AMD473 (ZD0473) exhibits marked *in vitro* anticancer activity in human tumor specimens taken directly from patients

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AMD473 (ZD0473; *cis*-amminedichloro[2-methylpyridine]-platinum [II]) is a new generation anticancer agent that, in preclinical studies, shows evidence of an extended spectrum of antitumor activity and overcomes platinum resistance mechanisms. Here we evaluate the activity of AMD473 (ZD0473) in a panel of 120 human tumor specimens using a soft agar cloning assay (human tumor colony-forming assay). When tumor cells were treated with 1.0, 4.0 or 16.0 μg/ml AMD473 (ZD0473) for 2 h, *in vitro* responses were observed in 18% (9/51), 33% (17/51) and 44% (19/43) of assessable specimens. Treatment of tumor cells with the same concentrations of AMD473 (ZD0473) for 24 h resulted in responses of 33% (16/48), 63% (30/48) and 85% (35/41). AMD473 (ZD0473) (16 μg/ml; 24 h) demonstrated activity towards 100% of the non-small cell lung (5/5) and ovarian (8/8) cancer specimens and 73% (8/11) of the breast cancer specimens treated. Low levels of cross-resistance to cisplatin cyclophosphamide, 5-flurouracil, etoposide and gemcitabine were observed. There was a positive relationship between AMD473 (ZD0473) concentration and effect, and a significant difference between response to 2- versus 24-h exposure to 4 or 16 μg/ml ($p = 0.003$ and $p = 0.001$, respectively). These responses demonstrate efficacy at pharmacologically relevant concentrations, suggesting AMD473 (ZD0473) deserves further evaluation. *Anti-Cancer Drugs* 14:275–280 © 2003 Lippincott Williams & Wilkins.

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Introduction

AMD473 (ZD0473; *cis*-amminedichloro[2-methylpyridine]platinum [II]) is a new generation anticancer agent that, in preclinical studies, shows evidence of an extended spectrum of antitumor activity and overcomes platinum resistance mechanisms (Fig. 1). AMD473 (ZD0473) was designed to have increased steric bulk, which was postulated to overcome inactivation by thiols [1]. AMD473 (ZD0473) has demonstrated *in vivo* antitumor activity against a variety of murine and human ovarian xenograft models, several of which were cisplatin resistant [2]. Holford *et al.* tested AMD473 (ZD0473) in human ovarian carcinoma cell lines that had cisplatin resistance via mechanisms of reduced drug transport, elevated glutathione or enhanced DNA repair/increased tolerance of platinum–DNA adducts and found that AMD473 (ZD0473) could circumvent acquired cisplatin resistance [3]. Phase I trials have demonstrated a predictable and favorable toxicity profile for AMD473 (ZD0473), comparable to that of carboplatin [4,5]. Studies to date have shown that the dose-limiting toxicity of AMD473 (ZD0473) is thrombocytopenia, while neutropenia and anemia are associated hematologic side effects [4,5]. To date, AMD473 (ZD0473) has not been associated with any clinically relevant nephro- or neurotoxicity [6,7].

The objectives of this study were to evaluate the cytotoxicity profile of AMD473 (ZD0473) at different concentrations and at two different durations of exposure. A panel of fresh human tumor specimens was taken both from untreated patients and from those who had received prior therapy; these were plated in a human tumor cloning system, similar to that previously described by Hamburger and Salmon [8]. The tumor specimens were exposed *in vitro* to AMD473 (ZD0473) and responses were evaluated by quantitating the formation of human tumor colony-forming units. Tumor specimens were from a range of tumors, including breast, non-small cell lung and ovarian cancers.

Materials and methods

Drugs

AMD473 (ZD0473) was provided by AstraZeneca (Alderley Park, UK) and was diluted in sterile saline (0.9%) to

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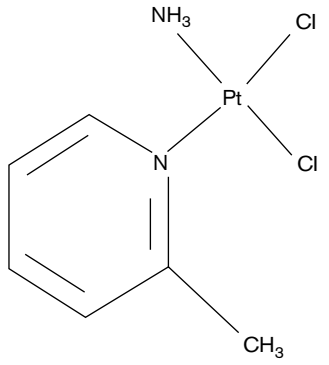
Fig. 1



Chemical structure of AMD473 (ZD0473).

create a stock concentration of 160 μg/ml and stored at 4°C. Serial dilutions from the stock concentration were prepared and frozen at –80°C. Normal sterile saline (0.9%) served as a negative (vehicle) control. Based on the previously reported *in vitro* IC_{50} of 8.1 μM [3], AMD473 (ZD0473) was tested at 1.0, 4.0 and 16.0 μg/ml. Tumor cells were exposed to AMD473 (ZD0473) for 2 or 24 h at 37°C. Comparator agents were used at concentrations based on the drug concentration achievable in patients, usually in the range of 0.1–1.0 × peak plasma levels [9,10]. Cisplatin was tested at 0.5 μg/ml, cyclophosphamide and etoposide were tested at 3.0 μg/ml, 5-flurouracil was tested at 6.0 μg/ml, and gemcitabine was tested at 20 μg/ml.

Human tumor cloning assay

Cell preparation

Methods for cell preparation were based on the human tumor cloning assay described by Hamburger and Salmon [8] and have been used previously in our laboratory [9–21]. Specimens were collected from patients undergoing tissue and/or fluid procurement procedures as part of their diagnostic work-up or as part of their routine therapeutic care. Specimens included malignant effusions, ascites and bone marrow aspirates containing tumor cells, as well as solid tumors. Solid tumors or malignant lymph nodes were placed in McCoy's 5A medium obtained from Mediatech-Cellgro (Herndon, VA) containing 10% heat-inactivated newborn calf serum and 1% penicillin/streptomycin. These solid tumors were mechanically disassociated with scissors, forced through number 100 stainless steel mesh, passed through 25-gauge needles and then washed with McCoy's 5A medium. Ascitic, pleural and pericardial fluids and bone marrow samples were obtained by the standard techniques used at each patient facility. The fluid or marrow specimens were placed in sterile vessels containing 10 U of preservative-free heparin (Sigma, St Louis, MO)/ml of fluid or marrow. After centrifugation at 150 *g* for 10 min, the cells were harvested and washed with McCoy's 5A medium containing 10% heat-inactivated fetal calf serum (HIFCS). The viability of cell suspensions was determined by Trypan blue exclusion cell counts.

In vitro *exposure of tumor cells to AMD473 (ZD0473)*

Tumor cells were exposed to 1.0, 4.0 and 16.0 μg/ml AMD473 (ZD0473) at 37°C for 2 or 24 h. Following exposure to AMD473 (ZD0473), tumor cells were washed with McCoy's 5A medium plus 10% HIFCS. Cells were centrifuged and resuspended in 0.3% agar in enriched CMRL 1066 medium, obtained from Gibco/BRL (Grand Island, NY), supplemented with 10% heat-inactivated horse serum, 7% HIFCS, 1% penicillin/streptomycin (5000 U/ml), 2% glutaMAX-1 (2 mM), insulin (2 U/ml) and 1.5% asparagine (6.6 mg/ml). AMD473 (ZD0473) was added to the cell suspension and plated in 35-mm Petri dishes as a top layer of agar over an underlayer of 0.3% agar, which supported tumor growth. Three plates were prepared for each data point and were incubated at 37°C for 14 days, at which point they were removed for a colony count; a colony was defined as a group of more than 50 cells. The number of colonies on the drug-treated plates was compared with the number of colonies formed on the untreated control plates and the percentage of colonies developing from cells treated with each concentration of AMD473 (ZD0473) was calculated. An evaluable test was defined as one averaging at least 20 colonies on the untreated control plates at day 14. For each tumor sample tested, three positive control plates were set up using 200 μg/ml of the non-specific cellular toxin, sodium orthovanadate, which inhibits colony growth [11]. For a sample test to be considered evaluable, sodium orthovanadate had to produce less than 30% survival of tumor colony-forming units when compared with untreated control plates. Methods for drug exposure, control parameters and assessment of results are based on the human tumor cloning assay described by Hamburger and Salmon [8] and have been used previously in our laboratory [9–21].

Statistical and data analysis

Data for each specimen were expressed as the percentage survival of tumor colony-forming units when treated with AMD473 (ZD0473), relative to the negative control (vehicle); this was calculated from the ratio of the average number of colonies formed after AMD473 (ZD0473) treatment to the average number of colonies formed in the negative control plates. A ratio of $\leq$50% denotes a positive response, suggesting significant inhibition of colony formation by the test drug, in this case, AMD473 (ZD0473) [12]. Pairwise comparisons of *in vitro* response rates were performed by McNemar's test for paired groups. A p value of < 0.05 was considered to indicate statistical significance.

Table 1 Tumor-specific concentration-dependent inhibition of colony formation by AMD473 (ZD0473)

	No. specimens inhibited[a]/no. specimens assessable (%)					
	2-h exposure			24-h exposure		
	1.0 μg/ml	4.0 μg/ml	16.0 μg/ml	1.0 μg/ml	4.0 μg/ml	16.0 μg/ml
Brain	–	–	–	1/1 (100)	1/1 (100)	1/1 (100)
Breast	3/10 (30)	4/10 (40)	4/10 (40)	4/11 (36)	7/11 (64)	8/11 (73)
Colon	1/6 (17)	1/6 (17)	2/5 (40)	1/5 (20)	3/5 (60)	2/3 (67)
Corpus uteri	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)
Head and neck	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)
Kidney	0/2 (0)	0/2 (0)	0/1 (0)	1/1 (100)	1/1 (100)	–
Melanoma	1/3 (33)	1/3 (33)	1/3 (33)	0/2 (0)	1/2 (50)	2/2 (100)
Neuroblastoma	0/0 (0)	0/0 (0)	0/0 (0)	1/2 (50)	2/2 (100)	2/2 (100)
Non-Hodgkin's lymphoma	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)
Non-small cell lung	2/11 (18)	4/11 (36)	3/7 (43)	2/8 (25)	4/8 (50)	5/5 (100)
Ovary	1/11 (9)	6/11 (55)	7/9 (78)	2/9 (22)	7/9 (78)	8/8 (100)
Peritoneum	0/1 (0)	0/1 (0)	1/1 (100)	1/2 (50)	1/2 (50)	2/2 (100)
Pleura (mesothelioma)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)
Prostate	–	–	–	1/1 (100)	1/1 (100)	1/1 (100)
Sarcoma	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)	1/1 (100)	0/1 (0)
Stomach	1/1 (100)	1/1 (100)	1/1 (100)	0/0 (0)	0/0 (0)	0/0 (0)
Unknown primary	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Total	9/51 (18)	17/51 (33)	19/43 (44)	16/48 (33)	30/48 (63)	35/41 (85)

[a]Inhibition: ≤50% survival.

Results

One hundred and twenty human tumor specimens taken directly from patients were treated with 1.0, 4.0 or 16.0 μg/ml AMD473 (ZD0473) for 2 or 24 h and the cytotoxicity of AMD473 (ZD0473) was measured using a soft agar-cloning assay. A range of 34% (41/120) to 43% (51/120) of specimens in the different treatment groups were evaluable, falling within the negative and positive control parameters defined in Materials and methods.

When cells were treated with 1.0, 4.0 or 16.0 μg/ml AMD473 (ZD0473) for 2 h, *in vitro* responses (defined as a ≤50% survival compared with untreated controls) [12] were observed in 18% (9/51), 33% (17/51) and 44% (19/43) of evaluable specimens, respectively. AMD473 (ZD0473) demonstrated activity in 33% (16/48), 63% (30/48) and 85% (35/41) of evaluable specimens treated with 1.0, 4.0 and 16.0 μg/ml AMD473 (ZD0473) for 24 h, respectively.

AMD473 (ZD0473) demonstrated inhibitory effects in multiple tumors, with notable activity seen in breast, non-small cell lung and ovarian cancer specimens. AMD473 (ZD0473) demonstrated activity towards 100% of the non-small cell lung (5/5) and ovarian (8/8) tumor specimens treated with 16.0 μg/ml for 24 h. Seventy-three percent (8/11) of the breast cancer specimens exhibited sensitivity to treatment with 16.0 μg/ml AMD473 (ZD0473) for 24 h (Table 1).

Response to AMD473 (ZD0473) was concentration dependent for both exposure periods (16.0 > 4.0 > 1.0 μg/ml; Table 1). Pairwise comparison of the *in vitro* response to different concentrations of AMD473 (ZD0473) was performed using McNemar's test for paired proportions and this indicated a significant dose–response relationship (Table 2). Exposure of samples to 1 μg/ml AMD473 (ZD0473) for 24 h resulted in greater response than exposure for 2 h ($p = 0.083$). Exposure of samples for 24 h resulted in significantly greater responses than for 2 h with both 4 μg/ml ($p = 0.003$) and 16 μg/ml ($p = 0.001$) AMD473 (ZD0473).

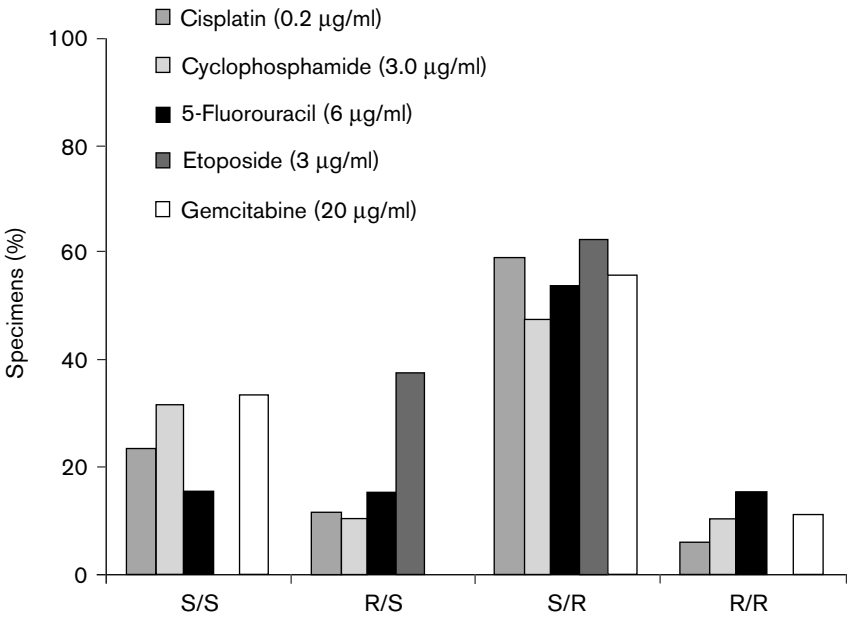
Table 2 Dose–response pairwise comparison by McNemar's test for paired proportions[a]

Comparison	Exposure		
	2 h		24 h
1.0 versus 4.0 μg/ml	$p=0.005$		$p=0.001$
4.0 versus 16.0 μg/ml	$p=0.317$		$p=0.013$
1.0 versus 16.0 μg/ml	$p=0.004$		$p=0.001$
1.0 μg/ml 2 versus 24 h		$p=0.083$	
4.0 μg/ml 2 versus 24 h		$p=0.003$	
16.0 μg/ml 2 versus 24 h		$p=0.001$	

[a]Statistical significance noted as $p<0.05$.

The activity of AMD473 (ZD0473) was compared with the activity of conventional agents that were used to treat the same patient specimens. Not all patient samples tested with AMD473 (ZD0473) were tested with each of the conventional agents. The selected concentrations of the conventional agents were based on the drug concentrations achievable in patients, usually in the range of 0.1–1.0 × peak plasma levels [9,10]. Specimens displaying ≤ 50% survival compared to negative controls were considered sensitive, while those displaying > 51% survival were classed as resistant to the drug being tested. A sizeable number of tumors that were resistant to

Fig. 2



Comparison of AMD473 (ZD0473) (at 16.0 μg/ml for 24 h) and conventional agents, in terms of sensitivity/resistance of tumor specimens. S/S = sensitive to AMD473 (ZD0473)/sensitive to conventional agent. R/S = resistant to AMD473 (ZD0473)/sensitive to conventional agent. S/R = sensitive to AMD473 (ZD0473)/resistant to conventional agent. R/R = resistant to AMD473 (ZD0473)/resistant to conventional agent.

certain conventional agents were sensitive to AMD473 (ZD0473) (at 16.0 μg/ml for 24 h) (Fig. 2): 59% of the specimens (10/17) were sensitive to AMD473 (ZD0473), but resistant to cisplatin; 47% of specimens (9/19) were sensitive to AMD473 (ZD0473), but resistant to cyclophosphamide; 54% of specimens (7/13) were sensitive to AMD473 (ZD0473), but resistant to 5-flurouracil; 63% of specimens (5/8) were sensitive to AMD473 (ZD0473), but resistant to etoposide; 56% of specimens (5/9) were sensitive to AMD473 (ZD0473), but resistant to gemcitabine.

Fewer tumor samples demonstrated resistance to AMD473 (ZD0473) (at 16.0 μg/ml for 24 h) and sensitivity to the conventional agents (Fig. 2): 12% of the specimens (2/17) were resistant to AMD473 (ZD0473), but sensitive to cisplatin; 11% of specimens (2/19) were resistant to AMD473 (ZD0473), but sensitive to cyclophosphamide; 15% of specimens (2/13) were resistant to AMD473 (ZD0473), but sensitive to 5-flurouracil; 38% of specimens (3/8) were resistant to AMD473 (ZD0473), but sensitive to etoposide. No tumor samples that displayed resistance to AMD473 (ZD0473) were sensitive to gemcitabine.

Discussion

AMD473 (ZD0473) was developed in an attempt to circumvent the mechanisms by which tumors are, or become, resistant to conventional platinum compounds such as cisplatin. The mechanisms that underlie tumor resistance to cisplatin include increased drug transport; greater cellular detoxification due to increased glutathione and metallothionein levels; changes in DNA repair; increased tolerance of DNA adducts; and alterations in apoptosis pathways [22]. AMD473 (ZD0473) was intended to have reduced susceptibility to inactivation by thiol-containing molecules, such as glutathione or the protein metallothionein, which covalently bind platinum-containing molecules, forming thiol–platinum adducts. To try and prevent inactivation by this mechanism, AMD473 (ZD0473) was designed with greater steric bulk at the platinum center, which resulted in a molecule that was less reactive with thiol compounds than cisplatin [23]. Tests of AMD473 (ZD0473) in a panel of cell lines with different cisplatin-resistance mechanisms demonstrated low levels of cross-resistance and, in particular, AMD473 (ZD0473) was significantly more effective than cisplatin ($p < 0.05$) in cells resistant to cisplatin due to enhanced glutathione levels [3].

In this study, we evaluated the effect of AMD473 (ZD0473) over two different exposure lengths (2 and 24 h) against a broad spectrum of primary human tumors taken directly from patients. Our data suggest that the duration of exposure significantly affects the toxicity of AMD473 (ZD0473) against human tumor colony-forming

units. When AMD473 (ZD0473) was evaluated at concentrations of 1.0, 4.0 or 16.0 μg/ml for 2 h, *in vitro* responses were observed in 18, 33 and 44% of all the specimens, respectively. When treated for 24 h with 1.0, 4.0 or 16.0 μg/ml AMD473 (ZD0473), activity was seen against 33, 63 and 85% of all the specimens tested, respectively.

The concentrations of AMD473 (ZD0473) used in this study were based on the drug concentrations achievable in patients, usually in the range of 0.1 to 1.0 $\times$ peak plasma levels [9,10]. Early clinical observations suggest that peak plasma concentrations are achievable in the ranges used here (Murakami *et al.*, personal communication). Activity was demonstrated at concentrations consistent with the previously reported *in vitro* IC_{50} [3] and although greatest activity (against 85% of specimens) was seen at the highest concentration tested for the longer exposure period, AMD473 (ZD0473) demonstrated significant activity (in 33% of specimens tested) when cells were treated with 4.0 μg/ml for 2 h (Table 2).

AMD473 (ZD0473) displayed activity towards a variety of primary human tumor samples, most notably in breast, non-small cell lung and ovarian tumor specimens. Comparison of the activity AMD473 (ZD0473) towards tumor cells with that of the conventional agents demonstrated relatively little cross-resistance. In particular, of the samples treated with cisplatin, 59% (10/17) were sensitive to AMD473 (ZD0473), but were resistant to cisplatin. If these *in vitro* observations translate into clinical efficacy, AMD473 (ZD0473) may be a good candidate for therapy in patients with a range of tumors that are cisplatin-resistant. AMD473 (ZD0473) has undergone phase II studies in a range of tumors [6,7,24,25], and preliminary results from phase II studies show AMD473 (ZD0473) is an active drug in a variety of tumors and has a manageable toxicity profile. Further work is ongoing to examine the efficacy and tolerability of AMD473 (ZD0473) in combination with other chemotherapeutic agents, including liposomal doxorubicin [26].

Conclusion

AMD473 (ZD0473) is clearly active *in vitro* against a variety of human tumors, including a subgroup of tumors resistant to conventional anticancer agents *in vitro*. AMD473 (ZD0473) demonstrated exposure time-dependent differences in activity as well as a clear trend in concentration-dependent activity. These data suggest that prolonged exposure may be important for maximum activity. *In vitro* responses demonstrate efficacy at physiologically relevant concentrations, which suggest that AMD473 (ZD0473) deserves further evaluation.

References

1 Kelland LR, Barnard CDJ. AMD473. *Drugs Future* 1998; **23**:1062–1065.
2 Raynaud FI, Boxall FE, Goddard PM, Valenti M, Jones M, Murrer BA, *et al.* *Cis*-amminedichloro(2-methylpyridine) platinum(II) (AMD473), a novel sterically hindered platinum complex: *in vivo* activity, toxicology, and pharmacokinetics in mice. *Clin Cancer Res* 1997; **3**:2063–2074.
3 Holford J, Sharp SY, Murrer BA, Abrams M, Kelland LR. *In vitro* circumvention of cisplatin resistance by the novel sterically hindered platinum complex AMD473. *Br J Cancer* 1998; **77**:366–373.
4 Kelland L, Judson I, Koehler M, Stevens T, Roberts D. Preclinical and clinical overview of the novel platinum complex, ZD0473 (*cis*-amminedichloro[2-methylpyridine] platinum [II]). *Lung Cancer* 2000; **29** (**suppl 1**):70 (abstr 227).
5 Stevenson JP, Blair IA, Redlinger M, Sun W, Oe T, Koehler M, *et al.* Pharmacokinetic (PK)/pharmacodynamic (PD) trial of the new generation platinum compound ZD0473 administered as an iv infusion every 21 days. *Eur J Cancer* 2001; **37** (**suppl 6**):72 (abstr 255).
6 Schiller J, Bonomi P, Modiano M, Cornett P, Koehler M. Activity of ZD0473 in small-cell lung cancer: an update in patients relapsing after one prior chemotherapy regimen. *Eur J Cancer* 2001; **37** (**suppl 6**):62 (abstr 219).
7 Gore M, Atkinson RJ, Dirix L, Rischin D, Beale P, Harnet P, *et al.* ZD0473 phase II monotherapy trial in second-line ovarian cancer. *Eur J Cancer* 2001; **37** (**suppl 6**):261 (abstr 967).
8 Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**:461–463.
9 Von Hoff DD, Casper J, Bradley E, Sandbach J, Jones D, Makuch R. Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. *Am J Med* 1981; **70**:1027–1041.
10 Scheithauer W, Clark GM, Salmon SE, Dorda W, Shoemaker RH, Von Hoff DD. Model for estimation of clinically achievable plasma concentrations for investigational anticancer drugs in man. *Cancer Treat Rep* 1986; **70**:1379–1382.
11 Hanauske U, Hanauske A-R, Marshall MH, Muggia VA, Von Hoff DD. Biphasic effects of vanadium salts on *in vitro* tumor colony growth. *Int J Cell Cloning* 1987; **5**:171–8.
12 Von Hoff DD, Clark GM, Stogdill BJ, Sarosdy MF, O'Brien MT, Casper JT, *et al.* Prospective clinical trial of a human tumor cloning system. *Cancer Res* 1983; **43**:1926–1931.
13 Shoemaker RH, Wolpert-Defillipes MK, Kern DH *et al.* Application of a human tumor colony-forming assay to new drug screening. *Cancer Res* 1985; **45**:2145–2153.
14 Browman GP, Levine MN, Robert RS. Editorial: bench to bedside research and the human tumor stem-cell assay—bridging the credibility gap. *J Clin Oncol* 1986; **4**:1730–1731.
15 Von Hoff DD. Human tumor stem cell assays. *Int J Cell Cloning* 1987; **5**:365–367.
16 Hanauske AR, Degen D, Hilsenbeck SG, Bissery MC, Von Hoff DD. Effects of taxotere and taxol on *in vitro* colony formation of freshly explanted human tumor cells. *Anticancer Drugs* 1992; **3**:121–124.
17 Eckardt JR, Burris III HA, Kuhn JG, Bissery MC, Klink-Alakl M, Clark GM, *et al.* Activity of intoplicine (RP60475), a new DNA topoisomerase I and II inhibitor, against human tumor colony-forming units *in vitro*. *J Natl Cancer Inst* 1994; **86**:30–33.
18 Raymond E, Hanauske A, Faivre S, Izbicka E, Clark G, Rowinsky EK, *et al.* Effects of prolonged versus short-term exposure paclitaxel (Taxol®) on human tumor colony-forming units. *Anticancer Drugs* 1997; **8**:379–385.
19 Hidalgo M, Izbicka E, Cerna C, Gomez L, Rowinsky EK, Weitman SK *et al.* Comparative activity of the cyclopropylpyrroloindole compounds adozelesin, bizelesin and carzelesin in a human tumor colony-forming assay. *Anticancer Drugs* 1999; **10**:295–302.
20 Diab SG, Hilsenbeck SG, Izbicka E, Weitman SD, Von Hoff DD. Significant activity of a novel cytotoxic agent, Ly295501, against a wide range of tumors in the human tumor cloning system. *Anticancer Drugs* 1999; **10**:303–307.
21 Petit T, Izbicka E, Lawrence RA, Bishop WR, Weitman SD, Von Hoff DD. Activity of SCH 66336, a tricyclic farnesyltransferase inhibitor, against human tumor colony-forming units. *Ann Oncol* 1999; **10**:449–453.
22 Johnson SW, Ferry KV, Hamilton TC. Recent insights into platinum drug resistance in cancer. *Drug Resist Update* 1998; **1**:243–254.
23 Holford J, Raynaud F, Murrer BA, Grimaldi K, Hartley JA, Abrams M, *et al.* Chemical, biochemical and pharmacological activity of the novel sterically hindered platinum co-ordination complex, *cis*-[amminedicholor(2-methylpyridine)] platinum(II) (AMD473). *Anticancer Drug Des* 1998; **13**: 1–18.

24 Treat J, Huang C, Damanjov N, Jahanzeb M, Edelman M, Koehler M. ZD0473 phase II monotherapy trial in second-line non-small cell lung cancer. *Eur J Cancer* 2001; **37** (**suppl 6**):59 (abstr 209).
25 Giaccone G, O'Brien M, Byrne M, Vansteenkiste J, Cosaert J. Current phase II data for ZD0473 in patients with mesothelioma who had relapsed following one prior chemotherapy regimen. *Eur J Cancer* 2001; **37** (**suppl 6**):45 (abstr 152).
26 Dizon DS, Maluf F, Aghajanian C, Daud A, Sabbatini P, Soignet S, *et al.* Phase I study of ZD0473 and liposomal doxorubicin in advanced refractory solid tumor malignancies. *Eur J Cancer* 2001; **37** (**suppl 6**):77 (abstr 273).