A Murine Model to Evaluate the Ability of in Vitro Clonogenic Assays to Predict the Response to Tumors in Vivo

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ABSTRACT

The use of the human tumor cloning assay as a predictor of clinical response of human tumors to drugs is predicated on the hypothesis that the in vivo response of a tumor to a drug can be correlated with the in vitro response of cells derived from the tumor. To test this hypothesis, we utilized a murine tumor model in which the in vivo and in vitro responses of a tumor can be accurately and reproducibly compared. Drug activity was assessed in P388 leukemia with the standard in vivo antitumor assay (i.p. tumor/i.p. drug administration) and an in vitro assay wherein the ascites tumor cells are removed from mice, treated with a drug, and directly cloned in soft agar to measure clonogenic capacity. The response of P388 cells to analogues within four separate classes of antitumor agents, anthracyclines, anthraquinones, platinum(II) coordination complexes, and phosphinegold(I) complexes was evaluated. The clonogenic assay failed to discriminate between highly active in vivo antitumor agents and analogues with only marginal in vivo efficacy (i.e., doxorubicin and daunorubicin versus rhodomycin A and B, ametrantone versus NSC 276740, cisplatin versus transplatin, [Au(dppe)2]Cl versus [Au(depe)2]PF6). Furthermore, the in vitro clonogenic assay failed to detect carboplatin which was a highly active agent in vivo. The basis for these discrepancies was explored by a more detailed comparison of doxorubicin and rhodomycin B. In vivo or in vitro drug exposure with subsequent measurement of cell kill by the in vitro clonogenic and in vivo tumorigenic assay demonstrated that the in vitro assay overestimated the cytotoxic potency of the drugs relative to the tumorigenic assay. Treatment of tumors in vivo with doxorubicin at doses below the maximally tolerated dose in mice resulted in multiple log cell kill as measured in vitro or in vivo; whereas rhodomycin B was cytotoxic only at dose levels exceeding its maximally tolerated dose. The results indicate that a subset of tumor stem cells capable of forming colonies in soft agar are significantly more sensitive to the cytotoxic effects of anthracyclines than are in vivo tumorigenic stem cells. Cytotoxic potency as measured by an in vitro soft agar clonogenic assay is not an accurate predictor of in vivo antitumor efficacy even in a model in which ascites tumor cells are directly exposed to i.p. drug. The in vitro cytotoxicity assay is useful only as a nonselective prescreen and must be used in combination with other indicators of tumor cell selectivity and dose-limiting organ toxicity.

INTRODUCTION

A major focus in oncological research has been the attempt to identify an in vitro or in vivo test system which can accurately predict for clinical antitumor activity. The demonstration by Hamburger and coworkers (1, 2) and others (3) that cells from human tumors could be selectively grown in primary culture and assayed for drug sensitivity suggested a new and relevant system which could potentially be used for drug screening. A number of laboratories have expended considerable effort to assess the utility of the human tumor stem cell assay in providing a system: (a) wherein the optimal chemotherapeutic strategy for an individual patient's tumor could be determined as a result of in vitro evaluation of sensitivity to a battery of drugs (4–9); and (b) for discovery and preclinical evaluation of new and potentially more disease-specific antitumor agents (10–15).

The use of the human tumor stem cell assay as a predictor of clinical response of human tumors to drugs is predicated on the hypothesis that the in vivo response of a tumor to a drug can be correlated with the in vitro response of cells derived from the tumor. It will be difficult to validate this hypothesis directly in humans because of the complexities of conducting conclusive prospective clinical trials (8). It is, however, possible to evaluate some aspects of the hypothesis in experimental animal tumor models. Towards this objective, we have utilized a murine model in which both the in vivo and in vitro responses of a particular tumor can be accurately and reproducibly measured. In evaluation of structure-activity relationships among different classes of oncolytic agents, it has been our experience (16, 17) and that of others (18, a review) that, even among close congeners, activity in in vitro cellular assays often does not correlate with antitumor activity in vivo. With the recently heightened emphasis (12, 14, 15, 19) on the use of in vitro antiproliferative assays for the detection of new antitumor drugs, we undertook to investigate the discrepancies between in vitro and in vivo endpoints in a widely used, chemosensitive animal tumor model, P388 leukemia. In the setting in which this tumor model is most commonly employed in preliminary drug screening, i.e., i.p. tumor implantation followed by i.p. treatment with drugs early during the course of disease, this in vivo assay most closely mimics an in vitro cytotoxicity screen. Three different treatment protocols were used in these studies directed at the comparison of sets of analogs with markedly different levels of in vivo antitumor activity to determine whether these differences are reflected in the in vitro clonogenic assay.

MATERIALS AND METHODS

Drugs and Chemical Reagents. Daunorubicin, rhodomycin A, rhodomycin B, ametrantone, NSC 276740, NSC 291924 and carboplatin were kindly provided by the Drug Synthesis and Design and Natural Products Branches of the National Cancer Institute, Bethesda, MD. Cisplatin and transplatin were provided by Johnson Matthey, Inc., Materials Technology Division, West Chester, PA. [Au(dppe)2]Cl and [Au(depe)2]PF6 were synthesized as described previously (20). Doxorubicin was purchased from Sigma Chemical Co., St. Louis, MO. Stock chemicals were purchased from Sigma or Thomas Scientific, Swedesboro, NJ.

Maintenance and Evaluation of Tumors. The P388 murine leukemia was maintained by serial i.p. passage in syngeneic B6D2F1 mice as described previously (21). The in vitro and in vivo chemosensitivities of P388 leukemia were determined using three separate protocols (Fig. 1).

Protocol A. In Vivo Treatment/Therapeutic Endpoint: In Vitro Treatment/in Vitro Clonogenic Assay. For in vivo studies, 106 P388 cells were inoculated i.p. in groups of eight female B6D2F1 mice. The drugs were

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2 The abbreviations used are: [Au(dppe)2]Cl, bis[1,2-bis(diphenylphosphino)-ethane]gold(I) chloride; [Au(depe)2]PF6, bis[1,2-bis(diethylphosphino)ethane]-gold(I) hexafluorophosphate; MTD, maximally tolerated dose; ILS, increase in lifespan; IC50 and IC90, drug concentration required to reduce in vitro clonogenic capacity by 50 and 50%, respectively.
Two-tenths ml (5 x 10^5 cells) were then mixed directly into tubes. The cell pellet was resuspended in PBS and the cell number determined by a hemocytometer. In order to separate the P388 cells from contaminating red blood cells, the cell suspension was centrifuged at 200 x g for 30 s at 4°C. The resulting cell suspension was centrifuged and washed in Hanks' balanced salt solution. After 1 h, ascites tumor cells from mice (three per group) were aseptically removed, centrifuged and washed in Hanks' balanced salt solution. Cells were resuspended in RPMI 1640 medium and were diluted into culture medium with a concentration of 10^6 per ml. One-tenth ml aliquots of cells were added to 0.9 ml of an appropriate drug dilution in RPMI 1640 medium and incubated for 1 h at 37°C. Two-tenths ml (5 x 10^5 cells) were then mixed directly into tubes containing 20 ml of RPMI 1640 medium with 0.1% agar, 20% fetal calf serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, 10 μg/ml 2-mercaptoethanol. Five ml aliquots of this 20-ml cell suspension were placed into 15-ml tubes and incubated at 37°C in a humidified 5% CO2 incubator. On Day 6, 1 ml of the metabolizable vital dye, 2-(p-iodophenyl)-3( p-nitrophenyl)-5-phenyl tetrazolium chloride (1 mg/ml), was added to the tubes. After 24 h, the stained viable colonies were counted with a Biotran III automatic count totalizer (New Brunswick Scientific Co., Edison, NJ). The mean surviving fraction ± SD of triplicate samples was determined for each drug concentration.

The concentrations of fetal calf serum and 2-mercaptoethanol used in these experiments were determined based on optimization of P388 ascites tumor cell cloning efficiency in soft agar. Under these conditions, plating efficiency was reproducibly measured to be 0.2% and linear through 5 x 10^5 cells inoculated per 5 ml soft agar growth medium.

Protocol B, In Vivo Treatment/Therapeutic Endpoint: In Vivo Treatment/In Vitro Clonogenic Assay. One million P388 cells were inoculated i.p. into mice as described in Protocol A. On Day 7, ascites cells were removed from five to 10 animals and diluted into culture medium with or without drugs as described in Protocol A. Following 1 h of drug treatment, the cell suspension was centrifuged and washed in Hanks’ balanced salt solution. Cells were resuspended in RPMI 1640 medium and were then either cloned in vitro as described in Protocol A, or 10^6 cells were inoculated into each of six mice per treatment group. Cell kill was determined as described above.

Protocol C: In Vivo Treatment/Therapeutic Endpoint: In Vivo Treatment/In Vivo Clonogenic Assay. One million P388 cells were implanted i.p. into B6D2F1 mice as described in Protocol A. On Day 7, groups of mice were treated i.p. with vehicle or drug at different dosage levels. After 1 h, ascites tumor cells from mice (three per group) were aseptically removed, centrifuged and washed in Hanks’ balanced salt solution. Cells were resuspended in RPMI 1640 medium and either cloned in vitro as described in Protocol A, or 10^6 cells from each of the donor mice were inoculated into each of six bioassay mice. The median survival time of the bioassay mice was determined and cell kill was calculated by comparison with a titration of tumor cells from vehicle-treated donors (22).

The cloning efficiency of P388 leukemia cells implanted in B6D2F1 mice is considerably greater than that determined by exploiting cells into soft agar. We have had considerable experience using this tumor model and have found in the course of 74 titrations of cells in this strain of mice that the TDo5 (number of cells which result in tumor death in 50% of inoculated mice) is 3.3 ± 0.7 cells/mouse. The in vivo doubling time of the tumor is 18.3 ± 1.2 h, and the time to death for a dilution equivalent to 10^6 cells/mouse is 26.5 ± 4.1 days. There is a linear relationship (V = 0.999) between log of tumor cell inoculum and median survival time which allows for determination of tumor cell kill from the survival time of animals inoculated with a known number of cells.

RESULTS

Comparison of the Response of P388 Tumor Cells to Different Classes of Cytotoxic Agents in the Standard i.p. Tumor Assay and the In Vitro Clonogenic Assay. The response of P388 to analogs within four separate classes of antitumor tumor agents was evaluated in Protocol A (Fig. 1, left). This protocol was designed to compare the response of the tumor to these agents in a standard in vivo model and in an in vitro soft agar cloning system.

Anthraccline Antibiotics. Doxorubicin and daunorubicin were clearly superior to rhodomycin A or B when evaluated for in vivo activity against i.p. P388 (Fig. 2, left). Doxorubicin was...
The therapeutic assay in vivo, the rhodomycins were more active against P388 cells in vitro (i.e., more cytotoxic) than were doxorubicin and daunorubicin. The IC₉₀ values for rhodomycin B, rhodomycin A, daunorubicin, and doxorubicin were 0.31, 0.35, 0.45, and 0.60 μM, respectively. The clonogenic assay failed to predict the vastly superior antitumor efficacy of doxorubicin and rhodomycin B compared to that of the rhodomycins. The in vitro assay was predictive for the rank order of the in vivo dose potency (i.e., MTD) but not the degree of activity.

To better simulate the actual ascites environment during in vitro drug exposure, ascites tumors were removed from animals and treated with doxorubicin and rhodomycin B before separation of the tumor cells from the ascites fluid and cloning in soft agar. Under these conditions, both drugs produced dose-response curves which were similar in shape to those shown in Fig. 2, right; however, both compounds were twofold less potent (IC₉₀ of 0.6 and 1.4 μM for rhodomycin B and doxorubicin, respectively).

Anthraquinones. Amantrantrone and two closely related 1,4-[(2-aminoethyl)amino]anthraquinones, NSC 276740 and NSC 291924 (21), were evaluated (Fig. 3). Amantrantrone was curative at a MTD of 16 mg/kg as well as at 8 mg/kg. This drug maintained a highly significant level of in vivo antitumor activity (>100% ILS) at 2 mg/kg. Both NSC 276740 and NSC 291924 were clearly inferior to amantrantrone against P388 in vivo; NSC 276740 produced only a 50% increase in ILS at a MTD of 16 mg/kg and NSC 291924 was inactive (i.e., ILS of <30%) at or below its MTD.

The superior antitumor activity of amantrantrone was not predicted in the clonogenic assay. Amantrantrone and NSC 276740 produced identical dose-response curves in vitro (Fig. 3, right). Although NSC 291924 was completely inactive in vivo, the compound was only about 10-fold less potent than amantrantrone in vitro, producing 90% cell kill at 7 μM.

Platinum(II) Coordination Complexes. Cisplatin was curative and carboplatin nearly so in i.p. P388 and were clearly superior to transplatin (Fig. 4, left). While cisplatin was more dose potent (i.e., lower MTD) than carboplatin in vivo, carboplatin demonstrated significant antitumor activity at doses at or below the MTD of cisplatin (8 mg/kg).

In vitro treatment of P388 tumor cells with cisplatin and transplatin produced monophasic and exponential decreases in the surviving clonogenic fraction with increases in drug concentration. Whereas the IC₉₀ of transplatin was only twofold higher...
than cisplatin, carboplatin produced no cell kill at a concentration of 20 μM. These results are inconsistent with the relative antitumor activities demonstrated by these drugs in vivo.

Phosphinogold(I) Complexes. We have previously reported on the in vivo antitumor activity of bis(diphenylphosphine)ethane and gold complexes thereof (e.g., [Au(dppe)₂]Cl) (20, 23). As shown in Fig. 5, left, [Au(dppe)₂]Cl produced 90% ILS at a MTD of 8 mg/kg, whereas the analog with ethyl-substituents on the phosphines, [Au(depe)₂]PF₆ had no significant in vivo antitumor activity. Although the phenyl-substituted analog demonstrated clearly superior antitumor activity, the compounds had identical activity in the clonogenic assay (Fig. 5, right).

Response of P388 Treated with Doxorubicin and Rhodomycin B in Vitro as Measured by the in Vitro Clonogenic Assay or in Vivo Tumorigenicity. The discrepancies noted above could result from either: (a) a nonequivalence in the methods used to assess cell kill (i.e., clonogenicity in soft agar as opposed to the ability of tumor stem cells to give rise to a progressively growing tumor which kills syngeneic mice); or (b) pharmacological differences associated with exposure to tumor cells to a drug in vivo and in vitro (i.e., rapid clearance of drug by distribution out of the peritoneal cavity or by binding to tissues).

To explore these possibilities, another protocol (B) was designed in which tumors were first removed from mice and then treated in vitro with doxorubicin and rhodomycin B; cell kill was determined by tumorigenicity in vivo or soft agar clonogenicity. In vitro clonogenicity was overpredictive relative to tumorigenicity for the amount of cell kill achieved by in vitro treatment with either doxorubicin or rhodomycin B. However, rhodomycin B was superior to doxorubicin when cells were treated with the drugs in vitro regardless of whether cell survival was determined by clonogenicity or tumorigenicity. The clonogenic IC₉₀ values for both doxorubicin and rhodomycin B are approximately threefold lower in the experiment described in Fig. 6 as compared to those in Fig. 2, right. These experiments were done using similar protocols but were done at different times. While the actual IC₉₀ varied from the two experiments, the relative difference between the two compounds was consistent in both experiments.

Response of P388 Treated with Doxorubicin and Rhodomycin B in Vivo as Measured by the in Vitro Clonogenic Assay or in Vivo Tumorigenicity. A modification of Protocol B was designed in which tumors were treated in vivo with an agent and the cells then removed from the treated mice. Tumor cell viability was subsequently measured by either reimplanting the tumors into mice and measuring tumorigenicity or cloning the cells in soft agar and measuring the surviving clonogenic fraction (Protocol C). Doxorubicin and rhodomycin B were compared in this experiment. As shown in Fig. 7, soft agar cloning of tumor cells treated in vivo with rhodomycin B or doxorubicin indicated a greater degree of cell kill at any given dose level than that measured in the tumorigenicity assay. This discrepancy between these two endpoints was much more pronounced for rhodomycin B than for doxorubicin. Doxorubicin at doses far below the MTD resulted in multiple log cell kill as measured by either tumorigenicity in vivo or clonogenicity in vitro. Doses of rhodomycin B at and below its MTD in mice resulted in no tumor cell kill when measured by tumorigenicity or clonogenicity.
DISCUSSION

The in vitro tumor clonogenic assay described in this paper was not a predictive measure of in vivo antitumor activity for the same tumor even though the tumor model was an i.p. implant/i.p. treatment system most closely mimicking the in vitro assay. This was demonstrated with groups of closely related analogs of agents with previously reported antitumor activity against P388 leukemia. The in vitro clonogenic assay failed to detect an active agent as exemplified by carboplatin (false negative). Although carboplatin was approximately 8-fold less potent than cisplatin with respect to in vivo MTD, the two agents had curative activity at their respective MTDs, and carboplatin had good activity at lower doses comparable to tolerated doses of cisplatin. This dose potency difference cannot account for the lack of activity of carboplatin in vitro, as even at 10-times, the IC50 of cisplatin, carboplatin had no effect on clonogenicity in the in vitro assay.

In the comparison of the anthraquinones, the in vitro assay did indicate that NSC 291924 was quantitatively less active than ametantrone. However, it is notable that, although NSC 291924 was devoid of antitumor activity, it was only slightly less toxic to the host animal than was ametantrone which had curative activity against the tumor. Although less active than ametantrone in vivo, NSC 291924 did have potent in vitro activity at concentrations below 10 μM. Thus, the complete lack of in vivo antitumor activity of NSC 291924 would not have been predicted by the in vitro clonogenic assay data for these compounds. The very minor structural modifications in the terminal side-chain substitutions in the anthraquinone series have a dramatic effect on tumor cell selectivity.

Our results indicate that it would be a typical finding for the tumor clonogenic assay to fail to discriminate between highly active antitumor agents and analogs with only marginal activity (e.g., doxorubicin versus the rhodomycins, ametantrone versus NSC 276740, [Au(dppe)2]Cl versus [Au(depe)2]PF6, and cisplatin versus transplatin). The inability to identify the most effective agent with the in vitro clonogenic assay would diminish the utility of such an assay in attempting to determine the most efficacious therapy for an individual patient's tumor.

The factors which should be taken into account when attempting to predict a clinical response based on an in vitro effect are complex. In vivo parameters such as compound distribution into tissues, serum levels, pharmacokinetics, and metabolism are difficult to simulate for established drugs in an in vitro system. These parameters become even more difficult to control when screening compounds for which little or no clinical or animal pharmacology data is available. Other complexities such as the relative cloning efficiency of tumor cells in vitro and in vivo, in vivo interactions between adjacent normal tissue and tumor tissue which cannot be simulated in vitro, the intrinsic differential sensitivity of normal and tumor cells to the cytotoxic effects of a particular agent, tumor cell heterogeneity, and phenotypic destabilization of tumor cell populations which can occur upon culturing in vitro (24) must be considered when...
attempting to correlate the response of tumors in in vitro and in vivo assays. We utilized the P388 leukemia in an attempt to minimize these variables by: (a) the use of a rapidly growing and chemosensitive tumor which could be routinely and reproducibly removed from mice and cloned in soft agar (using conditions similar to those used in the human tumor stem cell assay); (b) employing an in vivo dosing protocol in which the drug was administered at the site of the tumor; and (c) the use of a tumor in which in vivo and in vitro drug treatments were performed on ascites and suspension cells, respectively. However, as demonstrated in the comparison of doxorubicin and rhodomycin B, even in this most direct and sensitive in vivo model, the lack of predictive capability of the tumor clonogenic assay is likely due to a combination of factors. In vitro treatment of P388 cells resulted in a misleading conclusion of superiority of rhodomycin B regardless of whether subsequent assessment of cytotoxicity was carried out in vitro or in vivo (Figs. 2 and 6). Although rhodomycin B was able to kill P388 cells upon in vivo administration of the drug, this occurred only at dose levels which were toxic to mice (Fig. 7). An obvious major deficiency in the in vitro tumor clonogenic assay is its inability to estimate the differential sensitivity of normal and tumor cells to the toxic effects of a particular drug. However, if normal tissues in humans proved to be less sensitive to the toxicity of rhodomycin B than those of mice, this anthracycline discovered in the 1960s (25) could prove to have useful antitumor efficacy.

Differences in the in vitro and in vivo cloning efficiencies of P388 tumor cells appear to contribute to the lack of predictability of the clonogenic assay for in vivo antitumor activity. As shown in Fig. 6, the subset of P388 cells capable of forming colonies in soft agar (cloning efficiency of 0.2% for cells taken directly from ascites) may be more sensitive than the overall cell population capable of proliferating in vivo ("cloning" efficiency of approximately 50–100% on i.p. implantation). This difference in the degree of sensitivity of in vitro and in vivo populations of clonogenic cells appears to be a function of both the particular drug and the mode of treatment. The cytotoxicity of rhodomycin B and doxorubicin which resulted either from in vivo or in vitro treatment of P388 cells was greater when measured by the clonogenic assay than by the in vivo tumorigenicity assay. This difference was significantly greater for rhodomycin B following in vivo treatment (Fig. 7).

Previous studies with transplantable murine tumors have addressed the question of whether tumor stem cell cloning assays could be used to determine the sensitivity of the tumor to various anticancer drugs. In a report by Steel and Adams (26), the stem cell response of the in vivo Lewis lung carcinoma to single doses of cyclophosphamide was studied by three assay techniques: in vitro colony formation, lung colony formation, and the endpoint dilution assay. In these studies, the reductions in surviving fractions of the tumor stem cell population in response to this alkylating agent was equivalent when measured in all three assays. For doxorubicin, we found a reasonable correlation of in vitro colony formation and in vivo bioassay in determination of cell kill occurring in drug-treated mice. However, there was a clear-cut difference in the two endpoints for
Fig. 6. Dose-response of P388 leukemia treated with doxorubicin (A, △) and rhodomycin B (●, ○) in vitro as measured by the in vitro clonogenic assay (open symbols) or the in vivo tumorigenic assay (closed symbols) as described for protocol B.

Fig. 7. Dose-response of P388 leukemia treated with doxorubicin (A, △) and rhodomycin B (●, ○) in vivo as measured by the in vitro clonogenic assay (open symbols) or the in vivo tumorigenic assay (closed symbols) as described for protocol C.

Ogawa et al. (27, 28) studied a mouse myeloma and bone marrow stem cell sensitivity by in vitro clonogenic and spleen colony assays and found that the myeloma cells were much more sensitive to melphalan than were normal marrow progenitor cells; with the same differential sensitivity observed by both the in vitro and in vivo techniques. Furthermore, the differential effect of melphalan appeared to be dependent upon intrinsic properties of these cell classes rather than on differences in their proliferative states. The differential potency of melphalan against the tumor and hemopoietic cells predicted for the therapeutic responses of mice with advanced myeloma. More recently, Park et al. (29) have demonstrated the value of this model in the prediction of chemotherapy responses in human leukemia. The usefulness of these models as a predictive measure of therapeutic efficacy is limited to those drugs with activity against lymphomas and leukemias and in which hematopoietic toxicities are dose limiting. Other investigators have attempted to correlate the response of human solid tumors in in vitro and in vivo bioassays (14, review). For example, Bateman et al. (30) used malignant melanomas grown in culture and in immunosuppressed mice to test the correlation between the two methods of exposure of human tumor cells to eight chemotherapeutic agents. While the authors suggest a positive correlation between the in vivo and in vitro results taking into account differences in the relative in vitro toxic doses of the drugs, the studies were not sufficiently comprehensive to allow precise conclusions to be drawn. Zirvi et al. (31) reported a positive correlation between the in vitro and in vivo response of human colon adenocarcinoma cells to five anthracyclines and to 5-fluorouracil. As only one compound, 4-deoxydoxorubicin, had significant activity against the tumor in vivo, the conclusions from these studies were limited. In addition, the in vivo data which served as the basis of the authors' conclusions was extracted from previously reported results for four out of six of the compounds (i.e., the drugs were not tested in vitro and in vivo against the identical tumors).

Our findings confirm the necessity of demonstrating some measure of selective toxicity by an agent against normal versus tumor cells, either in vitro or in vivo, when attempting to predict in vivo antitumor activity based on in vitro cytotoxicity. As demonstrated here, in comparing either different classes of active compounds (e.g., cisplatin versus daunorubicin) or analogs of active compounds (e.g., doxorubicin versus rhodomycin B), relative cytotoxic potency in vitro is not a predictor of relative efficacy in vivo. The use of in vitro cytotoxicity assays to design clinical protocols, direct selection of appropriate drugs for individual patients, or to identify drugs as candidates for development must be carried out in conjunction with reliable indicators of tumor cell selectivity. Experience would indicate that adoption of in vitro cytotoxicity assays as the predominant method for identifying antineoplastic lead compounds will result in selection of many drugs which would fail to demonstrate activity in vivo against cell lines sensitive to their effects in vitro.

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