Cancer Chemother Pharmacol (2003) 51: 459–464
DOI 10.1007/s00280-003-0605-0

ORIGINAL ARTICLE

**Robert J. Morgan Jr · Edward M. Newman
Lawrence Sowers · Kevin Scanlon · Jonathan Harrison
Steven Akman · Lucille Leong · Kim Margolin
Joyce Niland · James Raschko · George Somlo
Mary Carroll · Warren Chow · Merry Tetef
Victor Hamasaki · Yun Yen · James H. Doroshow**

Phase I study of cisdiamminedichloroplatinum in combination with azidothymidine in the treatment of patients with advanced malignancies

Received: 24 September 2002 / Accepted: 10 February 2003 / Published online: 15 April 2003

Abstract *Purpose*: Azidothymidine (AZT, zidovudine) has been shown to reverse cisplatin resistance in cell culture. This phase I study was performed to determine the maximally tolerated dose (MTD) and dose-limiting toxicities of AZT when administered by continuous intravenous infusion in combination with cisplatin (CDDP), and to evaluate the pharmacokinetics of AZT in this setting. *Patients and methods*: Entered in the study were 61 patients with advanced, histologically confirmed malignancies which were unresponsive to or for which no "standard" chemotherapeutic regimen existed. AZT was administered as a 72-h infusion on days 1–3 and 14–16 of a 28-day cycle at dose levels from 400 through 14,364 mg/m^2 per day. CDDP at dose levels of 30, 45, or 60 mg/m^2 was administered at hour 36 of each AZT infusion. The plasma pharmacokinetics of AZT were determined in patients treated at representative dose levels. *Results*: Of the 61 patients who completed 125 courses of therapy, 21 had stable disease for a median of four cycles (range two to eight), 33 progressed on therapy, and 7 were not assessable for response. The major observed toxicity was myelosuppression. The MTD of AZT was 8135 mg/m^2 per day when administered on this schedule. Escalation of CDDP did not result in additive toxicity. The mean steady-state level of AZT at the MTD was 44 μ*M* (range 35–51 μ*M*). *Conclusions*: Steady-state concentrations of AZT increased with dose. The plasma levels achieved at the MTD exceeded those required for drug resistance reversal in vitro. The administration of CDDP had no effect on AZT steady-state levels. The dose-limiting toxicity of this drug combination is myelosuppression. AZT may be useful in further studies utilizing combination therapy to achieve increased chemotherapy effectiveness.

Keywords AZT · Cisplatin · Azidothymidine · Phase I trial

This work was presented in part at the Annual Meeting of the American Society of Clinical Oncology and reported in Proc ASCO 1994;1:146. The work was supported by NCI Cancer Center Core Grant CA 33572, and Burroughs Wellcome.

R. J. Morgan Jr (✉) · K. Scanlon · J. Harrison · S. Akman
L. Leong · K. Margolin · J. Raschko · G. Somlo · M. Carroll
W. Chow · M. Tetef · V. Hamasaki · Y. Yen · J. H. Doroshow
Department of Medical Oncology and Therapeutics Research,
City of Hope National Medical Center, 1500 E. Duarte Rd,
Duarte, CA 91010, USA
E-mail: rmorgan@coh.org
Tel.: +1-626-3598111
Fax: +1-626-3018233

E. M. Newman · L. Sowers
Division of Molecular Medicine,
City of Hope National Medical Center,
1500 E. Duarte Rd, Duarte, CA 91010, USA

J. Niland
Department of Biostatistics,
City of Hope National Medical Center,
1500 E. Duarte Rd, Duarte, CA 91010, USA

Introduction

Intrinsic and acquired cellular resistance to platinum compounds represents a significant impediment to the effective treatment of many malignancies. Augmented DNA repair pathways contribute to the development of cisplatin (CDDP) resistance. Strategies designed to overcome CDDP resistance include the coadministration of agents which interfere with DNA repair [14]. Scanlon et al. [15] have described the ability of azidothymidine (AZT, zidovudine) to reverse cellular resistance to CDDP in resistant cell lines in vitro. This process may be due to the incorporation of AZT into

DNA at sites of platinum-induced damage, acting to terminate DNA chain elongation, and ultimately to initiate cell death.

Clark et al. [5] have demonstrated the feasibility and potential in vivo effectiveness of AZT in modulating cellular resistance to chemotherapeutic agents by administration in combination with 5-fluorouracil and leucovorin. A 33% response rate was obtained in patients with metastatic colorectal cancer treated with AZT at 7 g/m^2, the dose derived from their previous phase I trial [1]. Toxicity in this trial was primarily limited to nausea and vomiting, with hypotension controlled by normal saline infusions; both toxicities were infrequent. The results of these preclinical and clinical studies encouraged us to perform a phase I trial of infusional AZT in combination with CDDP to determine the maximally tolerated dose (MTD) of AZT and to examine its pharmacokinetics when administered in combination with CDDP. The therapeutic regimen was chosen to take advantage of the previously described synergy between the two agents, which is independent of the sequence of administration. We report here the results of this trial.

Patients and methods

Patient selection

Entered into this phase I study were 61 patients. Eligibility requirements included: advanced inoperable malignancy which was histologically confirmed and unresponsive to previous chemotherapy regimens, or for which no "standard" chemotherapeutic regimen existed. Previous treatment with platinum compounds did not disqualify patients from treatment on this protocol if they had recovered from toxicity attributable to previous therapy. The presence of CNS metastases, either previously treated or asymptomatic, also did not exclude a patient from entry. Patients were required to be at least 18 years of age. Pregnant women were ineligible. Patients with any nonmalignant intercurrent illnesses (e.g., cardiovascular, pulmonary, or neurologic) which was either poorly controlled with currently available treatment or of such severity that the investigators deemed it unwise to enter the patient into this study were ineligible. Patients currently being treated for severe infection, or who were recovering from major surgery, were ineligible until recovery was complete. Required pretreatment tests were obtained within 2 weeks prior to the first course of chemotherapy, except for complete blood count and routine serum chemistries (SMA-7 and SMA-12) which were obtained within 2 days of initiating each administration of AZT.

Patients being treated with a calcium channel blocking agent were ineligible unless the medication could safely be withheld for 72 h prior to and during treatments due to the potential occurrence of hypotension associated with the administration of AZT in combination chemotherapy. Adequate renal and bone marrow function were defined as creatinine $\leq$ 1.6 mg/dl or measured creatinine clearance $\geq$60 ml/min, platelet count $\geq$150,000/μl, and absolute neutrophil count $\geq$3000/μl. In addition, bilirubin was required to be $\leq$ 1.5 mg/dl and hepatic transaminases were required to be less than three times the upper limit of normal. Patients had to have recovered from the toxicity of any previous chemotherapy or radiotherapy completed at least 4 weeks prior to enrollment in this study. All patients had a Karnofsky performance status of at least 60%, an estimated survival of at least 8 weeks, gave their voluntary informed consent, and signed an informed consent document approved by the Institutional Review Board of the City of Hope National Medical Center.

Pretreatment evaluation

Pretreatment evaluation included a complete history and physical examination, complete blood count with differential, chemistry panel including liver function tests and serum creatinine, urinalysis, 24-h urine creatinine clearance, electrocardiogram, chest radiography, serum magnesium, and radiographic examinations for tumor measurements in patients with measurable disease. Serum chemistries and blood counts were repeated weekly. Patients with bidimensionally measurable disease were required to have baseline evaluations within 2 weeks prior to the first course of treatment. Repeat radiographic evaluations were performed after every two courses (2 months) of therapy.

Treatment plan

Patients received AZT as a continuous intravenous infusion for 72 h at escalating doses according to a modified Fibonacci scheme from 400 to 14,364 mg/m^2 per day. At hour 36, the patients received CDDP at a fixed dose of 30 mg/m^2. One course was defined as two infusions administered beginning at hour 0 of day 1 and repeated at hour 0 of day 14. After determination of the MTD of AZT, the CDDP was then escalated to 45 and 60 mg/m^2 for the final two dose levels. The study was closed following these dose levels as the pharmacologic objectives of the study were achieved, and the protocol specified the maximum cisplatin dose to be 60 mg/m^2.

Intrapatient dosage escalations were not allowed during subsequent cycles at any dose level. However, dosage reductions of one dose level of AZT were allowed in patients experiencing grade 3 toxicity if resolution of that toxicity occurred within 21 days from the beginning of the last AZT infusion. If patients experienced grade 3 or higher toxicity persisting on or through the 22nd day from the start of the most recent AZT infusion, they were removed from treatment on this study.

Statistical considerations

This study was a phase I trial designed to establish the MTD and to describe the dose-limiting toxicities of the combination of CDDP and AZT. A minimum of three patients were entered at each dose level. If, after one complete course with a 14-day follow-up, no grade 3 or grade 4 toxicities were observed in any of the patients, drug doses were escalated by one level. Dose escalations continued until grade 3 or grade 4 toxicity was observed. A single instance of grade 4 toxicity would stop dose escalations, and patients would be accrued at one dose level lower. If a single patient at a dose level experienced a grade 3 toxicity, three additional patients were treated at the same dose level. If no further grade 3 or 4 toxicity was observed in the additional patients, drug doses continued to be escalated to the next level. If two or more of the six patients experienced a grade 3 or higher toxicity, the next lower dose level was expanded to six patients, if necessary. The study was closed and the MTD defined after six patients were accrued at the highest dose level at which no grade 4, and at most one grade 3 toxicity were observed. The MTD was first established for AZT, with the CDDP dose held at 30 mg/m^2. For the final two dose levels, the AZT dose was fixed and the CDDP dose was escalated.

AZT pharmacokinetics

Samples were analyzed for steady-state AZT concentrations from patients at representative dose levels. Blood samples (10 ml) were

obtained from a free-flowing heparin lock from the arm contralateral to the AZT infusion prior to treatment and at hours 30, 30.5, 38, and 38.5 of the AZT infusion (two samples prior to CDDP administration, and two samples after CDDP administration).

AZT concentrations in plasma were determined by reverse-phase HPLC with UV detection according to the method of Good et al. [8]. After the addition of the 3′-β-azido isomer of AZT (β-AZT) as an internal standard, samples were extracted onto C_{18} solid-phase extraction columns, eluted with methanol, evaporated to dryness, and redissolved in aqueous acetonitrile. The extracts were analyzed using isocratic elution (aqueous acetonitrile, buffered to pH 2.7 with ammonium phosphate) from a C_{18} analytical column with integration of the β-AZT and AZT peaks at 267 nm. Because of the characteristics of the analytical column used (Brownlee Spheri 5 RP-18), we decreased the concentration of acetonitrile in the mobile phase to 10% from the 15% used previously [8], with an Alltech Adsorbosphere column.

Table 1 Patient characteristics (values are number of patients, except age in years)

Gender	
Male	33
Female	28
Race	
Caucasian	45
Hispanic	7
Asian	4
African-American	2
Karnofsky performance status (%)	
90–100	29
70–80	31
60	1
Tumor types	
Lung	13
Gastrointestinal orhepatobiliary	21
Adenocarcinoma of unknown primary	5
Gynecologic	4
Breast	6
Head/neck	7
Kidney/adrenal	2
Soft tissue sarcoma	3
Age (years)	
Median	56
Range	28–78
Prior therapy	
Surgery	23
Radiation therapy	1
Chemotherapy	47
Platinum	27

Results

Patient characteristics

The 61 patients entered (33 male, 28 female) received 125 complete courses of chemotherapy (median 2, range 1–9) (Table 1). Their median age was 56 years (range 28–78 years). Their median Karnofsky performance status was 80% (range 60–100%), and the tumor types included: lung cancer (13), adenocarcinoma of unknown primary (5), gastrointestinal or hepatobiliary tract cancer (21), gynecologic cancer (4), breast cancer (6), head and neck cancer (7), kidney/adrenal cancer (2) and soft tissue sarcomas (3). All patients had received prior treatment which included various combinations of surgery, hormonal therapy, radiation, and/or chemotherapy (median number of prior chemotherapy regimens two, range none to five); 27 patients had received prior cisplatin (15) or carboplatin (12); 14 had received no prior chemotherapy; and 38 had received prior radiotherapy.

Toxicities of therapy

Standard Southwest Oncology Group response criteria [9] were used in patients with measurable disease. Toxicity was measured using the Southwest Oncology Group Toxicity Criteria. The dose levels, numbers of patients enrolled at each dose level, and toxicities of therapy are summarized in Tables 2 and 3. The toxicities were generally mild. Grade 3 granulocytopenia was noted in 10 of 125 complete courses of therapy and was the dose-limiting toxicity with two episodes during cycle 1 noted at dose level 11, and an expanded dose level 10. No fever or sequelae were noted in the patients

Table 2 Doses, numbers of patients enrolled/evaluable at each dose level, and dose-limiting toxicities

Dose level	Dose		No. of patients/ no. evaluable	No. of dose-limiting toxicities
	AZT (mg/m^2/day)	CDDP (mg/m^2)		
1	400	30	4/4	
2	800	30	4/3	
3	1,300	30	3/3	
4	2,000	30	7/6	1 (bone pain)
5	2,600	30	5/4	
6	3,458	30	3/3	
7	4,599	30	3/3	
8	6,166	30	8/6	1 (hemoglobin)
9	8,135	30	4/3	
10	10,820	30	5/5	2 (absolute granulocyte count)
11	14,364	30	4/4	2 (absolute granulocyte count)
12	8,135	45	4/3	
13	8,135	60	7/6	

Table 3 Grade 3 toxicities encountered (no.of episodes) in 125 complete courses

Toxicity	No. of episodes	Dose levels
Hematologic		
Granulocyte nadir 500–999/μl (grade 3)	10	8, 10, 11, 13, 5, 9
Hemoglobin 6.5–7.9 g/dl(grade 3)	3	9, 10
Miscellaneous		
Constipation	2	13
Alkaline phosphatase or bilirubin elevation	3	2, 10, 9
Fatigue	1	13
Nausea/emesis	4	8, 10, 13
Dermatitis	1	11
Pain	2	4

Table 4 Best response to protocol therapy

Response	No. of patients
Stable disease	21
Progressed	33
Inevaluable	7

suffering grade 3 toxicity and all showed granulocyte recovery within 1 week. No thrombocytopenia was noted at any dose level. One dose-limiting (first course occurrence) grade 3 bone pain was noted at dose level 4, and one patient on dose level 8 experienced grade 3 anemia (hemoglobin 6.5–7.9 g/dl) which necessitated the expansion of these dose levels. Other non-dose-limiting grade 3 toxicities included: anemia in two patients, nausea/emesis in four patients, transiently elevated bilirubin or alkaline phosphatase in three patients, fatigue in one patient, dermatitis in one patient, musculoskeletal pain in one patient, and constipation in two patients. No hypotension was noted.

Therapeutic responses

Therapeutic responses are summarized in Table 4. Of the 61 patients, 21 were observed to have stable disease for a median of four cycles (range two to eight), 33 progressed on therapy, and 7 were inevaluable for response. The inevaluable patients included two patients with sarcoma, one of whose chemotherapy was excessively delayed due to a bleeding episode unrelated to chemotherapy, and one with rapid tumor progression who died before the first cycle was complete. Two patients did not receive a complete cycle due to hematemesis unrelated to protocol therapy, and three patients, one each with breast cancer, pancreatic cancer, and adenocarcinoma of unknown primary, did not complete a full cycle of chemotherapy due to tumor progression immediately following the first week of chemotherapy.

Of the 14 previously untreated patients, 6 had stable disease for a median of four cycles (range two to six). Of 27 patients with previous exposure to a platinum-containing agent, 13 had stable disease for a median of four cycles (range two to eight).

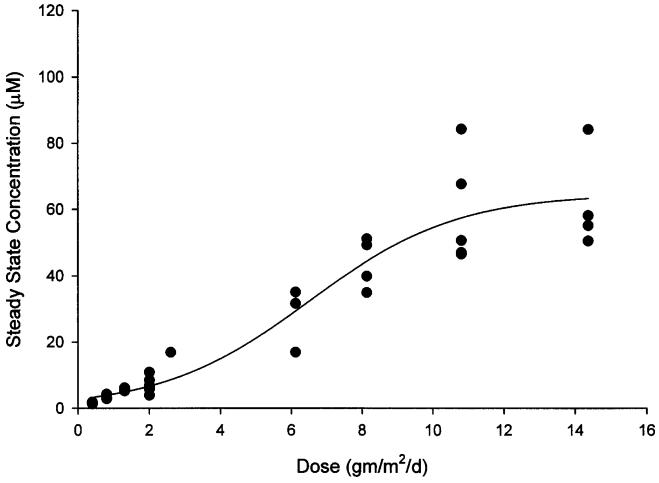

Fig. 1 Steady-state AZT concentrations increased monotonically with dose

AZT pharmacokinetics

The steady-state plasma concentrations of AZT increased with dose (Fig. 1) and were measured at 44 μ*M* (range 35–51 μ*M*) at the MTD. There was no significant difference in AZT levels before and after CDDP administration, suggesting that there was no effect of CDDP on AZT pharmacokinetics (Table 5). There was a statistically non-significant trend toward greater myelosuppression at higher doses and steady-state concentrations of AZT (Fig. 2).

Discussion

Resistance to platinum analogues remains a major limitation to curative chemotherapy in patients with advanced cancer. Preclinical investigations have demonstrated the ability of AZT, the pyrimidine nucleotide analogue 3′-azido-2′,3′-dideoxythymidine (azidothymidine), to reverse CDDP drug resistance. This has been found to be partially mediated by effects on DNA repair mechanisms and on oncogene expression in vitro [10].

Table 5 AZT concentrations before and afterCDDP in relation to AZT dose. Values are means (range)

AZT dose (g/m^2/day)	AZT concentration (μM)	
	Before CDDP	After CDDP
0.4	1.54 (1.25–175)	1.75 (1.31–2.11)
0.8	3.39 (2.65–4.49)	3.13 (1.38–5.17)
1.3	5.91 (5.47–7.02)	5.45 (4.67–5.96)
2.0	7.04 (3.98–10.7)	7.12 (4.80–11.69)
2.6	9.44 (9.40–9.48)	9.24 (9.16–9.72)
6.116	28.88 (18.07–40.10)	26.86 (15.83–37.01)
8.135	41.46 (29.28–54.69)	46.14 (37.97–53.62)
10.8	62.58 (43.45–87.82)	55.93 (38.83–83.06)
14.364	61.36 (36.70–80.25)	62.68 (43.05–89.45)

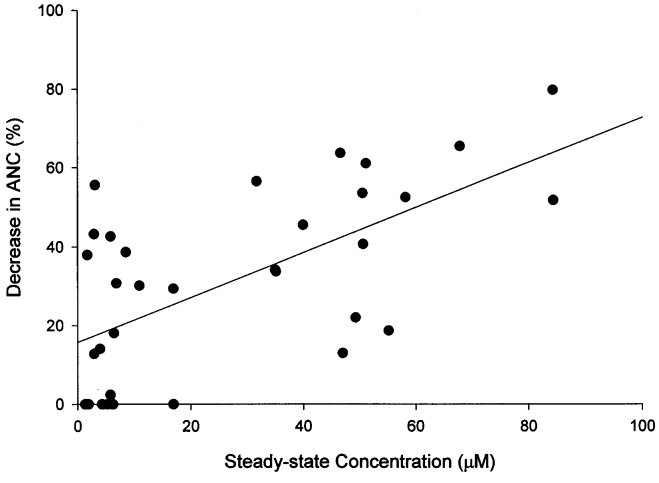

Fig. 2 A trend for an increased percentage change in absolute neutrophil count is seen with increasing steady-state concentrations of AZT

CDDP exerts its antineoplastic effects, at least in part, through the formation of DNA adducts, resulting in both intra- and interstrand DNA breaks [7]. Scanlon et al. [13, 14, 15] have demonstrated increased activity of DNA repair mechanisms in human ovarian and colon cell lines resistant to CDDP, including increased mRNA expression of DNA precursor synthesis enzymes (thymidylate synthase and thymidine kinase), and of DNA repair enzymes (DNA polymerases α and β). Increased steady-state levels of these mRNA species have also been demonstrated in tumor cells from patients resistant to CDDP [14]. The mechanism of CDDP resistance in these in vitro systems therefore appears to be through increased repair of the interstrand and intrastrand DNA breaks caused by the CDDP. Exposure to AZT has been postulated to restore CDDP sensitivity to the resistant tumor cells [13, 14] by incorporation of the AZT into the tumor cell DNA at the sites where platinum-induced damage has occurred. The AZT nucleotide subsequently results in chain termination of the DNA [13, 15], failure of DNA repair, and resultant cell death. In vitro, this effect has been achieved with AZT levels of 10 μ*M* [15].

The ability of AZT to potentiate the activity of chemotherapeutic agents has been investigated in phase I and II trials utilizing 5-fluorouracil and leucovorin, and methotrexate [3, 4]. We have shown that chemomodulating doses of AZT are achievable over a 72-h period with minimal toxicity when administered in combination with CDDP. Disease stabilization in patients previously refractory to platinum-containing agents suggests that this approach may have clinical utility.

The usual dose-limiting toxicity of single-agent AZT is myelosuppression [16]. CDDP usually exhibits nephrotoxicity and neurotoxicity as the dose-limiting toxicities [12]. In previous phase I and II clinical trials of AZT, symptomatic hypotension has been found to be a dose-limiting toxicity. We observed myelosuppression to be the dose-limiting toxicity in our trial, and did not note significant renal or neurotoxicity, or any evidence of hypotension. Phase I studies of AZT administered by continuous infusion in combination with 5-fluorouracil and leucovorin have achieved AZT doses of up to 12 g/m^2 per day, with no apparent potentiation of toxicity [6, 11].

For chemomodulation to be a clinically useful therapeutic strategy, it must be possible to deliver effective concentrations of a drug resistance-modifying agent at an acceptable level of toxicity. Browne et al. have reported linear pharmacokinetics with a peak plasma concentration of 601 μ*M* when AZT was administered at 1.5–7 g/m^2 over 2 h in combination with methotrexate [2]. We report that the steady-state plasma levels of AZT in all patients treated with a dose of 8135 mg/m^2 per day or above was >35 μ*M*, which exceeds concentrations required for modulation of platinum resistance in vitro.

In summary, in vivo AZT levels consistent with those required to achieve partial in vitro reversal of CDDP drug resistance are achievable with minimal toxicity when administered in combination with CDDP. AZT may be useful in further studies utilizing combination therapy to achieve increased chemotherapy effectiveness.

References

1. Beitz JG, Damowski JW, Cummings FJ, Browne MJ, Clark JW, Bigley JW, Weitberg AB (1995) Phase I trial of high-dose infused zidovudine combined with leucovorin plus fluorouracil. Cancer Invest 13:464–469
2. Browne MJ, Beitz J, Clark JW, Cummings FJ, Weitberg A, Murray C, Darnowski JW (1993) A phase I study of zidovudine (AZT) combined with methotrexate in patients with advanced cancer (meeting abstract). Proc ASCO 12:A451
3. Brunetti I, Darnowski JW, Falcone A, Johnson KA, Calabresi P (1989) Azidothymidine enhances fluorouracil and methotrexate antitumor and therapeutic activity. Proc Am Assoc Cancer Res 30:A2369
4. Brunetti I, Falcone A, Calabresi P, Goulette FA, Darnowski JW (1990) 5-Fluorouracil enhances azidothymidine cytotoxicity: in vitro, in vivo, and biochemical studies. Cancer Res 50:4026–4031

5. Clark J, Sikov W, Cummings F, Browne M, Akerley W, Wanebo H Weitberg A, Kennedy T, Cole B, Bigley J, Beitz J, Darnowski J (1996) Phase II study of 5-fluorouracil leucovorin and azidothymidine in patients with metastatic colorectal cancer. J Cancer Res Clin Oncol 122:554–558
6. DeLap R, Swain S, Ong D, Rosen N, Bodurian E, Steakley C, Nazzaro D, King D, Santore G (1991) A phase I study of zidovudine (azt), leucovorin (lv), and fluorouracil (fu) in patients with advanced cancer. Proc ASCO 10:A295
7. Eastman A (1985) Interstrand cross-links and sequence specificity in the reaction of cis-dichloro(ethylenediamine)platinum(II) with DNA. Biochemistry 24:5027–5032
8. Good SS, Reynolds DJ, de Miranda P (1988) Simultaneous quantification of zidovudine and its glucuronide in serum by high-performance liquid chromatography. J Chromatogr 431:123–133
9. Green S, Weiss GR (1992) Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. Invest New Drugs 10:239–253
10. Paietta E, Racevskis J, Heavey C, Lichtenstein A, Thomas D, Wiernik PH (1993) Modulation of multidrug resistance in de novo adult acute myeloid leukemia: variable efficacy of reverting agents in vitro. Proc ASCO 12:302
11. Posner MR, Darnowski JW, Weitberg AB, Dudley MN, Corvese D, Cummings FJ, Clark J, Murray C, Clendennin N, Bigley J, Calabresi P (1992) High-dose intravenous zidovudine with 5-fluorouracil and leucovorin. A phase I trial. Cancer 70:2929–2934
12. Rosenberg B (1985) Fundamental studies with cisplatin. Cancer 55:2303–2316
13. Scanlon KJ, Kashani-Sabet M, Miyachi H, Sowers LC, Rossi J (1989) Molecular basis of cisplatin resistance in human carcinomas: model systems and patients. Anticancer Res 9:1301–1312
14. Scanlon KJ, Kashani-Sabet M, Sowers LC (1989) Overexpression of DNA replication and repair enzymes in cisplatin-resistant human colon carcinoma HCT8 cells and circumvention by azidothymidine. Cancer Commun 1:269–275
15. Scanlon KJ, Funato T, Pezeshki B, Tone T, Sowers LC (1990) Potentiation of azidothymidine cytotoxicity in cisplatin-resistant human ovarian carcinoma cells. Cancer Commun 2:339–343
16. Yarchoan R, Mitsuya H, Myers CE, Broder S (1989) Clinical pharmacology of 3′-azido-2′,3′-dideoxythymidine (zidovudine) and related dideoxynucleosides. N Engl J Med 321:726–738