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In vitro chemosensitivity and radiosensitivity of an adriamycin-resistant subline of human small cell lung cancer cells.*

Hiroaki Miyamoto

Abstract

Using a cell line (SBC-3/ADM) of human small cell lung cancer, which is 30-fold more resistant to adriamycin than the parent cell line (SBC-3), the activity of a variety of anticancer agents was analyzed by soft agar clonogenic assay to search for a means of circumventing drug resistance. The SBC-3/ADM cells were markedly resistant to some anthracycline antibiotics in comparison with the SBC-3 cells: 28-fold for daunomycin, 26-fold for 4'-epiadriamycin, 18-fold for THP-adriamycin, and 8.4-fold for aclarubicin. However, the cells were as sensitive to mitoxantrone, one of the anthraquinone derivatives, as the parent cells. The cells were resistant to structurally or pharmacodynamically unrelated compounds such as vincristine, mitomycin C, and an active form of ifosfamide, whereas they were susceptible to cisplatin to some extent. The in vitro radiosensitivity of both cell lines was also evaluated, and they were found to be equally sensitive to X-ray. These results suggest that mitoxantrone and cisplatin may exert sufficient activity for small cell lung cancer which has acquired resistance to adriamycin, and that consolidative chest irradiation may be clinically useful after combination chemotherapy including adriamycin.

KEYWORDS: human small cell lung cancer cells, adriamycin-resistant subline, in vitro chemosensitivity, in vitro radiosensitivity

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***In Vitro* Chemosensitivity and Radiosensitivity of an Adriamycin-resistant Subline of Human Small Cell Lung Cancer Cells**

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Using a cell line (SBC-3/ADM) of human small cell lung cancer, which is 30-fold more resistant to adriamycin than the parent cell line (SBC-3), the activity of a variety of anticancer agents was analyzed by soft agar clonogenic assay to search for a means of circumventing drug resistance. The SBC-3/ADM cells were markedly resistant to some anthracycline antibiotics in comparison with the SBC-3 cells: 28-fold for daunomycin, 26-fold for 4'-epiadriamycin, 18-fold for THP-adriamycin, and 8.4-fold for aclarubicin. However, the cells were as sensitive to mitoxantrone, one of the anthraquinone derivatives, as the parent cells. The cells were resistant to structurally or pharmacodynamically unrelated compounds such as vincristine, mitomycin C, and an active form of ifosfamide, whereas they were susceptible to cisplatin to some extent. The *in vitro* radiosensitivity of both cell lines was also evaluated, and they were found to be equally sensitive to X-ray. These results suggest that mitoxantrone and cisplatin may exert sufficient activity for small cell lung cancer which has acquired resistance to adriamycin, and that consolidative chest irradiation may be clinically useful after combination chemotherapy including adriamycin.

Key words : human small cell lung cancer cells, adriamycin-resistant subline, *in vitro* chemosensitivity, *in vitro* radiosensitivity

Small cell lung cancer (SCLC) is highly sensitive to chemotherapeutic agents (1) and radiation therapy (2). Combination chemotherapy alone or combined chemotherapy and chest irradiation have produced significant palliation in nearly all patients with this neoplasm (3, 4). Many studies also show a small number of long-term survivors, suggesting the potential for cure (5). However, chemotherapy often fails after initial success and apparent remission despite continuing treatment. Furthermore, once the tumors become unresponsive to the first-line chemotherapy, they exhibit a wide range of resistance to drugs which are structurally dissimilar and have distinct cytotoxic targets.

Although the basis of this phenomenon remains obscure, one possible explanation is the selection and proliferation of multidrug resistant subpopulations of the tumor cells.

We previously established and characterized an adriamycin (ADM)-resistant subline of SCLC cells (SBC-3/ADM), as a tool to study the mechanism of drug resistance and to explore novel treatment strategies to combat the resistant cells (6). The purpose of this study was to elucidate cross-resistance patterns between ADM and a variety of chemotherapeutic agents using the SBC-3/ADM cells. The results could serve as a guide for drug selection where treatment has failed on account of the overgrowth

of drug-resistant cells in initially drug-sensitive tumors. Cross-resistance between ADM and X-ray irradiation was also reported.

Materials and Methods

Chemical agents. ADM and mitomycin C (MMC) were obtained from Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan; daunomycin (DM) and THP-adriamycin (THP), from Meiji Seika Co, Ltd, Tokyo, Japan; 4'-epiadriamycin (EPI), from Farmitalia Carlo Erba Co, Ltd, Tokyo, Japan; aclarubicin (ACR), from Yamanouchi Pharmaceutical Co, Ltd, Tokyo, Japan; mitoxantrone (MIT), from Lederle (Japan), Ltd, Tokyo, Japan; vincristine (VCR) and an active form of ifosfamide (40497S), from Shionogi Co, Ltd, Osaka, Japan; and cisplatin (CDDP), from Nippon Kayaku Co, Ltd, Tokyo, Japan. All agents were dissolved in 0.9% NaCl solution just before use.

Cell culture. The SBC-3 cells, established in our laboratory, were used as parent cell lines. The SBC-3/ADM cells were derived from the SBC-3 cells *in vitro* by continuous exposure to increasing concentrations of ADM and subsequent cloning in soft agar as described previously (6). Both cell lines were maintained in tissue culture flasks (Falcon 3013) containing RPMI 1640 medium (Grand Island Biological Co, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) at 37°C in an atmosphere with 5% CO₂ and subcultured 3 or 4 days.

Assay of drug sensitivity. The *in vitro* activity of a variety of anticancer drugs against the SBC-3 and SBC-3/ADM cells was determined by soft agar clonogenic assay. Briefly, single cell suspensions derived from both cell lines were exposed to the drug at graded concentrations for 1 h, and then plated in a double-layer soft agar system as mentioned previously (6). The plates were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. Colonies were counted 14 days after plating under an inverted microscope. Dose response curves of the two cell lines were obtained by calculating the percentage of surviving fractions at each drug concentration as compared to the number of colonies in control dishes. All experiments were carried out in triplicate and repeated

at least three times.

Measurement of radiosensitivity. The radiosensitivity of the parent and resistant cells was also examined by soft agar clonogenic assay. Singly dispersed cells prepared from both cell lines in culture tubes were irradiated with a Toshiba KXC-18 X-ray machine with graded doses, at a dose rate of 120 R/min, of X-rays delivered at 180 kVp and 25 mA. Medium was decanted after centrifugation, and the cells were plated in a double-layer soft agar system as mentioned above. Survival of the treated cultures was expressed as a fraction of the control cultures, which were given the value of 1.0. The means and standard deviations of the colony counts of triplicate cultures were calculated and plotted on a log scale against increasing concentrations of X-rays.

Results

Response to anticancer agents. Dose response curves of the SBC-3 and SBC-3/ADM cells to anthracycline-anthraquinone drugs are illustrated in Figs. 1 and 2. The degree of resistance (DR) was obtained from the ratio of the 70% lethal dose (LD₇₀) value of each agent against one cell line to that against the other. For instance, degree of resistance of the SBC-3/ADM cells to DM was calculated to be 28 from the LD₇₀ values of DM, 7.2×10^{-8} M for the parent cells and 200×10^{-8} M for the resistant cells. The SBC-3/ADM cells were found to be completely resistant to DM. As shown in

Table 1 Resistance of SBC-3/ADM cells to anthracycline-anthraquinone drugs

Drug	LD ₇₀ ($\times 10^{-8}$ M)		Degree of resistance ^a
	SBC-3	SBC-3/ADM	
Adriamycin	6.0	180	1 : 30
Daunomycin	7.2	200	1 : 28
4'-Epiadriamycin	13	340	1 : 26
THP-adriamycin	1.6	29	1 : 18
Aclarubicin	5.8	49	1 : 8.4
Mitoxantrone	1.8	2.1	1 : 1.2

a: Ratio of the LD₇₀ value against SBC-3/ADM cells to that against SBC-3 cells.

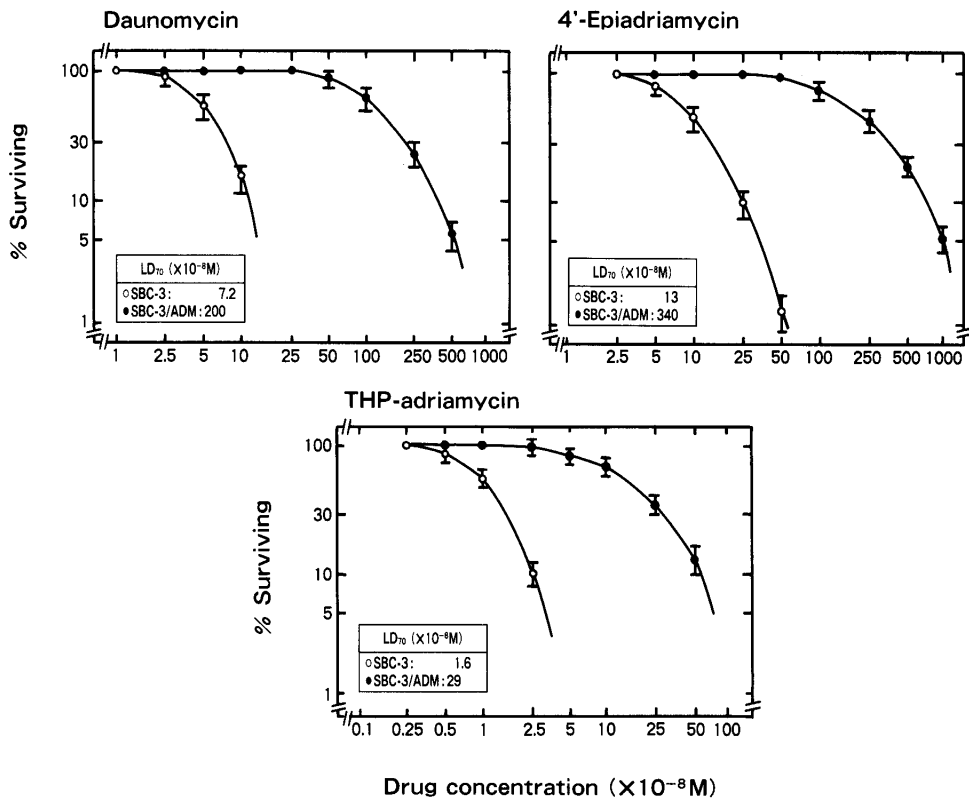


Fig. 1 Dose response curves of the SBC-3 and SBC-3/ADM cells to daunomycin, 4'-epiadriamycin, and THP-adriamycin. Cell suspensions of both cell lines were incubated in the presence of each drug at various concentrations for 1 h, and plated in a double-layer soft agar system as described in Materials and Methods. Each point represents the mean of three estimations $\pm$ SD.

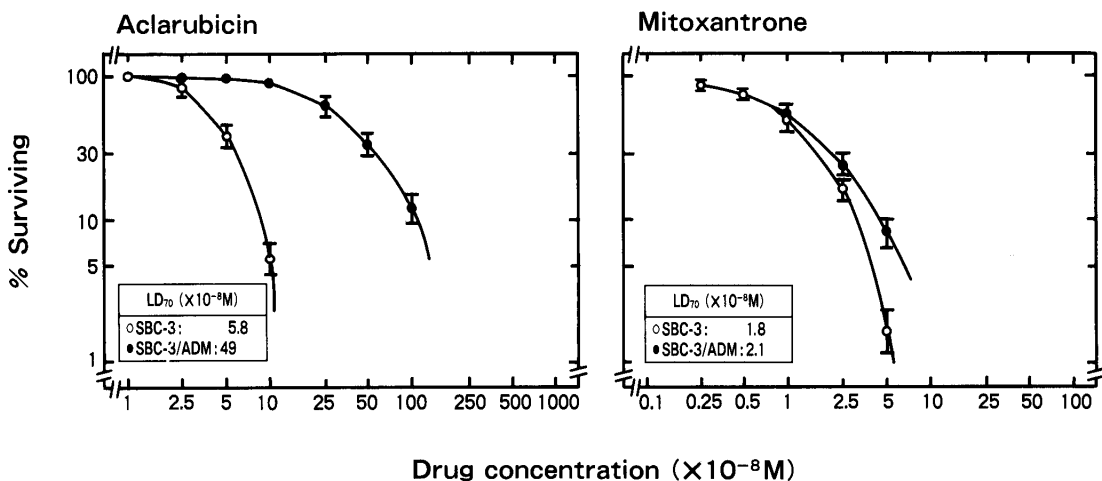


Fig. 2 Dose response curves of the SBC-3 and SBC-3/ADM cells to aclarubicin and mitoxantrone. Cell suspensions of both cell lines were incubated in the presence of each drug at various concentrations for 1 h, and plated in a double-layer soft agar system as described in Materials and Methods. Each point represents the mean of three estimations $\pm$ SD.

Table 1, the SBC-3/ADM cells were also markedly resistant to closely related anthracycline antibiotics including EPI (DR : 26) and THP (DR : 18), and were partially resistant to ACR (DR : 8.4). On the other hand, MIT, an anthraquinone derivative, had equivalent activity against both cell lines (DR : 1.2).

Dose response curves of the parent and resistant cells to other anticancer drugs are presented in Fig. 3, and the degree of resistance of the SBC-3/ADM cells to these experimental agents is summarized in Table 2. Resistance was clearly evident in the SBC-3/ADM cells to unrelated compounds, such as VCR (DR : 25) and MMC (DR : 19). Moreover, the cells exhibited a moderate

Table 2 Resistance of SBC-3/ADM cells to drugs other than anthracycline-anthraquinones

Drug	LD ₇₀ ($\times 10^{-8}$ M)		Degree of resistance ^a
	SBC-3	SBC-3/ADM	
Adriamycin	6.0	180	1 : 30
Vincristine	8.0	200	1 : 25
Mitomycin C	73	1400	1 : 19
40497S ^b	110	640	1 : 5.8
Cisplatin	1300	3200	1 : 2.5

a : Ratio of the LD₇₀ value against SBC-3/ADM cells to that against SBC-3 cells.

b : 40497S is an active form of ifosfamide.

degree of resistance to 40497S (DR : 5.8), but they were relatively sensitive to CDDP (DR : 2.5). Collateral sensitivity in the resistant sublines was not seen for any drug

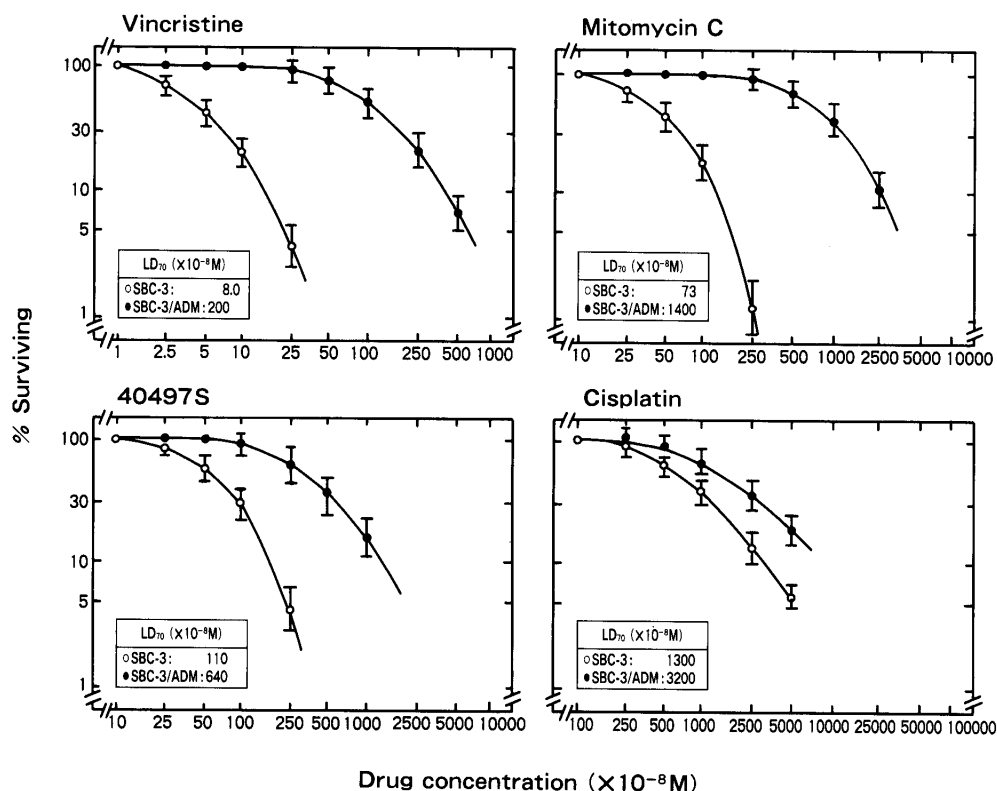


Fig. 3 Dose response curves of the SBC-3 and SBC-3/ADM cells to vincristine, mitomycin C, 40497S, and cisplatin. Cell suspensions of both cell lines were incubated in the presence of each drug at various concentrations for 1 h, and plated in a double-layer soft agar system as described in Materials and Methods. Each point represents the mean of three estimations $\pm$ SD.

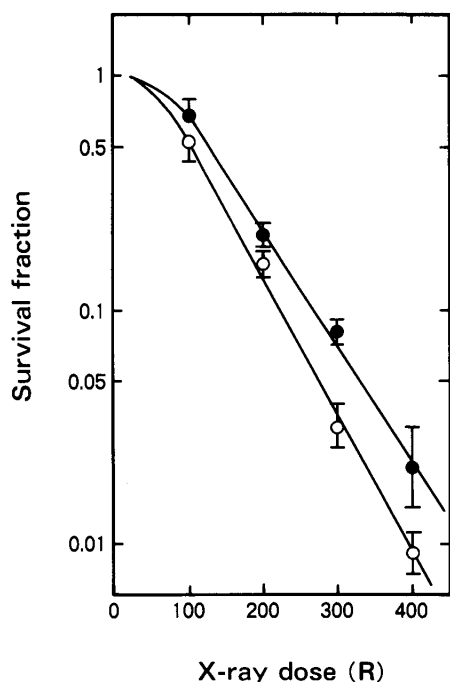


Fig. 4 Dose response curves of the SBC-3 (○) and SBC-3/ADM cells (●) against X-ray irradiation. Cells derived from both cell lines were irradiated with graded doses of X-rays, and plated in a double-layer soft agar system as described in Materials and Methods. Each point represents the mean of three estimations $\pm$ SD.

tested.

In vitro radiosensitivity. Fig. 4 shows the results of experiments on the survival of the SBC-3 and SBC-3/ADM cells irradiated with graded doses of X-rays. The parameters of the X-ray survival curve in the parent cells were 75 R for the mean lethal dose (D_0), 55 R for the quasithreshold dose (D_q), and 2.1 for the extrapolation number (n), whereas the parameters in the resistant cells were 85 R for D_0 , 65 R for D_q , and 2.2 for n . Accordingly, the SBC-3/ADM cells retained almost the same sensitivity to X-ray irradiation as the SBC-3 cells.

Discussion

Emergence of drug resistance is a major limiting factor for successful cancer chemo-

therapy. Even with the most drug-sensitive classes of tumor, such as SCLC, a significant proportion may relapse regardless of continuing therapy. Switching to new drugs only infrequently produces clinical responses that are achievable on the first application. These facts seem to indicate an occurrence of cell populations resistant to a wide range of anticancer agents during the course of treatment.

Biedler and Riehm (7, 8) found that actinomycin D-resistant Chinese hamster cells were resistant to DM, vinca alkaloids, and MMC, and that DM-resistant cells exhibited a considerable degree of resistance to ADM and actinomycin D. Other investigators reported that colchicine-resistant mutants of Chinese hamster ovary cells displayed extensive resistance to a number of apparently unrelated compounds including anthracycline antibiotics and vinblastine (9). Similarly, sublines of P388 leukemia resistant to ADM were also resistant to actinomycin D and emetine (10, 11). When a variant resistant to a single agent expresses resistance to multiple drugs with different structures or mechanisms of action, the phenomenon is known as pleiotropic drug resistance (12, 13). In this study, the SBC-3/ADM cells displayed complete or partial resistance to a variety of chemotherapeutic agents, which are chemically or pharmacodynamically dissimilar, as well as anthracycline antibiotics. Therefore, it seems likely that a pleiotropic drug-resistant phenotype would also occur in human tumors, leading to the emergence of a therapy-resistant disease.

The present studies using the SBC-3/ADM cells showed no cross-resistance between ADM and MIT and little cross-resistance with CDDP, while collateral sensitivity was not observed to any drug. Since alternations in membrane permeability are considered to be responsible for the development of resistance of the SBC-3/ADM

cells, the residual activities of MIT and CDDP against the resistant cells may be due to differences in the transport mechanism of these drugs as compared with ADM.

MIT, which was synthesized in an attempt to find a noncardiotoxic compound retaining its antitumor activity, has demonstrated a moderate degree of activity against ADM-resistant P388 leukemia cells (14, 15) or DM-resistant L1210 leukemia cells (16). In view of our data that the SBC-3 and SBC-3/ADM cells had an equivalent sensitivity to MIT, the drug seems to exert sufficient activity for SCLC developing resistance to ADM. Although a phase II study of MIT for SCLC revealed that partial responses were obtained only in two (response rate: 4%) out of 54 patients (17), additional studies are required for a concrete conclusion regarding the availability of MIT in the treatment of this type of neoplasm. The findings that the SBC-3/ADM cells demonstrated little cross-resistance between ADM and CDDP, coupled with the moderate activity in phase II clinical trials of the agent for SCLC (18-20), suggest that CDDP is worth evaluating as a drug in combination chemotherapy for tumors.

Prior chemotherapy often reduces the sensitivity to subsequent radiotherapy in patients with SCLC. However, the results of our experiments revealed that the parent and resistant cells were equally sensitive to X-ray irradiation, indicating that the emergence of drug-resistant tumor cells is not necessarily accompanied by decreased response to radiation. Current data regarding the role of radiation therapy in SCLC appear to support the value of cranial irradiation given to complete responders for the prophylaxis of occult CNS involvement, while several randomized studies suggest that adjuvant chest irradiation does not benefit any subgroup of patients in terms of median survival (4, 21). However, another promising

approach is to deliver consolidative chest irradiation to complete responders after chemotherapy has been administered for 4 to 6 months (22). The residual activity of X-ray irradiation against the SBC-3/ADM cells suggest that consolidative chest irradiation may be clinically useful in patients who have been treated with combination chemotherapy including ADM.

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References

1. Livingston RB: Small cell carcinoma of the lung. *Blood* (1980) **56**, 575-584.
2. Seydel HG, Creech RH, Mietlowski W and Perez C: Radiation therapy in small cell lung cancer. *Semin Oncol* (1978) **5**, 288-298.
3. Hansen M, Hansen H and Dombernowsky P: Long-term survival in small cell carcinoma of the lung. *J Am Med Assoc* (1980) **244**, 247-250.
4. Ohnoshi T, Hiraki S, Miyake K, Ozawa S, Kawahara S, Tamura M, Kimura I and Hiraki Y: Combination chemotherapy with multimodality approaches for small cell carcinoma of the lung. *Jpn J Cancer Clin* (1984) **30**, 734-740 (in Japanese).
5. Oldham RK and Greco FA: Small-cell lung cancer: a curable disease. *Cancer Chemother Pharmacol* (1980) **4**, 173-177.
6. Miyamoto H: Establishment and characterization of an adriamycin-resistant subline of human small cell lung cancer cells. *Acta Med Okayama* (1986) **40**, 65-73.
7. Biedler JL and Riehm H: Cellular resistance to actinomycin D in Chinese hamster cells *in vitro*: cross-resistance, radioautographic, and cytogenetic studies. *Cancer Res* (1970) **30**, 1174-1184.
8. Riehm H and Biedler JL: Cellular resistance to daunomycin in Chinese hamster cells *in vitro*. *Cancer Res* (1971) **31**, 409-412.
9. Beck-Hansen NT, Till JE and Ling V: Pleiotropic phenotype of colchicine-resistant CHO cells: cross-resistance and collateral sensitivity. *J Cell Physiol* (1976) **88**, 23-31.
10. Inaba M and Johnson RK: Decreased retention of actinomycin D as the basis for cross-resistance in anthracycline-resistant sublines of P388 leukemia. *Cancer Res* (1977) **37**, 4629-4634.

11. Chitnis MP and Johnson RK: Biochemical parameters of resistance of an adriamycin-resistant subline of P388 leukemia to emetine, an inhibitor of protein synthesis. *J Natl Cancer Inst* (1978) **60**, 1049-1054.
12. Aldrich CD: Pleiotropic phenotype of cultured murine cells resistant to maytansine, vincristine, colchicine, and adriamycin. *J Natl Cancer Inst* (1979) **63**, 751-757.
13. Goldie JH and Coldman AJ: The genetic origin of drug resistance in neoplasms: implications for systemic therapy. *Cancer Res* (1984) **44**, 3643-3653.
14. Johnson RK, Zee-Cheng RKY, Lee WW, Acton EM, Henry DW and Cheng CC: Experimental antitumor activity of aminoanthraquinones. *Cancer Treat Rep* (1979) **63**, 425-439.
15. Wallace RE, Murdock KC, Angier RB and Durr FE: Activity of a novel anthracenedione, 1,4-dihydroxy-5,8-bis [[2-[(2-hydroxyethyl) amino] ethyl] amino]-9,10-anthracenedione dihydrochloride, against experimental tumors in mice. *Cancer Res* (1979) **39**, 1570-1574.
16. Fujimoto S and Ogawa M: Antitumor activity of mitoxantrone against murine experimental tumors: comparative analysis against various antitumor antibiotics. *Cancer Chemother Pharmacol* (1982) **8**, 157-162.
17. Von Hoff DD, Chen T, Clark GM, Callahan SK, Livingston R and the Southwest Oncology Group: Mitoxantrone for treatment of patients with refractory small cell carcinoma of the lung: a Southwest Oncology Group study. *Cancer Treat Rep* (1983) **67**, 403-404.
18. Dombernowsky P, Sörenson S, Aisner J and Hansen HH: *Cis*-dichlorodiammineplatinum (II) in small cell anaplastic bronchogenic carcinoma: a phase II study. *Cancer Treat Rep* (1979) **63**, 543-545.
19. Cavalli F, Jungi WF, Sonntag RW, Nissen NI and Holland JF: Phase II trial of *cis*-dichlorodiammineplatinum (II) in advanced malignant lymphoma and small cell lung cancer: preliminary results. *Cancer Treat Rep* (1979) **63**, 1599-1603.
20. Levenson RM, Ihde DC, Huberman MS, Cohen MH, Bunn PA and Minna JD: Phase II trial of cisplatin in small cell carcinoma of the lung. *Cancer Treat Rep* (1981) **65**, 905-907.
21. Bleeher NM, Bunn PA, Cox JD, Dombernowsky P, Fox RM, Høst H, Joss R, White JE and Wittes RE: Role of radiation therapy in small cell anaplastic carcinoma of the lung. *Cancer Treat Rep* (1983) **67**, 11-19.
22. Byhardt RW, Cox JD, Holoye PY and Libnoch JA: The role of consolidation irradiation in combined modality therapy of small cell carcinoma of the lung. *Int J Radiat Oncol Biol Phys* (1982) **8**, 1271-1276.

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