human HCC cell lines. BEL-7402, BEL-7404 and Zip-177 were established by Shanghai Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences [12]. SMMC-7721 cell line was established by The Second Military Medical University [13] (Shanghai, China). Hep-G2 cell line was established by Baylor College of Medicine [14] and purchased from the ATCC (Rockville, MD).

Sulforhodamine B (SRB) assay

The *in vitro* cytotoxicity of PNU 154233 and other anticancer drugs were studied in five HCC cell lines using SRB assay. Exponentially growing cells, suspended with 0.25% trypsin and washed twice with medium, seeded into 96-well plates. Zip-177 and Hep-G2 cells were seeded with 1.5×10^4/well and the others were 9.0×10^3/well. After incubated at 37°C in a humidified, 5% CO_2 atmosphere for 24 h, cells were added with various concentrations of drugs. The plates were continuously incubated at same condition for 72 h. Inhibition of cell growth was evaluated as described previously [15]. The 50% inhibitory concentration (IC_{50}) was calculated on derived concentration–response curve by the Logit method [16]. Within each experiment, determinations were performed in triplicate and experiments were repeated at least 3 times.

Acute toxicity assay

KM mice were administered with PNU 152243 or ADM once i.v. The doses of PNU 152243 were 87.6, 105.2, 126.0, 152.3, 182.9 and 219.1 μg/kg; the doses of ADM were 11.1, 13.3, 15.9, 19.1, 22.8 and 27.4 mg/kg. The mice were observed for 30 days. The 10, 50 and 90% lethal doses (LD_{10}, LD_{50} and LD_{90}) were calculated on derived dose–response curves by the Bliss method [17].

Xenografts models and treatments

Human HCC BEL-7402 and Zip-177 xenografts were established by inoculated 2×10^6 cells s.c. in nude mice. The experiments were begun when the xenografts had 3 passages in nude mice. Under a sterilization condition, well growth tumors were cut into 1.5-mm^3 fragments and the fragments were transplanted s.c. into the right flank by trocar on nude mice. The sizes were measured with a caliper. When the tumor volume reached 150–500 mm^3, the mice were randomly divided into five groups and received a single i.v. injection of three doses of PNU 152243, ADM or saline (negative control) once a week for a period of 3–4 weeks. According to the results of acute toxicity, the high dose of PNU 152243 was 75 μg/kg, approximate to LD_{10}. The middle and low doses of PNU 152243 were 50 and 25 μg/kg, respectively. The dose of ADM was 5.5 mg/kg equivalent to the middle dose of PNU 152243. The lengths (A, mm) and widths (B, mm) of the tumors and the body weights of mice were measured twice weekly. Tumor volume (TV, mm^3) was calculated according to the following formula: $TV = 1/2AB^2$. Relative tumor volume (RTV) was calculated by the following formula: $RTV = V_t/V_0$, where V_t is the tumor volume on each day of measurement and V_0 is the tumor volume on the day of first drug treatment (day 0). Therapeutic effect of drug was expressed in terms of T/C (%) and the calculation formula is: $T/C\ (\%) = T_{RTV}/C_{RTV} \times 100\%$, where T_{RTV} is the RTV of treatment group and C_{RTV} is the RTV of negative control group [18].

Statistical analysis

Student's t-test was used for statistical analysis between the control and treated groups. $p < 0.05$ was considered significant.

Results

In vitro antitumor activity

In vitro cytotoxicity of PNU 152243 and other anticancer drugs on HCC cell lines was determined using the SRB assay (Table 1). Both PNU 152243 and five kinds of anticancer drugs dose-dependently inhibited the proliferation of five kinds of HCC cell lines. The average IC_{50}

value of PNU 152243 on HCC cell lines was 0.08 μM. In contrast, the average IC_{50} values of ADM, EDR, MMC, cisplatin and VP-16 were 0.96, 0.74, 2.81, 7.27 and 26.66 μM, respectively. The cytotoxicity of PNU 152243 was 13.7, 10.6, 40.1, 103.8 and 380.8 times more potent than ADM, EDR, MMC, cisplatin and VP-16 against HCC cell lines *in vitro*.

Acute toxicity

Between 1 and 2 days after administration, the mice appeared to show toxicity. The peak time of death was the day 5–8 day. The LD_{10}, LD_{50} and LD_{90} values of PNU on female mice were 90.7, 107.7 and 127.7 μg/kg and 91.3, 108.7 and 129.0 μg/kg on male mice. In contrast, the LD_{10}, LD_{50} and LD_{90} values of ADM on female mice were 9.9, 15.1 and 20.0 mg/kg and 9.6, 13.8 and 19.9 mg/kg on male mice.

In vivo antitumor activity

Tables 2 and 3 and Figures 2 and 3 present the results of the experimental therapeutic efficacy of PNU 152243 on human HCC xenografts BEL-7402 and Zip-177 in nude mice. Tables 2 and 3 show that the T/C (%) were 43.8 on BEL-7402 and 41.7 on Zip-177 at the dose of 25 μg/kg; the T/C (%) were 41.2 on BEL-7402 and 54.6 on Zip-177 at the dose of 50 μg/kg. The results showed that PNU 152243 had some growth inhibition of human HCC xenografts BEL-7402 and Zip-177 in nude mice. However, at the high dose (75 μg/kg), PNU152243 also showed obvious effects on the two xenografts, but the toxicity was high and results in the death of nude mice. The antitumor activity of positive control drug (ADM) was equivalent to or weaker than that of 25 μg/kg PUN 152243 against BEL-7402 or Zip-177 HCC xenografts, respectively.

Table 1 **IC_{50} values of PNU 152243 and other anticancer drugs on HCC cell lines cultured *in vitro***

Cell lines	IC_{50} (μM)					
	PNU	ADM	EDR	MMC	Cisplatin	VP-16
Zip-177	0.05	1.23	1.13	3.65	15.10	39.40
BEL-7402	0.04	0.49	0.10	2.24	5.47	16.20
BEL-7404	0.19	2.16	2.09	5.50	9.77	46.60
SMMC-7721	0.07	0.49	0.14	1.60	2.33	18.30
Hep-G2	0.05	0.42	0.24	1.08	3.62	12.80
Average IC_{50}	0.08	0.96	0.74	2.81	7.27	26.66

The cells were seeded into 96-well plates for 24 h and treated with various concentrations of different drugs for 72 h. Inhibition of cell growth was determined by SRB assay. IC_{50} was calculated as described in Materials and methods. Within each experiment, determinations were performed in triplicate and experiments were repeated at least 3 times.

Discussion

HCC is one of the most common malignancies in the world, about 560 000 cases of liver cancer, usually HCC, occur annually. More than 80% of cases occur in Asia and Africa where HCC is most frequently caused by hepatitis B or C (or both) virus infection, chronic liver disease and cirrhosis; concomitant dietary exposure to aflatoxins multiplies the risk [19]. The survival for the patients is poor due to local invasion or distant metastasis. At present, only complete surgical resection with either partial hepatectomy or a liver transplant is potentially curative, but this procedure is possible in only a small subset of patients. Even though there have been substantial advances in hepatic surgery, an operative approach may not be feasible because of co-morbid disease or intra- or extrahepatic metastatic diseases. In light of this situation, most lesions are not resectable for cure. Moreover, HCC is not sensitive to radiotherapy, so with the development of the hepatic arterial infusion chemotherapy and transcatheter hepatic arterial chemoembolization, chemotherapy is more and more important in HCC treatment [20].

Within the limits of chemotherapeutic agents currently available, an increasing number of approaches are being investigated in an effort to increase the survival and life quality of patients. Of all of the anti-neoplastic agents, doxorubicin initially was thought to have significant activity, although subsequent trials have failed to confirm single-agent response rates in excess of 20% [21–23]. Other agents alone or in combination, such as etoposide, cisplatin, mitocantrone, 5-fluorouracil with leucovorin, epirubicin, liposomal doxorubicin or AG-337 (Thymitaq), have failed to produce response rates that exceed 20–30% [24,25].

Table 2 **The experimental therapeutic efficacy of PNU 152243 on human HCC BEL-7402 tumor xenografted in BALB/cA nude mice**

Group	Doses	Route	Animal no.		Animal weight (g)		TV (mm^3, $x \pm SD$)		RTV ($x \pm SE$)	T/C (%)	*p*
			D_0	D_n	D_0	D_n	D_0	D_n			
NS		i.v.	16	16	20.5	27.6	504 ± 403	5015 ± 334	12.3 ± 7.3		
ADM	5.5 mg/kg	i.v.	8	8	21.0	26.8	480 ± 240	2028 ± 433	5.4 ± 2.9	43.6	0.011
PNU	25 μg/kg	i.v.	8	8	20.6	26.3	479 ± 129	2751 ± 830	5.4 ± 1.3	43.8	0.014
PNU	50 μg/kg	i.v.	8	8	21.6	25.6	504 ± 301	2258 ± 808	5.1 ± 1.8	41.2	0.007
PNU	75 μg/kg	i.v.	8	2	20.5	27.5	506 ± 353	2760 ± 515	4.1 ± 0.5	33.8	0.078

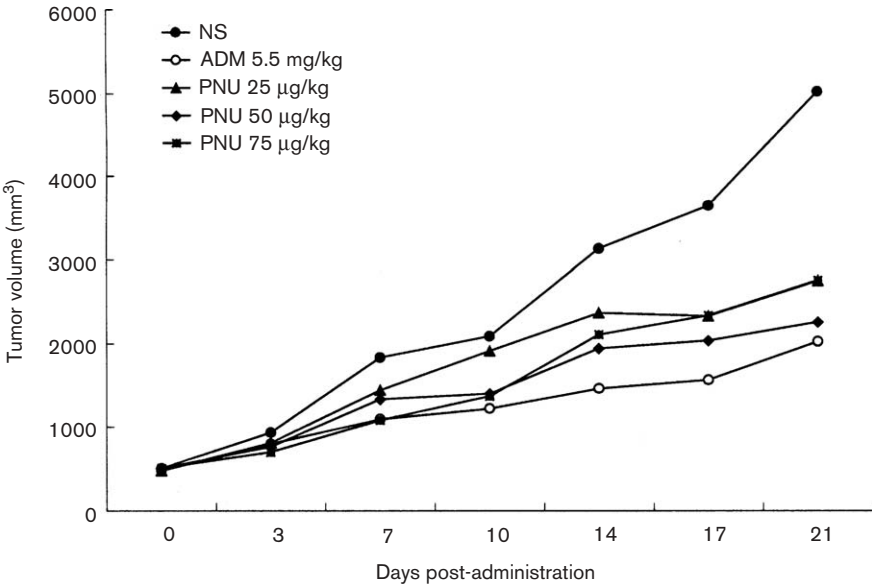
BEL-7402 tumor fragments (1.5 mm^3) were implanted s.c. into right flank by trocar on BALB/cA nude mice. The animals were randomly divided to five groups when the tumor volume reached 500 mm^3 and received a single i.v. injection of three doses of PNU 152243, ADM or saline once a week for a period of 3 weeks. Tumor growth was monitored as described in Materials and methods. Therapeutic effect of drugs was expressed in terms of T/C (%) and the calculation formula: T/C (%) = $T_{RTV}/C_{RTV} \times 100\%$, where T_{RTV} was the RTV of the treatment group and C_{RTV} was the RTV of the negative control group. D_0: day of the start of treatment. D_n: day of the optimal of treatment, which was the 21st day in the experiments. TV: tumor volume. RTV: relative tumor volume.

Table 3 The experimental therapeutic efficacy of PNU 152243 on human HCC Zip-177 tumor xenografted in BALB/cA nude mice

Group	Doses	Route	Animal no.		Animal weight (g)		TV (mm^3, $x \pm SD$)		RTV ($x \pm SE$)	T/C (%)	p
			D_0	D_n	D_0	D_n	D_0	D_n			
NS		i.v.	16	16	19.8	25.1	160 ± 56	1835 ± 1053	11.3 ± 5.4		
ADM	5.5 mg/kg	i.v.	8	8	20.8	23.5	160 ± 37	937 ± 382	5.9 ± 1.7	52.3	0.008
PNU	25 μg/kg	i.v.	8	8	20.0	24.4	163 ± 82	795 ± 471	5.4 ± 1.3	41.7	0.002
PNU	50 μg/kg	i.v.	8	8	20.4	24.6	163 ± 32	989 ± 318	6.2 ± 1.8	54.6	0.011
PNU	75 μg/kg	i.v.	8	6	20.5	24.5	160 ± 42	745 ± 239	4.7 ± 1.1	41.9	0.006

Zip-177 tumor fragments (1.5 mm^3) were implanted s.c. into right flank by trocar on BALB/cA nude mice. The animals were randomly divided to five groups when the tumor volume reached 160 mm^3 and received a single i.v. injection of three doses of PNU 152243, ADM or saline once a week for a period of 3 weeks. Tumor growth was monitored as described in Materials and methods. Therapeutic effect of drugs was expressed in terms of T/C (%) and the calculation formula: T/C (%) = $T_{RTV}/C_{RTV} \times 100\%$, where T_{RTV} was the RTV of the treatment group and C_{RTV} was the RTV of the negative control group. D_0: day of the start of treatment. D_n: day of the optimal of treatment, which was the 28th day in the experiments. TV: tumor volume. RTV: relative tumor volume.

Fig. 2

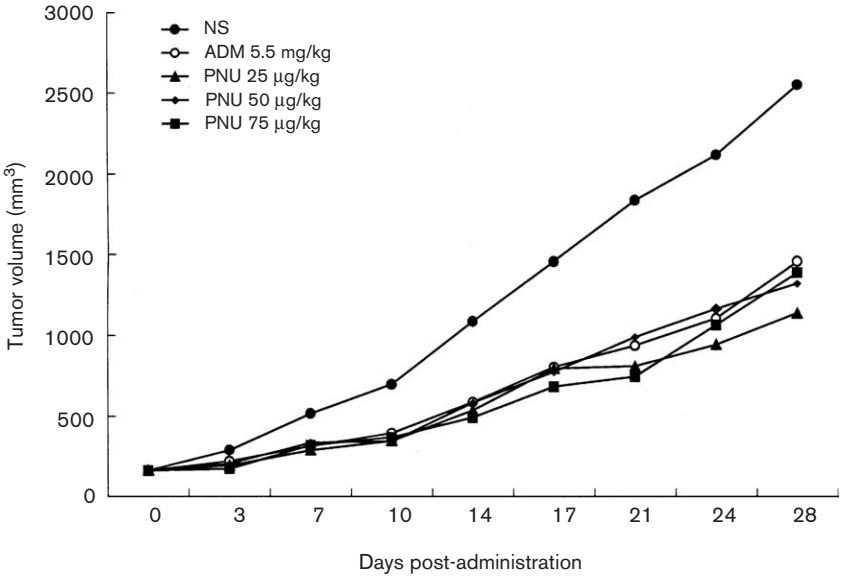


Growth inhibition of PNU 152243 on s.c. human hepatocarcinoma BEL-7402 tumors xenografted in nude mice. BEL-7402 tumor fragments (1.5 mm^3) were implanted s.c. into right flank by trocar on BALB/cA nude mice. The animals were randomly divided to five groups when the tumor volume reached 500 mm^3 and received a single i.v. injection of three doses of PNU 152243, ADM or saline once a week for a period of 3 weeks. Tumor volume was monitored as described in Materials and methods. Results are expressed as median tumor volumes.

PNU 152243 is a novel doxorubicin analog undergoing phase I and II clinical studies with three unique biological characteristics distinguishing this compound from doxorubicin [26–29]. First, in both *in vitro* and *in vivo* studies, PNU 152243 is much more potent than doxorubicin even against the tumor cells which are resistant to doxorubicin. Second, as compared with the topoisomerase II inhibition activity of doxorubicin, PNU 152243 is an inhibitor of both topoisomerases I and II. Lastly, PNU 152243 appears to be metabolized by human microsomal enzymes to a DNA cross-linking product with enhanced cytotoxicity. PNU 152243 undergoes hepatic biotransformation by cytochrome P450 (CYP) 3A into more cytotoxic metabolites and an active metabolite of PNU 152243, which contributes significantly to its *in vivo* anticancer activity and host toxicity [30,31].

HCC also is one of the most common malignancies in China, and most patients were HBV or/and HCV infection. Thus, liver transplantation is impossible for most patients. Although PNU 152243 maintains *in vitro* and *in vivo* cytotoxic activity against some human solid tumors, there were no reports on the anticancer activity of PNU 152243 on HCC up to now. As illustrated in this study, PNU 152243 is a potent cytotoxicity in human HCC cells. In our study, PNU 152243 was 13.7, 10.6, 40.1, 103.8 and 380.8 times more potent than ADM, EDR, MMC, cisplatin and VP-16 against human HCC *in vitro*. *In vivo*, compared with their LD_{10} doses, the 1/4 LD_{10} dose of PNU 152243 (25 μg/kg) possessed the same anti-HCC activity as the 1/2 LD_{10} dose of ADM (5.5 mg/kg). PNU 152243 appears to be less toxic and to have more anti-cancer activity against HCC *in vivo*, so it will be a more suitable and potent chemotherapy drug to

Fig. 3



The growth inhibition of PNU 152243 on s.c. human hepatocarcinoma Zip177 tumors xenografted in nude mice. Zip-177 tumor fragments (1.5 mm^3) were implanted s.c. into right flank by trocar on BALB/cA nude mice. The animals were randomly divided to five groups when the tumor volume reached 160 mm^3 and received a single i.v. injection of three doses of PNU 152243, ADM or saline once a week for a period of 4 weeks. Tumor volume was monitored as described in Materials and methods. Results are expressed as median tumor volumes.

HCC in the future. The preclinical experiments of PNU 152243 were completed and a phase II clinical trial against HCC is underway in China.

References

1 Blum RH, Carter SK. A new anticancer drug with significant clinical activity. *Ann Intern Med* 1974; **80**:249–259.

2 Bristow MR, Thompson PD, Martin RP, Mason JW, Billingham ME, Harrison DC, *et al.* Early anthracycline cardiotoxicity. *Am J Med* 1978; **65**:823–832.

3 Pastan I, Gottesman M. Multiple-drug resistance in human cancer. *N Engl J Med* 1987; **316**:1388–1393.

4 Sikic BI, Ehsan MN, Harker WG, Friend NF, Brown BW, Newman RA, *et al.* Dissociation of antitumor potency from anthracycline cardiotoxicity in a doxorubicin analog. *Science* 1985; **228**:1544–1546.

5 Danesi R, Agen C, Grandi M, Nardini V, Bevilacqua G, Del Tacca M. 3′-Deamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin (FCE 23762): a new anthracycline derivative with enhanced cytotoxicity and reduced cardiotoxicity. *Eur J Cancer* 1993; **29A**:1560–1565.

6 Streeter DG, Taylor DL, Acton EM, Peters JH. Comparative cytotoxicities of various morpholinyl anthracyclines. *Cancer Chemother Pharmacol* 1985; **14**:160–164.

7 Watanabe M, Komeshima N, Nakajima S, Tsuruo T. MX2, a morpholino anthracycline, as a new antitumor agent against drug-sensitive and multidrug-resistant human and murine tumor cells. *Cancer Res* 1988; **48**:6653–6657.

8 Geroni C, Pesenti E, Broggini M, Belvedere G, Tagliabue G, D'Incalci M, *et al.* L1210 cells selected for resistance to methoxymorpholinyl doxorubicin appear specifically resistant to this class of morpholinyl derivatives. *Br J Cancer* 1994; **69**:315–319.

9 Grandi M, Pezzoni G, Ballinari D, Capolongo L, Suarato A, Bargiotti A, *et al.* Novel anthracycline analogs. *Cancer Treat Rev* 1990; **17**:133–138.

10 Bakker M, Renes J, Groenhuijzen A, Visser P, Timmer-Bosscha H, Muller M, *et al.* Mechanisms for high methoxymorpholino doxorubicin cytotoxicity in doxorubicin-resistant tumor cell lines. *Int J Cancer* 1997; **73**:362–366.

11 Ripamonti M, Pezzoni G, Pesenti E, Pastori A, Farao M, Bargiotti A, *et al. In vivo* anti-tumor activity of FCE 23762, a methoxymorpholinyl derivative of doxorubicin active on doxorubicin-resistance tumor cells. *Br J Cancer* 1992; **65**:703–707.

12 Chen R, Zhu D, Ye X, Shen D, Lu R. Establishment of three human liver carcinoma cell lines and some of their biological characteristics *in vitro*. *Sci Sin* 1980; **23**:236–247.

13 Lu J, Xu RB, Doung RC. The glucocorticoid receptors and the induction of tyrosine aminotransferase by glucocorticoid in the human liver cancer cell line (SMMC-7721) *in vitro*. *Shih Yen Sheng Wu Hsueh Pao* 1985; **18**: 231–238.

14 Zannis VI, Breslow JL, SanGiacomo TR, Aden DP, Knowles BB. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry* 1981; **20**:7089–7096.

15 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990; **82**:1107–1112.

16 Eustice DC, Brittelli DR, Feldman PA, Brown LJ, Borkowski JJ, Slee AM. An automated pulse labelling method for structure–activity relationship studies with antibacterial oxazolidinones. *Drugs Exp Clin Res* 1990; **16**: 149–155.

17 Rosiello AP, Essignmann JM, Wogan GN. Rapid and accurate determination of the median lethal dose (LD_{50}) and its error with a small computer. *J Toxicol Environ Health* 1977; **3**:797–809.

18 Wand WR. *In vivo* methods. In: Teicher BA (editor): *Anticancer Drug Development Guide, Preclinical Screening, Clinical Trials and Approval*. Totowa, NJ: Humana Press; 1997, pp. 59–213.

19 Stewart BW, Kleihues P. Liver cancer. In: Stewart BW, Kleihues P (editors): *World Cancer Report*. Lyon: IARC Press; 2003, pp. 203–207.

20 Itsubo M. The present status of chemotherapy for HCC. *Jpn J Cancer Chemother* 1993; **20**:889–895.

21 Sciarrino E, Simonetti RG, Le Moli S, Pagliaro L. Adriamycin treatment for hepatocellular carcinoma: experience with 109 patients. *Cancer* 1985; **56**:2751–2755.

22 Chlebowski RT, Brzechwa-Adjukiewicz A, Cowden A, Block JB, Tong M, Chan KK. Doxorubicin (75 mg/m^2) for hepatocellular carcinoma: clinical and pharmacokinetic results. *Cancer Treat Rep* 1984; **68**:487–491.

23 Ihde DC, Kane RC, Cohen MH, McIntire KR, Minna JD. Adriamycin therapy in American patients with hepatocellular carcinoma. *Cancer Treat Rep* 1977; **61**:1385–1387.

24 Bing-hui Y, Zhao-you T. Randomized clinical trial of cisplatin in the treatment of hepatocellular carcinoma. In: Zhao-you T, Meng-chao W, Sui-sheng X (editors): *Primary Liver Cancer*. New York: Springer; 1989, pp. 434–437.

25 Falkson G, Moertel CG, Lavin P, Pretorius FJ, Carbone PP. Chemotherapy studied in primary liver cancer: a prospective randomized trial. *Cancer* 1978; **42**:2149–2156.
26 Vasey PA, Bissett D, Strolin-Benedetti M, Poggesi I, Breda M, Adams L, *et al.* Phase I clinical and pharmacokinetic study of 3′-deamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin (FCE 23762). *Cancer Res* 1995; **55**:2090–2096.
27 Sessa C, Zucchetti M, Ghielmini M, Bauer J, D'Incalci M, de Jong J, *et al.* Phase I clinical and pharmacological study of oral methoxymorpholinyl doxorubicin (PNU 152243). *Cancer Chemother Pharmacol* 1999; **44**:403–410.
28 Fokkema E, Verweij J, van Oosterom AT, Uges DR, Spinelli R, Valota O, *et al.* A prolonged methoxymorpholino doxorubicin (PNU-152243 or MMRDX) infusion schedule in patients with solid tumours: a phase 1 and pharmacokinetic study. *Br J Cancer* 2000; **82**:767–771.
29 Bakker M, Droz JP, Hanauske AR, Verweij J, van Oosterom AT, Groen HJ, *et al.* Broad phase II and pharmacokinetic study of methoxy-morpholino doxorubicin (FCE 23762-MMRDX) in non-small-cell lung cancer, renal cancer and other solid tumour patients. *Br J Cancer* 1998; **77**:139–146.
30 Lau DH, Duran GE, Lewis AD, Sikic BI. Metabolic conversion of methoxymorpholinyl doxorubicin: form a DNA strand breaker to a DNA cross-linker. *Br J Cancer* 1994; **70**:79–84.
31 Quintieri L, Rosato A, Napoli E, Sola F, Geroni C, Floreani M, *et al. In vivo* antitumor activity and host toxicity of methoxymorpholinyl doxorubicin: role of cytochrome P450 3A. *Cancer Res* 2000; **60**:3232–3238.