Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5029–5033, July 1987
Medical Sciences

In vivo-like drug responses of human tumors growing in three-dimensional gel-supported primary culture

(cancer drug development/individualized therapy)

ROBERT A. VESCIO*, CHARLES H. REDFERN*, THEODORA J. NELSON†, SCOTT UGORETZ†, PETER H. STERN*†, AND ROBERT M. HOFFMAN*†‡

*Laboratory of Cancer Biology, Department of Pediatrics M-009F, School of Medicine, University of California at San Diego, La Jolla, CA 92093; and †AntiCancer, Inc., 5325 Metro Street, San Diego, CA 92110

Communicated by Sheldon Penman, March 18, 1987

ABSTRACT **An *in vitro* test of cell sensitivity to drugs that indicates *in vivo* response is an important need in cancer therapy and cancer drug development. Toward this end, we previously developed a collagen gel-supported culture system for growth of human tumors. This three-dimensional culture system is general and grows tumors at high frequency directly from surgery or biopsy that maintain important *in vivo* properties *in vitro*, including tissue architecture. We report here that with autoradiographic techniques measuring cellular DNA synthesis the drug responses of individual cells within the tissue structure of *in vitro*-grown tumors can be determined. Twenty tumor classes, including all the major ones, have been measured *in toto* at >50% frequency. Quantitative and qualitative results show increasing cell kill with rising cytotoxic drug concentration, differential drug sensitivities of multiple cell types within individual cultured tumors, differential sensitivities of a series of tumors of the same histopathological classification to a single drug, differential sensitivities of individual tumors to a series of drugs, and sensitivity patterns of various tumor types similar to the sensitivities found *in vivo*. Therefore, the results indicate that potentially important therapeutic data can be obtained from tumor specimens growing *in vitro* for the individual cancer patient as well as for rational and relevant screening for new agents active against human solid tumors.**

A major clinical problem is that cancers that are classified as identical according to their histopathological characteristics are nonetheless highly individual in their drug sensitivities and there is currently no way to predict clinical outcome of chemotherapy for individual patients (1). A second major problem is that there is currently no relevant assay to screen for new human anticancer agents, especially for solid tumors (2). To overcome these problems, many attempts have been made to develop *in vitro* drug-sensitivity tests for individual cancer patients about to undergo chemotherapy and to screen for new anticancer agents. These attempts have suffered in one form or another from their inability to support growth of human tumors such that they reflect the *in vivo* situation. Plating of dissociated tumor cells in soft agar (3–12) and monolayer cultures (13–15) does not in many instances allow the growth of tumor cells. Often, when tumor cells do grow under these conditions, other cell types present in the original tumor probably have not grown. It is critical that cell types present in the original tumor be present in the assay since it has been shown that interactions between cell types can alter their drug sensitivities (16). Multicellular spheroids that are three-dimensional have been used recently for drug sensitivity testing (17, 18), but these also involve dissociation of cells from the tumor and reassociation into structures that do not resemble the original tissues. Short-term *in vitro* assays of drugs on noncultured nondissociated tumor specimens probably do not have tumors in representative physiological or growing conditions (19, 20) and, therefore, may not always yield valid drug-sensitivity results. Under these conditions, the tumors rapidly lose viability and long-term assays are not possible. When cells are dissociated from solid tumors and incubated for short periods in suspension in liquid medium, they are also not under *in vivo* conditions. Therefore, their drug responses determined by dye-exclusion assays to measure cell viability may not be relevant to the *in vivo* situation (21).

With regard to *in vivo* models, chemosensitivity testing of human tumors implanted in nude mice usually involves 30–50 weeks or more to obtain data and is, therefore, of limited use for most cancer patients (22). Another *in vivo* approach has been to implant human tumor tissue in the subrenal capsule of mice to determine drug sensitivity. This approach suffers from the possibility that cells do not always grow under these conditions and the requirement for large numbers of mice to test three or four drugs on even a single tumor (23). The limited number of tests possible with these systems in mice also precludes them from being useful for screening new drugs.

To overcome these problems, we have developed a three-dimensional *in vitro* human tumor culture system that is general and grows most human tumors obtained directly from surgery or biopsy. The culture system meets important criteria of *in vivo* growth, including maintenance of tissue structure (24). We report here that with the use of this tumor culture system, tumors respond to drugs in a manner that reflects the *in vivo* response.

MATERIALS AND METHODS

Tumors were explanted as described (24). Briefly, the tumors were surgically removed, minced into 2-mm diameter pieces, and placed on previously hydrated collagen gel matrices derived from pigskin (Health Design Industries, Rochester, NY). Eagle's minimal essential medium (MEM) containing Earle's salts, glutamine, 10% fetal calf serum, nonessential amino acids, and the antibiotics garamycin and claforan were added to culture dishes such that the upper part of the gel was not covered.

The drugs were used with the therapeutic concentrations and exposure times listed in Table 1 (25, 26). After a period

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Adr, doxorubicin hydrochloride; Cis, cisplatin; Mel, melphalan; MTX, methotrexate; FUra, 5-fluorouracil; IFN, interferon; dCof, deoxycoformycin.

‡To whom all reprint requests should be addressed at: Laboratory of Cancer Biology, M-009F, University of California, San Diego, La Jolla, CA 92093.

Table 1. Drug concentrations and exposure times used *in vitro*

Drug	Therapeutic (1×) concentration	Exposure time, hr
Adriamycin (Adr)*	29 ng/ml	24
BCNU	0.2 μg/ml	3–24
Cisplatin (Cis)	1.5 μg/ml	24
Melphalan (Mel)	0.5 μg/ml	5–24
Methotrexate (MTX)	2.25 μg/ml	24
Mitomycin C	100 ng/ml	1.5–24
5-Fluorouracil (FUra)	4.0 μg/ml	1–24
Vincristine	23 ng/ml	2.5–24
VP-16	4.8 μg/ml	24
Interferon (IFN)	3×10^3 units/ml	24
Deoxycoformycin (dCof)	26.8 μg/ml	5–24

The drug concentrations and exposure times were calculated from pharmacological data to simulate *in vitro* the drug concentrations achieved *in vivo*. Drug exposure times are based on the plasma half-life of the chemotherapeutic agents (25, 26). Drugs were given after tumors were cultured for at least 4 days.
*Generic name, doxorubicin hydrochloride.

of 3 days to recover from any transient effects of the drugs, cell proliferation was measured by administering a combination of [^{3}H]thymidine (2 μCi/ml; 1 Ci = 37 GBq) and [^{3}H]deoxyuridine (2 μCi/ml) (27) for 4 days. Cellular DNA is labeled in any cells undergoing replication within the tumors. Previous studies have correlated effects of drugs on incorporation of [^{3}H]thymidine into DNA with the degree of sensitivity of the cells themselves to the drugs tested (28). After 4 days of labeling, the cultures were washed with phosphate-buffered saline, placed in histology capsules, and fixed in 10% formalin. The cultures were then dehydrated, embedded in paraffin, and sectioned by standard methodology. After the slides were deparaffinized, they were prepared for autoradiography by coating with Kodak NTB-2 emulsion in the dark, exposed for 5 days, and then the slides were developed. After rinsing, the slides were stained with hematoxylin and eosin. The slides were then analyzed by determining the percentage of cells undergoing DNA synthesis in treated vs. untreated tumor cultures. Replicating cells were identified by the presence of black grains over their nuclei due to exposure of the NTB-2 emulsion to radioactive DNA.

RESULTS

Demonstration of Differential Effects of Various Drugs on Individual Tumors of Various Classes. Fig. 1 shows the responses to seven different drugs of the cultured ovarian cystadenoma of patient 414. The top row is an autoradiogram of the untreated control culture grown in the presence of [^{3}H]thymidine and [^{3}H]deoxyuridine. There are two populations of cells present. The large-nuclei-containing typical ovarian carcinoma cells (29) were actively dividing, as evidenced by the black grains over them; the small cells were not dividing. The effects of MTX, Adr, Cis, dCof, VP-16, and IFN are shown in the rows indicated. The drugs were given for the times listed in Table 1 and at 0.1×, 1×, and 10× the concentrations. It should be noted that throughout this discussion, the 1× concentrations of drugs correspond to usual clinically achievable plasma concentrations. By comparing the number of proliferating cells in the drug-treated cultures to the control, one can see that none of these drugs is effective for the tumor of patient 414. The resistance to Adr and Cis is not surprising in light of the patient's previous treatment failure with these drugs. However, as shown in the bottom row, the drug Mel seems highly effective for the tumor, with the 1× and 10× concentrations eliminating cellular proliferation and most of the cells themselves, respectively.

Fig. 2 shows the effect of three drugs on the cultured breast tumor of patient 289. In the top row are autoradiograms from untreated cultures. Note the large amount of radioactivity incorporated into the DNA of many but not all cells, as evidenced by the clusters of black grains over the purple-stained nuclei. In the middle row are parallel cultures of the tumor that were treated with 1× and 10× concentrations of Adr. The radioactively labeled nuclei indicate that there is a large population of cells in the tumor that are quite resistant

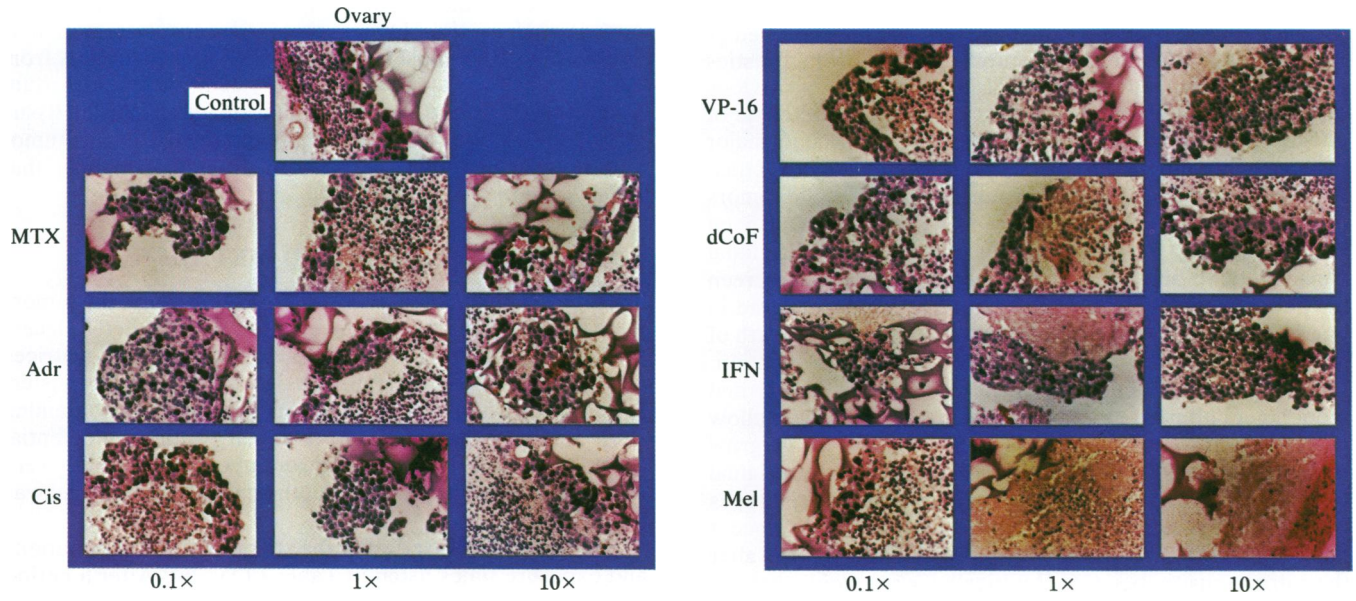

FIG. 1. Drug responses of cultures of ovarian cancer of patient 414. Autoradiograms were prepared from slides of cultures treated with the indicated drugs and labeled with [^{3}H]thymidine and [^{3}H]deoxyuridine as described. Autoradiograms were counterstained with hematoxylin and eosin. Black grains over purple-stained nuclei indicate uptake of radioactivity into the cells and DNA synthesis. Note resistance of cultured tumor to MTX (1× concentration, 2.25 μg/ml; 24-hr exposure), Adr (1× concentration, 29 ng/ml; 24-hr exposure), Cis (1× concentration, 1.5 μg/ml; 24-hr exposure), VP-16 (1× concentration, 4.8 μg/ml; 24-hr exposure), dCof (1× concentration, 26.8 μg/ml; 24-hr exposure), and IFN (1× concentration, 3×10^3 units/ml; 24-hr exposure), and the sensitivity to Mel (1× concentration, 0.5 μg/ml; 5-hr exposure). (×173.)

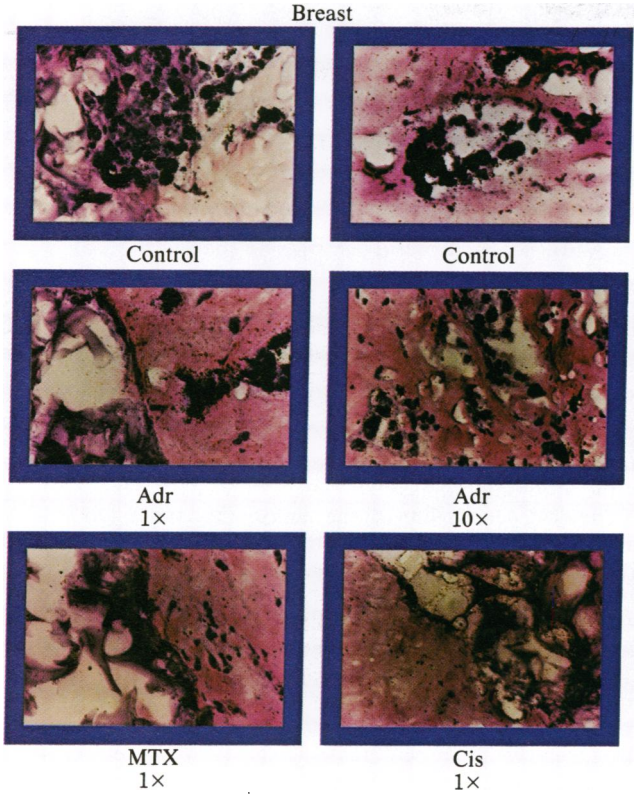

FIG. 2. Drug responses of cultures of breast cancer of patient 289. See Fig. 1 for details. Note resistance of cultured tumor to Adr and relative sensitivity to MTX and Cis. In the MTX-treated culture, there are darkly stained nuclei but very few autoradiographically labeled cells. (×377.)

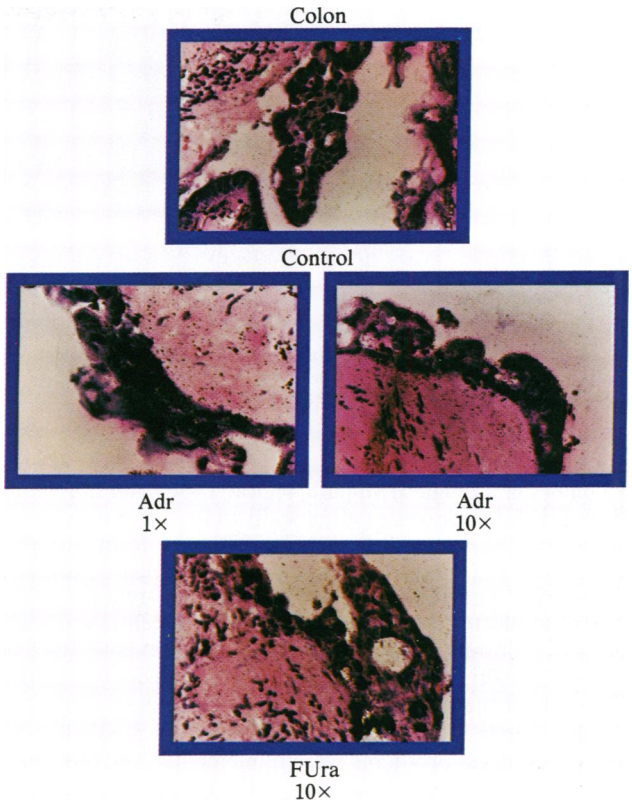

FIG. 3. Drug responses of cultures of liver metastasis of colon carcinoma of patient 337. See Fig. 1 for details. Note resistance of cultured tumor to high levels of Adr and FUra. (×400.)

to Adr. The bottom row, however, demonstrates that the 1× concentration of MTX and the 1× concentration of Cis were effective in reducing cell proliferation within the cultured tumor. Note that in the MTX-treated culture there are darkly stained nuclei but few autoradiographically labeled cells.

In Fig. 3, the cultured liver metastasis of the colon carcinoma from patient 337 is analyzed for drug sensitivity. The top row shows an untreated culture with a high degree of cellular proliferation. Note the organization of epithelial tissue attached to stromal structures. In the middle row are cultures treated with the 1× and 10× concentrations of Adr. One can see high rates of cellular proliferation indicating resistance to the drug even at the latter dose, which corresponds to 10 times the usual therapeutic level. There is a similar situation in the bottom row, where the autoradiogram indicates resistance to the 10× concentration of FUra. Thus, Figs. 1–3 show that it is possible to determine the proliferative capacity and the inhibition of this capacity by drugs of individual cells within the tissue structure of the cultured tumors.

Fig. 4 illustrates quantitatively the data in Fig. 1 for the drug responses of the cultured ovarian carcinoma of patient 414. It shows clearly that Mel is the only effective drug of the seven that were tried.

Demonstration of Differential Effects of a Single Drug on Different Cell Types Within a Single Tumor. Fig. 5 (*Left*) shows an autoradiogram from a culture of the axillary node lymphoma of patient 277 treated with the 1× concentration of Cis. It can be noted that there is a large degree of proliferation of the cells in the tumor explant tissue structure itself as well as in cells that have invaded the supporting collagen gel matrix, indicating resistance of both cell types to Cis at this level. The autoradiogram in the center is from a parallel culture of the same axillary node lymphoma of patient 277 treated with the 10× concentration of Adr, which causes cessation of proliferation of cells in the explant. The cells within the explant tissue are darkly stained but do not contain autoradiographic grains. However, as shown in the autoradiograms (*Center* and *Right*), Adr even at 10 times the therapeutic level does not preclude the proliferation of cells that have invaded the collagen gel matrix.

With the cultured colon cancer of patient 380, there is an opposite effect. The cells within the explant tissue structure are resistant to Adr as well as to FUra even at the 10× concentrations, while cells migrating into the gel are sensitive to these drugs (data not shown).

These data indicate that the cells that migrate into the supporting gel matrix may be of a different quality from those that do not, and their differences may include drug sensitivity.

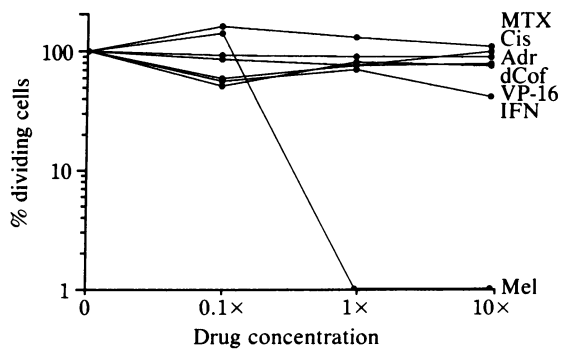

FIG. 4. Graphic representation of drug responses of cultures of ovarian carcinoma of patient 414. (See Fig. 1 for autoradiographic data of *in vitro* drug response for this patient.)

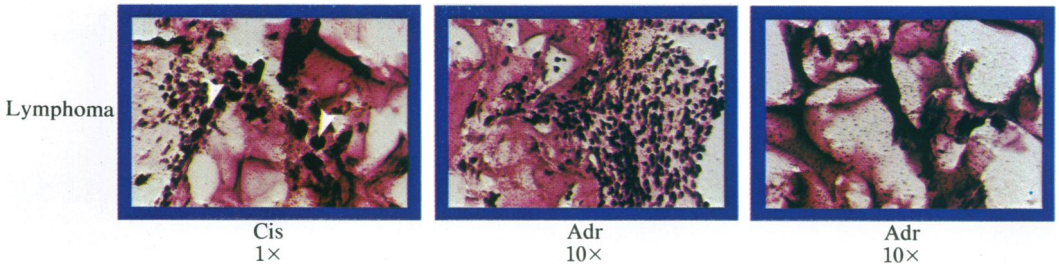

FIG. 5. Drug responses of different cell types of lymphoma of patient 277. See Fig. 1 and text for details. Note that cells within the tissue structure and those migrating into the gel matrix are both resistant to Cis (see arrows). However, only the cells migrating into the gel matrix are resistant to Adr. In the autoradiogram in the center, the nuclei within the tissue structure itself are darkly stained but not autoradiographically labeled and thereby responding to Adr as opposed to some of the nuclei of cells migrating into the gel matrix, which indeed are labeled autoradiographically and resistant to Adr. (×400.)

Demonstration of Differential Sensitivity of Members of a Set of Tumors of a Single Histopathological Type to an Individual Drug. Fig. 6 demonstrates graphically that a series of cultured tumors of the same histopathological type from different individuals can be very different in their sensitivities to a single drug. The percentage of dividing cells relative to the control is plotted as a function of Adr concentration for a series of cultured breast tumors from different patients. The range of sensitivity is quite large, which reflects the clinical situation for breast cancer patients in general.

Table 2 lists the total of tumor types explanted since the above-described protocols were implemented. At this point, >50% of explanted tumors of 20 different classes have yielded evaluatable results.

DISCUSSION

In vivo drug-response criteria, which are satisfied by our gel-supported three-dimensional primary culture (24) system, include differential sensitivities of individual tumors to different drugs (see Figs. 1–4), differential sensitivities of tumors of the same histopathological classification to the same drug (Fig. 6), and differential sensitivities of multiple cell types within individual tumors (Fig. 5). In particular, the cells that migrate from the original explant tissue structure to invade the supporting gel matrix may have, at least in some instances, specific drug sensitivities (Fig. 5). These migrating cells may also be more invasive *in vivo*, thereby making the identification of their drug sensitivities of potential clinical importance. Given the heterogeneous nature of individual tumors (30), it is important to be able to measure the drug sensitivities of specific cell types of cultured tumors, as shown here. This type of analysis is made possible by the fact that, in our culture system, three-dimensional tissue structure is maintained *in vivo* and can be observed histologically in the autoradiograms.

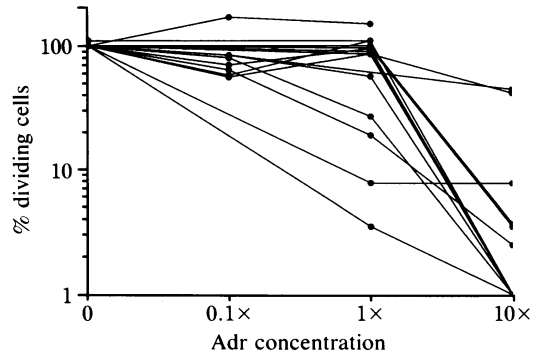

FIG. 6. Graphic responses of a series of cultured breast tumors to Adr (see text for details).

The drug sensitivities of the tumors thus far tested *in vitro* also resemble the overall clinical pattern (31) as revealed in Table 3, which indicates the *in vitro* drug sensitivities of 15 different types of cancers.

It is important to note that the system described here is a general one and allows drug response data to be obtained in all types of solid tumors at high frequency. The fact that tumors can be cultured for long periods (24) allows long-term testing, and cells that still retain proliferative capacity after drug treatment can be detected.

It is felt that the data presented demonstrate that tumors cultured in the three-dimensional gel-supported primary culture system respond to drugs in an *in vivo*-like way and justify the use of this *in vitro* system to develop clinically useful testing for individual human cancer patients and for the rational and relevant screening for agents active against human solid tumors.

Table 2. Frequency of evaluation of drug responses of cultured tumors

Tumor type	Number processed	Number evaluated	Percentage
Breast	32	10	31
Lung	14	9	64
Colon	14	7	50
Melanoma	7	6	86
Lymphoma	6	5	83
Ovary	9	6	66
Uterus	4	2	50
Stomach	3	2	67
Brain	3	1	33
Kidney	3	2	67
Sarcoma	2	1	50
Liver	2	0	0
Larynx	1	1	100
Testicle	1	1	100
Omentum	1	1	100
Osteosarcoma	1	0	0
Prostate	1	1	100
Pancreas	1	1	100
Duodenum	1	0	0
Scalp	1	0	0
Mediastinum	1	1	100
Thyroid	1	0	0
Thymoma	1	1	100
Neck	1	0	0
Bladder	1	0	0
Total	112	58	52

Tumors were cultured and drug responses were evaluated as described in the text.

Table 3. Frequency of drug response of various cultured tumor types to individual drugs

	Adr		MTX		Cis		Vin		FUra		Mito		Total response, %	
Tumor type	1×	10×	1×	10×	1×	10×	1×	10×	1×	10×	1×	10×	1×	10×
Breast	2/10	7/10	1/4	3/4	—	—	0/1	0/1	0/2	3/3	—	—	18	72
Kidney	0/1	0/1	—	—	0/2	1/2	0/2	1/2	—	—	—	—	0	40
Lung	1/6	4/7	0/1	0/1	1/7	5/7	0/2	1/2	0/1	1/1	0/4	3/4	10	64
Mediastinum	0/1	1/1	0/1	0/1	0/1	0/1	—	—	0/1	1/1	—	—	0	50
Larynx	0/1	1/1	1/1	1/1	0/1	0/1	—	—	—	—	—	—	33	66
Thymoma	1/1	1/1	1/1	1/1	0/1	0/1	—	—	—	—	—	—	66	66
Pancreas	—	—	—	—	—	—	—	—	0/1	1/1	0/1	1/1	0	100
Colon	1/2	1/2	0/1	1/1	1/2	—	—	—	3/6	6/6	2/4	1/2	47	82
Lymphoma	1/5	5/5	0/2	0/2	0/1	1/1	0/5	4/5	0/1	—	—	—	7	77
Omentum	1/1	1/1	—	—	—	—	—	—	0/1	1/1	—	—	50	100
Melanoma	0/1	0/1	—	—	1/5	2/5	1/6	4/6	0/1	1/1	0/1	1/1	14	57
Sarcoma	0/1	—	—	—	—	—	0/1	0/1	—	—	—	—	0	0
Uterus	0/1	0/1	—	—	1/2	1/2	—	—	0/1	0/1	—	—	25	25
Stomach	0/1	0/1	—	—	—	—	—	—	0/1	0/1	—	—	0	0
Brain	0/1	1/1	0/1	0/1	0/1	0/1	—	—	—	—	—	—	0	33
Prostate	—	—	—	—	0/1	1/1	—	—	0/1	0/1	—	—	0	50
Ovary	0/6	1/6	0/3	0/3	3/4	3/4	1/2	2/2	0/1	1/1	—	—	25	44
Testicle	—	—	—	—	0/1	0/1	0/1	0/1	—	—	—	—	0	0
Total	7/39	23/39	3/15	6/15	7/29	14/27	2/20	12/20	3/18	15/18	2/10	6/8		
Percentage	18%	59%	20%	40%	24%	52%	10%	60%	17%	83%	20%	75%		

A response indicates percent of dividing cells is <30% of control. See text for details on the culture of tumors and measurement of drug response. See Table 1 for 1× concentrations of each drug, which correspond to the clinical dose. Vin, vincristine; Mito, mitomycin C.

We thank Polly Pomeroy for the preparation of this manuscript. We thank the following for tumor specimens: Sharp Hospitals (Dr. H. R. Irwin, Dr. F. J. Luibel, Dr. H. Robin, and Ms. D. Bass), Scripps Clinic and Research Foundation (Dr. G. Bordin), Scripps Memorial Hospital (Dr. P. Pieslor), Mercy Hospital (Dr. T. Youngkin), University of California, San Diego (Dr. N. Varki), and Dr. J. Lamon. This research was supported by National Cancer Institute Grant RO1-CA27564-06 and Research Career Development Award KO4-CA00804-05; by Grant 1348AR2 from The Council for Tobacco Research–U.S.A., Inc., by the Cancer Research Coordinating Committee of the University of California, the United Cancer Council, the George A. Jacobs Memorial Fund for Cancer Research, the Pericles P. Stathas Memorial Fund for Cancer Research, the Bernard B. Hoffman Memorial Foundation, the Louis Sklarow M.D. Memorial Fund, and National Cancer Institute Small Business Innovation Research Grant R43-CA43411-01.

1. Chabner, B. A. ed. (1983) *Rational Basis for Chemotherapy* (Liss, New York).
2. Finlay, G. J. & Baguley, B. C. (1984) *Eur. J. Cancer Clin. Oncol.* **20,** 947–954.
3. Hamburger, A. W. & Salmon, S. E. (1977) *Science* **197,** 461–463.
4. Salmon, S. E., Hamburger, A., Soehnlen, B., Durie, B., Alberts, D. & Moon, T. (1978) *N. Engl. J. Med.* **298,** 1321–1327.
5. Twentyman, P. R. (1985) *Br. J. Cancer* **51,** 295–299.
6. Selby, P., Buick, R. & Tannock, I. (1983) *N. Engl. J. Med.* **308,** 129–134.
7. Von Hoff, D. D. (1983) *N. Engl. J. Med.* **308,** 154–155.
8. Pihl, A. (1986) *Int. J. Cancer* **37,** 1–5.
9. Hofmann, V., Berens, M. E. & Martz, G. (1984) *Predictive Drug Testing on Human Tumor Cells* (Springer, New York).
10. Dendy, P. & Hill, B. (1983) *Human Tumour Drug Sensitivity Testing In Vitro* (Academic, New York).
11. Salmon, S. & Trent, J. eds. (1984) *Human Tumor Cloning* (Grune & Stratton, Orlando, FL).
12. Singletary, S. E., Umback, G., Spitzer, G., Drewinko, B., Tomasovic, B., Ajani, J., Hug, V. & Blumenschein, G. (1985) *Int. J. Cell Cloning* **3,** 116–128.
13. Baker, F. L., Spitzer, G., Ajani, J. A., Brock, W. A., Lukeman, J., Pathak, S., Tomasovic, B., Thielvoldt, D., Williams, M., Vines, C. & Tofilon, P. (1986) *Cancer Res.* **46,** 1263–1274.
14. Wilson, A. P., Ford, C., Newman, C. & Howell, A. (1984) *Br. J. Cancer* **49,** 57–63.
15. Smith, H. S., Lippman, M. E., Hiller, A. J., Stampler, M. R. & Hackett, A. J. (1985) *J. Natl. Cancer Inst.* **74,** 341–347.
16. Miller, B. E., Miller, F. R. & Heppner, G. H. (1984) *J. Cell. Physiol.*, Suppl. 3, 105–116.
17. Tofilon, P. J., Buckley, N. & Deen, D. F. (1984) *Science* **226,** 862–864.
18. Erlichman, C. & Vidgen, D. (1984) *Cancer Res.* **44,** 5369–5375.
19. Zaffaroni, N., Silvestrini, R., Sanfilippo, O., Daidone, M. G. & Gasparini, G. (1985) *Tumori* **71,** 555–561.
20. Volm, M., Wayss, K., Kaufmann, M. & Mattern, J. (1979) *Eur. J. Cancer* **15,** 983–993.
21. Weisenthal, L. M., Marsden, J. A., Dill, P. L. & Macaluso, C. K. (1983) *Cancer Res.* **43,** 749–757.
22. Bailey, M. J., Jones, A. J., Shorthouse, A. J., Raghaven, D., Selby, P., Gibbs, J. & Peckham, M. J. (1984) *Br. J. Cancer* **50,** 721–724.
23. Zimmerman, R. J., VanWinkle, T. J., Mantel, N., Frei, E., III, & Goldin, A. (1986) *Cancer Res.* **46,** 694–700.
24. Freeman, A. E. & Hoffman, R. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2694–2698.
25. Chabner, B. (1982) *Pharmacologic Principles of Cancer Treatment* (Saunders, Philadelphia).
26. Alberts, D. S. & Chen, G. H.-S. (1980) in *Cloning of Human Tumor Stem Cells*, ed. Salmon, S. E. (Liss, New York), pp. 351–359.
27. Hamilton, E. & Dobbin, J. (1982) *J. Cell Tissue Kinet.* **15,** 405–411.
28. Brown, D. B. & Rao, P. N. (1984) *Yale Univ. J. Biol. Med.* **57,** 825–832.
29. Scully, R. E., ed. (1979) *Tumors of the Ovary and Maldeveloped Gonads*, Atlas of Tumor Pathology, Second Series Fascicle 16 (Armed Forces Inst. Pathol., Washington, DC).
30. Miller, B. E., Miller, F. R. & Heppner, G. H. (1981) *Cancer Res.* **41,** 4378–4381.
31. Haskell, C. M., ed. (1985) *Cancer Treatment* (Saunders, Philadelphia), 2nd Ed.