Cancer Chemother Pharmacol (2007) 60:589–600
DOI 10.1007/s00280-007-0502-z

ORIGINAL ARTICLE

Preclinical antitumor activity of the oral platinum analog satraplatin

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Received: 18 September 2006 / Accepted: 14 April 2007 / Published online: 31 May 2007

Abstract
Purpose Satraplatin is an orally available platinum analog. The purpose of this study was to better characterize satraplatin's preclinical antitumor efficacy in a variety of sensitive and resistant human tumor cell lines and in a prostate cancer xenograft model and to evaluate the effect of satraplatin on PSA expression and/or secretion in a prostate cancer cell line.
Methods Satraplatin and its primary metabolite JM-118 were preclinically tested for their cytotoxic activity in a range of cancer cells including: human prostate, those forming the NCI drug screening panel, and those resistant to anti-cancer drugs. Also, the antiproliferative efficacy of satraplatin was tested in vivo in a human prostate cancer model. The effect of satraplatin and JM-118 on PSA transcription was measured by quantitative real time PCR.
Results Satraplatin and JM-118 inhibited in vitro and in vivo the growth of prostate cancer cells in a dose-dependent fashion. The IC_{50} cytotoxicity values for satraplatin ranged from 1 to 3 μM for androgen-insensitive cells and was 11 μM for the androgen-sensitive cell line. Interestingly, JM-118 was up to 16-fold more potent than satraplatin. Oral administration of satraplatin to nude mouse PC-3 xenograft models inhibited the growth of these human tumors. Satraplatin had no direct effect on PSA transcription and the observed decrease in secreted PSA correlated with a decrease in cell number. When evaluated in the NCI drug-screening panel, satraplatin was most active in leukemia and small cell lung cancer cell lines.

Both satraplatin and JM-118 were tested on cells resistant to chemotherapeutic agents. Satraplatin and JM-118 were equally active in the cisplatin-resistant A129cp80 ovarian carcinoma cell line, with activity comparable to that observed in the parent line. Neither expression of MDR1, BCRP, MRP1, nor altered tubulin or topoisomerase I were found to mediate resistance to satraplatin or JM-118. Although these resistance mechanisms contribute to drug resistance for a number of chemotherapeutics, they do not appear to play a role in satraplatin resistance.
Conclusions These results demonstrate that satraplatin and JM-118 have preclinical antitumor activity in human prostate cancer and other tumor types as well, including several cell lines displaying drug resistance to cisplatin, docetaxel and mitoxantrone. In addition, the results suggest that PSA should be further evaluated as a relevant marker of clinical response in patients with prostate cancer treated with satraplatin.

Keywords Satraplatin · JM-118 · Platinum · Prostate cancer · Drug resistance

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Introduction

Satraplatin [formerly known as JM-216, or bis (acetato) ammine dichloro (cyclohexylamine) platinum (IV)] is a fourth-generation platinum analog that was rationally synthesized to be orally available. Many commonly used platinum analogs are associated with significant dose-limiting toxicities and/or drug resistance that can limit therapy and

negatively impact patient quality of life. For example, cisplatin therapy can result in nausea, vomiting, neurotoxicity, ototoxicity, and renal toxicity, while oxaliplatin may induce hematologic and gastrointestinal toxicity and peripheral neuropathy [1]. Although many patients with solid malignancies experience significant responses to cisplatin, acquired drug resistance often develops, necessitating changes in therapy. Thus there is a significant need for novel platinum analogs with lower toxicity, similar antitumor activity, and reduced cross-resistance to cisplatin. Satraplatin is not associated with the dose-limiting renal toxicity seen with cisplatin, nor the neurotoxicity seen with oxaliplatin. In addition, the results of a multicentre Phase III double blind randomized trial involving 950 patients with hormone-refractory prostate cancer showed that satraplatin significantly reduced the risk of disease progression in these patients [2]. In contrast to cisplatin, carboplatin, and oxaliplatin, which must all be given intravenously, oral administration of satraplatin is more convenient, eliminates the need and complications of venous access devices, and may improve patient acceptability of the treatment.

Satraplatin has a mechanism of action similar to that of cisplatin, inducing the formation of DNA adducts and inter- and intra-strand crosslinks and resulting in G_2 arrest and induction of apoptosis [3]. Adducts induced by satraplatin and other platinum analogs are repaired by the nucleotide excision repair pathway with comparable efficiency. Unlike cisplatin and carboplatin, however, satraplatin-induced adducts are not recognized by DNA mismatch repair proteins, which may play a role in its ability to obviate drug resistance due to loss of DNA mismatch repair [4].

Satraplatin has demonstrated significant in vitro antitumor activity against a variety of human tumor cell lines including ovarian [3, 5], cervical [6], and lung cancer [7] cell lines. Importantly, satraplatin has also shown activity in certain tumor cell lines with both acquired and intrinsic resistance to cisplatin, suggesting its potential in the treatment of cisplatin-resistant malignancies [7, 8].

Following oral administration, satraplatin is rapidly metabolized. JM-118 [ammine dichloro (cyclohexylamine) platinum (II)], is the primary metabolite detected in rat, dog, and human plasma ultrafiltrates. The studies presented here were designed to characterize the preclinical antitumor efficacy of satraplatin and/or JM-118 in a variety of cell lines, including those included in the NCI Drug Screening Panel, and in a prostate cancer xenograft model. Increased PSA level is often used clinically as an indicator of disease progression. It has been shown that the expression and/or secretion of PSA may be independent of the effect on cell growth [9]. Therefore, the effect of satraplatin on PSA expression and/or secretion in a prostate cancer cell line was evaluated.

Development of drug resistance is one of the primary reasons for treatment failure and can be a major obstacle to successful outcomes with chemotherapy. Since drug resistance frequently occurs during chemotherapy, studies were performed to better characterize the activity of satraplatin and JM-118 in cell lines expressing various defined mechanisms of drug resistance to commonly used chemotherapeutics.

Materials and methods

Cell culture

Hormone-insensitive human PC-3 metastatic prostate cancer cells (National Cancer Institute [NCI], passage 4) were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FCS, 1% glutamine and 1% penicillin/streptomycin. The sixth passage of cells with approximately 80–90% confluence in T-225 flasks was used in the xenograft study. Two additional prostate cancer cell lines, Du-145 (hormone-insensitive) and LNCaP (hormone-sensitive) were cultured in the same media. The human colon cancer cell line HT-29 and its mitoxantrone-resistant subline HT-29/MIT were the kind gift of Dr. Gabriella Pezzoni (Novuspharma, Bresso, Italy). The cells were grown in McCoy's 5A media supplemented with 2 mM glutamine, 10% FCS, and 20 mM HEPES buffer. The ovarian carcinoma cell lines A129 and its cisplatin-resistant subline A129cp80, and 1A9 and its paclitaxel-resistant subline PTX10 were kindly provided by Dr. Tito Fojo (National Institutes of Health, Bethesda, MD, USA). These cell lines were grown in RPMI 1640 media supplemented with 10% FCS. MCF-7 breast cancer cells and the etoposide-resistant cell line MCF-7/VP, a gift from Dr. Erasmus Schneider (Wadsworth Center, New York State Department of Health), were cultured in MEM (Eagle) supplemented with 20 μg/ml insulin and 10% FCS. The NCI-Adr-Res cell line was obtained from the NCI (Bethesda) and grown in RPMI 1640 media containing 10% FCS. The CCRF-CEM human T-lymphoblastic leukemia cell line and its camptothecin-resistant derivative CEM/C2 (American Type Culture Collection, Rockville, MD, USA) were grown in RPMI 1640 media with 10% FCS. The NCI-Adr-Res cells were evaluated in the presence and absence of the P-glycoprotein pump (Pgp) antagonist, verapamil (Sigma–Aldrich, St. Louis, MO, USA) at a concentration of 12.5 μg/ml for 4 h prior to addition of the test drugs.

Drug preparation

For the in vitro studies, satraplatin and JM-118 were prepared as 1 mM solutions in 50% ethanol. Satraplatin was

stored for up to 4 weeks at 4°C and JM-118 for a maximum of 2 weeks at −20°C. All solutions were protected from light. Docetaxel (Sigma–Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C.

For the in vivo study, satraplatin (purity 98.9%) was freshly formulated in peanut oil on each dosing day. Briefly, satraplatin was weighed in individual tubes for the different dosing groups and suspended in the proper amount of peanut oil (i.e., 5 mg/ml for 50 mg/kg, 4 mg/ml for 40 mg/kg, and 3 mg/ml for 30 mg/kg). The satraplatin suspensions were sonicated for 10 min, then vortexed for 10 s and administered within 30 min of preparation.

Mitoxantrone, etoposide, cisplatin, doxorubicin, and verapamil were purchased from Sigma–Aldrich. Mitoxantrone and etoposide were prepared as 10 mM solutions in DMSO. Cisplatin was dissolved in phosphate-buffered saline (PBS) to a final concentration of 3 mM. Doxorubicin and verapamil were prepared in distilled H_2O at concentrations of 10 mM and 12.5 mg/ml, respectively. Paclitaxel (EMD Biosciences, San Diego, CA, USA) and docetaxel (Fluka, Buchs, Switzerland) were prepared as 10 mM solutions in DMSO.

Cell proliferation assays

To determine the effects of test drugs on cell proliferation the drug resistant cell lines and their sensitive counterparts were harvested from sub-confluent plates and seeded in 96-well dishes (in triplicate for each dose tested) at a density of 2,000–4,000 cells/well. This density was previously determined to be in the linear range of the assay. After 24 h, cells were exposed to a range of drug concentrations and incubated for 48 h for determination of the IC_{50} (dose resulting in 50% growth inhibition). The effects of the compounds on cell proliferation were determined using the sulforhodamine B (SRB) assay [10]. Following drug exposure, cells were fixed with cold TCA to a final concentration of 10% and incubated at 4°C for 60 min. The plates were then washed five times with water and air-dried. SRB solution (0.4% [w/v] in 1% acetic acid) was added to each well and the plates were incubated for 10 min at room temperature. After washing five times with 1% acetic acid, the plates were air-dried. Protein-bound dye was solubilized with 10 mM Tris base (Sigma–Aldrich). Optical density was measured on a plate reader at 570 nm. Growth inhibition of the three human prostate carcinoma cell lines by satraplatin and other platinum analogs was measured using the XTT Cell Proliferation Kit II (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The cells were cultured in 25 or 75 cm^2 flasks, harvested with trypsin–EDTA (Sigma–Aldrich), counted in a Neubauer chamber, and seeded in 96-well plates (Becton Dickinson, San Jose, CA, USA) at a density of either 1×10^3 or 5×10^3 cells/well in 200 µl medium. The outer wells of the plates were filled with medium or PBS to minimize the drying effects in the wells.

The viability of CCRF-CEM and CEM/C2 human T-lymphoblastic leukemia cells was assayed by means of the Calcein-AM flow cytometric assay (Invitrogen). Suspension cell cultures (200,000 cells per T-25 flask) were treated with drug for 48 h, then recovered by centrifugation and washed twice in PBS. A 5 µM calcein-AM solution was diluted 1:16,000 in PBS, and 200 µl of this solution was added to each sample of cells. Following a 90-min incubation at 37°C, cells were washed once in PBS and fluorescence was quantified by flow cytometry (BD FACScan System, BD Biosciences, San Jose, CA, USA).

Effect of satraplatin or JM-118 on PSA mRNA levels

In order to determine whether satraplatin and JM-118 affected the rate of PSA transcription, RNA from drug-treated LNCaP cells was isolated and measured using quantitative real-time reverse-transcription PCR (RT-PCR). RNA was extracted from LNCaP cell extracts by means of the High Pure RNA Isolation Kit (Roche Diagnostics) and yield measured by UV/Vis spectrophotometry. Reverse transcription was performed with 1.5 µg RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics), and resulting cDNA was stored at −20°C. After diluting the cDNA solution 1:10 with H_2O, quantitative RT-PCR (LightCycler® system, Roche Diagnostics) was carried out using the LightCycler Fast-Start DNA Master SYBR Green I and LightCycler Capillaries. Quantitative PCR was performed for two genes in parallel: the PSA target gene and ß2-microglobulin as a reference. Additionally, a calibrator (RNA from untreated LNCaP cells) was used to compare the different quantitative PCR reactions to each other and to generate a standard curve. The primer for PSA was designed according to Straub et al. [11, 12]. Finasteride was used as a positive control since this drug can reduce PSA transcription and secretion without a significant loss of cell viability. Protein was measured using the BCA (bicinchoninic acid) assay (Pierce, Rockford, IL, USA).

NCI cell line panel screen

In vitro antitumor activity was determined using the NCI's drug screening program. This approach utilizes a panel of human tumor cell lines, representing nine tumor types, to characterize relative growth inhibition or cytotoxicity in various tumors [13, 14]. Relative growth inhibition was calculated for each cell line tested after staining for viability using the SRB assay described above. The logarithm of the GI_{50} values for responses measured for all cell lines was

calculated, and these data were displayed using a mean graph format, centered at the arithmetic mean value [15].

Xenograft studies

Athymic male NU/NU mice (6 to 8-weeks old) were obtained from Charles River Laboratories (Wilmington, MA, USA) and were allowed to acclimate for at least 5 days. They were housed under aseptic conditions and given irradiated rodent diet (Pharmaserv, Inc., Framingham, MA, USA) and sterile water ad libitum.

The xenograft studies utilized PC-3 cells (passage 6), which were grown to 80–90% confluence in T-225 flasks. After harvesting, cells were suspended in 50% matrigel (BD Biosciences, San Jose, CA, USA) in RPMI 1640 without supplements to a final concentration of 2×10^7 cells/ml. Mice ($N = 55$) were injected subcutaneously with 0.1 ml each of cell suspension containing a total of 2×10^6 cells. Injections were in the lower right flank area under light isoflurane anesthesia. Then days after transplantation, 40 animals with an average tumor size of 160 mm^3 were selected and randomly divided into four groups.

The mice were dosed daily for 5 consecutive days with 30, 40, or 50 mg/kg drug, followed by a 2-day interval, then for 5 additional days. The control group received peanut oil on the same schedule. Agents were administered orally by means of a 20-gauge gavage needle. The volume of administration for all oral treatments was 0.1 ml per 10 g body weight.

Tumor growth and body weight were monitored and recorded twice weekly. Tumors were measured by determining the length and width of the tumor with a digital caliper. Tumor volume was calculated using the following formula:

Tumor volume (mm^3) = $(w^2 \times l)/2$ where w = width and l = length (in mm) of the tumor. Percentage tumor growth inhibition (% TGI) was calculated as follows: %TGI = $100(1 - T/C)$ where T is the mean tumor size for a drug-treated group on a given day and C is the mean tumor size for the vehicle-treated group.

Mice were sacrificed when their tumors reached the 1,000 mm^3 endpoint volume. Treatment efficacy was determined as Log Cell Kill (LCK). This was defined as $(T-C)/(3.32)(Td)$ where T is the mean time required for the treatment group of mice to reach 1,000 mm^3 in size, C represents the mean time for the control group tumors to reach 1,000 mm^3 in size, Td equals the tumor doubling time (estimated from linear regression analysis of the control tumors during exponential growth), and *3.32* reflects the number of doublings required for a population to increase $1 - \log_{10}$ unit. Each LCK unit represents 1-$\log_{10}$ unit of cell killing (e.g., 1 LCK = 90% kill, 2 LCK = 99% kill).

Animals were weighed two times per week until the completion of the study. The mice were examined for overt signs of any adverse drug-related side effects. Acceptable toxicity for cancer drugs was defined using NCI guidelines as the group's mean body weight loss of <20% during the study period and not more than one toxic death among ten related animals [16].

Results

Growth inhibition of human prostate carcinoma cells

Satraplatin and JM-118 demonstrated activity in vitro against three human prostate carcinoma cell lines: the androgen-sensitive LNCaP cell line, and the androgen-insensitive PC-3 and Du-145 cell lines. IC_{50} values for in vitro cytotoxicity of satraplatin, JM-118, cisplatin, and oxaliplatin following 72-h incubation are summarized in Table 1. Results indicate that both satraplatin and JM-118 inhibit prostate cancer cell proliferation, but that JM-118 is up to 16-fold more active. Satraplatin and JM-118 were active in androgen-sensitive and androgen-insensitive prostate cancer cell lines. Satraplatin was least active in the LNCaP line, with an IC_{50} value 30-fold less potent than oxaliplatin. In the androgen-insensitive PC-3 line, oxaliplatin was weakest, whereas satraplatin, JM-118, and cisplatin all had equivalent activity. All compounds were equally active in the Du-145 cell line.

Effect of satraplatin or JM-118 on PSA mRNA and protein levels

To determine whether these agents affected PSA production, the hormone-sensitive, PSA expressing and secreting prostate cancer cell line LNCaP was incubated with satraplatin or JM-118. PSA concentrations in cell supernatants were measured in a clinical laboratory using standard methods, while PSA-specific transcription was assayed using total RNA isolated from treated cells.

Treatment of LNCaP cells with 12 μM satraplatin or 1.4 μM JM-118 resulted in a decrease in cell number (56 and 41.1% of control, respectively) and a decrease in secreted PSA protein level (58 and 45.5% of control,

Table 1 In vitro cytotoxicity against prostate cancer cell lines (72-h exposure)

Compound	IC_{50} (μM)		
	LNCaP	PC-3	Du-145
Satraplatin	10.9 (±0.6)	1.4 (±0.1)	2.8 (±0.2)
JM-118	0.7 (±0.6)	0.7 (±0.1)	0.9 (±0.2)
Cisplatin	3.7 (±0.4)	1.0 (±0.2)	1.7 (±0.3)
Oxaliplatin	0.3 (±0.1)	11.0 (±1.0)	1.8 (±0.5)

Values shown represent mean ± standard deviation

respectively (Fig. 1; Table 2). The decreases in PSA secretion correlated with declines in cell number as shown in Table 2 by the normalized secreted PSA protein amount per cell number. Finasteride was used as a reference compound since this drug reduces PSA transcription and secretion without loss of cell viability. Indeed, finasteride reduced PSA levels similar to that produced by JM-118 but without any significant decrease in cell number. Satraplatin and JM-118 had minimal effects on PSA transcription, ranging from 96% of control values with satraplatin (6 μM) to 84% with JM-118 (1.4 μM) (Fig. 1; Table 2). Therefore, treatment with satraplatin and JM-118 resulted in a decrease in cell number associated with a decrease in secretion of prostate-specific antigen (PSA) protein level that was not brought about by a reduction in PSA transcription.

Satraplatin xenograft studies

Satraplatin exhibited growth inhibition of PC-3 human prostate carcinoma xenografted in nude mice at all doses tested. A 30 mg/kg/day dose produced a tumor growth delay equivalent to 0.9 LCK, with a 10% drug-related mortality (Table 3). At the doses of 40 and 50 mg/kg, satraplatin was active but toxic, causing 30 and 50% mortality, respectively. Maximum tumor growth inhibition (relative to control animals) was achieved at day 26 for the 30 and 40 mg/kg doses and at day 31 for the 50-mg/kg dose (Fig. 2). No tumor regressions or cures were noted in this experiment.

Maximum body weight loss was approximately 13% for control animals and 18–20% for all satraplatin-treated groups. Decreases in body weight occurred within the first 10 days, then gradually increased towards baseline after cessation of therapy (Fig. 3).

In vitro antitumor activity in the panel human tumor cell lines of the NCI drug screen program

In vitro antitumor activity was also measured in a panel of human tumor cell lines using the NCI's drug screening

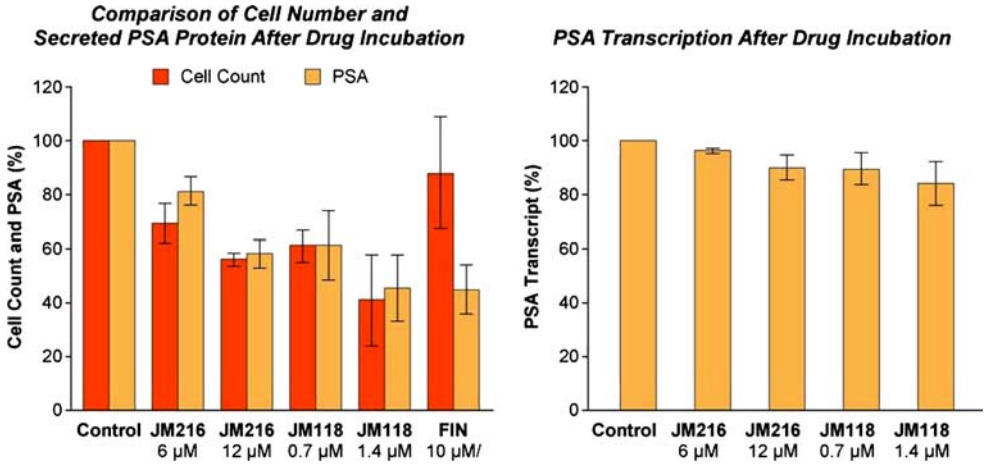

Fig. 1 Effects of Satraplatin (JM216) and JM-118 on Secreted PSA Protein Level and PSA Transcription. Cell cultures of the hormone-sensitive human prostate cancer cell line LNCaP were incubated with satraplatin (JM-216) or JM-118 for 42 h at the concentrations shown. Untreated cells and those treated with finasteride served as controls. Cell number and secreted PSA protein detected in cell supernatants were determined and plotted relative to control (±SD). PSA transcription was measured in drug-treated LNCaP cells using quantitative PCR as described in Materials and methods

Table 2 Drug induced effect on PSA Transcription, PSA protein secretion and cell number

Compound:	Control	Satraplatin		JM-118		Finasteride
Concentration:	–	6 μM	12 μM	0.7 μM	1.4 μM	10 μM
PSA transcript (%)	100	96.1 ± 1.1	89.8 ± 4.8	89.5 ± 5.8	83.9 ± 8.3	88.3 ± 1.0
Secreted PSA protein (%)	100	81.2 ± 5.6	58.0 ± 5.3	61.1 ± 12.7	45.5 ± 12.3	45.0 ± 9.5
Cell number (%)	100	69.3 ± 7.6	56.0 ± 2.5	61.1 ± 6.1	41.1 ± 17.1	88.0 ± 20.9
Relative secreted PSA protein/cell number	1	1.17	1.04	1	1.11	0.51

All values are means of 3 independent experiments (±SD)

Table 3 Antitumor activity of satraplatin in the mouse PC-3 human prostate carcinoma model

	Schedule	Dose		Max. BW Loss (%)	Decedents	Max TGI (Day)	LCK
		mg/kg	mg/m^2				
Control	qdx5qwx2	–	–	12.8	0/10	–	–
Satraplatin		30	90	18.0	1/10	58.5% (26)	0.91
		40	120	20.4	3/10	75.7% (26)	1.02
		50	150	19.8	5/10	86.2% (31)	1.33

BW body weight, *TGI* tumor growth inhibition: 100× (1−mean tumor size of treated mice/mean tumor size of the vehicle-treated mice); *LCK* log cell kill

qdx5qwx2 two cycles of 5 daily treatments, 2 days off-dose between cycles

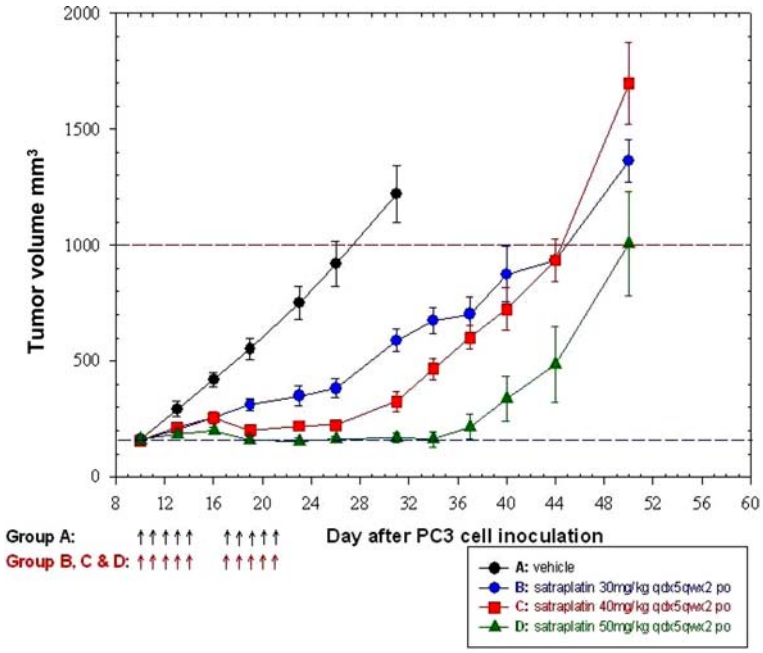

Fig. 2 Effects of satraplatin on growth of PC-3 human prostate xenografts. Athymic nude mice were used to establish xenografts of PC-3 human prostate tumor cells. Satraplatin (30, 40, or 50 mg/kg) or vehicle alone was administered orally for 5 days, followed by a 2-day rest period, then for an additional 5 days. Tumor weight was measured at the indicated time-points; arrows indicate satraplatin treatments

program. Two experiments were performed at the NCI: experiment 1 with 52 tumor cell lines and experiment 2 with 58 tumor cell lines. The sensitivity profile of experiment 1 is displayed in Fig. 4. Satraplatin GI_{50} values for all cell lines were in the micromolar concentration, ranging from a minimum of 1.6 μM for the NCI-H460 non small cell lung carcinoma line to a maximum of 28.8 μM for the TK10 renal carcinoma cell line. Satraplatin appeared particularly active in leukemia and small cell lung cancer cell lines, and less active in ovarian cancer cell lines (Table 4). The results of these experiments were compared to the historic sensitivity profiles of cisplatin (NSC 119875) and oxaliplatin (NSC 266046) available on the NCI server (http://www.dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp). Comparison of the sensitivity profiles of satraplatin, cisplatin and oxaliplatin demonstrated that the antiproliferative activity was lower for satraplatin (mean Log_{10} $GI_{50} = -5.09$) than for cisplatin (mean Log_{10} $GI_{50} = -6.15$) or oxaliplatin (mean Log_{10} $GI_{50} = -5.64$). JM-118 was not tested in this cell line panel but is be expected to be as or more active than cisplatin since JM-118 displayed a similar or stronger cytotoxic activity than cisplatin in other in vitro experiments (Tables 1, 6, 7).

Activity in drug-resistant tumor cell lines

Satraplatin and JM-118 were tested on cells resistant to chemotherapeutic agents including cisplatin, taxanes, doxorubicin, mitoxantrone, camptothecin, and etoposide. The cell lines and their resistant clones used in these studies are shown in Table 5.

Activity in cisplatin-resistant cells

To determine if satraplatin and JM-118 were cross resistant with the related platinum analog cisplatin, their activity was tested in ovarian carcinoma cells resistant to cisplatin. The A129cp80 cell line was selected for resistance to cisplatin through gradual exposure of an A2780 clone to this drug over 2–3 years [17]. Satraplatin, JM-118, and cisplatin

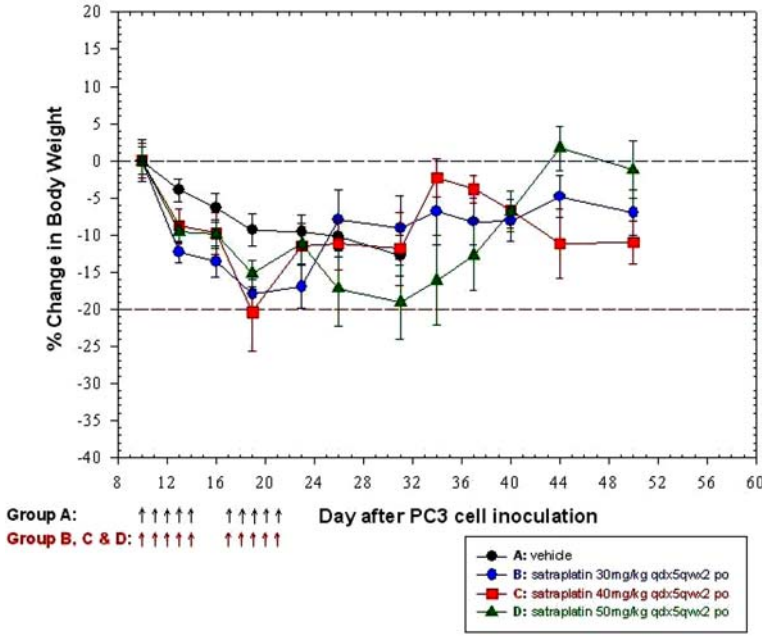

Fig. 3 Effects of satraplatin on body weight in mice bearing PC-3 Xenografts. Nude mice bearing established PC-3 xenografts were treated with satraplatin (30, 40, or 50 mg/kg) or vehicle. Body weight was found to decrease within the initial 10 days, then gradually returned towards baseline after cessation of therapy. Body weight was measured at the indicated timepoints; *arrows* indicate satraplatin treatments

were found to be equally active in the A129 parent cell line, with IC_{50} values of 0.23–0.30 μM. In the cisplatin-resistant A129cp80 derivative line, satraplatin and JM-118 displayed similar profiles, with both almost as active as in the parent line. In contrast, A129cp80 cells were nearly 70-fold less sensitive to cisplatin compared to the A129 parental line (Table 5).

Activity in cells with ß-tubulin mutation

Resistance to the taxanes (paclitaxel and docetaxel) can be achieved through different mechanisms (reviewed by Casazza and Fairchild [18]). One such mechanism is via the generation of multidrug resistance due to up-regulation of MDR1. Taxane resistance can also occur through accumulation of β-tubulin mutations that alter drug binding or changes in the intracellular level of tubulin [19–25]. Satraplatin and JM-118 were tested in 1A9PTX10 cells, which are taxane-resistant due to defects in tubulin polymerization caused by point mutations in the β-tubulin gene [25]. Satraplatin and JM-118 were both fully active, as was cisplatin (Table 5), suggesting that the cytotoxic activity of these platinum compounds is not dependent on alterations in tubulin polymerization.

Activity in cells with altered topoisomerase I enzyme

The camptothecins have found widespread use in the treatment of various malignancies, such as ovarian and lung cancers [26]. These agents inhibit DNA topoisomerase I (which maintains DNA topology by inducing transient single-strand DNA breaks in supercoiled DNA), resulting in accumulation of a DNA-topoisomerase "cleavable complex" reaction intermediate [27]. The CEM/C2 cell line, established through stepwise selection of human T-cell leukemia CCRF-CEM cells in camptothecin, has an altered topoisomerase I enzyme and displays >1,000-fold resistance to this drug relative to the parental line [28]. Similar to the pattern seen in etoposide-resistant cells, satraplatin, JM-118, and cisplatin were equipotent in CEM/C2 cells relative to the CCRF-CEM parental line (Table 6).

Activity in cells expressing multidrug resistance phenotype

The multidrug resistance (MDR) phenotype is characterized by cross-resistance to several structurally unrelated chemotherapeutic agents. This occurs via increased drug efflux and hence decreased intracellular accumulation. The MDR phenotype is associated with a family of membrane proteins known as ATP binding cassette (ABC) transporters [29]. Three members of these ABC drug transporters—MDR1 (ABCB1, Pgp), MRP1 (ABCC1), and BCRP (ABCG2, MXR1, ABCP)—were investigated to determine the activity of satraplatin and JM-118 in cells with these resistance mechanisms.

Activity in BCRP (ABCG2)-expressing cells

Because mitoxantrone is often used in the treatment of hormone-refractory prostate cancer, potentially inducing resistance, satraplatin and JM-118 were evaluated in cells resistant to this agent. Mitoxantrone resistance is generated through upregulation of the ABCG2 transporter (MXR, BRCP) [30]. In this study, colorectal carcinoma HT29/MIT

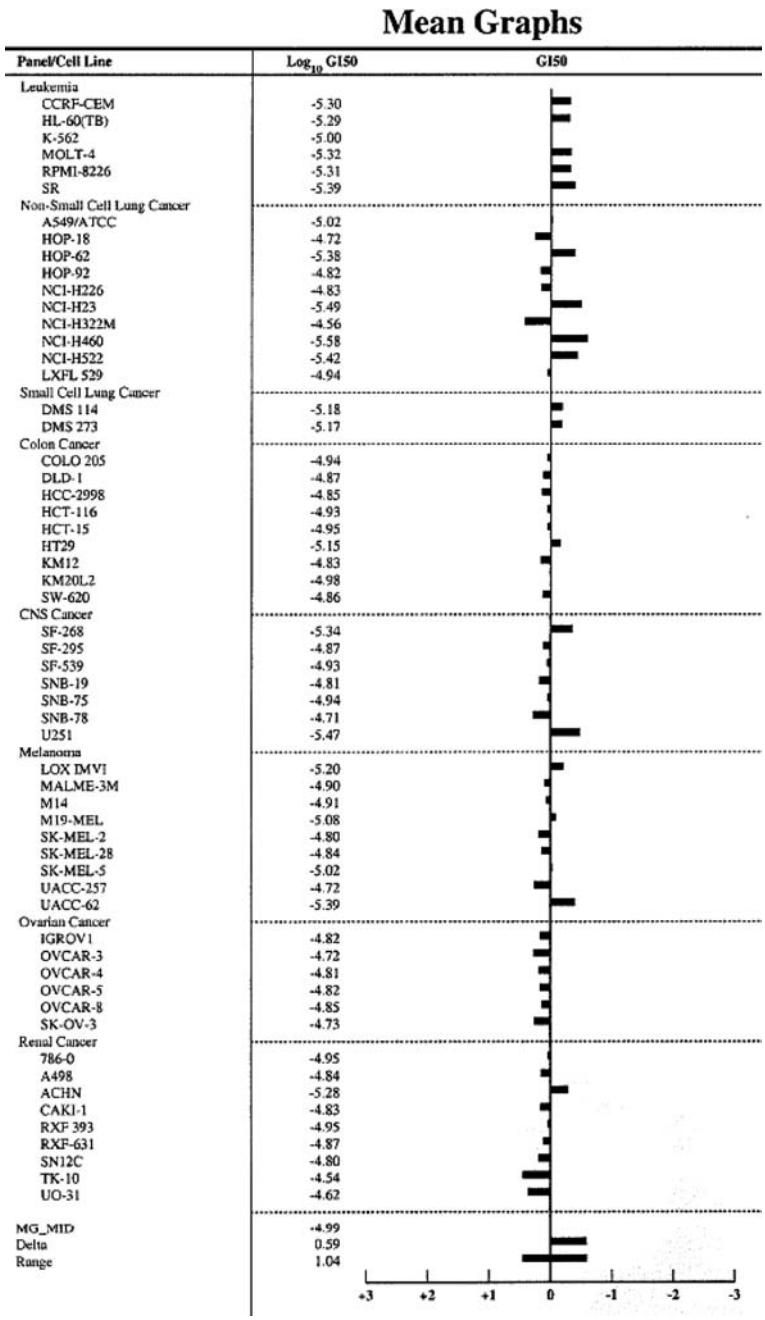

Fig. 4 Sensitivity Profile to Satraplatin derived from human tumor cell line panel of the NCI Drug Screen. Fifty-two tumor cell lines cells were exposed to a range of drug concentrations and incubated for 48 hours for determination of the GI_{50} values. The sensitivity profile of experiment 1 is displayed

cells were used, which display a 150-fold resistance to mitoxantrone relative to the parental HT29 cell line [31]. Satraplatin and JM-118 retained full activity in this cell line. Interestingly, although the parental HT29 cell line is inherently resistant to cisplatin, it is sensitive to satraplatin and JM-118 (Table 6), supporting the finding that the latter two compounds are active in cisplatin-resistant cells [32].

Activity in MRP1 (ABCC1)-expressing cells

The activity of satraplatin and JM-118 was determined in an MCF-7 subline (MCF-7/VP) selected for resistance to etoposide; these cells over-express the multidrug resistance-associated protein 1, MRP1 (ABCC1) [33]. While MCF-7/VP cells were 14-fold more resistant to etoposide than the parent cell line, satraplatin, JM-118, and cisplatin were equally active in both lines (Table 6). The cytotoxic activity of the platinum compounds tested was not dependent on the expression of MRP1, suggesting that these platinum compounds are not substrates of the MRP1 transporter.

Activity in MDR1 (ABCB1, Pgp)-expressing cells

The role of MDR1 in cellular resistance to satraplatin and JM-118 was explored through evaluation of the activity of these compounds in the NCI-Adr-Res breast carcinoma cell

Table 4 Sensitivity ranking of NCI tumor cell lines to growth inhibition by satraplatin, cisplatin and oxaliplatin

Tumor type	Mean Log_{10} GI_{50} exp. 1 (MG_MID: −5.19)	Mean Log_{10} GI_{50} exp. 2 (MG_MID: −4.99)	Sensitivity ranking of Satraplatin	Mean Log_{10} GI_{50} Oxaliplatin (MG_MID: −5.64)	Mean Log_{10} GI_{50} Cisplatin (MG_MID: −6.15)
Leukemia	−5.51	−5.27	High	−6.41	−6.60
SCLC	−5.52	−5.18	High	−5.85	−6.50
NSCLC	−5.21	−5.08	Moderate	−5.18	−6.21
CNS	−5.22	−5.01	Moderate	−5.15	−6.39
Melanoma	−5.23	−4.98	Moderate	−5.54	−6.31
Colon	−5.18	−4.93	Moderate	−6.67	−5.61
Renal	−5.09	−4.85	Low	−5.4	−6.14
Ovarian	−4.80	−4.79	Low	−5.31	−6.08

CNS central nervous system, *NSCLC* non-small cell lung cancer, *SCLC* small cell lung cancer

Table 5 Characteristics of parent tumor cell lines and drug-resistant lines used in studies

Parent cell line	Resistant line	Tumor type	Resistant to
A129	A129cp80	Ovarian	Cisplatin
NCI-Adr	NCI-Adr-Res	Breast	Doxorubicin
1A9	1A9PTX10	Ovarian	Paclitaxel
HT-29	HT-29/MIT	Colon	Mitoxantrone
MCF-7	MCF-7/VP	Breast	Etoposide
CCRF-CEM	CEM/C2	Leukemia	Camptothecin

line. This cell line displays resistance to the MDR1 substrates doxorubicin, paclitaxel and docetaxel, and this resistance can be reversed with the Pgp antagonist, verapamil [34]. Pretreatment with verapamil sensitizes these cells to compounds that undergo Pgp-mediated efflux. Verapamil increased the inhibition by doxorubicin and the taxanes 100-fold (Table 7). The activity of satraplatin and JM-118 was unchanged in the presence of verapamil, suggesting that these compounds are not substrates of MDR1.

Discussion

Both satraplatin and its primary metabolite, JM-118, have shown promising antitumor activity in preclinical studies. Data presented here demonstrate that both compounds inhibit the growth of human prostate carcinoma cells at micromolar concentrations. While the IC_{50} value for satraplatin was similar to that previously observed for cisplatin, JM-118 was up to 16-fold more active. In addition to inhibition of prostate cancer cell growth, results from the NCI drug screening profile reveal that micromolar concentrations of satraplatin are active in a variety of tumor cell

Table 6 Growth inhibition of satraplatin and JM-118 in human tumor cell lines with acquired drug resistance IC_{50} value (μM)

Cell Line	Acquired resistance to	Satraplatin	JM-118	Cisplatin	Paclitaxel	Docetaxel	Camptothecin	Mitoxantrone	Etoposide
A129 A129cp80 RR	Cisplatin	0.30 ± 0.24 1.54 ± 0.72 5.1	0.24 ± 0.14 0.69 ± 0.51 2.9	0.23 ± 0.17 15.1 ± 6.4 66					
1A9 1A9PTX10 RR	Paclitaxel	4.55 ± 2.57 7.61 ± 4.4 1.7	0.49 ± 0.23 1.54 ± 0.56 3.1	8.58 ± 3.44 15.9 ± 5.8 1.8	0.004 ± 0.003 0.235 ± 0.062 59	0.002 ± 0 0.179 ± 0.093 90			
CCRF-CEM CEM/C2 RR	Camptothecin	4.46 ± 1.73 3.46 ± 1.61 0.78	0.53 ± 0.23 0.59 ± 0.34 1.1	2.60 ± 1.10 2.20 ± 1.51 0.85			0.004 ± 0.0005 7.10 ± 0.68 1.775		
HT-29 HT-29/MIT RR	Mitoxantrone	6.9 ± 2.8 10.2 ± 2.2 1.5	4.51 ± 2.33 6.0 ± 3.3 1.3	>100 >100				1.3 ± 0.63 198.3 ± 14.6 152	
MCF-7 MCF-7/VP RR	Etoposide	3.0 ± 1.2 4.8 ± 1.4 1.6	1.2 ± 0.3 1.3 ± 0.3 1.1	28.7 ± 3.6 31.2 ± 7.0 1.1					7.2 ± 2.5 > 100 > 14

Relative resistance defined as ratio of activity in drug-resistant cell line and in parent line

RR relative resistance

Table 7 Activity of satraplatin and JM-118 in the MDR cell line NCI-Adr-Res

Compound	IC_{50} (μM)		Relative resistance[a]
	−Verapamil	+ Verapamil	
Doxorubicin	67 ± 11	0.69 ± 0.5	97
Paclitaxel	2.9 ± 2.3	0.03 ± 0.02	97
Docetaxel	3.3 ± 2.2	0.04 ± 0.02	83
Satraplatin	23 ± 1	20 ± 1	1.2
JM-118	3.8 ± 2.4	4.1 ± 1.7	0.9
Cisplatin	14.5 ± 3.6	16.7 ± 6.4	0.9

[a] Relative resistance defined as ratio of activity in absence and presence of verapamil

lines, particularly leukemia and small cell lung cancer. Results from clinical studies support its efficacy in prostate [35] and small cell lung cancer [36], although no trials have been conducted to date in leukemia. In vivo, satraplatin effectively inhibited the growth of PC-3 xenograft tumors in a dose-dependent manner. A 30 mg/kg/day dose produced a tumor growth delay equivalent to a LCK of 0.9. In support of these growth inhibition data, Sova and colleagues also found that satraplatin and another novel platinum (IV) complex, LA-12, had antitumor activity against PC-3 xenografts, as well as those derived from human colon and ovarian carcinoma cell lines, HCT-116 and A2780, respectively, when these agents were administered on a daily x5 schedule [37]. Satraplatin and LA-12 were both able to overcome cisplatin resistance, as evidenced by activity against the cisplatin-resistant A2780/cisR xenograft. Treatment of PSA-expressing prostate cancer cells with satraplatin or JM-118 resulted in a decrease in cell number that correlated with a decrease in secreted PSA protein level, which was not brought about by a reduction in PSA transcription. These data suggest that PSA may serve as a surrogate marker for clinical response of prostate cancer to satraplatin treatment.

Satraplatin and JM-118 were equally active in the cisplatin-resistant A129cp80 ovarian carcinoma cell line, with activity comparable to that of the parent line. Both drugs, however, were approximately 70-fold more potent than cisplatin, indicating that satraplatin and JM-118 are efficacious against selected platinum-resistant tumors. This lack of cross-resistance to cisplatin has been observed previously by other investigators in multiple tumor cell lines [3, 8, 38].

Several hypotheses have been postulated regarding the mechanism underlying the ability of satraplatin to circumvent cisplatin resistance. Due to its lipophilicity, uptake of satraplatin appears to be mediated primarily by passive diffusion [39]. Acquired cisplatin resistance in a panel of sublines of the ovarian carcinoma cell line 41 M was believed to be due to reduced active and/or facilitated transport and consequent reduction of intracellular platinum levels and platinum-DNA adducts [40]. The uptake of satraplatin and JM-118 was not altered in the cisplatin-resistant 41McisR6 cell line, and as a result, both the parent (i.e., 41 M) and resistant cell lines were equally sensitive to satraplatin and JM-118. The copper transporters CTR1, ATP7A, and ATP7B have been shown to modulate the pharmacology of cisplatin, oxaliplatin, and JM-118 by affecting cellular drug accumulation [41]. Down-regulation of CTR1 resulted in cisplatin resistance due to decreased accumulation of cisplatin. Loss of CTR1 function did not result in resistance to JM-118 and did not alter the extent of JM-118 accumulation in the cell. These results suggested that JM-118 would retain activity in cells in which cisplatin resistance is due to the loss of CTR1. Additionally, cisplatin resistance may be acquired through mechanisms unrelated to drug uptake. Fokkema and colleagues hypothesized that since satraplatin and JM-118 cytotoxicity did not correlate with platinum-DNA adduct formation (or p53 status), cisplatin cross-resistance may be mediated through other mechanisms such as DNA repair or tolerance to DNA damage [42].

MDR1 (as measured with the doxorubicin-resistant NCI-Adr-Res breast carcinoma cell line), BCRP (assessed with the mitoxantrone-resistant HT29/MIT colorectal carcinoma cell line), and MRP1 (using the etoposide-resistant MCF7/Vp breast cancer cell line) were not found to mediate resistance to satraplatin or JM-118. Consequently, although the family of ABC drug transporters contributes to multidrug resistance for a number of chemotherapeutics, it does not appear to play a role in satraplatin resistance. Also, the mutation in tubulin, which causes resistance to paclitaxel and docetaxel did not confer resistance to satraplatn. These results are clinically relevant when satraplatin is used as chemotherapy for the treatment of patients with hormone-refractory prostate cancer since mitoxantrone and docetaxel both have a role in treating this disease [43, 44].

The activity of satraplatin in PC-3 human prostate carcinoma cells in vitro and in mouse xenografts provides the rationale for clinical use of this oral agent in patients with prostate cancer. Clinical activity of satraplatin in this tumor type was seen in the randomized Phase II trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) in men with hormone-refractory prostate cancer [35]. These results were confirmed in a large Phase III SPARC (Satraplatin and Prednisone Against Refractory Cancer) trial as second-line therapy for this disease [2]. Additionally, satraplatin may also have clinical potential in combination therapy. Amorino and colleagues found that the combination of satraplatin followed by radiation therapy was additive in H460 human lung carcinoma cells, both in vitro and as subcutaneous xenografts [45]. Encouraging preliminary clinical results have been seen using this approach in a Phase I trial of satraplatin plus radiation therapy in

patients with non-small cell lung cancer or locally advanced squamous cell cancer of the head and neck [46]. Currently, several clinical trials are ongoing to evaluate other indications or combinations of satraplatin with other anticancer therapies.

References

1. Hartmann JT, Lipp HP (2003) Toxicity of platinum compounds. Expert Opin Pharmacother 4:889–901
2. Petrylak DP, Sartor O, Witjes F, Ferrero J, Berry WR, Koletsky A, Falcon S, Nathan FE, Petrone ME, Sternberg C (2007) A phase III, randomized, double-blind trial of satraplatin and prednisone vs placebo and prednisone for patients with hormone refractory prostate cancer. ASCO Prostate Cancer Proceedings: Abstract 145
3. Mellish KJ, Barnard CF, Murrer BA, Keller LR (1995) DNA-binding properties of novel *cis*- and *trans* platinum-based anticancer agents in 2 human ovarian carcinoma cell lines. Int J Cancer 62:717–723
4. Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehme A, Christen RD, Howell SB (1996) The role of DNA mismatch repair in platinum drug resistance. Cancer Res 56:4881–4886
5. Kelland LR, Abel G, McKeage MJ, Jones M, Goddard PM, Valenti M, Murrer BA, Harrap KR (1993) Preclinical antitumor evaluation of bis-acetato-ammine-dichloro-cyclohexylamine platinum (IV): an orally active platinum drug. Cancer Res 53:2581–2586
6. Mellish KJ, Kelland LR, Harrap KR (1993) In vitro platinum drug chemosensitivity of human cervical squamous cell carcinoma cell lines with intrinsic and acquired resistance to cisplatin. Br J Cancer 68:240–250
7. Twentyman PR, Wright KA, Mistry P, Kelland LR, Murrer BA (1992) Sensitivity to novel platinum compounds of panels of human lung cancer cell lines with acquired and inherent resistance to cisplatin. Cancer Res 52:5674–5680
8. O'Neill CF, Hunakova L, Kelland LR (1999) Cellular pharmacology of cis and trans pairs of platinum complexes in cisplatin-sensitive and -resistant human ovarian carcinoma cells. Chem Biol Interact 123:11–29
9. Dixon SC, Knopf KB, Figg WD (2001) The control of prostate-specific antigen expression and gene regulation by pharmacological agents. Pharmacol Rev 53:73–91
10. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst 82:1107–1112
11. Straub B, Müller M, Krause H, Schrader M, Goessl C, Heicappell R, Miller K (2001) Detection of prostate-specific antigen RNA before and after radical retropubic prostatectomy and transurethral resection of the prostate using "Light-cycler"-based quantitative real-time polymerase chain reaction. Urology 58:815–820
12. Straub B, Müller M, Krause H, Schrader M, Miller K (2003) Quantitative real-time RT-PCR for detection of circulating prostate-specific antigen mRNA using sequence-specific oligonucleotide hybridization probes in prostate cancer patients. Oncology 65(suppl 1):12–17
13. Grever MR, Schepartz SA, Chabner BA (1992) The National Cancer Institute: cancer drug discovery and development program. Semin Oncol 19:622–638
14. NCI/NIH Developmental Therapeutics Program human cell line screen Available at: http://www.dtp.nci.nih.gov
15. Paull KD, Shoemaker RH, Hodes L, Monks A, Scudiero DA, Rubinstein L, Plowman J, Boyd MR (1989) Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. J Natl Cancer Inst 81:1088–1092
16. Grieshaber CK, Marsoni S (1986) Relation of preclinical toxicology to findings in early clinical trials. Cancer Treat Rep 70:65–72
17. Rixe O, Ortuzar W, Alvarez M, Parker R, Reed E, Paull K, Fojo T (1996) Oxaliplatin, tetraplatin, cisplatin, and carboplatin: spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. Biochem Pharmacol 52:1855–1865
18. Casazza AM, Fairchild CR (1996) Paclitaxel (Taxol): mechanisms of resistance. Cancer Treat Res 87:149–171
19. Cabral F, Abraham I, Gottesman MM (1981) Isolation of a taxol-resistant Chinese hamster ovary cell mutant that has an alteration in alpha-tubulin. Proc Natl Acad Sci USA 78:4388–4391
20. Schibler MJ, Cabral F (1986) Taxol-dependent mutants of Chinese hamster ovary cells with alterations in alpha- and beta-tubulin. J Cell Biol 102:1522–1531
21. Murphy LD, Herzog CE, Rudick JB, Fojo AT, Bates SE (1990) Use of the polymerase chain reaction in the quantitation of mdr-1 gene expression. Biochemistry 29:10351–10356
22. Minotti AM, Barlow SB, Cabral F (1991) Resistance to antimitotic drugs in Chinese hamster ovary cells correlates with changes in the level of polymerized tubulin. J Biol Chem 266:3987–3994
23. Haber M, Burkhart CA, Regl DL, Madafiglio J, Norris MD, Horwitz SB (1995) Altered expression of M beta 2, the class II beta-tubulin isotype, in a murine J774.2 cell line with a high level of taxol resistance. J Biol Chem 270:31269–31275
24. Ohta S, Nishio K, Kubota N, Ohmori T, Funayama Y, Ohira T, Nakajima H, Adachi M, Saijo N (1994) Characterization of a taxol-resistant human small-cell lung cancer cell line. Jpn J Cancer Res 85:290–297
25. Giannakakou P, Sackett DL, Kang YK, Zhan Z, Buters JT, Fojo T, Poruchynsky MS (1997) Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization. J Biol Chem 272:17118–17125
26. Pizzolato JF, Saltz LB (2003) The camptothecins. Lancet 361:2235–2242
27. Liu LF, Desai SD, Li TK, Mao Y, Sun M, Sim SP (2000) Mechanism of action of camptothecin. Ann N Y Acad Sci 922:1–10
28. Kapoor R, Slade DL, Fujimori A, Pommier Y, Harker WG (1995) Altered topoisomerase I expression in two subclones of human CEM leukemia selected for resistance to camptothecin. Oncol Res 7:83–95
29. Glavinas H, Krajcsi P, Cserepes J, Sarkadi B (2004) The role of ABC transporters in drug resistance, metabolism and toxicity. Curr Drug Deliv 1:27–42
30. Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2:48–58
31. Perego P, De Cesare M, De Isabella P, Carenini N, Beggiolin G, Pezzoni G, Palumbo M, Tartaglia L, Pratesi G, Pisano C, Carminati P, Scheffer GL, Zunino F (2001) A novel 7-modified camptothecin analog overcomes breast cancer resistance protein-associated resistance in a mitoxantrone-selected colon carcinoma cell line. Cancer Res 61:6034–6037
32. Hector S, Bolanowska-Higdon W, Zdanowicz J, Hitt S, Pendyala L (2001) In vitro studies on the mechanisms of oxaliplatin resistance. Cancer Chemother Pharmacol 48:398–406
33. Schneider E, Horton JK, Yang CH, Nakagawa M, Cowan KH (1994) Multidrug resistance-associated protein gene overexpression and reduced drug sensitivity of topoisomerase II in a human breast carcinoma MCF7 cell line selected for etoposide resistance. Cancer Res 54:152–158
34. Alvarez M, Paull K, Monks A, Hose C, Lee JS, Weinstein J, Grever M, Bates S, Fojo T (1995) Generation of a drug resistance profile by quantitation of mdr-1/P-glycoprotein in the cell lines of the

National Cancer Institute Anticancer Drug Screen. J Clin Invest 95:2205–2214
35. Sternberg CN, Whelan P, Hetherington J, Paluchowska B, Slee PH, Vekemans K, Van Erps P, Theodore C, Koriakine O, Oliver T, Lebwohl D, Debois M, Zurlo A, Collette L, Genitourinary Tract Group of the EORTC (2005) Phase III trial of satraplatin, an oral platinum plus prednisone vs. prednisone alone in patients with hormone-refractory prostate cancer. Oncology 68:2–9
36. Fokkema E, Groen HJ, Bauer J, Uges DR, Weil C, Smith IE (1999) Phase II study of oral platinum drug JM216 as first-line treatment in patients with small-cell lung cancer. J Clin Oncol 17:3822–3827
37. Sova P, Mistr A, Kroutil A, Zak F, Pouckova P, Zadinova M (2006) Comparative anti-tumor efficacy of two orally administered platinum (IV) drugs in nude mice bearing human tumor xenografts. Anticancer Drugs 17:201–206
38. Raynaud FI, Boxall FE, Goddard P, Barnard CF, Murrer BA, Kelland LR (1996) Metabolism, protein binding and in vivo activity of the oral platinum drug JM216 and its biotransformation products. Anticancer Res 16:1857–1862
39. Loh SY, Mistry P, Kelland LR, Abel G, Harrap KR (1992) Reduced drug accumulation as a major mechanism of acquired resistance to cisplatin in a human ovarian carcinoma cell line: circumvention studies using novel platinum (II) and (IV) ammine/amine complexes. Br J Cancer 66:1109–1115
40. Kelland LR, Murrer BA, Abel G, Giandomenico CM, Mistry P, Harrap KR (1992) Ammine/amine platinum (IV) dicarboxylates: a novel class of platinum complex exhibiting selective cytotoxicity to intrinsically cisplatin-resistant human ovarian carcinoma cell lines. Cancer Res 52:822–828
41. Samimi G, Howell SB (2006) Modulation of the cellular pharmacology of JM118, the major metabolite of satraplatin, by copper influx and efflux transporters. Cancer Chemother Pharmacol 57:781–788
42. Fokkema E, Groen HJ, Helder MN, de Vries EG, Meijer C (2002) JM216-, JM118-, and cisplatin-induced cytotoxicity in relation to platinum-DNA adduct formation, glutathione levels and p53 status in human tumour cell lines with different sensitivities to cisplatin. Biochem Pharmacol 63:1989–1996
43. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA, TAX 327 Investigators (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 351:1502–1512
44. Dagher R, Li N, Abraham S, Rahman A, Sridhara R, Pazdur R (2004) Approval summary: Docetaxel in combination with prednisone for the treatment of androgen-independent hormone-refractory prostate cancer. Clin Cancer Res 10:8147–8151
45. Amorino GP, Mohr PJ, Hercules SK, Pyo H, Choy H (2000) Combined effects of the orally active cisplatin analog, JM216, and radiation in antitumor therapy. Cancer Chemother Pharmacol 46:423–426
46. Cmelak A, Choy H, Murphy B, DeVore R, Bria B, Day T, Porte K, Johnson D (1999) Phase I study of JM-216 with concurrent radiation in non-small cell lung cancer and squamous cell head and neck cancer. Proc Am Soc Clin Oncol 18 (abstract 1520)